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Insights into trehalose mediated physiological and biochemical mechanisms in *Zea mays* L. under chromium stress

Sadia Zafar[1*†], Inam Mehdi Khan[1†], Muhammad Arslan Ashraf[2], Muhammad Zafar[3], Mushtaq Ahmad[3], Rizwan Rasheed[2], Ansar Mehmood[4] and Khawaja Shafique Ahmad[4*]

Abstract

Background Chromium (Cr) toxicity significantly threatens agricultural ecosystems worldwide, adversely affecting plant growth and development and reducing crop productivity. Trehalose, a non-reducing sugar has been identified as a mitigator of toxic effects induced by abiotic stressors such as drought, salinity, and heavy metals. The primary objective of this study was to investigate the influence of exogenously applied trehalose on maize plants exposed to Cr stress.

Results Two maize varieties, FH-1046 and FH-1453, were subjected to two different Cr concentrations (0.3 mM, and 0.5 mM). The results revealed significant variations in growth and biochemical parameters for both maize varieties under Cr-induced stress conditions as compared to the control group. Foliar application of trehalose at a concentration of 30 mM was administered to both maize varieties, leading to a noteworthy reduction in the detrimental effects of Cr stress. Notably, the Cr (0.5 mM) stress more adversely affected the shoot length more than 0.3mM of Cr stress. Cr stress (0.5 mM) significantly reduced the shoot length by 12.4% in FH-1046 and 24.5% in FH-1453 while Trehalose increased shoot length by 30.19% and 4.75% in FH-1046 and FH-1453 respectively. Cr stress significantly constrained growth and biochemical processes, whereas trehalose notably improved plant growth by reducing Cr uptake and minimizing oxidative stress caused by Cr. This reduction in oxidative stress was evidenced by decreased production of proline, SOD, POD, MDA, H_2O_2, catalase, and APX. Trehalose also enhanced photosynthetic activities under Cr stress, as indicated by increased values of chlorophyll *a*, *b*, and carotenoids. Furthermore, the ameliorative potential of trehalose was demonstrated by increased contents of proteins and carbohydrates and a decrease in Cr uptake.

Conclusions The study demonstrates that trehalose application substantially improved growth and enhanced photosynthetic activities in both maize varieties. Trehalose (30 mM) significantly increased the plant biomass, reduced ROS production and enhanced resilience to Cr stress even at 0.5 mM.

†Sadia Zafar and Inam Mehdi Khan contributed equally to this work.

*Correspondence:
Sadia Zafar
sadia.zafar@ue.edu.pk
Khawaja Shafique Ahmad
ahmadks@upr.edu.pk

Full list of author information is available at the end of the article

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Introduction

The expanding global population has increased the need for food, alongside the rapid pace of industrialization and urban expansion [1, 2]. This surge in food demand has created immense pressure on agricultural systems worldwide [3]. Moreover, the rapid growth of modern industries has led to the influx of various heavy metals (HMs) into water and soil, posing lethal threats to living organisms [4]. Cr ranked 2nd amongst HMs pollution due to its extensive and widespread utilization in industries [5]. Therefore, Cr has become one of the researcher's top environmental concerns. Cr (III) and Cr (VI) are stable forms of Cr [6, 7]. Cr (VI) is more toxic than Cr (III) because toxicity mainly depends on the Cr oxidation state. Cr (VI) is more stable than Cr (III); thus, Cr (VI) has a deleterious effect on living organisms [8–10]. In soil, Cr (III), under oxidized conditions, is converted into Cr (VI), which is a much more toxic form of Cr and shows a negative effect on plants [11, 12]. Although Cr has a low bioavailability in natural settings it is absorbed and accumulated by plants from soil and water and then transferred to aboveground plant tissues. Cr entered into the human food chain by eating Cr-accumulated food and causing mild (skin irritation) to severe diseases like cancer [13].

Cr stress can significantly impact plant growth and development, particularly in plants like maize. Cr, especially in its hexavalent form (Cr(VI)), is highly toxic to plants and can induce various physiological and biochemical changes leading to oxidative stress, damage to cellular structures, inhibition of photosynthesis, and ultimately, impaired growth and yield. Cr stress can also disrupt nutrient uptake and metabolism, further exacerbating the detrimental effects on plant health [14]. Plants exposure to Cr stress can lead to symptoms such as chlorosis, stunted growth, reduced root development, and overall poor plant vigor [15, 16]. These effects can have significant consequences for agricultural productivity, as *Zea mays* L. (maize) is an essential crop worldwide [8, 9].

Trehalose, a non-reducing disaccharide, plays a crucial role in mitigating the adverse effects of Cr stress in maize plants [10]. Trehalose acts as a compatible solute, accumulating in plant cells in response to various environmental stresses, including HMs toxicity. Its protective mechanisms include acting as a stabilizer of biomolecules, maintaining cellular structure and integrity, scavenging reactive oxygen species (ROS), and regulating stress-responsive gene expression [10, 17].

This overproduction of ROS produces oxidative injury in plants. Oxidative stress of ROS causes structural and functional abnormalities [16, 17]. Cr stress disturbs the ion's homeostasis and interferes with enzymatic activities like cytochrome oxidase, nitrate reductase, catalase, and peroxidases [7]. Malondialdehyde (MDA) overproduction is the primary marker of stress and the degree of membrane peroxidation is directly correlated with MDA activity [15]. The oxidative damage caused by the accumulation of MDA and other ROS and the cellular redox homeostasis caused by Cr toxicity in maize plants has previously been documented [14, 17]. Plants utilize the ROS scavenging system as a defense mechanism in response to stress. This system involves the activation of various antioxidants, both enzymatic (like catalase, guaiacol peroxidase, ascorbate peroxidase, and superoxide dismutase) as well as non-enzymatic (Glutathione reductase and glutathione) to scavenge ROS and free radicals [18, 19].

Maize is considered the world's leading cereal crop due to its utilization as food, oral vaccines, and biofuel [20, 21]. Numerous studies documented Cr-induced phytotoxic in maize reflected by retarded plant growth, reduced photosynthetic activities, and abnormal physiological processes leading to poor grain quality and a drastic reduction in yield [22–24]. Moreover, exposure to Cr stress may result in an imbalance of nutrients in plants, as well as cellular membrane damage from oxidative stress and a hindrance to root growth and biomass production. Cr stress in maize plants typically manifests by oxidative stress caused by the overproduction of ROS and osmolyte accumulation which disrupts the photosynthetic efficiency and results in a shortage of plant length as well imbalance of ions homeostasis [25]. While it has been previously reported that several osmoprotectants play a mitigating role against a range of metal stresses in various plants, this is an environmentally friendly and economical approach [26, 27]. Trehalose is used to lessen the negative effects of Cr, which presently lowers maize productivity, to make up for this loss. Moreover, to assess how trehalose affects the morphology and physiology of early-growing maize under Cr stress. Trehalose's extraordinary efficacy in protecting plants from the detrimental impacts of various stresses brought international attention [28, 29]. Plants are protected from the damaging effects of heavy metal stress by foliar application of osmoprotectants [30]. Trehalose is a significant energy source stabilizing dehydrated proteins which maintain membranes. It protects the cell's structure and functions from oxidative damage due to its unique physio-chemical properties (glycosidic bond and increased hydrophilicity) [31]. Trehalose is a potent inducer of genes related to stress reactions and detoxification of reactive oxygen species [32]. However, most plants' natural ability to produce

trehalose is insufficient to lessen the detrimental impacts of various stresses. Thus, foliar applied trehalose raised endogenous trehalose concentrations and had been suggested as a crucial alternative to promote stress tolerance [33]. Exogenous trehalose modifies the enzymatic activities, thus decreasing the ROS level [10]. Exogenously applied trehalose enhanced biomass production in salinized environments by decreasing the accumulation of H_2O_2 and MDA [34], which also affected the activities of antioxidant enzymes and the Calvin cycle, which support the healthy growth of plants under Cd (35–36). Furthermore, Cr-Trehalose chelation could be beneficial similarly to Cd-Tre chelation, significantly minimizing Cd's toxicity and mobility to plant organs and enhancing growth under Cd stress [33]. Although trehalose's ability to mitigate a range of abiotic stresses in different plants has previously been documented as an economical and environmentally beneficial strategy, many studies have not been conducted on the exogenous application of trehalose against drought, salinity, and Cd stress. However, scarce data regarding the trehalose potential under Cr stress in maize is available. Thus, the aim of the current study is to demonstrate how exogenous trehalose (30 mM) reduces Cr stress in maize varieties in terms of antioxidants, photosynthesis, biomass, oxidative stress, and plant uptake of Cr.

Methods

The experiment was conducted under natural environmental conditions at research area of Botanical Garden, Department of Botany, University of Education Lahore Faisalabad Campus, Pakistan. Seeds of two maize cultivars: FH-1046 and FH-1453 were obtained from Ayub Agricultural Research Institute, Faisalabad, Pakistan. Seeds were surface sterilized with ethanol (30%) for 30 min and then seeds were thoroughly washed with distilled water for three times. Ten sterilized seeds were sown per pot (containing 1.8 kg soil) on 28th February 2022. The study was carried out by following Completely Randomized Design (CRD) in thrice replications for each treatment. One week after germination, plants were thinned to keep six plants per pot and full strength Hoagland's nutrient solution (68 ml of nutrients/L) was added in each pot along with irrigation. By following Mohammed et al. [34], Cr stress (0.3 mM and 0.5 mM) was applied after 2 weeks of seed germination at 2 leaf stage and after that foliar application of trehalose (30 mM) was applied after one week of stress application at 4 leaf stage [35, 36]. Harvesting of all the pots was done after 20 days of trehalose application. Growth related parameters were done of harvested plants and remaining plants were frozen with liquid nitrogen and stored at -20°C for further biochemical determination.

