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# Allicin improves gas exchange characteristics, redox balance, and ion homeostasis to mitigate chromium toxicity in wheat

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## Abstract

This research explored how exogenous application of allicin can assist wheat plants in coping with the stress caused by chromium (Cr). The findings indicate that Cr toxicity (30 mg kg$^{-1}$) conspicuously reduced biomass, chlorophyll content, and leaf relative water content (LRWC) in wheat plants. A significant surge in oxidative stress, as demonstrated by higher levels of hydrogen peroxide, superoxide radicals, and malondialdehyde alongside elevated lipoxygenase activity and electrolyte leakage percentage, was noticed in Cr-stressed plants. Chromium toxicity also induced higher methylglyoxal generation in plants. Allicin foliar administration (50, 100, and 200 µM) visibly curtailed Cr phytotoxicity and improved biomass. Allicin applied as 200 µM maximally subsided oxidative stress by augmenting the activities of superoxide dismutase (SOD), peroxidase (POD), catalase(CAT), and ascorbate peroxidase (APX), as well as the levels of ascorbate and reduced glutathione (GSH). Higher endogenous hydrogen sulfide ($H_2S$) and nitric oxide (NO) concentrations in allicin-treated plants further stimulated the antioxidant defense system of Cr-challenged plants. Allicin supplementation maintained GSH : GSSG ratio in Cr-stressed plants, suggesting a better redox balance. A noticeable accretion of soluble sugars, flavonoids, and phenolics due to allicin safeguarded plants from metal-induced damage and its associated oxidative stress. These findings highlight that exogenous application of allicin can be used as a promising strategy to alleviate Cr toxicity in wheat plants. Future studies should assess the effectiveness of exogenous allicin application in other cereal crops under controlled and field conditions. Additionally, exploring the molecular mechanisms underlying metal stress tolerance in allicin-treated plants will deepen our understanding.

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## Introduction

Elevated concentrations of heavy metal contaminants in agricultural soils have a profound effect on plant development, subsequently causing a marked reduction in crop yields (Alengebawy et al., 2021). As these heavy metals accumulate within plants, they can contribute to bioaccumulation, consequently moving down the food chain and creating major health concerns to both animals and humans as well as other living organisms (Sonone et al., 2020). Of different metals known, chromium (Cr) is a highly hazardous heavy metal with no known biological role in plants (Islam et al., 2023). In Pakistan, an estimated 32,500 hectares of land are irrigated with wastewater, which is potentially

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vulnerable to Cr contamination (Khanum et al., 2017). Chromium, which is widely used in various industrial applications, is prevalent in water, soil, and industrial waste (Jalil et al., 2024). This ubiquity poses significant environmental challenges as both a metal and a contaminant. Chromium exists in two stable oxidation states: trivalent ($Cr^{3+}$) and hexavalent ($Cr^{6+}$). These two forms differ considerably in their availability and toxicity, with hexavalent $Cr^{6+}$ being far more toxic than its trivalent counterpart (Ao et al., 2022). However, plants do not possess a dedicated mechanism for the Cr absorption. Rather, they assimilate Cr through sulfate transporters (Ferrari et al., 2022). Hexavalent Cr compounds, like chromate and dichromate, dissolve easily in water, whereas trivalent Cr is much less soluble (Ahammed et al., 2023).

Excessive buildup of Cr impairs plant metabolic functioning and membrane activities either directly or by inducing oxidative stress (Romero-Estévez et al., 2023). Plants exposed to Cr toxicity manifest higher ROS, including superoxide radicals ($O_2^{\bullet-}$) and hydrogen peroxide ($H_2O_2$), which can harm essential biomolecules (Ali et al., 2023; Zulfiqar et al., 2023). Various crops such as soybean (Basit et al., 2023), rice (Noor et al., 2023), sunflower (Ramzan et al., 2023), mungbean (Singh et al., 2023), wheat (Albqmi et al., 2023; Singh et al., 2023; Sun et al., 2023), and maize (Razzaq et al., 2024) showed diminished growth and biomass under Cr toxicity due to higher oxidative stress and impaired photosynthesis and antioxidant defense system.

In plants, dicarbonyl stress is due to higher production of cytotoxic methylglyoxal (MG) under Cr toxicity (Kharbech et al., 2020). Higher levels of MG react with nucleic acids, lipids, carbohydrates, and proteins, impairing their functionality (Lin et al., 2023). Reduced glutathione (GSH) is necessary for the glyoxalase system, which plants use to detoxify excess MG. Further, glyoxalase and antioxidant systems working in tandem is essential for MG detoxification (Kaya et al., 2023a). Notably, extensive research has focused on the alterations within the glyoxalase system in response to Cr stress in plants (Alam et al., 2021; Gupta and Seth, 2021; Kamran et al., 2021).

The detoxification of ROS in plants is achieved by utilizing the enzymatic and non-enzymatic antioxidant defense systems. These components work in concert to maintain redox balance in the plant system under normal and adverse environmental conditions. However, depending on the particular abiotic stressor and plant species, the antioxidant defense system can vary in functionality significantly (Li et al., 2023).

Wheat (*Triticum aestivum* L.) is the second most farmed cereal crop globally, following maize and rice, and it plays an essential role as a dietary staple food in numerous Asian countries and is crucial for food security in Pakistan (Shabnam et al., 2023). However, wheat is vulnerable to Cr contaminated conditions and manifests a significant decline in growth due to Cr phytotoxicity (Alam et al., 2023).

Allicin is an organosulfur compound abundantly present in garlic. It exhibits significant antioxidant properties and averts oxidative damage in the cellular environment (Salehi et al., 2019). It preserves mitochondrial function by curbing the generation of free radicals (Rais et al., 2023; Savairam et al., 2023). To date, no study has reported the role of exogenous allicin in alleviating chromium (Cr) stress in plants. Thus, the primary objective of this research was to evaluate the potential of foliar-applied allicin in mitigating Cr toxicity in wheat. The study specifically assessed the effects of Cr stress and allicin treatment on oxidative defense modulation, osmolyte accumulation, secondary metabolite production, and membrane lipid peroxidation in wheat plants.

## Materials and Methods

The study was conducted at the Government College University, Faisalabad, Pakistan. Wheat seeds (cv. Pasbaan-2021) were obtained from Ayub Agricultural Research Institute (AARI), Faisalabad, Pakistan. The seeds underwent a surface sterilization process involving a 3-minute immersion in a 2% hydrogen peroxide ($H_2O_2$) solution, followed by a thorough washing with distilled water (Banerjee et al. 2023). In pots each containing 8 kg of soil that had been deliberately contaminated with 30 mg $kg^{-1}$ Cr as $K_2Cr_2O_7$, seedlings were planted (Kumar et al. 2023). Throughout the experimental period, environmental conditions were characterized by an average *PAR* of 1031 $\mu mol\ m^{-2}\ s^{-1}$, relative humidity ranging from 31% to 67%, a mean temperature of 23 ± 2 °C, and average precipitation of 7.16 ± 1.29 mm. Seven days after germination, thinning was performed to maintain three plants per pot. At the 30-day interval following sowing, the plants received treatments with varying concentrations of allicin (0, 50, 100, and 200 µM) applied as a foliar spray. To improve allicin absorption, tween-20 (0.1%) was used as a surfactant. To avoid contamination of the soil medium by the foliar spray, aluminum foil was carefully wrapped around the base of the plants. The experimental treatment breakup was: (1) control + no spray of allicin, (2) control + allicin 50 µM, (3) control + allicin 100 µM, (4) control + allicin 200 µM, (5) Cr 30 mg kg$^{-1}$ + no spray of allicin, (6) Cr 30 mg kg$^{-1}$ + allicin 50 µM, (7) Cr 30 mg kg$^{-1}$ + allicin 100 µM, (8) Cr 30 mg kg$^{-1}$ + allicin 200 µM.

## Growth attributes

Plant shoot and root lengths were measured with a measuring scale. The fresh biomass of shoot and root was measured with a digital weighing balance. To determine dry mass, the samples of the shoots and roots were oven-dried at 75 °C for 48 h before weighing. The leaf area was calculated using the equation established by Gardner et al. (1985).

