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Exogenous application of trehalose minimizes cadmium toxicity and alleviates oxidative stress in wheat under cadmium stress

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Abstract: Practical and cost-effective food safety and production methods are essential, as huge areas of arable land are contaminated through heavy metals, including cadmium (Cd) pollution worldwide. This study was performed to assess impacts of dissimilar doses of trehalose (Tre) (0, 25, 50 mM) as foliar spray on wheat plants grown under different Cd stress levels (0, 2.5, 5, 10 mg/kg), considering different growth, photosynthetic attributes and antioxidative defense mechanism in relation with the uptake of Cd to different plant parts. A considerable reduction was detected in growth, biomass, and chlorophyll contents under Cd stress linked with boosted lipid peroxidation, electrolyte leakage (EL), and the uptake of Cd uptake to various parts of plants. Foliar-applied Tre enhanced the hostile possessions of Cd on all studied growing, photosynthetic pigments, and gas exchange attributes associated with reduced lipid peroxidation due to improved antioxidative defense mechanism and reduced Cd uptake to many parts of plant, as well as in grains. Foliar application of Tre reduced the uptake of Cd by 31% in roots, 33% in shoots, and 41% in grains as compared with respective control plants. The study findings propose Tre as a possible contender in reducing Cd stress in wheat with better growth by improving the physiobiochemical and antioxidative mechanisms and maintained better grain quality due to reduced accumulation of Cd that will help to reduce the Cd toxicity in wheat consumers.

Key words: Metal stress, oxidative damage, trehalose, antioxidant defense, toxicity alleviation

1. Introduction

Contaminated arable soils containing heavy metal toxicity have severe environmental concerns resulting from rapid and large-scale industrialization (Nafees et al., 2018; Shi et al., 2018; Ashraf et al., 2019; Ahammed and Yang, 2022). In this context, a large area of farmland soil has been contaminated with cadmium (Cd) toxicity globally (Wu et al., 2020). Cadmium pollution caused a considerable reduction in the production of essential crops worldwide, and further is being worsened due to its constant increase in agricultural soil (Abdulmajeed et al., 2022; Catav et al., 2020). Cadmium is a metal that is heavily toxic for human health and lethal for other living organisms (Alharby et al., 2021). It poses severe adverse effects on growth and development by disturbing the cellular metabolic activities (Gao et al., 2018). Typically, metal smelting, mining, excessive application of chemical fertilizers, sewage sludge, and atmospheric deposition are considered the primary anthropogenic sources of Cd contamination for agricultural soil (Rizwan et al., 2018; Liu et al., 2019). The anthropogenic and geogenic sources of Cd also resulted in groundwater contamination, soil, atmosphere, and agronomic products, leading to eventually the intimidated human health and as the derivative of food linkage (Hu et al., 2019; Kubier et al., 2019).

The available literature shows that Cd can cause acute and chronic health effects even with exposure to very low levels (Islam et al., 2017). Cadmium, a nonbiodegradable element, remains in circulation for longer periods once it discharges into the surrounding environment (Suhani et al., 2021). Regarding the deleterious effects, it is highly toxic for plants. It adversely affects seed germination necessary for better crop stand, biomass production, leaf photosynthetic activity and restricts antioxidative defense mechanism (Carvalho et al., 2018; El Rasafi et al., 2020). Cd caused the inhibition of photosynthesis due to the interference of heavy metals ion with photosynthesis and chloroplast membranes. Accumulation of heavy metals in leaves indirectly reduced photosynthesis which influences the functions

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of stomata and ultimately impacts on photosynthesis (Rady et al., 2023). Photosynthetic pigment decreased due to heavy metals impact indirectly on photosynthesis, consequently, the use of photocatalytic degradation and pigmentation to measure stress for regulatory conditions (Aggarwal et al., 2012). Cd toxicity decreased the carbon fixation during photosynthesis and ultimately decreased chlorophyll contents and photosynthesis (Gallego et al., 2012). Excessive accumulation of Cd in plant tissues results in ultrastructural changes and adversely affects the accumulation of necessary mineral elements required for proper plant growth (Shiyu et al., 2020). In plants, at the cellular level Cd toxicity induces oxidative stress through the overproduction of free radicals of O2 (ROS). These are in the form of reactive N2 species (RNS) and reactive O2 species (ROS) (Gallego and Benavides, 2019; Ahammed et al., 2020). Cd uptake leads to damage to the cell wall of the plasma membrane by damage to the membrane's lipid bilayers, biomolecules, and organelles (abbas et al., 2017), ultimately reduced the growth and physiological parameters by elevated production of electrolyte leakage (EL), malondialdehyde (MDA) and H2O2 (Ali et al., 2022). Cd toxicity also reduced rice growth and biomass by causing oxidative stress (Ahsan et al., 2007; Li et al., 2012a, b; Wang et al., 2014). In rice seedlings Cd stress cause enlarged oxidative stress level by overproduction of reactive oxygen species components as MDA, H2O2 and EL (Shah et al., 2001; Hassan et al., 2005; Wu et al., 2006; Yu et al., 2013; Srivastava et al., 2014). Cd stress adversely impacts the progression and growth of the plants primary to loss of quality and yield. At present, Cd pollution has developed as a major limiting factor for high-quality crop production (Hou et al., 2019).

Wheat is the predominant cereal and is regarded a staple food worldwide. Wheat provides many essential elements beneficial for health, including dietary fiber, proteins, vitamins, and phytochemicals (Kumar et al., 2011). Heavy metals accumulate in the human body irresistibly when they are part of their food chain. In the case of heavy contaminated staple food like that of wheat and its products, the accumulation is of paramount concern (Iqbal et al., 2011; Khan et al 2016). ROS causes the production of oxidative stress which ultimately leads to the destruction of plant progression and growth (Ahmad et al., 2022). It is well known that wheat-like other cereals can accumulate a substantial amount of Cd through roots that translocate to shoot and then accumulate in grains (Rizwan et al., 2017). So along with the wheat yield losses, Cd accumulated in wheat grains raises its levels in the human body by the food chain (Hussain et al., 2018). Thus, food safety measures for wheat grain because of human health should be considered at priority levels.

