Plant Health 03 (01) 2024. 121-134 DOI: 10.33687/planthealth.03.01.5372

Available Online at EScience Press
Plant Health
ISSN: 2305-6835
https://esciencepress.net/journals/planthealth

Efficacy of Sulfur Supplementation for Improving Chromium Tolerance in Canola (*Brassica napus* L.)

[a,b]Summera Jahan*, [b]Sumera Iqbal, [b]Khajista Jabeen
[a] *Institute of Botany, University of the Punjab Lahore, Pakistan.*
[b] *Department of Botany, Lahore College for Women University Lahore, Pakistan.*

ARTICLE INFO

Article History
Received: March 13, 2024
Revised: May 11, 2024
Accepted: May 19, 2024

Keywords
Phytohormones
Abiotic stress
Proline
Chlorophylls
Carotenoids

ABSTRACT

Canola (*Brassica napus* L.) is an important oil seed crop globally. Currently, the impact of sulphur feeding on physiological, phytohormonal, and yield aspects of canola (var. Pakola) under chromium (Cr) stress is being investigated. The pot trial was carried out in a completely randomized design (CRD). The pot soils were treated with two levels of chromium, low (40 ppm) and high (160 ppm), as well as two levels of sulphur, low (50 ppm) and high (150 ppm). Plants were sampled at both vegetative and reproductive phases. The results showed that chromium had a detrimental effect on relative water content, chlorophyll content, protein content, indole acetic acid (IAA), gibberellic acid (GA), 100 seed weight, and pod length compared to control plants. However, under chromium stress, sugar and proline levels, as well as abscisic acid (ABA), increased dramatically. However, sulphur supplementation, especially under low chromium stress, resulted in considerable improvements in relative water content, chlorophyll and carotenoid levels, protein content, number of branches/plants, number of pods/branches, number of seeds/pod, and 100 seed weight (g).The current study's findings show that sufur feeding helps to mitigate the negative effects of chromium stress on the physiological, biochemical, and yield aspects of canola.

Corresponding Author: Summera Jahan
Email: summera.botany@pu.edu.pk

INTRODUCTION

Extensive industrialization has led to the release of substantial substantial amounts of chromium into the soil and water bodies, posing a serious environmental threat due to its toxicity and persistance (Srivastava *et al*., 2021). Chromium contamination especially from industries like metal plating, tanning, and wood preservation, results in significant stress on crops, reducing growth and productivity through physiological disruptions, including impaired water relations, nutrient imbalances, and diminished photosynthetic capacity (Ghorbani *et al*., 2021; Junaid *et al*., 2017; Mushtaq *et al*., 2021). Industrial activities have resulted in the release of significant concentrations of chromium into the environment, contributing to soil and water contamination (Kotas *et al*., 2000; Sanjay *et al*., 2020). For instance, a report highlights that approximately 962,335 million gallons of wastewater from municipal and industrial sectors in Pakistan are discharged daily into water bodies (Muhammad and Usman, 2022). In Pakistan, for instance, chromium-laden watewater is frequently discharged into agricultural fields, affecting approximately 32,500 hectares and further exacerbating the contamination of croplands (Khalid *et al*., 2018; Qadir *et al*., 2000). While the detrimental effects of chromium on crops are well documented, strategies to mitigate these effects in oilseed crops, particularly canola, remains limited.

Canola (*Brassica napus* L.), an economically significant oilseed crop with high oil content (42-45%), is widely cultivated to meet domestic demand for edible oil (Chaganti *et al*., 2021). Its oil is rich in mono- and polyunsaturated fats (Hayward, 2012). However, its growth is heavily dependent on sulfur, a nutrient crucial not only for maximizing yeild but also for bolstering defense mechanism against abiotic stressors, including heavy metals (Capaldi *et al*., 2015; Hrivna *et al*., 2001). Sulfur enhances the synthesis of cysteine-rich peptides and glutathione, which play crucial roles in detoxifying heavy metals like cgromium, thereby improving plant stress tolerance (Singh *et al*., 2017). Despite the growing recognition of sulfur's role in heavy metal stress tolerance, there remains a noteable gap in understanding how sulfur application specifically impacts canola resilience to chromium-induced stress. Additionaly, sulfur deficiency in soils is a rising consern in agricultural systems, further highlighting the need for optimized nutrient strategies to support crop health under metal toxicity (Cobbett, 2000; Jahan *et al*., 2015).

Given the potential of sulfur to enhance plant tolerance to heavy metals, it is important to further explore its impact on canola, particularly under chromium stress (Zhong *et al*., 2011). This study addresses this research gap by systematically evaluating the effects of sulfur supplementation on canola under chromium stress. By examining physiological, phytohormonal, and yield-related parameters, this research provides insights into sulfur's role in enhancing chromium tolerance in canola, offering a potential strategy to improve crop resilience and productivity in contaminated soils. The findings could contribute significantly to reducing the edible oil supply-demand gap in Pakistan by increasing the yield of canola under adverse conditions.

MATERIALS AND METHODS

The pot experiment was carried out in triplicate with the following treatments: control (Cr0S0), chromium treated (Cr40S0 and Cr160S0), sulphur treated (Cr0S50 and Cr0S150), and sulphur supplementation under chromium stress (Cr40S50, Cr40S150, Cr160S50 and Cr160S150). The seeds of the Pakola variety of canola, which is a species of Brassica napus L., were received from the National Agriculture Research Centre (NARC) in Islamabad. The seeds were planted in 30 cm earthen pots with sandy loam soil, pH=7.5, EC= 0.86 dm/cm, organic matter=0.35%, sulfur=8 mg/kg. Before sowing, the soil was mixed with 40 ppm and 160 ppm chromium in the form of potassium dichromate salt, as well as 50 ppm and 150 ppm sulphur in the form of calcium sulphate. The plants were watered twice a week. Leaf sampling for physiological and biochemical analysis was performed during the vegetative and reproductive stages of canola, whereas yield analysis samples were collected at maturity.

The method of Wheatherley (1950) was employed to ascertain the relative water content (RWC) of leaves. For chlorophyll analysis, Arnon (1949) was used, while for carotenoids, Lichtenthaler and Wellburn's (1983) method was employed. The 25 mL of acetone (80%) was used to grind 0.5 g of leaf material. After filtration, the absorbance values at 470, 645, and 663 nm were measured using a spectrophotometer (BMS UV 2600).

The sugar content in canola leaves was determined using the method described by Dubois *et al*. (1956). The foliage material (0.5 g) was ground and subsequently homogenised with 10 mL of distilled water before being filtered. The filtrate (0.1 mL) was collected in a separate test tube and combined with 1 mL of phenol (5% v/v). The test tube was incubated at room temperature for 60 minutes, and 5 mL of 100% H_2SO_4 was added. The absorbance was measured at 420 nm.

The method developed by Bates *et al*. (1973) was used to determine the proline (Pro) content of the leaves. The leaf material (0.1 g) was pulverised and combined with 4 mL of sulfosalicylic acid (3%). The mixture was then stored at 4 °C overnight. Then the extract was centrifuged for 5 minutes. The supernatant (2 mL) was combined with 4 mL of acidic ninhydrin reagent and heated at 100°C for 1 hour. After cooling, 4 mL of toluene was added, agitated thoroughly, separated, and the absorbance was measured at 520 nm. The protein content of the canola leaf was analysed using the Lowry *et al*. (1951) method and the standard curve of bovine serum albumin (BSA).