Leaf area = leaf length × leaf width × 0.75

## Leaf relative water content (LRWC)

The LRWC was calculated using the formula provided by Barrs and Weatherley (1962).

$$\text{LRWC (\%)} = (\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight}) \times 100$$

## Leaf pigments

To determine chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, a 50 mg leaf sample was carefully cut, and soaked in 80% acetone for 24 h. This pigment extraction and quantification were adhered to the methodology established by Lichtenthaler (1987). A spectrophotometer was used for absorbance readings at wavelengths 480, 645, and 663 nm. The sample was processed following the protocol as set out by Sarker and Oba (2018a) for the measurement of β-cyanin and β-xanthin concentration. The absorbance values were also taken at 540 nm and 475 nm. Quantification of β-carotene was done through dissolving 0.5 g leaf samples in 80% methanol (in line with Jensen method 1978), and noting OD at 510 nm and 480 nm. A CL-01 chlorophyll meter was used to determine SPAD values.

## Anthocyanins

A mixture of leaf sample was developed using aqueous methanol solution having hydrochloric acid of 1% strength. The mixture was homogenized, and centrifuged to remove the supernatant. Following the steps provided by Mita et al. (1997), the supernatant was used to take absorbance measurements at 530 nm and 657 nm.

## Ascorbic acid

For the quantification of ascorbic acid, the protocol stated by Mukherjee and Choudhuri (1983) was followed. More precisely, 0.5 g of leaf tissue was triturated in 6% trichloroacetic solution. Finally, 1 mL of a 2% dinitrophenyl hydrazine solution, 1.5 mL of its obtained supernatant and one drop of 10% thiourea were combined. After that, the mixture was incubated for 40 minutes at 95 °C. The reaction was terminated by adding sulfuric acid (1.5 mL of 80%). The absorption of the final solution was read using a spectrophotometer at 530 nm wavelength.

## Estimation of phenolics and flavonoids

The methodology employed in this study was based on the Ribarova et al. (2005) method for the estimation of flavonoids contained in leaf samples. The total phenolic content was also estimated according to the protocol provided by Julkunen-Tiitto (1985).

## Quantification of cytosolutes

Quantification of soluble sugars was done as outlined by Yemm and Willis (1954). Reducing and non-reducing sugars were quantified by means of the techniques developed by Nelson (1944) and Loomis and Shull (1937), respectively. The of Bates et al. (1973) was employed to quantify the levels of free proline in the leaf samples. To analyze total free amino acids, the leaf sample weighed 0.5 g was homogenized in potassium phosphate (K-P) buffer at a pH of 7.8, as per Hamilton et al. (1943). The glycine betaine was estimated by the procedure described by Grieve and Grattan (1983).

## Oxidative stress markers

The protocol described by Velikova et al. (2000) was employed to determine the concentration of hydrogen peroxide ($H_2O_2$) in fresh leaf material. The procedure outlined by Yang et al. (2011) was followed for the quantification of superoxide ($O_2^{\bullet -}$) generation. For the estimation of malondialdehyde (MDA) content, the Heath and Packer (1968) method was undertaken. The electrolyte leakage (EL) was appraised according to the methodology proposed by Yang et al. (1996).

### Total soluble proteins and enzyme activities

Absorbance changes after addition of linoleic acid as a substrate in the presence of lipoxygenase (LOX) activity by the enzyme extract in the same reaction solution were measured as described by Doderer et al. (1992), and the method put forth by Bradford (1976), was used for the determination of total soluble proteins (TSP). Catalase (CAT) activity was measured by appraising the absorbance at 240 nm after 120 s of the reaction mixture containing 50 mM K-P buffer (pH 7.5) and 50 mM $H_2O_2$, (Qureshi et al., 2020). To determine APX activity, the reaction solution was prepared as described by Nakano and Asada (1981) with 50 mM K-P buffer at pH 7.5, 0.5 mM ascorbic acid, and 0.1 mM $H_2O_2$. The POD activity on guaiacol oxidation was assayed according to Chance and Maehly (1955) in 50 mM K-P buffer (pH 7.5), 100 µL of enzyme extract, 20 mM guaiacol, 5 mM $H_2O_2$, and 8 mL of distilled water. Absorbance was read at 470 nm. The superoxide dismutase (SOD) activity was determined using the method described by Lyons et al. (2023) and it requires a reaction mixture consisting of 75 nM EDTA, 1.3 mM riboflavin, 50 mM NBT, 50 mM K-P buffer (pH 7.5), 13 mM methionine, and 100 µL of the enzyme extract. Absorbance was measured at 560 nm.

### Glutathione metabolism

Fresh leaf tissue was analyzed for the levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) following the method proposed by Hasanuzzaman et al. (2011).

### DPPH activity determination

A quantitative analysis of the DPPH radical scavenging activity was carried out according to Sarker and Oba (2018a, b).

### Hydrogen sulfide ($H_2S$) and nitric oxide (NO) levels

The amount of $H_2S$ in fresh leaf tissues was measured based on the Nashef et al. (1977) protocol. NO was estimated according to the method outlined by Zhou et al. (2023).

### Methylglyoxal content

Five percent perchloric acid solution (10 mL) was used to homogenize 0.5 g of leaf tissue samples. The supernatant was then collected and centrifuged. After adding charcoal and sodium carbonate ($Na_2CO_3$), the supernatant was subjected to an N-acetyl-L-cysteine-based test. Using the technique outlined by Nahar et al. (2016), the optical density was measured at 288 nm.

### Statistics

The data from a completely randomized experiment with four replications of each treatment were analyzed using the analysis of variance technique with the help of Minitab statistical software (version 6.303). The data was visualized using the OriginPro 2021 software.

## Results

### Growth attributes and leaf relative water content (LRWC)

The study revealed a marked decrease ($P \leq 0.001$) in growth attributes. For example, shoot length (SL), root length (RL), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW), root dry weight (RDW), and LRWC decreased by 23.15%, 49.49%, 35.41%, 32.40%, 16.35%, 32.49%, 32.16%, respectively, when plants were exposed to Cr stress (30 mg $kg^{-1}$). Notably, the external application of allicin at concentrations of 50, 100, and 200 µM led to a significant improvement ($P \leq 0.001$) in fresh and dry weights of the plants subjected to Cr stress. In particular, the plants treated with 200 µM of allicin displayed a remarkable increase in SFW (58.80%), RFW (10.31%), and RDW (32.67%). On the other hand, the highest SDW increase was observed with 100 µM allicin, achieving a growth enhancement of 34.06% under the same Cr-induced stress conditions. Leaf area was notably ($P \leq 0.001$) abridged by 24.22% under 30 mg $kg^{-1}$ Cr stress. Plants treated with different concentrations of exogenous allicin (50, 100, and 200 µM) showed a significant increase ($P \leq 0.001$) in leaf area under Cr stress. In this context, a substantial improvement of 34.89% in leaf area was observed at the 200 µM level of allicin in the wheat plants subjected to Cr stress. Leaf relative water content exhibited a noticeable ($P \leq 0.001$) reduction of 32.16% in wheat plants exposed to 30 mg $kg^{-1}$ Cr toxicity**.** In contrast, plants under stress but treated with different concentrations of allicin (50, 100, and 200 µM) demonstrated higher leaf relative water content (LRWC) compared to the control. In this context, plants subjected to a 200 µM dose of allicin exhibited an improved LRWC with a significant increase of 26.18% (**Figure 1**).

