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Full Length Article

Foliar Application of Choline Chloride and Ferulic Acid Regulated Osmolyte Accumulation, Nutrient Acquisition and ROS Metabolism to Lessen Chromium Effects on Barley (*Hordeum vulgare*)

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Abstract

Chromium (Cr) toxicity negatively impacts plant growth, which can lead to decreased agricultural productivity and food security. This study investigated the potential effect of choline chloride (CC) and ferulic acid (FA) in mitigating the toxic effects of Cr on barley plants. The aim was to enhance growth, improve metabolite accumulation, strengthen antioxidant defense, reduce oxidative damage, enhance nutrient status, and minimize Cr uptake in two barley (*Hordeum vulgare* L.) varieties, Talbina-21 and Jau-17. A concentration of 150 μM of Cr significantly decreased growth parameters. However, foliar applications of CC (5 m*M*), FA (0.5 m*M*) and or a combination of both (CC+FA) improved growth traits, with the most significant benefits observed in Talbina-21. The experimental design was a completely randomized design with three replications of each treatment arranged in a factorial manner. Cr stress led to a reduction in chlorophyll content and relative water content, but CC, FA, and CC+FA treatments effectively enhanced these parameters, particularly in Talbina-21. The treatments also increased the accumulation of secondary metabolites, osmolytes, and antioxidants, such as phenolics, flavonoids, proline, glycine betaine, and ascorbic acid. Furthermore, CC and FA spray enhanced the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), which helped in scavenging reactive oxygen species (ROS) and preventing oxidative damage. The combination of CC and FA exhibited a more pronounced effect on the mitigation of Cr damage compared to CC and FA alone. Additionally, CC, FA, and CC+FA treatments substantially reduced Cr accumulation in the roots, stems, and leaves, with CC+FA showing the greatest reduction. Overall, the CC+FA treatment was the most effective in alleviating Cr-induced stress, promoting barley growth, enhancing antioxidant defense and minimizing Cr accumulation in plant tissues. This cost-effective and sustainable approach offers a promising strategy to improve crop productivity and ensure food safety in Cr-contaminated soils.

Keywords: Choline chloride; Ferulic acid; Oxidative stress; Antioxidant; Barley; Osmolytes; Chromium accumulation

Introduction

Metal toxicity is a growing concern in our environment, with industries and mining being major sources of metal pollution that can have widespread impacts on agriculture, human health, and ecosystems (Babaniyi *et al.* 2025). Chromium (Cr), primarily found in the form of chromite, is commonly used in metallurgical, leather, and chemical industries, leading to its release into the air and soil, contributing to pollution (Koleli and Demir 2016). Human activities release approximately 75,000 tonnes of Cr into the atmosphere annually, with about 33% of its existing as hexavalent Cr (Cr^{6+}). This form is highly soluble in water, making it prone to contaminating groundwater and surface water. While hexavalent Cr is toxic, non-essential, and carcinogenic, trivalent chromium (Cr^{3+}) has lower toxicity and bioavailability (Kieber *et al.* 2002). Excessive Cr exposure in humans can lead to oxidative stress, liver dysfunction, neurological impairments, and gastrointestinal issues (Davies *et al.* 2022). Elevated levels of Cr in plants can lead to toxicity, resulting in reduced growth, impaired photosynthesis, decreased uptake of essential minerals and lower crop quality and yield (Zaheer *et al.* 2022). Excessive Cr exposure can adversely affect human health, wildlife (Sharma *et al.* 2022), and induce plant stress, resulting in distinct ultrastructural changes in plant cells (Rehman *et al.* 2019). Research suggests that the elevated production of free radicals in response to plant stress can impede growth.

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Although the mechanism of Cr^{6+} uptake in plants remains not fully understood, various strategies, including conventional techniques and modern genetic approaches, are being used to protect plants from the phytotoxic effects of metals by enhancing plant defense mechanisms, such as increasing the biosynthesis of organic solutes (Rasheed *et al.* 2018).

Ferulic acid (FA) is an antioxidant that enhances plant tolerance to stressful conditions. Its antioxidant properties improve plant growth and photosynthesis rate, fortify cell walls, and influence various growth and physiological processes (Linić *et al.* 2021). FA also aids in mineral uptake, enhances chloroplast activity, and boosts photosynthetic rate. Additionally, it modulates the synthesis of biochemical metabolites (Bartwal *et al.* 2013), protects plants under stress, activates tolerance mechanisms, and signals transduction pathways (Linić *et al.* 2021). As a phenolic compound, FA helps prevent lipid peroxidation, promotes ascorbate regeneration, and stimulates catalase activity in plants (Yildiztugay *et al.* 2019).

Choline chloride (CC) is an organic compound that plays a crucial role in enhancing plant tolerance to stressful conditions. It acts as an osmoprotectant, helping cells maintain water balance and defend against osmotic stress (Riaz *et al.* 2021). Choline chloride also protects cellular components like proteins, lipids, and DNA from oxidative damage, enabling plants to better cope with environmental challenges (Tayebati *et al.* 2015). Moreover, it is essential for the formation of phospholipids, key components of cell membranes and can regulate the balance between stress-responsive and growth-promoting hormones to promote plant growth (Gou *et al.* 2015). Choline chloride is essential for plant growth and plays a key role in triggering a defensive response to stress. It also regulates the production and accumulation of plant osmolytes such as phenolics, proline, glycine betaine, and flavonoids, which are crucial for plant growth and stress resistance (Riaz *et al.* 2021). Studies have shown that applying different doses of choline and its derivatives ranging from 1.5 to 30 m*M* at various growth stages can help plants overcome severe environmental challenges (Huo *et al.* 2016; Riaz *et al.* 2021).

Barley (*Hordeum vulgare* L.) is a significant cereal crop globally, ranking fifth after wheat, rice and corn (Sato 2020). It plays a crucial role in human nutrition, animal feed, and the brewing industry, particularly in malt production. In Pakistan, barley, known as "Jao", is a staple crop rich in 80% carbohydrates, 12% protein, 2–3% lipids, 10% moisture, 2–3% minerals, 1% dietary fiber and vitamins (B and E), contributing to food security and economic growth (Das *et al.* 2016). Barley grains also contain a soluble fiber called *β*-glucan, which helps lower blood cholesterol (Ali *et al.* 2013). However, barley's growth and yield can be adversely affected by Cr stress, with concentrations as low as 100 μM kg^{-1} dry weight causing harm to plants (Ali *et al.* 2013; Davies *et al.* 2022). Cr stress has a detrimental impact on barley, reducing its growth attributes and decreasing biomass accumulation (Davies *et al.* 2022).

Despite their individual roles in enhancing plant stress tolerance, there is limited research on the combined effects of CC and FA in mitigating Cr stress in barley plants. Given the significant nutritional value of barley and its importance for food security in regions like Pakistan, improving its resilience to environmental stressors such as Cr toxicity is crucial. This study aims to explore the synergistic potential of CC and FA in enhancing barley's growth, improving antioxidant activity, reducing Cr uptake, and mitigating Cr-induced oxidative stress. We hypothesized that the combination of CC and FA may enhance barley growth and stress tolerance by improving physiological processes, antioxidant defense, and osmolyte accumulation, while simultaneously reducing Cr uptake and mitigating oxidative damage. This work studied the combined effects of CC and FA on barley plants under Cr stress, offering an opportunity to develop a more effective and environmentally friendly strategy for phytoremediation. The results of this study may not only enhance the understanding of barley's response to Cr toxicity but also have broader implications for improving the resilience of other crops exposed to similar abiotic stresses.

Materials and Methods

Experimental design and growth condition

Seeds of two improved barley varieties, Talbina-21 and Jau-17 (Afzal *et al.* 205), were obtained from the Agricultural Research Institute (AARI) in Faisalabad, Pakistan for a pot experiment conducted at the Botanica Garden, Department of Botany, Government College University, Faisalabad, Pakistan from November to March 2024. The study followed a completely randomized design with three replicates. The average maximum temperature during the experiment was 20 ± 2°C and the minimum was 11 ± 2°C. Soil physiochemical properties were analysed using Jackson's method (1962) before sowing (Table 1). Two weeks after germination, 150 μM Cr stress in the form of $K_2Cr_2O_7$ was applied to pots containing plants. Cr solution containing a 50 μM concentration was prepared and applied daily until reaching the desired Cr level (150 μM). Hoagland nutrient solution at 100% strength was used for watering purposes (Hoagland and Arnon 1950). Potassium was provided to the control plants to maintain normal nutrient levels for comparison. Thinning was done after plant emergence, with each pot containing three plants. After four weeks of germination, foliar application of FA at 0.5 *mM* (Afzal *et al.* 2025), CC at 5 m*M* (Riaz *et al.* 2021), or a combination of both (CC+FA) with 0.1% Tween-20 was done during the 21-day treatment period. Control plants were sprayed with 0.1% Tween-20 alone. Plants were harvested at the vegetative stage, and growth, physiological attributes, antioxidant activity, oxidative markers, osmolytes and inorganic mineral contents in both Talbina-21 and Jau-17 were analysed.

Table 1: Physicochemical properties of soil used in the study

Soil property	Values
Soil texture	Sandy loam
Sand (%)	70–85
Clay (%)	12–15
Organic matter (%)	0.31
Moisture Percentage (%)	23
Soil pH	7.49
ECe (dS m^{-1})	2.63
Potassium (mg kg^{-1})	120
Nitrogen (mg kg^{-1})	3.25
Phosphorus (mg kg^{-1})	2.45
Zinc (mg kg^{-1})	0.69
Chromium (μg g^{-1})	0.22

Measurements

Growth related traits: The plants were divided into root and shoot sections and their length were measured. Shoot fresh and dry biomass and leaf area were quantified from the harvested plant material. Following the measurement of fresh plant biomass, plants were dried in an oven at 65°C to measure dry biomass. The leaf area of the barley was calculated using a specific equation (Aldesuquy *et al.* 2014).

$$\text{Leaf area } (\text{cm}^2) = \text{length (L)} \times \text{width (W)} \times 0.75$$

Photosynthetic pigments: Fresh leaf tissue (0.1 g) was homogenized in 80% acetone utilized to determine photosynthetic leaf pigments, including chlorophyll *a*, *b*, total chlorophyll, and carotenoids, following the method described by Arnon method (1949). Absorbance was measured at 480, 645 and 663 nm using a spectrophotometer.

Relative water content of leaf: Cornic and Massacci (1996) method was used to measure the leaf relative water content of barley leaves by determining the fresh weight (FW), turgid weight (TW) and dry weight (DW). FW is measured right after leaf collection, TW is obtained by soaking the leaf in water for several h and DW is determined by drying the leaf at 60–70°C for 48 h. RWC is calculated using the specific equation:

$$\text{LRWC}(\%) = \left[\frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}}\right] \times 100$$

Total phenolics content: To determine the phenolic content, 0.15 g of fresh barley leaves were ground in acetone and centrifuged. The supernatant was then treated with 20% Na_2CO_3 and Folin-Ciocalteu phenol reagent. The optical density was measured at a wavelength of 750 nm (Julkunen-Tiitto 1985).

Total flavonoids content: To determine the flavonoid content, mix 1 mL of leaf extract in 80% ethanol with 300 μL of $NaNO_2$ and incubate at 27°C for 5 min. Then, add 2 mL of 1 *M* NaOH and 0.3 mL of 10% $AlCl_3$ and measure the optical density at 510 nm (Zhishen *et al*. 1999).

Anthocyanin content: A 0.15 g of fresh barley leaves were homogenized in 2 mL of acidified methanol. The mixture was heated in a water bath at 50°C for an hour before centrifugation at 12,000 rpm for 15 min. Anthocyanin levels were determined by calculating the optical density at 520 and 600 nm (Hodges and Nozzolillo 1996). The anthocyanin contents were determined by specific equation:

$$Anthocyanin = A530 - 0.25 \times A657$$

Total glutathione content: Glutathione levels were measured using a modified Griffith's method (1980). 0.25 g of fresh leaves was crushed in metaphosphoric acid with ethylenediaminetetraacetic acid. After centrifugation, the supernatant was used for GSH measurement. To determine GSH content, the supernatant was treated with DTNB and 2-vinylpyridine, and the optical density was measured at a wavelength of 412 nm. GSSG content was determined by treating the supernatant with 2-vinylpyridine and DTNB. GSH content was calculated by deducting GSSG from the total glutathione content.