Estimation of morphological parameters

The length of the shoot and root were measured using a measuring tape. The fresh weight of both the root and shoot were recorded by using an electronic weighing balance (model DM-305 F). After that, samples were kept in dry oven at 90 °C for 24 h. Dry weight was recorded after complete dry of samples. The moisture contents of both the shoot and root tissues were determined by following formula [37]:

$$Moisture\ \ contents\ \ (\%) = \frac{Freshweight - Dryweight}{DryWeight} \times 100$$

Determination of photosynthetic pigments

The quantification of leaf chlorophyll content was conducted following the method established by Arnon [38]. 0.5 g of leaf material was extracted with 70% ethanol. After centrifugation, the supernatant was used for chlorophyll contents (chlorophyll *a*, chlorophyll *b*, total chlorophyll, chlorophyll *a*/*b* ratio) and carotenoids analysis by spectrophotometer at 480 nm, 645 nm, and 663 nm. Obtained values were calculated described by Lichtenthaler [39].

Determination of total soluble proteins

The quantification of total soluble proteins involved the extraction of proteins from 0.5 g of leaf tissue by potassium phosphate buffer (50 mM) at a pH of 7.5 [40].

Determination of carbohydrates

Fresh leaf samples (0.5 g) were extracted with ethanol (80%) by centrifugation at 3500 g for 10 min [41]. After centrifugation, 100 µL of supernatant was mixed with 3 ml of anthrone reagent and vortexed for 2 min. The solution was incubated at 95 °C for 10 min in a water bath and cooled at room temperature. Absorbance was recorded at 625 nm by using a spectrophotometer.

Electrolyte conductivity estimation

Plant leaves weighing 0.5 g were cut into small fragments, and subsequently placed into test tubes. Following this, 10 mL of distilled water was added to the test tubes, and the samples were left to stand overnight. The electrical conductivity (EC-1) was determined by using an EC meter. Subsequently, the test tubes were subjected to a water bath maintained at 120 °C for a duration of 20 min. Post-heating, the electrical conductivity (EC-2) was determined, and these values were also recorded in accordance with the methodology described by [42].

Then final value of EC was recorded using the formula:

$$Electrolyte\ Conductivity\ \ (EC) = \frac{EC-1}{EC-2} \times\ 100$$

Determination of proline contents

The fresh leaf was extracted with 10 mL of sulfosalicylic acid (3%) and filtered [43]. Then, 2 mL of the filtrate was mixed with acid ninhydrin (2 mL) and glacial acetic acid (2 mL). This mixture was heated at 95 °C for 60 min in a water bath. The mixture was cooled at 4 °C, and after cooling, 4 mL of toluene was added to the solution and mixed well. The absorbance was examined at 520 nm by using a spectrophotometer.

Determination of antioxidants

Fresh leaf samples (0.5 g) were freeze dry by using liquid nitrogen and homogenized with 10 mL of potassium phosphate buffer (50 mM, pH 7.5). A portion of the resulting supernatant, obtained through centrifugation of the homogenate, was employed to assess the activity of antioxidant enzymes, including Superoxide Dismutase (SOD), Catalase (CAT), Peroxidase (POD), and Ascorbate Peroxidase (APX). SOD activity was assessed following the approach outlined by [44]. The activity of POD and CAT was determined using the methodology described by [45]. MDA content was quantified according to the method described by [46]. Hydrogen peroxide (H_2O_2) content was examined using the method explained by [47]. The assay developed by Nakano and Asada [48] was utilized to quantify APX activity.

Analysis of Cr contents

To determine Cr concentration, plant materials were divided into roots and shoots before being dried for 72 h at 90 °C in an oven and ground into powder. The powder was dry-ashed, and HNO_3-$HClO_4$ (3:1, v/v) was used for digestion. Inductively coupled plasma atomic emission spectroscopy was used to measure the Cr contents of the shoots and roots [49].

Statistical analysis

All analyses were conducted in triplicate to ensure robustness and reliability. Subsequently, a three way analysis of variance (ANOVA) using Statistix 8.1 (Analytical Software, USA). Post hoc analysis employed the least significant difference test to compare the means among distinct treatments, with significance considered at the $P \leq 0.05$ level.

Results

Morphological traits

Finding revealed the overall growth performance of maize variety FH-1046 was better as compared to FH-1453 (Fig. 1A-H). The exposure of Cr (0.3 mM and 0.5 mM) significantly reduced the shoot length by 12.4% and 5.7% in FH-1046 and 2.29% and 24.5% in FH-1453, respectively, as compared to the control. The Cr concentration of 0.5mM had more adversely effects on the shoot length than 0.3mM of Cr stress. However, trehalose application had a highly significant impact for reducing the damaging effects caused by Cr (Fig. 1A) while a non-significant difference was observed amongst the trehalose application, Cr stress and variety (Table 1). The shoot length was increased by 19.2% in FH-1046 and 1.5% in FH-1453 by trehalose. Under Cr concentrations of 0.3 mM and 0.5 mM, trehalose increased shoot length by 12.7% and 30.1% in FH-1046 and 0.93% and 4.75% in FH-1453, respectively.

Root length was significantly decreased by Cr stress and more prominent decrease was observed by Cr (0.5mM) as compared to the 0.3mM Cr (Fig. 1B). A significant increase was observed by trehalose (Table 1). Cr (0.3 mM and 0.5 mM) significantly reduces the root length by 9.58% and 18.12% in FH-1046 and 19.6% and 19.7% in FH-1453 respectively. Whereas root length was increased by 4.55% in FH-1046 and 3.49% in FH-1453 by trehalose. Under Cr concentrations of 0.3 mM and 0.5 mM, trehalose increased root length by 5.88% and 7.4% in FH-1046 and 11.8% and 15.24% in FH-1453 respectively. Overall root length was significantly greater in maize variety FH-1046 as compared to FH-1453.

The Cr concentration of 0.5 mM adversely affected the shoot fresh weight as compared to the Cr concentration of 0.3 mM (Fig. 1C). A highly significant difference was observed between Cr stress and trehalose (Table 1). The shoot fresh weight was increased by 12.8% and 3.93% by trehalose, while decreased by 11.86% and 46.20% and 33.3% and 46.77% by Cr (0.3 mM and 0.5 mM) in FH1046 and FH1453 respectively. Under Cr concentrations of 0.3mM and 0.5mM, trehalose increased shoot fresh weight by 8.54% and 25.3% in FH-1046 and 10.6% and 2.98% in FH-1453 respectively (Fig. 1C).

A highly significant difference was observed between variety, Cr stress and trehalose treatment and between their interactions (Table 1). The root fresh weight was significantly decreased by 20.39% and 34.21% in FH1046 and 40.78% and 35.93% in FH1453 by Cr (0.3 mM and 0.5 mM) respectively. However, a significant increase in root fresh weight of 21.4% in FH1046 and 41.8% in FH1453 was observed by trehalose. Under Cr concentrations of 0.3 mM and 0.5 mM, trehalose increased root fresh weight by 8.8% and 23.1% in FH-1046 and 7.18% and 16.7% in FH-1453 respectively. Root fresh weight was significantly greater in maize variety FH-1046 as compared to FH-1453. In addition to that, the Cr concentration of 0.5mM adversely affected the root fresh weight compared to the Cr concentration of 0.3 mM (Fig. 1D).

Shoot dry weight was significantly decreased by Cr 0.5 mM as compared to control (Fig. 1E). A highly significant difference was observed between variety, Cr stress and trehalose treatment and between their interactions (Table 1). The shoot dry weight was decreased by

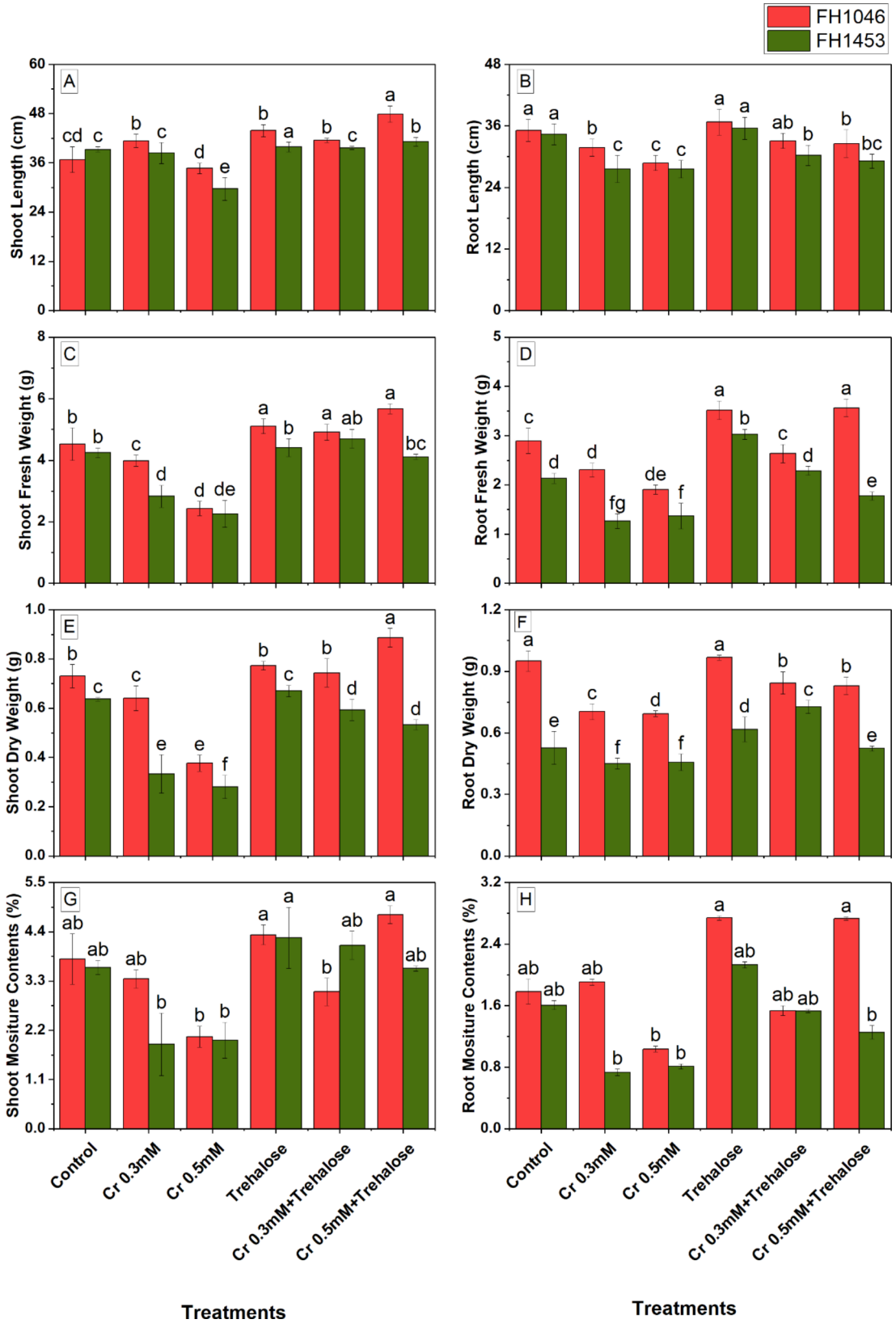

Fig. 1 Effect of maize foliar application of trehalose on length of shoot (**A**), length of root (**B**), Fresh weight of shoot (**C**), Fresh weight of root (**D**), Dry weight of shoot (**E**), Dry weight of root (**F**), moisture contents of shoot (**G**), and moisture contents of root (**H**) grown under induced Cr toxicity. Data is presented in means and standard errors were added while different letters for the different treatments represented a significant difference at $P < 0.05$