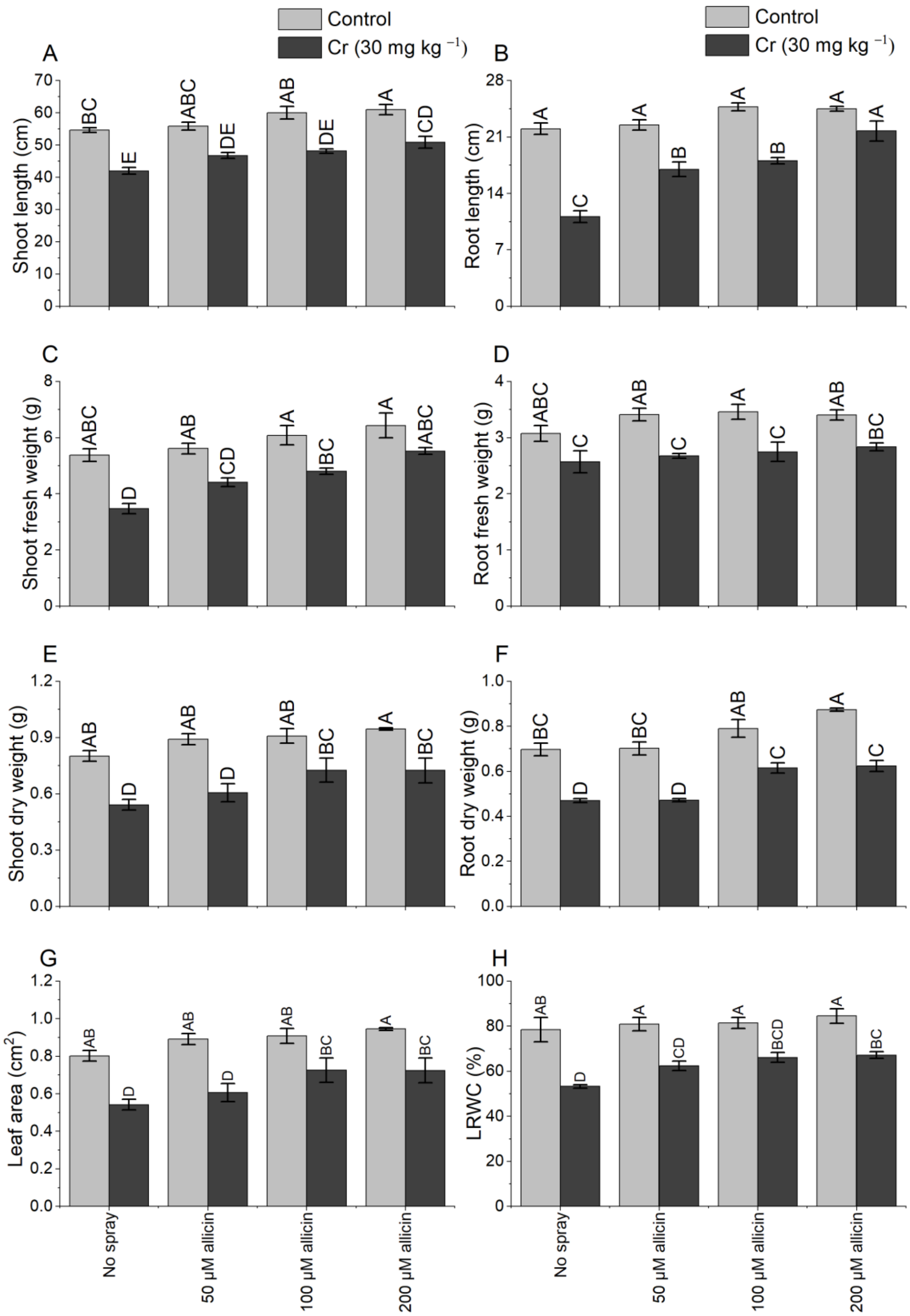

**Figure 1: Allicin-induced changes in growth parameters, shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, leaf area, and leaf relative water content (LRWC) in wheat (*Triticum aestivum* L.) plants under chromium (Cr) toxicity. Bars show means ± SE values of four replicates. Different letters on bars show statistically significant differences among means at *P* ≤ 0.05.**

## Photosynthetic pigments

There was a substantial ($P \leq 0.001$) decline of 46.35%, 39.62%, 43.71%, and 34.02% in chlorophyll *a*, *b*, T. chl, and carotenoids content, respectively, in wheat plants under 30 mg kg$^{-1}$ Cr stress. Foliar application of allicin (50, 100, and 200 µM) showed an improvement in leaf pigments in the Cr-stressed plants. However, plants treated with 200 µM allicin exhibited an increase ($P \leq 0.01$) in chlorophyll *a* content (31.73%) in wheat plants. Contrary to it, chl *b*, total chlorophyll, and carotenoid content showed enhancement ($P \leq 0.001$) in Cr-stressed plants by 82.31%, 53.10%, and 37.89% compared to unsprayed wheat plants (**Figure 2**).

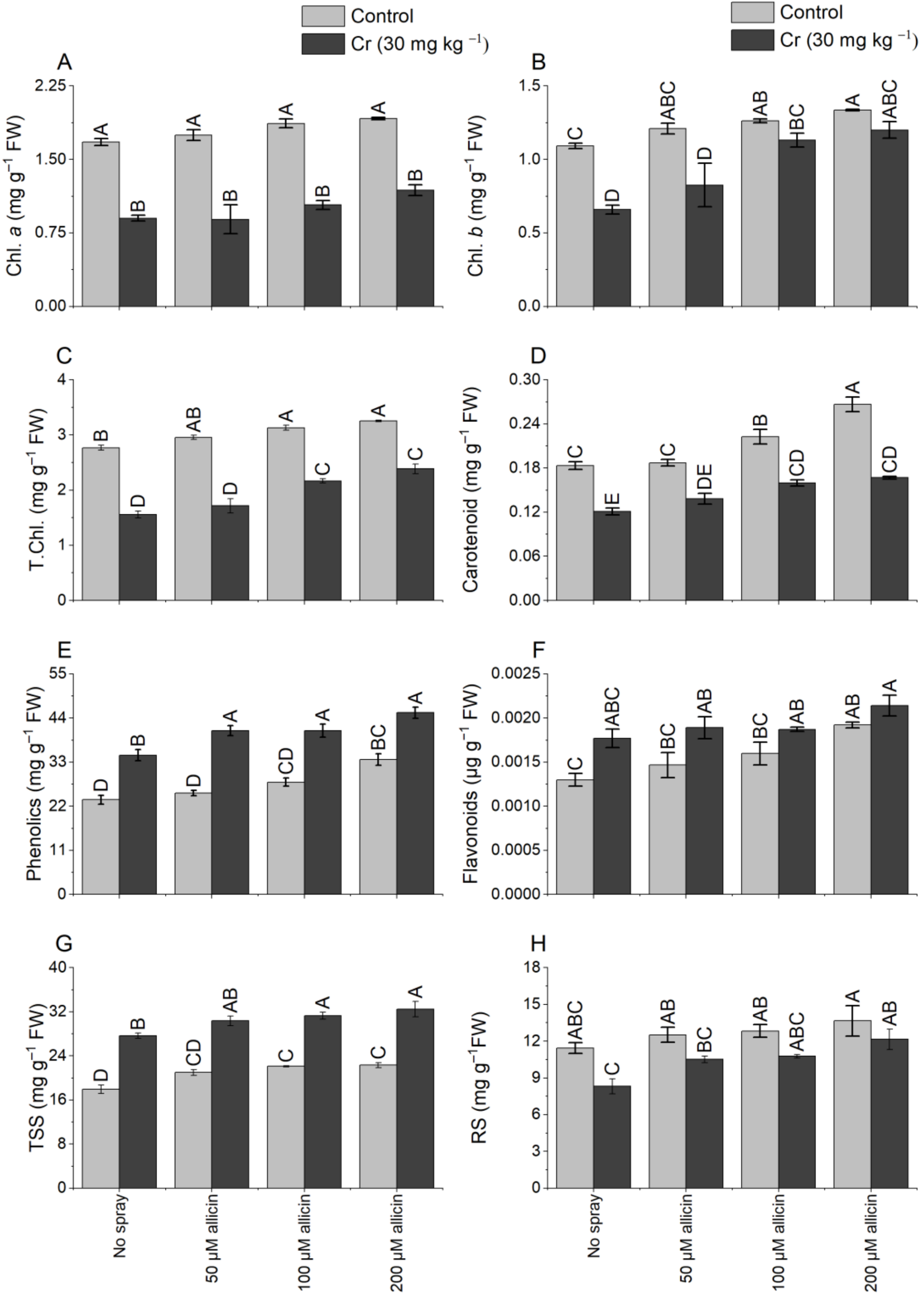

**Figure 2: Effect of allicin on chlorophyll *a* (chl. *a*), chlorophyll *b* (chl. *b*), total chlorophyll (T. chl), carotenoids, phenolics, flavonoids, total soluble sugars (TSS), and reducing sugars (RS) in wheat (*Triticum aestivum* L.) plants under chromium (Cr) toxicity. Bars show means ± SE values of four replicates. Different letters on bars show statistically significant differences among means at $P \leq 0.05$.**

