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Exogenously Applied Trehalose Augments Cadmium Stress Tolerance and Yield of Mung Bean (*Vigna radiata* L.) Grown in Soil and Hydroponic Systems through Reducing Cd Uptake and Enhancing Photosynthetic Efficiency and Antioxidant Defense Systems

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Abstract: Cadmium (Cd) toxicity is a serious environmental issue causing a significant reduction in crop growth and productivity globally. Trehalose (Tre) has emerged as an important reducing sugar that can reduce the adverse impacts of different abiotic stresses. Therefore, the present investigation was performed to determine the key role of Tre in alleviating Cd stress in the mung bean (*Vigna radiata* L.) crop. The study was comprised of different treatments of cadmium (0, 10, 20 mg kg^{-1} soil) and Tre (0, 15 and 30 mM). Cd stress significantly restricted the growth and yield of mung bean. However, Tre supplementation markedly improved growth and yield due to pronounced reductions in Cd uptake and Cd-induced oxidative stress as shown by the lower production of hydrogen peroxide (H_2O_2), electrolyte leakage (EL) and malondialdehyde (MDA) in Cd-stressed plants as well as by the enhanced activities of antioxidant enzymes (CAT, POD, APX and AsA). Moreover, the ameliorative role of Tre to Cd toxicity was also demonstrated by its ability to enhance chlorophyll contents, total soluble protein (TSP) and free amino acids (FAA). Taken together, Tre supplementation played a key beneficial role in improving Cd stress tolerance and yield traits of mung bean through restricting Cd uptake and enhancing photosynthetic capacity, osmolytes biosynthesis and antioxidant activities.

Keywords: cadmium; mung bean; photosynthetic pigments; antioxidants; ROS; trehalose

1. Introduction

Environmental pollution and ecological damages have been substantially increased in recent time owing to rapid industrialization. Heavy metal pollution in the soil has become a serious environmental issue nowadays and it negatively affects food production and human health [1]. Cadmium (Cd) is a non-essential and toxic element [2] readily absorbed by plant roots, causing serious structural and functional alternations as well as inhibition of the seed germination and root growth [3]. Cadmium inhibits the various physiological

processes including photosynthesis, respiration, water movement, leaf gas exchange and, therefore, impairs plant metabolism [4–7]. Additionally, Cd stress reduces the chlorophyll synthesis [8] and induces antioxidant activities by increasing the production of reactive oxygen species (ROS). Cd stress also decreases plant biomass production by inducing oxidative damage and decreasing the nutrient uptake and photosynthetic processes [9–11]. Moreover, Cd stress negatively affects the mineral uptake, transpiration rate and stomatal conductance [12]. Cd-induced injury is related to oxidative stress which causes damages to protein, DNA and lipids [13–18], and eventually leads to plant death [10]. However, plants respond to that and reduce the damages of heavy metals stress through activating their diverse enzymatic and non-enzymatic anti-oxidants and the accumulation of various osmolytes [10]. Cd easily accumulates in plant organs and in turn enters into the human food chain and causes chronic diseases [19]. Therefore, it is urgently need to find appropriate strategies to remediate the Cd contaminated soils to prevent its effects on plants and human health.

Globally, different techniques including leaching, stabilization and phytoremediation are used to remediate the Cd contaminated soils to prevent its impacts on the environment and humans [20,21]. Nonetheless, the current remediation practices to treat heavy metal-polluted soils have shown promising results; however, they are expensive [22]. Likewise, the use of different chelating agents to treat the heavy metal-polluted soils is also expensive and they also cause pollution owing to their artificial footprints and non-biodegradability [23]. Thus, to address these concerns the application of different osmo-protectants is suggested as an imperative strategy to ensure safe production and improve the plant tolerance against different abiotic stresses. Different protectants including proline, glycine betaine (GB) and trehalose achieved global attention due to their excellent efficiency in protecting plants from the deleterious effects of different stresses [24–27]. The selection of a suitable osmo-protectant is crucial to increase the plant's ability to cope with stresses. In this context, it is reported that exogenously applied osmo-protectants protect plants from the adverse effects of heavy metal stress [8].

Trehalose (Tre) is a non-reducing sugar and it is considered as an important osmo-protectant against different stresses [28,29]. Tre formation in plants involves the production of trehalose-6-phosphate (T6P) from glucose-6-phosphate and UDP-glucose by trehalose-6-phosphate synthase (TPS), and the subsequent dephosphorylation of T6P to Tre by trehalose-6-phosphate phosphatase (TPP) [30]. Two molecules of uridine diphosphate glucose (UDP-Glc) and glucose-6-phosphate (Glc-6-P) are used for the biosynthesis of Tre in plants. The enzyme TSP catalyzed UDP-Glc and Glc-6-P into T6P [31,32] whereas enzyme trehalose-6-phosphate phosphatase (TPP) catalyzed the T6P into Tre as a final product [33,34]. Tre is an important energy source and possesses special physio-chemical characteristics (glycosidic bond and higher hydrophilicity); therefore, it stabilizes dehydrated proteins, lipids and membranes and protects the biological structure from oxidative damages [23]. Trehalose acts as an imperious elicitor of the genes involved in stress responses and ROS detoxification [35]. Nonetheless, endogenous Tre production in most plants is not adequate to mitigate the effects of different stresses, therefore, in this case exogenously applied Tre increased the endogenous Tre levels and was recommended as an important alternate strategy to induce stress tolerance [36]. The exogenous Tre application alters the enzymatic activities and, therefore, reduces the ROS level [37]. Exogenously applied Tre increased the biomass production under salty conditions by reducing H_2O_2 and MDA accumulation [35]. Moreover, Tre application increased the activities of the anti-oxidants enzymes (CAT, SOD, POD) and the internal trehalose content in rice plants grown in hydroponic culture under Cd stress [23]. Moreover, formation of Cd–Tre chelation effectively reduces the Cd mobility and toxicity to rice plant organs and therefore improves rice growth under Cd stress [23].

Mung bean (*Vigna radiata* L.) is an important annual crop cultivated globally as grain, vegetable and livestock feed and it is also used for medicinal purposes [38,39]. Mung bean is considered to be sensitive to Cd stress; therefore, Cd stress can cause significant yield

losses in mung bean. Limited information is available about the mechanistic role of Tre in mitigating the deleterious impacts of Cd stress. Thus, this research was carried out to investigate and assess the impacts of exogenously applied Tre on the growth, physiological attributes and antioxidant systems of mung bean plants grown under Cd stress conditions. The present study provides interesting insights into the mechanistic role of Tre in improving Cd stress tolerance and yield of mung bean.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

The present study was conducted to determine the effect of trehalose (Tre) on mung bean (*Vigna radiata* L.) plants grown under cadmium stress. This experimental trial was carried out in pots containing soil collected from the upper soil layer (1–2 cm) from previously grown rice crop. The soil was sandy loam having pH of 7.82, organic matter of 0.82%, nitrogen of 0.042%, available phosphorus of 6.65 ppm and potassium of 160 ppm. The fertilizers di-ammonium phosphate (5.50 g) and sulfate of potash (1.82 g) were applied to each pot to fulfill nutrient needs. Silt and soil were mixed thoroughly (1:2) and pots contained 6 kg soil and silt. The study was comprised of different Cd stress levels, i.e., control (no Cd), 10 and 20 mg kg^{-1} soil and Tre application levels, i.e., control, 15 and 30 mM. Cadmium chloride was used as the cadmium source, and it was thoroughly mixed into the soil. Seeds of Azri-Mung-2006 were obtained from the Ayub Agricultural Research Institute (AARI) Faisalabad and used in the present study. Healthy seeds were sterilized by soaking in 5% sodium hypochlorite solution for 5 minutes and then carefully washed 2–3 times with water. Afterwards, 10 seeds were sown in every pot at 1 cm depth. After 15 days of germination, weeding and thinning were completed to maintain uniform and healthy seedlings (6 plants) in each pot. Moreover, pots were visited regularly and irrigation water was applied to pots according to the crop requirement on the basis of visual experience. Before flowering, the mung bean plants were subjected to the foliar application of Tre (0, 15 and 30 mM). The foliar application of Tre was completed only once using a hand sprayer. The plants were collected at the podding stage and used for the subsequent experimental analyses.

