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Full Length Article

Ferulic Acid-Induced Modulation in Photosynthesis, Redox Homeostasis, and Osmolyte Accumulation in Barley (*Hordeum vulgare*) under Chromium Stress

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Abstract

Chromium (Cr) is a significant limiting abiotic factor that negatively impacts agricultural productivity globally. In Pakistan, it is present in water and soil, posing a significant problem for both plants and humans. Therefore, the current study assessed the effectiveness of ferulic acid (FA) application (0.25, 0.5, 0.75, 1.0 mM) in reducing the phytotoxic effects of Cr (150 µM) in barley plants. Chromium stress led to a reduction in growth and chlorophyll content in plants, as well as oxidative damage. FA significantly improved growth and pigment content in plants under Cr stress, helping to mitigate oxidative stress by promoting redox homeostasis through enhanced detoxification of radicals. FA also increased the activities of superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), as well as the accumulation of osmolytes in barley plants under Cr toxicity. The accumulation of Cr content was minimal in plants administered with FA as foliar spray. Overall, FA enhanced tolerance in barley varieties under Cr stress, suggesting that foliar application of FA is a promising technique for mitigating Cr toxicity in barley plants and enabling their cultivation in soils with elevated Cr levels. In the future, FA may be used as an effective technique for stress management in other important crops facing abiotic stressors.

Keywords: Ferulic acid; Oxidative stress; Antioxidant; Barley; Osmolytes; Chromium

Introduction

Heavy metal toxicity is becoming increasingly severe globally. Among all heavy metals, hexavalent chromium (Cr^{6+}) is mobile, non-essential, toxic and carcinogenic, while the toxicity and bioavailability of trivalent chromium (Cr^{3+}) are low, but it is stable (Pan *et al.* 2022). Hexavalent Cr causes environmental pollution and is produced as a byproduct of industrial activities, automobile exhaust, and smoke (Sharma *et al.* 2022). Anthropogenic activities release 75,000 tonnes of Cr into the atmosphere, with 33% of it being in the form of Cr^{6+} (Kieber *et al.* 2002). Increased levels of Cr in plants cause toxicity by reducing growth, photosynthetic activities, uptake of mineral nutrients, crop quality, yield, biomass, and harming human health and wildlife (Picazo-Rodríguez *et al.* 2022). Primary plant response under stress increased the formation of reactive oxygen species (ROS), resulting in the reduction in plant development (López-Bucio *et al.* 2022). The mechanism of Cr^{6+} uptake via plants is still not fully understood (Qureshi *et al.* 2020). Chromium stress negatively affects stomatal conductance, the rate of photosynthesis, non-enzymatic antioxidants, and total flavonoid content, imposing serious threats such as shoot chlorosis, lipid peroxidation, and denaturation of proteins in plants (Sihag *et al.* 2019).

Ferulic acid (FA) is a natural hydroxycinnamic acid present in plant cell walls. It acts as a powerful antioxidant by scavenging reactive oxygen species (ROS) and regulating the activity of antioxidant enzymes (Cao *et al.* 2014). FA could reduce ROS levels and inhibit lung cancer cell proliferation (Zhang *et al.* 2018). Additionally, it helps protect plants from oxidative stress caused by heavy metals like cadmium, lead, and arsenic (Kumar and Pruthi 2014; Linić *et al.* 2021; Khan *et al.* 2024). Its antioxidant properties promote tolerance by stimulating plant growth, increasing photosynthesis rate, chlorophyll, and enzyme activity (Maurya and Devasagayam 2010). FA also strengthens cell walls, promotes the formation of organic compounds such as lignin (Vanholme *et al.* 2010); flavonoids (Murota and Terao 2003), anthocyanins (Li *et al.* 2021), Tannins (Watanabe *et al.* 2012), and coumarins (Shishido *et al.* 2000) and modulates various growth and biochemical developments in plants, leading to increased plant growth (Kumar and Pruthi 2014). Furthermore, FA

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facilitates the assimilation of important nutrients like nitrogen, phosphorus, and iron in plants (Kobza and Einhellig 1987) and enhances photosynthetic efficiency by regulating chloroplast and thylakoid membrane activity (Bartwal *et al.* 2013). As a phenolic compound, FA under heavy metal stress prevents lipid peroxidation, promotes the regeneration of ascorbate, and induces catalase action in plants (Yildiztugay *et al.* 2019).

Barley (*Hordeum vulgare* L.) belongs to the Poaceae family and has a large genome of 5.1 billion base pairs (Sato 2020). In Pakistan, barley, also locally known as "Jao," is a common cereal crop and is widely consumed globally. Barley is rich in starch, protein, dietary fibers, vitamins, lipids, minerals, and polyphenols (Das *et al.* 2016). According to Davies *et al.* (2002), Cr is toxic to plants at concentrations of 100 µmol kg^{-1} of dry weight. However, Cr stress has a detrimental impact on barley, reducing its growth attributes and decreasing biomass accumulation.

The physiological role of FA in alleviating Cr stress in barley has not been extensively studied. We hypothesized that foliar application of FA could alleviate Cr toxicity in barley by enhancing antioxidant enzyme activities, reducing Cr accumulation, and ultimately improving growth and stress tolerance. This study was aimed at to assess the efficacy of FA in promoting growth, enhancing the antioxidant system, increasing biomass, scavenging ROS, and promoting cytosolic solute accumulation in barley plants under Cr intoxication, and exploring possible Cr toxicity mechanisms under the exogenous application of FA.

Materials and Methods

Experimental site, growth condition and design

For this research, seeds of two improved varieties of barley named Talbina-21 and Jau-17 were obtained from Agricultural Agricultural Research Institute (AARI), Faisalabad, Pakistan. The study was conducted in pots from November to February 2023 at the Botanica Garden, Department of Botany, Government College University, Faisalabad. A completely randomized design with three replicates was used. The average maximum temperature was 28°C with a variation of ± 2°C, and the minimum temperature was 13°C with a variation of ± 2°C during the experiment. The soil's physicochemical properties were assessed using Jackson's method (1962) before sowing the seeds (Table 1). Two weeks after germination, 150 µM chromium stress in the form of $K_2Cr_2O_7$ was applied to pots containing plants. For this purpose, a chromium solution containing a 50 µM concentration was prepared, which was applied to each pot daily until the desired chromium level (150 µM) was achieved. Potassium was provided to the control plants to maintain their normal nutrient levels, ensuring they serve as a baseline for comparison. Thinning was done after the emergence of plants, with each pot containing three plants. Hoagland's nutrient solution at

Table 1: Physicochemical properties of soil used in the study

Soil property	Values
Soil texture	Sandy loam
Sand (%)	71-82
Clay (%)	13-17
Moisture Percentage (%)	20
Soil pH	7.8
ECe (dS m^{-1})	0.45-0.52
Organic matter (%)	0.30-0.65
N (mg/ kg)	1.25-4.8
P (mg/ kg)	7-15
K (mg/ kg)	115-135
Ca (mg/ kg)	103
Cr (µg/g)	0.30-1.00

100% strength was used for watering the plants (Hoagland and Arnon 1950). After four weeks of germination, FA (0.25, 0.5, 0.75 and 1.0 mM) were sprayed. FA-treated plants (both with and without Cr) were sprayed with a mixture of FA and 0.1% Tween-20 three times during 21 days of treatment. The control plants were also sprayed with 0.1% Tween-20 alone. After 21 days of spray treatment, the plants were harvested, and various parameters including growth, physiological attributes, antioxidants, oxidative markers, osmolytes, and ascorbic acid in Talbina-21 and Jau-17 varieties were analysed.