## Phenolic and flavonoid contents

In wheat plants, the contents of phenolics and flavonoids significantly increased ($P \leq 0.001$) by 47.08% and 36.17%, respectively, in response to 30 mg kg Cr toxicity when compared to the control. Further, the foliar application of allicin (50, 100, and 200 µM) showed conspicuous improvement ($P \leq 0.001$) in phenolic and flavonoid contents exposed to Cr toxicity. In this context, the 200 µM allicin application exhibited a maximum enhancement in phenolic and flavonoid contents (30.15% and 21.02%, respectively) under Cr toxicity in wheat plants compared to the control (**Figure 2**).

## Soluble sugars

We observed a significant increase ($P \leqq 0.001$) in total soluble sugar content, with a notable enhancement of 54.07% in wheat plants exposed to Cr stress at a concentration of 30 mg kg$^{-1}$. Additionally, the application of allicin at concentrations of 50, 100, and 200 µM resulted in a marked improvement ($P \leq 0.001$) in total soluble sugar levels in plants subjected to Cr stress. Among these treatments, the wheat plants receiving 200 µM of allicin displayed the most considerable enhancement in total soluble sugar content, exhibiting a 17.51% increase relative to the other allicin concentrations. In contrast, Cr stress at 30 mg kg$^{-1}$ exerted a detrimental effect ($P \leq 0.001$) on the reducing sugar content of wheat plants, leading to a reduction of as much as 27.31%. Conversely, the application of varying allicin concentrations (50, 100, and 200 µM) resulted in a significant increase ($P \leq 0.01$) in the accumulation of reducing sugars in the wheat plants. Notably, our findings demonstrated that the application of 200 µM allicin significantly enhanced reducing sugar content by 46.02% in the wheat plants subjected to Cr stress (**Figure 2**).

The highest recorded level of non-reducing sugars (NRS), reaching 197.27%, was observed in plants subjected to chromium stress at 30 mg kg$^{-1}$. Furthermore, the administration of exogenous allicin treatment led to a non-significant increase in NRS content. Specifically, the application of 200 µM allicin resulted in a 5.24% increase in NRS in wheat plants under Cr stress (**Figure 3**).

## Ascorbic acid

The content of ascorbic acid shows a notable increase (47.50%) when the wheat plants are exposed to chromium at a concentration of 30 mg kg$^{-1}$. Additionally, the administration of allicin at concentrations of 50, 100, and 200 µM resulted in a marked enhancement of ascorbic acid levels in the wheat plants subjected to chromium-induced stress. Furthermore, the exposure of the wheat plants to Cr toxicity led to a significant rise (41.01%) in ascorbic acid content compared to the control treatment (**Figure 3**).

## Anthocyanin content

The anthocyanin content in the wheat plants significantly increased ($P \leq 0.001$) by 23.96% under chromium stress at 30 mg kg$^{-1}$ compared to the control. Additionally, various doses of allicin (50, 100, and 200 µM) further enhanced the anthocyanin content. Specifically, the plants treated with 200 µM of allicin showed a notable increase in anthocyanin levels, with a rise of 26.30% in the wheat plants experiencing chromium-induced stress (**Figure 3**).

## Antioxidant pigments

The wheat plants' exposure to Cr significantly increased the levels of different pigments. Specifically, there was a notable increase of 32.55% in β-carotenes, 69.44% in β-cyanins, and 77.78% in β-xanthin when compared to those of the control plants. Additionally, the application of allicin at concentrations of 50, 100, and 200 µM showed a significant effect ($P \leq 0.001$) on the contents of β-carotenes and β-cyanins in the wheat plants under Cr stress. For instance, both β-carotenes and β-cyanins increased by 32.70% and 35.40%, respectively, due to Cr stress. Moreover, the use of allicin at these concentrations led to a significant increase ($P \leq 0.01$) in β-xanthin levels in response to Cr stress. Notably, the wheat plants treated with 200 µM of allicin showed an improvement in β-xanthin levels, which rose by 14.03% when subjected to Cr stress (**Figure 3**).

## Total soluble proteins (TSP) and total free amino acids (TFAA)

Total soluble protein content significantly decreased ($P \leq 0.001$) in the wheat plants subjected to Cr stress at 30 mg kg$^{-1}$. Chromium stress caused a 40.16% reduction in TSP levels. Allicin (50, 100, and 200 µM) partially alleviated the detrimental effects of Cr stress, and the most favorable response (36.69%) was observed with the application of 200 µM allicin under Cr stress. We observed a 34.85% increase in TFAA content ($P \leq 0.001$) in the wheat plants subjected to Cr stress. Additionally, the application of allicin at concentrations of 50, 100, and 200 µM significantly enhanced ($P \leq 0.001$) TFAA levels in the wheat plants under Cr stress. The highest TFAA contents were obtained with the dose of 200 µM allicin, showing a significant increase of 42.96% under Cr stress compared to the control (**Figure 3**).