A hydroponic experiment has also been carried out to estimate the morphological and growth traits of mung bean plants grown under natural and Cd stress conditions in order to validate and compare the recorded morphological data with those recorded for plants grown in the above-mentioned soil. The sterilized mung bean seeds were allowed to grow on wet filter papers at 23 °C for 7 days. The germinated mung bean seedlings were then transferred into hydroponic plastic pots, containing Hoagland plant nutrient solution and left to grow in a growth chamber having constant conditions (27/20 °C (day/night) and 70% humidity). At the third leaf stage, uniform seedlings were selected and treated in solutions containing diverse levels of Cd and Tre for four days. The concentrations of Cd and Tre used to treat mung bean plants were as follows: Cd (0, 200, and 400 µM) and Tre (0, 15 and 30 mM). Following treatment for four days, the seedlings were harvested and used for measuring their morphological traits.

2.2. Growth Traits

Three plants per soil and hydroponic pots were used for growth traits' measurement. Roots and shoots were separated and their lengths were measured. Roots and shoots were also weighed to determine their fresh weights. Roots and shoots were then oven-dried (72 °C) for three hours to determine their dry weights. Moreover, the number of leaves per plant were counted and averaged.

2.3. Measurement of Photosynthetic Pigments Contents in Soil-Grown Plants

The standard procedure of Arnon [40] was used to determine the contents of photosynthetic pigments in soil-grown plants. Approximately 0.5 g of plant samples were taken and ground in 5 mL of methanol solution for 24 h. The mixture was then centrifuged for

10 min at 10,000 rpm. The absorbance of the extract was then recorded at 663, 645 and 480 nm to determine chlorophyll (a, b) and carotenoid contents. The following standard formula were used to compute the levels of chlorophylls (a, b) and carotenoids:

$$\text{chlorophyll a} = (12.7\ (OD663) - 2.69\ (OD645)) \times V/1000 \times W$$

$$\text{chlorophyll b} = (22.9\ (OD645) - 4.68\ (OD663)) \times V/1000 \times W$$

$$\text{Carotenoid} = [(OD480) + 0.114\ (OD663) - 0.638\ (OD645)]/2500$$

where V is the volume of sample supernatant and W is the weight of the sample.

For the determination of anthocyanin content, 0.5 g of fresh leaves' sample was homogenized in potassium buffer (10 mL). The mixture was then centrifuged for 15 min at 15,000 rpm. The absorbance of the supernatant was recorded at 600 nm to determine anthocyanin content.

2.4. Determination of Relative Water Content (RWC) in Soil-Grown Plants

For the measurement of RWC in soil-grown plants, the standard protocol of Mostofa and Fujita [41] was used. The second leaf was plucked from different mung bean seedlings and weighed to determine the fresh weight (FW). The leaves were dipped in distilled water and kept in the dark for 24 h. Leaves were left in the air to dry and then weighed to determine their turgid weight. Samples were then oven-dried (70 °C) for two hours to determine dry weight. RWC was then estimated using the following standard formula:

$$\text{RWC}(\%) = \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} \times 100$$

where FW is the fresh weight, DW is the dry weight, and TW is the turgid weight.

2.5. Determination of Electrolyte Leakage (EL), Malondialdehyde (MDA) and Hydrogen Peroxide (H_2O_2) Contents in Soil-Grown Plants

To estimate the electrolyte leakage (*EL*) in soil-grown plants, fresh leaves were collected from each treatment and washed carefully with distilled water. Approximately 0.5 g of leaf sample was cut into pieces and placed in a test tube having distilled water (50 mL) and then first electrical conductivity (EC_1) was read on EC meter after 3 h. Afterwards, test tubes were incubated at 120 °C for 20 min and the second value of EC_2 was recorded. EL was estimated using the following standard formula:

$$EL\ (\%) = (EC_1 \div EC_2) \times 100$$

For determination of the MDA content in soil-grown plants, 0.5 g mung bean leaves were homogenized in 5 mL of 5% TCA. The mixture was then centrifuged for 15 min at 15,000 rpm and the supernatant was collected. A total of 1 mL TCA (0.5%) and 1 mL of TBA (thiobutyric acid: 20%) were then added into the supernatant and placed at 90 °C for 50 min. Absorbance was noted at 532 nm and 600 nm to determine MDA content following Cakmak and Horst [42]. For measuring the H_2O_2 content in soil-grown plants, 0.25 g of plant leaves were ground in 5 mL of (0.1%) w/v trichloroacetic acid (TCA) using a pestle and mortar under chilled conditions. The extract was then centrifuged at 15,000 rpm at 4 °C for 10 min and the supernatant was collected. Approximately, 1 mL of supernatant, 100 µL potassium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide were mixed well and the absorbance was recorded at 390 nm following Velikova et al. [43].

2.6. Determination of the Total Soluble Proteins (TSP) and Free Amino Acids (FAA) in Soil-Grown Plants

Plant leaf samples (0.5 g) were homogenized in 5 mL potassium phosphate (50 mM). The mixture was then centrifuged at 12,000 rpm for 15 min. A mixture of 100 µL of fresh plant extract and 100 µL of Bradford reagent were then prepared. Protein intensity

in leaf tissue was spectrophotometrically measured at 595 nm following Bradford [44]. Moreover, the protocol of the Van Slyke technique [45] was followed to estimate the FAA content of mung bean plants. Fresh leaves (0.5 g) of mung bean were ground in 5 mL potassium phosphate buffer (50 mM) in an ice bath. The homogenate was then centrifuged at 15,000 rpm for 15 min at 4 °C. Plant extract was then treated with 1 mL of ninhydrine (2%) and pyridine (10%) solutions in a test tube. The samples were then heated up at 90 °C for 30 min. After heating, the volume of the mixture was brought up to 20 mL and absorbance was noted at 570 nm.

2.7. Antioxidant Activities Assay in Soil-Grown Plants

To analyze the catalase (CAT) activity of mung bean plants, the procedure of Chance and Maehly [46] was followed. Plant material (0.5 g) was ground in 5 mL of potassium buffer (50 mM). The mixture was then centrifuged at 4 °C and 10,000 rpm for 15 min. Approximately, 2.5 mL of potassium phosphate buffer was added into a test tube containing 100 µL of H_2O_2. After that, 100 µL of plant crude extract was rapidly added into the reaction mixture and absorbance was noted at 240 nm. Peroxidase (POD) activity was estimated following the method described by Guan et al. [47]. Approximately, 0.5 g of leaf sample was ground in 5 mL potassium buffer (50 mM) under ice cold conditions and centrifuged for 15 min at 15,000 rpm and the supernatant was then collected. POD reaction contained 100 µL of H_2O_2, 100 µL guaiacol and 100 µL of enzyme extract, and the absorbance was then read at 470 nm. Ascorbate peroxidase (APX) activity was also assessed following the method of Nakano and Asada [48]. Approximately, 0.5 g of plant samples were ground into 5 mL of potassium buffer (50 mL) and centrifuged for 15 min at 15,000 rpm to collect the supernatant. The reaction medium contained 600 µL H_2O_2, 100 µL ascorbic acid, 1 mL potassium buffer and 100 µL of enzyme extract. Absorbance was then noted at 290 nm. The method of Mukherjee and Choudhri [49] was used to assess the ascorbic acid activity. An amount of 0.5 g of plant leaves were ground in 5 mL of 10 % trichloroacetic acid. The mixture was then centrifuged at 15,000 rpm for 15 min at 4 °C and was then kept for 30 min at 30 °C and absorbance was noted at 520 mM to determine ascorbic acid content.

