Science of the Total Environment 908 (2024) 168208

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Mitigating chromium toxicity in rice (*Oryza sativa* L.) via ABA and 6-BAP: Unveiling synergistic benefits on morphophysiological traits and ASA-GSH cycle

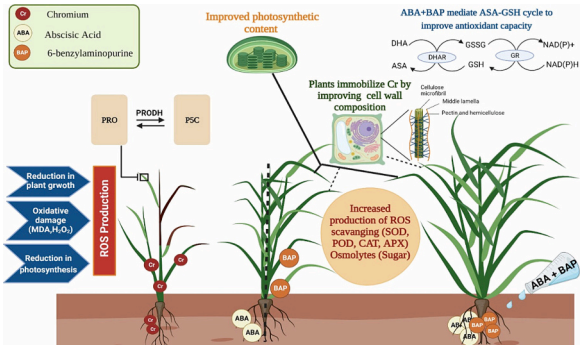
Khairiah Mubarak Alwutayd [a], Suliman Mohammed Suliman Alghanem [b], Rahaf Alwutayd [c], Sameera A. Alghamdi [d], Nadiyah M. Alabdallah [e,f], Rahmah N. Al-Qthanin [g,h], Wajiha Sarfraz [i], Noreen Khalid [i], Nayab Naeem [i], Baber Ali [j], Muhammad Hamzah Saleem [k], Sadia Javed [l,*], Leobardo Manuel Gómez-Oliván [m,*], Amany H.A. Abeed [n]

[a] *Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, Riyadh 11671, Saudi Arabia*
[b] *Department of Biology, College of Science, Qassim University, Burydah 52571, Saudi Arabia*
[c] *Department of Information of Technology, College of Computer and Information Science, Princess Nourah bint Abdulrahman University, Riyadh 11671, Saudi Arabia*
[d] *Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah 21589, Saudi Arabia*
[e] *Department of Biology, College of Science, Imam Abdulrahman Bin Faisal University, 31441 Dammam, Saudi Arabia*
[f] *Basic & Applied Scientific Research Centre, Imam Abdulrahman Bin Faisal University, Dammam 31441, Saudi Arabia*
[g] *Department of Biology, College of Sciences, King Khalid University, Abha 61413, Saudi Arabia*
[h] *Prince Sultan Bin Abdelaziz for Environmental Research and Natural Resources Sustainability Center, King Khalid University, Abha 61421, Saudi Arabia*
[i] *Department of Botany, Government College Women University, Sialkot, Pakistan*
[j] *Department of Plant Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan*
[k] *Office of Academic Research, Office of VP for Research & Graduate Studies, Qatar University, Doha 2713, Qatar*
[l] *Department of Biochemistry, Government College University, Faisalabad 38000, Pakistan*
[m] *Universidad Autónoma del Estado de México, Paseo Colón, intersección Paseo Tollocan Col. Universidad, CP 50120 Toluca, Estado de México, México*
[n] *Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut 71516, Egypt*

HIGHLIGHTS

- Individual and combinatorial application of ABA and 6-BAP was applied to Cr stressed rice.
- Cr stress decreased eco-physiology of plants.
- Cr stress affected proline metabolism and AsA-GSH cycle.
- Application of ABA and 6-BAP reduced Cr stress and increase plant development.

GRAPHICAL ABSTRACT



* Corresponding authors.
E-mail addresses: kmalwateed@pnu.edu.sa (K.M. Alwutayd), su.alghanem@qu.edu.sa (S.M.S. Alghanem), saalghamdy1@kau.edu.sa (S.A. Alghamdi), nmalabdallah@iau.edu.sa (N.M. Alabdallah), rngerse@kku.edu.sa (R.N. Al-Qthanin), wajiha.sarfraz@gcwus.edu.pk (W. Sarfraz), noreen.khalid@gcwus.edu.pk (N. Khalid), baberali@bs.qau.edu.pk (B. Ali), saleemhamza312@webmail.hzau.edu.cn (M.H. Saleem), sadiajaved@gcuf.edu.pk (S. Javed), lmgomezo@uaemex.mx (L.M. Gómez-Oliván), dramany2015@aun.edu.eg (A.H.A. Abeed).

https://doi.org/10.1016/j.scitotenv.2023.168208
Received 15 September 2023; Received in revised form 26 October 2023; Accepted 27 October 2023
Available online 31 October 2023

ARTICLE INFO

Editor: Damià Barceló

Keywords:
Phytohormones
Plant growth regulators
Abscisic acid
6-Benzylaminopurine
Chromium stress
Cell wall compartments
Relative gene expression

ABSTRACT

In recent years, the use of plant hormones, such as abscisic acid (ABA) and 6-benzylaminopurine (6-BAP), has gained significant attention for their role in mitigating abiotic stresses across various plant species. These hormones have been shown to play a vital role in enhancing the ascorbate-glutathione cycle and eliciting a wide range of plant growth and biomass, photosynthetic efficiency, oxidative stress and response of antioxidants and other physiological responses. While previous research has been conducted on the individual impact of ABA and 6-BAP in metal stress resistance among various crop species, their combined effects in the context of heavy metal-stressed conditions remain underexplored. The current investigation is to assess the beneficial effects of single and combined ABA (5 and 10 μM L^{-1}) and 6-BAP (5 and 10 μM L^{-1}) applications in rice (*Oryza sativa* L.) cultivated in chromium (Cr)-contaminated soil (100 μM). Our results showed that the Cr toxicity in the soil showed a significant declined in the growth, gas exchange attributes, sugars, AsA-GSH cycle, cellular fractionation, proline metabolism in *O. sativa*. However, Cr toxicity significantly increased oxidative stress biomarkers, organic acids, enzymatic and non-enzymatic antioxidants including their gene expression in *O. sativa* seedlings. Although, the application of ABA and 6-BAP showed a significant increase in the plant growth and biomass, gas exchange characteristics, enzymatic and non-enzymatic compounds and their gene expression and also decreased the oxidative stress,

And Cr uptake. In addition, individual or combined application of ABA and 6-BAP enhanced the cellular fractionation and decreases the proline metabolism and AsA-GSH cycle in rice plants. These results open new insights for sustainable agriculture practices and hold immense promise in addressing the pressing challenges of heavy metal contamination in agricultural soils.

1. Introduction

In recent decades, rapid increases in urbanization and industrialization have caused the excessive release of heavy metals in farmlands with damaging effects on ecosystems (Abeed et al., 2022a; Sarker et al., 2023; Singh et al., 2023b). Heavy metals are of high ecological significance due to their persistence, toxicity, and bioaccumulation capacity, and also the excess amount of heavy metals in soil significantly reduces plant production and yield (Krivokapić, 2021; Alam et al., 2023). Cr is a major toxic element discharged into the environment through various industries, such as tanning, electroplating, manufacturing of pigments, production of nuclear weapons, and corrosion control (Danish et al., 2019; Gautam et al., 2020). This extensive industrial use of Cr composites and their subsequent release, without prior treatment, into the surrounding environment contaminates the entire ecosystem and can lead to catastrophic health risks (Abeed and Salama, 2022; Basit et al., 2023; Singh et al., 2023a). Higher Cr levels in plants cause ultrastructural alterations (Shahid et al., 2017; Jobby et al., 2018), oxidative stress in plants, and increased electrolyte leakage (EL) and malondialdehyde (MDA) concentrations, whereas induced alterations in antioxidant enzyme activities such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) and ascorbate peroxidase (APX) (Alsafran et al., 2022; Salama et al., 2022; Ali et al., 2022). Previously, antioxidative enzymes played a significant role in the reduction of Cr phytotoxicity in *Lemna minor* (Sallah-Ud-Din et al., 2017), *Brassica napus* (Li et al., 2018), *Vigna radiata* (Singh et al., 2023a), *Spinacia oleracea* (Zaheer et al., 2020), and *Triticum aestivum* (Basit et al., 2022) grown under excessive Cr concentrations. Hence, it is immensely required to safeguard plant from Cr toxicity to counter the phytotoxicity and oxidative stress triggered by the uptake of Cr in plants (Abeed et al., 2022b).

Rice (*Oryza sativa* L.) is a cereal grain, it is the most widely consumed staple food for a large part of the world's human population, especially in Asia and Africa (Panhwar et al., 2015; AbdElgawad et al., 2023). It is the agricultural commodity with the third-highest worldwide production (*O. sativa*, 741.5 million ton in 2014), after *Saccharum officinarum* (1.9 billion ton) and *Zea mays* (1.0 billion ton) (FAOSTAT, 2018). Metal stress can hinder growth and reduce yields, which can have a severe impact on food security across the globe (Abeed et al., 2022a). *O. sativa* cultivation is well-suited to countries and regions with low labor costs and high rainfall, as it is labor-intensive to cultivate and requires ample water. However, *O. sativa* can be grown practically anywhere, even on a steep hill or mountain area with the use of water-controlling terrace systems (Fahad et al., 2019; Mao et al., 2022). Cr is one of the main pollutants in paddy fields, and its accumulation in *O. sativa* and subsequent transfer to food chain is a global environmental issue (Zeng et al., 2011; Tripathi et al., 2012). Although, it was also reported that Cr can be readily taken up by *O. sativa* and translocated to shoot and then to grains (Basit et al., 2022; AbdElgawad et al., 2023). Thus, Cr can enter into the food chain through *O. sativa* consumption, even at low Cr concentrations in the soils, and cause toxicities to humans (Abeed and Salama, 2022; Sarfraz et al., 2022; Zhu et al., 2022).

Plants have evolved various mechanisms i.e., adaptive and/or constitutive to tolerate the heavy metals toxicity (Mfarrej et al., 2023). The strategies involved in these tolerance mechanisms might consist of the reduction of heavy metal uptake, induced expression of specific transporter genes, promote accumulation in vacuoles, and increasing the signaling molecules (antioxidant enzymes) to reduce the ROS-induced toxicity (Basharat et al., 2023; Ejaz et al., 2023). The plant growth modulators, comprising of a group of plant growth phytohormones such as abscisic acid (ABA), and 6-benzyl aminopurine (6-BAP), regulate plant responses under heavy metals toxicity by mediating numerous biochemical and physiological processes (Kamran et al., 2020; Emamverdian et al., 2022). ABA is a key stress phytohormone and has been recognized as chemical messenger, involved in the activation of antioxidative defense machinery to check overproduction of ROS and signal transduction in plants under adverse conditions (Chen et al., 2022) such as Cr toxicity (Choudhary et al., 2011). Likewise, 6-benzylaminopurine (6-BAP) is known as a master growth modulator, can effectively promote survival ability and resistance capacity by acting as a free radicle scavenger in various crops against abiotic stresses (Aderholt et al., 2017) and also against Cr stress (Emamverdian et al., 2022). Furthermore, these hormone interactions not only improve apical dominance in shoots and overall growth of plants but also enhance antioxidant defenses, regulate stomata opening, and optimize the photosynthesis process, collectively enhancing the plant's ability to overcome the harmful effects of metal stress (Chen et al., 2020; Kamran et al., 2021; Emamverdian et al., 2022).

Despite the many useful advantages related to the individual applications of ABA and 6-BAP, but the combined application to these phytohormones still remains unclear in combating the metal stress. Our study hypothesized the individual and combined application of ABA and 6-BAP to *O. sativa* seedlings under Cr stress. The results from this study would add to our knowledge about (I) the morphological and photosynthetic efficiency (II) oxidative stress and response of enzymatic and non-enzymatic responses along with their specific gene expression (III)

proline metabolism, AsA-GSH cycle, cellular fractionation, organic acids exudation pattern and Cr uptake in different parts of the plants under the Cr stress with the individual or combined application of ABA and 6-BAP.

2. Materials and methods

2.1. Experimental setup

A pot experiment was conducted in the Botanical Garden of the department of Biology, College of Science, Princess Nourah bint Abdulrahman, Riyadh 11,671, Saudi Arabia, under the glass house. Pots were placed under glass house environment where they received natural sunlight, day/night humidity (60/70 %) and day/night temperature (24/12 °C), respectively. "Muthmira" or "Saudi Baladi" rice cultivar were used as a test plant, and *O. sativa* seeds were taken from the Princess Nourah bint Abdulrahman. Before sowing, the seeds were carefully washed and sterilized in 0.1 % $HgCl_2$ solution for 1 min and then washed three times with distilled water. Uncontaminated soil, obtained from the research field of Princess Nourah bint Abdulrahman, was air dried and passed through a 2-mm sieve. The initial physio-chemical properties of natural soil are provided in Supplementary Table 1. After contamination of soil with Cr using potassium dichromate ($K_2Cr_2O_7$), pots (30-cm-tall * 40-cm-wide) were filled with 10 kg of amended soil and undergone at four different cycles of water equilibrated for two months and then air. The similar level of Cr in the soil was also used in our previous study by (Ma et al., 2022). The pots used in this study were rotated regularly in order to avoid environmental effects on the plants. The total duration of experimental treatments was two months under controlled conditions. The chemical reagents such as 6-Benzylaminopurine (6-BAP), Abscisic Acid (ABA) were purchased from the local chemical suppliers in Riyadh 11,671, Saudi Arabia. The 6-BAP used in the experiment have 99 % purity level, while ABA was of 98 %. The application of ABA and 6-BAP was applied as foliar application when seedlings were germinated to 14 days old. The same concentrations of ABA and 6-BAP were used in our previous study (Kamran et al., 2020). Whereas, Cr stressed *O. sativa* plants were supplied with two plant growth modulators of analytical grade (ABA and 6-BAP) at two levels (5 and 10 μM L^{-1}). There were 12 treatments and the detailed experimental treatment details are as follow: **CK:** control (no Cr + ABA + 6-BAP), **T_1**: ABA (5 μM L^{-1}), **T_2**: ABA (10 μM L^{-1}), **T_3**: 6-BAP (5 μM L^{-1}), **T_4**: 6-BAP (10 μM L^{-1}), **T_5:** Cr (100 μM), **T_6:** Cr (100 μM) + ABA (5 μM L^{-1}), **T_7**: Cr (100 μM) + ABA (10 μM L^{-1}), **T_8**: Cr (100 μM) + 6-BAP (5 μM L^{-1}), **T_9**: Cr (100 μM) + 6-BAP (10 μM L^{-1}), **T_{10}**: Cr (100 μM) + ABA (5 μM L^{-1}) + 6-BAP (5 μM L^{-1}), and **T_{11}**: Cr (100 μM) + ABA (10 μM L^{-1}) + 6-BAP (10 μM L^{-1}).

2.2. Sampling and data collection

The plants were picked up after two months of treatments, and various morphological traits were measured, including root and shoot length (cm), and their fresh and dry weights in grams (g). The root and shoot length were calculated on a scale measuring meter, and the fresh weight (FW) of the roots and shoots was determined separately for each replicate using a weighting digital balance. Later, root and shoot were dried in an oven at 105 °C for 1 h, then at 70 °C for 72 h to determine their dry weight. Roots were immersed in 20 mM Na_2EDTA for 15–20 min to remove Cr adhered to the surface of roots. Then, roots were washed thrice with distilled water and finally once with deionized water and dried for further analysis. Although this experiment was conducted in pots, for the collection of organic acids, two seedlings were transferred to the rhizoboxes which consist of plastic sheet, nylon net, and wet soil (Javed et al., 2013). After 48 h, plants were taken from the rhizoboxes and the roots were washed with redistilled water to collect the exudates from root surface. The samples were filtered through a 0.45-μm filter (MillexHA, Millipore) and collected in eppendorf tubes (Greger and Landberg, 2008). The collected samples were mixed with NaOH (0.01 M) in order to analyze the organic acids. However, the samples used for analysis of oxalic acid were not treated with NaOH (Javed et al., 2013).