Regulation of Cd homeostasis is crucial to maintain its intracellular levels to avoid its elevated toxicity. Plants espoused different physiological and molecular mechanisms to limit the toxicity of cadmium. These include the intake and increase the level of metals by combining with cell wall components to extracellular matrix. Complication of metals ions inside the cell wall with organic acids, amino acids, phytochelatins, and metallothioneins and regulating the activities of antioxidative enzymes. Additionally, inhibition of Cd accumulation through sequential binding with root exudates, intracellular sequestration, and chelation and the exclusion of Cd from the cell via sugar alcohol molecules such as Tre also minimize cadmium venomousness in plants (Yu et al., 2020; Wang et al., 2020). Tre is a nonreducing sugar compound comprising two glucose molecules (1-α-D-glucopyranoside, and α-D-glucopyranosyl-1). It is extensively found in several organisms, including yeast, bacteria, fungi, and higher plants (Banfalvi, 2019; Kosar et al., 2019). It is an efficient growth regulator that is extensively used to increase the tolerance of plants in contradiction of various A-biotic stresses (Liu et al., 2020), including salinity (Sadak, 2019), drought (Yu et al., 2019), heat (Lyu et al., 2018), and chilling stress (Fu et al., 2020). Tre plays a significant role in reducing membrane lipid oxidation by averting the development of several superoxide radicals, malondialdehyde (MDA), and ROS by augmenting the happenings of antioxidant enzymes underneath stressful conditions (Luo et al., 2018). However, Tre beneficial impacts under heavy metal stresses and its linked mechanism are still not fully discovered.

This study was designed to scrutinize the impacts of cadmium stress on growth and development of economically important wheat crop. More importantly, it is useful to attain an insight in understanding the modulations in biochemical and physiological mechanisms. When Tre was used exogenously to increase the tolerance in wheat grown under Cd stress. To the best of our knowledge, the current study is the first one for the alleviation of Cd toxicity by Tre in most consumable food crop (wheat) in Pakistan. In this experiment, we analyzed the effects of Tre on the accumulation and uptake of cadmium in wheat concerning different growth parameters, membrane lipid peroxidation, electrolyte leakage as well as the modulations in antioxidative defense mechanism. It was hypothesized that Cd toxicity could be minimized by the exogenous application of Tre through enhancement of wheat defense system.

2. Materials and methods

2.1. Soil sampling and soil analysis

Field soil samples were taken at a depth of 0–15cm (Arif et al., 2018). The soil was sieved with 2-mm mesh. Debris was removed, and a homogenized sample was prepared. The soil was characterized for texture, pH, EC, organic matter, cations and anions, along with different metal

Table. Physicochemical properties of soil used for the pot experiment.

Physicochemical properties	
Texture	Clay loam
Sand %	26
Silt %	21
Clay %	52
pH	7.6
EC (dS m^{-1})	2.9
SAR $(mmol^{-1})^{1/2}$	6.6
Organic matter (%)	0.33
Available P (mg kg^{-1})	2.21
SO_4^{2-} (mmol l^{-1})	6.56
HCO_3 (mmol l^{-1})	3.47
Cl– (mmol l^{-1})	2.28
$Ca^{2++}Mg^{2+}$ (mmol l^{-1})	3.4
K^+ (mmol l^{-1})	0.07
Na^{2+} (mmol l^{-1})	3.6
Available Cu^{2+} (mg kg^{-1})	0.32
Available Cd^{2+} (mg kg^{-1})	0.001

contents (Allison and Richard, 1954; Page et al., 1982). The phytochemical screening of soil is expressed in Table.

2.2. Wheat sowing and harvesting

A pot study was run under ambient environment friendly conditions (day night temperature vs. 28/20 °C, relative humidity vs. 67 ± 5%) in warehouse of Department of Environmental Sciences and Engineering at Government College University Faisalabad, Pakistan (31°24'53.7"N, 73°04'04.7"E). Plastic pots were filled with 6.0 kg soil spiked with various Cd concentrations (0, 2.5, 5, 10 mg/kg). In contrast, control pots were irrigated with filtered water. The study was designed completely random design (CRD) with three replications of each treatment. Uniform healthy wheat seeds of cultivar Lasani-2008 were used for the experimentation. Before sowing, the seeds were rinsed with hydrogen peroxide solution (15% v/v) trailed by washing with distilled and tap water. Six healthy and uniform seeds were sown in every plastic pot. Water to the pots was provided as per requirement and to avoid the nutrients deficiencies then fertilizers (N, P, and K) were applied at recommended doses (120:50:25 kg ha^{-1}). After one week of seed growth and development, thinning was done to maintain only three healthy uniform plants in each pot (Hussain et al., 2019). The uprooted plants were crushed into individual pots. Foliar spray of Tre (0, 25, 50 mM) was done just after the thinning process, and the control plants were sprayed with filtered water. Total five foliar sprays of Tre were given with an interval of 1 week. After 130 days of propagating and elongation, the plants were harvested and divided into different parts. Roots were washed with distilled water and dilute acid to confiscate the debris. All plant parts were dried in oven at 70 °C for 72 h, dry weight of shoot (g pot^{-1}), root (g pot^{-1}) and grain (g pot^{-1}) and length of shoot (cm), root (cm) and spike length (cm) were recorded by weighing balance and measuring rod and stored for further analysis.

2.3. Photosynthesis measurement

After 60 days of sowing, fresh leaf samples (0.1 g) were taken, and the extract was made in 80% acetone solution to measure carotenoid contents and chlorophyll contents. Samples were centrifuged, and readings were taken at various wavelengths. After that, carotenoids and chlorophyll contents in leaf tissues were measured using a standard equation (Lichtenthaler, 1987; Lichtenthaler and Buschmann, 2001) by spectrophotometer (Halo DB-20/DB-20S, Dynamica Company, London, UK). To estimate leaf gas exchange attributes, Infrared Gas Analyzer (IRGA, LCA-4, Analytical Development Company, Hoddesdon, UK) was castoff. The conductance of stomata, water use efficiency, photosynthetic rate and rate of transpiration were recorded at noon as at that time the plants metabolism was fully functional.

2.4. Oxidative stress markers and antioxidants enzymes

To determine electrolyte leakage (EL) the fully expanded leaf blade of selected plants was collected, immediately weighed, and cut into small segments (1 cm). Segments from each leaf were put into 20 mL of deionized water in a test tube and washed slowly at room temperature using a rotary shaker to remove the damaged cells due to cutting and leaf surface's solutes. After vortex, the electric conductivity of the solution in the test tube was measured and labeled it as EC0. Then put the samples in a defreeze for 24 h and measured the EC and termed it as EC1. Autoclave all the samples for 30 min, and EC2 was noted after cooling samples at room temperature. The final measurement of EL was recorded accordingly to the procedure of Dionisio-Sese and Tobita (Dionisio-Sese and Tobita, 1998). To determine the malondialdehyde (MDA) contents, homogenized 5mL fresh leaves from each treatment were add up into 10% trichloroacetic acid (TCA) by using mortar and pestle. Centrifugation of homogenates was done for 20 min. Then 2 mL of 0.6% thiobarbituric acid (TBA) prepared in 10% TCA was added to each 2 mL aliquot of the supernatant. The solution was then impassioned for 15 min at 100 °C and immediately chilled in the ice bath. Supernatant's absorbance was measured at 532 after centrifugation at 10,000×g for 20 min. (Zhang and Choudhuri, 1981; Li et al., 2013). To measure the leaf H2O2 contents, the mixture was prepared in phosphate buffer, and the supernatant's absorbance was recorded at 410 nm as described by Jana and Choudhuri (1981). For the estimation of antioxidant enzymes actions, samples were collected in liquid nitrogen and ground in standardized 0.5M phosphate buffer at a pH of 7.8. The supernatant was obtained after crushing 0.1 g plant sample in 10 mL phosphate buffer with pH 7.0. To obtain the supernatant, centrifugation was done for 10 min at 12000×g at 4 °C. Superoxide dismutase and POD activities were estimated following the Zhang method (Zhang, 1992). The activity of CAT from the same buffer extract used for SOD and POD was measured by checking solution absorbance at 240 nm (Aebi, 1984), and contents of APX were checked by the following method of Nakano and Asada (Nakano and Asada, 1981).