2.8. Determination of Yield Components in Soil-Grown Plants

Plants' pods were collected and counted and their lengths were measured. Plants were then harvested to determine grains' yield and 100 grain weight.

2.9. Determination of Cadmium Concentration in Organs of Soil-Grown Plants

The plant samples (roots, stems, leaves and grains) were collected, dried and stored. Afterward, 0.5 g of each plant was ground into powder and digested by adding HNO_3: $HClO_4$ in 2:1 ratio [50]. After digestion, the concentration of Cd in plant organs was measured using atomic absorption spectrometry and calculated following the formula: Cd concentration = (reading of AAS × dilution factor)/dry weight of root/shoot/seed and expressed in µg g^{-1} of D.M.

2.10. Statistical Analysis

The collected data were subjected to ANOVA using Statistix 8.1 software and the significant difference among means was computed by LSD test ($p < 0.05$) [51]. Moreover, sigma-plot 10 was used to prepare graphs.

3. Results

3.1. Growth Traits

The findings indicated that the different levels of Tre application and Cd stress had significant impacts on the growth traits of mung bean plants grown in soil and hydroponic systems (Tables 1 and 2). Cd stress significantly decreased the growth and biomass production; however, it was dose-dependent and a higher Cd concentration caused more reduction as compared to a lower dose of Cd stress. Nonetheless, Tre application induced a markedly

increase in both growth and biomass production under control and Cd stress conditions (Tables 1 and 2). The highest RL (6.67 cm) and SL (28.10) were noted under no Cd and 30 mM Tre, and the lowest RL (3.17 cm) and SL (19.20 cm) was noted under highest Cd level and without Tre supplementation (Table 1). Cd stress significantly reduced the RFW, SFW, RDW and SDW of mung bean plants (Tables 1 and 2). Nonetheless, Tre application (30 mM) significantly increased the RFW, SFW, RDW and SDW under Cd stress and control conditions (Tables 1 and 2). Likewise, a decrease in the number of LPP was recorded under both Cd stress levels, whereas Tre application induced a significant increase in LPP under control and both Cd stress levels (Table 1). The application of 30 mM Tre significantly increased the number of LPP as compared to control and 15 mM Tre application under control and Cd stress (10 and 20 mg kg^{-1}) (Table 1).

Table 1. Effect of Tre supply on the growth and biomass traits of mung bean grown in soil contaminated with Cd.

Treatments		Root Length (cm)	Shoot Length (cm)	Root Fresh Weight (g)	Shoot Fresh Weight (g)	Root Dry Weight (g)	Shoot Dry Weight (g)	Leaves Per Plant
Cd_0	Tre_0	4.89 ± 0.029 ^{cd}	25.40 ± 0.036 ^{c}	0.76 ± 0.004 ^{c}	5.23 ± 0.111 ^{de}	0.27 ± 0.003 ^{c}	0.71 ± 0.007 ^{c}	11.0 ± 0.559 ^{ab}
Cd_0	Tre_1	5.90 ± 0.158 ^{b}	27.13 ± 0.059 ^{ab}	1.16 ± 0.044 ^{a}	6.96 ± 0.076 ^{b}	0.34 ± 0.007 ^{b}	0.83 ± 0.005 ^{b}	12.0 ± 0.211 ^{ab}
Cd_0	Tre_2	6.67 ± 0.075 ^{a}	28.10 ± 0.073 ^{a}	1.13 ± 0.021 ^{a}	7.67 ± 0.055 ^{a}	0.41 ± 0.004 ^{a}	1.03 ± 0.020 ^{a}	13.0 ± 0.366 ^{a}
Cd_1	Tre_0	3.43 ± 0.045 ^{f}	22.29 ± 0.124 ^{e}	0.63 ± 0.004 ^{d}	4.73 ± 0.021 ^{f}	0.26 ± 0.004 ^{c}	0.69 ± 0.005 ^{c}	11.0 ± 0.422 ^{bc}
Cd_1	Tre_1	4.13 ± 0.049 ^{e}	23.72 ± 0.039 ^{d}	1.00 ± 0.013 ^{b}	4.93 ± 0.055 ^{ef}	0.26 ± 0.004 ^{c}	0.73 ± 0.007 ^{c}	11.0 ± 0.559 ^{bc}
Cd_1	Tre_2	5.33 ± 0.042 ^{c}	26.57 ± 0.055 ^{bc}	0.84 ± 0.004 ^{c}	6.17 ± 0.042 ^{c}	0.32 ± 0.007 ^{b}	0.88 ± 0.009 ^{b}	10.0 ± 0.366 ^{cd}
Cd_2	Tre_0	3.17 ± 0.091 ^{f}	19.20 ± 0.036 ^{f}	0.49 ± 0.004 ^{e}	3.83 ± 0.092 ^{g}	0.15 ± 0.004 ^{e}	0.57 ± 0.002 ^{e}	9.0 ± 0.634 ^{d}
Cd_2	Tre_1	4.67 ± 0.046 ^{d}	22.97 ± 0.046 ^{de}	0.60 ± 0.005 ^{d}	4.83 ± 0.057 ^{ef}	0.21 ± 0.004 ^{d}	0.64 ± 0.005 ^{d}	9.0 ± 0.332 ^{d}
Cd_2	Tre_2	4.97 ± 0.062 ^{cd}	24.13 ± 0.107 ^{d}	0.77 ± 0.006 ^{c}	5.56 ± 0.112 ^{d}	0.25 ± 0.004 ^{c}	0.70 ± 0.004 ^{c}	10.0 ± 0.211 ^{cd}

The values given in table show the mean of three replicates with ± S.E and different letters show the significant differences at $p < 0.05$. Cd_0, Cd_1 and Cd_2 indicate 0, 10 and 20 mg of Cd kg^{-1} of soil and Tre_0, Tre_1 and Tre_2 indicate 0, 15 and 30 mM Trehalose.

Table 2. Effect of Tre supply on the growth and biomass traits of mung bean grown in hydroponic culture under normal and Cd stress conditions.

Treatments		Root Length (cm)	Shoot Length (cm)	Root Fresh Weight (g)	Shoot Fresh Weight (g)	Root Dry Weight (g)	Shoot Dry Weight (g)
Cd_0	Tre_0	3.55 ± 0.048 ^{e}	20.56 ± 0.077 ^{c}	0.61 ± 0.005 ^{c}	4.71 ± 0.087 ^{d}	0.21 ± 0.004 ^{d}	0.53 ± 0.006 ^{d}
Cd_0	Tre_1	4.88 ± 0.097 ^{b}	21.68 ± 0.062 ^{b}	0.80 ± 0.053 ^{b}	5.11 ± 0.082 ^{c}	0.28 ± 0.008 ^{b}	0.63 ± 0.007 ^{c}
Cd_0	Tre_2	5.79 ± 0.112 ^{a}	22.52 ± 0.054 ^{a}	0.96 ± 0.016 ^{a}	6.15 ± 0.075 ^{a}	0.35 ± 0.006 ^{a}	0.91 ± 0.011 ^{a}
Cd_1	Tre_0	3.06 ± 0.083 ^{f}	17.11 ± 0.086 ^{f}	0.44 ± 0.006 ^{d}	3.61 ± 0.048 ^{f}	0.17 ± 0.007 ^{e}	0.45 ± 0.008 ^{e}
Cd_1	Tre_1	3.95 ± 0.091 ^{d}	18.42 ± 0.104 ^{e}	0.62 ± 0.023 ^{c}	4.62 ± 0.062 ^{d}	0.24 ± 0.005 ^{c}	0.53 ± 0.006 ^{d}
Cd_1	Tre_2	4.42 ± 0.065 ^{c}	19.51 ± 0.113 ^{d}	0.79 ± 0.008 ^{b}	5.58 ± 0.051 ^{b}	0.29 ± 0.006 ^{b}	0.69 ± 0.008 ^{b}
Cd_2	Tre_0	2.81 ± 0.088 ^{g}	14.01 ± 0.098 ^{h}	0.31 ± 0.005 ^{e}	3.01 ± 0.063 ^{g}	0.12 ± 0.006 ^{f}	0.43 ± 0.004 ^{e}
Cd_2	Tre_1	3.56 ± 0.057 ^{e}	15.89 ± 0.087 ^{g}	0.46 ± 0.008 ^{d}	4.19 ± 0.071 ^{e}	0.17 ± 0.005 ^{e}	0.61 ± 0.006 ^{c}
Cd_2	Tre_2	4.34 ± 0.091 ^{c}	17.08 ± 0.111 ^{f}	0.60 ± 0.009 ^{c}	5.07 ± 0.092 ^{c}	0.21 ± 0.006 ^{d}	0.63 ± 0.005 ^{c}