2.3. Determination of photosynthetic pigments and gas exchange characteristics

Leaves were collected for the determination of chlorophyll and carotenoid contents. For chlorophylls, 0.1 g of fresh leaf sample was extracted with 8 mL of 95 % acetone for 24 h at 4 °C in the dark. The absorbance was measured by a spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan) at 646.6, 663.6, and 450 nm. Chlorophyll content was calculated by the standard method of (Arnon, 1949).

Net photosynthesis (*Pn*), leaf stomatal conductance *(Gs)*, transpiration rate (*Ts*), and intercellular carbon dioxide concentration (*Ci*) were measured from four different plants in each treatment group. Measurements were conducted between 11:30 and 13:30 on days with a clear sky. Rates of leaf *Pn*, *Gs, Ts*, and *Ci* were measured with a LI-COR gas-exchange system (LI-6400; LI-COR Biosciences, Lincoln, NE, USA) with a red-blue LED light source on the leaf chamber. In the LI-COR cuvette, CO_2 concentration was set as 380 mmol mol^{-1} and LED light intensity was set at 1000 mmol m^{-2} s^{-1}, which was the average saturation intensity for photosynthesis in *S. oleracea* (Austin, 1990).

2.4. Determination of oxidative stress indicators

The degree of lipid peroxidation was evaluated as malondialdehyde (MDA) contents. Briefly, 0.1 g of frozen leaves were ground at 4 °C in a mortar with 25 mL of 50 mM phosphate buffer solution (pH 7.8) containing 1 % polyethene pyrrole. The homogenate was centrifuged at 10,000 ×*g* at 4 °C for 15 min. The mixtures were heated at 100 °C for 15–30 min and then quickly cooled in an ice bath. The absorbance of the supernatant was recorded by using a spectrophotometer (xMark™ Microplate Absorbance Spectrophotometer; Bio-Rad, United States) at wavelengths of 532, 600, and 450 nm. Lipid peroxidation was expressed as l mol g^{-1} by using the formula: 6.45 (A532-A600)-0.56 A450. Lipid peroxidation was measured by using a method previously published by (Heath and Packer, 1968).

To estimate H_2O_2 content of plant tissues (root and leaf), 3 mL of sample extract was mixed with 1 mL of 0.1 % titanium sulfate in 20 % (v/v) H_2SO_4 and centrifuged at 6000 ×*g* for 15 min. The yellow colour intensity was evaluated at 410 nm. The H_2O_2 level was computed by the extinction coefficient of 0.28 $mmol^{-1}$ cm^{-1}. The contents of H_2O_2 were measured by the method presented by (Jana and Choudhuri, 1981).

Stress-induced electrolyte leakage (EL) of the uppermost stretched leaves was determined by using the methodology of (Dionisio-Sese and Tobita, 1998). The leaves were cut into minor slices (5 mm length) and placed in test tubes having 8 mL distilled water. These tubes were incubated and transferred into a water bath for 2 h prior to measuring the initial electrical conductivity (EC_1). The samples were autoclaved at 121 °C for 20 min and then cooled down to 25 °C before measuring the final electrical conductivity (EC_2). Electrolyte leakage was calculated by the following formula;

$$EL = (EC_1/EC_2) \times 100.$$

2.5. Determination of antioxidant enzyme activities and relative gene expression

To evaluate enzyme activities, fresh leaves (0.5 g) were homogenized in liquid nitrogen and 5 mL of 50 mmol sodium phosphate buffer (pH 7.0), including 0.5 mmol EDTA and 0.15 mol NaCl. The homogenate was centrifuged at 12,000 ×*g* for 10 min at 4 °C, and the supernatant was used for measurement of superoxidase dismutase (SOD) and peroxidase (POD) activities. SOD activity was assayed in 3 mL reaction mixture containing 50 mM sodium phosphate buffer (pH 7), 56 mM nitro blue

tetrazolium, 1.17 mM riboflavin, 10 mM methionine, and 100 μL enzyme extract. Finally, the sample was measured by using a spectrophotometer (xMark™ Microplate Absorbance Spectrophotometer; Bio-Rad). Enzyme activity was measured by using a method by (Chen and Pan, 1996) and expressed as U g^{-1} FW.

POD activity in the leaves was estimated by using the method of (Sakharov and Ardila, 1999) by using guaiacol as the substrate. A reaction mixture (3 mL) containing 0.05 mL of enzyme extract, 2.75 mL of 50 mM phosphate buffer (pH 7.0), 0.1 mL of 1 % H_2O_2, and 0.1 mL of 4 % guaiacol solution was prepared. Increases in the absorbance at 470 nm because of guaiacol oxidation was recorded for 2 min. One unit of enzyme activity was defined as the amount of the enzyme.

Catalase (CAT) activity was analyzed according to (Aebi, 1984). The assay mixture (3.0 mL) was comprised of 100 μL enzyme extract, 100 μL H_2O_2 (300 mM), and 2.8 mL 50 mM phosphate buffer with 2 mM ETDA (pH 7.0). The CAT activity was measured from the decline in absorbance at 240 nm as a result of H_2O_2 loss ($\varepsilon = 39.4$ mM^{-1} cm^{-1}).

Ascorbate peroxidase (APX) activity was measured according to (Nakano and Asada, 1981). The mixture containing 100 μL enzyme extract, 100 μL ascorbate (7.5 mM), 100 μL H_2O_2 (300 mM), and 2.7 mL 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0) was used for measuring APX activity. The oxidation pattern of ascorbate was estimated from the variations in wavelength at 290 nm ($\varepsilon = 2.8$ mM^{-1} cm^{-1}).

Quantitative real-time PCR (RT-qPCR) assay was applied to investigate the expression levels of 4 stress-related genes, including Fe-SOD, POD, CAT and APX. Total RNA was extracted from leaf tissue samples using RNeasy Plant Mini kits (Qiagen, Manchester, UK). Contaminating DNA was then removed and first-strand cDNAs were prepared using Reverse Transcription kits (Qiagen, Manchester, UK). RT-qPCR analysis was conducted as reported in the protocol of QuantiTect SYBR Green PCR kit (Qiagen, Manchester, UK). Reaction volume and PCR amplification conditions were adjusted as mentioned by (El-Esawi et al., 2020). The gene amplifications of (Sirhindi et al., 2016) of the following genes are given in Table 2S.

2.6. Determination of non-enzymatic antioxidants, and sugar

Plant ethanol extracts were prepared for the determination of non-enzymatic antioxidants and some key osmolytes. For this purpose, 50 mg of dry plant material was homogenized with 10 mL ethanol (80 %) and filtered through Whatman No. 41 filter paper. The residue was re-extracted with ethanol, and the 2 extracts were pooled together to a final volume of 20 mL. The determination of flavonoids (Pękal and Pyrzynska, 2014), phenolics (Bray and Thorpe, 1954), anthocyanin (Lewis et al., 1998), and total sugars (Dubois et al., 1956) was performed from the extracts.

2.7. Measuring proline, P5CR, ProDH, and P5C enzyme activity

To measure proline concentrations, 0.5 g of shoot tissues were ground in sulfosalicylic acid and then centrifuged, and the supernatant was collected from each sample. The proline concentration in each sample was measured (Bates et al., 1973). Specifically, the supernatant from each sample was reacted with acid ninhydrin, and the resulting colorimetric reaction was measured to determine the proline concentration by "UV-1700 pharmaSpec spectrophotometer".

The ProDH "proline dehydrogenase", P5CR "pyrroline-5-carboxylate reductase", and P5C "pyrroline-5-carboxylate" were measured using kits provided by Jiangsu Meibiao Biological Technology Co., Ltd. Enzyme activities were accurately measured using these reagent kits, which include all chemicals and related instructions by "UV-1700 pharmaSpec spectrophotometer".

2.8. Biochemical assay of GSH and AsA in plant tissues

Glutathione (GSH), glutathione disulfide (GSSH), DHA (dehydroascorbic acid), and ascorbic acid (AsA) were determined in fresh leaves (Singh et al., 2015) and were extracted by homogenizing 0.2 g of leaves in TCA and then collecting the supernatant by centrifugation. GSH concentration was measured in a solution including phosphate buffer, supernatant, and DTNB reagent (PBS, pH 7.0) (Singh et al., 2015). The amount of GSH was determined by a spectrophotometer. To measure the AsA content, NaH_2PO_4 solution, enzyme extract, distilled water, and 10 % TCA were mixed to determine the concentration of AsA in the samples (Singh et al., 2015). After a 30-s incubation period, $FeCl_3$ solution, H_3PO_4, and 2,2′ -dipyridine were added to the reaction mixture. The $FeCl_3$ and 2,2′ -dipyridine reacted with the AsA to produce a red-colored complex that can be measured spectrophotometrically at 525 nm. The amount of AsA present in the sample was calculated.

2.9. Cell wall component fractionation

Cell wall isolation was done as reported by (Yang et al., 2011). Shoots (4 g) were placed in a mortar and ground with liquid nitrogen. The homogenized samples were transferred to centrifuge tubes and 75 % ethanol was added and incubated at 25 °C. The samples were centrifuged. The bottom sediment was further homogenized in 10 mL of each of acetone, chloroform, and methanol (v:v = 1:1) for 30 min each, with shaking at room temperature. The homogenate was centrifuged. The remaining cell wall components were lyophilized until dry sediment was obtained. The lyophilized cell wall components were analyzed for biochemical assays. Subsequently, the separation of the hemicellulose fraction was carried out (Yang et al., 2011). Approximately 3 mg CW was mixed with water in an Eppendorf tube. The mixture was boiled for 1 h using a heating block or hot plate set at 100 °C and centrifuged. The above procedure was repeated for duplicate samples. After 12 h, the precipitate was extracted twice with 1 mL of KOH (24 %, *w/v*) at room temperature. After each extraction, centrifugation was done. The hemicellulose concentration was measured at 540 nm absorbance.

Pectin Assay Kit was used to detect pectin. Pectinesterase Assay Kit was used to detect PME activity. The Cellulose Assay Kit was used to detect cellulose concentrations using kits provided by Jiangsu Meibiao Biological Technology Co., Ltd. Enzyme activities were accurately measured using these reagent kits, which include all chemicals and related instructions. DM was calculated using the formula: demethylation degree = 100 - DM, where DM is the degree of methylation.

2.10. Measuring proline, P5CR, ProDH, and P5C enzyme activity in rice seedlings

A solution from 0.5 g of tissue mixture crushed in $C_7H_6O_6S$ and spun up was used to quantify proline levels. Each sample's proline level was assessed (Bates et al., 1973). Proline concentrations were determined using a "UV-1700 pharmaSpec spectrophotometer" by treating residual solutions with acid ninhydrin. Jiangsu Meibiao Biological Technology Co., Ltd. specified the kits to analyze ProDH (proline dehydrogenase), P5CR (pyrroline-5-carboxylate reductase), and P5C (pyrroline-5-carboxylate). The enzyme procedures were precisely determined by the "UV-1700 pharmaSpec spectrophotometer" via such reagent kits, comprising every chemical with guidelines.

2.11. Organic acids exudation pattern and Cr uptake

In order to determine the concentration of organic acids, freeze-dried exudates were mixed with ethanol (80 %), and 20 μL of the solutions were injected into the C18 column (Brownlee Analytical C-183 μm; length 150 mm × 4.6 mm^2, USA). Quantitative analysis of organic acids in root exudates was executed with high-performance liquid chromatography (HPLC), having a Flexer FX-10 UHPLC isocratic pump

(PerkinElmer, MA, USA). The mobile phase used in HPLC was comprised of an acidic solution of aceto-nitrile containing aceto-nitrile:H_2SO_4: acetic acid in ratios of 15:4:1, respectively, and pH of 4.9. The samples were analyzed at a flow rate of 1.0 mL min^{-1} for a time period of 10 min. The inner temperature of the column was fixed at 45 °C, and quantification of organic acids was carried out at 214 nm wavelength with the help of a detector (UV–VIS Series 200, USA) as described by (UdDin et al., 2015). Freeze-dried samples were dissolved in redistilled water, and the pH of the exudates was recorded with LL micro-pH glass electrode by using a pH meter (ISTEK Model 4005–08007 Seoul, South Korea).

Plant samples were vigilantly digested via di-acid (HNO_3-$HClO_4$) technique. 0.5 g dry sample of roots and shoots of the plants were taken into the flask having 10 mL of HNO_3-$HClO_4$ (3:1, v:v); this collection was then retained overnight. Final digestion of these plants' samples was completed after the addition of HNO_3 (5 mL) and then placed on the hot plate for complete digestion as described by (Rehman et al., 2015). Atomic absorption spectrophotometer (AAS) was used to investigate the exact amount of Cr in shoots and roots of the plant.

2.12. Statistical analysis

The normality of data was analyzed using Statistix 8.1 through a multivariate post-hoc test, followed by Duncan's test to determine the interaction among significant values. Thus, the differences between treatments were determined by using one-way analysis of variance (ANOVA), and the least significant difference test ($P < 0.05$) was used for multiple comparisons between treatment mean values where significant. The experiment was performed in a complete randomized design (CRD) with three replications. We have used the Cr as an independent factor and phytohormones a dependent factor. Tukey's HSD post-hoc test was used to compare the multiple comparisons of mean values. The analysis showed that the data in this study were almost normally distributed. The graphical presentation was carried out using