2.5. Total phenolics, ascorbic acid, and total soluble proteins

Contents of total phenolic in fresh leaves were measured as described by Ainsworth and Gillespie (Ainsworth and Gillespie, 2007). The DCIP conversion into DCIPH2 was recorded by spectrophotometer at 520 nm to measure ascorbic acid contents. Then, the ascorbic acid concentration in fresh leaves was measured through linear regression equation (Hameed et al., 2005). At the same time, total soluble protein contents in fresh leaves were measured by the dye-binding method (Bradford, 1976; Chang and Zhang, 2017).

2.6. Cadmium concentration

Dry plant samples were used for the estimation of Cd. After grinding, plant samples (0.2 g) were put in HNO3 and HClO4 (3:1) solution overnight, then heated on a hot plate until complete digestion. The solution was then cooled down, made a final volume 50 mL, and filtered. Finally, the atomic absorption spectrophotometer measured metal concentration (novAA 350–Analytik Jena, Germany).

2.7. Statistical analysis

For result interpretations, one-way ANOVA was used to find out the variances and significant values at $p \leq 0.05$ of all groups treatments using a SPSS 21.0 software for Windows. The least significance difference (LSD) posthoc test was used for significant comparison among treatment means. The hierarchical clustering and heatmap analysis were prepared using RStudio 2022, for Mac.

3. Results

3.1. The effect of Cd and Tre on plant growth

Cadmium toxicity expressively affected plant growth attributes such as shoot length, root length, and spike length over the control treatment. The declining tendency in growth attributes was augmented with the subsequent increase in the cadmium levels in the growth medium. Spike length, shoot length, and root length of the plants average reduced by 26%, 46%, and 59%, respectively, at the highest concentration of Cd application (10 mg/kg) over the control treatment as shown in Figure 1. Foliar spray of Tre significantly reduced the hostile effects of Cd on studied plant development attributes compared to Cd application alone. Combining Tre and Cd (50 mM + 10 mg/kg) significantly enhanced the shoot length, root length, and spike length by 33%, 48%, and 28%, respectively, compared to Cd (10 mg/kg) treatment alone. Likewise, Cd stress reduced shoot, root, and grain dry weight compared to the control treatment (Tre 0 + Cd 0). The highest decrease in these attributes was detected at a maximum Cd concentration (10 mg/kg). The maximum decline in dry weight of shoot, root, and grain was 37%, 58%, and 56%, respectively, compared to the control treatment (Figure 1). While, the application of Tre significantly improved the dry weights of these attributes than Cd treatments alone. The maximum increase in dry biomass of shoot, roots, and grain was observed by 26%, 53% and 58%, respectively, under the combined application of Tre and Cd (50 mM + 10 mg/kg) compared to Cd (10 mg/kg) treatment alone.

3.2. Effect of cadmium and Tre on chlorophyll contents and gas exchange characteristics

Considerable declines in chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid concentrations were found under all cadmium treatments associated to control ones. The extreme reduction in chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids were 54%, 63%, 56%, and 50%, respectively, under maximum cadmium concentration (10 mg/kg) as depicted in Figure 2. However, the foliar application of Tre significantly reduced Cd's adverse effects on these parameters compared to Cd-only treatment. Combined application of Tre and Cd (50 mM + 10 mg/kg) considerably improved the chlorophyll a, chlorophyll b, and total chlorophyll by 47%, 53%, and 48%, respectively, compared to maximum Cd alone treatment of 10 mg/kg. Under Cd-only stress, a gradually decreasing tendency was observed in gas exchange traits over control treatment. Whereas the exogenous application of Tre ominously ($p \leq 0.05$) reduced the deleterious properties of Cd stress on this gas-exchange attributes (Figure 3). The Tre application significantly improved the transpiration rate, photosynthetic rate, stomatal conductance and water use efficiency in wheat plants. The maximum increase in leaf carotenoid contents due to Tre foliar treatment was 68% with the combined application of Tre and Cd (50 mM + 5 mg/kg) compared to the Cd (5 mg/kg) treatment alone (Figure 3).

3.3. Effect of Cd and Tre on oxidative stress and antioxidant enzymes

A gradual increase in EL, MDA levels, and H2O2 concentration was found in wheat plants with the increase in Cd concentration (0, 2.5, 5, 10 mg/kg) compared to the control treatment. Plant exposure with maximum Cd stress (10 mg/kg), MDA, EL and H2O2 contents significantly increased in wheat plants in leaves and roots. Application of Tre as foliar spray with both levels (25, 50

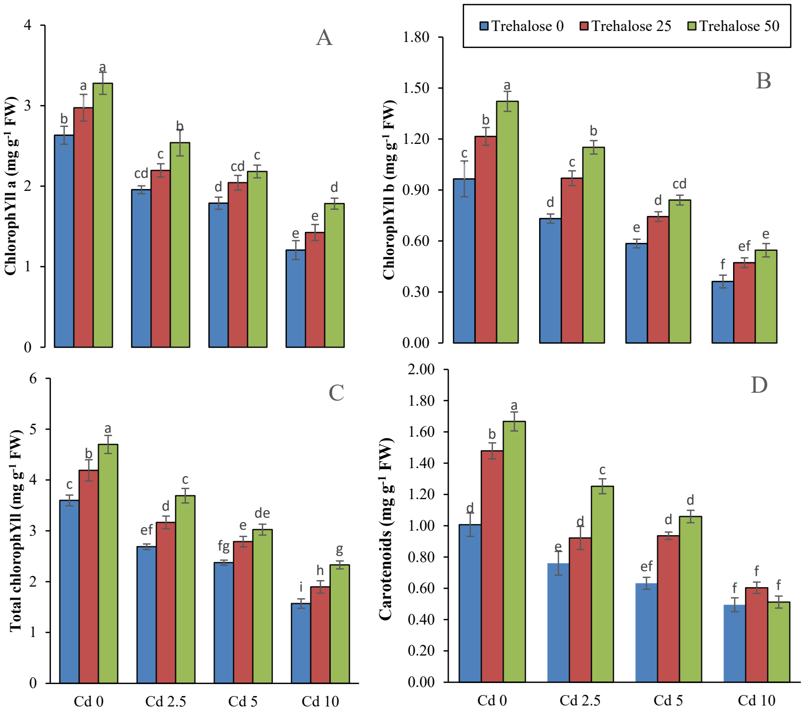

Figure 1. Impact of Cd (0, 2.5, 5, 10 mg/kg) and trehalose (0, 25, 50 mM) on shoot length (A), root length (B), spikes length (C), shoot dry weight (D), root dry weight (E), and grain dry weights (F) of wheat plants. Given values are means of three replications with standard deviation at p ≤ 0.05 level.