The values given in the table show the mean of three replicates with ± S.E and different letters show the significant differences at $p < 0.05$. Cd_0, Cd_1 and Cd_2 indicate 0, 200 and 400 µM of Cd and Tre_0, Tre_1 and Tre_2 indicate 0, 15 and 30 mM Trehalose.

3.2. Photosynthetic Pigments and Anthocyanin Contents

Cd stress significantly reduced the biosynthesis of photosynthetic pigments (chlorophyll and carotenoid) of mung bean plants. Conversely, Tre application significantly increased chlorophyll and carotenoid contents under control and Cd stress. The highest foliar spray of Tre (30 mM) significantly increased chlorophyll and carotenoid contents under Cd stress (Figure 1). Cd stress also significantly reduced the carotenoid and anthocyanin contents (Figure 1). Nonetheless, Tre application (30 mM) improved carotenoid contents by 22% and 55%, and anthocyanin contents by 13% and 50% under Cd stress (10 and 20 mg kg^{-1}) (Figure 1).

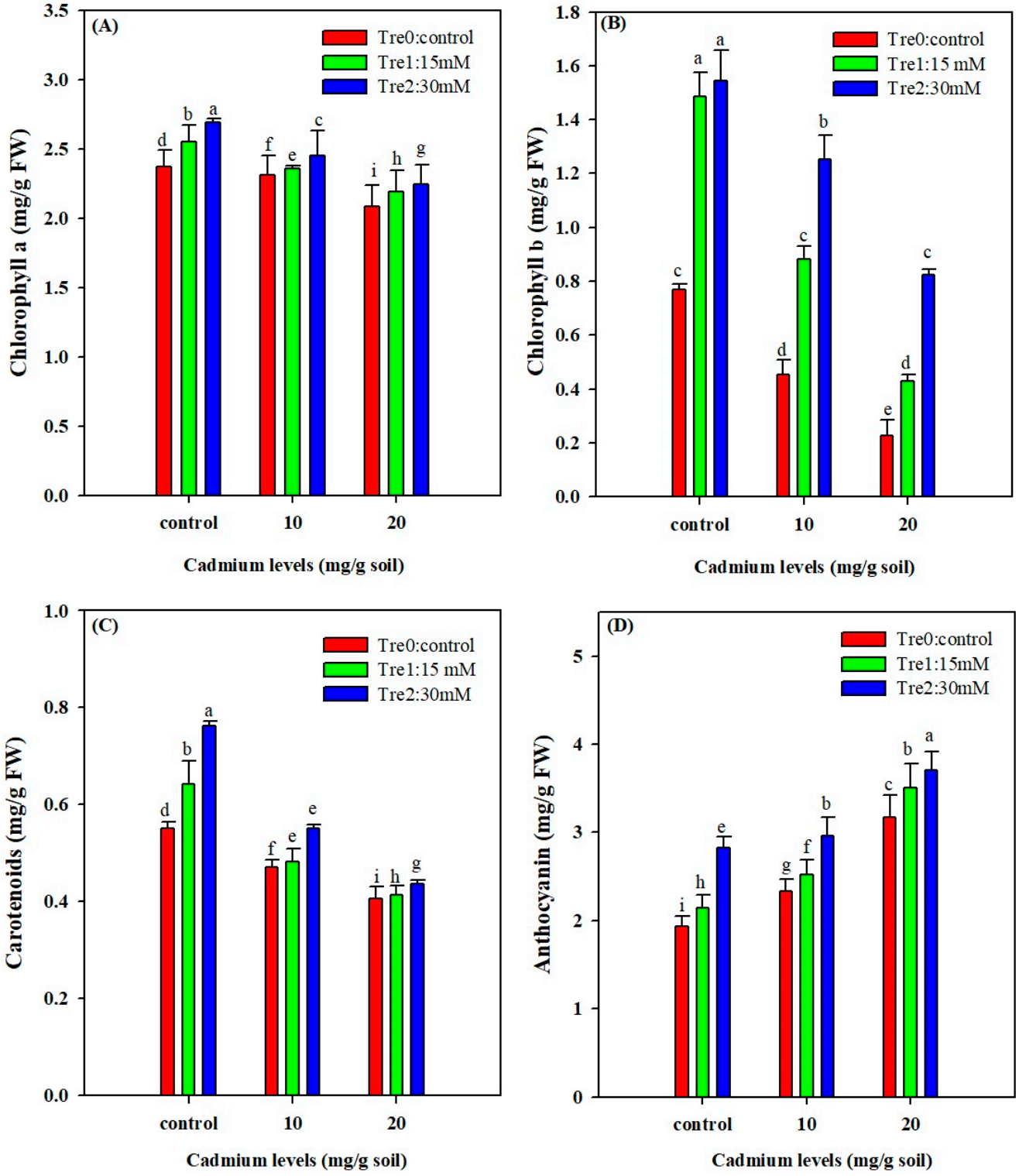

Figure 1. Effect of foliar application of Tre on chlorophyll a (**A**); chlorophyll b (**B**); carotenoid (**C**); and anthocyanin (**D**) contents of mung bean plants grown under Cd stress. The values given in vertical bars are showing the mean of three replicates with ± S.E and the different letters above the bars under the same treatment show the significant differences at $p < 0.05$.

3.3. Relative Water Content

The RWC was significantly reduced under Cd stress. However, the foliar application of Tre markedly improved the RWC under normal and Cd stress conditions. Both levels of Cd stress reduced the RWC and the maximum decrease was recorded under 20 mg kg^{-1}

Cd stress (Figure 2). On the other hand, Tre application (30 mM) enhanced the RWC of mung bean plants by 3% and 9% at the lowest (10 mg kg^{-1}) and the highest (20 mg kg^{-1}) Cd stress levels (Figure 2).