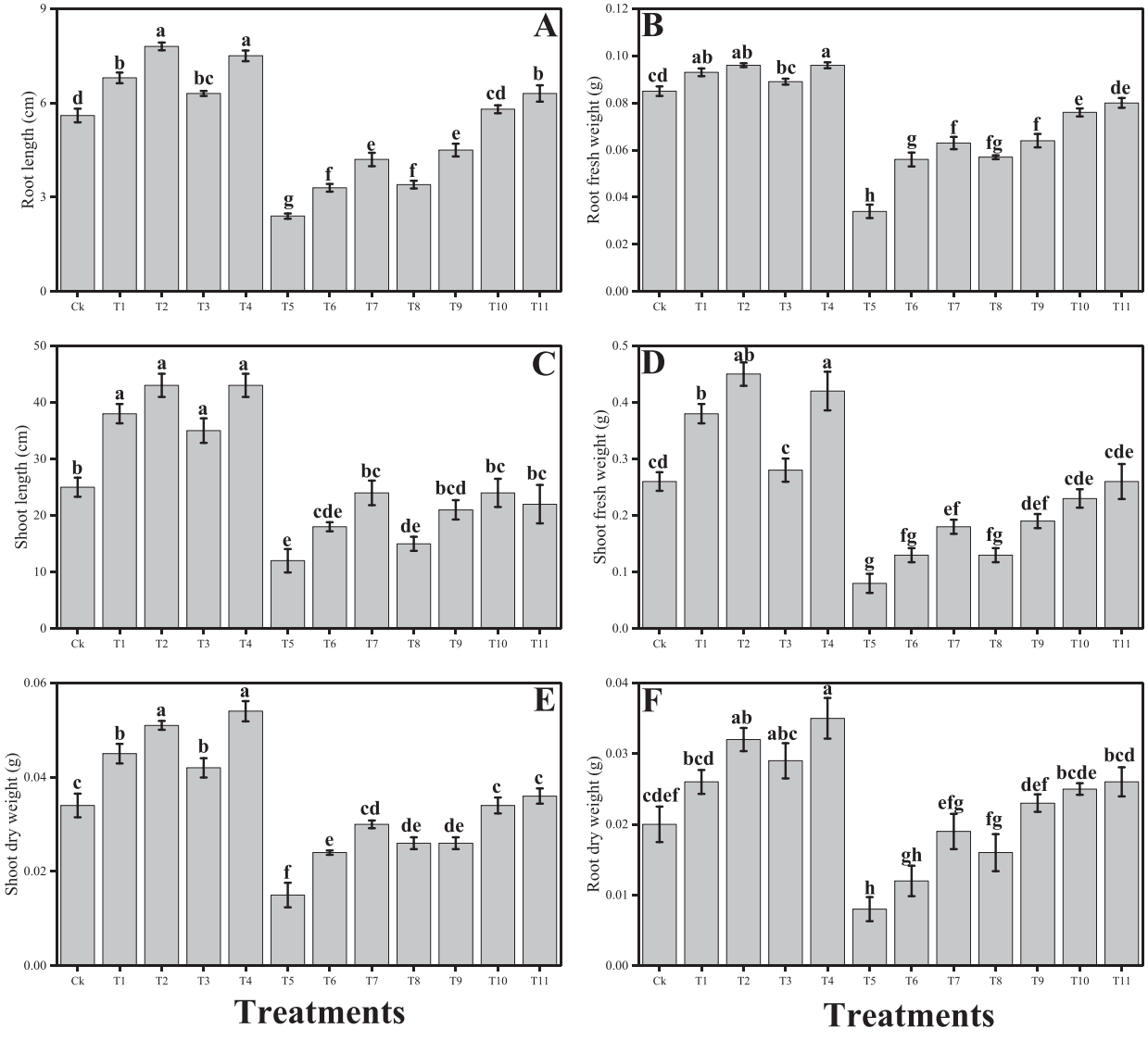


Fig. 1. Effect of alone and/or combinatorial application of abscisic acid (ABA) and 6-benzylaminopurine (6-BAP) on morphological attributes i.e., root length (A), root fresh weight (B), shoot length (C), shoot fresh weight (D), shoot dry weight (E), root dry weight (F) of rice (*O. sativa* L.) seedlings grown under the Cr stress. Values in the figures indicate just one harvest. Mean ± SD (n = 3). One-way ANOVA was performed and mean differences were tested by HSD ($P < 0.05$). Different lowercase letters on the error bars indicate significant differences between the treatments. The treatments abbreviations are as following: **CK:** control (no Cr + ABA + 6-BAP), $\mathbf{T_1}$: ABA (5 μM L^{-1}), $\mathbf{T_2}$: ABA (10 μM L^{-1}), $\mathbf{T_3}$: 6-BAP (5 μM L^{-1}), $\mathbf{T_4}$: 6-BAP (10 μM L^{-1}), $\mathbf{T_5}$: Cr (100 μM), $\mathbf{T_6}$: Cr (100 μM) + ABA (5 μM L^{-1}), $\mathbf{T_7}$: Cr (100 μM) + ABA (10 μM L^{-1}), $\mathbf{T_8}$: Cr (100 μM) + 6-BAP (5 μM L^{-1}), $\mathbf{T_9}$: Cr (100 μM) + 6-BAP (10 μM L^{-1}), $\mathbf{T_{10}}$: Cr (100 μM) + ABA (5 μM L^{-1}) + 6-BAP (5 μM L^{-1}), and $\mathbf{T_{11}}$: Cr (100 μM) + ABA (10 μM L^{-1}) + 6-BAP (10 μM L^{-1}).

Origin-Pro 2017. The plots of principal component analysis on O. sativa parameters were carried out using the RStudio software.

3. Results

3.1. Effects of Cr stress on growth and photosynthesis on O. sativa under the application of ABA and 6-BAP

In the present study, various growth parameters and photosynthetic pigments and also the gas exchange parameters in *O. sativa* under the Cr stress with the application of ABA and 6-BAP were measured. Growth and biomasses of *O. sativa* are presented in Fig. 1 and Table 1, while gas exchange attributes are presented in Fig. 2 and Table 1. According to the given results, Cr stress caused a significant toxicity in *O. sativa* and decreases the root length, root fresh weight, shoot length, shoot fresh weight, shoot dry weight, root dry weight, chlorophyll-a, chlorophyll *b*, chlorophyll *a*/b, carotenoid, net photosynthesis, stomatal conductance, transpiration rate and intercellular CO_2. However, application of ABA and 6-BAP caused increase in root length, root fresh weight, shoot length, shoot fresh weight, shoot dry weight, root dry weight, chlorophyll-a, chlorophyll *b*, chlorophyll *a*/b, carotenoid, net photosynthesis, stomatal conductance, transpiration rate and intercellular CO_2 compared to the plants which were grown in the control. The application of ABA and 6-BAP, when applied to the plants which were not treated with Cr stress, there was a significant increase in in root length, root fresh weight, shoot length, shoot fresh weight, shoot dry weight, root dry weight, chlorophyll-a, chlorophyll *b*, chlorophyll *a*/b, carotenoid, net photosynthesis, stomatal conductance, transpiration rate and intercellular CO_2 was observed in the plants compared to those which were not treated with the application of ABA and 6-BAP. Although the combined application of ABA and 6-BAP induced higher growth and biomass and gas exchange attributes, compared to the individual applications of ABA and 6-BAP.

Table 1
Mean square values from ANOVA of data for application with ABA and 6-BAP modulate growth, photosynthetic pigments, gaseous exchange parameters, oxidative stress and response of antioxidant and their gene expression, AsA-GSH cycle, cellular fractionation and Cr uptake *O. sativa* grown under various concentrations of Cr-contaminated soil. Where * is significant at $P \leq 0.05$; ** significant at $P \leq 0.01$; *** significant at $P \leq 0.001$.

Source of variations	Treatment df = 11	Error df = 24
Root length	9.164***	0.04222
Root fresh weight	0.00115***	0.00001
Shoot length	327.020***	6.333
Shoot fresh weight	0.04320***	0.00059
Shoot dry weight	0.000083***	0.0000045
Root dry weight	0.0000184***	0.000000667
Chlorophyll a	1.61271***	0.00359
Chlorophyll b	0.29033***	0.00264
Total chlorophyll	3.11431***	0.00695
Carotenoid	0.29057***	0.00431
Net photosynthesis	33.3703***	0.1359
Stomatal conductance	0.04527***	0.00101
Transpiration rate	47.6467***	0.5964
Intercellular CO_2	671.020*	233.583
SOD	10,135.8***	109.5
APX	1404000***	31.66
POD	1404000*	1043
CAT	101559***	872
Hydrogen peroxide	8751.46***	52.69
MDA	1488.82***	11.44
Total phenolic	20,637.5***	79.0
Total flavonoid	21,637.4***	143.7
Ascorbic acid	11.4244***	0.0292
Anthocyanin	0.15053***	0.00087
Total sugar	198.432***	4.500
Reducing sugar	406.475***	7.944
Gene expression of SOD	4.17733***	0.00311
Gene expression of APX	0.33816***	0.00056
Gene expression of POD	7.98084***	0.00534
Gene expression of CAT	0.51599***	0.00196
Cr in roots	2091.79***	10.72
Cr in shoots	403.967***	5.500
Proline	0.09663***	0.00101
P5C	1.40707***	0.00328
P5CR	118.659***	2.893
PRODH	407.475***	6.944
GSH	0.08814***	0.00181
Ascorbate	0.12901***	0.00176
GSSG	0.01004***	0.00004
DHA	0.04418***	0.00121
PME	0.02854***	0.00040
Uronic acid	11.4175***	0.4565
Hemicellulose I	262.331***	5.989
Hemicellulose II	24.4966***	1.0750
Cellulose	27.0418***	1.1421
pectin methylesterase	238.657***	9.833

3.2. Effects of Cr stress on oxidative stress and response of antioxidants on O. sativa under the application of ABA and 6-BAP

In the present study different oxidative stress biomarkers i.e., malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) were measured from the leaves of *O. sativa* as presented in Fig. 3E-F. According to the results, it was observed that the Cr stress caused a significant increase in MDA and H_2O_2 content in the leaves of the plants compared to the control. Although the application of ABA and 6-BAP decreases the MDA and H_2O_2 content in the leaves of *O. sativa*. We have also noticed that the individual application of ABA and 6-BAP also decreases MDA and H_2O_2 content, when plants were grown without the contamination of Cr in the soil. In addition, combined application of ABA and 6-BAP showed more severe results when compared to the plants which grown in the alone application of ABA and 6-BAP.

Different enzymatic antioxidants i.e., superoxidase dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), catalase (CAT) and non-enzymatic compounds i.e., phenolic, anthocyanin, flavonoids, ascorbic acid and flavonoid and also their relevant gene expression i.e., SOD, POD, CAT and APX were also measured from the leaves of *O. sativa*. The results regarding the enzymatic antioxidants are presented in Fig. 3, while the results regarding their relevant gene expression are presented in Fig. 4 and the results regarding the non-enzymatic compounds are presented in Fig. 5. According to the results, we have noticed that the Cr stress in the soil significantly increases the enzymatic antioxidants i.e., SOD, POD, CAT and APX and their relevant gene expression and also the non-enzymatic compounds i.e., phenolic, anthocyanin, flavonoids, ascorbic acid and flavonoid compared to the plants grown in the control treatment. Present findings also showed that the alone application of either ABA or 6-BAP also increases the activity of SOD, POD, CAT and APX and their relevant gene expression and also the non-enzymatic compounds i.e., phenolic, anthocyanin, flavonoids, ascorbic acid and flavonoid compared to the plants which were not treatment with the application of ABA or 6-BAP in Cr stressed soil and also the normal soil i.e., which was not spiked with Cr. In addition, the maximum activity of SOD, POD, CAT and APX and their relevant gene expression and also the non-enzymatic compounds i.e., phenolic, anthocyanin, flavonoids, ascorbic acid and flavonoid were observed in the plants which grown in the combined application of ABA and 6-BAP application.

3.3. Effects of Cr stress on sugar, proline metabolism and glutathione-ascorbate cycle on O. sativa under the application of ABA and 6-BAP

In the present study, total sugar and reducing sugar were also measured from the *O. sativa* under the Cr stress and presented in Fig. 5. The proline metabolism is presented in Fig. 6 and AsA-GSH cycle is presented in Fig. 7. According to the given results, it was noticed that the Cr toxicity in the soil significantly decreased the total sugar and reducing sugar compared to the control. However, the alone application

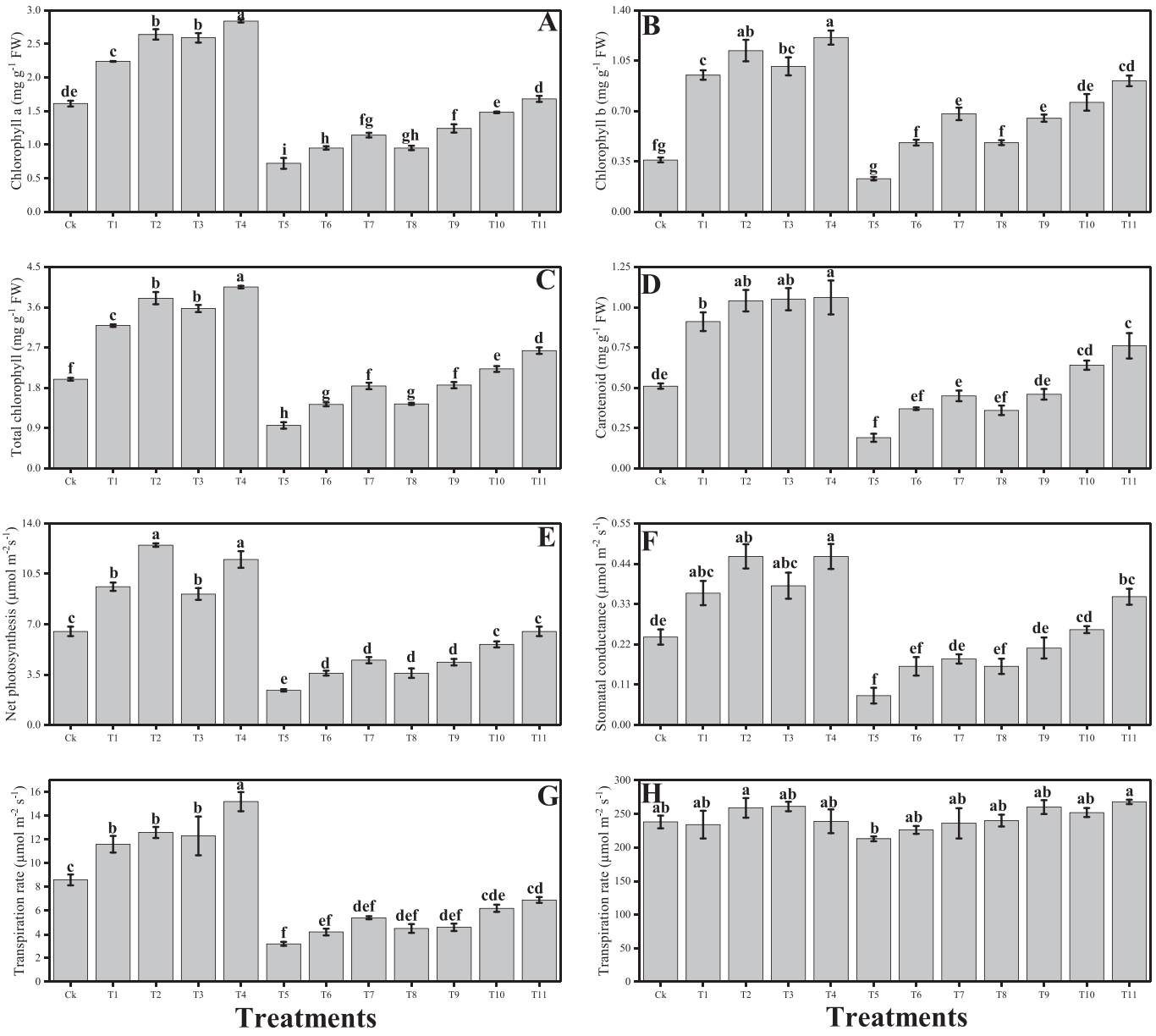


Fig. 2. Effect of alone and/or combinatorial application of abscisic acid (ABA) and 6-benzylaminopurine (6-BAP) on photosynthetic pigments and gas exchange attributes i.e., chlorophyll-a (A), chlorophyll-b (C), chlorophyll *a*/b (C), carotenoid (D), net photosynthesis (E), stomatal conductance (F), transpiration rate (G) and intercellular CO_2 (H) of rice (*O. sativa* L.) seedlings grown under the Cr stress. Values in the figures indicate just one harvest. Mean ± SD (n = 3). One-way ANOVA was performed and mean differences were tested by HSD ($P < 0.05$). Different lowercase letters on the error bars indicate significant differences between the treatments. The treatments abbreviations are as following: **CK:** control (no Cr + ABA + 6-BAP), $\mathbf{T_1}$: ABA (5 µM L^{-1}), $\mathbf{T_2}$: ABA (10 µM L^{-1}), $\mathbf{T_3}$: 6-BAP (5 µM L^{-1}), $\mathbf{T_4}$: 6-BAP (10 µM L^{-1}), $\mathbf{T_5}$: Cr (100 µM), $\mathbf{T_6}$: Cr (100 µM) + ABA (5 µM L^{-1}), $\mathbf{T_7}$: Cr (100 µM) + ABA (10 µM L^{-1}), $\mathbf{T_8}$: Cr (100 µM) + 6-BAP (5 µM L^{-1}), $\mathbf{T_9}$: Cr (100 µM) + 6-BAP (10 µM L^{-1}), $\mathbf{T_{10}}$: Cr (100 µM) + ABA (5 µM L^{-1}) + 6-BAP (5 µM L^{-1}), and $\mathbf{T_{11}}$: Cr (100 µM) + ABA (10 µM L^{-1}) + 6-BAP (10 µM L^{-1}).

of either ABA or 6-BAP caused a significant increase in the total sugar and reducing sugar compared to those plants which were grown in the control in the plants which were grown in the soil which was spiked with Cr and also the soil which was not spiked with the Cr. The maximum increase was observed in the plants which were grown in the combined application of ABA and 6-BAP compared to the single treatment of ABA and 6-BAP.