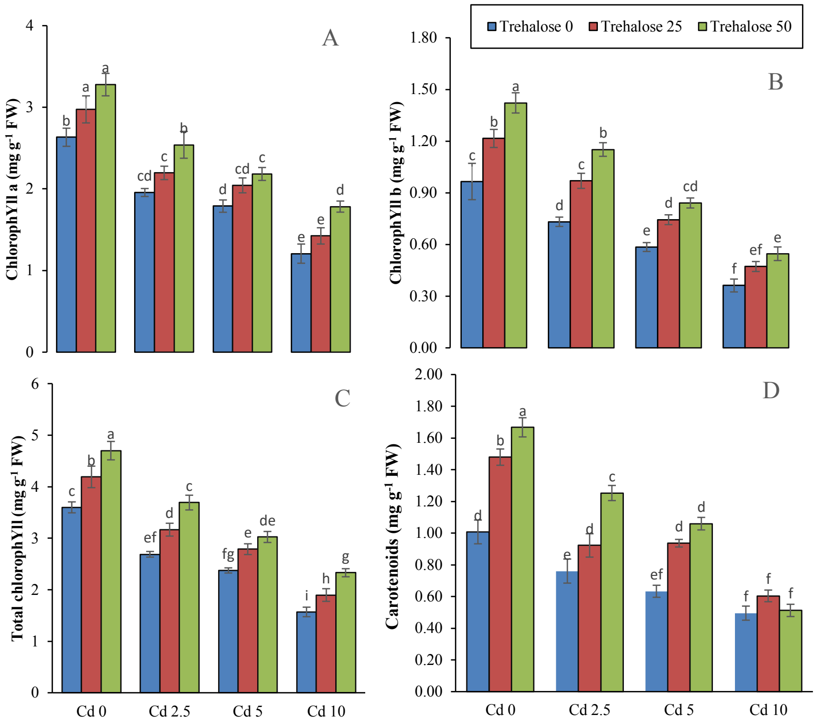

Figure 2. Impact of Cd (0, 2.5, 5, 10 mg/kg) and trehalose (0, 25, 50mM) on concentrations of chlorophyll a (A), chlorophyll b (B), total chlorophyll (C) and carotenoid contents (D) of wheat plants. Given values are means of three replications with standard deviation at p ≤ 0.05 level.

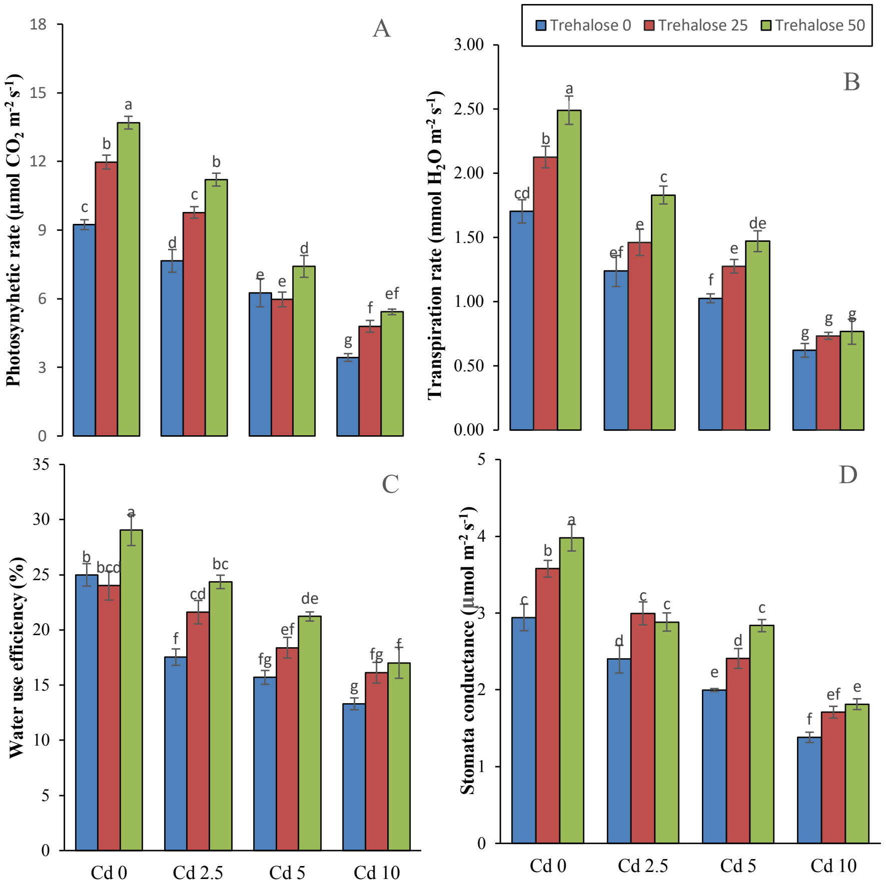

Figure 3. Impact of Cd (0, 2.5, 5, 10 mg/kg) and trehalose (0, 25, 50 mM) on photosynthetic rate (A), transpiration rate (B), water use efficiency (C) and stomatal conductance (D) of wheat plants. Given values are means of three replications with standard deviation at $p \leq 0.05$.

mM) significantly reduced the oxidative damages induced by Cd stress. The maximum amelioration was found in plants sprayed with 50 mM treatment of Tre compared with 25 mM. The reductions in MDA, EL and H2O2 levels in wheat leaves by combined Tre and Cd (50 mM + 5 mg/kg) were 33%, 31%, and 28%, respectively. In contrast, in the wheat roots, this decline was 33%, 28%, and 21%, respectively, compared to the Cd (5 mg/kg) treatment alone (Figure 4).