Enzymatic antioxidants: To assess enzymatic antioxidants, 0.5 g of leaf was homogenized in phosphate buffer and centrifuged at 13,000 rpm for 10 min at 40°C. The resultant extract was utilized to assess activity of superoxide dismutase (SOD) enzyme (Giannopolitis and Ries 1977). The supernatant was mixed with Nitro-blue Tetrazolium (NBT), L-methionine, EDTA, H_2O_2, riboflavin, phosphate buffer, and Triton-X, and the optical density was measured at 560 nm. Catalase (CAT) and peroxidase (POD) activities were measured following the method by Chance and Maehly (1954). Phosphate buffer, hydrogen peroxide, and Guaiacol were added to the extract, and absorbance was measured at 470 nm for POD and 240 nm for CAT. Ascorbate peroxidase (APX) activity was measured with the method of Krivosheeva *et al.* (1996). The reaction mixture consisted of PBS, H_2O_2, ascorbic acid, and enzyme extract from plant material. The absorbance of the mixture was monitored at 290 nm.

Glutathione S-transferase (GST) and Glutathione reductase (GR): GST and GR enzyme activities were measured using the method described by Hasanuzzaman *et al*. (2017). For GST estimation, a mixture of enzyme extract, GSH, Tris-HCl buffer and 2, 4-dinitrophenyl chloride was prepared and the optical density was monitored at 340 nm. For the GR enzyme assay, the mixture contained potassium phosphate buffer saline, EDTA, NADPH and GSSG, with absorbance measured at 340 nm.

Metallothionein (MT): Metallothionein (MT) levels were measured according to Viarengo *et al*. (1997). Fresh leaf tissues were ground in PBS, centrifuged at 4°C for 10 min at 6000 g, and supernatant was combined with NaCl, HCl, EDTA and Ellman's reagent. The absorbance was determined using a microplate reader, with reduced glutathione serving as a standard substance.

Hydrogen peroxide content: To determine hydrogen peroxide levels in fresh leaves, 0.15 g of leaf sample was ground in 10 mL of 5% trichloroacetic acid and centrifuged. Then extract (0.5 mL) was combined with potassium iodide and potassium phosphate buffer. Absorbance was measured at 390 nm (Velikova *et al*. 2000).

Electrolyte leakage: Korkmaz *et al*. (2010) developed a method to assess electrolyte leakage through the measurement of electrical conductivity. Ten leaf discs (1.2 cm) were rinsed with distilled water to eliminate debris and then soaked in 10 mL of distilled water overnight at room temperature to determine the initial electrical conductivity (EC1). The leaf discs were autoclaved at 120°C for 15 min to release all electrolytes into the water. After cooling, the electrical conductivity of the solution (EC2) was measured to estimate the proportion of electrolyte leakage using the equation by Huo *et al*. (2016).

$$Electrolyte\ leakage(\%) = [(EC1)/(EC2) \times 100)]$$

Malondialdehyde (MDA) content: To determine MDA content, we followed the assay by Dhindsa *et al*. (1981). Fresh leaf sample (0.15 g) was ground in 1.5 mL of 20% TCA and centrifuged at 15,000 rpm for 15 min. The resulting mixture (2 mL supernatant + 2 mL 0.5% TBA) was incubated for 25 min. After centrifuging for 10 min at 4,800 rpm, spectrophotometer readings were taken at 532 and 600 nm. The MDA content was measured using an absorption coefficient of 155,000 nmol/mol fresh weight.

$$MDA\ (nmol\ mL^{-1}\ FW) = [(Abs532 - Abs600)/(155000) \times 10^6]$$

Proline content: Proline content in fresh leaf samples was measured following the method outlined by Bates *et al*. (1973). Leaf samples (0.25 g) were homogenized in 3% sulfosalicylic acid, filtered and reacted with acid ninhydrin and acetic acid. The supernatant was incubated, and the absorbance was read at 520 nm.

Glycine betaine: A solution was prepared by dissolving 10 g of KI and 7.5 g of resublimed iodine in 1 M HCl for the measurement of glycine betaine. Filtration was then performed to obtain the acid potassium triiodide solution for total QACs analysis. The filtrate was treated with 1N sulfuric acid and KI, and the resulting solution was cooled in a cooling bath for one hour. Subsequently, 1, 2-dichloromethane and cooled distilled water were added. After the formation of two layers of solution, the lower layer was removed, and the optical density was measured at 365 nm (Grieve and Grattan 1983).

Total soluble proteins: To measure the soluble protein content, 0.15 g of fresh leaf was homogenized in chilled 50 m*M* phosphate buffer at pH 7.5 and centrifuged at 12,000 rpm for 20 min. The extract was then combined with the Bradford reagent, and the optical density was read at 595 nm (Bradford 1976).

Total free amino acids: Fresh leaf 0.15 g were pulverized in 10 mL of 50 m*M* potassium phosphate buffer and centrifuged at 12,000 rpm for 10 min. 1 mL of the supernatant was treated with 1 mL of pyridine and 1 mL of acid ninhydrin, then boiled in a water bath for 30 min. The absorbance was then measured at 570 nm to calculate total free amino acids (Hamilton and Slyke 1943).

Ascorbic acid: 0.15 g of barley leaf was homogenized in a 5% TCA solution, centrifuged and then treated with 1 mL of 2, 4-dinitrophenyl hydrazine and a drop of thiourea. The solution was boiled for 20 min, cooled, and 5 mL of sulfuric acid (80%) was added. The optical density was measured at 530 nm using a spectrophotometer (Mukherjee and Choudhuri 1983).

Salicylic acid: Salicylic acid was extracted and determined using the method described by Malamy and Klessig (1992). Leaf samples weighing 0.5 g were ground with 1.5 mL of 90% methanol and centrifuged at 10,000 rpm for 15 min. The pellets were re-extracted with 1.5 mL of 100% methanol and centrifuged again. The supernatants from both extractions were combined and evaporated under vacuum at 35°C until the methanol was completely removed. The aqueous phases were then mixed with 2.5 mL of distilled water and heated at 80°C in a water bath for 2 min. Subsequently, the samples were incubated with 2.5 mL of sodium acetate buffer (pH 4.5) at 37°C for 10 min, followed by extraction with 10 mL of ethyl acetate/cyclopentane/2-propanol. The absorbance of the resulting solution was measured at 540 nm using a spectrophotometer.

Nutrients analysis: Barley roots, stems, and leaves (0.1 g) were ground and digested with a mixture of H_2SO_4 and HNO_3 (1:3 ratio) in glass test tubes following Wolf's method (1982). The test tubes were heated on a hot plate at 250°C for 2–3 h until the sample turned black. Then, 0.5 mL of $HClO_4$ was added, and the solution was heated until colourless. After cooling, the solution was diluted with dH_2O_2 to 50 mL, filtered, and used for measuring Fe, Zn, and Mg contents. Metal analysis was done using an atomic absorption spectrophotometer (novAA400, Analytik Jena, Germany).

Cr analysis: Plant material was digested according to Allen *et al*. (1986) and total Cr content was measured using atomic absorption (novAA400, Analytik Jena, Germany).

Statistical analysis

The data were analyzed using Statistix (Version 8.0) software with a three-way ANOVA. Significant mean differences between treatments were determined using Tukey's HSD test at a significance level of $P < 0.05$. The PCA was performed using SPSS software. Pearson's correlation matrix was prepared using R studio.

Results

CC, FA and their combined spray effects on growth attributes in barley under Cr stress

The growth parameters of two barley varieties under Cr stress are presented in Table 3. Cr toxicity (150 μM) led to a significant ($P \leq 0.001$) decrease in leaf area, shoot dry weight, root fresh and dry weight in both Talbina-21 and Jau-17 (Table 2). Cr stress also produced a non-significant drop in shoot fresh weight, plant height, root and shoot length in both Talbina-21 and Jau-17 barley varieties

Table 2: ANOVA summary regarding effect of CC, FA and CC+FA spray on growth, redox balance, secondary metabolism and nutrient acquisition in barley under chromium stress

Source of variance	Variety (V), df = 1	Chromium (C), df = 1	Spray (S), df = 3	V × C df = 1	V × S df = 3	C × S df = 3	V × C × S df = 3	Error df = 32	CV
Shoot length	2434.47***	3208.62***	686.77***	6.28ns	109.57***	24.74ns	22.26**	5.92	6.77
Root length	412.165***	411.584***	97.087***	0.444ns	20.666***	2.279ns	5.071**	1.3229	8.19
Shoot fresh weight	990.565***	591.078***	287.348 ***	0.063ns	36.866***	10.805**	8.408**	2.096	5.57
Shoot dry weight	59.7600***	30.6822***	16.7419***	3.7523***	2.5077***	0.1325ns	0.5139*	0.1839	6.75
Leaf area	1614.53***	415.66***	125.48***	34.57***	22.55***	6.60***	2.94*	0.97	3.47
Root fresh weight	272.226***	197.810***	57.060***	40.438***	10.338***	0.809ns	1.255ns	0.971	8.24
Root dry weight	32.6566***	4.8018***	1.7539***	0.2234***	0.4422***	0.0228ns	0.0095ns	0.0275	7.31
Chlorophyll a	6.67943***	2.14463***	0.67745***	0.05088ns	0.09121*	0.02219ns	0.02247ns	0.02951	5.42
Chlorophyll b	10.9838***	2.4911***	0.2598***	0.0007ns	0.0532**	0.0016ns	0.0016ns	0.0133	5.38
Total chlorophyll	34.7939***	9.2585***	1.7760***	0.0632ns	0.2817**	0.0280ns	0.0302ns	0.0639	4.76
Chlorophyll a/b	1.39323***	0.23104***	0.00123ns	0.02099ns	0.00076ns	0.00358ns	0.00193ns	0.00579	4.98
Total carotenoid	0.60259***	0.08875***	0.01730***	0.00690***	0.00357***	0.00068ns	0.00018ns	0.00028	4.48
Relative water content	11164.6***	5073.7***	1069.1***	0.6ns	230.0***	44.0ns	6.8ns	16.9	7.87
Phenolics	3.4532***	32.6742***	1.2462***	0.6260***	0.0834ns	0.6444***	0.1008ns	0.0492	8.53
Flavonoids	3.1270***	24.3153***	1.4695 ***	2.0778***	0.1772**	0.6067***	0.1712**	0.0414	9.48
Anthocyanin	9.5841***	37.6987***	2.5694***	4.9416***	0.1407ns	0.9030***	0.2979**	0.0598	7.12
Proline	32.9899***	50.2030***	3.4956 ***	7.6547***	0.7060**	1.6159***	0.6973**	0.1704	12.33
Glycine betaine	49.3301***	68.0164***	10.4906***	6.9299***	1.7132***	2.8668***	0.9533 ***	0.1533	8.18
Total soluble sugar	514.568***	719.094***	67.326***	141.409 ***	11.822**	19.336***	9.039*	2.748	10.77
Total free amino acid	28.8548***	19.6353***	2.7983***	1.9056***	0.0564ns	0.0716ns	0.2625***	0.0446	6.76
Total soluble protein	226.690***	118.361***	23.515***	8.079***	4.049***	0.255ns	0.212ns	0.440	7.88
Salicylic acid	39.4036 ***	21.9318 ***	2.1969***	6.8075***	0.5541***	0.8293***	0.3741***	0.0791	11.67
Ascorbic acid	75.7117***	47.5041***	2.1349***	11.3209***	0.6510**	1.3631***	0.6245***	0.1586	11.21
Malondialdehyde	446.604***	361.686***	81.966***	48.946***	3.131ns	6.731*	1.454ns	2.160	5.77
Hydrogen peroxide	501.064***	370.867***	40.398***	86.533***	0.746ns	0.85ns	0.433ns	1.956	5.60
Electrolytes leakage	3246.09***	1032.77***	268.19***	83.07***	7.21*	4.48 ns	4.00ns	2.57	4.04
Reduced glutathione	50149.9 ***	36422.9***	1799.0***	4704.2***	351.5*	807.2***	356.6*	114.2	9.33
Oxidized glutathione	114.216***	136.908***	17.404***	15.963***	4.470*	3.592*	0.632ns	1.267	10.38
GSH+2GSSG	60183.0***	45903.0***	1251.7***	5862.9***	232.5ns	635.8**	324.8ns	163.8	9.39
Superoxide dismutase	2213.76***	2787.91***	294.03***	754.86***	49.20***	147.31***	58.67***	7.52	8.63
Peroxidase	1745.1***	21121.1***	1294.1***	1083.0***	28.9ns	604.4***	97.5*	35.4	7.72
Catalase	13499.5***	37882.1***	5701.7***	4095.7***	330.6***	1970.7***	207.1**	38.3	5.11
Ascorbate peroxidase	71046***	622885***	45437***	40164***	2883*	20992***	5183**	844	8.95
GST	4623.05***	1839.18***	220.81***	460.62***	1.47ns	40.18*	40.06*	11.12	6.07
Glutathione reductase	1377.57***	493.71***	84.79***	88.53***	16.82***	22.95***	6.44*	2.11	9.34
Metallothionein	13499.5***	37882.1***	5701.7***	4095.7***	330.6***	1970.7***	207.1**	38.3	5.58
Root Mg	11823.7***	60101.7***	13337.1***	664.4*	729.7**	24.1ns	284.7ns	140.2	2.59
Stem Mg	22809.2***	12751.3***	8221.2***	809.8**	1258.8***	54.9ns	180.5ns	76.2	4.35
Leaf Mg	6003.50***	5609.42***	1571.52***	651.33**	187.70ns	41.86ns	17.41ns	70.07	7.00
Root Zn	2158.02***	194.32***	188.08***	49.38**	21.42**	1.44ns	0.19ns	4.39	5.58
Stem Zn	670.722***	152.059***	39.293***	20.797***	4.732**	0.275ns	0.219ns	1.218	4.36
Leaf Zn	28.0679***	7.4824***	3.5990***	1.1284*	0.5211ns	0.1956ns	0.1273ns	0.2210	3.85
Root Fe	9296.2***	79369.1***	6264.8***	3744.6***	456.7ns	322.3ns	130.9ns	241.5	3.88
Stem Fe	2645.9***	10022.5***	3622.8***	72.1ns	142.1ns	60.0ns	39.8ns	74.1	3.78
Leaf Fe	10512.5***	6631.5***	820.0***	38.8ns	90.5*	34.3ns	0.9ns	32.0	6.13
Root Cr	60.35***	1098.95***	10.15***	83.92***	1.03ns	3.29ns	2.45ns	1.78	25.01
Stem Cr	63.561***	719.501***	9.075***	80.218***	0.905ns	3.734*	1.986ns	1.185	25.55
Leaf Cr	66.670***	419.643***	8.041***	76.804***	0.874ns	4.297**	1.581ns	0.755	27.29