The proline metabolism such as proline, pyrroline-5-carboxylate, pyrroline-5-carboxylate reductase and pyrroline-5-carboxylate dehydrogenase were also measured from the *O. sativa* tissue and results showed that the Cr toxicity induced a significant decrease in the proline, pyrroline-5-carboxylate, pyrroline-5-carboxylate reductase and pyrroline-5-carboxylate dehydrogenase compared to the control (Fig. 6). However, the application of ABA and 6-BAP induced a significant increase in the content of proline, pyrroline-5-carboxylate, pyrroline-5-carboxylate reductase and pyrroline-5-carboxylate dehydrogenase compared to those plants which were grown without the application of ABA or 6-BAP. We have also noticed that the individual applications of ABA and 6-BAP in the normal soil (which was not spiked with the Cr), the levels of proline, pyrroline-5-carboxylate, pyrroline-5-carboxylate reductase and pyrroline-5-carboxylate dehydrogenase was decreased when compared to the plants which were grown in the control. In addition, the combined application of ABA or 6-BAP induced a maximum increase in the content of proline, pyrroline-5-carboxylate, pyrroline-5-carboxylate reductase and pyrroline-5-carboxylate dehydrogenase compared to the individual application of ABA or 6-BAP in the Cr stressed soil.

AsA-GSH cycle including the contents of glutathione, ascorbate,

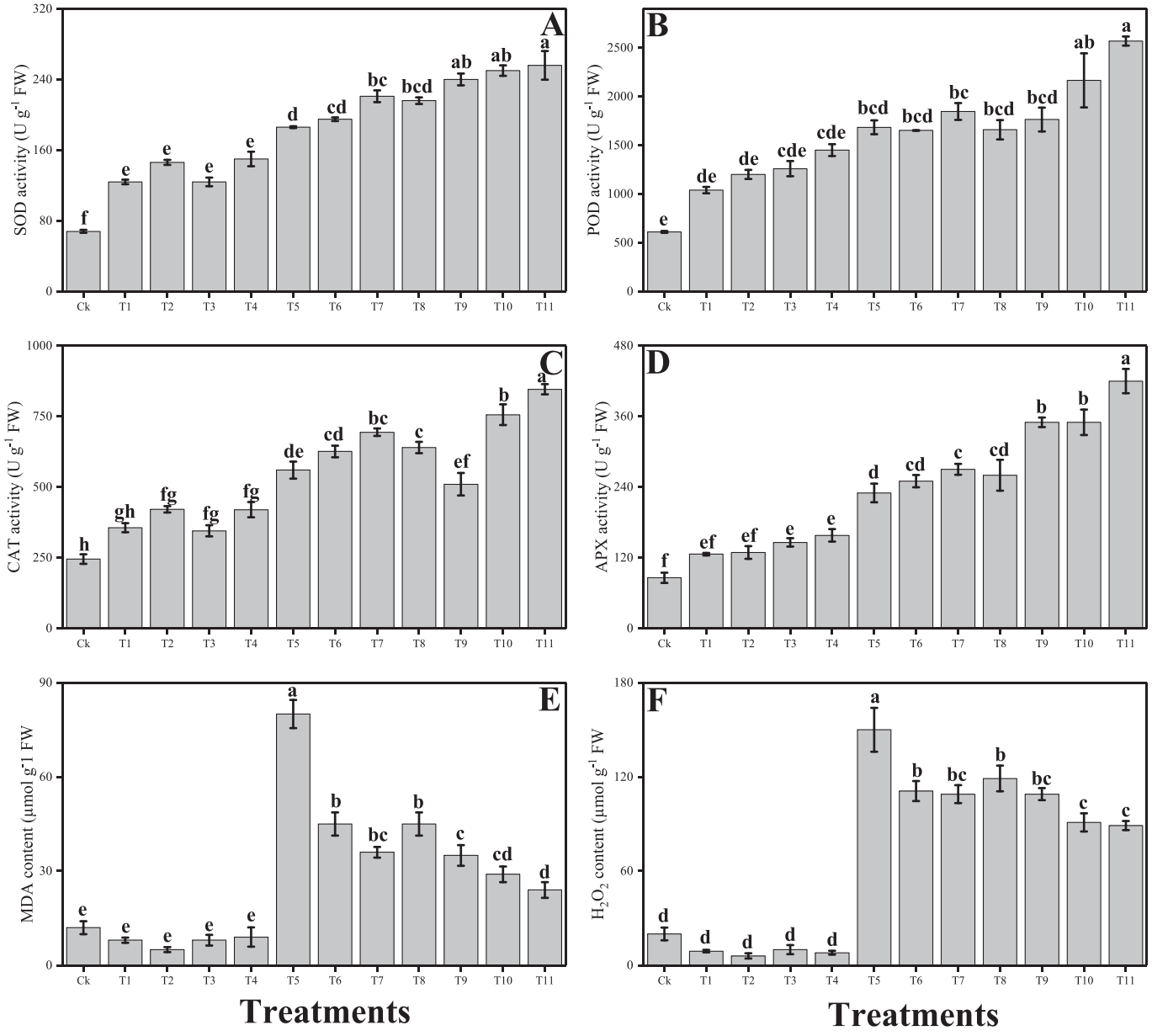


Fig. 3. Effect of alone and/or combinatorial application of abscisic acid (ABA) and 6-benzylaminopurine (6-BAP) on enzymatic antioxidants and oxidative stress biomarkers i.e., superoxidase dismutase (A), ascorbate peroxidase (B), peroxidase (C), catalase (D), hydrogen peroxide (E) and malondialdehyde (F) of rice (*O. sativa* L.) seedlings grown under the Cr stress. Values in the figures indicate just one harvest. Mean ± SD (n = 3). One-way ANOVA was performed and mean differences were tested by HSD ($P < 0.05$). Different lowercase letters on the error bars indicate significant differences between the treatments. The treatments abbreviations are as following: **CK:** control (no Cr + ABA +6-BAP), $\mathbf{T_1}$: ABA (5 µM L^{-1}), $\mathbf{T_2}$: ABA (10 µM L^{-1}), $\mathbf{T_3}$: 6-BAP (5 µM L^{-1}), $\mathbf{T_4}$: 6-BAP (10 µM L^{-1}), $\mathbf{T_5}$: Cr (100 µM), $\mathbf{T_6}$: Cr (100 µM) + ABA (5 µM L^{-1}), $\mathbf{T_7}$: Cr (100 µM) + ABA (10 µM L^{-1}), $\mathbf{T_8}$: Cr (100 µM) + 6-BAP (5 µM L^{-1}), $\mathbf{T_9}$: Cr (100 µM) + 6-BAP (10 µM L^{-1}), $\mathbf{T_{10}}$: Cr (100 µM) + ABA (5 µM L^{-1}) + 6-BAP (5 µM L^{-1}), and $\mathbf{T_{11}}$: Cr (100 µM) + ABA (10 µM L^{-1}) + 6-BAP (10 µM L^{-1}).

glutathione disulfide, dehydroascorbic acid was also measured and we have noticed that the Cr toxicity significantly decreases the content of glutathione, ascorbate and dehydroascorbic acid while increases the content of glutathione disulfide from the tissues of the *O. sativa* (Fig. 7). We have also noticed that the plants which were not contaminated with the Cr and applied with the individual application of ABA or 6-BAP showed a significant ($P < 0.05$) increases in the content of glutathione, ascorbate, dehydroascorbic acid and glutathione disulfide when compared to the plants which were not applied with the application of ABA or 6-BAP. However, the application of ABA or 6-BAP in alone increases the contents of glutathione, ascorbate and dehydroascorbic acid compared to the plants which were grown without the application of ABA or 6-BAP. Although, the application of ABA or 6-BAP decreases the content of glutathione disulfide when applied in alone or in combined treatment.

3.4. Effects of Cr stress on cellular fractionation and Cr uptake on O. sativa under the application of ABA and 6-BAP

In the present study the Cr concentration from the roots and shoots of *O. sativa* was also determined and presented in the Fig. 6 (A, B). According to the results we have noticed that the Cr stress in the soil significantly increases the Cr concentration in the roots and shoots of the plants. We have also noticed that Cr concentration was decreases when the plants applied with the singular application of ABA or 6-BAP in the control treatments which were not spiked with the Cr. Although, the

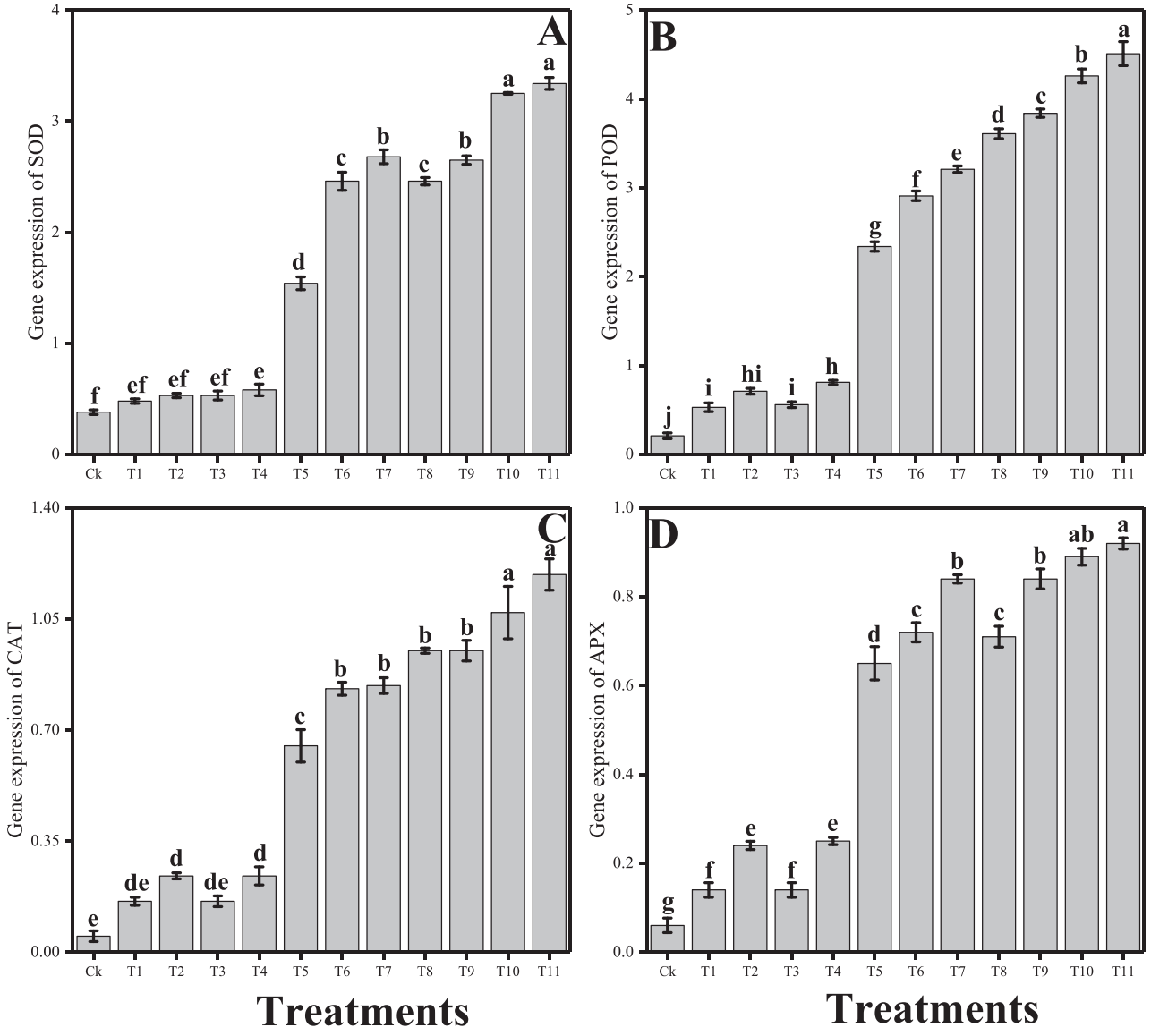


Fig. 4. Effect of alone and/or combinatorial application of abscisic acid (ABA) and 6-benzylaminopurine (6-BAP) on relevant gene expression i.e., superoxidase dismutase (A), ascorbate peroxidase (B), peroxidase (C) and catalase (D) of rice (*O. sativa* L.) seedlings grown under the Cr stress. Values in the figures indicate just one harvest. Mean ± SD (n = 3). One-way ANOVA was performed and mean differences were tested by HSD ($P < 0.05$). Different lowercase letters on the error bars indicate significant differences between the treatments. The treatments abbreviations are as following: **CK:** control (no Cr + ABA + 6-BAP), $\mathbf{T_1}$: ABA (5 μM L^{-1}), $\mathbf{T_2}$: ABA (10 μM L^{-1}), $\mathbf{T_3}$: 6-BAP (5 μM L^{-1}), $\mathbf{T_4}$: 6-BAP (10 μM L^{-1}), $\mathbf{T_5}$: Cr (100 μM), $\mathbf{T_6}$: Cr (100 μM) + ABA (5 μM L^{-1}), $\mathbf{T_7}$: Cr (100 μM) + ABA (10 μM L^{-1}), $\mathbf{T_8}$: Cr (100 μM) + 6-BAP (5 μM L^{-1}), $\mathbf{T_9}$: Cr (100 μM) + 6-BAP (10 μM L^{-1}), $\mathbf{T_{10}}$: Cr (100 μM) + ABA (5 μM L^{-1}) + 6-BAP (5 μM L^{-1}), and $\mathbf{T_{11}}$: Cr (100 μM) + ABA (10 μM L^{-1}) + 6-BAP (10 μM L^{-1}).

application of ABA or 6-BAP decreases Cr concentration in roots and shoots of the plants, while maximum decrease was observed where plants were applied with the combined application of ABA or 6-BAP in the plants which were spiked with the Cr toxicity.