Effect of cadmium stress (0, 2.5, 5, 10 mg/kg) and Tre (0, 25, 50 mM) alone or in combination with the antioxidant enzymes activities in leaves and roots of the wheat plant are given in Figure 5. Considerable reductions in all these antioxidant enzymes activities were found with Cd treatments (0, 2.5, 5, 10 mg/kg) alone as compared to nontreated ones (Cd 0, Tre 0). A higher concentration of Cd stress (10 mg/kg) resulted in more decline in the activities of studied enzymes as compared with lower Cd stress levels (0, 2.5, 5 mg/kg) (Figure 5). Application of Tre (50 mM) in combination with Cd (2.5 mg/kg) significantly improved the POD activities by 65% and 58% in roots and leaves of the plants over the Cd (2.5 mg/kg) treatment alone, respectively. Similarly, at Cd (5 mg/kg) treatment, Tre (50 mM) improved the POD activity and produced 68% and 47% improvement in roots and leaves of the Cd stressed wheat plant, respectively. Likewise, the maximum increase in SOD activities in wheat plants' leaves was 33% and 46% in plants supplied with Tre (50 mM) at Cd levels (5 and 25 mg/kg), respectively. In comparison, POD activities in roots increased by 35% and 27% under the same application of Tre (50 mM) at Cd levels (2.5 and 5 mg/kg), respectively. The maximum decline in the activities of the APX was observed 93% and 88% in roots and leaves at Cd levels (10 mg/kg) compared to the control ones. While combined application of Tre (50 mM) with Cd levels (2.5, 5 mg/kg) considerably improved the activities of APX by 43% and 48% in leaves and roots as compared to the Cd (2.5, 5 mg/kg) treatment, alone. Similarly, APX activities were improved by 43% and 38% in roots and leaves with the subsequent application of Tre (50 mM). Supplementation of Tre (50 mM) also enhanced the activities of CAT in wheat tissues. A maximum increase

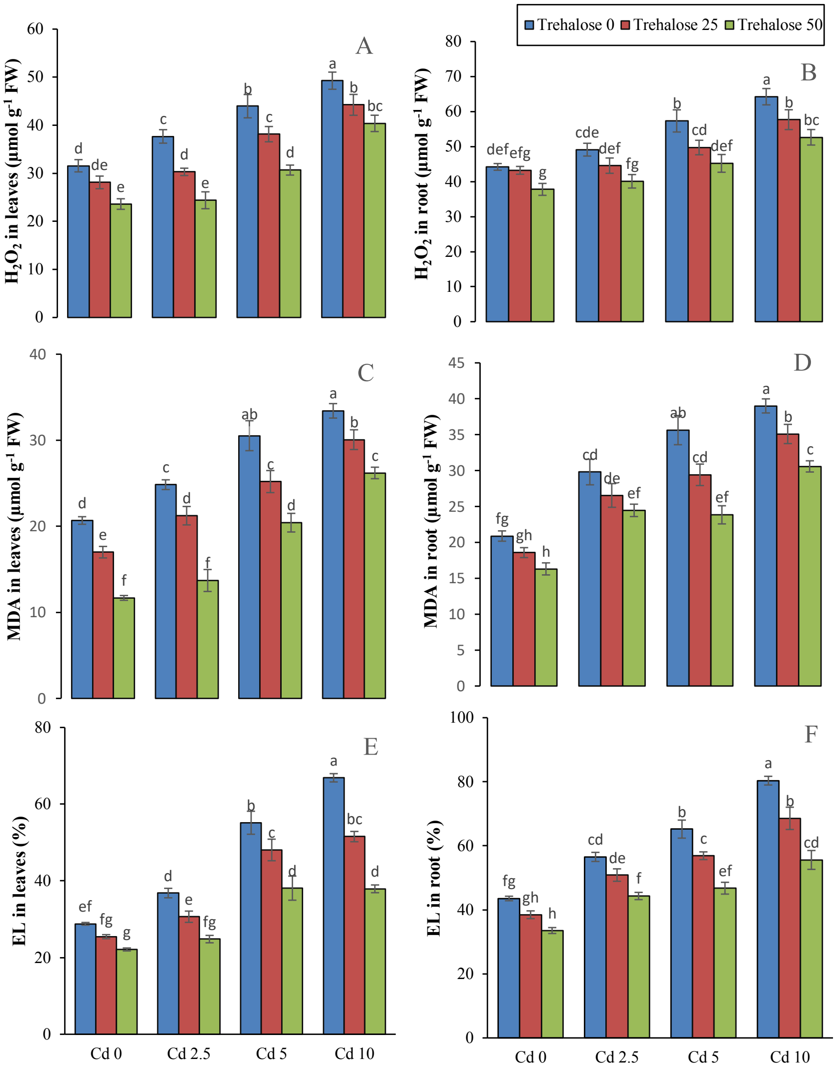

Figure 4. Impact of Cd (0, 2.5, 5, 10 mg/kg) and trehalose (0, 25, 50 mM) on H_2O_2 in leaves (A), H_2O_2 in roots (B), MDA in leaves (C), MDA in roots (D), EL in leaves (E) and EL in roots (F) of wheat plants. Given values are means of three replications with standard deviation at $p \leq 0.05$.

of 48% and 56% in the CAT activities were observed in the roots and leaves as compared to the Cd (2.5 mg/kg) treatment alone as compared with combined application of Tre and Cd (50 mM + 2.5 mg/kg) as given in Figure 5.

3.4. Total phenolics, ascorbic acids, and total soluble proteins

Effect of Cd stress (0, 2.5, 5, 10 mg/kg) and foliar application of Tre (0, 25, 50 mM) alone or in combination on total soluble proteins, total phenolics, and ascorbic acid contents in leaves of the wheat plant has been given in Figure 6. Considerable effects of Cd stress and Tre application have been found on the contents of these metabolites. Leaf total soluble proteins decreased significantly with Cd stress, and the maximum decrease was at 10 mg/kg. However, leaf ascorbic acid and total phenolic contents increased with Cd stress, and the maximum increase was at 5 and 10 mg/kg levels, respectively. Foliar spray of Tre significantly increased the leaf total soluble proteins and leaf ascorbic acid contents, and a maximum increase was at 50 mM level of Tre at all Cd levels. However, leaf total phenolics contents decreased significantly due to Tre foliar spray under Cd stress and nonstressed conditions. Among all Tre levels, maximum increase in leaf ascorbic acid content was found at 50 mM level at all Cd levels.