Measurements

Growth attributes: Data were collected from each replicate to measure morphological traits such as shoot and root length, fresh and dry weight, and leaf area. The plants were dried in an oven until completely dehydrated, and their dry weight was recorded using a balance. The leaf area of the barley was calculated using the following equation (Aldesuquy *et al.* 2014):

$$\text{Leaf area (cm2)} = \text{length (L)} \times \text{Width (W)} \times 0.75$$

Photosynthetic pigments: To measure the photosynthetic pigments, 0.1 g of fresh tissue was homogenized in 80% acetone following the Arnon method (1949). Absorbance was then recorded at 480 nm, 663 nm, and 645 nm using a spectrophotometer.

Proline estimation: The method described by Bates *et al.* (1973) was employed to determine the proline content. This involved homogenizing 0.25 g of fresh leaf samples in 3% sulfosalicylic acid, filtering the mixture, and then combining it with acetic acid and ninhydrin. The supernatant was incubated, and the absorbance was measured at 520 nm.

Total soluble sugars: According to the method described by Yemm and Willis (1954), 0.15 g of leaf sample was homogenized in 80% ethanol and reacted with an anthrone reagent. The mixture was vortexed and incubated for ten minutes at 97°C. Subsequently, the absorbance was measured at 620 nm after cooling.

Total free amino acids: To measure the total free amino acids, 0.15 g of fresh leaf samples were ground in 10 mL of

50 mM potassium phosphate buffer (pH 7.5), and the mixture was centrifuged for 10 min at 12,000 rpm. One mL of the supernatant was treated with 1 mL of 10% pyridine and 1 mL of 2% acid ninhydrin, then boiled for 30 min in a water bath. The absorbance was measured at 570 nm (Hamilton and Van Slyke 1943).

Phenolics content: To determine the phenolic content, a 0.15 g sample of fresh leaves was homogenized in acetone and then centrifuged. One mL of the supernatant was mixed with 5 mL of Na_2CO_3 (20%) and 1 mL of Folin-Ciocalteau's phenol reagent, and the absorbance was measured at 750 nm (Julkunen-Tiitto 1985).

Anthocyanin content: A 0.15 g of fresh leaves were homogenized in 2 mL of acidified methanol. After 1 h of warming at 50°C in a water bath, the mixture was centrifuged at 12,000 rpm for 15 min. To measure anthocyanin levels, the optical density at 520 and 600 nm were calculated (Hodges and Nozzolillo 1996).

Flavonoids content: To measure the flavonoid contents, 1 mL of leaf extract was mixed with 80% ethanol and 300 µL of $NaNO_2$, and then incubated at room temperature for 5 min. After the addition of 2 mL of 1 M NaOH and 0.3 mL of 10% $AlCl_3$, the optical density was measured at 510 nm (Zhishen *et al.* 1999).

Hydrogen peroxide content: To measure the hydrogen peroxide content in fresh leaves, a 0.15 g leaf sample was ground in 10 mL of 5% TCA (trichloroacetic acid) and then centrifuged. The resulting extract (0.5 mL) was treated with potassium iodide (1.0 M) and 0.5 mL of potassium phosphate buffer. The absorbance was measured at 390 nm (Velikova *et al.* 2000).

Malondialdehyde content: To determine the MDA content, we used the assay developed by Dhindsa *et al.* (1981). A 0.15 g fresh leaf sample was ground in 1.5 mL of 20% TCA and then centrifuged for 15 min at 15,000 rpm. The resulting mixture, composed of 2 mL of supernatant and 2 mL of 0.5% thiobarbituric acid, was incubated for 25 minutes. Subsequently, after centrifugation at 4,800 rpm for 10 min, readings were taken using a spectrophotometer at 532 and 600 nm. The MDA content was calculated using an absorption coefficient of 155,000 nmol mol^{-1} fresh weight.

$$\text{MDA}\left(\frac{\text{nmol}}{\text{mL}}\ \text{FW}\right) = [(\text{Abs}532 - \text{Abs}600)/(155000) \times 10^6]$$

Ascorbic acid: The 0.15 g leaf was homogenized in a 5% TCA solution, centrifuged, and then treated with 1 mL of 2,4-dinitrophenyl hydrazine (2%) and one drop of thiourea (10%). The mixture was heated to boiling for 20 min and then 5 mL of sulfuric acid (80%) was added upon cooling. The optical density was measured at 530 nm using a spectrophotometer (Mukherjee and Choudhuri 1983).

Glycine betaine: To determine the glycine betaine content, 10 g of KI and 7.5 g of resublimed iodine were dissolved in 1 M HCl. Filtration was performed to obtain the acid potassium triiodide solution for total QACs. The filtrate was then mixed with 1 N sulfuric acid and KI, and the resulting mixture was cooled in a cooling bath for one hour. Subsequently, 1,2-dichloromethane and cooled distilled water were added to the mixture, resulting in the formation of two layers. The bottom layer was aspirated, and the optical density was observed at 365 nm (Grieve and Grattan 1983).

Total soluble proteins: To determine the soluble protein content, 0.15 g of fresh leaf was homogenized in chilled phosphate buffer (pH 7.5; 50 mM) and then centrifuged at 12,000 rpm for 20 min. The extract was mixed with Bradford reagent, and the absorbance was measured at 595 nm (Bradford 1976).

Enzymatic antioxidants: To determine the enzymatic antioxidants, 0.5 g of leaf was homogenized in phosphate buffer. The mixture was then centrifuged for 10 min at 13,000 rpm at 40°C. The resulting extract was used to measure the superoxide dismutase enzyme activity (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. The optical density was measured at 560 nm. Catalase and peroxidase activities were measured using the method described by Chance and Maehly (1954). For this analysis, phosphate buffer, hydrogen peroxide, and Guaiacol were added to the extract. Absorbance was then measured using a spectrophotometer at 470 nm for POD and 240 nm for CAT.

Chromium analysis: The dry plant material was digested following the protocol of Allen *et al.* (1986). The total chromium contents were determined using atomic absorption (novAA400, Analytik Jena, Germany).

Statistical analysis

The collected data were analysed using Statistix (Version 8.0) software with a three-way ANOVA. Significant ($P < 0.05$) mean differences between treatments were tested using Tukey's HSD test.

Results

Effect of FA spray on barley growth related attributes in presence of chromium

Chromium (150 µM) toxicity induced a remarkable drop in shoot and root lengths, fresh and dry weight and leaf area in barley varieties. In this context, a significant ($P \leq 0.001$) decline in several growth-related parameters in both Talbina-21 and Jau-17 varieties. Specifically, Cr stress decreased shoot and root lengths, shoot and root fresh and dry weight and leaf area in both varieties compared to control plants. FA (0.25, 0.50, 0.75 and 1.0 mM) significantly ($P \leq 0.001$) enhanced plant growth characteristics in barley plants, particularly evident in plants fed with 0.50 mM FA under Cr stress. In this context, FA (0.50 mM) improved shoot and root lengths, shoot and root fresh and dry weight and leaf area in both Talbina-21 and Jau-17, under Cr stress relative to control (Table 2 and 3).