For hormone extraction, the leaves (1g) were pulverised in a clean pestle and mortar and stored in an ice box at 4°C. Some BHT crystals were added as an antioxidant. The leaf material was then mixed with 10 ml of methanol (80%) and homogenised. The suspension was transferred to the test tubes. The extraction of phytohormones was conducted at 4 °C for a period of 72 hours using 10 mL of methanol (80%). The methanol was removed every 24 hours. The methanolic extract was centrifuged, and the supernatant was collected. The

supernatant (30 mL) was converted to an aqueous phase at 35 °C using a rotary thin film evaporator (RFE). The film was then mechanically shaken for 1 minute with 10 mL of distilled water added. To estimate ABA, GA, and IAA, pH was standardised to 2.5-3.0 using 0.1 N HCl. The mixture was then partitioned four times by ½ volume of ethyl acetate until a translucent layer was achieved. A rotary thin film evaporator was then used to completely dry up the ethyl acetate. 1 mL of 100% methanol dissolved the thin film and was put into Eppendorf tubes and refrigerated until HPLC analysis (Kettner and Doerffling, 1995). The C-18 column was employed to evaluate the samples using HPLC. For HPLC, the mobile phase was made up of 30 parts methanol and 70 parts double-distilled water.

For IAA and GA detection, isocratic elution was used. The wavelength used to find IAA was 280 nm (Sarwar *et al*., 1992), and the wavelength used to find GA was 254 nm. Abscisic acid (ABA) was eluted using gradient elution for 30 minutes, at a wavelength of 254 nm (Li *et al*., 1994). Growth hormones were classified according to their retention time and peak area. Pure IAA, GA, and ABA were used as standards to find and measure phytohormones. Plants were harvested (165-180 DAYS) from all nine treatment trials, and yield-related characteristics such as the number of branches per plant (BPP), pod length (PL), number of pods per branch (PPB), number of seeds per pod (SPP), and 100 seed weight (HSW) were determined. ANOVA was employed to statistically analyse the data, and the mean values were contrasted using Duncan's Multiple Range Test (DMRT) in COSTAS software (Duncan, 1955).

RESULTS

The carotenoid (Caro) content in the leaves of plants (Figure 1 a and b) grown with $Cr_{40}S_0$ and $Cr_{160}S_0$ was 1.2-1.6-folds higher at the vegetative stage and 2-2.6% higher at the reproductive stage than the plants under control treatment. While plants grown under $Cr_{40}S_{50}$, $Cr_{40}S_{150}$, $Cr_{160}S_{50}$, and $Cr_{160}S_{150}$ had significantly lower Caro content, i.e., 20%-24% reduced at vegetative stage and 13%-21% reduced at reproductive stage than in the non-sulfur supplemented soil under $Cr_{40}S_0$ and $Cr_{160}S_0$ treatment. Chl a (chlorophyll a) content (µg/ g) (Figure 1 c and d) was significantly lowered (48%-58%) in plants treated by both concentrations of chromium (40 and 160 ppm) at both growth stages. While Chl a content was significantly higher (34-68%) in $Cr_{40}S_{50}$, $Cr_{40}S_{150}$ in contrast to $Cr_{40}S_0$. Similarly, Chl a content in the leaves of plants grown in $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$ treated soil was significantly higher (36% to 52%) compared to non-sulfur-supplemented soil plants under $Cr_{160}S_0$ at both growth stages.

The Chl b (chlorophyll b) content (Figure 1 e and f) in plants treated with $Cr_{40}S_{50}$ and $Cr_{40}S_{150}$ was considerably higher, i.e., 15.2-48% at the vegetative stage and 20-27% at the reproductive stage, than the plants without sulfur supplementation under $Cr_{40}S_0$ stress. While it had significantly higher Chl b content than $Cr_{40}S_0$ treated plants at the reproductive stage. Likewise, Chl b content in the plants grown in $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$ treated soil was significantly higher (46-48% at vegetative stage and 20-24% at reproductive stage) compared to plants of non-sulfur-supplemented soil under $Cr_{160}S_0$ stress.

The T Chl (total chlorophyll) content (Figure 1 g and h) was found to be significantly lower under chromium stress in canola plants, i.e., 46-59% decreased at the vegetative stage, and 47-57% decreased at the reproductive stage under $Cr_{40}S_0$ and $Cr_{160}S_0$, respectively than the plants grown under control conditions. However, T Chl content was significantly enhanced by 55-64% at the vegetative stage and 40-44% at the reproductive stage with sulfur supplantation in $Cr_{40}S_{50}$ and $Cr_{40}S_{150}$ treatments, respectively, than in the plants grown with sulfur supplantation in $Cr_{40}S_0$ treatment. Likewise, in contrast to plants grown only in $Cr_{160}S_0$ stress, T Chl content improved in the plants of $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$ treated soil, i.e., 38-44% at the vegetative stage and 32-35% at the reproductive stage, respectively.

Leaf osmotic potential (Figure 2 a, b) was significantly more negative under chromium stress (43-91% at the vegetative stage; 59-75% at the reproductive stage with $Cr_{40}S_0$ and $Cr_{160}S_0$, respectively) than control grown. While plants grown under $Cr_{40}S_{50}$, and $Cr_{40}S_{150}$ had less negative osmotic potential, i.e., 25% and16% less negative at the vegetative stage and 15% and 8% less negative at the reproductive stage. Similarly, plants grown in $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$ had 15% and 8% less negative osmotic potential at the vegetative stage and 10% and 40% less negative osmotic potential at the reproductive stage in contrast to the plants under $Cr_{160}S_0$ stress.