*, **, *** = significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$ levels, respectively. ns = non-significant; df = degree of freedom; C = chromium; CV = coefficient of variation; CC = choline chloride; FA = ferulic acid; GSH + 2GSSG = total glutathione; GST = glutathione S-transferase; Mg = magnesium; Zn = zinc; Fe = iron

compared to control plants. Application of CC (5 m*M*), FA (0.50 m*M*) and their combined spray significantly increased plant height, shoot and root length, shoot fresh and dry weight, and leaf area while showing a non-significant increase in root fresh weight and dry weight under Cr stress in both Talbina-21 and Jau-17 barley varieties. The maximum increase was observed in Talbina-21 plants compared to Jau-17 plants. Furthermore, CC, FA and CC+FA application improved the growth-related traits in the following order: CC+FA > CC > FA spray in both barley varieties under control and Cr toxicity conditions (Table 3).

CC, FA and their combined spray effects on chlorophyll and relative water content in barley under Cr stress

The results for photosynthetic pigments and relative water content of Talbia-21 and Jau-17 varieties of barley under Cr toxicity are presented in Table 4. The data indicated that Cr toxicity (150 μM) significantly ($P \leq 0.001$) reduced total carotenoid content and showed a non-significant decrease in chlorophyll a, chlorophyll b, total chlorophyll, chlorophyll a and b ratio, and relative water content in both Talbia-21 and Jau-17 (Table 2). Foliar application of CC, FA and their

Table 3: Effect of choline chloride, ferulic acid and their combined spray on growth attributes in barley under chromium stress

Treatments	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	Leaf area (cm^2)
Talbina-2021							
NS	36.67 ± 0.88^{f}	14.00 ± 0.57^{f-h}	25.00 ± 0.57^{e-g}	5.77 ± 0.28^{e}	11.00 ± 0.57^{fg}	2.76 ± 0.08^{cd}	29.50 ± 0.28^{de}
CC-5 m*M*	57.33 ± 0.88^{ab}	22.16 ± 0.44^{ab}	37.33 ± 0.88^{b}	8.80 ± 0.15^{ab}	17.16 ± 0.60^{ab}	3.53 ± 0.14^{ab}	37.70 ± 0.88^{b}
FA-0.50 m*M*	51.83 ± 1.09^{bc}	20.16 ± 0.72^{bc}	31.83 ± 1.09^{c}	8.13 ± 0.18^{bc}	16.16 ± 0.44^{a-d}	3.13 ± 0.12^{bc}	35.10 ± 0.55^{bc}
CC+FA	63.67 ± 0.88^{a}	25.00 ± 0.57^{a}	43.67 ± 0.88^{a}	9.50 ± 0.28^{a}	18.06 ± 0.58^{a}	4.04 ± 0.12^{a}	43.06 ± 0.52^{a}
Cr-150 μM	25.00 ± 0.57^{gh}	10.10 ± 0.49^{g-i}	20.00 ± 0.57^{g-i}	5.10 ± 0.10^{e-g}	9.20 ± 0.64^{gh}	2.30 ± 0.11^{de}	28.16 ± 0.60^{d-f}
Cr + CC-5 m*M*	35.00 ± 1.15^{f}	15.00 ± 0.57^{d-f}	29.00 ± 0.57^{cd}	7.50 ± 0.35^{cd}	14.13 ± 0.59^{c-e}	2.90 ± 0.05^{ab}	34.06 ± 0.58^{c}
Cr + FA-0.50 m*M*	32.00 ± 1.15^{fg}	12.67 ± 0.72^{f-h}	27.00 ± 0.57^{de}	6.36 ± 0.38^{de}	13.20 ± 0.64^{d-f}	2.66 ± 0.08^{c}	30.10 ± 0.60^{d}
Cr + CC+FA	46.50 ± 1.04^{cd}	18.13 ± 0.46^{cd}	32.00 ± 1.15^{c}	8.80 ± 0.17^{ab}	16.30 ± 0.65^{a-c}	3.63 ± 0.08^{fg}	36.06 ± 0.63^{bc}
Jau-2017							
NS	30.67 ± 1.20^{fg}	12.50 ± 0.76^{f-h}	21.00 ± 0.57^{g-i}	5.63 ± 0.23^{e}	10.63 ± 0.63^{fg}	1.56 ± 0.12^{fg}	24.00 ± 0.57^{g}
CC-5 m*M*	37.67 ± 0.92^{ef}	14.00 ± 0.57^{e-g}	25.67 ± 0.44^{d-f}	6.17 ± 0.28^{e}	13.06 ± 0.52^{ef}	1.93 ± 0.08^{ef}	27.10 ± 0.58^{ef}
FA-0.50 m*M*	35.00 ± 0.57^{f}	13.16 ± 0.60^{e-h}	24.00 ± 0.58^{e-g}	5.80 ± 0.15^{e}	11.83 ± 0.44^{e-g}	1.70 ± 0.11^{e-g}	25.10 ± 0.58^{fg}
CC+FA	43.16 ± 1.69^{de}	16.26 ± 0.37^{de}	29.00 ± 0.58^{cd}	7.67 ± 0.17^{bc}	14.56 ± 0.34^{b-e}	2.10 ± 0.05^{e}	29.00 ± 0.57^{de}
Cr-150 μM	15.83 ± 0.72^{i}	6.20 ± 0.41^{j}	14.16 ± 0.44^{j}	3.33 ± 0.20^{h}	5.20 ± 0.52^{i}	0.90 ± 0.05^{h}	16.56 ± 0.23^{i}
Cr + CC-5 m*M*	22.50 ± 1.04^{hi}	8.63 ± 0.47^{ij}	19.00 ± 0.57^{hi}	4.26 ± 0.27^{f-h}	7.16 ± 0.46^{hi}	1.03 ± 0.06^{h}	19.80 ± 0.36^{h}
Cr + FA-0.50 m*M*	19.67 ± 0.88^{hi}	8.13 ± 0.46^{ij}	17.00 ± 0.57^{ij}	3.80 ± 0.17^{gh}	6.43 ± 0.50^{hi}	1.00 ± 0.05^{h}	18.13 ± 0.61^{hi}
Cr + CC+FA	25.50 ± 1.44^{gh}	10.13 ± 0.59^{hi}	21.50 ± 0.76^{f-h}	5.16 ± 0.20^{ef}	7.53 ± 0.35^{hi}	1.26 ± 0.08^{gh}	20.13 ± 0.46^{h}

Each column displays the mean values with their standard errors of three replicates. The significant differences between the treatments show by different letters at $P < 0.05$ using the Tukey test. Abbreviations: NS = no spray; Cr = chromium; CC = choline chloride; FA = ferulic acid; cm = centimetre; g = gram

Table 4: Effect of choline chloride, ferulic acid and their combined spray on photosynthetic pigments and relative water content in barley under chromium stress

Treatments	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Chlorophyll a/b ratio	Total carotenoids (mg g^{-1} FW)	Relative water content (%)
Talbina-2021						
NS	3.36 ± 0.08^{c-e}	2.63 ± 0.03^{cd}	6.00 ± 0.05^{cd}	1.27 ± 0.04^{d}	0.45 ± 0.008^{de}	59.96 ± 1.51^{ef}
CC-5 m*M*	3.80 ± 0.11^{ab}	2.94 ± 0.08^{ab}	6.74 ± 0.15^{ab}	1.29 ± 0.04^{d}	0.52 ± 0.005^{b}	86.22 ± 1.85^{ab}
FA-0.50 m*M*	3.60 ± 0.05^{bc}	2.80 ± 0.05^{a-c}	6.40 ± 0.10^{bc}	1.28 ± 0.02^{d}	0.49 ± 0.008^{bc}	76.23 ± 1.85^{bc}
CC+FA	4.23 ± 0.14^{a}	3.10 ± 0.05^{a}	7.33 ± 0.13^{a}	1.36 ± 0.06^{cd}	0.60 ± 0.011^{a}	93.23 ± 1.33^{a}
Cr-150 μM	3.00 ± 0.05^{d-h}	2.20 ± 0.05^{ef}	5.20 ± 0.05^{de}	1.36 ± 0.05^{b-d}	0.40 ± 0.005^{ef}	42.18 ± 1.92^{g-i}
Cr + CC-5 m*M*	3.43 ± 0.14^{b-d}	2.43 ± 0.08^{de}	5.86 ± 0.23^{cd}	1.41 ± 0.09^{b-d}	0.46 ± 0.008^{cd}	63.64 ± 1.57^{de}
Cr + FA-0.50 m*M*	3.40 ± 0.11^{b-d}	2.36 ± 0.03^{de}	5.76 ± 0.14^{cd}	1.43 ± 0.03^{b-d}	0.43 ± 0.012^{de}	55.08 ± 2.66^{e-g}
Cr + CC+FA	3.63 ± 0.09^{bc}	2.63 ± 0.09^{b-d}	6.27 ± 0.18^{bc}	1.38 ± 0.01^{cd}	0.50 ± 0.012^{bc}	68.59 ± 1.69^{cd}
Jau-2017						
NS	2.86 ± 0.08^{e-i}	1.80 ± 0.05^{fg}	4.67 ± 0.13^{e-g}	1.59 ± 0.04^{a-c}	0.28 ± 0.008^{h}	39.43 ± 0.86^{hi}
CC-5 m*M*	3.06 ± 0.08^{d-g}	1.90 ± 0.05^{f}	4.96 ± 0.12^{ef}	1.61 ± 0.05^{ab}	0.31 ± 0.005^{gh}	51.66 ± 2.00^{e-h}
FA-0.50 m*M*	3.00 ± 0.05^{d-h}	1.86 ± 0.06^{fg}	4.86 ± 0.08^{ef}	1.61 ± 0.06^{ab}	0.30 ± 0.006^{gh}	43.99 ± 1.98^{f-i}
CC+FA	3.23 ± 0.08^{c-f}	2.00 ± 0.05^{f}	5.23 ± 0.14^{de}	1.62 ± 0.09^{ab}	0.34 ± 0.008^{fg}	54.17 ± 2.14^{e-g}
Cr-150 μM	2.36 ± 0.08^{i}	1.33 ± 0.06^{h}	3.70 ± 0.15^{h}	1.77 ± 0.03^{a}	0.18 ± 0.005^{i}	22.11 ± 1.91^{j}
Cr + CC-5 m*M*	2.60 ± 0.05^{g-i}	1.43 ± 0.03^{h}	4.03 ± 0.06^{gh}	1.81 ± 0.05^{a}	0.20 ± 0.003^{i}	26.66 ± 1.64^{ij}
Cr + FA-0.50 m*M*	2.50 ± 0.05^{hi}	1.40 ± 0.05^{h}	3.90 ± 0.10^{h}	1.79 ± 0.06^{a}	0.20 ± 0.005^{i}	24.99 ± 1.13^{ij}
Cr + CC+FA	2.73 ± 0.08^{f-i}	1.53 ± 0.03^{gh}	4.26 ± 0.12^{f-h}	1.78 ± 0.02^{a}	0.22 ± 0.008^{i}	31.66 ± 1.99^{ij}