Table 2: ANOVA summary for growth and physio-biochemical attributes of barley varieties treated with ferulic acid under chromium stress

Source of variance	Variety (V), df = 1	Chromium (C), df = 1	Spray (S), df = 4	V × C, df = 1	V ×S, df = 4	C ×S, df = 4	V× C ×S, df = 4	Error, df = 38	CV
Shoot length	601.67***	1972.27***	416.07***	8.82***	7.53***	20.63***	2.74***	0.13	1.66
Root length	256.267***	232.067***	35.933***	24.067***	3.892***	0.171ns	1.421***	0.179	5.72
Shoot fresh weight	870.204***	418.704***	236.631***	116.204***	12.652***	10.673***	8.735***	0.221	3.16
Shoot dry weight	19.4940***	14.8007***	3.9503***	0.0167 ns	0.2519***	0.5394***	0.0771***	0.0127	2.54
Leaf area	163.35***	2343.75***	214.89***	10.42***	3.97**	16.88***	6.79***	0.82	3.36
Root fresh weight	190.817***	86.400***	11.708***	0.417ns	0.150ns	0.171ns	0.104ns	0.161	8.02
Root dry weight	11.4407***	14.0167***	1.7694 ***	0.0667**	0.4036***	0.0554***	0.0146***	0.0100	7.62
Chlorophyll *a*	0.70294***	6.76289***	0.83137***	0.00011ns	0.00547ns	0.01051**	0.00345ns	0.00305	2.47
Chlorophyll *b*	0.54345***	1.82631***	0.20823***	0.02462***	0.00326ns	0.00138ns	0.00082ns	0.00158	2.80
Total chlorophyll	5.47296***	8.13394***	1.32160***	0.04090***	0.00343**	0.01107***	0.01778***	0.00103	0.85
Total carotenoid	0.02371***	0.22444***	0.02586***	0.00008ns	0.00017ns	0.00020ns	0.00064***	0.00011	4.05
Glycine betaine	2.0823***	34.3433***	7.2570***	0.2718***	0.2616***	1.3850***	0.0406***	0.0025	1.06
Hydrogen peroxide	307.262***	252.264***	51.641 ***	5.113**	2.160**	2.113**	1.374*	0.508	2.50
Malondialdehyde	38.754***	640.443***	146.058***	3.402*	10.661***	3.843***	5.670***	0.635	3.48
Ascorbic acid	13.5912***	29.9934***	17.2874***	2.3714***	0.6364***	0.8060***	0.0896***	0.0112	2.24
Total soluble protein	140.091***	259.939***	24.859***	0.885***	0.257***	0.277***	0.917 ***	0.044	2.34
Total free amino acids	18.5603***	24.2631 ***	10.0215***	1.2908***	0.2259***	0.0582***	0.2594***	0.0108	3.07
Total soluble sugar	151.775***	603.787***	399.088 ***	0.014ns	3.958***	23.669***	1.209**	0.282	3.61
Anthocyanin	1.88230***	7.98969***	6.90841***	0.63976***	0.17403***	0.34778***	0.13080***	0.00216	1.95
Flavonoids	11.4642 ***	4.6371 ***	2.0520***	0.4173***	0.0308ns	0.0181ns	0.0701*	0.0284	7.00
Phenolics	5.1693***	13.7008***	3.2392***	0.6946**	0.0554***	0.1002***	0.0434***	0.0072	3.43
Proline	84.9533***	48.5392***	14.8640***	0.9040**	2.5695***	1.1527***	0.0990ns	0.1070	7.21
Superoxide dismutase	2774.68***	3362.87***	909.06***	435.40***	58.15***	2.10ns	6.01ns	5.20	6.60
Peroxidase	4731.9***	12247.0***	2450.1***	1719.6***	82.0***	10.5 ns	65.8***	8.0	3.46
Catalase	4755.8***	12453.1***	2454.4***	1646.2***	77.1***	9.7ns	77.3***	6.2	2.18
Root chromium	46.32***	1589.27***	9.00***	44.15***	0.35ns	8.16***	0.37ns	0.50	13.22
Shoot chromium	43.327***	596.559***	3.553***	40.647***	0.215ns	3.277***	0.213ns	0.243	15.13

*, **, ***- significant at 0.05, 0.01, and 0.001 levels, respectively. Means ± SE (*n = 3*). Three-way ANOVA was performed and means differences were tested using Tukey's HSD test (P < 0.05). Significant differences between the treatments show by different letters

Table 3: Effect of ferulic acid on growth attributes of two barley varieties 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	21.16 ± 0.16^{gh}	9.17 ± 0.17^{ef}	13.50 ± 0.29^{gh}	4.53 ± 0.03^{df}	$6.66 \pm 0.17^{c-e}$	1.43 ± 0.03^{ef}	28.00 ± 0.58^{ef}
FA1-0.25 mM	30.83 ± 0.16^{d}	11.66 ± 0.17^{bc}	22.00 ± 0.29^{c}	5.10 ± 0.18^{bc}	7.50 ± 0.29^{bc}	2.00 ± 0.06^{c}	33.00 ± 0.58^{c}
FA2-0.50 mM	41.66 ± 0.16^{a}	16.33 ± 0.33^{a}	31.33 ± 0.44^{a}	7.00 ± 0.06^{a}	9.66 ± 0.17^{a}	3.00 ± 0.06^{a}	43.00 ± 0.58^{a}
FA3-0.75 mM	35.33 ± 0.16^{b}	12.50 ± 0.29^{b}	27.00 ± 0.29^{b}	6.00 ± 0.06^{ab}	8.50 ± 0.29^{ab}	2.66 ± 0.09^{b}	37.00 ± 0.33^{b}
FA4-1.0 mM	25.66 ± 0.33^{e}	10.66 ± 0.33^{cd}	19.00 ± 0.29^{de}	4.90 ± 0.06^{cd}	7.16 ± 0.17^{cd}	1.90 ± 0.06^{cd}	31.00 ± 0.58^{b}
Cr-150 µM	12.50 ± 0.28^{l}	4.50 ± 0.29^{kl}	9.50 ± 0.29^{k}	$4.13 \pm 0.03^{g-i}$	$4.50 \pm 0.29^{g-i}$	0.70 ± 0.06^{ij}	19.00 ± 0.58^{jk}
Cr + FA1	17.00 ± 0.284^{j}	$6.33 \pm 0.29^{h-j}$	14.66 ± 0.17^{fg}	$4.30 \pm 0.03^{e-h}$	$5.50 \pm 0.29^{e-g}$	$1.33 \pm 0.03^{e-g}$	23.00 ± 0.58^{hi}
Cr + FA2	26.33 ± 0.16^{e}	9.66 ± 0.17^{de}	20.20 ± 0.17^{d}	5.23 ± 0.03^{cd}	7.20 ± 0.17^{cd}	1.90 ± 0.09^{c}	27.00 ± 0.58^{b}
Cr + FA3	20.83 ± 0.16^{h}	7.50 ± 0.29^{gh}	15.50 ± 0.29^{f}	$4.53 \pm 0.03^{d-f}$	$6.00 \pm 0.29^{d-f}$	1.63 ± 0.03^{de}	24.00 ± 0.58^{gh}
Cr + FA4	16.83 ± 0.16^{j}	6.16 ± 0.17^{ij}	13.50 ± 0.29^{gh}	$4.20 \pm 0.06^{e-g}$	$5.16 \pm 0.17^{f-h}$	0.90 ± 0.06^{hi}	21.00 ± 0.58^{ij}
Jau-2017							
NS	15.00 ± 0.28^{k}	$5.66 \pm 0.17^{j-k}$	7.50 ± 0.29^{l}	$3.70 \pm 0.12^{f-i}$	$3.20 \pm 0.17^{h-j}$	1.10 ± 0.03^{gh}	26.00 ± 0.58^{fg}
FA1-0.25 mM	23.83 ± 0.17^{f}	$6.66 \pm 0.17^{h-j}$	12.00 ± 0.29^{ij}	$4.33 \pm 0.03^{d-f}$	$4.50 \pm 0.29^{e-g}$	$1.33 \pm 0.03^{e-g}$	32.00 ± 0.58^{cd}
FA2-0.50 mM	32.33 ± 0.17^{c}	8.00 ± 0.29^{fg}	17.66 ± 0.17^{e}	5.10 ± 0.03^{bc}	5.83 ± 0.17^{bc}	1.76 ± 0.03^{c}	39.00 ± 0.58^{b}
FA3-0.75 mM	26.00 ± 0.28^{e}	$7.00 \pm 0.29^{g-i}$	13.16 ± 0.17^{hi}	$4.63 \pm 0.03^{c-e}$	$5.00 \pm 0.29^{d-f}$	1.53 ± 0.03^{ef}	33.00 ± 0.58^{c}
FA4-1.0 mM	22.00 ± 0.28^{g}	6.00 ± 0.29^{ij}	11.33 ± 0.17^{j}	$3.90 \pm 0.06^{e-g}$	$4.00 \pm 0.29^{f-h}$	$1.20 \pm 0.06^{f-h}$	30.00 ± 0.58^{de}
Cr-150 µM	8.17 ± 0.16^{n}	2.50 ± 0.29^{m}	5.50 ± 0.29^{m}	3.10 ± 0.06^{i}	1.16 ± 0.17^{j}	0.26 ± 0.12^{k}	13.00 ± 0.58^{l}
Cr + FA1	12.33 ± 0.16^{lm}	3.66 ± 0.17^{lm}	9.50 ± 0.29^{k}	$3.30 \pm 0.06^{g-i}$	1.66 ± 0.44^{ij}	0.33 ± 0.06^{k}	$17.00 \pm 0.5`8^{k}$
Cr + FA2	19.66 ± 0.33^{i}	6.16 ± 0.17^{ij}	$14.50 \pm 0.29^{f-h}$	$3.90 \pm 0.06^{d-f}$	$3.16 \pm 0.17^{f-h}$	0.63 ± 0.09^{hi}	22.00 ± 0.58^{hi}
Cr + FA3	14.16 ± 0.16^{k}	4.66 ± 0.17^{kl}	11.00 ± 0.29^{j}	$3.40 \pm 0.06^{f-i}$	$2.16 \pm 0.17^{h-j}$	0.40 ± 0.06^{jk}	19.00 ± 0.33^{hi}
Cr + FA4	11.33 ± 0.16^{m}	3.00 ± 0.29^{m}	$8.66 \pm 0.17k^{l}$	3.20 ± 0.06^{hi}	1.50 ± 0.29^{ij}	0.20 ± 0.06^{k}	15.00 ± 0.58^{jk}