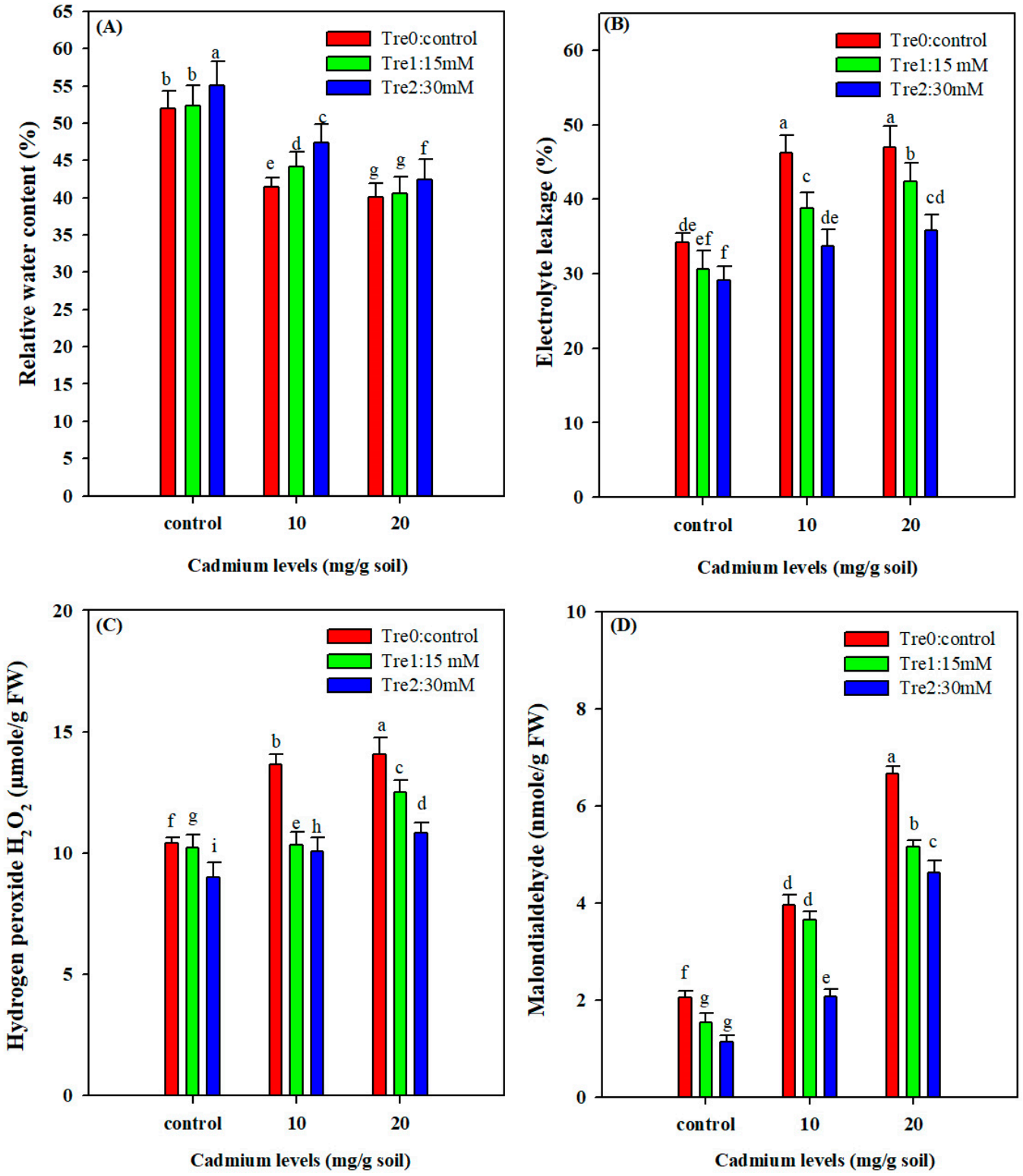

Figure 2. Effect of foliar application of Tre on RWC (**A**); electrolyte leakage (**B**); H_2O_2 (**C**); and MDA (**D**) contents of mung bean grown under Cd stress. The values are the means of three replicates with ± S.E and the different letters above the bars under the same treatment show the significant differences at $p < 0.05$.

3.4. Electrolyte Leakage, MDA and H_2O_2

Electrolyte leakage (EL) showed a significant increase under Cd stress conditions (Figure 2). The maximum EL was recorded in the highest Cd (20 mg kg^{-1}) level without Tre application and the lowest EL was recorded in control (no Cd) with Tre application of 30 mM (Figure 2). As such, Tre supplementation significantly reduced the EL (Figure 2). Foliar spray of Tre (30 mM) minimized the negative effects of EL and reduced the EL by 14% and 29% under the 10 and 20 mg kg^{-1} Cd stress (Figure 2). Cd stress considerably induced the MDA and H_2O_2 contents and their maximum increase was recorded under 20 mg kg^{-1} Cd stress (Figure 2). Conversely, Tre application (30 mM) markedly reduced MDA and H_2O_2 accumulation, indicating its important role in ameliorating Cd stress (Figure 2).

3.5. Total Soluble Proteins and Free Amino Acids

The TSP and FAA showed a marked reduction with an increased in the Cd stress; however, Tre application showed a marked improvement in TSP and FAA under both Cd stress and normal conditions (Figure 3). The TSP was reduced by 46% and 89% whereas FAA was decreased by 51% and 35% under Cd (10 and 20 mg kg^{-1}). The foliar supplementation of Tre (30 mM) significantly enhanced the TSP and FAA contents as compared to control and foliar spray of 15 mM Tre under normal and Cd stress conditions (Figure 3).

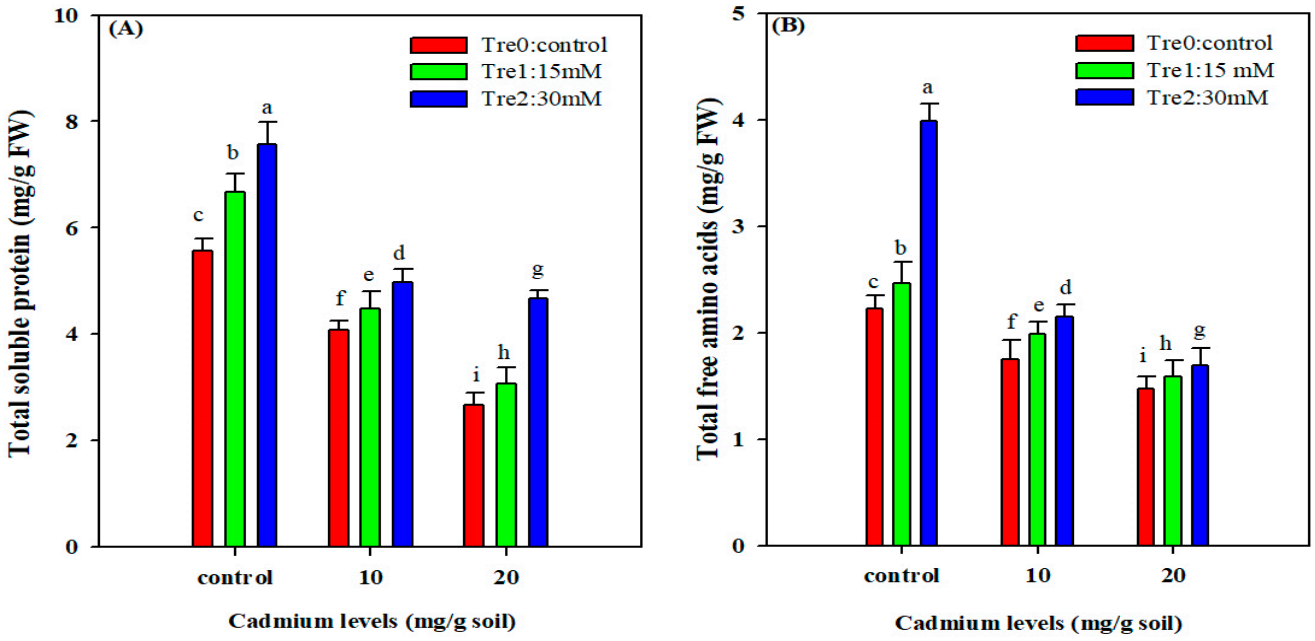

Figure 3. Effect of foliar application of Tre on TSP (**A**); and FAA (**B**) of mung bean plants grown under Cd stress. The values given in vertical bars are showing the mean of three replicates with ± S.E and the different letters above the bars under the same treatment show the significant differences at $p < 0.05$.