Each column displays the mean values with their standard errors of three replicates. The significant differences between the treatments show by different letters at $P < 0.05$ using the Tukey test. Abbreviations: NS = no spray; Cr = chromium; CC = choline chloride; FA = ferulic acid; FW = fresh weight

combination increased chlorophyll *a*, chlorophyll *b*, total chlorophyll, chlorophyll *a*/*b* ratio, carotenoids, and relative water content in both barley varieties. The enhancement was more pronounced in Talbia-21 compared to Jau-17. The order of increase in photosynthetic pigments and relative water content was CC+FA > CC > FA spray for Talbia-21 and Jau-17 plants under control and Cr toxicity conditions (Table 4).

CC, FA, and their combined spray effects on barley secondary metabolites, osmolytes, salicylic acid and ascorbic acid under Cr toxicity

Results show that when the Talbina-21 and Jau-17 barley varieties were subjected to Cr stress, phenolic content were enhanced significantly ($P \leq 0.001$) compared to control plants (Table 2). CC (5 m*M*), FA (0.50 m*M*) and their combined spray also boosted the phenolic content in both Talbina-21 and Jau-17 varieties (Table 5). Flavonoid content also increased noticeably ($P \leq 0.001$) under Cr toxicity in both Talbina-21 and Jau-17 barley varieties (Table 2). After the application of CC, FA, and their combined spray, flavonoid content was significantly enhanced in both barley varieties (Table 5). A substantial increase ($P \leq 0.001$) was observed in anthocyanin content in both Talbina-21 and Jau-17 barley varieties under Cr toxicity (Table 2). When CC, FA and their combined spray were applied, anthocyanin content was significantly enhanced in plants subjected to Cr stress in both barley varieties (Table 5).

Table 5: Effect of choline chloride, ferulic acid and their combined spray on biochemical parameters in barley under chromium toxicity

Treatments	Phenolics (mg g^{-1} FW)	Flavonoid (mg g^{-1} FW)	Anthocyanin (mg g^{-1} FW)	Glycine betaine (μmol g^{-1} DW)	Total soluble sugar (mg g^{-1} FW)	Total free amino acid (mg g^{-1} FW)	TSP (mg g^{-1} FW)	Proline (μmol g^{-1} FW)
Talbina-2021								
NS	1.57 ± 0.03^{fg}	1.07 ± 0.02^{h}	2.26 ± 0.06^{gh}	$3.50 \pm 0.05^{d-f}$	10.42 ± 0.39^{gh}	$2.47 \pm 0.11^{f-h}$	10.38 ± 0.23^{cd}	$2.22 \pm 0.15^{d-h}$
CC-5 m*M*	2.02 ± 0.08^{fg}	1.50 ± 0.05^{gh}	2.54 ± 0.05^{gh}	$4.32 \pm 0.07^{c-e}$	12.44 ± 0.33^{gh}	2.99 ± 0.06^{fg}	12.28 ± 0.18^{ab}	$2.57 \pm 0.26^{e-h}$
FA-0.50 m*M*	1.67 ± 0.06^{g}	1.33 ± 0.03^{h}	2.47 ± 0.07^{gh}	$3.58 \pm 0.24^{e-g}$	$11.77 \pm 0.25^{g-i}$	$2.80 \pm 0.04^{f-h}$	11.17 ± 0.14^{bc}	$2.52 \pm 0.10^{e-h}$
CC+FA	2.08 ± 0.05^{fg}	$1.70 \pm 0.01^{e-h}$	$3.03 \pm 0.06^{e-g}$	5.07 ± 0.24^{cd}	$15.43 \pm 0.39^{d-f}$	3.66 ± 0.04^{de}	14.18 ± 0.12^{a}	$3.14 \pm 0.21^{d-f}$
Cr-150 μM	2.87 ± 0.05^{de}	$2.22 \pm 0.10^{d-f}$	3.84 ± 0.07^{cd}	$5.13 \pm 0.10^{b-d}$	18.39 ± 0.35^{d}	3.81 ± 0.08^{cd}	6.76 ± 0.10^{ef}	4.12 ± 0.12^{cd}
Cr + CC-5 m*M*	4.03 ± 0.06^{ab}	3.71 ± 0.03^{ab}	5.59 ± 0.07^{a}	8.56 ± 0.22^{a}	25.79 ± 0.78^{b}	5.08 ± 0.07^{ab}	9.51 ± 0.18^{cd}	5.92 ± 0.09^{b}
Cr+FA-0.50 mM	3.75 ± 0.04^{bc}	3.27 ± 0.06^{bc}	4.74 ± 0.06^{b}	6.26 ± 0.13^{b}	22.46 ± 0.45^{c}	4.56 ± 0.06^{bc}	9.51 ± 0.18^{cd}	5.22 ± 0.19^{bc}
Cr + CC+FA	4.59 ± 0.05^{a}	4.12 ± 0.07^{a}	6.15 ± 0.05^{a}	9.60 ± 0.22^{a}	30.99 ± 1.09^{a}	5.45 ± 0.06^{a}	11.84 ± 0.42^{b}	7.31 ± 0.17^{a}
Jau-2017								
NS	1.48 ± 0.04^{g}	1.25 ± 0.03^{h}	2.15 ± 0.04^{h}	2.59 ± 0.18^{g}	9.21 ± 0.77^{i}	1.14 ± 0.06^{j}	6.87 ± 0.11^{ef}	1.64 ± 0.18^{h}
CC-5 m*M*	1.67 ± 0.02^{g}	1.41 ± 0.04^{gh}	2.48 ± 0.05^{gh}	3.10 ± 0.12^{fg}	9.64 ± 0.34^{i}	2.14 ± 0.05^{i}	8.61 ± 0.13^{de}	$1.92 \pm 0.05^{f-h}$
FA-0.50 m*M*	1.57 ± 0.03^{g}	1.30 ± 0.05^{h}	2.35 ± 0.07^{gh}	2.64 ± 0.10^{g}	9.31 ± 0.23^{i}	1.94 ± 0.03^{i}	8.15 ± 0.18^{de}	1.85 ± 0.08^{gh}
CC+FA	1.71 ± 0.05^{g}	$1.55 \pm 0.04^{f-h}$	$2.66 \pm 0.06^{f-h}$	$3.44 \pm 0.14^{e-g}$	11.51 ± 0.30^{hi}	2.35 ± 0.06^{hi}	9.22 ± 0.11^{cd}	$2.10 \pm 0.03^{f-h}$
Cr-150 μM	2.52 ± 0.06^{ef}	$1.96 \pm 0.05^{e-g}$	$3.00 \pm 0.05^{e-g}$	$3.45 \pm 0.14^{e-g}$	12.46 ± 0.31^{gh}	2.34 ± 0.06^{hi}	3.25 ± 0.14^{g}	$2.59 \pm 0.11^{e-h}$
Cr + CC-5 m*M*	3.20 ± 0.04^{cd}	2.58 ± 0.05^{d}	$3.55 \pm 0.04^{c-e}$	4.94 ± 0.09^{cd}	$14.50 \pm 0.36^{e-g}$	$3.02 \pm 0.10^{e-g}$	4.47 ± 0.12^{g}	3.41 ± 0.12^{de}
Cr+FA-0.50 m*M*	2.98 ± 0.05^{de}	2.30 ± 0.03^{de}	$3.39 \pm 0.04^{d-f}$	$4.45 \pm 0.20^{c-e}$	$13.50 \pm 0.80^{f-h}$	$2.51 \pm 0.05^{g-i}$	4.23 ± 0.13^{g}	$2.94 \pm 0.16^{d-g}$
Cr + CC+FA	$3.45 \pm 0.04^{b-d}$	2.73 ± 0.03^{cd}	4.26 ± 0.06^{bc}	5.37 ± 0.23^{bc}	16.58 ± 0.98^{de}	$3.26 \pm 0.05^{d-f}$	4.94 ± 0.11^{fg}	3.60 ± 0.06^{de}

Each column displays the mean values with their standard errors of three replicates. The significant differences between the treatments show by different letters at $P < 0.05$ using the Tukey test. Abbreviations: NS = no spray; Cr = chromium; CC = choline chloride; FA = ferulic acid; TSP = total soluble proteins; FW = fresh weight; DW = dry weight

Cr stress noticeably ($P \leq 0.001$) augmented proline accumulation in both Talbina-21 and Jau-17 varieties (Table 2). After the application of CC, FA, and their combined spray, proline accumulation was significantly enhanced in both barley varieties under Cr stress (Table 5). Glycine betaine was significantly enhanced ($P \leq 0.001$) in both Talbina-21 and Jau-17 varieties under Cr toxicity (Table 2). Furthermore, the application of CC, FA and their combined spray significantly ($P \leq 0.001$) enhanced glycine betaine in both barley varieties under Cr toxicity (Table 2, 5). Cr stress substantially ($P \leq 0.001$) enhanced the total soluble sugar content in Talbina-21 and Jau-17 varieties (Table 2). After CC, FA and their combined spray, the total soluble sugar content was significantly enhanced under stress in both barley varieties (Table 5). A markedly improvement ($P \leq 0.001$) was noticed in total free amino acids in both Talbina-21 and Jau-17 varieties under Cr stress (Table 2). Additionally, these contents were noticeably enhanced in both varieties after CC, FA, and CC+FA spray (Table 5).

Cr stress considerably decreased the total soluble protein in Talbina-21 and Jau-17 barley varieties (Table 2). After the application of CC, FA, and their combined spray, total soluble protein was enhanced in both varieties (Table 5). Salicylic acid and ascorbic acid were significantly enhanced ($P \leq 0.001$) in both Talbina-21 and Jau-17 varieties under Cr stress (Table 2). Additionally, the application of CC, FA and their combined spray further significantly enhanced both salicylic acid and ascorbic acid in barley plants treated with Cr (Table 5). The enhancement was more pronounced in Talbina-21 compared to Jau-17. Furthermore, the order of increase in abovementioned parameters under both control and stress condition was CC+FA > CC > FA spray for Talbina-21 and Jau-17 plants (Table 5).