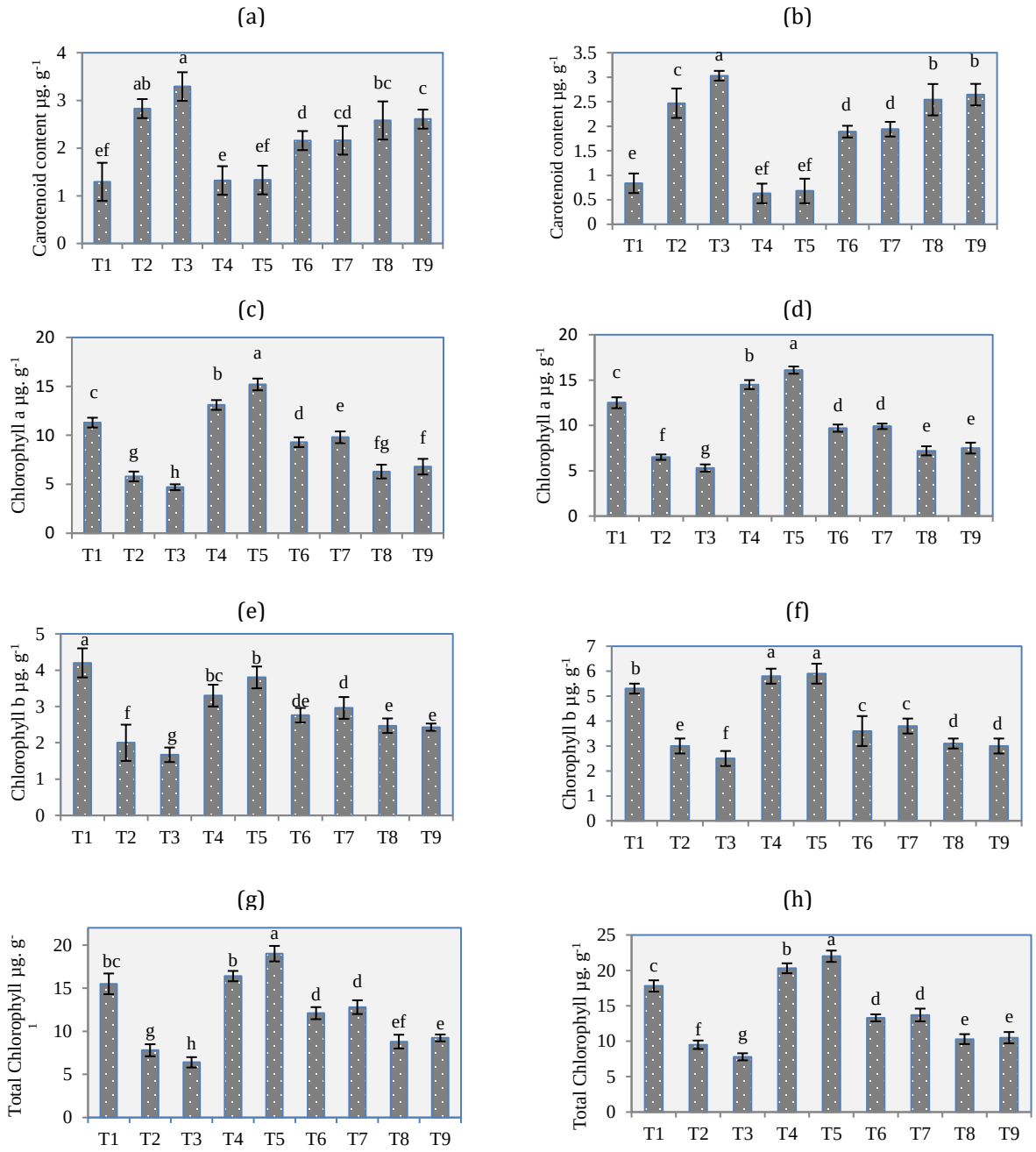


Figure 1. Influence of Sulfur on photosynthetic pigments a) Carotenoid content at vegetative b) Carotenoid content at reproductive stage c) Chlorophyll a at vegetative d) Chlorophyll a at reproductive e) Chlorophyll b at vegetative f) Chlorophyll b at reproductive g) Total chlorophyll at vegetative h) Total chlorophyll at reproductive stage of Canola (*Brassica napus* L.) under Chromium Stress.

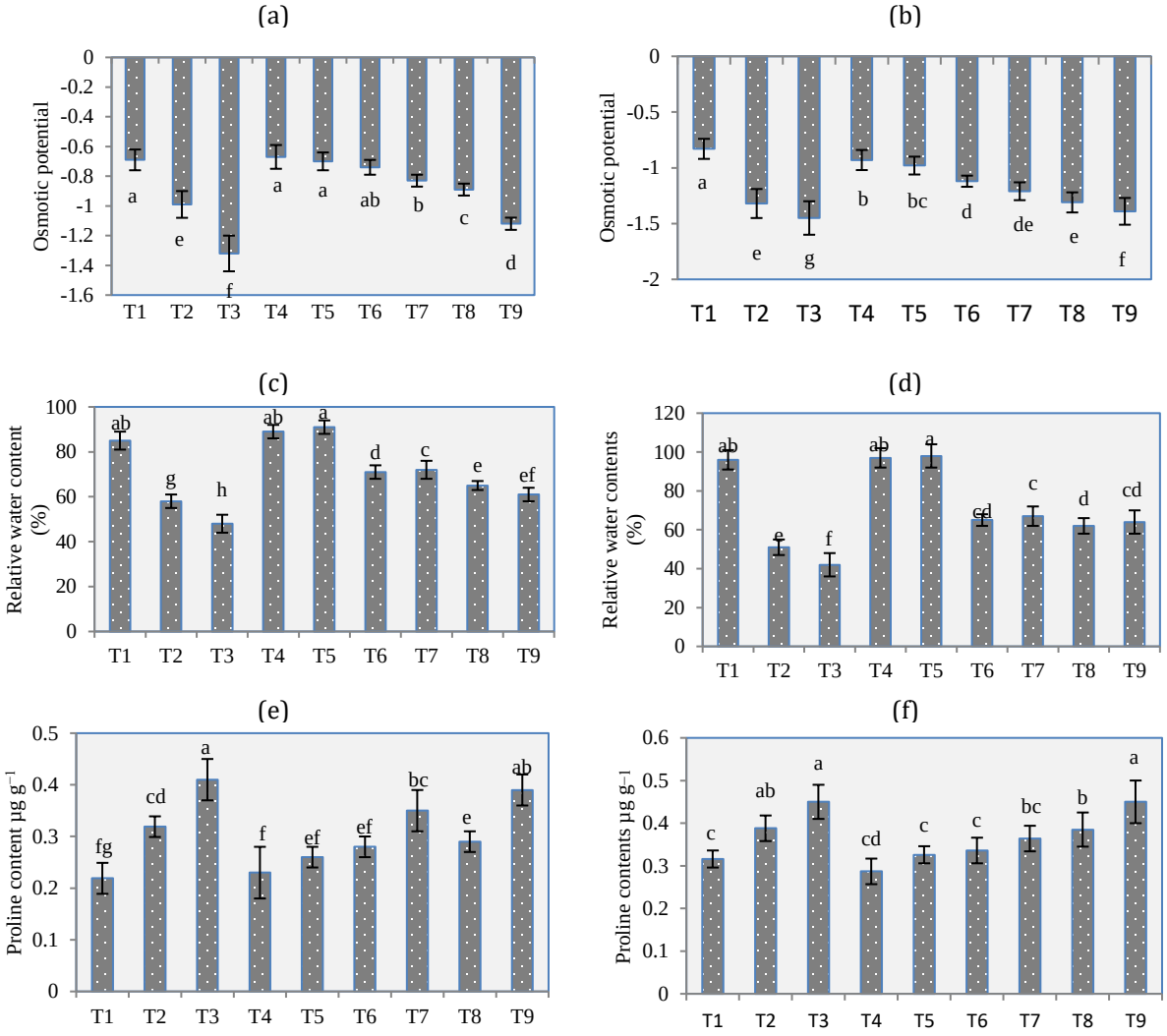
Where, T1= Cr_0S_0, T2= $Cr_{40}S_0$, T3= $Cr_{160}S_0$, T4= Cr_0S_{50}, T5= Cr_0S_{150}, T6= $Cr_{40}S_{50}$, T7= $Cr_{40}S_{150}$, T8= $Cr_{160}S_{50}$, T9= $Cr_{160}S_{150}$.

At the vegetative growth stage (Figure 2c and d), plants grown with chromium 40 ppm ($Cr_{40}S_0$) and chromium 160 ppm ($Cr_{160}S_0$) had significantly ($P< 0.05$) low relative water content respectively in comparison to control. Plants grown with sulfur 50 ppm (S_{50}) under chromium 40 ppm ($Cr_{40}S_{50}$), sulfur 150 ppm (S_{150}) under chromium 40 ppm ($Cr_{40}S_{150}$) had significantly higher (22%-24%) relative water content than $Cr_{40}S_0$ treated plants. The $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$ had significantly increased relative water content, i.e., 35.4% and 27%

higher in contrast to Cr_{160}. Similarly, at the reproductive stage (Figure 2d), relative water content was increased in $Cr_{40}S_{50}$ and $Cr_{40}S_{150}$ from 27-32%, in contrast to non-sulfur-supplemented soil under chromium stress. Similarly, $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$ treatment had significantly improved relative water content, i.e., 48-52% enhanced than plants of non-sulfur-supplemented soil under Cr_{160}.

Chromium stress in treatments $Cr_{40}S_0$ and $Cr_{160}S_0$ resulted in significantly high proline content (46-87% at vegetative and 23-42% at reproductive stage, respectively) than in control plants (Figure 2 e and f). At the vegetative stage, sulfur supplementation under chromium stress in treatments $Cr_{40}S_{50}$ caused a reduction in proline content by 12%, while $Cr_{40}S_{150}$ treatment resulted in significantly higher leaf proline content (9.7% increased) in comparison to plants under $Cr_{40}S_0$ stress. However, at the reproductive stage, a considerable decline in leaf proline content was observed, i.e., 29% and 5% lower in $Cr_{40}S_{50}$ and $Cr_{40}S_{150}$, respectively, than in the plants exposed to chromium stress without sulfur supplementation. Similarly, proline content was also lower in plants grown under $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$ in contrast to those under $Cr_{160}S_0$ alone.

Plants with $Cr_{40}S_0$ and $Cr_{160}S_0$ had significantly higher sugar content (59-17.4% enhanced at the vegetative stage and 28-66% enhanced at the reproductive stage) in contrast to the plants under control treatment (Figure 2 g and h). $Cr_{40}S_{50}$ and $Cr_{40}S_{150}$ had significantly lower leaf sugar content, i.e., 11-17.4% when compared to the treatment of $Cr_{40}S_0$ at both growth stages. Similarly, the leaf sugar content was 8-24% lower in $Cr_{160}S_{50}$, and $Cr_{160}S_{150}$ treatments, respectively, compared to $Cr_{160}S_0$ treated plants.