Table 1 Effect of exogenous trehalose on plant length, shoot and root length, plant fresh and dry weight, shoot and root fresh and dry weight and moisture contents of shoot and root in maize seedlings with or without cr stress

Source of variation	df	Shoot length	Root length	Shoot fresh weight	Root fresh weight	Shoot dry weight	Root dry weight	Moisture content shoot	Moisture content root
Varieties (Var)	1	0.0074 **	0.1841 ^{ns}	0.0441 *	0.0000 ***	0.0000 ***	0.0000 ***	0.0137 *	0.0000 ***
Cr Stress (Cr)	2	0.2915 ^{ns}	0.0455 *	0.0016 **	0.0016 **	0.0007 ***	0.0165 *	0.0018 **	0.0005 ***
Trehalose (T)	1	0.0000 ***	0.8915 ^{ns}	0.0000 ***	0.0004 ***	0.0000 ***	0.2259 ^{ns}	0.0000 ***	0.0020 **
Var × Cr	2	0.1351 ^{ns}	0.8657 ^{ns}	0.5672 ^{ns}	0.8097 ^{ns}	0.5810 ^{ns}	0.2286 ^{ns}	0.5198 ^{ns}	0.5182 ^{ns}
Var × T	1	0.2654 ^{ns}	0.4263 ^{ns}	0.3363 ^{ns}	0.9562 ^{ns}	0.6204 ^{ns}	0.1928 ^{ns}	0.6477 ^{ns}	0.3793 ^{ns}
Cr ×T	2	0.0003 ***	0.2752 ^{ns}	0.0076 **	0.1056 ^{ns}	0.0001 ***	0.3435 ^{ns}	0.0464 *	0.0052 **
Var × Cr × T	2	0.3295 ^{ns}	0.7743 ^{ns}	0.0055 **	0.0117 *	0.0060 **	0.2501 ^{ns}	0.0063 **	0.0243 *

ns: non significant; *,** and *** significant at 0.05, 0.01, 0.001 levels respectively

Table 2 Effect of exogenous trehalose on electrical conductivity (EC), chlorophyll a (Chl. A), chlorophyll b (Chl. B), chlorophyll a/b (Chl. a/b), total chlorophyll (total chl.), total soluble protein (TSP), carbohydrates and proline in maize seedlings with and without cr stress

Source of Variation	df	EC	Chl. a	Chl. b	Chl. ab	Total Chl.	TSP	Carbohydrates	Proline
Varieties (Var)	1	0.0000 ***	0.6978 ^{ns}	0.7099 ^{ns}	0.5128 ^{ns}	0.2750 ^{ns}	0.0153 *	0.0000 ***	0.5637 ^{ns}
Cr Stress (Cr)	2	0.0194 *	0.1298 ^{ns}	0.3196 ^{ns}	0.1043 ^{ns}	0.0002 ***	0.0000 ***	0.0000 ***	0.0000 ***
Trehalose (T)	1	0.0006 ***	0.8681 ^{ns}	0.5286 ^{ns}	0.7024 ^{ns}	0.0001 ***	0.0000 ***	0.0000 ***	0.0000 ***
Var × Cr	2	0.0149 *	0.1378 ^{ns}	0.3622 ^{ns}	0.0845 ^{ns}	0.0009 ***	0.2443 ^{ns}	0.0352 *	0.0586 ^{ns}
Var × T	1	0.1873 ^{ns}	0.7675 ^{ns}	0.4889 ^{ns}	0.5928 ^{ns}	0.5239 ^{ns}	0.5810 ^{ns}	0.0048 **	0.5637 ^{ns}
Cr ×T	2	0.7811 ^{ns}	0.4141 ^{ns}	0.8491 ^{ns}	0.2887 ^{ns}	0.0403 *	0.6416 ^{ns}	0.0045 **	0.2638 ^{ns}
Var × Cr × T	2	0.0639 ^{ns}	0.6334 ^{ns}	0.4614 ^{ns}	0.2324 ^{ns}	0.7702 ^{ns}	0.0381 *	0.5637 ^{ns}	0.6385 ^{ns}

ns: non significant; *,** and *** significant at 0.05, 0.01, 0.001 levels respectively

12.32% and 48.4% in FH1046 and 47.64% and 56.02% in FH1453 by Cr (0.3 mM and 0.5 mM) respectively, while an increased 5.93% in FH1046 and 5.23% in FH1453 was observed by trehalose. Under Cr concentrations of 0.3 mM and 0.5 mM, trehalose increased shoot dry weight by 1.8% and 21.4% in FH-1046 and 6.8% and 16.2% in FH-1453 respectively.

The Cr concentration of 0.5 mM adversely affected the root dry weight compared to the Cr concentration of 0.3 mM (Fig. 1F). Cr (0.3 mM and 0.5 mM) exposure decreased root dry weight by 25.96% and 27.01% in FH1046 and 14.55% and 13.29% in FH1453 respectively. A significant increase of 1.75% in FH1046 and 17.1% in FH1453 was observed by trehalose. Under Cr concentrations of 0.3 mM and 0.5 mM, trehalose increased root dry weight by 11.2% and 12.6% in FH-1046 and 37.9% and 0.6% in FH-1453 respectively. A highly significant difference was observed between variety, Cr stress and trehalose treatment (Table 1).

Shoot moisture content was significantly greater in maize variety FH-1046 as compared to FH-1453 (Table 1). Shoot moisture content was significantly decreased by Cr 0.5 mM as compared to control. The Cr concentration of 0.5 mM adversely affected the root dry weight compared to the Cr concentration of 0.3 mM. Moreover, Cr (0.3 mM and 0.5 mM) stress significantly reduces the shoot moisture contents by 11.77% and 45.78% in FH-1046 and 47.64% and 45.14% in FH-1453 respectively (Fig. 1G). A significant increase of 14.23% in FH-1046 and 18.4% in FH-1453 was observed by trehalose. Under Cr, with concentrations of 0.3mM and 0.5mM, trehalose increased shoot moisture content by 19.3% and 26.1% in FH-1046 and 13.7% and 0.65% in FH-1453 respectively (Fig. 1G).

The Cr exposure adversely affected the root dry weight, and a greater decrease was observed by 0.5 mM as compared to the Cr concentration of 0.3 mM. A significant increase was observed by trehalose (Table 1). The root moisture content was increased by 53.6% in FH-1046 and 32.5% in FH-1453 by trehalose, while decreased by 6.91% and 41.8% in FH-1046 and 54.35% and 49.5% in FH-1453 by Cr (0.3 mM and 0.5 mM) respectively (Fig. 1H). Under Cr concentrations of 0.3 mM and 0.5 mM, trehalose increased root moisture content by 13.8% and 53.2% in FH-1046 and 4.77% and 21.9% in FH-1453 respectively. Trehalose significantly reduces the damaging impact of Cr stress by overall increasing the length and biomass of plant.

Photosynthetic pigments

Overall, the chlorophyll activities were significantly greater in maize variety FH-1046 as compared to FH-1453 (Table 2). Chlorophyll a was significantly decreased by 25.3% and 46.8% in FH1046 and 14.1% and 31.3% in FH1453 by Cr (0.3 mM and 0.5 mM) stress respectively as compared to the control. Whereas trehalose significantly reduced the damaging effect of Cr by improving the chlorophyll by 4.49% in FH1046 and 16.64% in FH1453. Under Cr concentrations of 0.3 mM

and 0.5 mM, trehalose increased chlorophyll a 20.5% and 43.3% in FH-1046 and 32.4% and 28.1% in FH-1453 respectively (Fig. 2A).

Chlorophyll *b* was significantly greater in maize variety FH-1046 as compared to FH-1453 (Table 2). Chlorophyll b content was significantly decreased by Cr (0.3 mM and 0.5 mM) stress by 15.5% and 25.4% in FH1046 and 9.70% and 23.5% in FH1453 respectively. However, a significant increase of 2.90% in FH-1046 and 11.3% in FH1453 was observed by foliar application of trehalose. Under Cr concentrations of 0.3mM and 0.5 mM, trehalose increased Chlorophyll b by 4.8% and 6.7% in FH-1046 and 11.4% and 13.7% in FH-1453 respectively (Fig. 2B).

Chlorophyll *a*/*b* content was significantly decreased by 19.6% and 59.1% in FH1046 and 10.5% and 25.3% in FH1453 by Cr (0.3 mM and 0.5 mM) respectively as compared to the control. However, a highly significant increase 16.6% in FH1046 and 4.64% in FH1453 was observed by the foliar application of trehalose (Fig. 2C). Under Cr concentration of 0.3 mM and 0.5 mM, trehalose increased Chlorophyll a/b 13.8% and 39.5% in FH-1046 and 6.2% and 10.8% in FH-1453 respectively. (Fig. 2C). The Cr concentration of 0.5 mM adversely affected the chlorophyll *a*/*b* compared to Cr concentration of 0.3 mM (Table 2).