Means ± SE (*n = 3*). Three-way ANOVA was performed and means differences were tested using Tukey's HSD test (P < 0.05). Significant differences between the treatments show by different letters. Abbreviations: FA-ferulic acid; NS-no spray; Cr-chromium

Effect of FA spray on barley photosynthetic pigments in presence of chromium

Chromium (150 µM) stress caused a significant (P ≤ 0.001) decline in chlorophyll contents in both Talbina-21 and Jau-17 varieties. Specifically, chlorophyll *a*, chlorophyll *b*, and total chlorophyll levels were lower in both barley varieties under Cr toxicity compared to the control plants. FA (0.25, 0.50, 0.75 and 1.0 mM) remarkably decreased Cr effects on photosynthetic pigments in barley. In this context, FA (0.50 mM) noticeably (P ≤ 0.001) raised the chlorophyll content in both varieties. The highest increase in chlorophyll

Table 4: Effect of ferulic acid on photosynthetic pigments of two barley varieties under chromium stress

Treatments	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Total carotenoids (mg g^{-1} FW)
Talbina-2021				
NS	2.35 ± 0.02^{d}	1.49 ± 0.01^{fg}	4.05 ± 0.02^{ef}	0.27 ± 0.004^{ef}
FA1-0.25 mM	2.62 ± 0.03^{c}	1.71 ± 0.04^{bc}	4.43 ± 0.02^{c}	0.32 ± 0.002^{c}
FA2-0.50 mM	3.14 ± 0.07^{a}	1.91 ± 0.03^{a}	4.92 ± 0.02^{a}	0.40 ± 0.006^{a}
FA3-0.75 mM	2.81 ± 0.04^{b}	1.79 ± 0.01^{ab}	4.63 ± 0.03^{b}	0.36 ± 0.003^{b}
FA4-1.0 mM	2.43 ± 0.03^{d}	1.62 ± 0.02^{c-e}	4.25 ± 0.02^{d}	0.31 ± 0.004^{cd}
Cr-150 µM	1.75 ± 0.03^{hi}	1.15 ± 0.02^{kl}	3.31 ± 0.01^{m}	0.15 ± 0.002^{lm}
Cr + FA1	1.99 ± 0.07^{fg}	1.29 ± 0.01^{h-j}	3.65 ± 0.02^{i}	0.19c ± 0.004^{jk}
Cr + FA2	2.34 ± 0.03^{d}	1.50 ± 0.01^{e-g}	4.11 ± 0.02^{e}	0.28 ± 0.005^{d-f}
Cr + FA3	2.13 ± 0.02^{ef}	1.38 ± 0.01^{gh}	3.89 ± 0.02^{g}	0.24 ± 0.05^{gh}
Cr + FA4	1.83 ± 0.03^{g-i}	1.25 ± 0.03^{i-k}	3.41 ± 0.02^{kl}	0.21 ± 0.01^{ij}
Jau-2017				
NS	2.15 ± 0.02^{ef}	1.32 ± 0.02^{h-j}	3.45 ± 0.02^{k}	0.23 ± 0.008^{hi}
FA1-0.25 mM	2.44 ± 0.03^{d}	1.49 ± 0.02^{fg}	3.72 ± 0.02^{h}	0.27 ± 0.008^{fg}
FA2-0.50 mM	2.82 ± 0.02^{b}	1.63 ± 0.01^{cd}	4.32 ± 0.03^{d}	0.36 ± 0.005^{b}
FA3-0.75 mM	2.62 ± 0.01^{c}	1.51 ± 0.01^{d-f}	3.96 ± 0.03^{fg}	0.32 ± 0.006^{c}
FA4-1.0 mM	2.29 ± 0.01^{de}	1.42 ± 0.02^{f-h}	3.55 ± 0.01^{ij}	0.30 ± 0.006^{c-e}
Cr-150 µM	1.52 ± 0.02^{j}	1.01 ± 0.04^{m}	2.63 ± 0.02^{p}	0.12 ± 0.005^{m}
Cr + FA1	1.72 ± 0.02^{i}	1.15 ± 0.02^{kl}	3.11 ± 0.01^{n}	0.17 ± 0.004^{kl}
Cr + FA2	2.12 ± 0.02^{ef}	1.33 ± 0.02^{hi}	3.49 ± 0.03^{jk}	0.22 ± 0.007^{h-j}
Cr + FA3	1.92 ± 0.02^{gh}	1.21 ± 0.02^{j-l}	3.33 ± 0.01^{lm}	0.19 ± 0.005^{jk}
Cr + FA4	1.66 ± 0.03^{ij}	1.11 ± 0.02^{lm}	3.01 ± 0.02^{o}	0.15 ± 0.004^{lm}

Means ± SE (*n = 3*). Three-way ANOVA was performed and means differences were tested using Tukey's HSD test (P < 0.05). Significant differences between the treatments showed by different letters. Abbreviations: NS-no spray; FA-ferulic acid; Cr-chromium

Table 5: Effect of FA on biochemical attributes of two barley varieties under chromium stress