3.6. Antioxidant Enzymes Activities

The results demonstrated that the activities of antioxidant enzymes (CAT, POD, APX and AsA) were significantly enhanced under Cd stress (Figure 4). Interestingly, Tre application further enhanced the antioxidant enzymes' activities. The foliar spray of Tre (30 mM) increased CAT, POD, APX and AsA activities under both the Cd stress levels (10 and 20 mg kg^{-1}) (Figure 4).

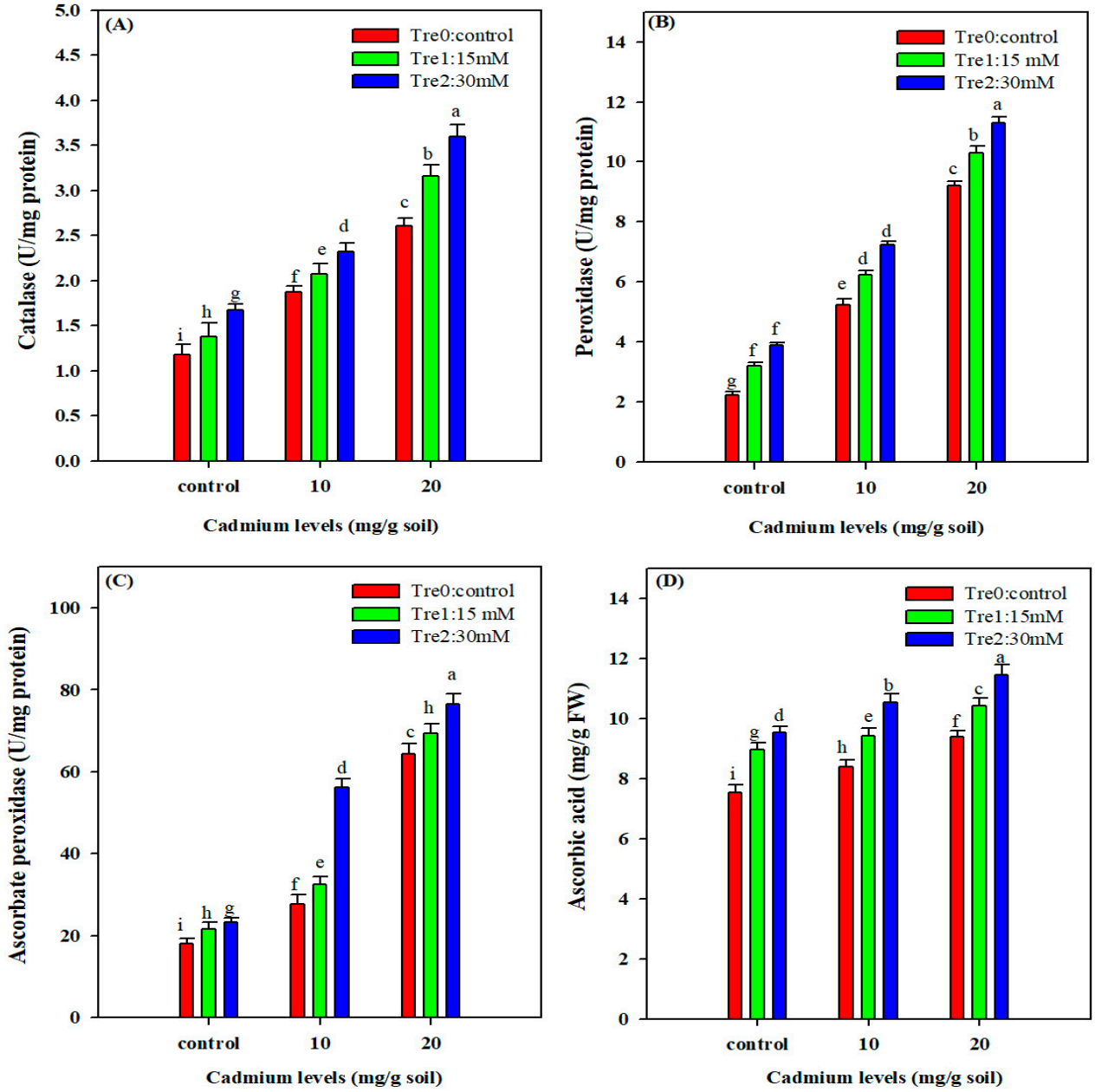

Figure 4. Effect of foliar spray of Tre on activities of CAT (**A**), POD (**B**), APX (**C**) and AsA (**D**) in mung bean plants grown under Cd stress. The values given in vertical bars are showing the mean of three replicates with ± S.E and the different letters above the bars under the same treatment show the significant differences at $p < 0.05$.

3.7. Cd Concentration in Different Plant Organs

The concentration of Cd in the tested plant organs was significantly increased under Cd. On the other hand, Tre application significantly decreased the Cd accretion in plant organs (Figure 5). The maximum Cd concentration in roots and stems was recorded in the highest Cd stress (20 mg kg^{-1}) without Tre application, and the lowest Cd concentration in root and stem was recorded in control (no Cd) with highest Tre application (30 mM) (Figure 5). Likewise, in the leaves and grains, the maximum Cd concentration was recorded under the 20 mg kg^{-1} Cd stress (Figure 5). Tre application (30 mM) significantly reduced Cd accumulation in mung bean seeds and leaves (Figure 5).