Total chlorophyll contents were significantly decreased by 26.5% and 70.9% in FH-1046 and 13.6% and 20.1% in FH-1453 by Cr (0.3 mM and 0.5 mM) stress respectively. Moreover, a significant increase of 2.81% in FH-1046 and 19.2% in FH-1453 was observed by foliar application of trehalose. Under Cr concentrations of 0.3 mM and 0.5 mM, trehalose increased total chlorophyll contents by 12.3% and 31.02% in FH-1046 and 15.3% and 27.5% in FH-1453 respectively (Fig. 2D). The non-significant difference was observed in variety, Cr stress and trehalose treatment and between their interactions (Table 2).

Carotenoid contents were also significantly decreased by 3.18% and 5.54% in FH-1046 and 24.3% and 2.14% in FH-1453 by Cr (0.3 mM and 0.5 mM) respectively as compared to control. In addition to that, Cr 0.5 mM had more prominent damaging effects on the carotenoids contents as compared to the Cr 0.3 mM. A significant increase of 0.81% in FH-1046 and 3.09% in FH-1453 was observed by foliar application of trehalose. Under Cr concentrations of 0.3 mM and 0.5 mM, trehalose increased Carotenoids by 11.3% and 17.34% in FH-1046 and 5.4% and 8.6% in FH-1453 respectively (Fig. 2E). Trehalose (30 mM) foliar application significantly enhances photosynthetic pigments in maize plants which help the plant improve the growth under Cr stress.

Total soluble proteins

Total soluble proteins also decreased by exposure to Cr (0.3 mM and 0.5 mM) stress by 13.15% and 18.42% in FH-1046 and 13.5% and 25.6% in FH-1453 respectively. Whereas trehalose (30 mM) significantly increased the total soluble protein levels in both maize varieties by 28.94% in FH-1046 and 10.81% in FH-1453. Under Cr concentrations of 0.3 mM and 0.5 mM, trehalose increased Total soluble proteins by 3.9% and 10.5% in FH-1046 and 1.35% and 4.05% in FH-1453 respectively (Fig. 2F). Trehalose (30 mM) foliar application increases total soluble proteins, strengthening enzymatic activities and stabilizing the cell wall's structure.

Carbohydrates

Cr stress and trehalose also significantly altered the carbohydrates (Table 2). Trehalose (30 mM) foliar application resulted in a noticeable rise in carbohydrates 2.73% in FH-1046 and 9.48% in FH-1453 (Fig. 2G). While Cr (0.3 mM and 0.5 mM) significantly decreased by 10.15% and 16.79% 7.75% and 11.20% in FH-1046 and FH-1453 respectively. Under Cr concentrations of 0.3 mM and 0.5 mM, trehalose increased carbohydrates by 9.7% and 8.5% in FH-1046 respectively (Fig. 2G). Trehalose application significantly increases the carbohydrates of maize plants under Cr stress.

Electrolyte conductivity

Electrolyte conductivity (EC) was significantly increased by 23.1% and 38.2% in FH-1046 and 26.5% and 37.3% in FH-1453 by Cr (0.3 mM and 0.5 mM) respectively whereas trehalose significantly decreased 7.23% in FH1046 and 12.5% in FH1453 EC. Under Cr concentrations of 0.3 mM and 0.5 mM, trehalose decreased EC by 11.02% and 27.5% in FH-1046 and 15.8% and 23.4% in FH-1453 respectively (Fig. 2H). A highly difference was observed between variety and trehalose treatment (Table 2). Trehalose (30 mM) foliar application reduces EC in both varieties of maize, whereas Cr (0.5 mM) exposure significantly boosted it.

Proline content

Cr stress caused a significant rise in the proline level in both maize varieties. The proline content was decreased by 51.02% and 75.51% in FH-1046 and 19.29% and 47.36% in FH-1453 by Cr (0.3 mM and 0.5 mM) respectively. However, trehalose markedly increases by 32.65% in FH-1046 and 17.54% in FH-1453 (Fig. 3A). Under Cr concentrations of 0.3mM and 0.5mM, trehalose increased proline 89.79% and 92.7% in FH-1046 and 56.1% and 75.4% in FH-1453 respectively (Fig. 3A). The proline content was increased by trehalose, while respectively (Table 3). Trehalose (30 mM) foliar application increases proline in both maize varieties, while exposure to Cr (0.5 mM) significantly reduces it.

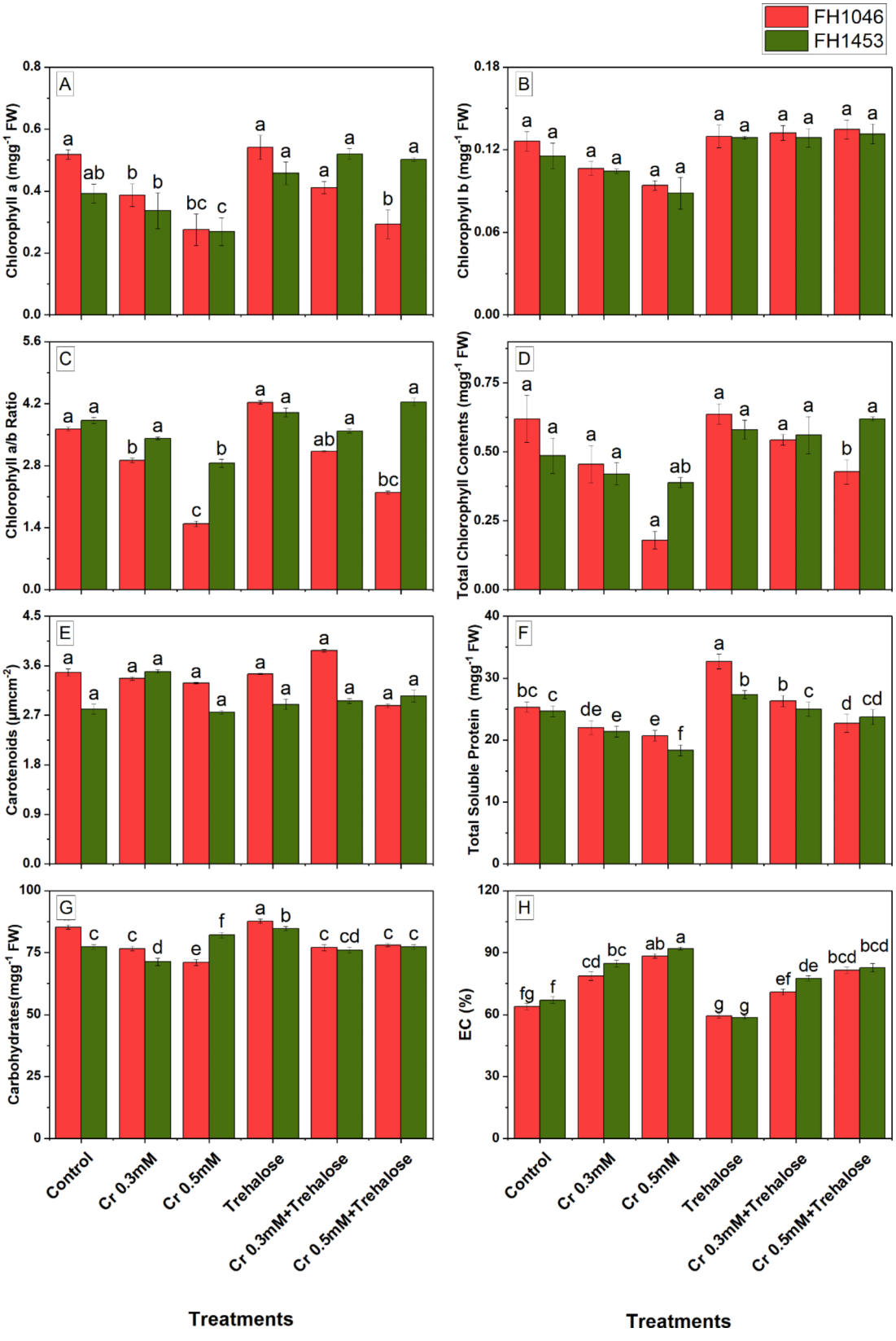

Fig. 2 Effect of maize foliar application of trehalose on Chlorophyll a (**A**), Chlorophyll b (**B**), Chlorophyll a/b (**C**), Total Chlorophyll contents (**D**), Carotenoids (**E**), Total soluble Proteins (**F**), Carbohydrates (**G**) and EC (**H**) grown under induced Cr toxicity. Data is presented in means and standard errors were added while different letters for the different treatments represented a significant difference at $P < 0.05$