Treatments	Total amino acids (mg g^{-1} FW)	Soluble protein (mg g^{-1} FW)	Anthocyanin (mg g^{-1} FW)	Total flavonoids (mg g^{-1} FW)	Total Phenolics (mg g^{-1} FW)	Soluble sugar (mg g^{-1} FW)	Glycine betaine (µmol g^{-1} DW)	Ascorbic acid (mg g^{-1} FW)
Talbina-2021								
NS	1.99 ± 0.04^{j-l}	10.38 ± 0.23^{e}	1.15 ± 0.02^{j}	1.75 ± 0.06^{f-h}	1.64 ± 0.04^{kl}	9.42 ± 0.34^{ij}	2.50 ± 0.05^{j}	2.71 ± 0.06^{n}
FA1-0.25 mM	2.94 ± 0.06^{fg}	12.20 ± 0.15^{c}	2.17 ± 0.03^{fg}	2.58 ± 0.11^{c-e}	2.47 ± 0.08^{g}	12.44 ± 0.34^{g}	4.23 ± 0.02^{h}	4.21 ± 0.05^{ij}
FA2-0.50 mM	4.70 ± 0.04^{c}	15.19 ± 0.13^{a}	3.20 ± 0.03^{c}	3.19 ± 0.09^{ab}	3.02 ± 0.04^{de}	20.40 ± 0.33^{d}	5.17 ± 0.02^{e}	5.72 ± 0.02^{e}
FA3-0.75 mM	3.99 ± 0.07^{d}	13.29 ± 0.18^{b}	2.41 ± 0.02^{e}	2.91 ± 0.16^{b-d}	2.74 ± 0.09^{f}	15.44 ± 0.40^{f}	4.75 ± 0.03^{f}	5.04 ± 0.03^{g}
FA4-1.0 mM	2.24 ± 0.05^{i-k}	11.16 ± 0.14^{d}	1.55 ± 0.03^{i}	2.82 ± 0.14^{b-d}	2.15 ± 0.02^{i}	8.00 ± 0.32^{jk}	3.99 ± 0.04^{i}	3.81 ± 0.09^{l}
Cr-150 µM	3.81 ± 0.09^{d}	7.13 ± 0.11^{h}	2.04 ± 0.03^{g}	2.53 ± 0.12^{c-e}	2.17 ± 0.0^{hi}	15.21 ± 0.41^{f}	5.12 ± 0.02^{e}	3.89 ± 0.07^{kl}
Cr + FA1	4.55 ± 0.06^{c}	8.51 ± 0.19^{g}	3.10 ± 0.03^{c}	2.85 ± 0.04^{b-d}	3.22 ± 0.06^{b-d}	18.40 ± 0.35^{e}	5.55 ± 0.05^{d}	6.11 ± 0.07^{d}
Cr + FA2	6.08 ± 0.08^{a}	10.10 ± 0.14^{e}	4.19 ± 0.02^{a}	3.51 ± 0.09^{a}	3.83 ± 0.02^{a}	30.66 ± 0.37^{a}	6.88 ± 0.02^{a}	8.12 ± 0.07^{a}
Cr + FA3	5.22 ± 0.06^{b}	9.23 ± 0.20^{g}	3.55 ± 0.02^{b}	3.28 ± 0.12^{ab}	3.47 ± 0.04^{b}	22.46 ± 0.45^{c}	6.12 ± 0.01^{b}	7.11 ± 0.07^{fg}
Cr + FA4	3.96 ± 0.07^{d}	7.23 ± 0.19^{h}	2.30 ± 0.04^{ef}	3.02 ± 0.10^{a-c}	3.03 ± 0.04^{de}	10.56 ± 0.34^{hi}	5.22 ± 0.04^{e}	5.27 ± 0.08^{f}
Jau-2017								
NS	1.67 ± 0.08^{l}	8.30 ± 0.18^{g}	1.06 ± 0.01^{j}	1.15 ± 0.04^{i}	1.15 ± 0.04^{m}	7.55 ± 0.35^{k}	1.90 ± 0.03^{k}	2.72 ± 0.05^{n}
FA1-0.25 mM	2.23 ± 0.02^{ij}	9.18 ± 0.16^{f}	1.75 ± 0.02^{h}	1.42 ± 0.06^{hi}	1.65 ± 0.06^{kl}	9.65 ± 0.35^{ij}	4.21 ± 0.02^{h}	3.71 ± 0.06^{lm}
FA2-0.50 mM	3.19 ± 0.05^{ef}	11.30 ± 0.17^{d}	2.71 ± 0.03^{d}	2.05 ± 0.05^{e-g}	1.99 ± 0.03^{ij}	15.46 ± 0.33^{f}	4.67 ± 0.02^{f}	4.72 ± 0.04^{gh}
FA3-0.75 mM	2.65 ± 0.07^{gh}	10.12 ± 0.11^{e}	2.11 ± 0.04^{g}	1.84 ± 0.06^{f-h}	1.79 ± 0.03^{jk}	11.51 ± 0.31^{gh}	4.44 ± 0.03^{g}	4.12 ± 0.07^{jk}
FA4-1.0 mM	1.94 ± 0.04^{kl}	9.23 ± 0.12^{f}	2.13 ± 0.004^{g}	1.58 ± 0.07^{g-i}	1.42 ± 0.04^{l}	5.49 ± 0.36^{l}	4.17 ± 0.02^{h}	3.41 ± 0.02^{m}
Cr-150 µM	2.34 ± 0.06^{hi}	3.25 ± 0.14^{l}	1.60 ± 0.04^{i}	1.75 ± 0.11^{f-h}	1.93 ± 0.04^{ij}	12.50 ± 0.32^{g}	4.71 ± 0.01^{f}	3.41 ± 0.04^{m}
Cr + FA1	3.26 ± 0.05^{e}	5.23 ± 0.13^{j}	2.33 ± 0.02^{e}	2.15 ± 0.11^{ef}	2.87 ± 0.09^{ef}	14.51 ± 0.37^{f}	5.14 ± 0.03^{d}	4.52 ± 0.05^{hi}
Cr + FA2	4.76 ± 0.07^{c}	7.16 ± 0.12^{h}	3.58 ± 0.04^{b}	2.79 ± 0.11^{b-d}	3.45 ± 0.02^{bc}	25.46 ± 0.32^{b}	5.96 ± 0.02^{c}	6.77 ± 0.09^{c}
Cr + FA3	3.75 ± 0.03^{d}	6.24 ± 0.14^{i}	3.13 ± 0.01^{c}	2.53 ± 0.11^{c-e}	3.19 ± 0.04^{cd}	20.58 ± 0.35^{d}	5.41 ± 0.02^{d}	5.12 ± 0.06^{f}
Cr + FA4	2.51 ± 0.05^{hi}	4.23 ± 0.15^{k}	1.74 ± 0.03^{h}	2.43 ± 0.09^{de}	2.42 ± 0.08^{gh}	8.53 ± 0.38 ^{jk}	5.11 ± 0.02^{e}	3.94 ± 0.02^{j-l}

Means ± SE (*n = 3*). Three-way ANOVA was performed and means differences were tested using Tukey's HSD test (P < 0.05). Significant differences between the treatments show by different letters. Abbreviations: FA-ferulic acid; Cr-chromium; NS-no spray

a, chlorophyll *b*, and total chlorophyll was observed with a 0.5 mM FA spray under Cr stress conditions in both varieties Talbina-21 and Jau-17 relative to control (Table 2 and 4).

Chromium toxicity induced a considerable (P ≤ 0.001) rise in anthocyanins in both barley varieties Talbina-21 and Jau-17. However, the exogenous application of various concentrations (0.25, 0.50, 0.75 and 1.0 mM) of FA significantly enhanced carotenoid levels in both varieties. The greatest increase (P ≤ 0.001) in carotenoid content was observed when plants were treated with 0.5 mM FA under both control and Cr stress conditions (Table 2 and 4).

Interactive effect of FA and Cr on secondary metabolites, osmolytes and ascorbic acid accumulation

Barley plants of both varieties Talbina-21 and Jau-17 manifested a significant (P ≤ 0.001) increase in total free amino acids (TFAA) under Cr toxicity compared to control plants. Further, plants administered FA (0.25, 0.50, 0.75 and 1.0 mM) further enhanced TFAA levels in both Talbina-21 and Jau-17 varieties, with the highest (P ≤ 0.001) increases observed when 0.50 mM FA was applied under both control and Cr stress conditions (Table 2, 5).

A significant ($P \leq 0.001$) increase in TTS was observed in both Talbina-21 and Jau-17 in relation to control plants under Cr toxicity. The application of FA (0.25, 0.50, 0.75 and 1.0 mM) also promoted an increase in soluble sugars, particularly at the 0.5 mM concentration, which resulted in the most significant ($P \leq 0.01$) enhancement under both conditions. However, a decrease in TSS content was noted in both Talbina-21 and Jau-17 varieties when treated with 1.0 mM FA under stress conditions (Table 2 and 5).