CC, FA, and their combined spray effects on oxidative stress markers

Results showed that Cr toxicity noticeably ($P \leq 0.001$) increased the MDA and H_2O_2 levels due to oxidative damage to both Talbina-21 and Jau-17 varieties (Table 2). Application of CC, FA and their combined spray, decreased MDA and H_2O_2 in both varieties. However, a maximum decrease was noted in Talbina-21 plants compared to Jau-17 plants (Fig. 1). Electrolyte leakage also considerably ($P \leq 0.001$) enhanced in both varieties under Cr toxicity (Table 2). Foliar spray of CC, FA and their combination decreased the electrolyte leakage in both Talbina-21 and Jau-17 varieties (Fig. 1). Additionally, CC, FA and CC+FA spray decreased the oxidative stress indicators and electrolyte leakage in the following order: CC+FA > CC > FA spray in both barley varieties under control and Cr toxicity conditions.

CC, FA and their combined spray effects on barley non-enzymatic antioxidants activities

Cr stress substantially ($P \leq 0.001$) enhanced the reduced glutathione (GSH) content in Talbina-21 and Jau-17 barley varieties (Table 2). Foliar-applied CC, FA and CC+FA demonstrated a noticeable enhancement in GSH content in both varieties under Cr stress conditions (Fig. 4). Oxidized glutathione (GSSG) content also significantly ($P \leq 0.001$) increased in both Talbina-21 and Jau-17 varieties under Cr toxicity (Table 2). GSSG content decreased after foliar spray of CC, FA and CC+FA under Cr stress conditions in both varieties (Fig. 4). Cr stress markedly ($P \leq 0.001$) boosted the GSH+2GSSG content in both Talbina-21 and Jau-17 varieties (Table 2). GSH+2GSSG content was further enhanced after foliar application of CC, FA and CC+FA in

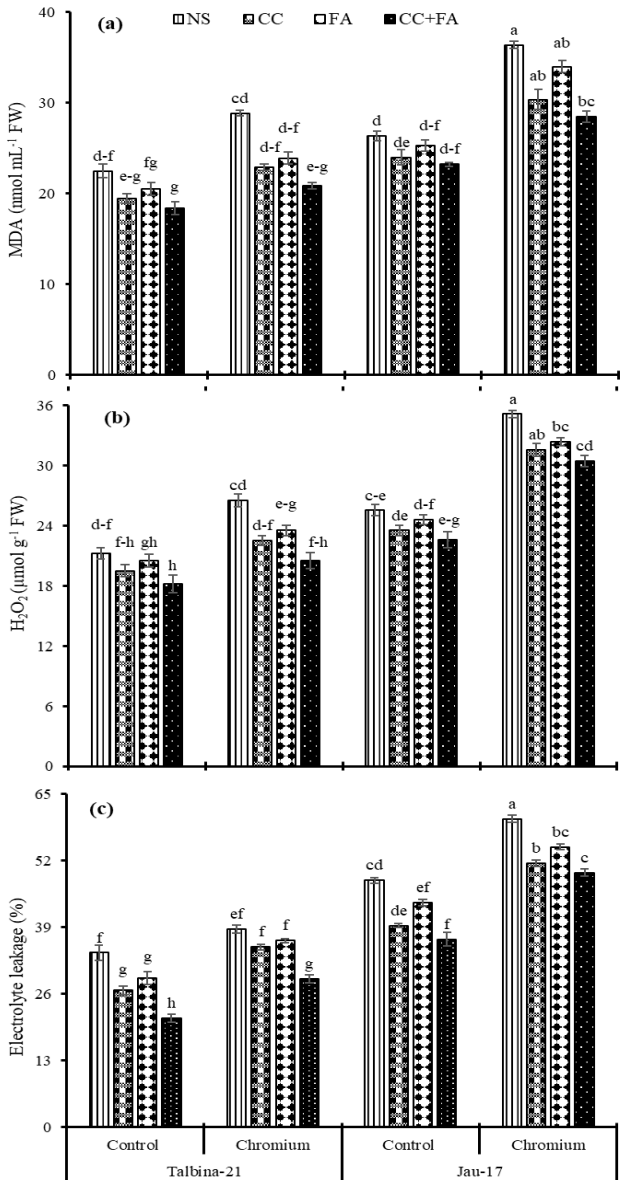

Fig. 1: The effect of choline chloride, ferulic acid and their combined foliar spray on oxidative stress markers (**a, b**) and electrolyte leakage (**c**) in barley plants of Talbina-21 and Jau-17 subjected to chromium stress. The highest significant mean differences are represented with bars, along with different letters at $P < 0.05$ using the Tukey test. Abbreviations: MDA = malondialdehyde; H_2O_2 = hydrogen peroxide; NS = no spray; CC = choline chloride; FA = ferulic acid

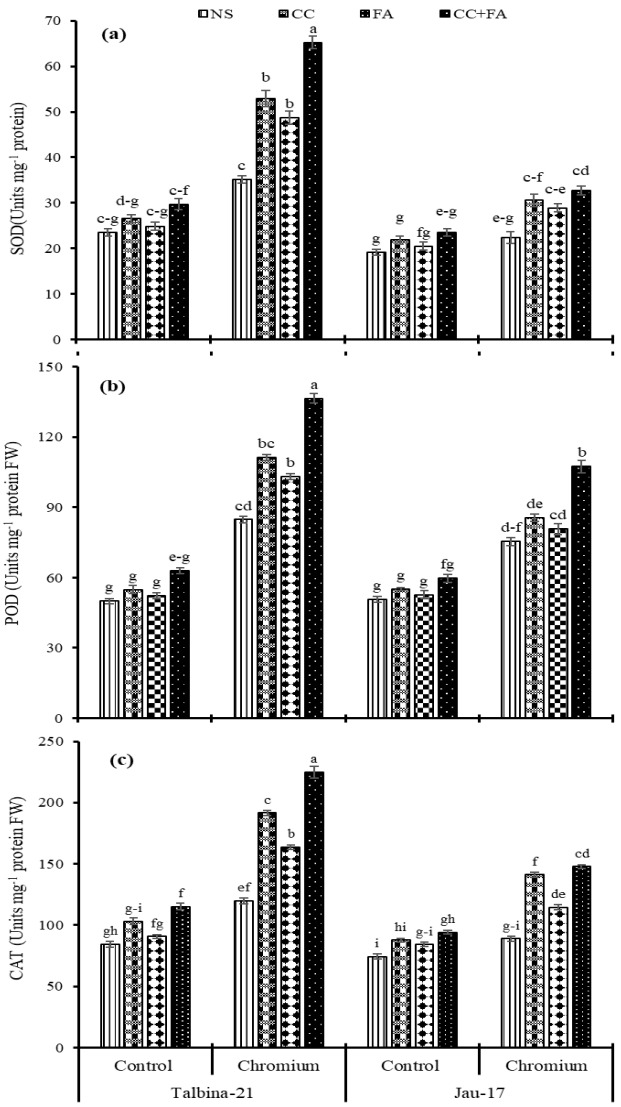

Fig. 2: The effect of choline chloride, ferulic acid and their combined foliar spray on superoxide dismutase activity (**a**), peroxidase (**b**) and catalase activities (**c**) in barley plants of Talbina-21 and Jau-17 subjected to chromium stress. The highest significant mean differences are represented with bars, along with different letters at $P < 0.05$ using the Tukey test. Abbreviations: SOD = superoxide dismutase; POD = peroxidase; CAT = catalase; NS = no spray; CC = choline chloride; FA = ferulic acid

both varieties under Cr toxicity (Fig. 4). Additionally, CC, FA, and CC+FA spray increased or decreased these non-enzymatic antioxidant activities in the following order: CC+FA > FA > CC spray in barley plants under control and Cr toxicity.

Metallothionein significantly ($P \leq 0.001$) increased in both Talbina-21 and Jau-17 varieties under Cr stress (Table 2). Foliar application of CC, FA and CC+FA significantly increased the metallothionein content. And this enhancement was more pronounced in Talbina-21 compared to Jau-17 (Fig. 5). Furthermore, the order of increase in metallothionein was CC+FA > CC > FA spray for Talbina-21 and Jau-17 plants under control and Cr toxicity condition.

CC, FA and their combined spray effects on barley enzymatic antioxidants activities under Cr stress

Our study demonstrated a considerable ($P \leq 0.001$) enhancement in SOD activity in both Talbina-21 and Jau-17

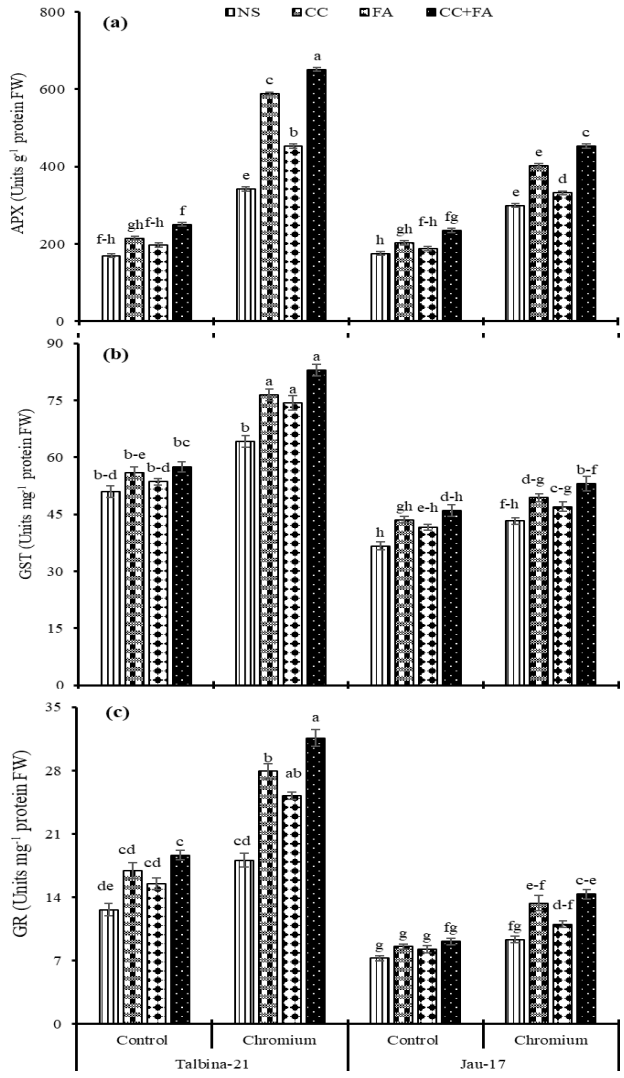

Fig. 3: The effect of choline chloride, ferulic acid and their combined foliar spray on ascorbate peroxidase activity (**a**), glutathione s-transferase (**b**) and glutathione reductase activities (**c**) in barley plants of Talbina-21 and Jau-17 subjected to chromium stress. The highest significant mean differences are represented with bars, along with different letters at $P < 0.05$ using the Tukey test. Abbreviations: APX = ascorbate peroxidase; GST = glutathione S-transferase; GR = glutathione reductase; NS = no spray; CC = choline chloride; FA = ferulic acid