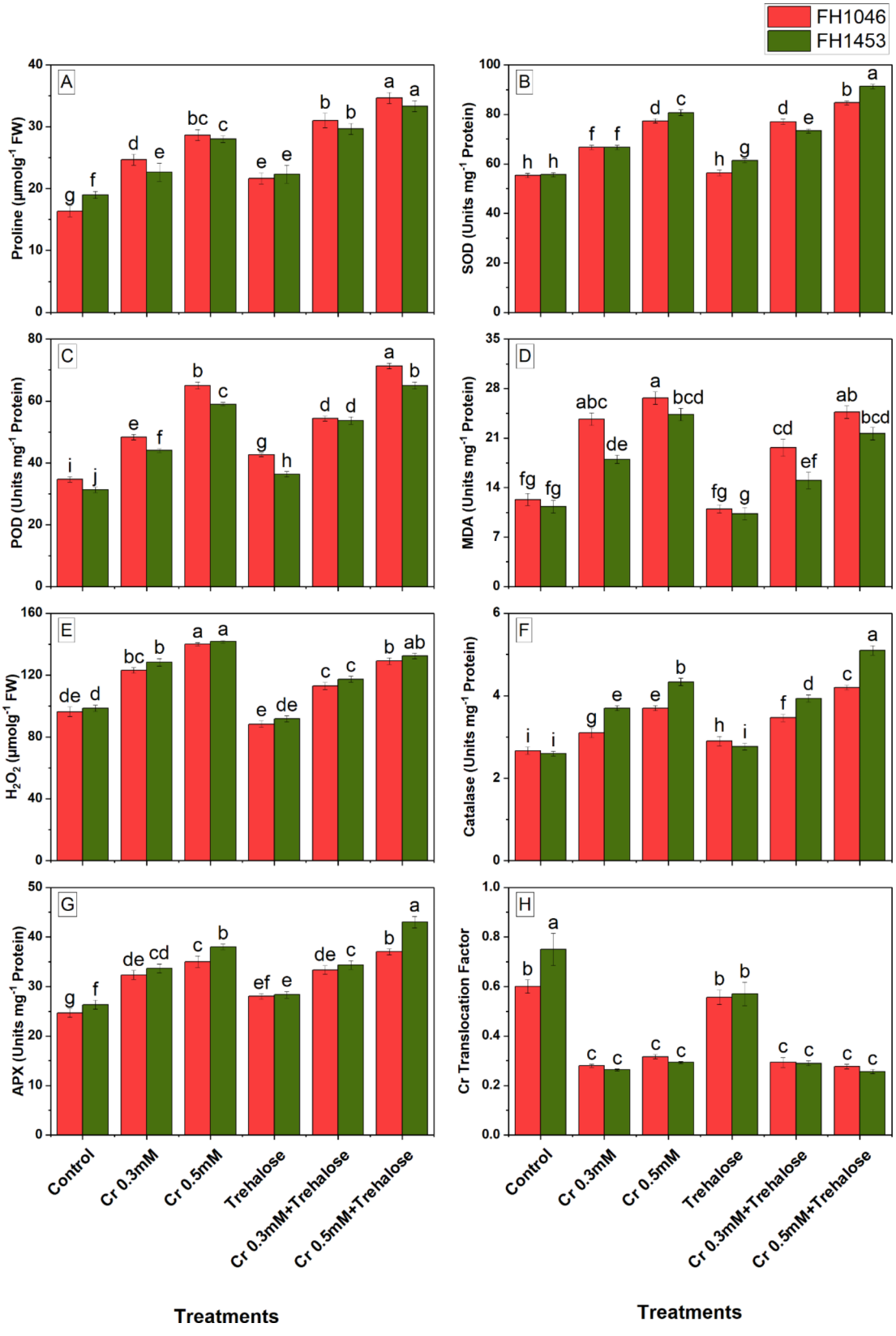

Fig. 3 Effect of maize foliar application of trehalose on Proline (**A**), SOD (**B**), POD (**C**), MDA (**D**), H_2O_2 (**E**), Catalase (**F**), APX (**G**) and Cr Translocation Factor (**H**) grown under induced Cr toxicity. Data is presented in means and standard errors were added while different letters for the different treatments represented a significant difference at $P < 0.05$

Table 3 Effect of exogenous trehalose on super oxide dismutase(SOD), per oxide dismutase(POD), catalases(CAT), ascorbate peroxidase (APX), malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) and also concentration in maize seedlings with or without cr stress

Source of Variation	df	SOD	POD	CAT	APX	MDA	H_2O_2	Cr in Root	Cr in Shoot	Cr Translocation factor
Varieties (Var)	1	0.0019 **	0.0000 ***	0.0000 ***	0.0001 ***	0.0003 ***	0.0048 **	0.0000 ***	0.0000 ***	0.9202 ^{ns}
Cr Stress (Cr)	2	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***
Trehalose (T)	1	0.0000 ***	0.0000 ***	0.0000 ***	0.0001 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0000 ***	0.0064 **
Var × Cr	2	0.0002 ***	0.0277 *	0.0000 ***	0.0122 *	0.0005 ***	0.0005 ***	0.0000 ***	0.0006 ***	0.5002 ^{ns}
Var × T	1	0.2081 ^{ns}	0.9165 ^{ns}	0.8281 ^{ns}	0.6567 ^{ns}	0.2396 ^{ns}	0.0003 ***	0.2612 ^{ns}	. 0.9466 ^{ns}	0.2825 ^{ns}
Cr ×T	2	0.0005 ***	0.4031 ^{ns}	0.0049 **	0.0995 ^{ns}	0.5699 ^{ns}	0.4350 ^{ns}	0.0000 ***	0.0000 ***	0.0026 **
Var × Cr × T	2	0.0119 *	0.0495 *	0.2446 ^{ns}	0.1936 ^{ns}	0.0836 ^{ns}	0.0013 **	0.0124 *	0.0664 ^{ns}	0.1477 ^{ns}

ns: non significant; *,** and *** significant at 0.05, 0.01, 0.001 levels respectively

Enzymatic activities

The activities of antioxidant enzymes were significantly altered by Cr stress and trehalose. Plants exposed to Cr stress have shown greater SOD activity as compared to control group. Trehalose significantly increased SOD activity by 1.80% in FH-1046 and 10.17% in FH-1453 (Fig. 3B). While SOD activity significantly decreased by 20.4% and 39.7% in FH-1046 and 19.76% and 44.9% in FH-1453 under Cr (0.3 mM and 0.5 mM) stress respectively. Under Cr, concentrations of 0.3 mM and 0.5 mM, trehalose increased SOD 39.1% and 53.01% in FH-1046 and 31.7% and 64.1% in FH-1453 respectively. (Fig. 3B). While SOD activity significantly increased by 20.48-39.75% in FH-1046 and 19.76-44.91% in FH-1453 under Cr stress.

In plants growing under Cr (0.3 mM and 0.5 mM) stress, POD activity was reduced noticeably by 39.42% and 87.5% in FH-1046 and 40.42% and 88.29% in FH-1453 respectively (Fig. 3C). Maximum POD activity levels were noticed in FH-1046. POD activity significantly increased in plants treated with 30 mM trehalose, and this rise was more pronounced in FH-1046 under Cr stress. The POD was increased by 23.07% in FH-1046 and 15.95% in FH-1453 by trehalose. Under Cr, the concentration of (0.3 mM), trehalose increased POD 56.7% in FH-1046 and 71.2% in FH-1453 (Fig. 3C).

The levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) are crucial indicators for determining the extent of oxidative damage in plants. Results depicted the substantial rise in MDA (Fig. 3D) and H_2O_2 (Fig. 3E) and levels in the cells of plants exposed to Cr stress. Regarding MDA and H_2O_2 concentration, varieties differed significantly from one another with respect to trehalose treatment (Table 3). The MDA was decreased by 10.81% and 8.82% by trehalose. Wheras, increased by 91.89% and 58.82% was observed by Cr (0.3 mM) in FH-1046 and FH-1453 respectively. Under Cr concentrations of (0.3 mM), trehalose decreased MDA 59.4% in FH-1046 and 32.3% in FH-1453 respectively (Fig. 3D). The H_2O_2 was decreased by 8.30% and 7.09% by trehalose, while increased by 27.68% and 45.32% and 30.06% and 43.58% under Cr (0.3 mM and 0.5 mM) in FH-1046 and FH-1453 respectively. Under Cr concentrations of 0.3 mM and 0.5 mM, trehalose decreased H_2O_2 by 17.3% and 33.9% in FH-1046 and 18.9% and 34.1% in FH-1453, respectively (Fig. 3E).

CAT activity was significantly greater in maize variety FH-1453 as compared to FH-1046 (Table 3). The CAT was increased by 8.75% and 6.41% by trehalose, while decreased by 16.25% 38.75%, and 42.30%, and 66.66% by Cr (0.3 mM and 0.5 mM) in FH-1046 and FH-1453 respectively. Under Cr, the concentration of 0.3 mM and 0.5 mM, trehalose increased CAT 30% and 57.5% in FH-1046 and 51.28% and 96.15% in FH-1453 respectively (Fig. 3F). Both maize cultivars significantly increased their APX activity in response to Cr stress.

APX activity was greater in maize variety FH-1453 as compared to FH-1046. The application of trehalose increased APX activity by13.51% in FH-1046 and 7.59% in FH-1453. Whereas APX decreased by 31.08% and 41.89% in FH-1046 and 27.84% and 44.30% in FH-1453 under Cr stress (0.3 mM and 0.5 mM) respectively. Under Cr, the concentration of 0.3 mM and 0.5 mM, trehalose increased APX 35.1% and 50% in FH-1046 and 30.37% and 63.2% in FH-1453 respectively (Fig. 3G). The oxidative stress produced by Cr stress is greatly reduced by foliar application of trehalose (30 mM), as evidenced by a decrease in SOD, POD, Catalase, APX, and other such markers (Fig. 3).

Cr in root and shoot

The findings showed that 30 mM trehalose prevented Cr from being transported in the aerial part of the plant (Fig. 3H). The application of trehalose decreased the translocation factor by 7.22% in FH-1046 and 24% in FH-1453. Whereas translocation factor increased by 53.3% and 47.22% in FH-1046 and 64.88% and 60.88% in FH-1453 under Cr stress (0.3 mM and 0.5 mM) respectively. Under Cr concentrations of 0.3 mM and 0.5 mM, trehalose decreased translocation factor 51.1% and 53.8% in FH-1046 and 61.3% and 65.7% in FH-1453 respectively (Fig. 3H).

The root Cr accumulation in maize plants was considerable and more pronounced in FH-1453. Application of trehalose (30 mM) resulted in an even greater rise in root Cr (Fig. 4A). The Cr in root was increased 15.14-95.23% in FH-1046 and 20.83-91.16% in FH-1453 under Cr stress, while decreased in Cr concentration was observed 12.23-65.71% and 15.6-57.5% by trehalose in FH-1046 and FH-1453 respectively (Fig. 4A). It was determined that plants exposed to Cr toxicity had their stem Cr concentrations significantly increased. Regarding shoot Cr accumulation (Fig. 4B), there was a noticeable variation across varieties, whereas the reaction of varieties varied for root Cr accumulation. The Cr in shoot increased 14.18-71.2% and 22.11-97.5% by Cr, while decreased 20.2-41.42% and 28.84-56.9% by trehalose in FH-1046 and FH-1453 respectively. In contrast to the accumulation of root Cr, which decreased in plants treated with trehalose. Thus, trehalose significantly reduced shoot Cr concentrations.

Positive correlation was observed among growth parameters and photosynthetic pigments whereas there was strong positive correlation was observed between antioxidants and Cr concentration in root and shoot (Fig. 5). Trehalose significantly reduces the damaging effect by improving the growth which improves the biomass and increases the osmolytes accumulation. Principal component analysis clearly revealed the negative interaction between the antioxidants and growth-related parameters as well as for the photosynthetic pigments (Fig. 6). This is evident that increase in the photosynthetic activities improves the growth and biomass of the plant. However, increase in the antioxidants is related with stress that's why there is a strong negative correlation between antioxidants and growth-related attributes. That's findings clearly reflect the stress indicator increase in the antioxidants and decrease the growth and photosynthetic activities which may be reversed by trehalose application (30 mM).