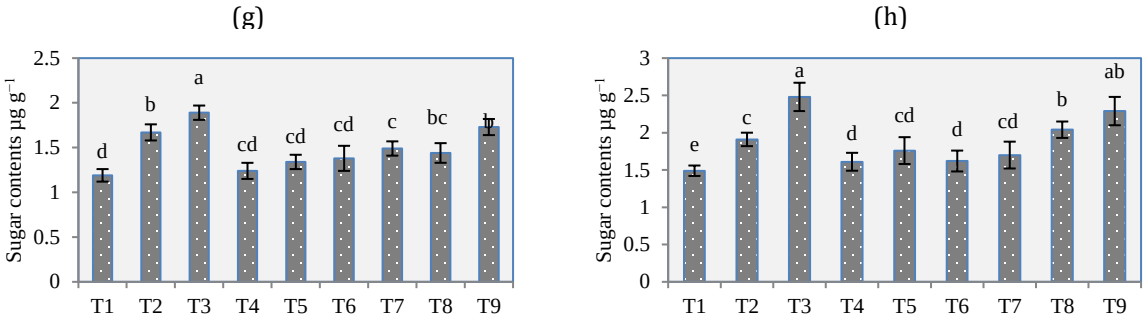


Figure 2. Influence of Sulfur on photosynthetic pigments a) osmotic potential at vegetative b) osmotic potential at reproductive stage c) relative water content at vegetative d) relative water content at reproductive e) proline content at vegetative f) proline content at reproductive g) sugar content at vegetative h) sugar content at reproductive stage of Canola (*Brassica napus* L.) under Chromium Stress.

Where, T1= Cr_0S_0, T2= $Cr_{40}S_0$, T3= $Cr_{160}S_0$, T4= Cr_0S_{50}, T5= Cr_0S_{150}, T6= $Cr_{40}S_{50}$, T7= $Cr_{40}S_{150}$, T8= $Cr_{160}S_{50}$, T9= $Cr_{160}S_{150}$.

At both growth stages (Figure 3 a, b), there was significantly lower protein content (44.5-67% reduced at the vegetative stage and 12-21% reduced at the reproductive stage) in treatment $Cr_{40}S_0$ and Cr_{160}, respectively, in comparison to the control. Considerably higher leaf protein content was observed in plants treated with $Cr_{40}S_{50}$, $Cr_{40}S_{50}$ (2-3-folds enhanced at vegetative stage and 15-20% enhanced at reproductive stage); Cr_{160}S50 and $Cr_{160}S_{150}$ (5-6-folds higher at vegetative stage and 22-23% enhanced at reproductive stage) in comparison to those with $Cr_{40}S_0$ as well as $Cr_{160}S_0$ treatment.

Abscisic acid (ABA) content was found to be significantly higher under chromium stress in canola plants, i.e., 1-2-folds higher at both growth stages under $Cr_{40}S_0$ and $Cr_{160}S_0$, respectively, than the plants grown under control conditions (Figure 3 c and d). However, leaf ABA content was significantly enhanced by 70-75% at the vegetative stage while declined by 33% and 23% at the reproductive stage with sulfur supplantation in $Cr_{40}S_{50}$ and $Cr_{40}S_{150}$ treatments, respectively, compared with $Cr_{40}S_0$ treatment. In contrast to plants grown only in $Cr_{160}S_0$ stress, ABA content significantly declined by 56-66% at the vegetative stage and by 11-16% at the reproductive stage, respectively improved in the plants of $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$ treated soil.

Chromium stress in treatments $Cr_{40}S_0$ and $Cr_{160}S_0$ resulted in significantly reduced leaf gibberellic acid (GA) content (26-50% at vegetative and 87% and 71% lower at reproductive stage, respectively) than in control plants (Figure 3 e and f). At the vegetative stage, GA content was significantly enhanced by 7-57% in $Cr_{40}S_{50}$ and $Cr_{40}S_{150}$, 2-folds and 45% $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$ in comparison to plants under $Cr_{40}S_0$ and $Cr_{160}S_0$, respectively. However, at the reproductive stage, a considerable decline in leaf GA content was observed, i.e., 32-675% in $Cr_{40}S_{150}$, $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$, than in the plants exposed to $Cr_{40}S_0$ and $Cr_{160}S_0$ stress alone.

There was significantly lower indole acetic acid (IAA) content by 90-94% at the vegetative stage and by 75-86% lower at the reproductive stage in $Cr_{40}S_0$ and Cr_{160}, respectively, in comparison to the control (Figure 3 g and h). However, significantly enhanced leaf IAA content was observed in plants treated with $Cr_{40}S_{50}$, $Cr_{40}S_{50}$ (4-folds and 44% 2higher at the vegetative stage and 1.1-1.2-folds enhanced at reproductive stage); $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$ (1-3.4-folds higher at vegetative stage and 4-86% enhanced at reproductive stage) in comparison to those with $Cr_{40}S_0$ as well as $Cr_{160}S_0$ treatment.

Regarding yield attributing parameters at the harvesting stage (Table 1), both treatments, $Cr_{40}S_0$ and $Cr_{160}S_0$, significantly reduced the number of branches per plant (i.e., 71-88% lower) in contrast to the control. Sulfur supplementation in treatments $Cr_{40}S_{50}$ and $Cr_{40}S_{150}$ caused a considerable improvement in the number of branches per plant, i.e., 73-120%, in contrast to non-sulfur-supplemented plants under $Cr_{40}S_0$ stress. Similarly, in $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$ treatments, there was an improvement of 1.2-folds, and 80%, respectively, compared to the plants of non-sulfur-supplemented soil under $Cr_{160}S_0$ stress.