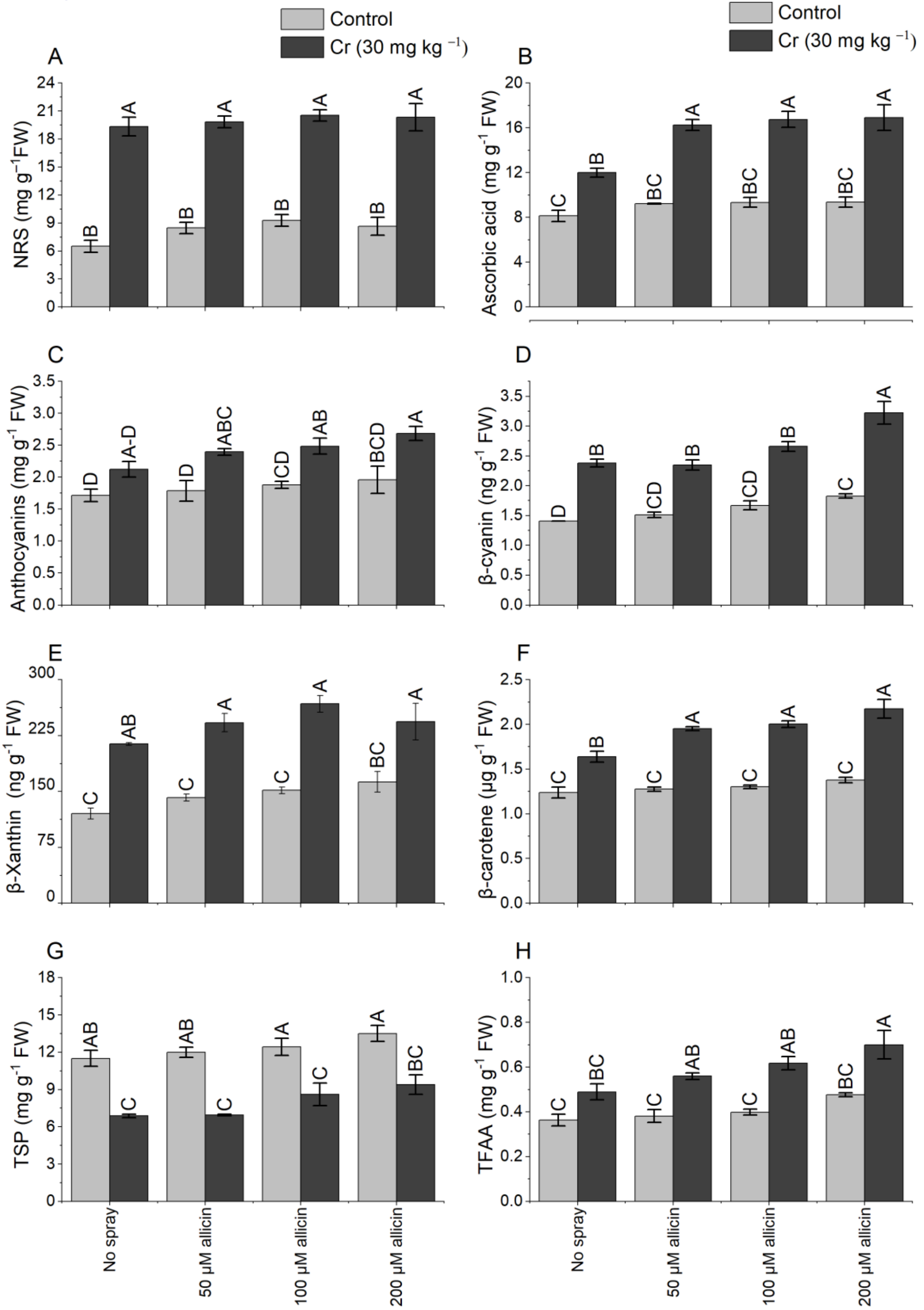

**Figure 3: Effect of allicin on non-reducing sugars (NRS), ascorbic acid, anthocyanins, ascorbic acid (ASA), β-cyanin, β-xanthin, β-carotene, total soluble proteins (TSP), and total free amino acids (TFAA) in wheat (*Triticum aestivum* L.) plants under chromium (Cr) toxicity. Bars show means ± SE values of four replicates. Different letters on bars show statistically significant differences among means at $P \leq 0.05$.**

## Oxidative stress indicators

Chromium (30 mg kg $^{-1}$) toxicity led to a notable upsurge ($P \leq 0.001$) in MDA, $H_2O_2$, $O_2^{\bullet-}$, LOX, MG, and EL in the wheat plants by 104.53%, 58.06%, 193.03%, 182.82%, 160.30%, and 616.84%, respectively, in comparison to those of the controls. In addition, exogenously supplemented allicin (50, 100, and 200 µM) doses visibly reduced the toxic effect of Cr toxicity to the wheat plants in all stress markers. However, 200 µM allicin administration notably reduced MDA, $H_2O_2$, $O_2^{\bullet-}$, lipoxygenase (LOX), methylglyoxal (MG), and electrolyte leakage (EL) by 51.07%, 27.39%, 40.20%, 46.23%, 40.08%, and 67.22%, respectively, in the wheat plants under Cr-induced stress (**Figure 4**).

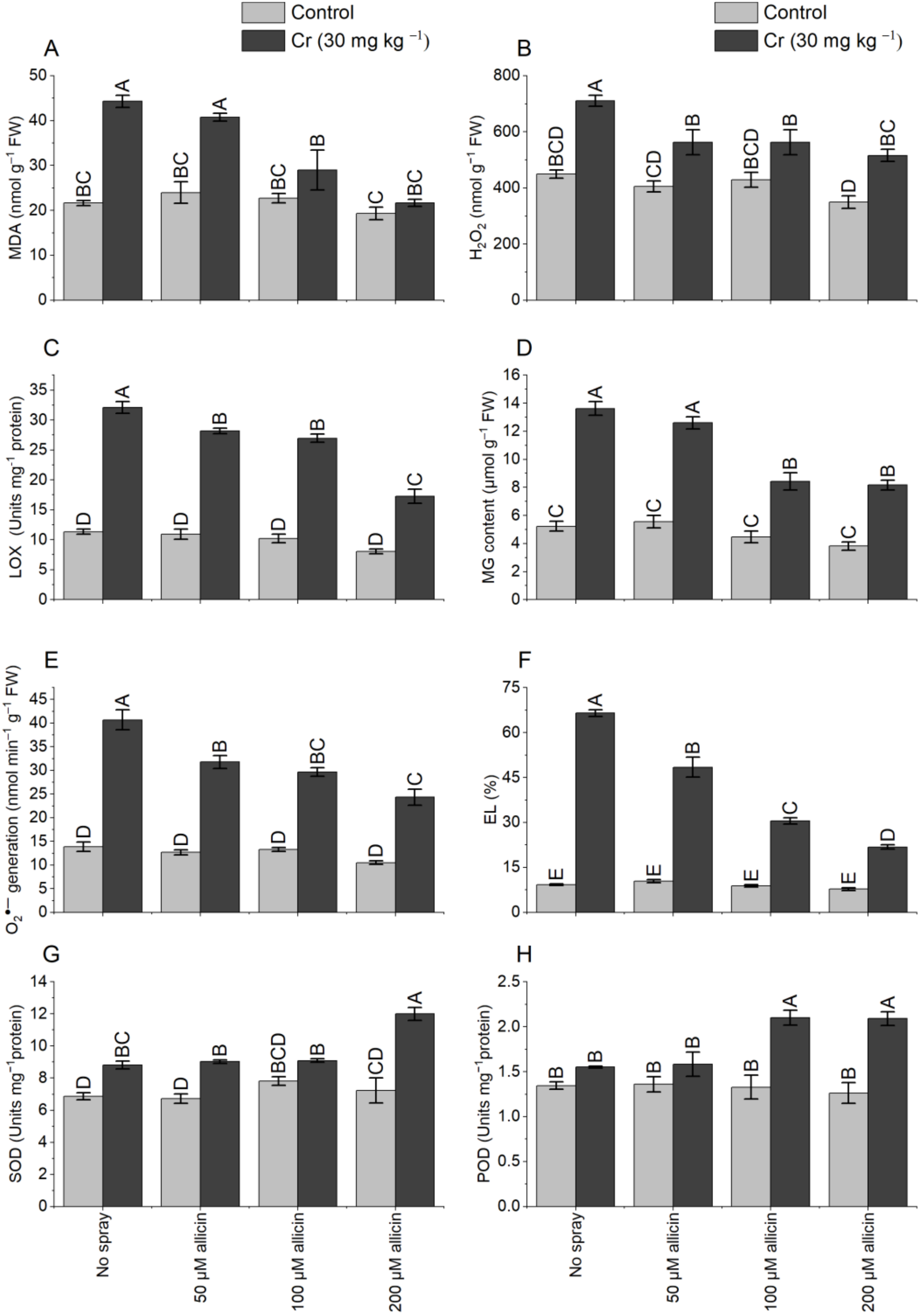

**Figure 4: Allicin-induced modulations in oxidative stress indicators such as malondialdehyde (MDA), hydrogen peroxide ($H_2O_2$), lipoxygenase (LOX), methylglyoxal (MG), superoxide radical ($O_2^{\bullet-}$), superoxide dismutase (SOD), peroxide dismutase (POD) activity as well as electrolyte leakage (EL) in wheat (*Triticum aestivum* L.) plants under chromium (Cr) toxicity. Bars show means ± SE values of four replicates. Different letters on bars show statistically significant differences among means at $P \leq 0.05$.**

## Enzymatic antioxidants

The activities of SOD and POD increased significantly ($P \leq 0.001$) in the wheat plants (28.46% and 15.29%, respectively), under 30 mg kg$^{-1}$ of Cr, when compared to the controls. Furthermore, the allicin (50, 100, and 200 µM) application further enhanced the activities of these enzymes (**Figure 4G, H**). Chromium applied as 30 mg kg$^{-1}$ visibly enhanced the CAT and APX activities by 44.37% and 35.39%, respectively, in the wheat plants compared to the controls. However, application of allicin (50, 100, and 200 µM) showed enhancement in CAT activity, whereas the APX activity showed a non-significant improvement under Cr stress. In this context, the foliar application of 200 µM allicin showed a maximum increase in CAT and APX activities by 43.01% and 27.79%, respectively (**Figure 5**).