Cellular compartments fractionation i.e., pectin methylesterase activity, uronic acid, hemicellulose I, hemicellulose II, cellulose and pectin methylesterase were also determined from the *O. sativa* and presented as Fig. 8. Results from the present study showed that the Cr stress causes a significant increase in the pectin methylesterase activity, uronic acid, hemicellulose I, hemicellulose II, cellulose and pectin methylesterase when compared to the control. In addition, the plants grown without the concentration of Cr in the soil decreases the pectin methylesterase activity, uronic acid, hemicellulose I, hemicellulose II, cellulose and pectin methylesterase compared to those which were not applied with the application of ABA or 6-BAP. However, application of ABA or 6-BAP to the plants which were spiked with the Cr concentration, further increases the content of pectin methylesterase activity, uronic acid, hemicellulose I, hemicellulose II, cellulose and pectin methylesterase in *O. sativa*.

3.5. Principal component analysis

The loading plots of the principal component analysis (PCA) performed to evaluate the effects of phytohormones treatment on some attributes of *O. sativa* seedlings are presented in Fig. 9. Among all components, the Dim1 and Dim2 components exhibited the maximum contribution in the database. Fig. 9 shows that Cr toxicity had negative impacts on the growth and ecophysiology of *O. sativa* seedlings and that the application of phytohormones reduced the metal toxicity. The first group of variables with which Dim2 was positively correlated includes Cr concentration in the roots and Cr concentration in the shoots. In contrast, significant negative correlations of Dim1 variables with the

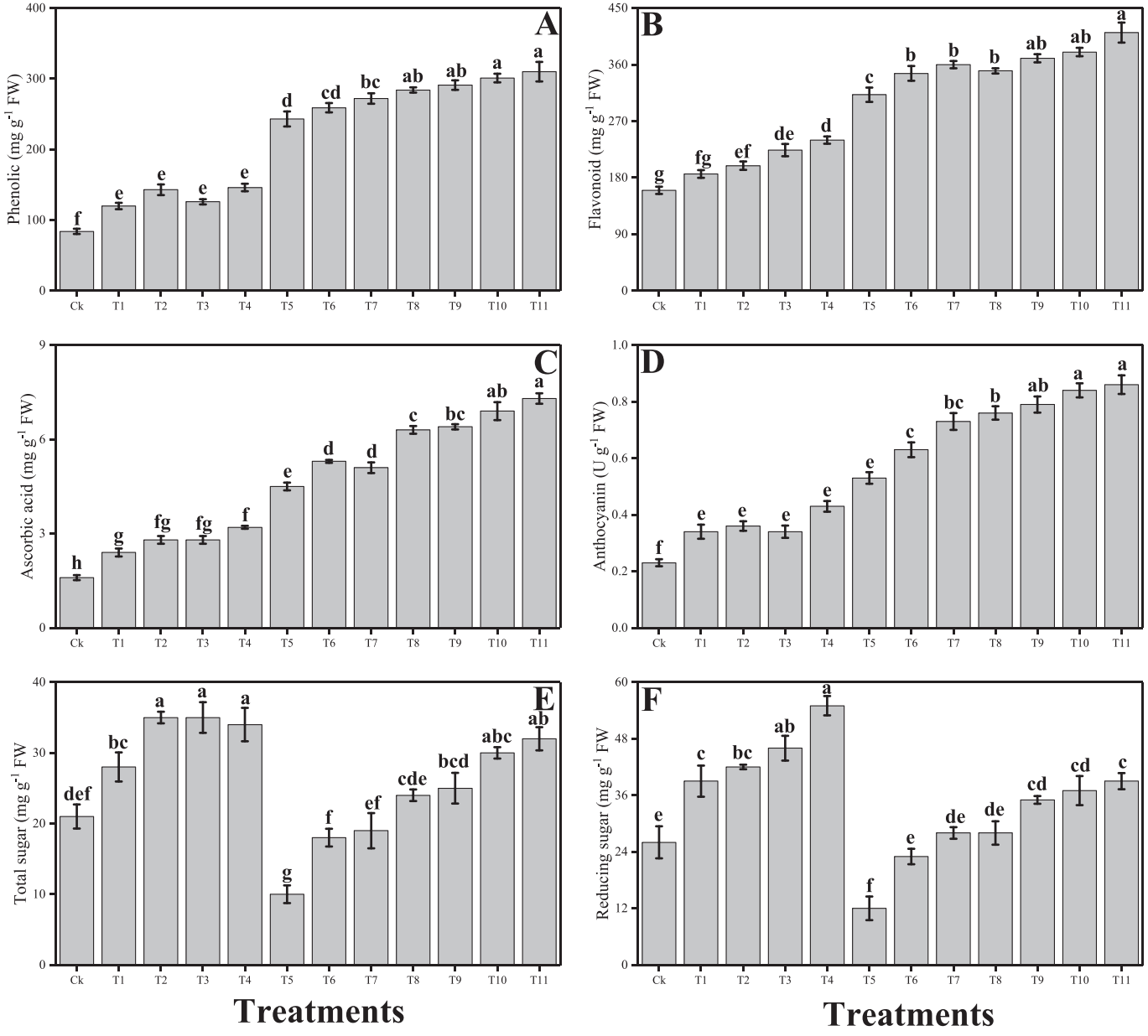


Fig. 5. Effect of alone and/or combinatorial application of abscisic acid (ABA) and 6-benzylaminopurine (6-BAP) on non-enzymatic compounds and sugar content i.e., phenolic (A), flavonoid (B), ascorbic acid (C), anthocyanin (D), total sugar (E) and reducing sugar (F) of rice (*O. sativa* L.) seedlings grown under the Cr stress. Values in the figures indicate just one harvest. Mean ± SD (n = 3). One-way ANOVA was performed and mean differences were tested by HSD ($P < 0.05$). Different lowercase letters on the error bars indicate significant differences between the treatments. The treatments abbreviations are as following: **CK:** control (no Cr + ABA + 6-BAP), **T_1**: ABA (5 μM L^{-1}), **T_2**: ABA (10 μM L^{-1}), **T_3**: 6-BAP (5 μM L^{-1}), **T_4**: 6-BAP (10 μM L^{-1}), **T_5**: Cr (100 μM), **T_6**: Cr (100 μM) + ABA (5 μM L^{-1}), **T_7**: Cr (100 μM) + ABA (10 μM L^{-1}), **T_8**: Cr (100 μM) + 6-BAP (5 μM L^{-1}), **T_9**: Cr (100 μM) + 6-BAP (10 μM L^{-1}), **T_{10}**: Cr (100 μM) + ABA (5 μM L^{-1}) + 6-BAP (5 μM L^{-1}), and **T_{11}**: Cr (100 μM) + ABA (10 μM L^{-1}) + 6-BAP (10 μM L^{-1}).

variables aligned with Dim2 were observed: root length, root fresh weight, shoot length, shoot fresh weight, shoot dry weight, root dry weight, chlorophyll-a, chlorophyll *b*, chlorophyll *a*/b, carotenoid, net photosynthesis, stomatal conductance, transpiration rate and intercellular CO_2.

4. Discussion

Cr is among the most toxic trace elements present in agricultural soils and is being released through a variety of anthropogenic activities such as electroplating and leather tanning (Hashem et al., 2020; Sarfraz et al., 2022). However, Cr is a well-known toxic metal and is harmful to the growth and development of the plant and it was also reported that it provokes adverse effects on biochemistry and physiology of various important crops (Rana et al., 2020; Javed et al., 2021). Exposure to Cr may induce toxic effects in several biochemical processes in plants, such as plant germination, root growth and length, stem growth, and leaf development (Maqbool et al., 2018; Ashraf et al., 2022). It has been previously shown that Cr stress negatively affects the plant biomass and photosynthetic efficiency in different plant species which depends upon a number of factors including plant species, dose, and duration of Cr application (Ali et al., 2013; Danish et al., 2019; Hussain et al., 2021). Cr stress can disturb the dynamic equilibrium of reactive oxygen species (ROS) production and elimination under normal growth in plants (Naz et al., 2021; Tang et al., 2021), which promote ROS accumulation and membrane lipid peroxidation, and disrupt the structure and function of

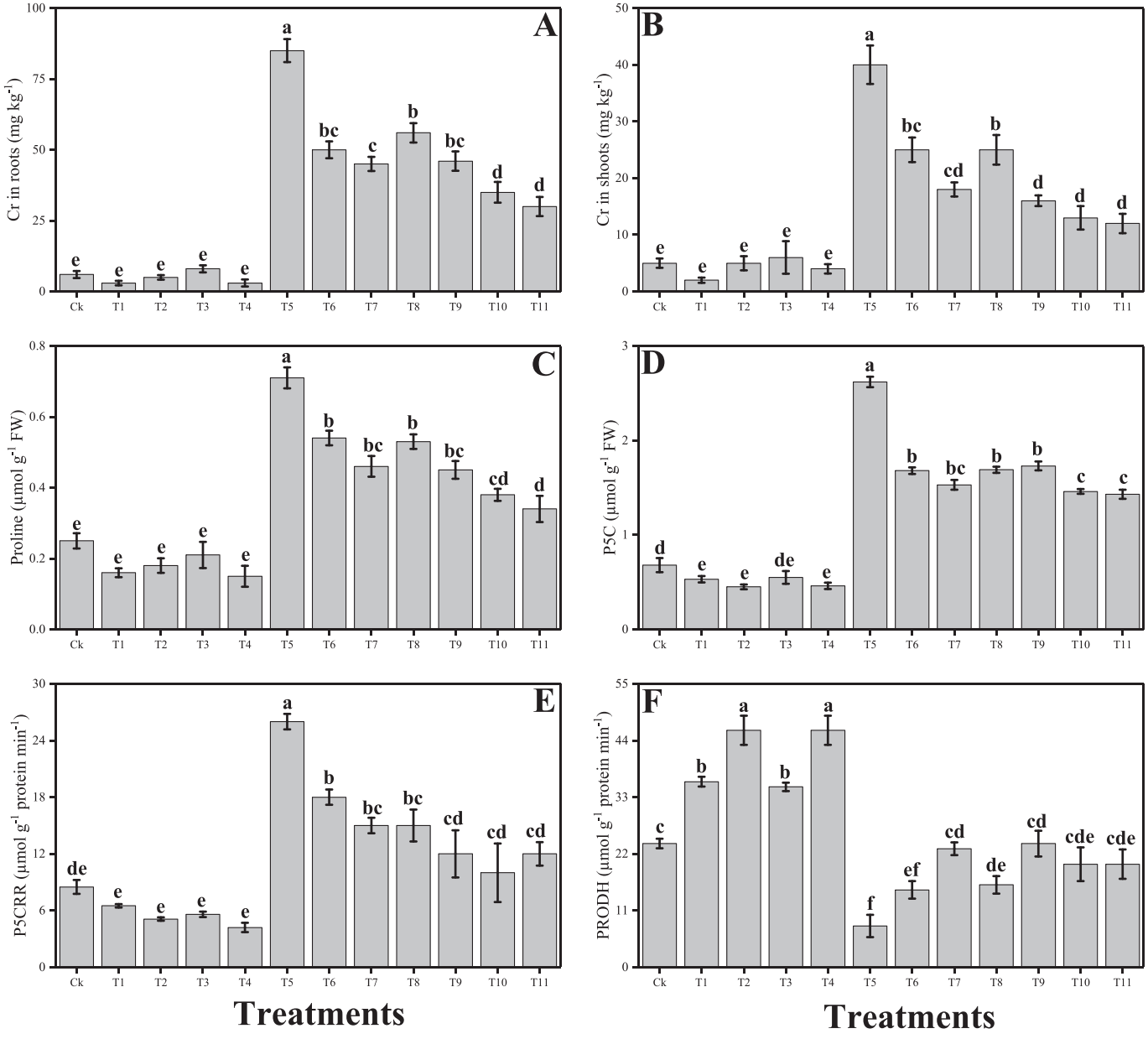


Fig. 6. Effect of alone and/or combinatorial application of abscisic acid (ABA) and 6-benzylaminopurine (6-BAP) on Cr uptake and proline metabolism i.e., Cr concentration in the roots (A), Cr concentration in the shoots (B), proline (C), pyrroline-5-carboxylate (D), pyrroline-5-carboxylate reductase (E) and pyrroline-5-carboxylate dehydrogenase (F) of rice (*O. sativa* L.) seedlings grown under the Cr stress. Values in the figures indicate just one harvest. Mean ± SD (n = 3). One-way ANOVA was performed and mean differences were tested by HSD ($P < 0.05$). Different lowercase letters on the error bars indicate significant differences between the treatments. The treatments abbreviations are as following: **CK:** control (no Cr + ABA + 6-BAP), $\mathbf{T_1}$: ABA (5 µM L^{-1}), $\mathbf{T_2}$: ABA (10 µM L^{-1}), $\mathbf{T_3}$: 6-BAP (5 µM L^{-1}), $\mathbf{T_4}$: 6-BAP (10 µM L^{-1}), $\mathbf{T_5}$: Cr (100 µM), $\mathbf{T_6}$: Cr (100 µM) + ABA (5 µM L^{-1}), $\mathbf{T_7}$: Cr (100 µM) + ABA (10 µM L^{-1}), $\mathbf{T_8}$: Cr (100 µM) + 6-BAP (5 µM L^{-1}), $\mathbf{T_9}$: Cr (100 µM) + 6-BAP (10 µM L^{-1}), $\mathbf{T_{10}}$: Cr (100 µM) + ABA (5 µM L^{-1}) + 6-BAP (5 µM L^{-1}), and $\mathbf{T_{11}}$: Cr (100 µM) + ABA (10 µM L^{-1}) + 6-BAP (10 µM L^{-1}).

cell membrane system (Hussain et al., 2021; Vishnupradeep et al., 2022). It is well documented that Cr toxicity directly caused oxidative injury in the plants through the Fetone and Haber–Weiss reactions which also helps in the generation of large amount of ROS which is toxic to the plant (Basit et al., 2023; Khan et al., 2023). This ROS accumulation in plants is removed by a variety of antioxidant enzymes such as SOD, POD, CAT, and APX (Fig. 3) and non-enzymatic antioxidant (Fig. 5) and their gene expression (Fig. 4). However, the expression of antioxidative enzymes, such as SOD, POD, CAT, and APX under Cr stressed environment plays a significant role in reducing Cr toxicity, which was reported in a number of studies under various plant species (Ali et al., 2013; Farid et al., 2019).