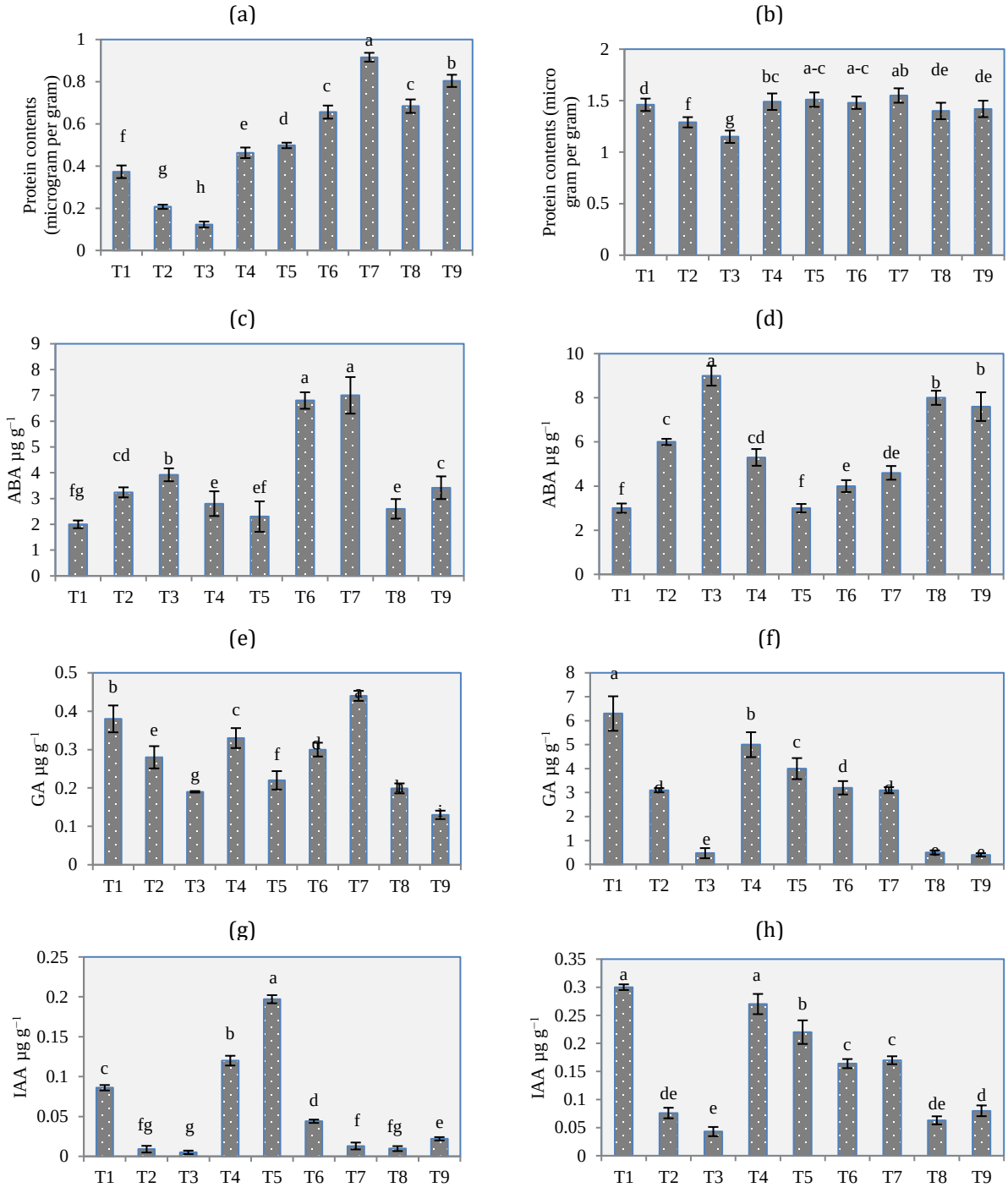


Figure 3. Influence of Sulfur on photosynthetic pigments a) protein content at vegetative b) protein content at reproductive stage c) abscisic acid at vegetative d) abscisic acid at reproductive e) gibberllic acid at vegetative f) gibberllic acid at reproductive g) indole acetic acid at vegetative h) indole acetic acid at reproductive stage of canola (*Brassica napus* L.) under chromium Stress.

Where, T1= Cr_0S_0, T2= $Cr_{40}S_0$, T3= $Cr_{160}S_0$, T4= Cr_0S_{50}, T5= Cr_0S_{150}, T6= $Cr_{40}S_{50}$, T7= $Cr_{40}S_{150}$, T8= $Cr_{160}S_{50}$, T9= $Cr_{160}S_{150}$.

Table 1. Influence of Sulfur on Yield attributes i.e., Number of Branches per plant, Number of pods per branch, Number seeds per pod, Pod length and 100 seeds Weight (g) of Canola (*Brassica napus* L.) under Chromium Stress.

Treatments	Number of branches per plant	Number of pods per branch	Number seeds per pod	Pod length (cm)	100 seeds weight (g)
Cr_0S_0	8 ± 0.12 c	21 ± 2.2 c	26 ± 1.8 c	7.5 ± 0.4 b	0.35 ± 0.21 b
$Cr_{40}S_0$	2.3 ± 0.15 f	13 ± 1.5 ef	20 ± 2.1 f	5.7 ± 0.3 e	0.18 ± 0.14 f
$Cr_{160}S_0$	1 ± 0.13 h	8 ± 0.9 g	5 ± 1.3 h	3.4 ± 0.1 g	0.15 ± 0.18 g
Cr_0S_{50}	8.5 ± 0.11 b	34 ± 2.1 b	29 ± 2.3 b	7.8 ± 0.3 b	0.37 ± 0.28 b
Cr_0S_{150}	9.6 ± 0.25 a	41 ± 2.5 a	34 ± 2.1 a	8.5 ± 0.2 a	0.42 ± 0.31 a
$Cr_{40}S_{50}$	4 ± 0.38 e	19 ± 2.4 cd	23 ± 1.9 de	6.5 ± 0.2 d	0.29 ± 0. 18 cd
$Cr_{40}S_{150}$	5 ± 0.45 d	21 ± 1.6 c	24 ± 1.5 d	6.9 ± 0.4 c	0.31 ± 0.21 c
$Cr_{160}S_{50}$	2.2 ± 0.14 f	15 ± 2.3 e	14 ± 1.3 g	5.5 ± 0.2 ef	0.22 ± 0.26 e
$Cr_{160}S_{150}$	1.8 ± 0.23 fg	13 ± 1.3 ef	15 ± 1.9 g	5.6 ± 0.7 e	0.21 ± 0.28 e

Different alphabets (a-h) with standard deviation values indicates level of significance at 95% confidence interval.

The number of pods per branch (PPB) was also reduced significantly, i.e., 38-62% lower with $Cr_{40}S_0$ and $Cr_{160}S_0$ treatments compared to control. However, sulfur supplementation caused a considerable improvement in the PPB in canola, i.e., 2.1-folds and 46% higher $Cr_{40}S_{50}$ and $Cr_{40}S_{150}$ in contrast to $Cr_{40}S_0$ stress. Similarly, 160% and 88% improvement was observed in the PPB in $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$ treatments compared to the non-sulfur-supplemented plants under $Cr_{160}S_0$ stress.

In contrast, to control, $Cr_{40}S_0$ and $Cr_{160}S_0$ treatments caused a significant decline in the number of seeds per pod (SPP), i.e., 23-81% lower, respectively. However, the SPP improved considerably in $Cr_{40}S_{50}$ and $Cr_{40}S_{150}$ treatments (i.e., 15-20% higher) than in the non-sulfur-supplemented plants in the $Cr_{40}S_0$ treatment. Likewise, in $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$, an improvement of 1.2-2-folds in SPP was noticed respectively than alone $Cr_{160}S_0$ plants.

Pod length (PL) was 24-55% lower in the plants exposed to $Cr_{40}S_0$ and $Cr_{160}S_0$ stress than in the control plants. However, sulfur supplementation in $Cr_{40}S_{50}$ and $Cr_{40}S_{150}$ treatments caused an improvement of 14-21% in PL, respectively, in contrast to plants that received no sulfur supplementation and were exposed to $Cr_{40}S_0$ stress. Similarly, $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$ treatments caused a considerable improvement of 62% and 41% in PL, respectively, compared with the plants of sulfur supplementation under chromium stress (Cr_{160}).

In $Cr_{40}S_0$ and $Cr_{160}S_0$ treatments, a significant decline in HSW was recorded, i.e., 49-57% lower, respectively, than in the control plants. In $Cr_{160}S_{50}$ and $Cr_{160}S_{150}$, an improvement of 47% and 40% was noticed in HSW, respectively, than the non-sulfur supplemented plants under $Cr_{160}S_0$ stress.

Principal component analysis (PCA) described the relationship between treatments and physiological, phytohormonal, and yield attributes of canola (Figure 4). The first component (PC-1) described a total of 79.4% variance, while the second component (PC-II) described a variance of 8%. In PC-I, IAA, PPB, BPP, T Chl, Chl b, T Chl, Chl a, RWC, SPP, HSW, OP, PL, GA, and protein content are positively correlated for enhancing growth in control plants (Cr0S0), plants with sulfur supplementation (Cr0S50 and Cr0S150) without chromium stress. While ABA, protein, and GA content are positively correlated with the plants of Cr40S50 and Cr40S150 treatments. However, PC-II's Caro, Pro, SC, ABA, and Prot content negatively correlates with all other physiological and yield attributes and treatments Cr0S0, Cr0S50, and Cr0S150. However, these attributes positively correlate with Cr40, Cr160, Cr160S50, and Cr160S150.