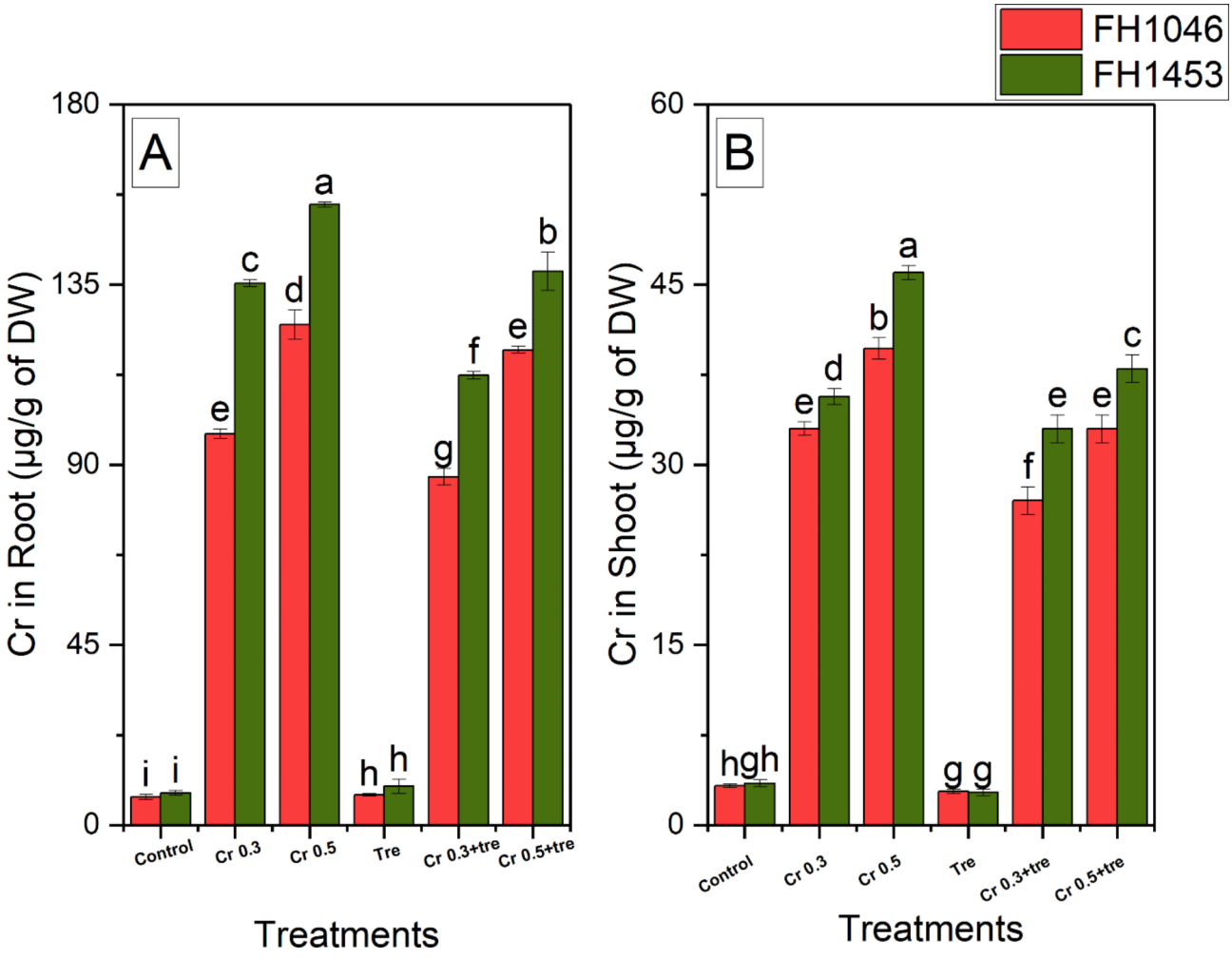

Fig. 4 Effect of maize foliar application of trehalose (30 mM) on Cr in Root (**A**) and Cr in Shoot (**B**) grown under induced Cr toxicity (0.3 mM & 0.5 mM). Data is presented in means and standard errors were added while different letters for the different treatments represented a significant difference at $P < 0.05$

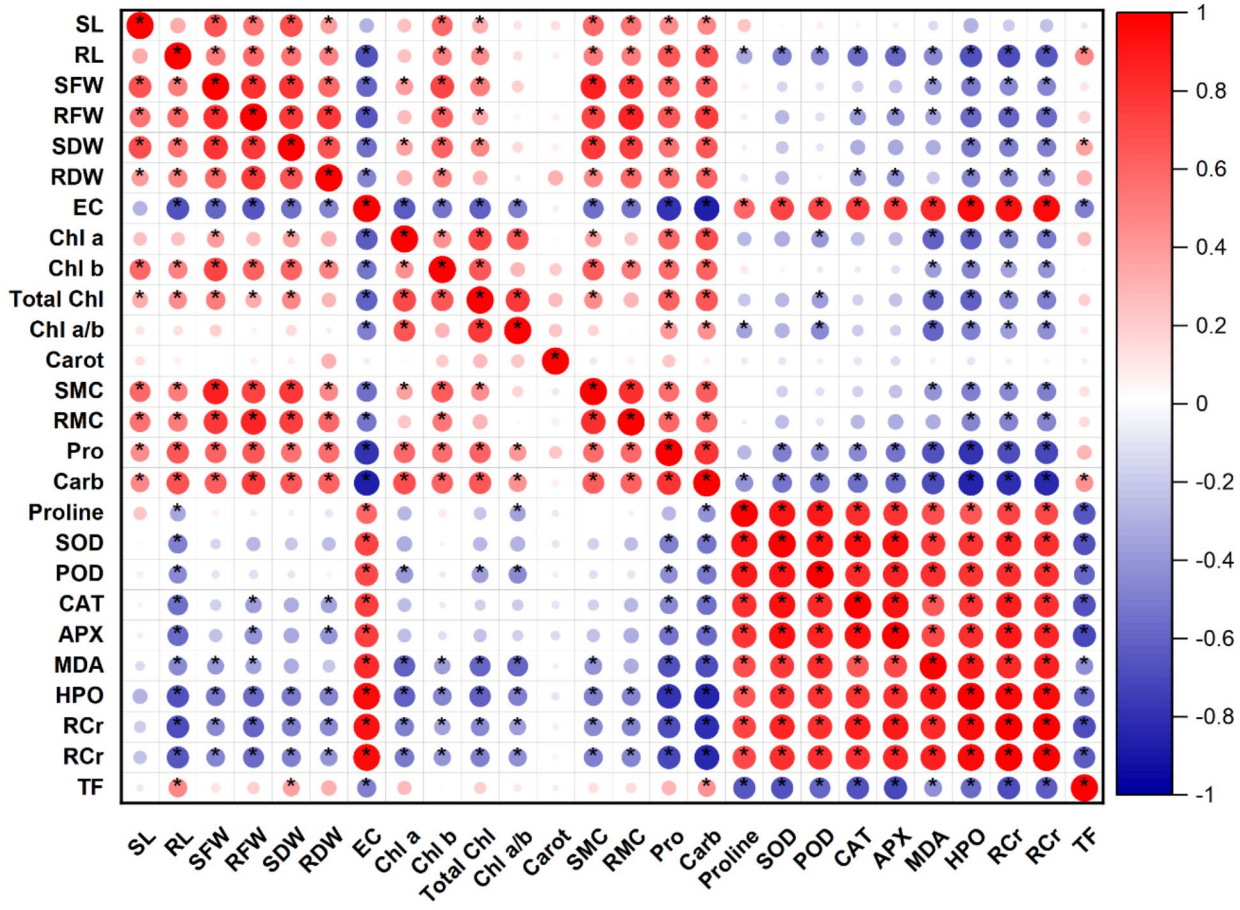

Fig. 5 Pearson correlations among various growth and biochemical parameters of maize plants treated with trehalose under Cr toxicity. * shows the significant difference at $P < 0.05$ among different parameters SL: Shoot Length, RL: Root Length, SFW: Shoot Fresh Weight, RFW: Root Fresh Weight, SDW: Shoot Dry Weight, RDW: Root Dry Weight, MCS: Moisture Contents of Shoot, MCR: Moisture Contents of Root, EC: Electrolytic Leakage, Chl a: Chlorophyll a, Chl b: Chlorophyll b, T. Chl: Total Chlorophyll Contents, Chl a/b: Chlorophyll a/b ratio, Pro: Protein Contents, Carb: Carbohydrates, Proline: Proline Contents, SOD: Superoxide Dismutase, POD: Peroxidase, CAT: Catalase, APX: Ascorbate Peroxidase, MDA: Malondialdehyde, HPO: Hydrogen peroxide, RCr: Cr Concentration in Root, SCr: Cr Concentration in shoot, TF: Translocation Factors of Cr

Discussion

The bioavailability of metals in the rhizosphere is crucial because it directly influences the uptake of these metals by plant roots, determining the extent of stress imposed on plants by heavy metals [50]. Various soil factors such as pH, organic matter content, redox potential, and the presence of competing ions significantly affect the solubility and mobility of heavy metals [26]. Soluble heavy metals in the soil are more readily taken up by plant roots, leading to toxicity and stress symptoms in plants. Conversely, if heavy metals are in insoluble forms or tightly bound to soil particles, their bioavailability decreases, reducing plant uptake and subsequent stress [29]. Accelerated industrial activity has exacerbated chromium (Cr) toxicity in plants, posing significant concerns for both plant health and human well-being [4]. Cr toxicity affects various aspects of plant physiology, including growth, biomass production, and yield. Toxicity symptoms vary depending on Cr concentration and exposure duration [51]. Elevated Cr concentrations in plant cells can impede nutrient absorption, constraining plant development [10].