Proline accumulation was significantly ($P \leq 0.001$) increased in both Talbina-21 and Jau-17 under Cr (150 µM) stress. The application of FA at concentrations of 0.25, 0.50, 0.75 and 1.00 mM also enhanced proline accumulation in a concentration-dependent manner in both varieties, with 0.50 mM FA showing the greatest increase. This treatment notably ($P \leq 0.001$) enhanced proline levels in both Talbina-21 and Jau-17 under control and Cr stress conditions (Table 2 and Fig. 1).

A noticeable ($P \leq 0.001$) increased in TSP content was noted in plants in Talbina-21 and Jau-17 under Cr stress. The supplementation of FA at various concentrations (0.25, 0.50, 0.75 and 1.0 mM) significantly ($P \leq 0.001$) increased TSP levels. FA (0.5 mM) raised TSP in Talbina-21 and Jau-17 maximally in compared to control plants under Cr stressed (Table 2 and 5).

Chromium toxicity significantly ($P \leq 0.001$) increased phenolics compared to control plants in Talbina-21 and Jau-17, respectively. FA (0.25, 0.50, 0.75 and 1.0 mM) markedly enhanced ($P \leq 0.001$) phenolic contents in barley plants subjected to Cr toxicity. Further, plants administered 0.5 mM FA manifested markedly greater values of phenolics in Talbina-21 and Jau-17, compared to control plants under Cr toxicity (Table 2 and 5).

Flavonoids remarkably ($P \leq 0.001$) increased in both Talbina-21 and Jau-17, in relation to control plants under Cr toxicity. FA (0.25, 0.50, 0.75 and 1.0 mM) significantly further improved flavonoid contents in two barley varieties exposed to Cr toxicity. In this context, the greatest improvement ($P \leq 0.001$) was observed at 0.50 mM FA in Talbina-21 and Jau-17 compared to control plants under Cr toxicity (Table 2 and 5).

Anthocyanin contents increased significantly ($P \leq 0.001$) in both Talbina-21 and Jau-17 under Cr stress. FA (0.25, 0.50, 0.75 and 1.0 mM) resulted in a noticeable rise ($P \leq 0.001$) in anthocyanin contents in a dose dependent manner. The maximal value of anthocyanins was present in plant administered with 0.50 mM FA compared to control plants under Cr toxicity (Table 2 and 5).

Chromium toxicity significantly ($P \leq 0.001$) increased glycine betaine in both Talbina-21 and Jau-17 compared to control plants. Further, FA (0.25, 0.50, 0.75 and 1.0 mM) notably enhanced glycine betaine contents in barley varieties under Cr toxicity. In this context, the maximum ($P \leq 0.001$) values of GB in Talbina-21 and Jau-17 were evident when administered 0.50 mM FA, under both control and stressed plants (Table 2 and 5). Talbina-21 accumulated more glycine betaine than control plants in response to 0.50 mM FA application under Cr toxicity (Table 2 and 5).

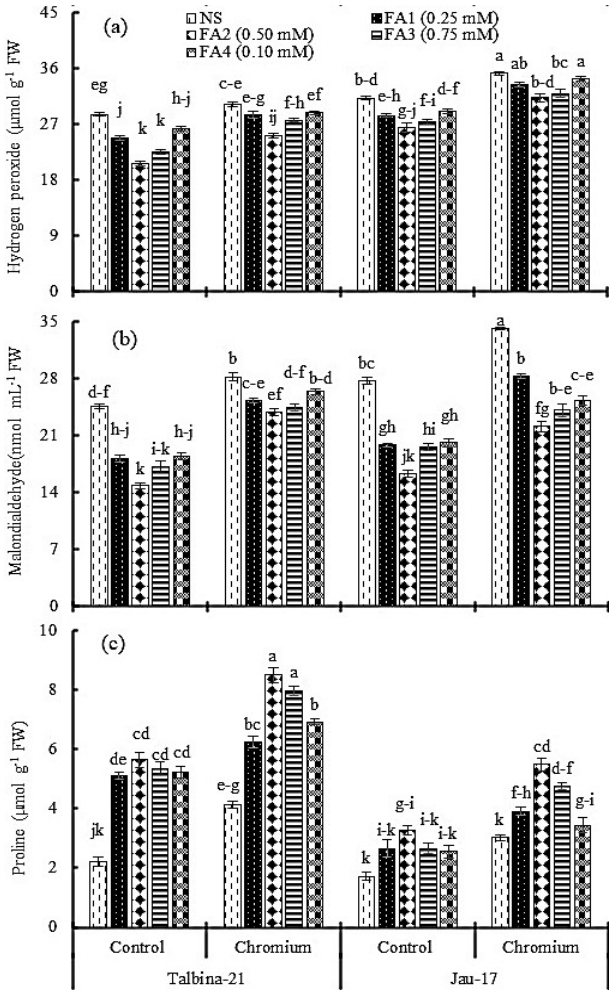

Fig. 1: Effect of ferulic acid spray on oxidative stress indicators (**a**, **b**) and proline content (**c**) of barley plants of Talbina-21 and Jau-17 subjected to chromium stress. Bars with different lowercase letters represents the significant differences between means at $P < 0.05$ using Tukey's HSD test

Ascorbic acid levels substantially ($P \leq 0.001$) increased under Cr stress in both barley varieties Talbina-21 and Jau-17. The application of various level of FA further enhanced the ascorbic acid content, with the 0.50 mM concentration showing the most significant ($P \leq 0.001$) increase in both control and stressed plants of Talbina-21 and Jau-17 (Table 2 and 5).

Cr and FA interaction on oxidative stress markers

Chromium toxicity led to oxidative damage in both Talbina-21 and Jau-17, with significantly ($P \leq 0.001$) higher levels of H_2O_2 and MDA compared to the control plants. The application of FA at various concentrations (0.25, 0.50, 0.75 and 1.00 mM) resulted in considerably subsided in both H_2O_2 and MDA concentrations in both varieties subjected to Cr

stress. Specifically, the 0.5 mM FA treatment effectively ($P \leq 0.001$) lowered H_2O_2 levels in Talbina-21 and Jau-17 under stress conditions compared to control plants. Similarly, MDA content was significantly ($P \leq 0.001$) reduced in both Talbina-21 and Jau-17 varieties after treatment with 0.5 mM FA under stress relative to control (Table 2; Fig. 1).

FA and Cr interaction on enzymatic antioxidant activities

Cr (150 µM) toxicity significantly ($P \leq 0.001$) altered the activities of antioxidant enzymes in both Talbina-21 and Jau-17 plants. The activities of SOD, POD, and CAT were notably elevated in both barley varieties under Cr stress. Foliar application of FA at concentrations of 0.25, 0.50, 0.75 and 1.00 mM further boosted the antioxidant activities in both varieties under both conditions. Specifically, treatment with 0.50 mM FA led to a significant ($P \leq 0.001$) increase in SOD activity in both Talbina-21 and Jau-17 under control and stressed conditions. Similarly, POD activity was significantly ($P \leq 0.001$) enhanced by 0.50 mM FA in both varieties, with more pronounced effects observed in Talbina-21. Furthermore, CAT activity showed a substantial ($P \leq 0.001$) increase in both Talbina-21 and Jau-17 varieties under control and stress conditions when treated with 0.50 mM FA. These findings suggest that Talbina-21 possesses a vigorous antioxidant defense system capable of mitigating oxidative damage caused by Cr toxicity, while Jau-17 appears to have a less efficient enzymatic response, making it more susceptible to Cr-induced stress (Table 2 and Fig. 2).