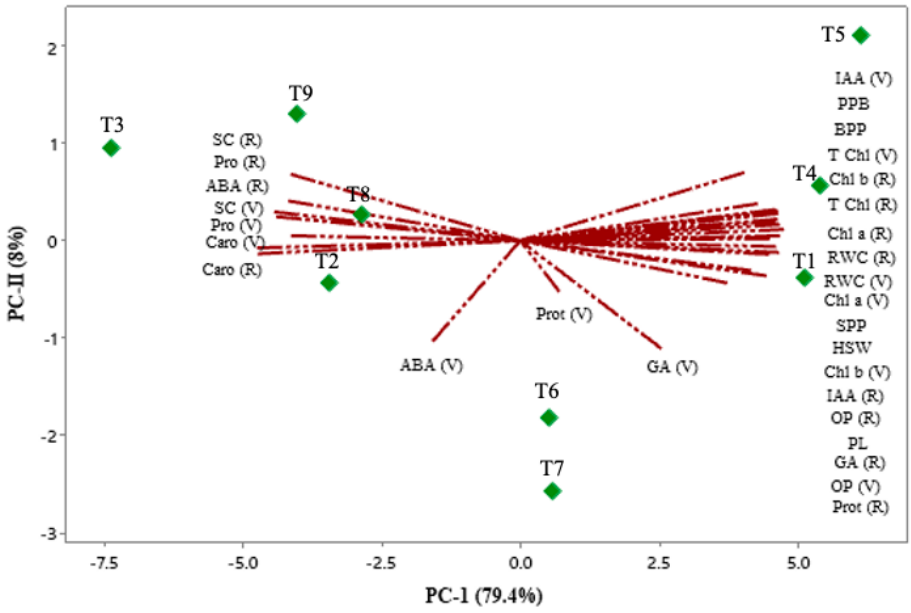


Figure 4. Biplot of principle component analysis showing relationship between treatments and canola physiological, biochemical and yield parameters.

Where, T1= Cr_0S_0, T2= $Cr_{40}S_0$, T3= $Cr_{160}S_0$, T4= Cr_0S_{50}, T5= Cr_0S_{150}, T6= $Cr_{40}S_{50}$, T7= $Cr_{40}S_{150}$, T8= $Cr_{160}S_{50}$, T9= $Cr_{160}S_{150}$ V= vegetative stage, R= reproductive stage, SC= sugar content, Pro= proline content, ABA= abscissic acid, Caro= carotenoid content, IAA= indole acetic acid, PPB= pods per branch, BPP= branches per plant, T Chl= total chlorophyll content, Chl b= chlorophyll b, Chl a = chlorophyll a, RWC= relative water content, SPP= seeds per pod, HSW= hundred seed weight, OP= osmotic potential, PL= pod length, GA= gibberellic acid, Prot= protein content.

DISCUSSION

The present study evidenced the more negative leaf osmotic potential and decrease in relative water content due to chromium stress, and this decline increases with chromium concentration in soil (Vishnupradeep *et al*., 2022). The reason might be that heavy metals can reduce water uptake in plants by modulating osmotic potential. Moreover, root damage caused by chromium is another reason for impeding water uptake in plants and decreasing leaf tissue water content which triggers plasmolysis in the tissues (Fahr *et al*., 2013). However, sulfur nutrition under chromium stress in Cr40S50 and Cr40S150 facilitated relatively high water content during both stages of growth. This increase in relative water content could be because of increased ABA under stress conditions in plants as a defense mechanism. Sulfur also leads to glutathione, cysteine, methionine, and biotin (Sanità di Toppi *et al.*, 2002). These compounds play a critical role during stress conditions such as glutathione in combination with ABA, reducing the stomatal aperture size, minimizing the excessive loss of water through transpiration, and preventing the plant from dehydration (Chen *et al.,* 2012).

Chromium stress caused a reduction in chlorophyll and carotenoid content of canola. This decline in the chlorophyll content caused by chromium might be due to the shrinkage of the marginal part of the antenna complex of photosystems. Besides, Cr also triggers protein deterioration and collapse in the antenna complex's peripheral part, which might be responsible for reduced chlorophyll content. Moreover, chromium stress also affects the chlorophyll biosynthetic machinery through enzyme inactivation, such as δ-aminolevulinic acid dehydratase. This enzymatic inactivation is mainly responsible for most plants' substantially reduced chlorophyll content (Ashraf *et al*., 2022; Diwan *et al.,* 2012).

Furthermore, hexavalent chromium usually substitutes Mg ions from the active sites of many essential enzymes involved in the synthesis of chlorophyll content and eventually reduces chlorophyll content in the leaves (Vajpayee *et al.,* 2000). Sulfur interaction with chromium

might have increased the chlorophyll content in canola. Similar results have been reported by Schiavon *et al.,* (2008) that sulfur supplementation under chromium stress in *B. junceae* plants enhanced chlorophyll a and chlorophyll b content (Chandel *et al.,* 2002).

An increase in proline content in canola under chromium stress might be due to the reduction in proline degradation, an increased biosynthesis of proline, a lapse in the production of protein, or their utilization in the hydrolysis of proteins (Charest and Phan 1990). Sulfur supplementation under chromium stress in plants also enhanced sugar concentrations.

Chromium stress also triggered a reduction in the protein content of canola plants; it might be due to the chromium-associated reduction in nitrate reductase activity, which might affect the production of amino acids which ultimately caused a decline in protein content (Panda and Choudhary 2004). The increase in protein content under sulfur nutrition with or without chromium stress in canola might be due to the sulfur-mediated biosynthesis of cysteine (Cys), which is also a vital amino acid in the synthesis of proteins and impart stress tolerance in plants.

Plants exposed to chromium stress accumulated high levels of ABA; this might be due to the role of ABA in stress tolerance, and its metabolism becomes higher during stress leading to increased production of ABA (Iqbal and Ashraf 2007). The slightly increased production of ABA under sulfur supplementation might be caused by sulfur metabolism, which ABA regulates, and it is also involved in sulfur homeostasis by enhancing the concentration of GSH, an intermediate in sulfur metabolism (Chen *et al.,* 2012). It was noted that gibberellic acid (GA) levels declined under Cr stress. This decline in GA might be linked with a decline in growth due to a decline in physiological functioning (low photosynthetic pigments and relative water content) of plants, as evidenced at present under Cr stress (Hamayun *et al.,* 2010). Similarly, sulfur supplementation also enhanced the IAA level in canola plants. This pattern of phytohormone under chromium stress in canola might be due to the central relationship of phytohormones with sulfur metabolism for stress tolerance. The sulfur metabolism produces secondary metabolites such as cysteine and glutathione, which reduce the reactive oxygen species produced through heavy metal stress and assist in maintaining the levels of GA and IAA (Szalai *et al.,* 2009).

In the present study, there was found a decrease in No. of branches per plant, No. of pods per branch, No. of seeds per pod, pod length, 100 seeds weight in canola under chromium stress. Chromium toxicity in the plant developmental processes during the early growth period resulted in yield reduction because of the reduced production of photo-assimilates and their translocation and transport to the plant's reproductive parts (Barcelo *et al.,* 1986; Jahan *et al.,* 2015).

Additionally, the selective inorganic nutrient uptake mechanism may have been disturbed by the larger quantities of hexavalent chromium in the soil. Its large quantities were then transported to the shoot, producing oxidative damage in the photosynthetic mitochondrial machinery, finally ending in poor growth. However, sulfur supplementation enhanced the yield attributes of canola. This increase in yield parameters in canola might be due to its large requirement for synthesizing cysteine and methionine content (Hrivna *et al.,* 2001). Sulfur is also an essential constituent of seed and enhances the oil percentage of the seed (Chaudhary *et al.,* 1992).