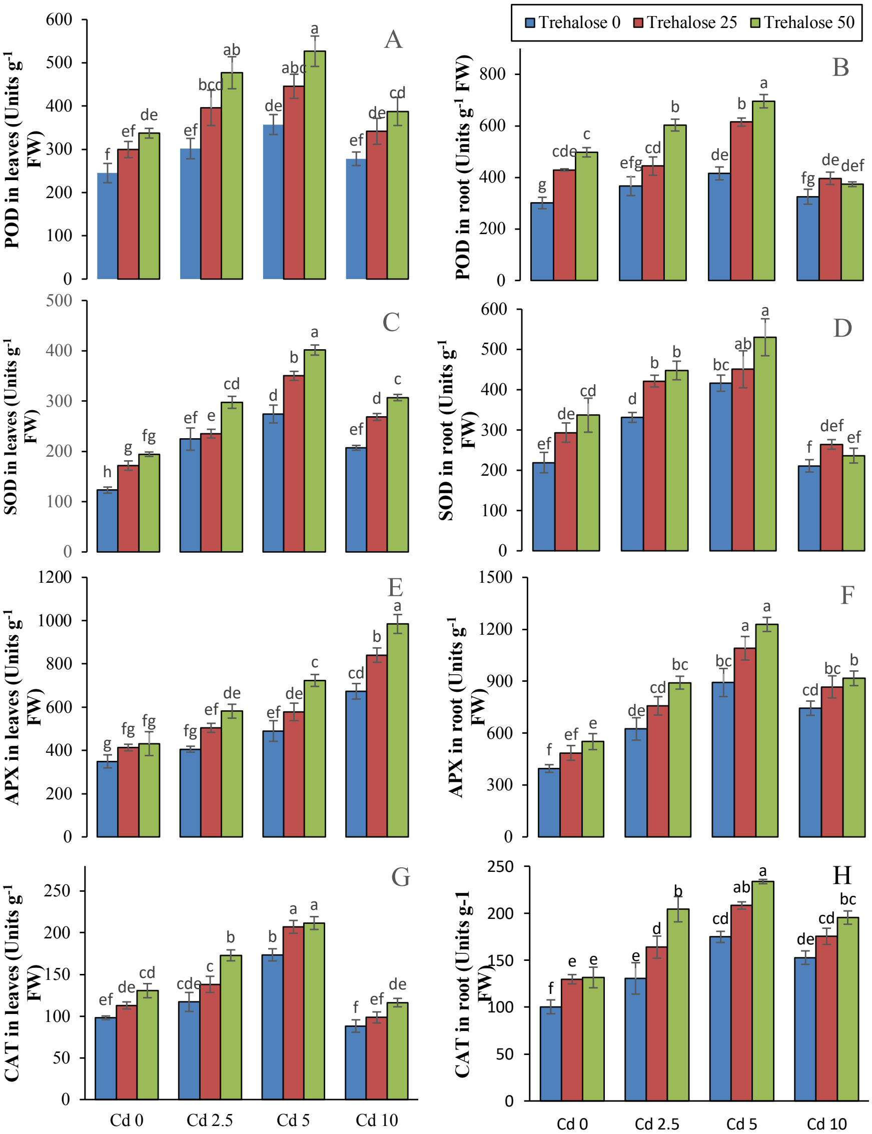

Figure 5. Impact of Cd (0, 2.5, 5, 10 mg/kg) and trehalose (0, 25, 50 mM) on antioxidants activities such as POD in leaves (A), POD in roots (B), SOD in leaves (C), SOD in roots (D), APX in leaves (E), APX in roots (F) and CAT in leaves (G) as well as CAT in roots (H) of wheat plants. Given values are means of three replications with standard deviation at $p \leq 0.05$.

3.5. Cadmium concentration and uptake in wheat

The absorption of cadmium observed in the wheat plants' shoots, roots, and grains was significantly improved by the absorption of Cd doses in the growth medium. The most concentration of Cd absorbed by the wheat plant was reserved in roots as the nature of cadmium is mobile i, a considerable amount was transferred to the plant's shoots and grain. The higher the Cd absorption in growth media, the more its concentration was found in wheat tissues, as presented in Figure 6. Foliar application of Tre along and in combination with Cd (2.5, 5 mg/kg) incredibly declined the concentration of Cd by 31% and 33% in shoots, and 48% and 41% in grains, and by 33% and 31% in roots over Cd-only treatments (2.5 mg Cd/kg + Tre 50 mM vs. 2.5 mg Cd/kg, 5 mg Cd/kg + Tre 50 mM vs. 5 mg Cd/kg). The decline of Cd concentration in aboveground parts of the plant was more than the roots of the wheat, which is beneficial to decrease the Cd concentration in the harvestable grains.

Hierarchical clustering analysis

Hierarchical clustering and heatmap was constructed among all the growth, physiological parameters (A), oxidants and antioxidative as well as Cd uptake in

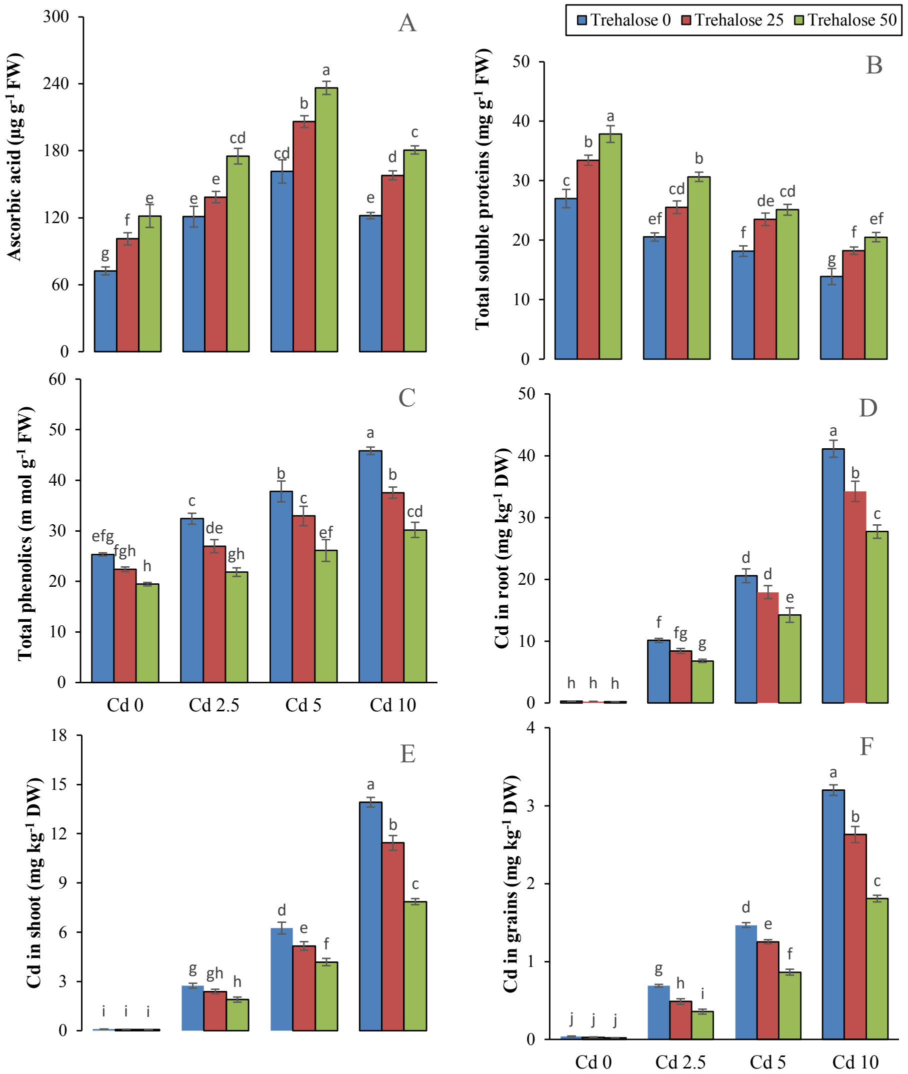

Figure 6. Impact of Cd (0, 2.5, 5, 10 mg/kg) and trehalose (0, 25, 50 mM) on ascorbic acid (A), total soluble proteins (B), and total phenolics (C) of wheat fresh leaves as well as Cd uptake in roots (D), shoots (E) and grain (F). Given values are means of three replications with standard deviation at $p \leq 0.05$ levels.