Effect of FA spray on tissue chromium content

The accumulation of Cr content was many folds greater in root and shoot in Talbina-21 and Jau-17, under Cr toxicity. The aerial translocation of Cr was significantly ($P \leq 0.001$) greater in Jau-17 compared to Talbina-21 under Cr toxicity. FA (0.25, 0.50, 0.75 and 1.00 mM) substantially ($P \leq 0.001$) abridged root Cr and shoot Cr in Talbina-21 and Jau-17, respectively, under Cr toxicity. A considerable reduction in Cr accumulation was observed when 0.50 mM FA was applied, particularly under Cr stress, with a notable decrease in roots and shoots of both Talbina-21 and Jau-17 varieties. These results highlight the potential of FA to mitigate Cr uptake in plants subjected to Cr stress (Table 2 and Fig. 3).

Principal Component Analysis

The PCA loading plot was used to evaluate the relationship between Cr stress and FA on the growth, physiological, and biochemical attributes of barley. The two main components, Component 1 (Dim 1) and Component 2 (Dim 2), explained 68.7 and 23% of the total variation, respectively with a combined total of 91.7%. PC1 showed a positive correlation with growth-related traits, chlorophyll content, osmolytes, and antioxidant activities, while a negative correlation was observed with PC2 variables, including MDA and H_2O_2

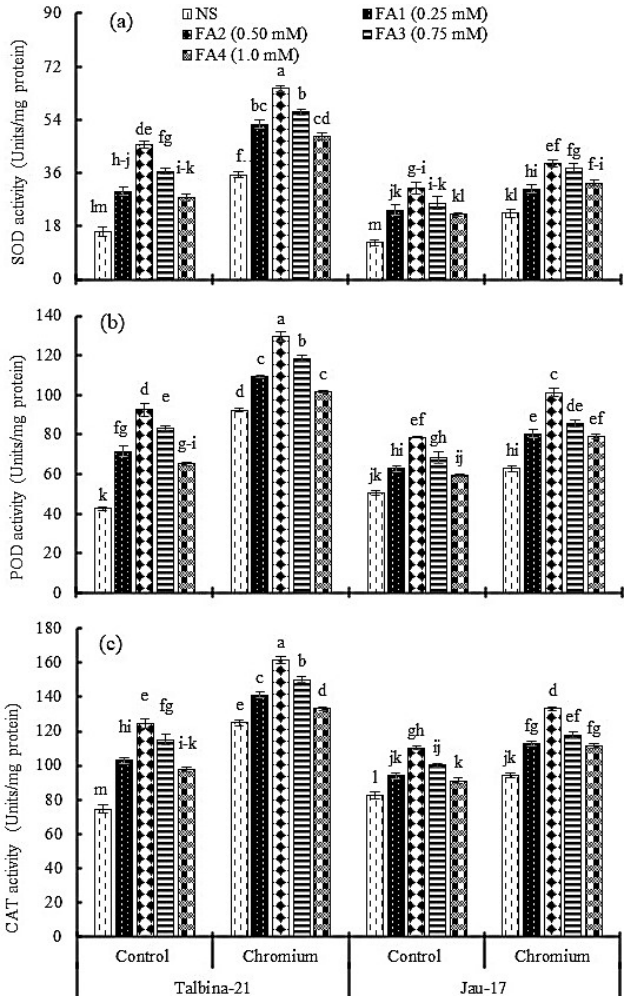

Fig. 2: Effect of ferulic acid spray on superoxide dismutase activity (**a**), peroxidase (**b**) and catalase activities (**c**) in barley plants of Talbina-21 and Jau-17 subjected to chromium stress. Bars with different lowercase letters represents the significant differences between means at $P < 0.05$ using Tukey's HSD test

contents, and Cr accumulation in the root and shoot of barley plants (Fig. 4).

Pearson correlation

A Pearson correlation matrix graph was used to examine the relationships between parameters affected by treatments. The results showed that Cr treatment had a positive correlation with oxidative stress markers and antioxidant activities, but a negative correlation with growth parameters, photosynthetic pigments, and total soluble protein contents (Fig. 5).

Discussion

These results demonstrate the detrimental effects of Cr toxicity on barley growth, leading to oxidative stress and reduced plant development, in line with previous studies

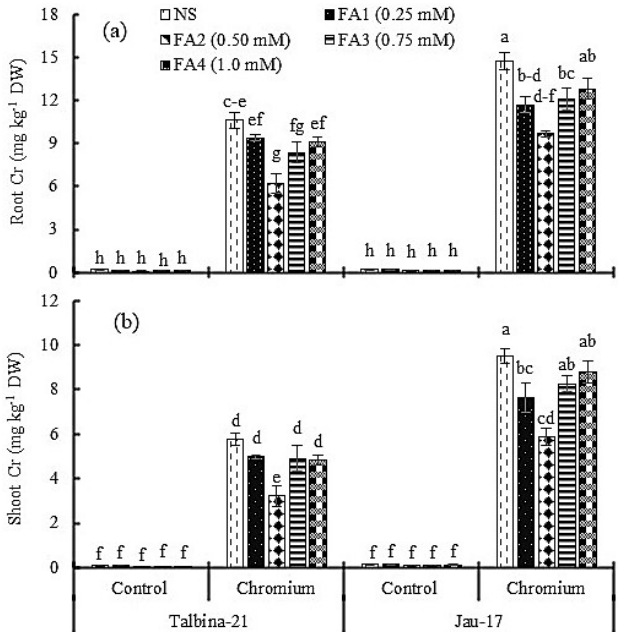

Fig. 3: Effect of ferulic acid spray on chromium uptake in root (**a**) and shoot (**b**) of barley plants of Talbina-21 and Jau-17 subjected to chromium stress. Bars with different lowercase letters represents the significant differences between means at $P < 0.05$ using Tukey's HSD test

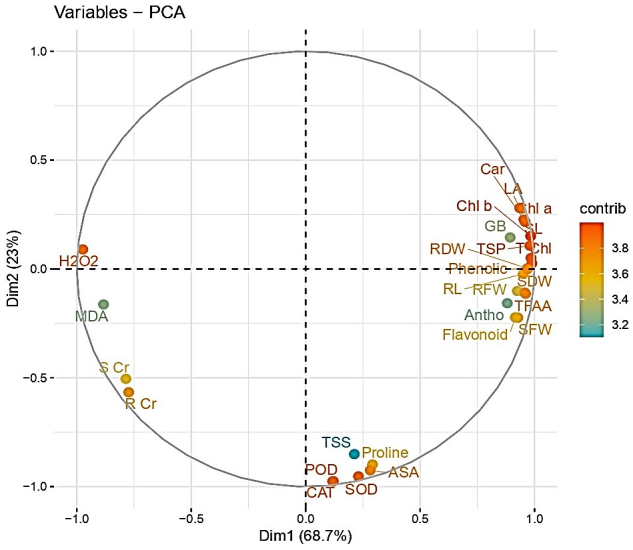

Fig. 4: Principal component analysis loading plots related to growth and physio-biochemical attributes of plants of Talbina-21 and Jau-17 under chromium stress. Abbreviations: SL-shoot length; RL-root length; SFW-shoot fresh weight; LA-leaf area; Chl *a*-chlorophyll *a*; Chl *b*-chlorophyll *b*; TSP- total soluble protein; TFAA-total free amino acids; SOD-superoxide dismutase; MDA- malondialdehyde; H_2O_2- hydrogen peroxide; TSS-total soluble sugar; GB-glycine betaine; RCr-root chromium; SCr-shoot chromium