The PCA analysis shows that different physiological and yield-related traits respond differently to chromium stress and sulphur supplementation, which is consistent with previous research on plant physiological responses to heavy metal stress and the modulatory role of sulphur (Yang *et al*., 2020; Hussain *et al*., 2021). Sulfur-supplemented, non-stressed plants have better chlorophyll production, water relations, and phytohormonal levels, which are essential for plant health and productivity (Shah *et al*., 2022; Wei 2020). Moderate sulphur supplementation during low chromium stress has been found to preserve development by increasing ABA and GA levels, all of which play important roles in stress adaption and growth control (Zhang *et al*., 2019). However, at greater chromium levels, an increase in stress markers such as carotenoids and proline indicates a shift in defence from growth to survival, emphasising the function of sulphur in antioxidant synthesis while simultaneously demonstrating its limitations in stress tolerance (Mehmood *et al*., 2021; Ahmad *et al*., 2022). These findings indicate that sulphur serves a dual function: as a growth enhancer in non-stressful conditions and as a protective factor in mild stress. However, it is insufficient to protect against severe chromium toxicity, thereby highlighting the necessity of integrated stress management in agriculture (Yang *et al*., 2020; Hussain *et*

al., 2021).

CONCLUSION

The results of this study demonstrate that sulfur supplementation significantly mitigates the detrimental effects of chromium stress in canola plants across both vegetative and reproductive growth stages. Specifically, sulfur supplementation in treatments Cr40S50, Cr40S150, Cr160S50, and Cr160S150 led to notable improvements in chlorophyll a, chlorophyll b, total chlorophyll content, and relative water content compared to plants under chromium stress without sulfur. Sulfur supplementation also lowered the accumulation of carotenoids and proline, which are typically elevated under chromium stress, while enhancing leaf protein content, leaf gibberellic acid, and indole acetic acid levels. These physiological adjustments facilitated improved water retention and osmotic regulation, ultimately enhancing plant growth under chromium stress conditions. The PCA analysis further confirmed the positive correlation of sulfur-supplemented treatments with critical physiological and yield parameters, indicating that sulfur supplementation not only alleviates chromium-induced stress but also enhances crop productivity. These findings underscore the potential of sulfur supplementation as a practical approach for improving chromium tolerance and overall yield in canola cultivation under chromium-contaminated soils. Moreover, optimum sulfur levels required for this variety should be investigated under field conditions and recommended to the farmers to obtain maximum yields under normal and chromium-contaminated soils.

CONFLICT OF INTEREST

The authors affirm that the research was conducted without any commercial or financial affiliations that could be perceived as potential conflicts of interest.

ACKNOWLEDGMENTS

The authors extend their appreciation to the Minhaj University Lahore for all types of support.

REFERENCES

Ahmad, P., M.N. Alyemeni, and L. Wijaya. 2022. Role of sulfur in modulating plant responses to heavy metal stress. *Plant Stress*, 4(2), 117-125. https://doi.org/10.1016/j.stress.2022.117125.

Arnon, D.I. 1949. Copper enzyme in isolated chloroplast polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, 24, 1-15. https://doi.org/10.1104/pp.24.1.1.

Ashraf, M.A., R. Rasheed, I. Hussain, A. Hafeez, M. Adrees, M.Z. Rehman, and S. Ali. 2022. Effect of different seed priming agents on chromium accumulation, oxidative defense, glyoxalase system, and mineral nutrition in canola (*Brassica napus* L.) cultivars. *Environmental Pollution*,309, 119769. https://doi.org/10.1016/j.envpol.2022.119769.

Barcelo, J., C. Poschenrieder, and B. Gunse. 1986. Water relations of chromium VI treated bush bean plants (*Phaseolus vulgaris* L. cv. Contender) under both normal and water stress conditions. *Journal of Experimental Botany*, 37, 178-187. https://doi.org/10.1093/jxb/37.2.178.

Bates, L.S., R.P. Waldren, and I.D. Teare. 1973. Rapid determination of free proline for water stress studies. *Plant and Soil,* 39, 205-207. https://doi.org/10.1007/BF00018060.

Capaldi, F.R., P.L. Gratão, A.R. Reis, L.W. Lima, and R.A. Azevedo. 2015. Sulfur metabolism and stress defense responses in plants. *Tropical Plant Biology*, 8, 60-73.

Chaganti, V.N., G. Ganjegunte, G. Niu, A. Ulery, J.M. Enciso, R. Flynn, and J.R. Kiniry. 2021. Yield response of canola as a biofuel feedstock and soil quality changes under treated urban wastewater irrigation and soil amendment application. *Industrial Crops and Products*, 170, 113659. https://doi.org/10.1016/j.indcrop.2021.113659.

Chandel, R.S., P.C. Sudhakar, and K. Singh. 2002. Direct and residual effect of sulphur on Indian mustard (*Brassica juncea* L.) in rice (*Oryza sativa* L.) cropping system. *Indian Journal of Agricultural Sciences*, 72, 230-232.

Charest, C., and C.T. Phan. 1990. Cold acclimation of wheat (*Triticum aestivum* L.): Properties of enzymes involved in proline metabolism. *Physiologia Plantarum*, 80, 159-168. https://doi.org/10.1111/j.1399-3054.1990.tb04391.x.

Chaudhary, S.K., N.M. Gogulwar, and A.K. Singh. 1992. Effect of sulphur and nitrogen on seed yield and oil content of mustard (*Brassica juncea* L.). *Indian Journal of Agronomy,* 37, 839-840.

Chen, J., H. Jiang, E. Hsieh, H. Chen, C. Chien, H. Hsieh, and T. Lin. 2012. Drought and salt stress tolerance of an Arabidopsis glutathione S-transferase U17 knockout mutant attributed to the combined effect of glutathione and abscisic acid. *Plant Physiology*, 158, 340-351. https://doi.org/10.1104/pp.111.181875

Cobbett, C.S. 2000. Phytochelatins and their roles in heavy metal detoxification. *Journal of Plant Physiology,* 123, 825-832. https://doi.org/10.1104/pp.123.3.825

Diwan, H., A. Ahmad, and M. Iqbal. 2012. Chromium-induced alterations in photosynthesis and associated attributes in Indian mustard. *Journal of Environmental Biology*, 33, 239-244.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and F. Smith. 1956. Colorimetric method for the determination of sugars and related substances. *Journal of Analytical Chemistry*, 28, 350-356. https://doi.org/10.1021/ac60111a017.

Duncan, D.B. 1955. Multiple range and multiple F-test. *Biometrics*, 11, 1-42.

Fahr, M., L. Laplaze, N. Bendaou, V. Hocher, M. El Mzibri, D. Bogusz, and A. Smouni. 2013. Effect of lead on root growth. *Frontiers in Plant Science*, 4, 175. https://doi.org/10.3389/fpls.2013.00175.

Ghorbani, A., L. Pishkar, N. Roodbari, N. Pehlivan, and C. Wu. 2021. Nitric oxide alleviates arsenic phytotoxicity in tomato (*Solanum lycopersicum* L.) by modulating photosynthetic pigments, phytochelatin metabolism, molecular redox status, and arsenic sequestration. *Plant Physiology and Biochemistry*, 167, 337-348. https://doi.org/10.1016/j.plaphy.2021.08.019

Hamayun, M., E. Sohni, S.A. Khan, Z.K. Shinwari, A.L. Khan, and I. Lee. 2010. Silicon alleviates the adverse effects of salinity and drought stress on growth and endogenous plant growth hormones of soybean (*Glycine max* L.). *Pakistan Journal of Botany,* 42(3), 1713-1722.

Hayward, A. 2012. Introduction-oilseed brassicas. In: Edwards, D., J. Batley, I. Parkin, and C. Kole (Eds), *Genetics, Genomics and Breeding of Oilseed Brassicas*. CRC Press, Boca Raton, FL, USA, pp. 1-13.

Hrivna, L., R. Richter, and T. Losak. 2001. The effect of the content of water-soluble sulphur in the soil on the utilisation of nitrogen and on the yields and quality of winter rape. *Rostlinná Výroba,* 47, 18-22.

Hussain, A., M. Ali, and Z. Rahman. 2021. Sulfur supplementation alleviates chromium-induced oxidative stress in canola plants by improving physiological and biochemical traits. *Environmental and Experimental Botany*, 185,104428. https://doi.org/10.1016/j.envexpbot.2021.104428.