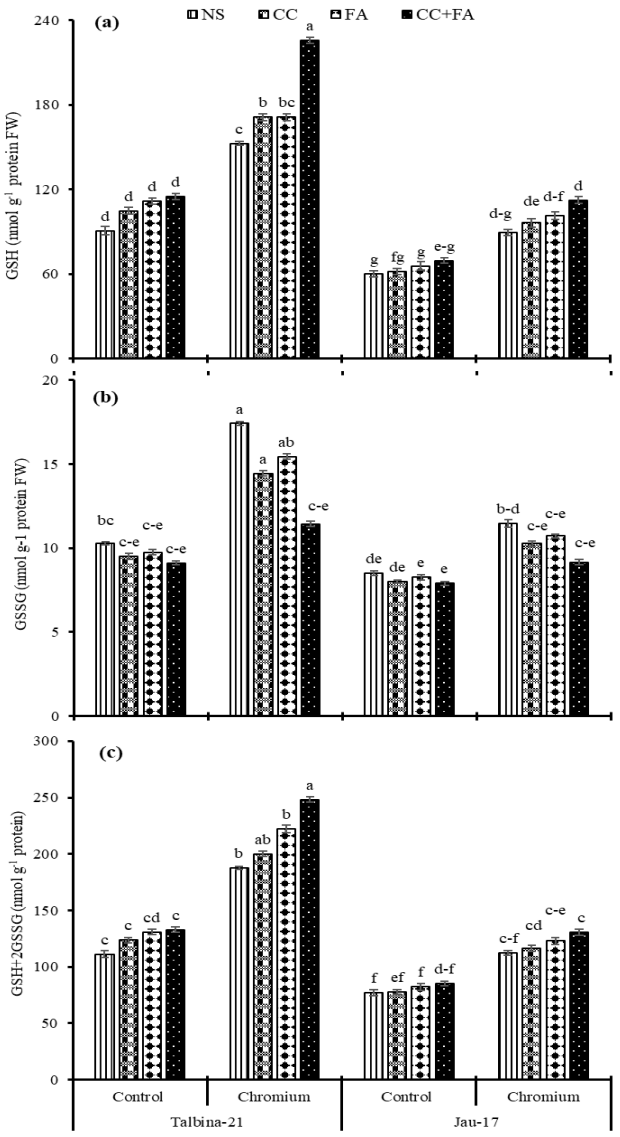

Fig. 4: The effect of choline chloride, ferulic acid and their combined foliar spray on reduced glutathione (**a**), oxidized glutathione (**b**) and total glutathione (**c**) in barley plants of Talbina-21 and Jau-17 subjected to chromium stress. The highest significant mean differences are represented with bars, along with different letters at $P < 0.05$ using the Tukey test. Abbreviations: GSH = reduced glutathione; GSSG = glutathione disulfide; GSH+2GSSG = total glutathione; NS = no spray; CC = choline chloride; FA = ferulic acid

varieties of plants under Cr stress (Table 2). The application of CC, FA, and CC+FA spray led to a noteworthy ($P \leq 0.001$) enhancement in SOD activity in both varieties under Cr stress (Fig. 2). The levels of SOD were increased in both control and stress plants in the order of CC+FA > CC > FA in both barley varieties. Cr stress noticeably ($P \leq 0.001$) rise the POD activity in both Talbina-21 and Jau-17 varieties (Table 2). The foliar spray of CC, FA and CC+FA considerably enhanced the POD activity in Talbina-21 and Jau-17 barley varieties in Cr toxicity (Fig. 2). Both Talbina-21 and Jau-17 plants showed a considerably ($P \leq 0.001$) enhancement in CAT activity under Cr stress (Table 2). The foliar spray of CC, FA, and CC+FA significantly enhanced the CAT activity in both barley varieties (Fig. 2). Our findings indicate a noticeable ($P \leq 0.001$) enhanced in APX activity in both Talbina-21 and Jau-17 plants under Cr stress (Table 2). The application of CC, FA and CC+FA spray induced a significant enhancement in APX activity in both varieties under Cr stress (Fig. 3). Cr stress considerably ($P \leq 0.001$) raised the GST activity in Talbina-21 and Jau-17

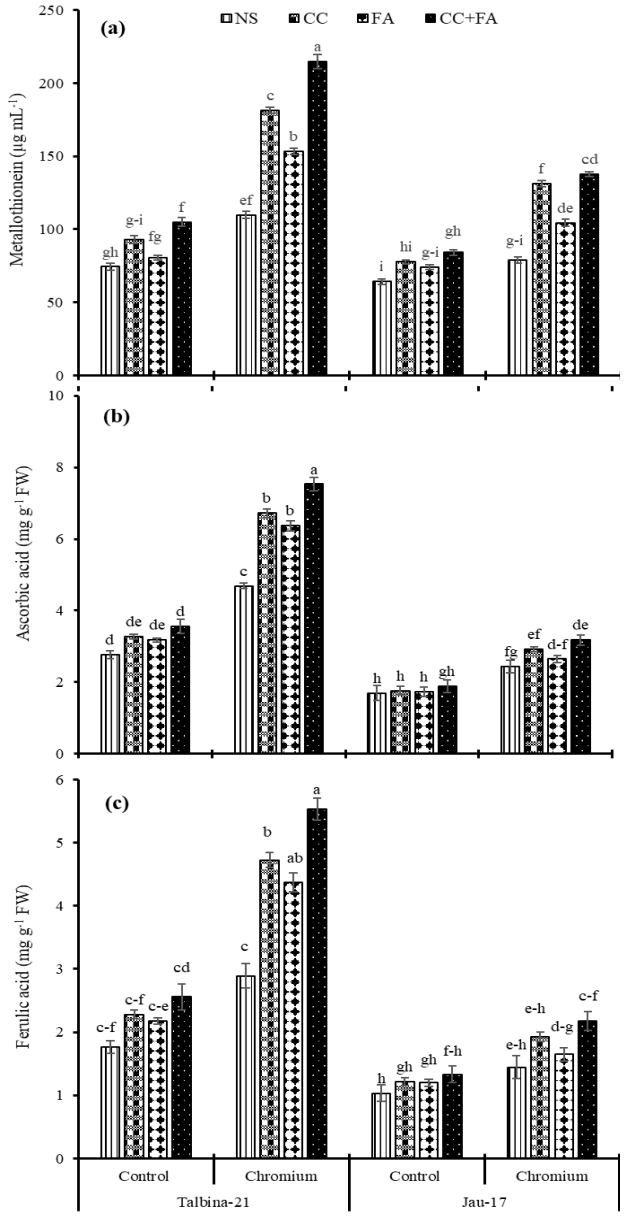

Fig. 5: The effect of choline chloride, ferulic acid and their combined foliar spray on metallothionein (**a**), ascorbic acid (**b**) and salicylic acid (**c**) in barley plants of Talbina-21 and Jau-17 subjected to chromium stress. The highest significant mean differences are represented with bars, along with different letters at $P < 0.05$ using the Tukey test. Abbreviations: NS = no spray; CC = choline chloride; FA = ferulic acid; FW = fresh weight

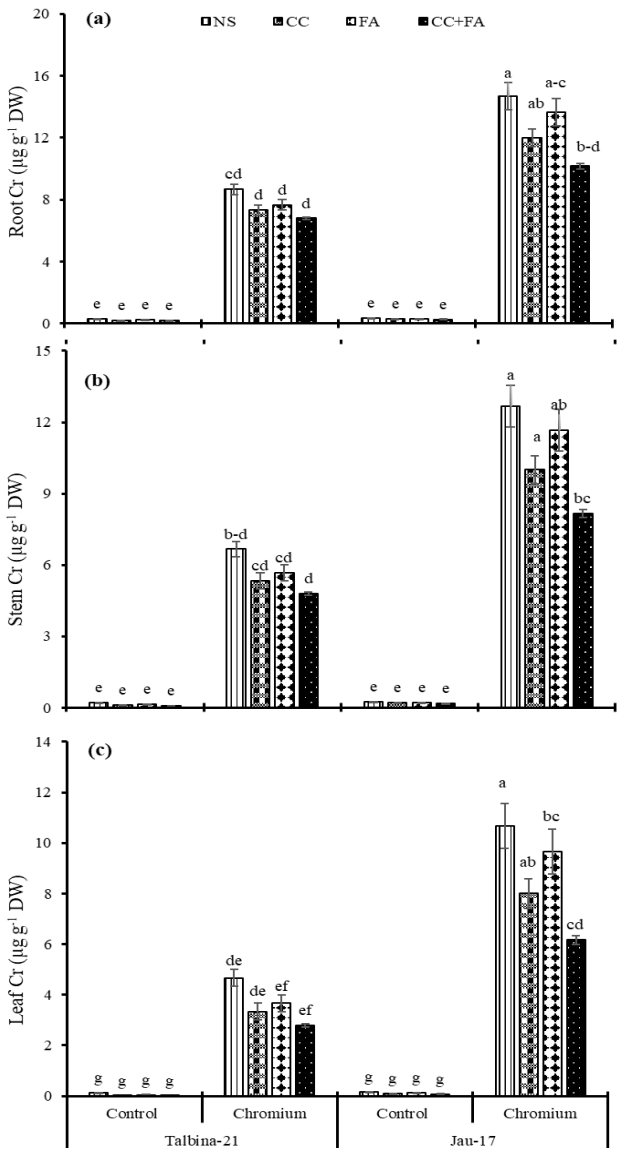

Fig. 6: The effect of choline chloride, ferulic acid and their combined foliar spray on chromium uptake in root (**a**), stem (**b**) and leaves (**c**) in barley plants of Talbina-21 and Jau-17 subjected to chromium stress. The highest significant mean differences are represented with bars, along with different letters at $P < 0.05$ using the Tukey test. Abbreviations: Cr = chromium; NS = no spray; CC = choline chloride; FA = ferulic acid; DW = dry weight

barley varieties (Table 2). The spray of CC, FA, and CC+FA noticeably boosted the GST activity in barley varieties under both Cr toxicity and control condition (Fig. 3). Both Talbina-21 and Jau-17 plants exhibited a considerable ($P \leq 0.001$) enhanced in GR activity under Cr toxicity (Table 2). CC, FA and CC+FA foliar spray considerably raised the activity of the GR enzyme in both barley varieties (Fig. 3).

CC, FA and their combined spray effects on barley minerals accumulation in roots, stems and leaves under Cr stress

Cr toxicity induced a considerable ($P \leq 0.001$) drop in magnesium (Mg) content of the roots, stems, and leaves of both Talbina-21 and Jau-17 barley varieties (Table 2). In contrast, foliar applied CC, FA, and their combination enhanced the Mg content in the root, stem, and leaf in the order of CC+FA > CC > FA in both varieties under control

Table 6: Effect of choline chloride, ferulic acid and their combined spray on magnesium, zinc and iron content in barley under chromium stress