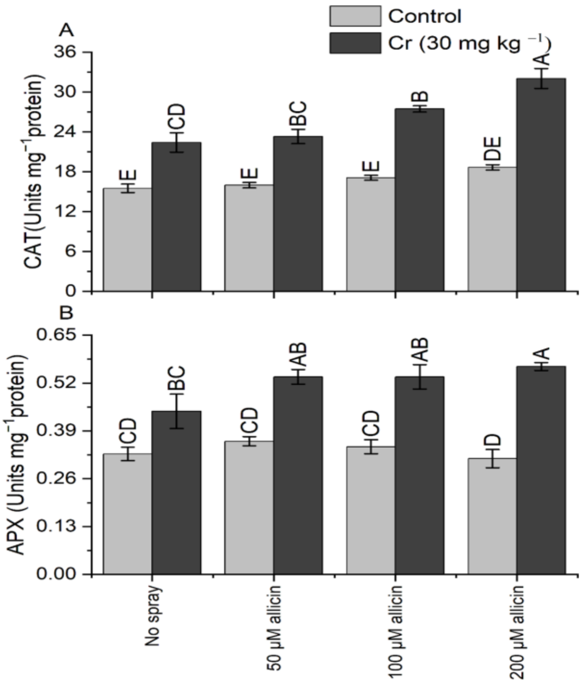

**Figure 5: Allicin-induced changes in the activities of catalase (CAT) and ascorbate peroxidase (APX) in wheat (*Triticum aestivum* L.) plants under chromium (Cr) toxicity. Bars show means ± SE values of four replicates. Different letters on bars show statistically significant differences among means at $P \leq 0.05$.**

## Proline and glycine betaine

A significant increase in proline content, reaching 61.16%, was observed in the wheat plants exposed to chromium (30 mg kg$^{-1}$) stress. Additionally, the treatment with allicin at concentrations of 50, 100, and 200 µM also resulted in a notable increase in proline levels under chromium stress. Higher accumulation in proline content by 57.84% was noticed in the wheat plants experiencing 200 µM allicin application under Cr-induced stress. A notable upsurge in GB contents by 42.57% was discerned in the wheat plants subjected to Cr (30 mg kg$^{-1}$) stress. Allicin (50, 100, and 200 µM) doses also prompted a significant ($P \leq 0.001$) surge in GB content. Additionally, a visible rise (52.60%) in GB content was observed in the wheat plants, receiving 200 µM allicin treatment under Cr toxicity (**Figure 6**).

## Endogenous hydrogen sulfide ($H_2S$) and nitric oxide (NO) levels

Hydrogen sulfide and nitric oxide supplied with 30 mg kg$^{-1}$ Cr exhibited a visible ($P \leq 0.001$) enhancement by 131.79% and 93.62% in the wheat plants. Moreover, allicin (50, 100, and 200 µM) application further increased $H_2S$ and NO levels in the wheat plants under Cr-induced stress. Likewise, allicin application at 100 µM significantly upsurged $H_2S$ by 32.10% and NO level by 36.12% with 200 µM allicin application in the wheat plants under Cr toxicity (**Figure 6**).

## Glutathione metabolism

Reduced and oxidized glutathione (GSH and GSSG) tremendously increased in the wheat plants by 43.13% and 93.86%, respectively, when exposed to 30 mg kg$^{-1}$ Cr stress. Besides, the exogenous application of allicin (50, 100, and 200 µM) visibly increased GSH content under Cr toxicity. The maximum enhancement was observed in the wheat plants when 200 µM allicin was applied. Contrary to this, GSSG content was notably reduced by the allicin (50, 100, and 200 µM) doses. The most noticeable decline was observed during 200 µM allicin treatment by 24.85% compared to that in the unsprayed wheat plants. While the ratio of GSH: GSSG showed a visible decrease in the wheat plants when supplied with 30 mg kg$^{-1}$ Cr by 25.95% compared to the controls. Exogenous application of allicin (50, 100, and 200 µM) showed a pronounced enhancement under Cr stress. In addition, 200 µM allicin displayed a pronounced augmentation by 62.35% in the GSH: GSSG ratio of the wheat plants (**Figure 6**).

## DPPH radical scavenging activity

The results demonstrated a significant increase in DPPH activity, with an enhancement of 43.23% under Cr stress at a concentration of 30 mg kg$^{-1}$. Additionally, even greater increases ($P \leq$

0.001) in DPPH• activity were noted in the plants exposed to different doses of exogenously applied allicin (50, 100, and 200 µM) under Cr stress. Specifically, the application of 200 µM of allicin resulted in a maximum reduction of DPPH• activity by 39.14% when subjected to Cr-induced stress (**Figure 6**).

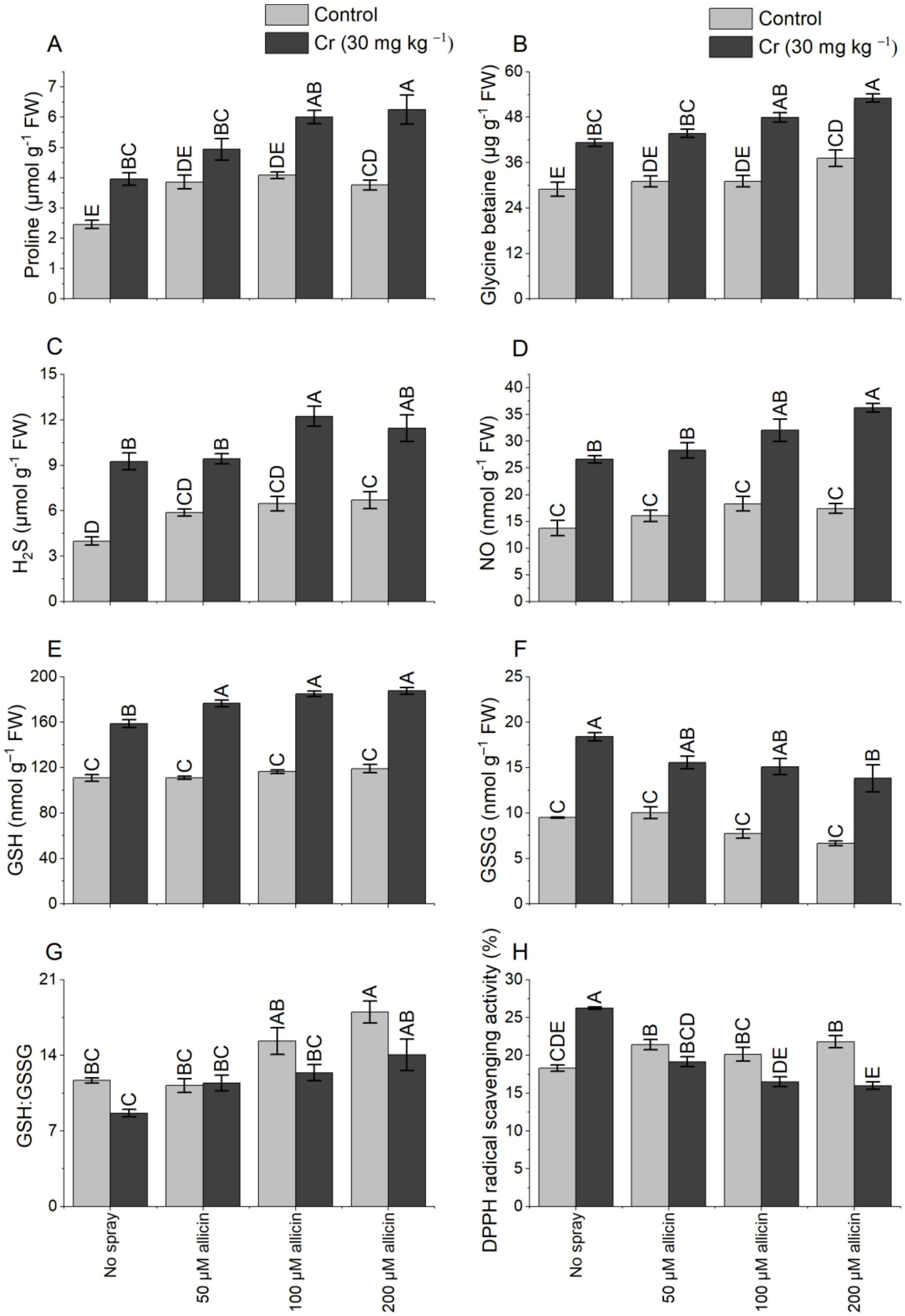

**Figure 6: Allicin-induced changes in proline, glycine betaine, hydrogen sulfide ($H_2S$), nitric oxide (NO), glutathione reduced (GSH), glutathione oxidized (GSSG), glutathione ratio (GSH: GSSG), and DPPH scavenging activity in wheat (*Triticum aestivum* L.) plants under chromium (Cr) toxicity. Bars show means ± SE values of four replicates. Different letters on bars show statistically significant differences among means at $P \leq 0.05$.**