Iqbal, M., and M. Ashraf. 2007. Seed treatment with auxins modulates growth and ion partitioning in salt-stressed wheat plants. *Journal of Integrative Plant Biology*, 49,1003-1015. https://doi.org/10.1111/j.1672-9072.2007.00488.x.

Jahan, S., S. Iqbal, K. Jabeen, and S. Sadaf. 2015. Ameliorating influence of sulfur on germination attributes of canola (*Brassica napus* L.) under chromium stress. *Pakistan Journal of Botany*, 47,407-411.

Junaid, M., M.Z. Hashmi, Y.M. Tang, R.N. Malik, and D.S. Pei. 2017. Potential health risk of heavy metals in the leather manufacturing industries in Sialkot, Pakistan. *Scientific Reports*, 7,1-13. https://doi.org/10.13057/biodiv/d230440.

Kettner, J., and K. Doerffling. 1995. Biosynthesis and metabolism of abscisic acid in tomato leaves infected with *Botrytis cinerae*. *Planta*, 196,627-634. https://doi.org/10.1007/BF01106753.

Khalid, S., M. Shahid, I. Bibi, T. Sarwar, A.H. Shah, and N.K. Niazi. 2018. A review of environmental contamination and health risk assessment of wastewater use for crop irrigation with a focus on low- and high-income countries. *International Journal of Environmental Research and Public Health*, 15,895. https://doi.org/10.3390/ijerph15050895.

Kotas, J., and Z. Stasicka. 2000. Chromium occurrence in the environment and methods of its speciation. *Environmental Pollution,* 107,263-283. https://doi.org/10.1016/S0269-7491(99)00168-2.

Li, J.C., J. Shi, X.L. Zhao, G. Wang, H.F. Yu, and Y.J. Ren. 1994. Separation and determination of three kinds of plant hormones by high-performance liquid chromatography. *Fenxi Huaxue,* 22,801-804.

Lichtenthaler, H.K., and W.R. Wellburn. 1983. Determination of total carotenoids and

chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions*, 11,591-592.

Lowry, O.H., N.J. Rosebrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry,* 193,265-275.

Mehmood, M., S.H. Shah, and A. Raza. 2021. Effect of sulfur on proline and carotenoid synthesis under chromium stress in canola. *Journal of Plant Physiology*, 267,153570. https://doi.org/10.1016/j.jplph.2021.153570.

Muhammad, S., and Q.A. Usman. 2022. Heavy metal contamination in water of Indus River and its tributaries, Northern Pakistan: Evaluation for potential risk and source apportionment. *Toxin Reviews*, 41,380-388. https://doi.org/10.1080/15569543.2021.1882499.

Mushtaq, Z., H.N. Asghar, and Z.A. Zahir. 2021. Comparative growth analysis of okra (*Abelmoschus esculentus*) in the presence of PGPR and press mud in chromium-contaminated soil. *Chemosphere*, 262,127865. https://doi.org/10.1016/j.chemosphere.2020.127865.

Panda, S.K., and S. Choudhury. 2004. Changes in nitrate reductase activity, lipid peroxidation, and antioxidant system in moss *Polytrichium* subjected to hexavalent chromium stress. *Brazilian Journal of Plant Physiology*, 31,179-184. https://doi.org/10.1590/S1677-04202005000200001.

Qadir, M., A. Ghafoor, and G. Murtaza. 2000. Cadmium concentration in vegetables grown on urban soils irrigated with untreated municipal sewage. *Environment, Development and Sustainability*, 2,11-19. https://doi.org/10.1023/A:1010061711331

Sanità di Toppi, L., F. Fossati, R. Musetti, I. Mikerezi, and M.A. Favali. 2002. Effects of hexavalent chromium on maize, tomato, and cauliflower plants. *Journal of Plant Nutrition,* 25,701-717. https://doi.org/10.1081/PLN-120002953.

Sanjay, M.S., D. Sudarsanam, G.A. Raj, and K. Baskar. 2020. Isolation and identification of chromium-reducing bacteria from tannery effluent. *Journal of King Saud University - Science,* 32,265-271. https://doi.org/10.1016/j.jksus.2018.05.001.

Sarwar, M., M. Arshad, D.A. Martens, and W.T. Frankenberger Jr. 1992. Tryptophan-dependent biosynthesis of auxin in soil. *Plant and Soil*, 147,207-215.

Schiavon, M., E.A.H. Pilon-Smits, M. Wirtz, and R.H.M. Malagoli. 2008. Interactions between chromium and sulfur metabolism in *Brassica juncea*. *Journal of Environmental Quality*, 37,1536-1545. https://doi.org/10.2134/jeq2007.0032.

Shah, S.T., N. Qamar, and M. Khan. 2022. Growth and yield responses of *Brassica* under sulfur supplementation and chromium stress. *Environmental Science and Pollution Research*, 29(5),7420-7432. https://doi.org/10.1007/s11356-021-16218-1

Singh, M., B.K. Kushwaha, S. Singh, V. Kumar, V.P. Singh, and S.M. Prasad. 2017. Sulfur alters chromium (VI) toxicity in *Solanum melongena* seedlings: Role of sulfur assimilation and sulfur-containing antioxidants. *Plant Physiology and Biochemistry,* 112,183-192. https://doi.org/10.1016/j.plaphy.2016.12.024

Srivastava, D., M. Tiwari, P. Dutta, P. Singh, K. Chawda, M. Kumari, and D. Chakrabarty. 2021. Chromium stress in plants: Toxicity, tolerance, and phytoremediation. *Sustainability*, 13,4629. https://doi.org/10.3390/su13094629.

Szalai, G., T. Kellos, G. Galiba, and G. Kocsy. 2009. Glutathione as an antioxidant and regulatory molecule in plants under abiotic stress conditions. *Journal of Plant Growth Regulation*, 28,66-80. https://doi.org/10.1007/s00344-008-9075-2.

Vajpayee, P., R.D. Tripathi, U.N. Rai, M.B. Ali, and S.N. Singh. 2000. Chromium (VI) accumulation reduces chlorophyll biosynthesis, nitrate reductase activity, and protein content in *Nymphaea alba* L. *Chemosphere*, 41,1075-1082. https://doi.org/10.1016/S0045-535(99)00426-9.

Vishnupradeep, R., L.B. Bruno, Z. Taj, C. Karthik, D. Challabathula, A. Kumar, H. Freitas, and M. Rajkumar. 2022. Plant growth-promoting bacteria improve growth and phytostabilization potential of *Zea mays* under chromium and drought stress by altering photosynthetic and antioxidant responses. *Environmental Technology &*

Innovation, 25,102154. https://doi.org/10.1016/j.eti.2021.102154.

Wei, Y., J. Zhu, and L. Gao. 2020. Sulfur-induced modulation of chlorophyll and hormone levels in canola plants under chromium stress. *Plant Physiology Reports*, 25(3),414-422. https://doi.org/10.1007/s40502-020-00513-4

Wheatherley, P.E. 1950. Studies in water relations of cotton plants: The field measurement of water deficit in leaves. *New Phytologist*, 49, 81–87. http://dx.doi.org/10.1111/j.1469-8137.1950.tb05146.x

Yang, Y., Y. Feng and J. Zhang. 2020. Principal component analysis of physiological and hormonal responses to chromium and sulfur treatments in crops. *Agronomy*, 10(9), 1341. https://doi.org/10.3390/agronomy10091341.

Zhang, X., Z. Liu and P. Wang. 2019. Impact of sulfur supplementation on abscisic acid and gibberellic acid levels in chromium-stressed plants. *Journal of Plant Growth Regulation*, 38(1), 87–96. https://doi.org/10.1007/s00344-018-9822-6.

Zhong, L., C. Hu, Q. Tan, J. Liu and X. Sun. 2011. Effects of sulfur application on sulfur and arsenic absorption by rapeseed in arsenic-contaminated soil. *Plant, Soil and Environment,* 57, 429–434. https://doi.org/10.17221/224/2011-PSE.