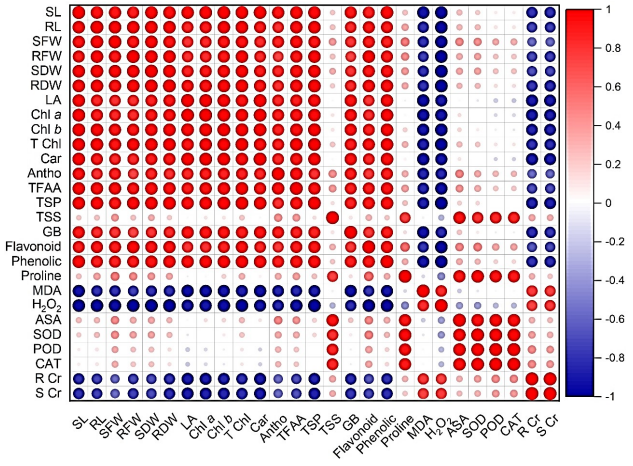

Fig. 5: The correlation matrix between several growth and physio-chemical aspects in barley plants of Talbina-21 and Jau-17 under chromium stress. Abbreviations: RL-root length; SFW-shoot fresh weight; RFW-root fresh weight; Chl *a*-chlorophyll *a*; Chl *b*-chlorophyll *b*; TSP-total soluble protein; POD-peroxidase; TFAA-total free amino acids; GB-glycine betaine; SOD-superoxide dismutase; MDA-malondialdehyde; H_2O_2-hydrogen peroxide; TSS-total soluble sugar; CAT-catalase; RCr-root chromium; SCr-shoot chromium

such as Dotaniya *et al.* (2014). Chromium-induced oxidative damage impairs key physiological processes, including photosynthesis, and decreases plant growth potential. However, foliar supplementation with FA was found to alleviate Cr-induced cytotoxicity and promoting improved growth in barley (Table 3). These findings are consistent with the research of Yildiztugay *et al.* (2019), who showed that FA could detoxify harmful compounds and reduce oxidative stress, ultimately enhancing plant growth (Table 3). The Pearson's correlation analysis also revealed a negative relationship between growth parameters and oxidative stress markers, supporting the protective role of FA against Cr-induced damage (Fig. 5).

Chromium toxicity is known to decrease chlorophyll content by interfering with key biochemical processes involved in chlorophyll biosynthesis. Chromium competes with iron for uptake, limiting its availability for chlorophyll production (Sharma *et al.* 2022). Moreover, Cr disrupts photosystem II (PSII) in thylakoid membranes, which impedes electron transport necessary for the splitting of water molecules and reduces the production of ATP and NADPH, essential molecules for chlorophyll synthesis (Anjum *et al.* 2016). Additionally, Cr induces oxidative stress by generating ROS, which damage cellular structures, including DNA, pigments, and membranes, leading to chloroplast dysfunction (Garcia-Caparros *et al.* 2021). Furthermore, Cr inhibits the activity of δ-aminolevulinic acid dehydratase (ALAD), a key enzyme in chlorophyll biosynthesis, further reducing chlorophyll production (Qureshi *et al.* 2020). This study also showed a significant reduction in chlorophyll content under Cr toxicity, in line with findings by Anjum *et al.* (2016). However, FA application significantly mitigated this effect, enhancing

chlorophyll content and biomass in both Talbina-21 and Jau-17 varieties, with Talbina-21 showing higher resilience (Table 4). The principal component analysis (PCA) confirmed the negative correlation between chlorophyll content and oxidative stress markers (Fig. 4).

Chromium toxicity induces excessive ROS production, leading to lipid peroxidation, as evidenced by increased MDA levels, a marker of oxidative damage (Qureshi *et al.* 2020). Lipids containing unsaturated fatty acids are especially vulnerable to oxidative damage, which disrupts membrane structure and function, contributing to cell damage (Hussain *et al.* 2023). In this study, FA treatment effectively reduced MDA levels, suggesting that FA plays a key role in protecting cell membranes from oxidative damage under Cr stress (Fig. 1).

To counteract ROS, plants rely on antioxidant enzymes such as SOD, CAT, and POD. These enzymes scavenge superoxide radicals and H_2O_2, preventing cellular damage (Garcia-Caparros *et al.* 2021). The study found that under Cr stress, barley plants exhibited increased oxidative damage, with higher MDA and H_2O_2 levels. The Jau-17 variety experienced more severe oxidative damage compared to Talbina-21, which showed improved antioxidant enzyme activity, particularly in CAT and POD, under Cr stress (Fig. 2). These results are consistent with previous studies on *Zea mays*, where Cr exposure induced antioxidant enzymes to mitigate oxidative stress (Mishra *et al.* 2012). FA treatment significantly enhanced antioxidant enzyme activity in both barley varieties, effectively reducing ROS accumulation and protecting plants from oxidative stress. The Pearson's correlation plot further confirmed that enzymatic antioxidants were less positively correlated with oxidative stress markers, reinforcing the protective role of FA in modulating the antioxidant defense system (Fig. 5).

Chromium toxicity also induces the accumulation of cytosolic solutes like proline, glycine betaine, and soluble sugars, which help plants cope with oxidative stress by acting as osmoprotectants, stabilizing cellular structures, and scavenging ROS (Qureshi *et al.* 2020). Proline has been shown to maintain cellular redox balance and protect cellular integrity under stress conditions (Rasheed *et al.* 2018). FA application significantly increased the accumulation of these osmolytes, contributing to improved plant survival under Cr toxicity. Additionally, FA treatment elevated phenolic and flavonoid levels, which are known to neutralize free radicals and chelate toxic metal ions, further enhancing plant stress tolerance (Anjitha *et al.* 2021). Free amino acids can accumulate in plant cells under stress conditions as a source of nitrogen and energy, acting as osmolytes and antioxidants, scavenging ROS, and protecting cellular structures and enzymes from damage caused by oxidative damage (Anjum *et al.* 2016). Soluble sugars accumulate in plant cells under stress conditions, acting as a source of energy and osmoprotectants, stabilizing cellular structures and enzymes, preventing water loss from cells, scavenging ROS, and protecting cellular structures and enzymes from damage caused by oxidative stress (Qureshi *et al.* 2020). The results of the present study show that foliar application of FA substantially increased the levels of osmolytes, which may have helped plants survive under Cr toxicity, as indicated by growth attributes (Table 5). These metabolites are produced through the phenylpropanoid pathway, with phenylalanine ammonia lyase (PAL) catalyzing key steps in their biosynthesis (Sharma *et al.* 2019). In the current study, FA treatment not only increased phenolic and flavonoid content but also enhanced PAL activity, contributing to improved Cr stress tolerance (Table 5). These findings are consistent with previous research indicating that FA helps maintain osmotic balance and redox equilibrium under metal stress (Qureshi *et al.* 2020). A Pearson's correlation plot further indicated a negative correlation between osmolyte accumulation and oxidative stress markers (Fig. 5).

Chromium toxicity leads to the accumulation of toxic Cr in plant tissues, particularly in the roots and leaves, where it can replace essential elements, disrupting biochemical processes and causing growth inhibition (Habiba *et al.* 2019). Excessive Cr exposure leads to symptoms such as chlorosis and necrosis, which are attributed to oxidative stress (Anjum *et al.* 2016). In this study, FA treatment reduced Cr accumulation in the shoots and roots by enhancing the activity of polyphenol oxidase and PAL, enzymes involved in detoxifying Cr through chelation. FA also mitigated oxidative stress-induced membrane damage by boosting the antioxidant defense system, thereby reducing the toxic effects of Cr (Fig. 3). Pearson's correlation analysis revealed a positive correlation between Cr content and oxidative stress markers, suggesting that reducing Cr uptake via FA application helped alleviate stress (Fig. 5). Furthermore, FA treatment improved growth and biomass in barley plants, demonstrating its potential as a strategy to reduce Cr toxicity and improve plant resilience.

Conclusion

Cr intoxication inhibited plant biomass and photosynthetic pigments in barley leading to oxidative damage. However, foliar spray of FA effectively prevented the Cr toxicity. FA spray limited the aerial translocation of Cr by reducing the production of ROS. Additionally, FA improved osmoregulation by enhancing osmolyte accumulation as well as promoting the activities of antioxidants and secondary metabolites synthesis and also improving photosynthetic pigment content. These properties of FA make it a promising option for phytoremediation in Cr polluted areas.

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Author Contributions

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

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Data Availability

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

Ethical Approval

This article does not involve any studies with animals or human participants as subjects of research.

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