## Discussion

Chromium is classified as a heavy metal and is widely acknowledged for its toxic effects on both plants and humans (Sharma et al., 2020). Elevated concentrations of Cr in soil can be absorbed by plants, leading to its accumulation in various tissues and adversely affecting overall plant health. In plants, Cr toxicity is known to suppress key growth parameters, including reductions in both shoot and root biomass (**Figure 7**), as well as a decline in relative water content (Malik et al., 2021; Prasad et al., 2021). Chromium's disruption of respiration, photosynthesis, protein synthesis, and enzymatic activities (Patra et al., 2024) is primarily responsible for these negative outcomes. Additionally, exposure to Cr has been shown to increase oxidative stress by generating excessively high levels of ROS (**Figure 7**), which significantly damaged crucial cellular components that might result in cell death (Mansoor et al., 2023). Other recent studies also confirmed that, generally, chromium toxicity leads to a noticeable decrease in both growth and water retention in wheat plants and that it does adversely impact plant development (Jalil et al., 2024). Significant oxidative damage, owing to the generation of ROS and MG with concomitant distortions in oxidative defense mechanisms, was correlated with the decline in plant growth attributes and water content. Interestingly, allicin presence greatly amplified the plant growth attributes in the presence of Cr toxicity (**Figure 7**). Allicin conferred amelioration of plant growth under Cr toxicity due to decreased chlorophyll degradation. Furthermore, the plants treated with allicin exhibited lower Cr accumulation under conditions of Cr toxicity. Within this context, Singh et al. (2023) reported that mungbean plants exhibiting reduced Cr accumulation demonstrated enhanced growth under conditions of Cr toxicity. Similarly, Razzaq et al. (2024) observed diminished growth in *Zea mays* plants subjected to Cr stress. Additionally, oxidative injury was identified as a detrimental factor negatively impacting plant growth under Cr toxicity, as documented elsewhere (Kaya et al., 2023b) (**Figure 1**). The mechanistic representation of Cr toxicity and its mitigation by exogenous allicin in wheat is provided in **Figure 7**.

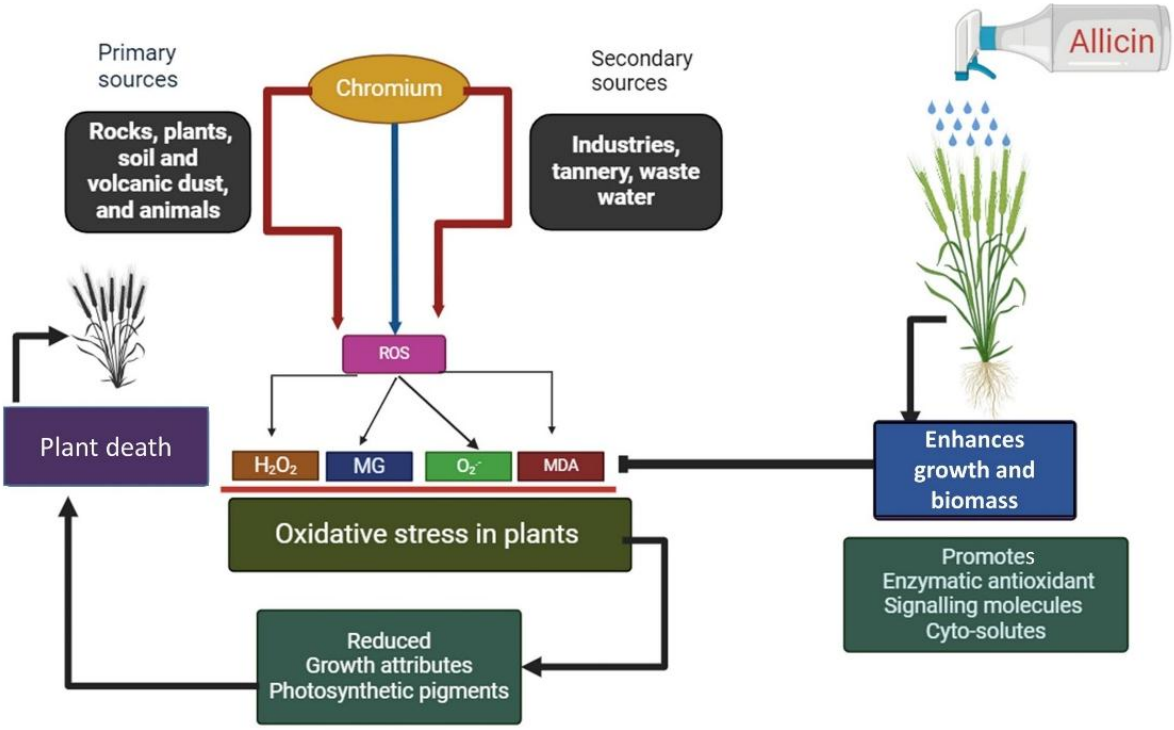

**Figure 7: Mechanistic illustration of allicin-mediated chromium tolerance in wheat (*Triticum aestivum* L.). Abbreviations: $H_2O_2$, hydrogen peroxide; MG, methylglyoxal; $O_2^{\bullet-}$, superoxide radicals; MDA, malondialdehyde**

Chromium can diminish chlorophyll content in plants by impairing chlorophyll biosynthesis and possibly by enhancing chlorophyll degradation (Qin et al., 2024). The chloroplast is recognized as the primary site affected by Cr stress in plants (Fan et al., 2020; Wakeel et al., 2020). The present experiment showed that Cr markedly reduced chlorophyll content in the wheat plants. Similarly, Cr stress led to reduced chlorophyll levels in sunflower plants (Farid et al., 2020). Furthermore, the accumulation of Cr in thylakoid membranes leads to ultrastructural changes (Anjum et al., 2017). As a result, there is a disruption in electron transport along the photosystems, which inhibits photosynthesis (Zsiros et al., 2020). The administration of allicin to plants mitigated chlorophyll degradation under Cr toxicity. The role of allicin in diminishing oxidative damage likely contributed to the observed reduction in chlorophyll degradation under Cr stress (**Figure 2**).

Chromium toxicity induced increased levels of $H_2O_2$, $O_2^{\bullet-}$, and MG alongside LOX activity, which increased MDA levels and EL. Intriguingly, antioxidant enzyme activities exhibited an augmentation by Cr toxicity in the wheat plants. Chromium appears to potentially diminish the defense mechanisms of wheat plants, leading to an escalation in oxidative stress. This phenomenon can give rise to lipid peroxidation, wherein radicals initiate a chain reaction by attacking membrane lipids, potentially compromising the integrity and functionality of the cellular membrane (Ashraf et al., 2023; Hafeez et al., 2024). This aligns with the findings wherein Cr stress caused an elevated production of ROS in rice (Ma et al., 2016), faba bean (Alam et al., 2022), and oilseed rape (Zhang et al., 2018). The upsurge in antioxidant enzyme activities observed in our study under Cr stress aligns with consistent findings reported in previous investigations (Raja et al., 2023; Singh et al., 2023).