This study investigated changes in physiological and biochemical characteristics of maize plants following the application of trehalose and exposure to Cr stress. Trehalose has been demonstrated to improve plant growth and physiological responses under different abiotic stresses [52], such as drought [53, 54], salt stress [55, 56], copper [30], cadmium [33], and Cr [10]. Trehalose is recognized for its ability to mitigate the toxic effects of heavy metals [57, 58].

The current study revealed a significant decrease in growth attributes such as shoot and root length, and

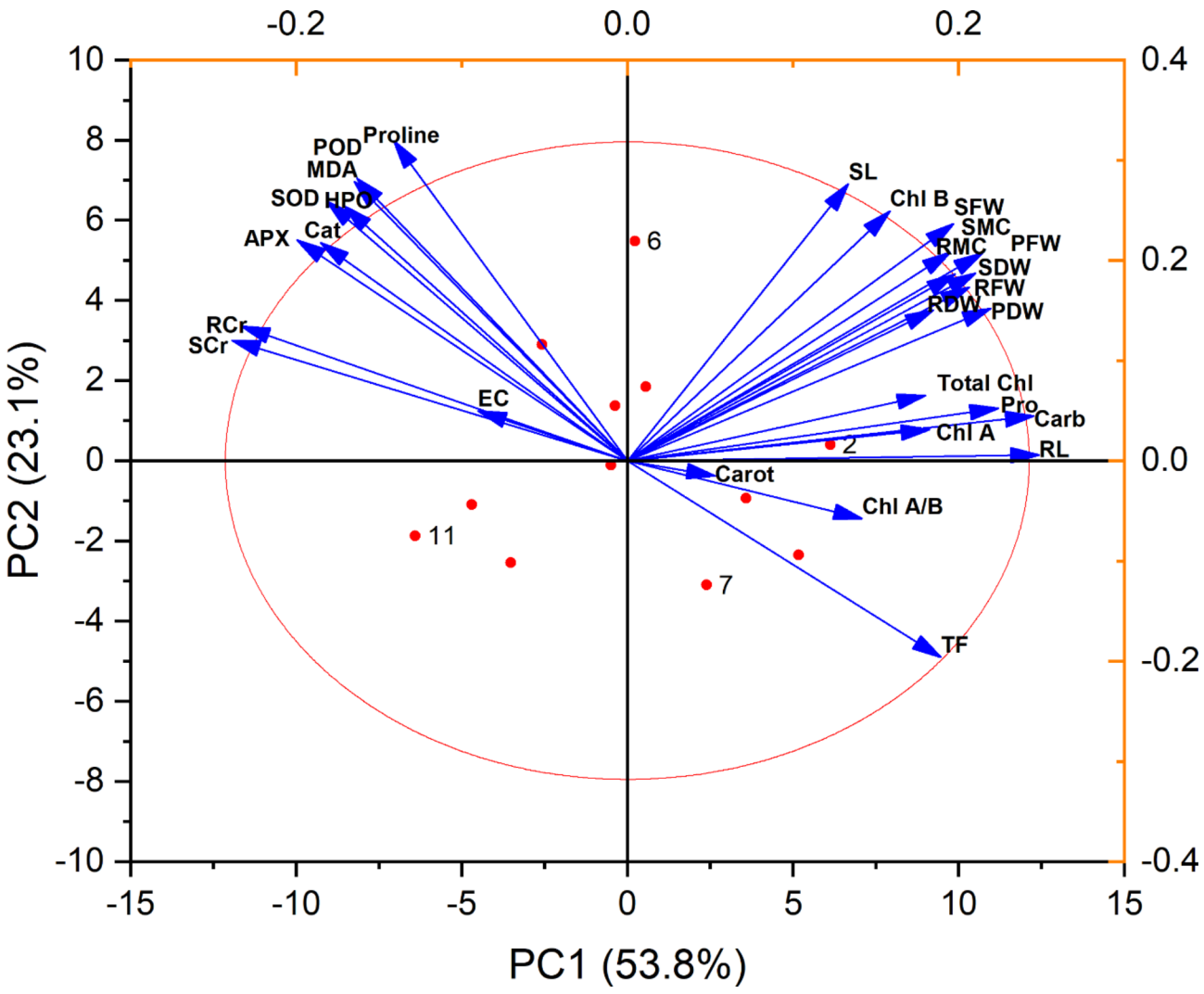

Fig. 6 PCA between growth and biochemical parameters of maize plants treated with trehalose (30 mM) under Cr toxicity (0.3 mM & 0.5 mM). SL: Shoot Length, RL: Root Length, SFW: Shoot Fresh Weight, RFW: Root Fresh Weight, SDW: Shoot Dry Weight, RDW: Root Dry Weight, MCS: Moisture Contents of Shoot, MCR: Moisture Contents of Root, EC: Electrolytic Leakage, Chl a: Chlorophyll a, Chl b: Chlorophyll b, T. Chl: Total Chlorophyll Contents, Chl a/b: Chlorophyll a/b ratio, Pro: Protein Contents, Carb: Carbohydrates, Proline: Proline Contents, SOD: Superoxide Dismutase, POD: Peroxidase, CAT: Catalase, APX: Ascorbate Peroxidase, MDA: Malondialdehyde, HPO: Hydrogen peroxide, RCr: Cr Concentration in Root, SCr: Cr Concentration in shoot, TF: Translocation Factors of Cr

shoot and root fresh and dry weight of both maize cultivars under Cr stress. This reduction was more pronounced in the FH-1046 maize variety compared to FH-1453. Cr-induced growth reduction has been documented in many plants, including maize [10, 59], and is likely due to disrupted nutritional homeostasis and lower mineral absorption [60]. Heavy metals precipitated in xylem cells of vegetative organs cause the endoderm and hypoderm to harden, leading to withered and shrunken morphology [36]. Evidence indicates Cr stress causes morphological and ultrastructural alterations in various plant species, including maize [61] and *Triticum aestivum* (wheat) [62]. The application of trehalose significantly enhanced maize plant development under Cr stress, as seen in previous research on Cr-stressed maize [10] and Cu-toxicity in *Oryza sativa* (rice) [63].

Photosynthesis, a primary mechanism for ATP synthesis and other energy-rich molecules, is adversely affected by heavy metal stress, impacting leaf ultrastructure, carbon fixation, gene expression, and photosynthetic pigments [64]. Heavy metals increase reactive oxygen species (ROS), leading to oxidative stress and alterations in genetic material, gene expression, enzyme activities, and pigment synthesis [65]. Cr toxicity disrupts chloroplasts and degenerates photosynthetic pigments [66]. Photosynthetic pigment status indicates plant health under abiotic stresses like Cr stress, which affects photosynthetic efficiency and growth [67, 68]. This study found

significant adverse effects of Cr exposure on maize [69], including reduced chlorophyll content and photosynthetic capacity, especially at higher Cr concentrations (0.5 mM). Similar reductions in photosynthetic pigments have been reported in other plants under metal toxicity including rice [70] and wheat [71], though some, like *Pisum sativum* (pea), showed increased carotenoids and photosynthetic pigments in response to Cr stress [68].

The experimental findings indicate a decline in leaf chlorophyll content with increasing concentrations of Cr application, particularly evident at higher Cr concentrations, notably at 0.5 mM. This reduction in chlorophyll content consequently led to decreased photosynthetic capacity in both maize cultivars. These results are consistent with prior studies, which highlighted the significant reduction in photosynthetic pigments (chlorophyll a and b) in maize and *Helianthus annuus* (sunflower) plants due to Cr exposure [10, 72, 73]. Likewise, wheat, *Brassica oleracea* (brassica), and *Vigna radiata* (mung bean) plants subjected to metal toxicity also exhibited decreased photosynthetic contents [10, 74–76]. However, Pea demonstrated increased levels of carotenoids and photosynthetic pigments in response to Cr stress [77]. The application of trehalose to the leaves resulted in an enhancement of the photosynthetic rate, which was accompanied by a decrease in lipid peroxidation of cellular membranes and leakage of electrolytes in plants under Cr-induced stress. These modifications led to the preservation of the water content inside the cells, resulting in enhanced photosynthetic activity and improved growth and biomass output of maize plants. Furthermore, the data suggest that the application of trehalose reduces lipid peroxidation in cellular membranes, particularly in chloroplast membranes. This helps to preserve the integrity of the cellular membranes, resulting in improved functioning of the photosynthetic electron transport chain. The findings are consistent with the results of Duman [78], who found that applying trehalose with glycine betaine enhanced leaf photosynthesis in *Lemna gibba* L. (duckweed) by reducing the negative effects of Cd stress.

In this research, the application of trehalose demonstrated a significant positive influence on photosynthesis and various gas exchange parameters under Cr stress