different wheat parts (B) under Cd toxicity (0, 2.5, 5, 10 mg/kg) with different Tre levels (0, 25, 50 mM) as shown in Figure 7. In the heatmap, red color intensity on scale showed that all growth and physiological parameters increased as Cd decreased. On the other hand, it is prominent that as higher Cd toxicity increases MDA, H2O2, EL and total phenolic in roots then in leaves as shown intensity in color. While the intensity of color decreased towards yellow and blue showed that the application of Tre decreased MDA, H2O2, EL and total phenolics as well as Cd concentration in leaves and roots. But the intensity toward blue color showed that SOD, POD, CAT and APX decreased as Cd increased similarly the application of Tre increased the antioxidants activity as compared to Cd toxicity.

4. Discussion

In this investigate, submission of Cd resulted in a subsequent lessening in all the studied growth attributes such as shoot, root and spike length, shoot, root and grains dry weight in wheat plants. Furthermore, a gradual increase in cadmium echelons in the development medium resulted in more decline in these growth parameters. Several research reports also have reported that with an increase in Cd stress harshness, the growth and total biomass production further decreases as reported in various crop plants, including cotton (An et al., 2020), rice (Barman et al., 2020), maize (Liu et al., 2020), and wheat (Rehman et al., 2015). The decrease in the growth parameters might be due to the limiting uptake of nutrients by the plant because Cd-induced stress significantly disturbs the nutrient homeostasis by decreasing the plant mineral uptake (Rahmen et al., 2016). It is testified that cadmium stress grades in morphological and ultrastructural changes in several plant species, including rice (Hussain et al., 2018) and maize (Figlioli et al., 2019). The use of Tre significantly improved the growth attributes of Cd-stressed wheat plants than the respective Cd-only treatment. Such promotive role of Tre has previously been reported in Cd-stressed Lemna gibba (L.) (Duman et al., 2011) and in rice under Cu toxicity (Mostofa et al., 2015). The above studies depicted that trehalose's application can significantly enhance the growth and biomass of the plants by successfully ameliorating the stress-induced adverse effects generated by heavy metals.

Amongst between different physiological processes and biochemical activities, photosynthesis is the main process that occurs in plants in production of ATP and energy rich compounds. It performs a vital role in maintaining the ecosystem by managing production and utilization rate of energy in the environment (Brestic et al., 2015). Heavy metal stress causes adversative effects in leaf ultrastructure, carbon fixation, leaf size, gene expression, photosynthetic pigments. Enzymes and phytohormones are also associated with photosynthetic pathways (Khan et al., 2019). The elevated level of heavy metals which cause

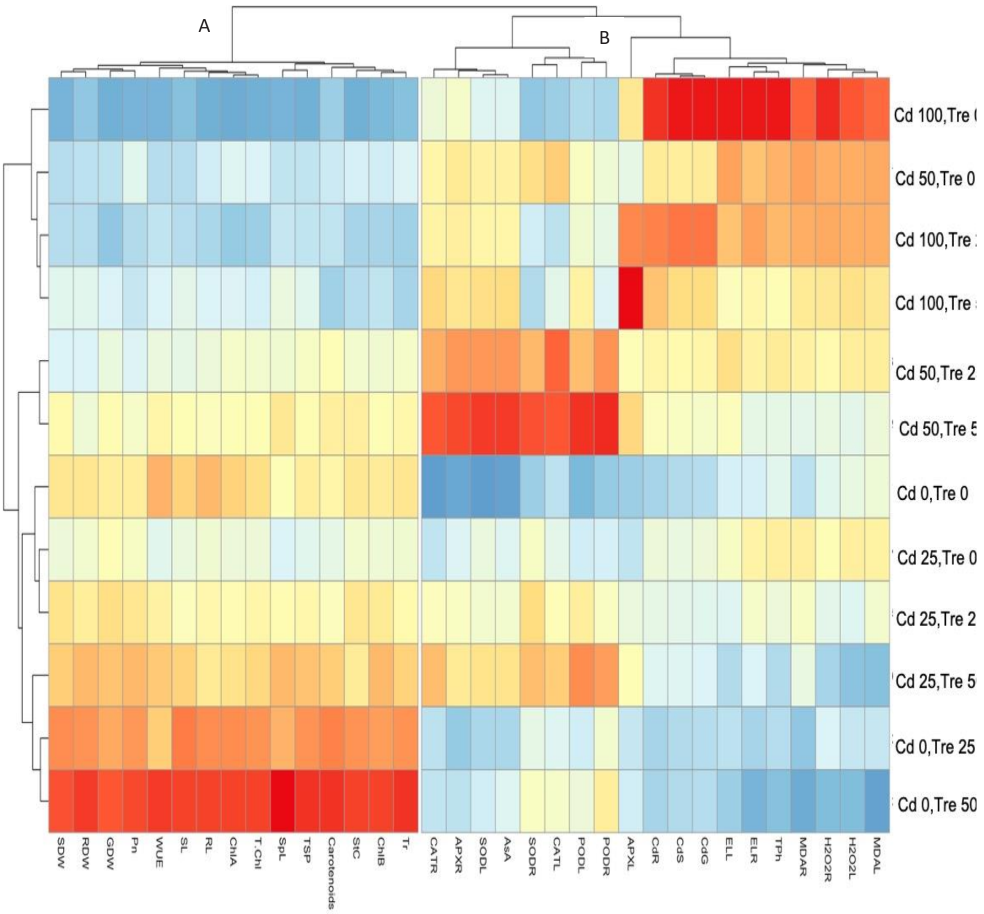

Figure 7. Hierarchical clustering and heatmap analysis about the shoot length (SL), root length (RL), shoot dry weight (SDW), root dry weight (RDW), spike length (SpL), grain dry weight (GDW), chlorophyll a, b, and total chlorophyll (Chl a, Chl b, T Chl), carotenoids, transpiration rate (*Tr*), photosynthesis rate (*Pn*), stomatal conductance (*gs*), water use efficiency (*WUE*) and total soluble proteins (TSP) are shown in cluster A. MDA, H_2O_2, EL in leaves and roots (MDAL, H_2O_2L, ELL and MDR, H_2O_2R, ELR), SOD, POD, CAT, APX in leaves and roots (SODL, PODL, CATL, APXL and SODR, PODR, CATR, APXR), total phenolics (TPh), ascorbic acid (AsA) and cadmium in roots, shoots and grains (CdR, CdS, CdG) are shown in cluster B. The color scale showed the intensity of normalized mean values of different parameters.