During stress conditions, plants compensate for the excess ROS by activating complex enzymatic and non-enzymatic antioxidant networks. On the other hand, Cr toxicity is known to modify enzymatic antioxidant activities in a number of species, including mustard (Asgher et al., 2023), and bamboo (Emamverdian et al., 2023). However, SOD visibly surged in the plants under severe Cr stress, while POD, CAT, and APX activities were also significantly higher in the wheat plants under severe Cr stress. Moreover, it was noted that the levels of superoxide ($O_2^{\bullet-}$) and $H_2O_2$ remained high, signifying an insufficiency in the capacity of the antioxidant defense system to effectively detoxify these ROS. Certainly, allicin treatment not only raised the SOD, POD, CAT, and APX activities associated with control but also caused a notable reduction in $O_2^{\bullet-}$ and $H_2O_2$ levels (Ashraf et al., 2023). Plants, therefore, become endowed with a strategy to cope with oxidative damage through increasing antioxidant enzymes, for example, SOD, POD, CAT, and APX activities (Ashraf et al., 2017). To further understand the endogenous signaling mechanism of allicin in response to Cr toxicity, we carried out the analysis of antioxidant enzymes in stressed and non-stressed wheat plants in the absence or presence of allicin (**Figure 4**).

Proline and GB can help osmotic regulation and stabilization of cellular redox state, protecting membranes and proteins, and reducing the effects of free radicals and metal ions in plants under stressful conditions. In the current study, wheat plants showed an augmented accumulation of proline and GB under chromium (Cr) stress, which confirms findings from other investigations (Ali et al., 2015; Ahmad et al., 2020; Kumar, 2021). Furthermore, Cr-stressed plants showed augmented levels of amino acids and phenolics. The free amino acids and phenolics play an important role in osmoprotection, metal detoxification, ion transport, and redox homeostasis of damaged plants (Meena et al., 2019). Moreover, Cr stress increased proline, GB, total free amino acids, total soluble proteins, and phenolics in the wheat plants, which were further improved with exogenous allicin application. This can be related to the relevant function of allicin in osmoregulation due to its function as an organic osmolyte and modulator of a wide range of cytosolutes (Borlinghaus et al., 2014). The dual purpose of such a search is useful for improving leaf water status and protecting the membrane system (Ashraf et al., 2023). Exhibiting a similar positive trend, allicin supplementation positively affected Cr-stressed pea plants as well (Pandey et al., 2023) (**Figure 5**).

Glutathione (GSH) plays an important part in safeguarding plant cells against oxidative damage produced by ROS, especially during stress imposition such as heavy metal toxicity. As a major intracellular antioxidant, GSH participates in detoxifying ROS by directly scavenging free radicals and acting as a substrate for antioxidant enzymes like glutathione peroxidase and glutathione *S*-transferase (Chai and Mieyal, 2023). During stress, such as chromium (Cr), ROS production in plants increases significantly, threatening cellular structures and metabolic processes. In response, plants often boost GSH synthesis to restore redox balance and minimize oxidative injury. In addition, GSH participates in the regeneration of other antioxidants such as ascorbate via the ascorbate-glutathione cycle, thus strengthening the defensive system of the plant. Glutathione is a vital molecule for plants to survive under environmental stress as it maintains cellular redox homeostasis and supports detoxification pathways by combating the elevated ROS level, and thus enables them to remain healthy and achieve proper growth (Zulfiqar et al., 2023). Cellular redox state is commonly monitored using the GSH/GSSG ratio. The cell is in a reducing environment when there is a higher GSH/GSSG ratio and in an oxidative state when there is a lower value of the ratio (Vašková et al., 2023). As cells tend towards a more oxidized environment, this leads to the consumption of more GSH, an increase in GSSG, resulting in a reduction of this ratio under Cr stress. Correlation of an enhanced level of GSH, GSSG, and a lowered GSH/GSSG ratio with oxidative damage caused by ROS activity can be observed in Cr-stressed plants (Singh et al., 2023). Extreme or prolonged exposure to these stressors may induce irreversible damage to plant metabolism and impair growth and plant death (Ashraf et al., 2023; Saleem et al., 2023) (**Figure 5**).

Anthocyanins participate importantly in plant defense responses, particularly under environmental stress, serving as a capacity of biomarker of stress, scavenging of ROS, and binding with metal ions (Kaur et al., 2023). In this analysis, it was also found that plants exposed to Cr stress showed greater anthocyanin levels. In addition, allicin treatment also brought about a higher accumulation of anthocyanin that may play a more effective role in the detoxification of ROS induced by Cr stress, which is beneficial to plant growth. In wheat, chromium exposure also resulted in an increase in antioxidant pigments such as β-cyanin and β-xanthin. The pigment levels further rose after administration of allicin, fortifying this plant's antioxidant defense. These outcomes are in line with the reports of Qureshi et al. (2020) that also showed increased levels of antioxidant plant pigments, like β-carotene, β-xanthin, and β-cyanin in bitter-gourd plant subjected to abiotic stress.

Hydrogen sulfide ($H_2S$) is viewed as an important naturally occurring gaseous signal in plants that has significantly enhanced the resistance of plants to several abiotic stressors. The major function, as its protective role, is its ability to control both enzymatic and nonenzymatic antioxidant defense systems (Sahariya et al., 2023). Recent studies of recent have recently shown a conspicuous increase in $H_2S$ concentration in plants subjected to Cr toxicity after allicin application (**Figure 5**). $H_2S$ plays an important role in decreasing metal uptake and internal buildup, thereby rendering the plant more resistant to metal stress. Moreover, since $H_2S$ also has a reducing ability, it acts as a good ROS scavenger (Kaya et al., 2023a).

The negative impacts of MG are documented by previous studies, and, in particular, increased abiotic stressors such as metal stressors have been shown to exacerbate these effects (Bless et al., 2023; Guo et al., 2023). However, our investigation suggests that plants under chromium stress had a greater level of MG. Under such conditions, MG does not have in place essential protective mechanisms; thus, it potentially interacts adversely with proteins, lipids, and nucleic acids. These interactions can then serve as a cause of the inactivation of essential defense systems, which can produce irreversible metabolic disruptions, bring about genetic mutations, and eventually lead to cell death (Bless, 2016). MG is highly detoxified and converted into non-toxic products through the activity of Gly I and Gly II within the glyoxalase system with the aid of GSH (Hasanuzzaman et al., 2017). It was found that the supplementation with exogenous allicin in Cr affected seedlings resulted in a significantly better functioning of the glyoxalase system when compared with the case of wheat seedlings in Cr stress. It was evidenced by increased GSH levels and enhanced activities of glyoxalase enzymes to scavenge out the excessive MG production that occurred due to chromium stress (**Figure 4**). For example, methylglyoxal toxicity is alleviated by the glyoxalase system under chromium stress by stimulating the enzyme activity in *Oryza sativa* (Pan et al., 2023) and *Brassica parachinensis* (Kamran et al., 2021).

## Conclusion

Chromium toxicity caused a metabolic perturbation and inhibited plant growth. Plants suffered a marked surge in oxidative stress, which caused lipid peroxidation, leading to higher membrane damage and electrolyte leakage. Chlorophyll levels and gas exchange attributes were also decreased notably in chromium-stressed plants. Allicin administration decreased oxidative stress by strengthening the antioxidant system alongside hydrogen sulfide and nitric oxide levels. Allicin maintained redox balance by elevating the endogenous glutathione levels. Exogenously supplemented allicin regulated ion homeostasis by improving nutrient uptake under chromium toxicity. These findings highlighted the potential of exogenous allicin administration in the alleviation of metal toxicity in plants. This research provides important insights into using natural, plant-based compounds for more sustainable farming practices, especially in regions affected by heavy metal pollution. Future research on the molecular level is needed to elucidate how allicin modulates critical plant physiological and biochemical processes to impart metal stress tolerance.

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### Permissions and ethical compliance
This study does not involve human/animal subjects, and thus no ethical approval is required.

### Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

### Supplementary material
No supplementary material is included with this manuscript.

### Conflict of interest
The authors declare no conflict of interest.

### Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

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All authors have critically read this manuscript and agreed to publish in IJAaEB.

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It is declared that the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

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