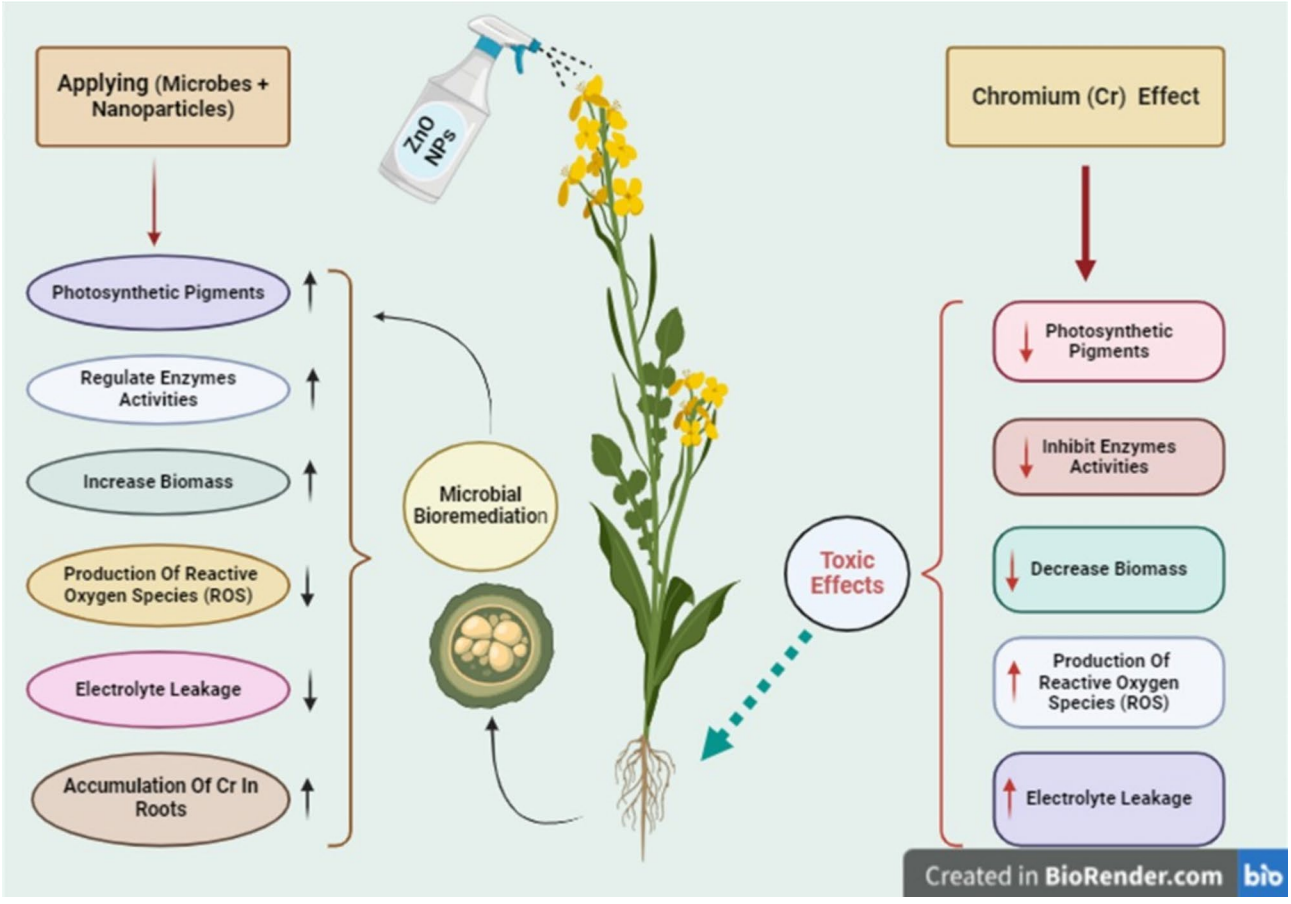

Fig. 7 Mechanism of chromium toxicity and maize plant response trehalose application under Cr toxicity. This image was prepared by using online software Biorender (https://www.biorender.com)

conditions. Specifically, trehalose application boosted the leaf photosynthetic rate, which can be attributed to the decrease in lipid peroxidation in cellular membranes and the subsequent reduction in electrolyte leakage observed in Cr-stressed plants. These changes collectively aided in maintaining cellular water content, leading to improved photosynthetic activity, enhanced growth, and increased biomass production in maize plants. Trehalose application enhanced photosynthetic rate, decreased lipid peroxidation, and reduced electrolyte leakage in Cr-stressed plants, preserving cellular water content and improving photosynthetic activity, growth, and biomass output. Trehalose reduced lipid peroxidation in chloroplast membranes, preserving the photosynthetic electron transport chain's function [74, 76]. Similar findings were reported in mung bean under zinc stress [79].

Carbohydrate levels decreased in both maize varieties, contrasting with observations in wheat exposed to industrial effluent containing high levels of Pb, Cr, and Hg [80], and in the roots of *Spinacia oleracea* L. (spinach) under As stress [81, 82]. Cr stress notably diminished the fresh and dry masses of shoots and roots in two maize cultivars, with FH-1046 showing the most significant reduction, which was attributed to a decrease in carbohydrate content. The decline in carbohydrate levels may be due to the loss of photosynthetic pigments, leading to reduced biomass. Exogenous administration of trehalose enhances starch buildup in Arabidopsis by increasing the activity of ADP-glucose pyrophosphorylase, a key enzyme governing starch synthesis, and preventing starch breakdown [83]. Trehalose potentially influences glucose metabolism indirectly by disrupting photosynthetic capability and utilizing alternative sugars [84].

Cr stress in plants leads to an overproduction of reactive oxygen species (ROS), causing lipid peroxidation. This process destroys lipid molecules, increasing levels of cholesterol and fatty acids, which can promote plaque development in blood arteries and veins [85]. In this study, Cr stress-induced excessive oxidative stress in maize plants was demonstrated by elevated levels of hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and electrolyte conductivity (EC). The findings revealed that ROS formation under Cr stress could be measured by the increased MDA and H_2O_2 levels. Conversely, the application of trehalose to Cr-stressed plants significantly reduced EL, H_2O_2, and MDA levels compared to Cr treatment alone. Elevated MDA levels, linked to heavy metal stress, indicate oxidative stress caused by excess ROS. The study found that heavy metal-contaminated soil positively correlated with H_2O_2 production, leading to increased lipid peroxidation. Trehalose application led to a significant decrease in H_2O_2 levels and subsequent lipid peroxidation. These results suggest that trehalose plays a promotive role in reducing oxidative stress in Cr-stressed maize plants. Assessing ROS levels, plant extracts can detoxify heavy metals like Cr in the body [86].

Plants have developed defense mechanisms to repair and minimize damage caused by excessive reactive oxygen species (ROS) through the activation of various antioxidant enzymes [87]. Enzymes such as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), and catalase (CAT), along with their associated protein content, play crucial roles in maintaining the defense system against oxidative stress. In this study, Cr toxicity was observed to decrease the activities of these antioxidant enzymes in plant tissues, a response that is both species-specific and dependent on the level of stress. The rapid decline in antioxidant enzyme activity under Cr treatment is likely due to severe oxidative stress. However, the application of trehalose significantly increased the activities of these antioxidant enzymes in Cr-stressed plants compared to those without trehalose treatment. Present findings align with previous studies, which have demonstrated that exogenous trehalose application helps mitigate the harmful effects of oxidative stress by enhancing the activities of antioxidant enzymes, as observed in Cd-challenged wheat plants [10, 35, 53].

Osmoprotectants like proline play a vital role in safeguarding plants under stress conditions [4, 88]. In maize plants subjected to Cr stress, proline levels increased, a response similar to that observed in rapeseed [10, 89]. Furthermore, the application of trehalose spray elevated proline concentration in maize plants exposed to toxic Cr conditions, consistent with previous findings in trehalose-treated Brassica plants under water deficit [90]. Trehalose acts as a regulatory and signaling molecule in response to chromium toxicity [10]. Additionally, trehalose spray increased the overall levels of free amino acids in plants, aligning with findings in *Raphanus sativus* (radish) under Cr stress reported by Kocaman [91]. This highlights the enhanced amino acid production as a key adaptive response to Cr toxicity in plants.

Cr stress significantly impairs nutrient absorption in plants. Trehalose application has been shown to enhance the uptake of essential nutrients under Cd toxicity conditions [33]. Moreover, trehalose treatment affects the absorption and distribution of heavy metals within plants, restricting Cr to the roots and reducing its accumulation in aerial parts [29, 30].

In this study, elevated Cr concentrations in the growth medium led to maize roots absorbing and depositing Cr in both underground and aboveground parts, consistent with previous findings [10]. However, roots retained higher Cr levels compared to leaves. Conversely, exogenous trehalose application significantly reduced Cr translocation from roots to aboveground segments. This reduction could be attributed to trehalose's protective effect against adverse environmental conditions.

Research by Habiba [69] further indicates that mannitol application enhances metal accumulation and translocation in maize plants.

The overall mechanism of foliar trehalose application under chromium toxicity involves changes in maize seedlings' morphological, physiological, and biochemical traits (Fig. 7). Trehalose triggers osmoprotection in response to Cr stress, stabilizing biological macromolecules like proteins and carbohydrates, and maintaining membrane integrity. This stabilization protects plants against severe environmental stressors. Although trehalose is naturally produced in low concentrations in plants, it is crucial for various metabolic stresses and its levels should be increased due to its protective functions. The concentration of trehalose can be augmented through external application, which enhances photosynthetic efficiency, thus boosting plant growth and physiological attributes [8]. Trehalose can be readily absorbed from the soil and translocated from plant roots to aerial parts. By enhancing chlorophyll pigments, osmoprotectants, and antioxidant enzyme activities, foliar-applied trehalose significantly improved maize tolerance to Cr stress. The exogenous application of trehalose also lowered Cr concentration in the shoots and roots. Trehalose spray markedly increased maize growth by inhibiting the levels of MDA and H_2O_2 in Cr-stressed plants [10, 30].

Conclusion

This study reveals that Cr toxicity (0.3 mM & 0.5 mM) significantly hinders maize seedling growth and photosynthesis. Trehalose application (30 mM) effectively ameliorated these adverse effects by reducing oxidative stress, enhancing antioxidant enzyme activity, and improving water status. By limiting Cr translocation to shoots, trehalose protected the photosynthetic apparatus. Our findings highlight trehalose as a promising agent for mitigating Cr toxicity in maize and underscore its potential for improving crop resilience under Cr-contaminated conditions. Further investigations are necessary to optimize trehalose application strategies for different maize cultivars and to explore its interactive effects with other stress-mitigating compounds.

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Author contributions
SZ and KSA devised the study framework and oversaw the experimental work. IMK carried out all investigations and initially drafted the manuscript. MAA and RR conducted the statistical analysis. MA, AM and MZ helped in editing. The manuscript was critically reviewed and revised by all authors and finally approved.

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Data availability
The primary data generated in this study are available with the first author and can be requested if needed to reproduce data visualizations and modeling.

Declarations

Ethical approval
The study does not include any animal or human subjects and no specific ethical approval is needed. Other necessary guidelines set by University of Education Lahore for handling of plant material during conduction of laboratory work were followed. All samplings were done with the least possible disturbances to plant communities and environment. After completion of study, all experimental materials were properly discarded/incinerated in a controlled environment to avoid bio-contamination.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Botany, Division of Science and Technology, University of Education Lahore, Punjab 54770, Pakistan
[2]Department of Botany, Government College University, Faisalabad 38000, Pakistan
[3]Department of Plant Systematics and Biodiversity Lab, Quaid-i-Azam University, Islamabad 45320, Pakistan
[4]Department of Botany, University of Poonch Rawalakot, Rawalakot 12350, Pakistan

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