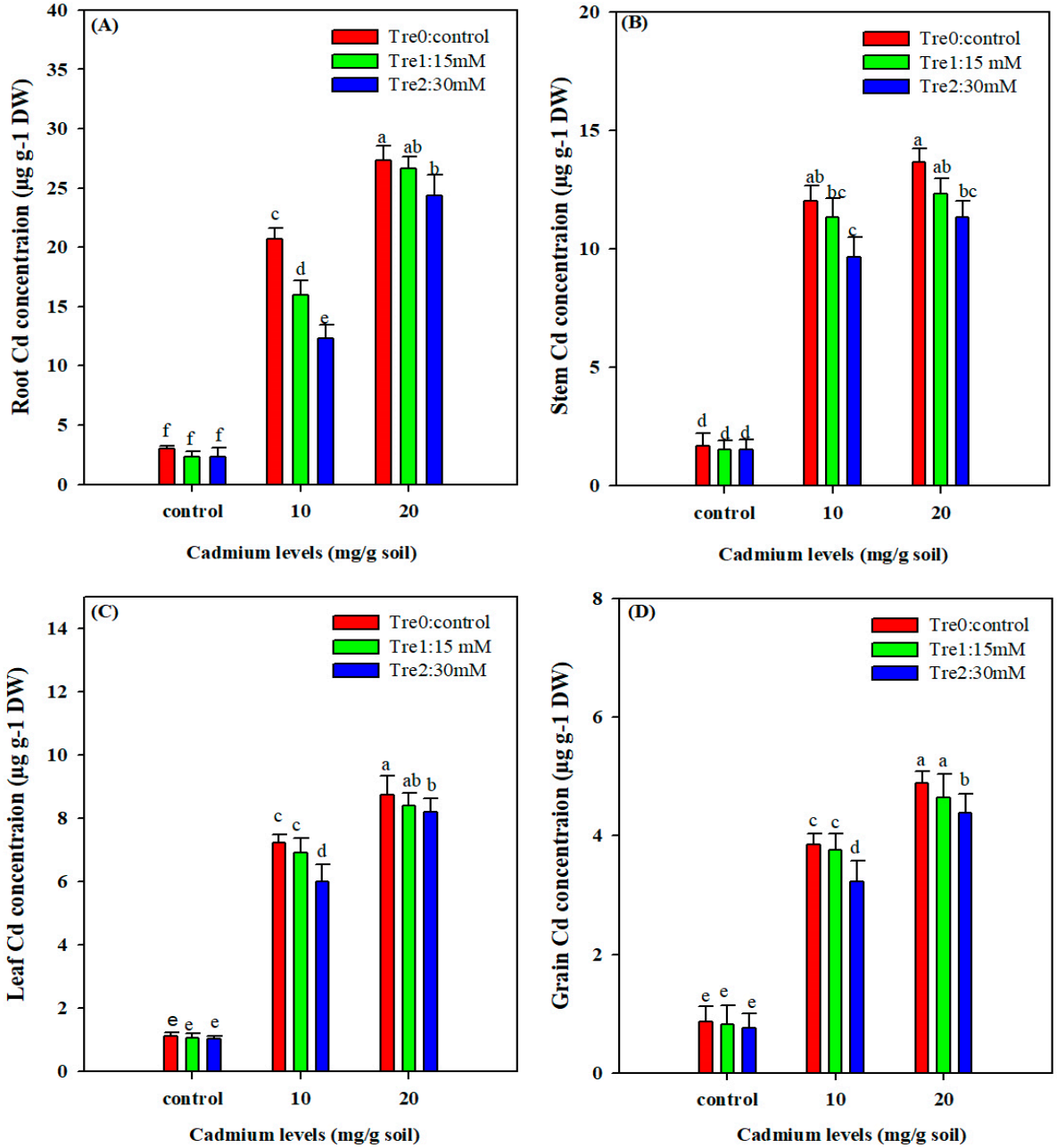

Figure 5. Effect of foliar spray of Tre on Cd concentration in the root (**A**); stem (**B**); leaf (**C**); and grain (**D**). Cd contents of mung bean plant grown under Cd stress. The values given in vertical bars are showing the mean of three replicates with ± S.E and the different letters above the bars under the same treatment show the significant differences at $p < 0.05$.

3.8. Yield Components

Cd stress significantly reduced the yield components of mung bean plants. However, Tre ameliorated the negative effects of Cd stress and improved yield of mung bean plants under normal and Cd stress conditions (Table 3). The longer pods (12.20 cm) with more grains (12.67) were recorded under no Cd stress with Tre application of 30 mM, but the shorter pods (8.02 cm) with minimum grains (8.33) were noted under Cd stress (20 mg kg^{-1}) without Tre application (Table 3). Cd stress levels also significantly reduced the grain weight and grain yield (Table 3) and the maximum reduction was noted under 20 mg kg^{-1} Cd stress conditions. Conversely Tre supply (30 mM) markedly improved the 100-grain weight and grain yield under Cd stress (Table 3).

Table 3. Effect of trehalose supply on yield traits of mung bean crop under different levels of Cd stress.

Treatments		Pod Length (cm)	Grains/Pod	100 Seed Weight (g)	Grain Yield (g pot^{-1})
Cd_0	Tre_0	11.13 ± 0.14 ^{bc}	11.33 ± 0.27 ^{abc}	6.24 ± 0.034 ^{b}	37.67 ± 1.29 ^{bc}
Cd_0	Tre_1	11.46 ± 0.21 ^{b}	12.33 ± 0.29 ^{ab}	6.33 ± 0.053 ^{b}	39.67 ± 1.49 ^{ab}
Cd_0	Tre_2	12.20 ± 0.16 ^{a}	12.67 ± 0.54 ^{a}	6.65 ± 0.062 ^{a}	44.00 ± 1.41 ^{a}
Cd_1	Tre_0	10.20 ± 0.17 ^{e}	9.67 d ± 0.53 ^{de}	5.03 ± 0.030 ^{e}	33.00 ± 1.25 ^{d}
Cd_1	Tre_1	10.62 ± 0.12 ^{de}	10.67 ± 0.27 ^{bcd}	5.47 ± 0.110 ^{d}	32.67 ± 1.18 ^{d}
Cd_1	Tre_2	10.87 ± 0.11 ^{cd}	11.00 ± 0.47 ^{bcd}	5.92 ± 0.072 ^{c}	35.00 ± 0.94 ^{cd}
Cd_2	Tre_0	8.02 ± 0.03 ^{g}	8.33 ± 0.29 ^{f}	4.13 ± 0.064 ^{g}	25.67 ± 0.98 ^{f}
Cd_2	Tre_1	8.60 ± 0.14 ^{f}	8.33 ± 0.30 ^{f}	4.44 ± 0.047 ^{f}	26.67 ± 0.72 ^{e}
Cd_2	Tre_2	9.07 ± 0.07 ^{f}	9.00 ± 0.48 ^{e}	4.61 ± 0.072 ^{f}	28.00 ± 1.69 ^{e}

The values given in the table are showing the mean of three replicates with ± S.E and different letters showing the significant differences at $p < 0.05$. Cd_0, Cd_1 and Cd_2 showing 0, 10 and 20 mg of Cd kg^{-1} of soil and Tre_0, Tre_1 and Tre_2 indicating trehalose application at different levels, i.e., control (0 mM), 15 mM and 30 mM.

3.9. Pearson's Correlation Analysis

The data of diverse traits recorded for soil-grown plants were subjected to Pearson's correlation analysis to determine the relationship among different parameters (Figure 3.9). The results indicated that Cd concentration had positive linking with EL, MDA and H_2O_2 accumulation while it had negative correlations with growth, yield, TSA, FAA and RWC and photosynthetic pigments. Moreover, a strong positive correlation was also noted between Cd concentration and antioxidant activities. Conversely, Tre was positively correlated with biomass, yield, photosynthetic pigments, TSP, FAA, RWC and anti-oxidant enzymes while it had negative correlations with EL, MDA and H_2O_2 and Cd accumulation in different plants' organs.

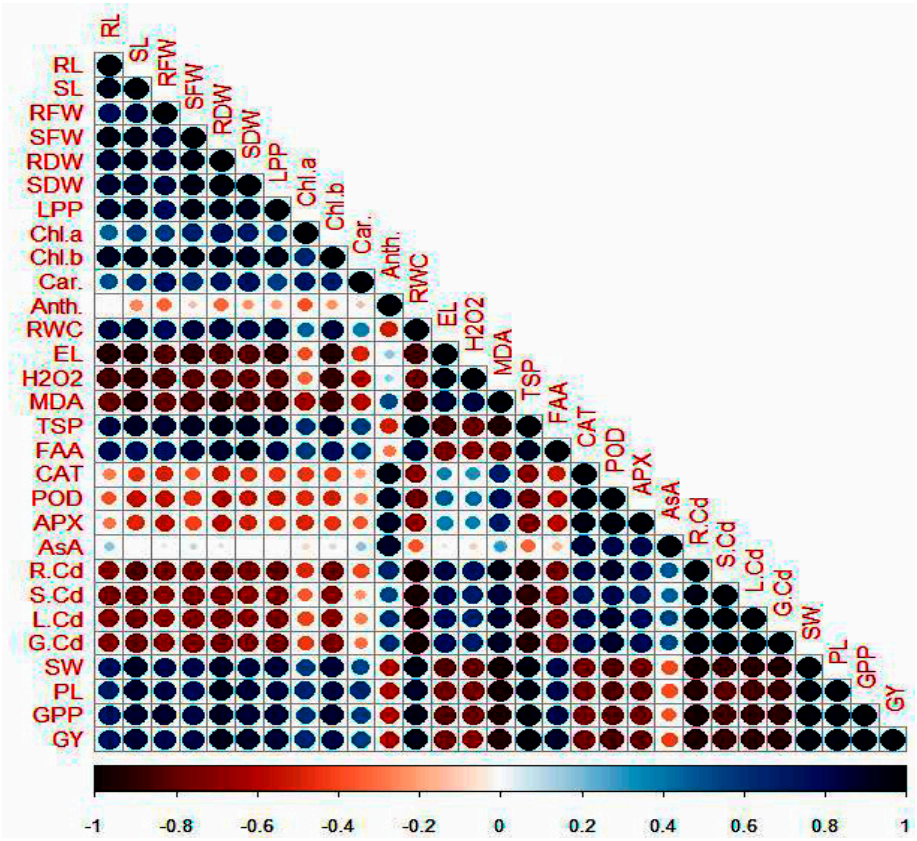

Figure 6. Pearson's correlation between the studied traits of mung bean. Anth: anthocyanin; APX: ascorbate peroxidase; AsA: ascorbic acid; Chl a: chlorophyll a; Chl b: chlorophyll b; Car: carotenoid;

CAT: catalase; EL: electrolyte leakage; FAA: free amino acid; GPP: grains/pod; G.Cd: grain Cd concentration; GY: grain yield; H_2O_2: hydrogen peroxide; LPP: leavers per plant; L.Cd: leaf Cd concentration; MDA: malondialdehyde; PL; pod length; POD: peroxidase; RFW: root fresh weight; RL: root length; RDW: root dry weight; RWC: relative water content; R.Cd: root Cd concentration; SL: shoot length; SFW: shoot fresh weight; SDW: shoot dry weight; S.Cd: stem Cd concentration; SW: 100 seed weight; TSP: total soluble protein.