Treatments	Root Mg ($\mu g\ g^{-1}$ DW)	Stem Mg ($\mu g\ g^{-1}$ DW)	Leaf Mg ($\mu g\ g^{-1}$ DW)	Root Zn ($\mu g\ g^{-1}$ DW)	Stem Zn ($\mu g\ g^{-1}$ DW)	Leaf Zn ($\mu g\ g^{-1}$ DW)	Root Fe ($\mu g\ g^{-1}$ DW)	Stem Fe ($\mu g\ g^{-1}$ DW)	Leaf Fe ($\mu g\ g^{-1}$ DW)
Talbina-2021									
NS	457.27 ± 4.69^{fg}	192.94 ± 3.66^{de}	122.94 ± 3.73^{c-e}	39.94 ± 0.84^{cd}	27.61 ± 0.89^{d-f}	12.61 ± 0.27cd	426.00 ± 5.03^{c-e}	225.66 ± 4.77^{d-f}	107.00 ± 3.57^{c-e}
CC-5 m*M*	531.52 ± 4.18^{ab}	254.52 ± 3.02^{a}	144.52 ± 3.02^{ab}	47.38 ± 1.23^{ab}	31.72 ± 0.69^{ab}	13.52 ± 0.30a-c	456.12 ± 4.78^{ab}	256.12 ± 4.78^{ab}	122.27 ± 3.99^{ab}
FA-0.50 m*M*	502.72 ± 4.76^{bc}	223.05 ± 4.39^{b}	133.05 ± 4.39^{a-c}	44.72 ± 1.10^{ab}	29.72 ± 0.64^{a-d}	13.11 ± 0.32a-d	448.93 ± 4.38^{ab}	248.93 ± 4.38^{ab}	115.66 ± 4.76^{a-c}
CC+FA	540.19 ± 5.40^{a}	272.19 ± 4.58^{a}	157.19 ± 5.71^{a}	50.52 ± 1.55^{a}	32.52 ± 0.51^{a}	14.19 ± 0.20a	476.59 ± 5.55^{a}	270.93 ± 5.20^{a}	131.46 ± 3.66^{a}
Cr-150 μM	391.44 ± 6.76^{ij}	173.10 ± 4.45^{ef}	106.44 ± 3.87^{e-g}	35.44 ± 0.66^{e-f}	24.61 ± 0.27^{e-g}	11.94 ± 0.30c-f	349.75 ± 4.83^{f-h}	199.63 ± 4.77^{fg}	86.29 ± 2.80^{fg}
Cr + CC-5 m*M*	451.89 ± 4.22^{d-f}	221.89 ± 4.27^{b}	121.89 ± 4.27^{b-d}	45.21 ± 1.51^{ab}	28.72 ± 0.52^{b-d}	12.52 ± 0.30b-d	380.84 ± 5.88^{d-f}	231.84 ± 6.25^{b-e}	96.17 ± 2.87^{d-f}
Cr + FA-0.50 m*M*	443.84 ± 5.82^{e-g}	193.17 ± 3.93^{c-e}	119.84 ± 4.45^{b-e}	42.87 ± 1.00^{bc}	27.72 ± 0.64^{c-e}	12.26 ± 0.28cd	370.84 ± 5.88^{e-g}	211.51 ± 4.53^{e-g}	94.84 ± 2.41^{d-f}
Cr + CC+FA	481.84 ± 4.06^{cd}	256.84 ± 4.77^{a}	143.51 ± 4.08^{ab}	49.17 ± 0.49^{ab}	30.52 ± 0.51^{a-c}	13.86 ± 0.37ab	423.84 ± 4.14^{b-d}	246.17 ± 3.69^{a-c}	109.51 ± 2.49^{b-d}
Jau-2017									
NS	415.85 ± 5.00^{g-i}	162.86 ± 4.45^{f}	95.52 ± 3.05^{e-g}	28.85 ± 1.02^{f-h}	19.52 ± 0.65^{ij}	10.88 ± 0.16e-g	339.18 ± 4.05^{f-h}	214.18 ± 3.87^{ef}	64.51 ± 2.26^{hi}
CC-5 m*M*	482.17 ± 4.29^{cd}	202.17 ± 7.42^{b-d}	127.17 ± 5.83^{b-d}	34.51 ± 0.98^{d-f}	24.51 ± 0.47^{e-g}	12.12 ± 0.24c-f	441.52 ± 4.05^{a-c}	241.52 ± 4.05^{b-d}	94.84 ± 2.41^{d-f}
FA-0.50 m*M*	479.30 ± 4.45^{c-e}	190.88 ± 5.73^{de}	122.54 ± 4.25^{b-d}	32.49 ± 1.21^{e-g}	23.83 ± 0.41^{f-h}	11.93 ± 0.09d-f	431.05 ± 5.36^{a-c}	231.05 ± 5.36^{b-e}	88.17 ± 1.85^{e-g}
CC+FA	508.81 ± 7.23^{a-c}	218.81 ± 5.90^{bc}	130.14 ± 5.41^{bc}	36.89 ± 1.10^{c-e}	25.22 ± 0.25^{e-g}	12.26 ± 0.28c-e	451.52 ± 5.00^{ab}	251.52 ± 5.00^{ab}	96.17 ± 2.87^{d-f}
Cr-150 μM	361.03 ± 6.02^{j}	131.36 ± 6.02^{g}	83.03 ± 5.05^{g}	24.03 ± 1.07^{h}	17.70 ± 0.65^{j}	10.41 ± 0.29g	315.27 ± 5.72^{h}	186.61 ± 3.56^{g}	59.86 ± 2.39^{i}
Cr + CC-5 m*M*	415.85 ± 5.00^{g-i}	162.86 ± 4.45^{f}	95.52 ± 3.05^{e-g}	28.85 ± 1.02^{f-h}	19.52 ± 0.65^{ij}	10.88 ± 0.16e-g	339.18 ± 4.05^{f-h}	214.18 ± 3.87^{ef}	64.51 ± 2.26^{hi}
Cr + FA-0.50 m*M*	398.85 ± 6.18^{hi}	160.86 ± 5.42^{f}	93.52 ± 3.77^{fg}	26.52 ± 1.68^{gh}	18.52 ± 0.70^{j}	10.75 ± 0.15fg	325.67 ± 5.30^{gh}	197.00 ± 3.29^{fg}	62.51 ± 2.46^{i}
Cr + CC+FA	430.70 ± 3.00^{f-h}	177.37 ± 5.90^{d-f}	102.70 ± 6.27^{d-g}	31.52 ± 0.54^{e-g}	20.52 ± 0.54^{h-j}	11.51 ± 0.18d-g	366.19 ± 5.98^{e-g}	221.19 ± 3.41^{c-f}	71.29 ± 2.49^{g-i}

Each column displays the mean values with their standard errors of three replicates. The significant differences between the treatments show by different letters at $P < 0.05$ using the Tukey test. Abbreviations: NS = no spray; Cr = chromium; CC = choline chloride; FA = ferulic acid; Mg = magnesium; Zn = Zinc; Fe = iron; DW = dry weight

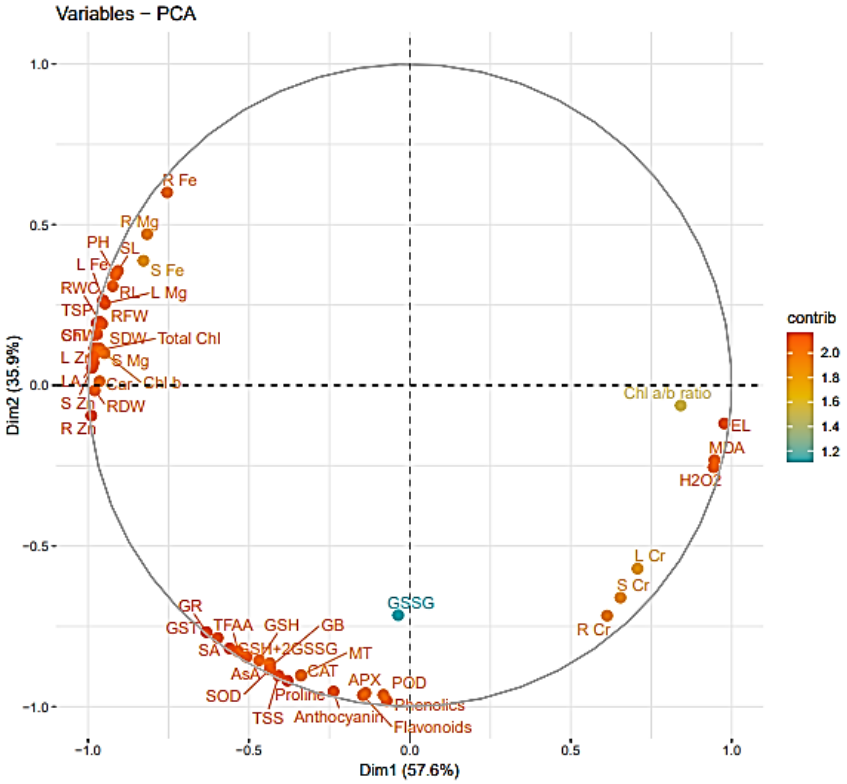

Fig. 7: Principal component analysis loading plots related to growth and physio-biochemical attributes of plants of Talbina-21 and Jau-17 under chromium stress. Abbreviations: SL-shoot length; RL-root length; SFW-shoot fresh weight; SL-shoot length; RL-root length; SFW-shoot fresh weight; RFW-root fresh weight; SDW-shoot dry weight; RDW-root dry weight; LA-leaf area; PH-plant height; Chl *a*-chlorophyll *a*; Chl *b*-chlorophyll *b*; Chl a/b ratio-chlorophyll a/b ratio; Total Chl-total chlorophyll; Car-carotenoids; RWC-relative water content; SMg-shoot magnesium; RMg-root magnesium; L-magnesium-leaf magnesium; SFe-shoot iron; RFe-root iron; LFe-leaf iron; SZn-shoot zinc; RZn-root zinc; LZn-leaf zinc; EL-electrolyte leakage; MDA-malondialdehyde; H_2O_2-hydrogen peroxide; GR-glutathione reductase; GSH-reduced glutathione; GSSG-glutathione disulfide; GSH+2GSSG-total glutathione; GST-glutathione S-transferase; FA; ferulic acid; ASA-ascorbic acid; TFAA-total free amino acids; TSP-total soluble protein; MT-metallothionein; GB-glycine betaine; TSS-total soluble sugar; SOD-superoxide dismutase; POD-peroxidase; CAT-catalase; APX-ascorbate peroxidase; RCr-root chromium; SCr-shoot chromium; LCr-leaf chromium

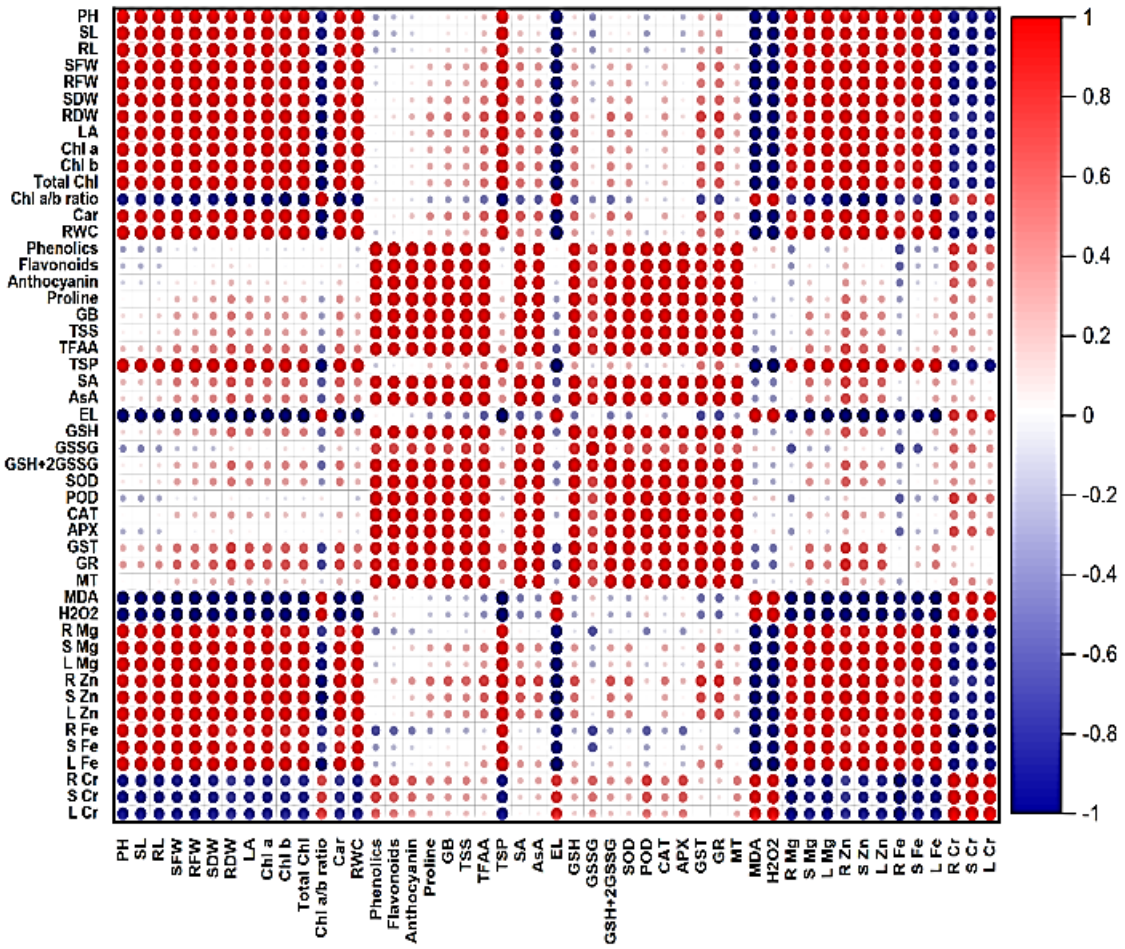

Fig. 8: The correlation matrix between growth and physio-biochemical attributes of Talbina-21 and Jau-17 plants under chromium stress. Abbreviations: SL-shoot length; RL-root length; SFW-shoot fresh weight; RFW-root fresh weight; SDW-shoot dry weight; RDW-root dry weight; LA-leaf area; PH-plant height; Chl *a*-chlorophyll *a*; Chl *b*-chlorophyll *b*; Total Chl-total chlorophyll; Chl a/b ratio-chlorophyll a/b ratio; Car-carotenoids; RWC-relative water content; GB-glycine betaine; TSS-total soluble sugar; TFAA-total free amino acids; TSP-total soluble protein; FA; ferulic acid; AsA-ascorbic acid; EL-electrolyte leakage; GSH-reduced glutathione; GSSG-glutathione disulfide; GSH+2GSSG-total glutathione; SOD-superoxide dismutase; POD-peroxidase; CAT-catalase; APX-ascorbate peroxidase;; GST-glutathione S-transferase; GR-glutathione reductase;MT-metallothionein; MDA-malondialdehyde; H_2O_2-hydrogen peroxide; RMg-root magnesium; SMg-shoot magnesium; L-magnesium-leaf magnesium; RZn-root zinc; SZn-shoot zinc; LZn-leaf zinc; RFe-root iron;SFe-shoot iron; LFe-leaf iron; RCr-root chromium; SCr-shoot chromium; LCr-leaf chromium

and Cr toxicity conditions (Table 6). Similarly, a significant ($P \leq 0.001$) reduction was observed in the zinc (Zn) content in the root, stem, and leaf of plants of both Talbina-21 and Jau-17 varieties under Cr toxicity (Table 2). However, CC, FA and CC+FA applications improved the Zn content in root, stem and leaf tissues of both barley varieties in the order: CC+FA > CC > FA under control and Cr toxicity, respectively. Iron (Fe) content significantly ($P \leq 0.001$) decreased in plants of both Talbina-21 and Jau-17 varieties under Cr toxicity (Table 2). However, foliar treatment of CC, FA and CC+FA enhanced Fe content in both stressed and control plants in the following order: CC+FA > CC > FA in both Talbina-21 and Jau-17 varieties (Table 6).