The maintenance of cellular redox equilibrium by these antioxidant systems is necessary for plant growth and survival under a variety of stress circumstances (Ali et al., 2022). Lipid peroxidation takes place when ROS damages the polyunsaturated fatty acids in cellular membranes (Wang et al., 2015; Ashraf et al., 2022). This process has the potential to compromise the integrity of the cell membrane, interfere with cellular processes, and eventually cause cell death (Azooz et al., 2012; Li et al., 2013). Cell wall in plants is responsible for the development of stress tolerance to metals and non-metals (Kareem et al., 2022). Moreover, cell wall can form a hard structure that prevents toxic ions from entering the cytoplasm (Ulhassan et al., 2022; Li et al., 2023). Polysaccharides found in cell wall include cellulose, hemicellulose, and pectin, all of which can change in response to environmental stress (Gao et al., 2019). For example, pectin methyl esterase can change the level of

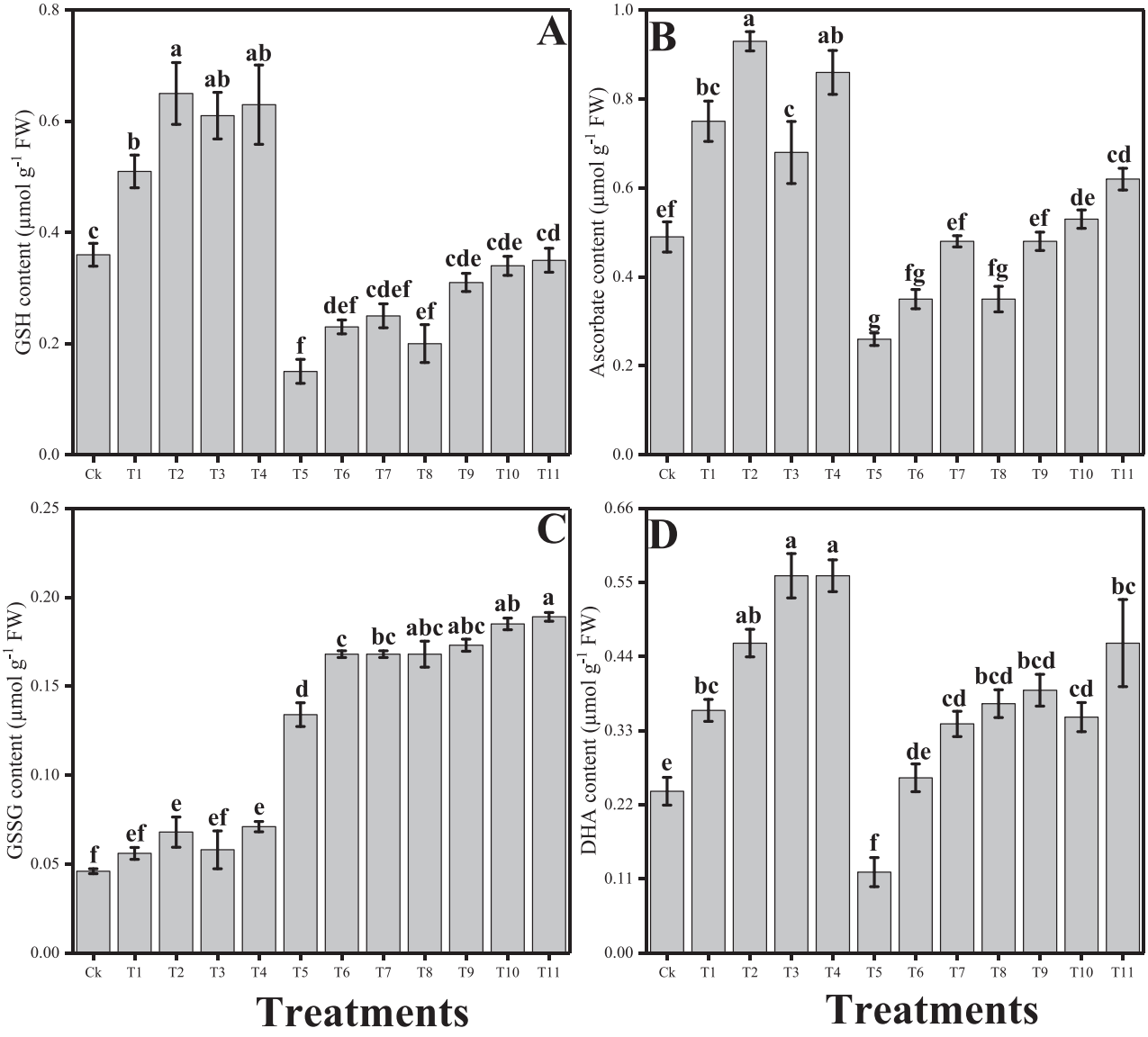


Fig. 7. Effect of alone and/or combinatorial application of abscisic acid (ABA) and 6-benzylaminopurine (6-BAP) on AsA-GSH cycle i.e., glutathione (A), ascorbate (B), glutathione disulfide (C) and dehydroascorbic acid (D) of rice (*O. sativa* L.) seedlings grown under the Cr stress. Values in the figures indicate just one harvest. Mean ± SD (n = 3). One-way ANOVA was performed and mean differences were tested by HSD ($P < 0.05$). Different lowercase letters on the error bars indicate significant differences between the treatments. The treatments abbreviations are as following: **CK:** control (no Cr + ABA + 6-BAP), $\mathbf{T_1}$: ABA (5 μM L^{-1}), $\mathbf{T_2}$: ABA (10 μM L^{-1}), $\mathbf{T_3}$: 6-BAP (5 μM L^{-1}), $\mathbf{T_4}$: 6-BAP (10 μM L^{-1}), $\mathbf{T_5}$: Cr (100 μM), $\mathbf{T_6}$: Cr (100 μM) + ABA (5 μM L^{-1}), $\mathbf{T_7}$: Cr (100 μM) + ABA (10 μM L^{-1}), $\mathbf{T_8}$: Cr (100 μM) + 6-BAP (5 μM L^{-1}), $\mathbf{T_9}$: Cr (100 μM) + 6-BAP (10 μM L^{-1}), $\mathbf{T_{10}}$: Cr (100 μM) + ABA (5 μM L^{-1}) + 6-BAP (5 μM L^{-1}), and $\mathbf{T_{11}}$: Cr (100 μM) + ABA (10 μM L^{-1}) + 6-BAP (10 μM L^{-1}).

pectin methylation and thus the number of metal ion bound to the cell wall (Yang et al., 2020). In addition, Cr stress causes the accumulation of lignin in cell wall, which changes its chemical composition and properties (Caldelas et al., 2012; Ali et al., 2013).

Several previous investigations showed that plant growth modulators application i.e., ABA and 6-BAP, prominently ameliorated the inhibition of plant growth caused by various abiotic stresses (Aderholt et al., 2017; Kamran et al., 2020). The plant growth modulators-induced improvements in *O. sativa* growth and root morphology might be due to the reduced Cr uptake (Pérez-León et al., 2023) by deactivating the metals due to various modifications in phytochelatins (Najmanova et al., 2012; Ulhassan et al., 2022). The other possible explanations of alleviation of heavy metal-induced phytotoxicity could be due to increased photosynthetic activity, transpiration rate, accumulation of amino acids (Matysiak et al., 2020) and decreased cell membrane damage (Gill et al., 2015). Also, the single or combined application of ABA and 6-BAP further enhanced the activities of antioxidant enzymes (APX, SOD, POD, and CAT) in the control and also the plants grown in the Cr toxicity, reduced the Cr-induced oxidative damage in *O. sativa* leaves. The alone application of ABA or 6-BAP increases plant growth and biomass and increases gas exchange attributes by decreases oxidative damage and increases the activities of enzymatic and non-enzymatic compounds thereby preventing cellular damage and promoting growth as suggested by (Kamran et al., 2020). Previously, many studies reported that lower levels of ABA application enhanced the scavenging capacity by boosting antioxidants activities of tomato (Kamran et al., 2020), thereby alleviated B-induced ROS effects. Similarly, 6-BAP has also been recognized as an oxidative stress alleviator and membrane stabilizer to enhance the antioxidant enzyme activities of *Panicum virgatum* (Aderholt et al., 2017) exposed to various abiotic stresses. Plant growth modulators have been shown to enhance the activities of enzymes involved in photosynthesis, carbon metabolism, and carbohydrate metabolism, as

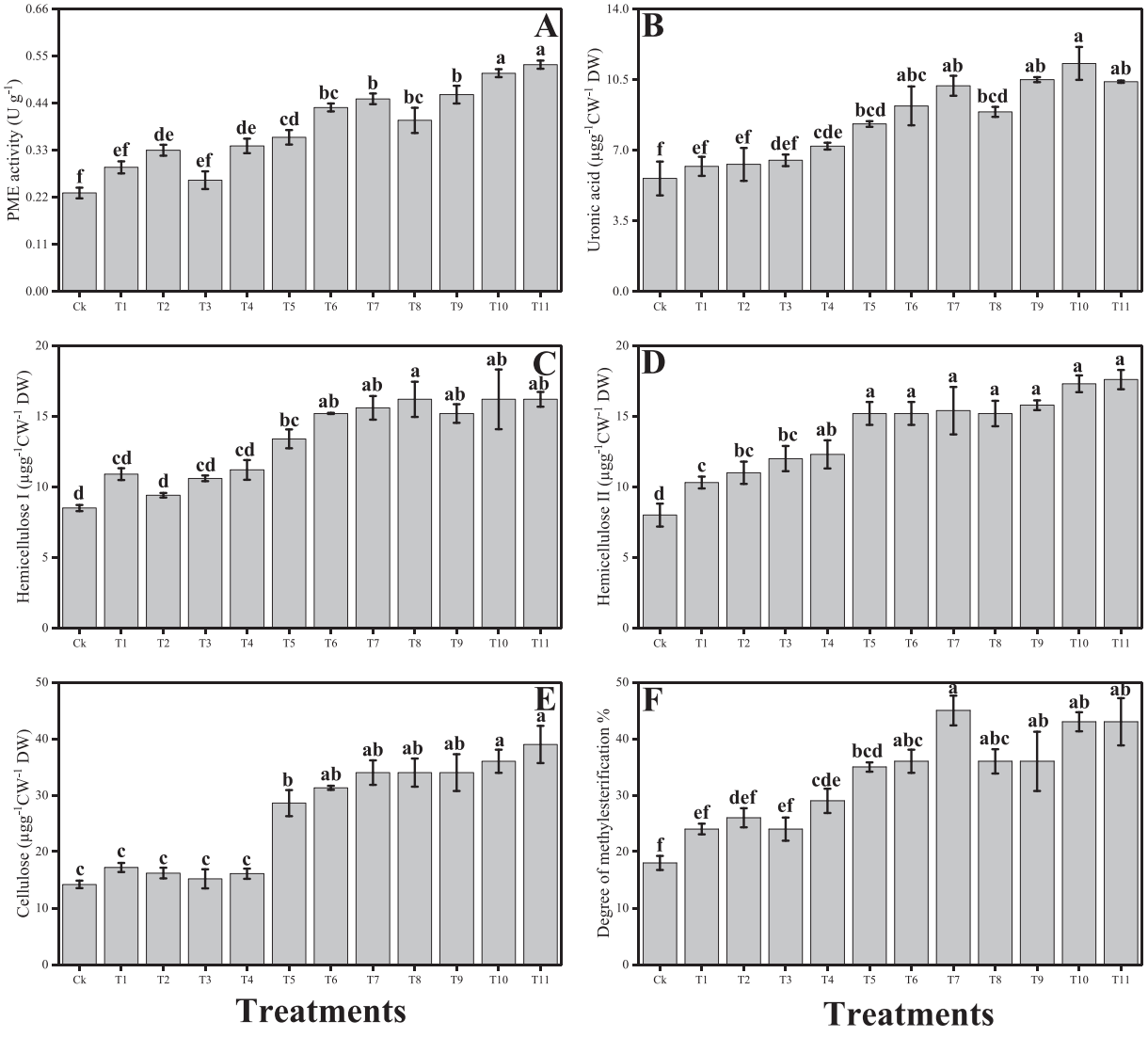


Fig. 8. Effect of alone and/or combinatorial application of abscisic acid (ABA) and 6-benzylaminopurine (6-BAP) on cellular fractionation i.e., pectin methylesterase activity (A), uronic acid (B), hemicellulose I (C), hemicellulose II (D), cellulose (E) and pectin methylesterase (F) of rice (*O. sativa* L.) seedlings grown under the Cr stress. Values in the figures indicate just one harvest. Mean ± SD (n = 3). One-way ANOVA was performed and mean differences were tested by HSD ($P < 0.05$). Different lowercase letters on the error bars indicate significant differences between the treatments. The treatments abbreviations are as following: **CK:** control (no Cr + ABA + 6-BAP), $\mathbf{T_1}$: ABA (5 µM L^{-1}), $\mathbf{T_2}$: ABA (10 µM L^{-1}), $\mathbf{T_3}$: 6-BAP (5 µM L^{-1}), $\mathbf{T_4}$: 6-BAP (10 µM L^{-1}), $\mathbf{T_5}$: Cr (100 µM), $\mathbf{T_6}$: Cr (100 µM) + ABA (5 µM L^{-1}), $\mathbf{T_7}$: Cr (100 µM) + ABA (10 µM L^{-1}), $\mathbf{T_8}$: Cr (100 µM) + 6-BAP (5 µM L^{-1}), $\mathbf{T_9}$: Cr (100 µM) + 6-BAP (10 µM L^{-1}), $\mathbf{T_{10}}$: Cr (100 µM) + ABA (5 µM L^{-1}) + 6-BAP (5 µM L^{-1}), and $\mathbf{T_{11}}$: Cr (100 µM) + ABA (10 µM L^{-1}) + 6-BAP (10 µM L^{-1}).

well as upregulate the activity of Δ1-pyrroline-5-carboxylate synthetase, which is responsible for Pro synthesis (Bisht et al., 2020; Sun et al., 2021). Correspondingly, Plant growth modulators application promoted PME activity, which could enhance cell wall polysaccharides and adsorption of more extra Cr onto the cell wall by increasing pectin methylation (Yang et al., 2020; Pan et al., 2021). The findings of this study strongly advocate for the simultaneous combination of 6-BAP and ABA as effective strategies to alleviate Cr-induced stress in *O. sativa* plants. These strategies operate through several well-defined mechanisms: (1) enhancing of both antioxidant and non-antioxidant activities within the plant, (2) increasing of proline and glutathione content, vital components in stress tolerance and, (3) significant reduction in the translocation and accumulation of Cr within the root and shoot systems.