production of oxidative stress by producing ROS which ultimately change in genetic makeup, gene expression and enzymes activities and phytochemicals for production of pigments associated to photosynthesis (Silva et al., 2017). Disruption of chloroplast results under Cd toxicity and photosynthetic pigments deterioration in plants (Lee and Back, 2017). It is known that the status of photosynthetic pigments reveals the health of plants under abiotic stresses, including Cd stress that also affects plant photosynthetic efficiency due to alterations in leaf photosynthetic pigments and alters plant growth efficiency as found in the present study (Hussain et al., 2018). In this study, the Cd toxicity also remarkably reduced the leaf photosynthetic pigments, photosynthetic rate, transpiration rate, water use efficacy, and stomatal conductance in the wheat plant. These results are similar to the finding of available published reports in which Cd stress-induced reduction in photosynthesis in cotton (Farooq et al., 2016), maize (Qu et al., 2019; Malkowski et al., 2020), rice (Liu et al., 2020) as well as wheat (Qin et al., 2018; Rizwan et al., 2019).

The chlorophyll in the chloroplast is positively correlated with the rate of photosynthesis in the plant (Zhang et al., 2020). The experimental results depict that leaf chlorophyll contents generally reduce with an increase in the concentration of Cd application, especially at a higher concentration of Cd (10 mg/kg). In the present study, exogenous Tre application significantly improved the photosynthesis and other gas exchange characteristics under Cd stress over control containing only cadmium. The leaf photosynthetic rate was improved due to foliar application of Tre, associated with reduced lipid peroxidation due to cellular membranes and electrolyte leakage in Cd stressed plants. These changes resulted in maintaining the cellular water content leading to better photosynthetic activity and better growth and biomass production of wheat plants. Moreover, from the results it can also be inferred that due to reduced lipid peroxidation of cellular membranes by Tre application especially the chloroplast membranes maintain the integrity of the cellular membranes leading to better functioning of photosynthetic electron transport chain. The findings are in line with the results of Duman et al. (2011) in L. gibba (L.), who reported that the application of Tre with glycine betaine improved the leaf photosynthesis by alleviating the Cd stress. The findings revealed that the Tre application protects the plant's photosynthetic machinery and improves its growth under Cd stress.

Cd stress in body causes overproduction of reactive oxygen species which causes lipid peroxidation which cause degradation of lipid molecules resulting increase he level cholesterol and fatty acid which causes plaque formation into blood arteries and veins (Dar et al., 2017). In this study, Cd stress-induced excessive oxidative stress in plants is revealed by the escalation in the generation of H2O2, MDA, EL and total phenolic acids. Present study results depict that ROS production might have occurred in the wheat plant under Cd stress that can be assessed from the enhanced levels of MDA and H2O2. By contrast, the application of Tre to Cd stressed plants significantly reduced the EL, and accumulation of H2O2 and MDA compared to the Cd treatment alone. These findings suggest a promotive role of Tre in inhibiting oxidative stress in Cd-stressed wheat plants.

To appraise the level of ROS plant extracts are used having the ability to detoxify the concentration of heavy metals like Cd into the body (Bhaduri and Fulekar, 2012). Plants have defensive mechanisms of repairing and mitigating the damage induced by the overly produced ROS by activating several antioxidant enzymes (Shahbaz et al., 2017). SOD, POD, APX, CAT activities and contents of ascorbic acid, phenolics, and proteins play crucial role in maintaining the defensive system against oxidative pressure. In this investigate, Cd toxicity minimized the activities of these antioxidant enzymes in tissues, but this mechanism is plant species-specific and stress level specific. The rapid decline in the activities of antioxidant enzymes under Cd treatment valor happen due to severe oxidative stress. In the present study, Tre application considerably improved the activities of antioxidants enzymes in the Cd stressed plants compared to the Cd, stressed plants without Tre application. The findings can be correlated well with earlier findings where the exogenous application of Tre was found effective in reducing the adverse effects of oxidative stress by improving the activities of antioxidant enzymes in heavy metals stressed crop plants (Bánfalvi, 2019).

In this investigate, wheat plants grown-up underneath Cd stress with foliar application of Tre showed noteworthy distinctions in Cd accumulation in grains, roots and shoots. Plant life have the capability to mineralize the toxic effect of Cd because it accumulated and caused a destruction of biomolecules and cells as well (Nazar et al., 2012). In the present study, an increase in Cd concentration in the growth medium consequently increased the total concentration of Cd in numerous portions of the plants. The accumulation of Cd in various plant parts was as root > shoots > grains. It depicts that more Cd accumulation in roots clearly shows the Cd sequestration in roots, a tolerate mechanism that inhibited the translocation of Cd to upper parts like shoots to avoid Cd toxicity. Similar findings regarding the limited uptake of Cd from root to shoot under Cd stress have previously been stated by Ali et al. (2019). The exogenous submission of Tre suggestively restricted the Cd's accumulation in roots and translocation to the upper parts of the plants compared to plants Cd treatment alone. The findings are similar to the studies of Duman et al. (2011), who demonstrated that exogenous application of Tre found considerably effective in reducing the Cd uptake to various parts of the plants and reduced its adverse effects on the uptake of other essential nutrients (Duman et al., 2011). Thus, exogenous application of Tre is seemed to be very effective in restricting the accumulation of Cd in plants.

5. Conclusion

The present study findings showed that the foliar submission of Tre has increased the tolerance level in wheat plants under cadmium stress. Tre application significantly reduced the uptake of Cd from root to upper plant parts. Prominent decrease in Cd uptake with Tre application that resulted in reduced damaging effects of overly produced ROS in terms of reduced lipid peroxidation. It was also found that the application of Tre effectively maintained the normal functioning of photosynthetic membranes and chlorophyll contents due to improved photosynthetic activity in Cd stressed wheat plants. Furthermore, Tre application found effective for reduction in Cd uptake to grains, showing its role in maintaining grain quality with fewer health risks. The study will be more helpful to increase wheat quality and yield as the application of Tre improved growth, physiology and defense system of wheat plants by decreasing Cd uptake and its translocation to various plant parts. Therefore, additional research is desired to analyze the beneficial protagonist of exogenous Tre application on other crops under field conditions in Cd polluted soil.

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