CC, FA and their combined spray effects on Cr partitioning in barley under Cr toxicity

Cr toxicity markedly ($P \leq 0.001$) raised the Cr accumulation in the roots of both Talbina-21 and Jau-17 barley varieties (Table 2). In addition, foliar application of CC, FA and CC+FA considerably decreased the Cr accumulation in the roots of both Talbina-21 and Jau-17 varieties under Cr toxicity (Fig. 6) Cr toxicity triggered a considerable ($P \leq 0.001$) rise in Cr accumulation in the stems of both barley varieties (Table 2). After foliar application of CC, FA, and CC+FA, the Cr content in barley plant stems decreased (Fig. 2). Leaf Cr accumulation also significantly ($P \leq 0.001$) increased due to Cr stress in barley plants of both Talbina-21

and Jau-17 varieties (Table 2). Foliar spray of CC, FA and CC+FA drop the Cr accumulation in the leaves of both Talbina-21 and Jau-17 varieties (Fig. 6). Likewise, the addition of CC, FA, and CC+FA, to stressed plants showed a drop in Cr accumulation of roots, stems, and leaves in the following order: CC+FA > CC > FA in both barley varieties.

Principal component analysis

The PCA loading plot was used to assess the impact of Cr toxicity on Talbina-21 and Jau-17 barley varieties in terms of growth, physiological, and biochemical attributes. The plot showed that most of the variation was explained by Dim 1 and Dim 2, accounting for 57.6% and 35.9% of the total data, respectively, totalling 93.5%. PC1 was negatively correlated with growth parameters, photosynthetic pigments and nutrient acquisition. PC2 showed a positive correlation with variables such as oxidative stress indicators and Cr content in the roots, stems and leaves of barley plants (Fig. 7).

Pearson's correlation

Pearson's correlation matrix was created to determine the relationships between the variables analyzed and investigate the correlations between parameters influenced by foliar treatments under Cr toxicity. The foliar spray treatments showed positive correlations with growth, pigments, osmolytes, enzymatic and non-enzymatic antioxidants, and nutrient acquisition, while they exhibited negative correlations with oxidative stress markers under Cr toxicity (Fig. 8).

Discussion

As indicated by our results Cr toxicity hindered barley growth by increasing oxidative stress, which impairs physiological processes such as photosynthesis, cell turgor maintenance, and mineral uptake due to the impaired ultrastructure of root cells, in line with previous studies such as Hayat *et al*. (2012). In contrast, foliar sprays of FA, CC, and CC+FA were found to alleviate Cr toxicity and enhance barley growth (Table 3). In line with this, Riaz *et al*. (2021) and Yildiztugay *et al*. (2019), showed that FA and CC could improve plant growth by reducing oxidative damage (Table 3). A Pearson's correlation plot similarly revealed a negative association between growth parameters and oxidative stress markers under Cr stress, supporting the protective role of FA, CC and CC+FA treatments (Fig. 8).

Cr toxicity also decreases photosynthetic pigments by altering the ultrastructure of thylakoid membranes and the antenna size of chloroplasts, negatively impacting the photosynthesis process and chlorophyll biosynthesis. Cr stress inhibits the electron transport chain by denaturing enzyme structures in chloroplasts. Additionally, Cr induces the production of reactive oxygen species, leading to oxidative damage (Qureshi *et al.* 2020). This study also showed that Cr reduces chlorophyll content in plants, consistent with findings by Hadif *et al*. (2015). However, FA, CC, and CC+FA applications enhanced the photosynthetic pigments in both Talbina-21 and Jau-17 varieties. The maximum increase was noted in Talbina-21 plants compared to Jau-17 plants (Table 4). A Pearson's correlation plot confirmed the positive association between foliar spray and chlorophyll content (Fig. 8).

Cr stress increases oxidative stress markers because of lipid peroxidation in cells, enhancing the production of free radicals (Sharma *et al*. 2022). Lipids containing unsaturated fatty acids are particularly vulnerable to oxidative damage, disrupting membrane structure and function, contributing to cell damage (Mansoor *et al*. 2023). In this study, foliar spray of FA, CC, and CC+FA treatments effectively reduced MDA content in barley varieties, suggesting that FA, CC, and their combination play an important role in protecting cell membranes from free radicals under Cr stress (Fig. 1). A Pearson's correlation plot confirmed the negative association between foliar spray and oxidative stress indicators (Fig. 8).

Glutathione (GSH) show a crucial role in enhancing plant tolerance to metal stress by forming chelates with metals through its sulfhydryl group in cysteine. This process helps reduce the formation of free radicals in plant cells, leading to increased detoxification and sequestration of heavy metals (Barrameda-Medina *et al*. 2014). GSSG is formed after the binding of two GSH molecules under Cr stress. The ratio of GSH to GSSG reflects the cellular redox state, with a higher ratio indicating lower oxidative stress and greater plant resilience (Hasanuzzaman *et al*. 2017). Barley plants exposed to Cr toxicity, foliar application of FA, CC, and a combination of FA and CC increased the GSH/GSSG ratio, thereby enhancing plant tolerance (Fig. 4). A Pearson's correlation plot further confirmed a positive correlation between foliar spray treatment and GSH content under Cr stress (Fig. 8).

Enzymatic antioxidants such as SOD, POD, CAT, APX, GST, and GR, along with non-enzymatic antioxidants like MT, play a crucial role in enhancing plant tolerance to Cr stress by scavenging free radicals and protecting plants from oxidative damage (Raza *et al*. 2021). Our study demonstrated a considerable increase in antioxidants in both Talbina-21 and Jau-17 barley varieties, with Talbina-21 showing higher tolerance compared to Jau-17 after foliar application of FA, CC treatment, and their combination under both normal and Cr stress conditions. These findings align with previous research on wheat, where Cr stress led to a rise in antioxidant enzymes to counteract oxidative damage (Askari *et al*. 2021). A Pearson's correlation study revealed a beneficial link between foliar spray treatments and antioxidant levels in the context of Cr poisoning (Fig. 8).

Osmo-protectants, such as total soluble sugar (TSS) proline, phenolics, flavonoids, and glycine betaine, are essential for enhancing plant survival under metal toxicity. Proline maintains the integrity of plant cells by accumulating in cells to maintain cell turgor and perform

cellular activities during stress conditions (Afzal *et al*. 2025). Spray of FA, CC, and CC+FA significantly increased the accumulation of proline content to increase tolerance in both Talbina-21 and Jau-17 barley varieties under Cr stress conditions (Table 5). Soluble sugars protect plant cells by maintaining turgor, stabilizing the cell membrane, and providing energy for cellular processes (Qureshi *et al*. 2020). When FA, CC and CC+FA sprays were applied to Cr-stressed plants, total soluble sugar content significantly increased to enhance tolerance in both barley varieties (Table 5). Additionally, FA and CC treatments increased phenolics and flavonoids contents in plants, which help scavenge free radicals and activate phenylpropanoid metabolism for strain tolerance (Riaz *et al*. 2021). Under Cr toxicity, free amino acids can raise in plant cells, serving as osmolytes and antioxidants. They scavenge reactive oxygen species and protect cellular structures and enzymes from oxidative damage (Anjum *et al*. 2016). The current study discovered that foliar application of FA, CC and CC+FA increased osmolyte levels. This enhancement may have contributed to plant survival under Cr toxicity, as indicated by growth characteristics (Table 5). A Pearson's correlation plot confirmed the positive correlation between foliar spray and osmolyte accumulation in barley plants under Cr toxicity (Fig. 8).

Cr metal competes with other plant minerals such as P, S, Mg, Zn, and Fe due to structural similarity, reducing adsorption sites and forming insoluble compounds in the soil rhizosphere (Zulfiqar *et al*. 2023). Foliar applications of FA, CC, and CC+FA spray improved the uptake of other important minerals in barley plants under Cr stress (Table 6). A Pearson's correlation plot confirmed a positive relationship between foliar spray and nutrient acquisition in barley plants under Cr toxicity (Fig. 8).

Cr toxicity leads to the accumulation of toxic Cr in plant tissues, particularly in the roots, stem, and leaves, where it can replace essential components, disrupt biochemical processes, and hinder growth (Habiba *et al*. 2019). Excessive Cr exposure results in symptoms such as chlorosis and necrosis, which are associated with free radical stress (Anjum *et al*. 2016). In this research, FA, CC, and CC+FA treatments reduced Cr accumulation in the leaves, stem, and roots by enhancing the activity of polyphenol oxidase and PAL, enzymes involved in detoxifying Cr through chelation. FA and CC also mitigated oxidative stress-induced membrane damage by boosting the antioxidant defense system, thereby mitigating the harmful effects of Cr (Fig. 2-3). A Pearson's correlation plot demonstrated a positive relationship between Cr content and oxidative stress indicators, suggesting that lowering Cr absorption with FA, CC, and CC+FA administration reduced stress (Fig. 8). Furthermore, FA, CC and their combination treatments increased growth and biomass in barley plants, indicating their promise as a method for reducing Cr toxicity and improving plant tolerance.

Conclusion

The Cr stress significantly reduced various growth attributes in barley plants, such as shoot and root length, fresh and dry weight, and photosynthetic parameters. However, foliar application of CC (5 m*M*), FA (0.5 m*M*) and their combination notably alleviated these detrimental effects, improving growth characteristics and photosynthetic pigment content. These treatments resulted in enhanced growth parameters, leaf area, and photosynthetic pigments under Cr stress. Furthermore, CC, FA, and their combination increased the accumulation of antioxidants, osmolytes, secondary metabolites, and essential nutrients, while reducing oxidative stress markers, aerial translocation of Cr, and improving nutrient uptake, especially magnesium, zinc and iron. These findings suggest that CC, FA, and their combination have significant potential to mitigate the adverse effects of Cr, improving plant growth and stress tolerance. These properties make CC and FA promising options for phytoremediation in Cr-polluted areas, leading to improved crop yield and quality in contaminated soils. The use of CC and FA offers a sustainable solution for addressing Cr stress and enhancing agricultural resilience.

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Author Contributions

SA, IH, RR and NAA contributed equally to the design, conceptualization, analysis and drafting of the manuscript. All authors reviewed and approved the final draft.

Conflict of Interest

The authors declare no conflicts of interest.

Data Availability

The data presented in this study will be available upon request to the corresponding author.

Ethical Approval

Not applicable to this study.

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