5. Conclusion

Exogenous application of phytohormones like ABA and 6-BPA can mitigate the adverse effects of Cr stress in *O. sativa*. This study showed that toxic concentration of Cr toxicity in the soil showed a significant declined in the growth, gas exchange attributes, sugars, AsA-GSH cycle, cellular fractionation, proline metabolism in *O. sativa*. However, Cr toxicity significantly increased oxidative stress biomarkers, organic acids, enzymatic and non-enzymatic antioxidants including their gene expression in *O. sativa* seedlings. Plant growth regulators such as 6-BAP and ABA, either individually or in combination, decreased oxidative stress in the *O. sativa* plant under Cr stress. This might safeguard the cell wall compartment and reduce oxidative stress by increasing the antioxidant and non-antioxidant activity and also their gene expression and regulating glutathione levels. We have noticed that combining ABA and 6-BAP would increase plant resistance to Cr toxicity through different mechanisms. These mechanisms include triggering defensive responses, upregulation of gene expression related to antioxidant enzymes, modulation of secondary metabolism compounds, decline the uptake of Cr concentration from roots to the shoots, and improvement of gas exchange attributes. Furthermore, combined application of ABA and 6-BAP showed more severe results and enhance plant tolerance

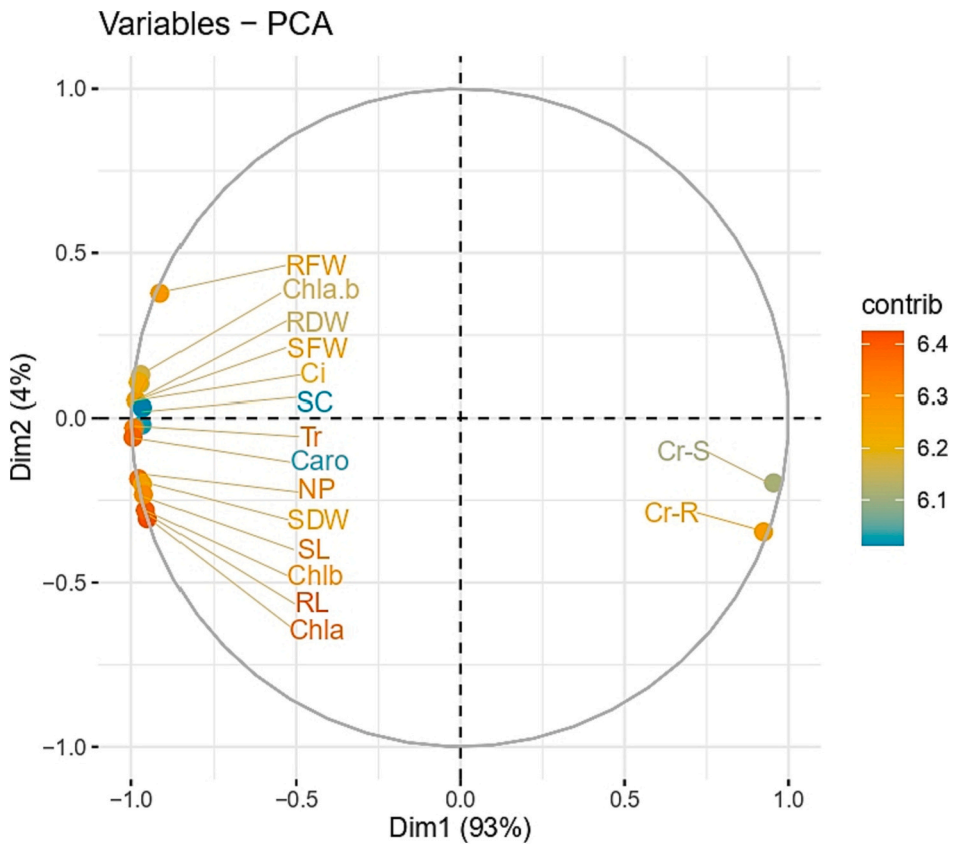


Fig. 9. Loading plots of PCA on different studied attributes of *O. sativa* seedlings grown in Cr contaminated soil with individual and combined application of ABA and 6-BAP. Different parameters studied in the PCA are as follow: root length, root fresh weight, shoot length, shoot fresh weight, shoot dry weight, root dry weight, chlorophyll-a, chlorophyll *b*, chlorophyll *a*/b, carotenoid, net photosynthesis, stomatal conductance, transpiration rate, intercellular CO_2, Cr concentration in the roots and Cr concentration in the shoots.

compared to the single application. This is an initial investigation, and more research using different species in this field will be needed to identify the ideal dosages of various phytohormones in single and combination forms.

CRediT authorship contribution statement

Conceptualization, Khairiah Mubarak Alwutayd; Data curation, Sameera A. Alghamdi; formal analysis, Nayab Naeem; funding acquisition, Nadiyah M. Alabdallah, Rahmah N. Al-Qthanin, Suliman Mohammed Suliman Alghanem Rahaf Alwutayd; investigation, Wajiha Sarfraz; methodology, Sadia Javed, Rahmah N. Al-Qthanin, Amany H. A. Abeed; project administration, Baber Ali; resources, Muhammad Hamzah Saleem; software, Baber Ali, Sadia Javed, Leobardo Manuel Gómez-Oliván, Muhammad Hamzah Saleem, Khairiah Mubarak Alwutayd, Rahaf Alwutayd, Wajiha Sarfraz; validation, Muhammad Hamzah Saleem, Baber Ali; visualization, Noreen Khalid, Nadiyah M. Alabdallah; writing—original draft, Sadia Javed, Muhammad Hamzah Saleem, Baber Ali, Khairiah Mubarak Alwutayd, Leobardo Manuel Gómez-Oliván, Rahaf Alwutayd, Wajiha Sarfraz, Amany H.A. Abeed, Nadiyah M. Alabdallah, Rahmah N. Al-Qthanin; writing—review and editing, Suliman Mohammed Suliman Alghanem, Nadiyah M. Alabdallah, Rahmah N. Al-Qthanin Muhammad Hamzah Saleem, Sameera A. Alghamdi, Suliman Mohammed Suliman Alghanem, Khairiah Mubarak Alwutayd, Baber Ali, Amany H.A. Abeed, Rahaf Alwutayd. All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2024R402), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia. The authors are grateful to the deanship of scientific research at King Khalid University for supporting this work under the grant number (R.G.P2/326/44).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.168208.

References

AbdElgawad, H., Mohammed, A.E., van Dijk, J.R., Beemster, G.T., Alotaibi, M.O., Saleh, A.M., 2023. The impact of chromium toxicity on the yield and quality of rice grains produced under ambient and elevated levels of CO2. Front. Plant Sci. 14, 1019859.

Abeed, A.H., Salama, F.M., 2022. Attenuating effect of an extract of Cd-Hyperaccumulator Solanum nigrum on the growth and physio-chemical changes of Datura innoxia under Cd stress. J. Soil Sci. Plant Nutr. 22, 4868–4882.

Abeed, A.H., Mahdy, R.E., Alshehri, D., Hammami, I., Eissa, M.A., Abdel Latef, A.A.H., Mahmoud, G.A.-E., 2022a. Induction of resilience strategies against biochemical deteriorations prompted by severe cadmium stress in sunflower plant when

Trichoderma and bacterial inoculation were used as biofertilizers. Front. Plant Sci. 13, 1004173.
Abeed, A.H., Tammam, S.A., El-Mahdy, M., 2022b. Hydrogen peroxide pretreatment assisted phytoremediation of sodium dodecyl sulfate by Juncus acutus L. BMC Plant Biol. 22 (591), 1–16.
Aderholt, M., Vogelien, D.L., Koether, M., Greipsson, S., 2017. Phytoextraction of contaminated urban soils by Panicum virgatum L. enhanced with application of a plant growth regulator (BAP) and citric acid. Chemosphere 175, 85–96.
Aebi, H., 1984. [13] Catalase In Vitro. Methods in Enzymology. Elsevier, pp. 121–126.
Alam, M., Rohani, M.F., Hossain, M.S., 2023. Heavy metals accumulation in some important fish species cultured in commercial fish farm of Natore, Bangladesh and possible health risk evaluation. Emerg. Contam. 100254.
Ali, S., Farooq, M.A., Yasmeen, T., Hussain, S., Arif, M.S., Abbas, F., Bharwana, S.A., Zhang, G., 2013. The influence of silicon on barley growth, photosynthesis and ultra-structure under chromium stress. Ecotoxicol. Environ. Saf. 89, 66–72.
Ali, Q., Zheng, H., Rao, M.J., Ali, M., Hussain, A., Saleem, M.H., Nehela, Y., Sohail, M.A., Ahmed, A.M., Kubar, K.A., Ali, S., Usman, K., Manghwar, H., Zhou, L., 2022. Advances, limitations, and prospects of biosensing technology for detecting phytopathogenic bacteria. Chemosphere 133773.
Alsafran, M., Usman, K., Ahmed, B., Rizwan, M., Saleem, M.H., Al Jabri, H., 2022. Understanding the phytoremediation mechanisms of potentially toxic elements: a proteomic overview of recent advances. Front. Plant Sci. 13.
Arnon, D.I., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24, 1.
Ashraf, M.A., Rasheed, R., Hussain, I., Iqbal, M., Farooq, M.U., Saleem, M.H., Ali, S., 2022. Taurine modulates dynamics of oxidative defense, secondary metabolism, and nutrient relation to mitigate boron and chromium toxicity in Triticum aestivum L. plants. Environ. Sci. Pollut. Res. 29 (30), 45527–45548.
Austin, R.B., 1990. Prospects for Genetically Increasing the Photosynthetic Capacity of Crops.
Azooz, M.M., Abou-Elhamd, M.F., Al-Fredan, M.A., 2012. Biphasic effect of copper on growth, proline, lipid peroxidation and antioxidant enzyme activities of wheat ('Triticum aestivum'cv. Hasaawi) at early growing stage. Aust. J. Crop. Sci. 6, 688.
Basharat, S., Ahmad, F., Hameed, M., Naeem, M.S., 2023. Defense through foliar anatomical alterations in large spike buffel grass [Cenchrus prieurii (kunth) maire] against multiple environmental stresses. Pak. J. Bot. 55, 2067–2079.
Basit, F., Bhat, J.A., Ulhassan, Z., Noman, M., Zhao, B., Zhou, W., Kaushik, P., Ahmad, A., Ahmad, P., Guan, Y., 2022. Seed priming with spermine mitigates chromium stress in rice by modifying the ion homeostasis, cellular ultrastructure and phytohormones balance. Antioxidants 11, 1704.
Basit, F., Tao, J., An, J., Song, X., Sheteiwy, M.S., Holford, P., Hu, J., Jośko, I., Guan, Y., 2023. Nitric oxide and brassinosteroids enhance chromium stress tolerance in Glycine max L.(Merr.) by modulating antioxidative defense and glyoxalase systems. Environ. Sci. Pollut. Res. 30, 51638–51653.
Bates, L.S., Waldren, R.A., Teare, I., 1973. Rapid determination of free proline for water-stress studies. Plant Soil 39, 205–207.
Bisht, N., Mishra, S.K., Chauhan, P.S., 2020. Bacillus amyloliquefaciens inoculation alters physiology of rice (Oryza sativa L. var. IR-36) through modulating carbohydrate metabolism to mitigate stress induced by nutrient starvation. Int. J. Biol. Macromol. 143, 937–951.
Bray, H., Thorpe, W., 1954. Analysis of phenolic compounds of interest in metabolism. Methods Biochem. Anal. 27–52.
Caldelas, C., Bort, J., Febrero, A., 2012. Ultrastructure and subcellular distribution of Cr in Iris pseudacorus L. using TEM and X-ray microanalysis. Cell Biol. Toxicol. 28, 57–68.
Chen, C.-N., Pan, S.-M., 1996. Assay of superoxide dismutase activity by combining electrophoresis and densitometry. Bot. Bull. Acad. Sin. 37.
Chen, L., Long, C., Wang, D., Yang, J., 2020. Phytoremediation of cadmium (Cd) and uranium (U) contaminated soils by Brassica juncea L. enhanced with exogenous application of plant growth regulators. Chemosphere 242, 125112.
Chen, P., Yang, R., Bartels, D., Dong, T., Duan, H., 2022. Roles of abscisic acid and gibberellins in stem/root tuber development. Int. J. Mol. Sci. 23, 4955.
Choudhary, S.P., Kanwar, M., Bhardwaj, R., Gupta, B., Gupta, R., 2011. Epibrassinolide ameliorates Cr (VI) stress via influencing the levels of indole-3-acetic acid, abscisic acid, polyamines and antioxidant system of radish seedlings. Chemosphere 84, 592–600.
Danish, S., Kiran, S., Fahad, S., Ahmad, N., Ali, M.A., Tahir, F.A., Rasheed, M.K., Shahzad, K., Li, X., Wang, D., 2019. Alleviation of chromium toxicity in maize by Fe fortification and chromium tolerant ACC deaminase producing plant growth promoting rhizobacteria. Ecotoxicol. Environ. Saf. 185, 109706.
Dionisio-Sese, M.L., Tobita, S., 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Sci. 135, 1–9.
Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.T., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.
Ejaz, M., Raja, N.I., Khan, S.A., Mashwani, Z.-U.-R., Hanif, A., Iqbal, M., Hussain, M., Syed, A., Iqbal, R.K., Qureshi, H., 2023. Biosynthesized silver nanoparticles ameliorate biotic stress in rice (Oryza sativa) by intricating biochemical and mineral profile. Pak. J. Bot. 55, 2019–2028.
El-Esawi, M.A., Elkelish, A., Soliman, M., Elansary, H.O., Zaid, A., Wani, S.H., 2020. Serratia marcescens BM1 enhances cadmium stress tolerance and phytoremediation potential of soybean through modulation of osmolytes, leaf gas exchange, antioxidant machinery, and stress-responsive genes expression. Antioxidants 9, 43.
Emamverdian, A., Ding, Y., Alyemeni, M.N., Barker, J., Liu, G., Li, Y., Mokhberdoran, F., Ahmad, P., 2022. Benzylaminopurine and abscisic acid mitigates cadmium and copper toxicity by boosting plant growth, antioxidant capacity, reducing metal accumulation and translocation in bamboo [Pleioblastus pygmaeus (Miq.)] plants. Antioxidants 11, 2328.
Fahad, S., Rehman, A., Shahzad, B., Tanveer, M., Saud, S., Kamran, M., Ihtisham, M., Khan, S.U., Turan, V., ur Rahman, M.H., 2019. Rice Responses and Tolerance to Metal/Metalloid Toxicity. Advances in Rice Research for Abiotic Stress Tolerance. Elsevier, pp. 299–312.
FAOSTAT, 2018. Rice production data for 2018. FAOSTAT database. https://www.fao.org/faostat/en/#home.
Farid, M., Ali, S., Saeed, R., Rizwan, M., Bukhari, S.A.H., Abbasi, G.H., Hussain, A., Ali, B., Zamir, M.S.I., Ahmad, I., 2019. Combined application of citric acid and 5-aminolevulinic acid improved biomass, photosynthesis and gas exchange attributes of sunflower (Helianthus annuus L.) grown on chromium contaminated soil. Int. J. Phytoremediation 1–8.
Gao, W., Huang, Y., Zeng, X.-A., Brennan, M.A., 2019. Effect of soluble soybean polysaccharides on freeze-denaturation and structure of myofibrillar protein of bighead carp surimi with liquid nitrogen freezing. Int. J. Biol. Macromol. 135, 839–844.
Gautam, V., Kohli, S.K., Kapoor, D., Bakshi, P., Sharma, P., Arora, S., Bhardwaj, R., Ahmad, P., 2020. Stress protective effect of Rhododendron arboreum leaves (MEL) on chromium-treated Vigna radiata plants. J. Plant Growth Regul. 1–13.
Gill, S.S., Anjum, N.A., Gill, R., Yadav, S., Hasanuzzaman, M., Fujita, M., Mishra, P., Sabat, S.C., Tuteja, N., 2015. Superoxide dismutase–mentor of abiotic stress tolerance in crop plants. Environ. Sci. Pollut. Res. Int. 22, 10375–10394.
Greger, M., Landberg, T., 2008. Role of rhizosphere mechanisms in Cd uptake by various wheat cultivars. Plant Soil 312, 195–205.
Hashem, I.A., Abbas, A.Y., Abd El, A.E.-N.H., Salem, H.M., El-Hosseiny, O.E., Abdel-Salam, M.A., Saleem, M.H., Zhou, W., Hu, R., 2020. Potential of rice straw biochar, sulfur and ryegrass (Lolium perenne L.) in remediating soil contaminated with nickel through irrigation with untreated wastewater. PeerJ 8, e9267.
Heath, R.L., Packer, L., 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125, 189–198.
Hussain, I., Saleem, M.H., Mumtaz, S., Rasheed, R., Ashraf, M.A., Maqsood, F., Rehman, M., Yasmin, H., Ahmed, S., Ishtiaq, M., 2021. Choline chloride mediates chromium tolerance in spinach (Spinacia oleracea L.) by restricting its uptake in relation to morpho-physio-biochemical attributes. J. Plant Growth Regul. 1–21.
Jana, S., Choudhuri, M.A., 1981. Glycolate metabolism of three submersed aquatic angiosperms: effect of heavy metals. Aquat. Bot. 11, 67–77.
Javed, M.T., Stoltz, E., Lindberg, S., Greger, M., 2013. Changes in pH and organic acids in mucilage of Eriophorum angustifolium roots after exposure to elevated concentrations of toxic elements. Environ. Sci. Pollut. Res. 20, 1876–1880.
Javed, M.T., Tanwir, K., Abbas, S., Saleem, M.H., Iqbal, R., Chaudhary, H.J., 2021. Chromium retention potential of two contrasting Solanum lycopersicum Mill. cultivars as deciphered by altered pH dynamics, growth, and organic acid exudation under Cr stress. Environ. Sci. Pollut. Res. 1–13.
Jobby, R., Jha, P., Yadav, A.K., Desai, N., 2018. Biosorption and biotransformation of hexavalent chromium [Cr (VI)]: a comprehensive review. Chemosphere 207, 255–266.
Kamran, M., Danish, M., Saleem, M.H., Malik, Z., Parveen, A., Abbasi, G.H., Jamil, M., Ali, S., Afzal, S., Riaz, M., 2020. Application of abscisic acid and 6-benzylamino-purine modulated morpho-physiological and antioxidative defense responses of tomato (Solanum lycopersicum L.) by minimizing cobalt uptake. Chemosphere 128169.
Kamran, M., Danish, M., Saleem, M.H., Malik, Z., Parveen, A., Abbasi, G.H., Jamil, M., Ali, S., Afzal, S., Riaz, M., 2021. Application of abscisic acid and 6-benzylamino-purine modulated morpho-physiological and antioxidative defense responses of tomato (Solanum lycopersicum L.) by minimizing cobalt uptake. Chemosphere 263, 128169.
Kareem, H.A., Hassan, M.U., Zain, M., Irshad, A., Shakoor, N., Saleem, S., Niu, J., Skalicky, M., Chen, Z., Guo, Z., Wang, Q., 2022. Nanosized zinc oxide (n-ZnO) particles pretreatment to alfalfa seedlings alleviate heat-induced morpho-physiological and ultrastructural damages. Environ. Pollut. 303, 119069.
Khan, M.A., Kumar, S., Wang, Q., Wang, M., Fahad, S., Nizamani, M.M., Chang, K., Khan, S., Huang, Q., Zhu, G., 2023. Influence of polyvinyl chloride microplastic on chromium uptake and toxicity in sweet potato. Ecotoxicol. Environ. Saf. 251, 114526.
Krivokapić, M., 2021. Study on the evaluation of (heavy) metals in water and sediment of Skadar Lake (Montenegro), with BCF assessment and translocation ability (TA) by Trapa natans and a review of SDGs. Water 13, 876.
Lewis, C.E., Walker, J.R., Lancaster, J.E., Sutton, K.H., 1998. Determination of anthocyanins, flavonoids and phenolic acids in potatoes. I: coloured cultivars of Solanum tuberosum L. J. Sci. Food Agric. 77, 45–57.
Li, F.-T., Qi, J.-M., Zhang, G.-Y., Lin, L.-H., Fang, P.-P., Tao, A.-F., Xu, J.-T., 2013. Effect of cadmium stress on the growth, antioxidative enzymes and lipid peroxidation in two kenaf (Hibiscus cannabinus L.) plant seedlings. J. Integr. Agric. 12, 610–620.
Li, L., Zhang, K., Gill, R.A., Islam, F., Farooq, M.A., Wang, J., Zhou, W., 2018. Ecotoxicological and interactive effects of copper and chromium on physiochemical, ultrastructural, and molecular profiling in Brassica napus L. Biomed. Res. Int. 2018.
Li, X., Kamran, M., Saleem, M.H., Al-Ghamdi, A.A., Al-Hemaid, F.M., Elshikh, M.S., Zhao, S., Riaz, M., 2023. Potential application of melatonin in reducing boron toxicity in rice seedlings through improved growth, cell wall composition, proline, and defense mechanisms. Chemosphere 139068.
Ma, J., Saleem, M.H., Yasin, G., Mumtaz, S., Qureshi, F.F., Ali, B., Ercisli, S., Alhag, S.K., Ahmed, A.E., Vodnar, D.C., Hussain, I., Marc, R.A., Chen, F., 2022. Individual and combinatorial effects of SNP and NaHS on morpho-physio-biochemical attributes and phytoextraction of chromium through Cr-stressed spinach (Spinacia oleracea L.). Front. Plant Sci. 13.