4. Discussion

Heavy metals induced serious alterations in the physiological and biochemical processes, growth, photosynthetic pigments and antioxidant defenses of plants [52]. Heavy metals' pollution is a serious concern to plant growth and human health; therefore, proper technologies must be adopted to control the heavy metal pollution in order to ensure better crop productivity and human health. Cadmium is a toxic and easily absorbed metal by plants and causes negative impacts on plant growth and development [53]. In the present study, Cd stress induced oxidative stress in mung bean plants, resulting in significant reductions in growth and biomass production [54]. Cd stress also inhibited the nutrients acquisition and photosynthesis which reduced the assimilates' production and thereby caused significant reductions in growth [55,56]. On the other hand, Tre supply significantly increased the growth and biomass production. This increase in plant growth and biomass production by Tre could be attributed to the Tre-mediated increases in RWC and photosynthetic performance and reductions in MDA and H_2O_2 accumulation due to the significant increases in antioxidant activities. We also hypothesized that Tre might also reduce the Cd uptake by forming Tre-Cd complexes and therefore improved the plant growth and biomass production. Trehalose possesses a relatively low surface potential and it readily interacts with Cd, resulting in reduced Cd uptake and improved growth and biomass [57].

Photosynthesis is an imperative plant physiological process that could be severely inhibited by Cd stress, based on the Cd doses applied [10,58]. In the present study, Cd stress markedly reduced the photosynthetic pigments. This was in line with the previous reports which revealed that Cd stress could reduce the absorption of Mg, Fe, K and P from soil and reduce the formation of leaf porphyrin rings, resulting in a marked reduction in chlorophyll synthesis [59] owing to the fact that Mg is a building block in the formation of chlorophyll contents. However, Tre supplementation significantly increased the synthesis of photosynthetic pigments, indicating that Trehalose supply might protect the membrane integrity and photosynthetic apparatus from oxidative stress via the reduction of the activity of chlorophyll degrading enzymes under stress conditions [60].

In the present study, RWC was significantly reduced under Cd stress. This could be attributed to the fact that Cd stress could reduce the osmotic potential and water uptake and subsequently reduce RWC [61]. Moreover, ABA-mediated stomatal closure due to Cd stress could reduce the transpiration pull and led to limited water uptake by plant roots and entails lower RWC in mung bean plants. On the other hand, Tre supply significantly improved RWC under both normal and Cd stress conditions, indicating that Tre supplementation protects the membrane and osmotic potential and scavenges the ROS formation and, therefore, could improve the water retention in plant organs under stress conditions [62]. Moreover, it is also hypothesized that Tre might reduce the ABA-mediated stomata closure and enhance the water uptake and RWC under Cd stress. In the present study, EL, H_2O_2 and MDA also showed a significant increase with increasing the Cd stress. Such increase is possibly due to alterations in membrane structure and changes in cellular homeostasis. However, Tre supply reduced the electrolyte leakage which might be due to the maintenance of membrane integrity and scavenging of ROS due to the increases in antioxidant activities [63]. The exposure of plants to Cd stress induced the production of ROS which damages the membrane [5]. However, in the present study, Tre supplied by foliar application markedly reduced the oxidative stress by decreasing the production of H_2O_2 and MDA contents under Cd stress. The reduction in MDA was due to the decrease in membrane damage. Tre supplementation might maintain the appreciable levels of K^+ and Ca^{2+} under Cd which protect the membranes from oxidative stress and thereby could

reduce MDA and EL under stress conditions. Tre might be participating in stress signaling and triggers osmolytes' accumulation and antioxidant activities and therefore reduces the ROS production under Cd stress.

The exposure of plants to heavy metals might increase the activity of anti-oxidants; however, the very high metal concentration might destroy the protective enzymes system, and thereby decreases the antioxidant activities [64]. The antioxidant enzymes (CAT, POD, and APX) activity was markedly increased under Cd stress. Furthermore, Tre application further increased the antioxidant enzyme activities. Similarly, Tre supply also enhanced the activity of antioxidants which play a crucial role in counteracting the heavy metal-induced stress [65]. Therefore, it can also be concluded that Tre directly mediates the signaling transduction network or indirectly improves osmolytes' accumulations which trigger antioxidant activities and encounter Cd-induced toxic effects.

Proteins play multipurpose functions in plants; however, their activity is degraded under different metal stresses. In the current study, TSP and FAA were significantly reduced under Cd stress. This could be due to the fact that Cd stress toxicity increases protease activity, which triggers the degradation of proteins [66], resulting in reduced protein accumulation under Cd stress. Cd toxicity disrupts the metabolism of amino acids, and changes in amino acid level could play a crucial role in plant responsiveness to Cd stress [67,68]. Tre supply increased the concentrations of FAA and TSP under Cd stress. This increase could be attributed to the ability of Tre to stabilize proteins and dehydrated enzymes involved in protein and amino acid synthesis. Plant species varied in their ability to absorb Cd from the soil and transport it into different plant organs [69]. The quantity of Cd absorbed by plants and its translocation to shoots depend on its bonding with the extracellular matrix, roots efflux, complexion within cells and transport efficiency [70]. In the current study, it was noted that the roots accumulated more Cd as compared to stems, leaves and grains, which could be attributed to the fact that the roots came into direct contact with Cd. However, Tre supplementation had an inhibitory effect on Cd accumulation and significantly reduced Cd accumulation in mung bean plant organs. A possible reason for this reduction could be that Tre acts as barrier to the Cd uptake in plant root which, therefore, tends to reduce the Cd transportation and accumulation in upper plant organs. Furthermore, Tre might also form complexes with Cd and reduce its uptake by plant roots and therefore reduce the Cd accumulation.

Cd toxicity significantly reduced the yield traits of mung bean. This reduction in yield could be attributed to the elevated oxidative stress levels, accumulation of MDA and H_2O_2, Cd uptake and pronounced decreases in photosynthetic pigments, RWC, and protein and amino acids' accumulation. Tre supplementation considerably increased the yield traits under normal and Cd stress conditions. Tre protected the cell membrane and proteins and ensured better chlorophyll synthesis, leaf RWC, TSP and FAA accumulation, antioxidant activities, this markedly improved the yield traits [71].

5. Conclusions

Tre supplementation significantly enhanced Cd stress tolerance of mung bean crop. Cd stress markedly reduced the mung bean growth and yield which was associated with significant reduction in photosynthetic pigments and increases in ROS production and MDA accumulation. Nonetheless, Tre supply markedly improved mung bean yield by decreasing the Cd uptake, improving photosynthetic pigments, TSP and FAA and scavenging ROS by triggering the anti-oxidant enzymes. Therefore, these findings concluded that Tre supply could strengthen mung bean anti-oxidant defenses and ameliorate the Cd-induced deleterious effects on mung bean growth and yield.

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