Mao, Y., Tan, H., Wang, M., Jiang, T., Wei, H., Xu, W., Jiang, Q., Bao, H., Ding, Y., Wang, F., 2022. Research progress of soil microorganisms in response to heavy metals in rice. J. Agric. Food Chem. 70, 8513–8522.
Maqbool, A., Ali, S., Rizwan, M., Ishaque, W., Rasool, N., ur Rehman, M.Z., Bashir, A., Abid, M., Wu, L., 2018. Management of tannery wastewater for improving growth attributes and reducing chromium uptake in spinach through citric acid application. Environ. Sci. Pollut. Res. 25, 10848–10856.
Matysiak, K., Kierzek, R., Siatkowski, I., Kowalska, J., Krawczyk, R., Miziniak, W., 2020. Effect of exogenous application of amino acids l-arginine and glycine on maize under temperature stress. Agronomy 10, 769.
Mfarrej, M.F.B., Wang, X., Fahid, M., Saleem, M.H., Alatawi, A., Ali, S., Shabir, G., Zafar, R., Afzal, M., Fahad, S., 2023. Floating treatment wetlands (FTWs) is an innovative approach for the remediation of petroleum hydrocarbons-contaminated water. J. Plant Growth Regul. 42 (3), 1402–1420.
Najmanova, J., Neumannova, E., Leonhardt, T., Zitka, O., Kizek, R., Macek, T., Mackova, M., Kotrba, P., 2012. Cadmium-induced production of phytochelatins and speciation of intracellular cadmium in organs of Linum usitatissimum seedlings. Ind. Crop. Prod. 36, 536–542.
Nakano, Y., Asada, K., 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22, 867–880.
Naz, R., Sarfraz, A., Anwar, Z., Yasmin, H., Nosheen, A., Keyani, R., Roberts, T.H., 2021. Combined ability of salicylic acid and spermidine to mitigate the individual and interactive effects of drought and chromium stress in maize (Zea mays L.). Plant Physiol. Biochem. 159, 285–300.
Pan, J., Guan, M., Xu, P., Chen, M., Cao, Z., 2021. Salicylic acid reduces cadmium (Cd) accumulation in rice (Oryza sativa L.) by regulating root cell wall composition via nitric oxide signaling. Sci. Total Environ. 797, 149202.
Panhwar, Q., Naher, U., Radziah, O., Shamshuddin, J., Razi, I.M., Dipti, S., Aghamolki, M.K., 2015. Quality and antioxidant activity of rice grown on alluvial soil amended with Zn, Cu and Mo. S. Afr. J. Bot. 98, 77–83.
Pękal, A., Pyrzynska, K., 2014. Evaluation of aluminium complexation reaction for flavonoid content assay. Food Anal. Methods 7, 1776–1782.
Pérez-León, M.I., González-Fuentes, J.A., Valdez-Aguilar, L.A., Benavides-Mendoza, A., Alvarado-Camarillo, D., Castillo-Chacón, C.E., 2023. Effect of glutamic acid and 6-benzylaminopurine on flower bud biostimulation, fruit quality and antioxidant activity in blueberry. Plants 12, 2363.
Rana, M.S., Hu, C.X., Shaaban, M., Imran, M., Afzal, J., Moussa, M.G., Elyamine, A.M., Bhantana, P., Saleem, M.H., Syaifudin, M., 2020. Soil phosphorus transformation characteristics in response to molybdenum supply in leguminous crops. J. Environ. Manag. 268, 110610.
Rehman, M.Z.-U., Rizwan, M., Ghafoor, A., Naeem, A., Ali, S., Sabir, M., Qayyum, M.F., 2015. Effect of inorganic amendments for in situ stabilization of cadmium in contaminated soils and its phyto-availability to wheat and rice under rotation. Environ. Sci. Pollut. Res. 22, 16897–16906.
Sakharov, I.Y., Ardila, G.B., 1999. Variations of peroxidase activity in cocoa (Theobroma cacao L.) beans during their ripening, fermentation and drying. Food Chem. 65, 51–54.
Salama, F.M., Al-Huqail, A.A., Ali, M., Abeed, A.H., 2022. Cd Phytoextraction potential in halophyte Salicornia fruticosa: salinity impact. Plants 11, 2556.
Sallah-Ud-Din, R., Farid, M., Saeed, R., Ali, S., Rizwan, M., Tauqeer, H.M., Bukhari, S.A. H., 2017. Citric acid enhanced the antioxidant defense system and chromium uptake by Lemna minor L. grown in hydroponics under Cr stress. Environ. Sci. Pollut. Res. 24, 17669–17678.
Sarfraz, W., Farid, M., Khalid, N., Rizvi, Z.F., Asam, Z.U.Z., Nazir, A., Naeem, N., Farid, S., Ali, S., 2022. Ameliorative role of foliar Zn-lysine application on wheat (Triticum aestivum L.) stressed by Tannery Wastewater. Physiol. Mol. Biol. Plants 28, 2099–2110.
Sarker, A., Al Masud, M.A., Deepo, D.M., Das, K., Nandi, R., Ansary, M.W.R., Islam, A.R. M.T., Islam, T., 2023. Biological and green remediation of heavy metal contaminated water and soils: a state-of-the-art review. Chemosphere 138861.
Shahid, M., Shamshad, S., Rafiq, M., Khalid, S., Bibi, I., Niazi, N.K., Dumat, C., Rashid, M. I., 2017. Chromium speciation, bioavailability, uptake, toxicity and detoxification in soil-plant system: a review. Chemosphere 178, 513–533.
Singh, M.K., Chand, T., Kumar, M., Singh, K., Lodhi, S., Singh, V., Sirohi, V.S., 2015. Response of different doses of NPK and boron on growth and yield of broccoli (Brassicaoleracea L. var. italica). Int. J. Bio-Resour. Stress Manag. 6, 108–112.
Singh, D., Sharma, N.L., Singh, D., Siddiqui, M.H., Taunk, J., Sarkar, S.K., Rathore, A., Singh, C.K., Al-Amri, A.A., Alansi, S., 2023a. Exogenous hydrogen sulfide alleviates chromium toxicity by modulating chromium, nutrients and reactive oxygen species accumulation, and antioxidant defence system in mungbean (Vigna radiata L.) seedlings. Plant Physiol. Biochem. 200, 107767.
Singh, V., Singh, N., Rai, S.N., Kumar, A., Singh, A.K., Singh, M.P., Sahoo, A., Shekhar, S., Vamanu, E., Mishra, V., 2023b. Heavy metal contamination in the aquatic ecosystem: toxicity and its remediation using eco-friendly approaches. Toxics 11, 147.
Sirhindi, G., Mir, M.A., Abd-Allah, E.F., Ahmad, P., Gucel, S., 2016. Jasmonic acid modulates the physio-biochemical attributes, antioxidant enzyme activity, and gene expression in Glycine max under nickel toxicity. Front. Plant Sci. 7, 591.
Sun, L., Song, F., Zhu, X., Liu, S., Liu, F., Wang, Y., Li, X., 2021. Nano-ZnO alleviates drought stress via modulating the plant water use and carbohydrate metabolism in maize. Arch. Agron. Soil Sci. 67, 245–259.
Tang, X., Huang, Y., Li, Y., Wang, L., Pei, X., Zhou, D., He, P., Hughes, S.S., 2021. Study on detoxification and removal mechanisms of hexavalent chromium by microorganisms. Ecotoxicol. Environ. Saf. 208, 111699.
Tripathi, D.K., Singh, V.P., Kumar, D., Chauhan, D.K., 2012. Impact of exogenous silicon addition on chromium uptake, growth, mineral elements, oxidative stress, antioxidant capacity, and leaf and root structures in rice seedlings exposed to hexavalent chromium. Acta Physiol. Plant. 34, 279–289.
UdDin, I., Bano, A., Masood, S., 2015. Chromium toxicity tolerance of Solanum nigrum L. and Parthenium hysterophorus L. plants with reference to ion pattern, antioxidation activity and root exudation. Ecotoxicol. Environ. Saf. 113, 271–278.
Ulhassan, Z., Bhat, J.A., Zhou, W., Senan, A.M., Alam, P., Ahmad, P., 2022. Attenuation mechanisms of arsenic induced toxicity and its accumulation in plants by engineered nanoparticles: a review. Environ. Pollut. 302, 119038.
Vishnupradeep, R., Bruno, L.B., Taj, Z., Karthik, C., Challabathula, D., Kumar, A., Freitas, H., Rajkumar, M., 2022. Plant growth promoting bacteria improve growth and phytostabilization potential of Zea mays under chromium and drought stress by altering photosynthetic and antioxidant responses. Environ. Technol. Innov. 25, 102154.
Wang, H., Wang, T., Ahmad, I., 2015. Involvement of phosphate supplies in different transcriptional regulation pathway of *Oryza sativa* L.'s antioxidative system in response to arsenite and cadmium stress. Ecotoxicology 24, 1259–1268.
Yang, Q.-W., Xu, Y., Liu, S.-J., He, J.-F., Long, F.-Y., 2011. Concentration and potential health risk of heavy metals in market vegetables in Chongqing, China. Ecotoxicol. Environ. Saf. 74, 1664–1669.
Yang, C., Qiu, W., Chen, Z., Chen, W., Li, Y., Zhu, J., Rahman, S.U., Han, Z., Jiang, Y., Yang, G., 2020. Phosphorus influence Cd phytoextraction in Populus stems via modulating xylem development, cell wall Cd storage and antioxidant defense. Chemosphere 242, 125154.
Zaheer, I.E., Ali, S., Saleem, M.H., Noor, I., El-Esawi, M.A., Hayat, K., Rizwan, M., Abbas, Z., El-Sheikh, M.A., Alyemeni, M.N., 2020. Iron–lysine mediated alleviation of chromium toxicity in spinach (*Spinacia oleracea* L.) plants in relation to morpho-physiological traits and iron uptake when irrigated with tannery wastewater. Sustainability 12, 6690.
Zaheer, I.E., Ali, S., Saleem, M.H., Yousaf, H.S., Malik, A., Abbas, Z., Rizwan, M., Abualreesh, M.H., Alatawi, A., Wang, X., 2022. Combined application of zinc and iron-lysine and its effects on morpho-physiological traits, antioxidant capacity and chromium uptake in rapeseed (Brassica napus L.). PLoS One 17, e0262140.
Zeng, F.-R., Zhao, F.-S., Qiu, B.-Y., Ouyang, Y.-N., Wu, F.-B., Zhang, G.-P., 2011. Alleviation of chromium toxicity by silicon addition in rice plants. Agric. Sci. China 10, 1188–1196.
Zhu, A., Li, J., Fu, W., Wang, W., Tao, L., Fu, G., Chen, T., Feng, B., 2022. Abscisic acid improves rice thermo-tolerance by affecting trehalose metabolism. Int. J. Mol. Sci. 23, 10615.