Pak. J. Bot., 55(5): 1633-1646, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-5(6)

SELENIUM APPLICATION REDUCES CADMIUM UPTAKE IN TOMATO (*LYCOPERSICUM ESCULENTUM* MILL.) BY MODULATING GROWTH, NUTRIENT UPTAKE, GAS EXCHANGE, ROOT EXUDATES AND ANTIOXIDANT PROFILE

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Abstract

In the recent past, the application of trace elements/micronutrients has gained a considerable attention to mitigate harmful impacts of cadmium (Cd) and other heavy metals. In current study we investigated the effects of selenium (Se) in tomato (*Lycopersicum esculentum* Mill.) for mitigating Cd toxicity. Different applications of Se i.e., 2.0, 4.0 and 6.0 µM were employed under 20.0 µM Cd Stress. A significant ($p \leq 0.001$) decrease in physiological, nutrient uptake and plant growth parameters was observed under Cd stress. A decrease of 34% in root length, 21% in shoot length, 40% in shoot fresh weight, 31% in root fresh weight, 24% in shoot dry weight and 20% in root dry weight as compared to control under Cd stress was recorded. Higher levels of antioxidants (enzymatic, non-enzymatic) indicated a higher production of oxidative stress indicators. An increase in SOD (34%), POD (65%), CAT (58%) and proline (81%) was recorded in plants treated with Cd as compared to control (Cd non treated ones). The MDA and H_2O_2 contents in Cd-treated plants likewise exhibited a significant increase. An augmentation of 23% in Chl A/B ratio, 39% in carotenoids, 50% in transpiration rate and 28% in net photosynthesis was recorded as compared to control under Se application. Plants recovery from Cd stress was evident after application of Se. Results indicated a decrease of 18 and 15% in MDA and H_2O_2 contents respectively, due to Se application. The crux of the present investigation revealed that Se application mitigated Cd stress in tomato cultivars used in this study. Tomato var. Nagina proved to be more Cd stress tolerant with lower up take of Cd to aerial parts than Roma. It is concluded from the outcomes of this study that Se improved Cd toxicity tolerance in tomato in terms of its low uptake, enhanced growth, better nutrient uptake, improved photosynthetic and antioxidant attributes. This will pave the futuristic path for exploration of the underlying molecular mechanism for Cd toxicity tolerance with Se application in tomato.

Key words: Abiotic stress; Heavy metal toxicity; Selenate; Photosynthesis; Oxidative stress.

Introduction

Abiotic stress triggers a substantial decrease in plant development and yield (Gull *et al*., 2019). The environment is being polluted by human activities which are carried out to fulfill the ever-increasing demands of a luxurious lifestyle. Different toxic substances are being added into the natural environment including toxic heavy metals (Khan *et al*., 2019). Heavy metal toxicity is a serious inputs demanding issue of the present time causing critical losses in crop yield universally (Branco-Neves *et al*., 2017; Alyemeni *et al*., 2018; Alamri *et al*., 2020). The amount of Cd in the soil is constantly increasing due to undue rocks weathering and industrial operations such as smelting and mining. The Cd contamination is caused by different sources like natural and anthropogenic sources (Ahmad *et al*., 2018). Phosphate fertilizers might be the major source of Cd enrichment in soil. Almost 40-45% of Cd is absorbed and translocated to leaves, shoots, and grains of the plants and may cause adverse effects on human health directly or indirectly (Retamal-Salgado *et al*., 2017; Dinu *et al*., 2021).

Rate of photosynthesis, nitrogen assimilation and absorption are downregulated, moreover chlorophyll fluorescence and antioxidant enzyme activity are altered in plants due to toxic effects of Cd stress (Khan *et al*., 2015; Ahmad *et al*., 2016). It also has a substantial affinity for the sulfhydryl moiety of enzymes, inhibiting their activity deteriorating plant metabolism. The uptake of Cd increases ROS (reactive oxygen species) production, which damage metabolism such as photosynthesis (Khan *et al*., 2015; Khan *et al*., 2019). Important molecules like nucleic acids and proteins are damaged under oxidative stress caused by ROS generation. Heavy metals stress induced ROS production, particularly affecs the plant membranes and nucleic acids thus negatively affecting growth, development (Ahanger *et al*., 2017; Alyemeni *et al*., 2018) and yield (Abass, 2018; Javed *et al*., 2021; Ofoe *et al*., 2022). ROS production reduces the photosynthetic pigments (Rodriguez *et al*., 2012) mineral uptake (Rizwan *et al*., 2017; Lima *et al*., 2019) and water-related phenomena in plants (Mukhopadhyay & Mondal, 2015). Toxic effects of the heavy metals are countered by different types of the defense mechanisms such as enzymatic and non-enzymatic antioxidants (Hashem *et al*., 2016). Antioxidant activity and boosted production of osmolytes all help together to mitigate stress induced damages in plant cells directly or indirectly. Furthermore, metallothioneins and phytochelatins activities along with the compartmentation of harmful metalic ions to fewer sensitive tissues help to alleviate the negative consequences of heavy metals stress including Cd toxicity (Liu *et al*., 2014; Alves *et al*., 2020). In plants, the transport of Cd takes place through the root system into the shoot owing to high solubility in water and its mobility (Lux *et al*., 2011). Its uptake and storage are species specific in plants (Vitória *et al*., 2001; Pereira *et al*., 2002).

Different strategies and supplementation with mineral elements are being used for Cd toxicity ameliorations in plants (Alyemeni *et al*., 2018). According to different researchers, Se fosters plant growth by minimizing the negative effects of metal stress (Khan *et al*., 2015; Ahmad *et al*., 2016).

Tomato (*Lycopersicon esculantum* Mill) is a member of Solanaceae and is a major food crop in the world. It is grown in different areas of Pakistan and is available whole the year to consumers. According to a survey Pakistan is ranked at 34th position in tomato production (Qasim *et al*., 2018). The constituents of tomatoes are vitamin A, C and potassium. The main antioxidant present in tomatoes is lycopene which is used to treat many types of cancer (Adenuga *et al*., 2013). The consumption of tomatoes is increasing because they are an important constituent of human food items. Soil contamination with heavy metals is one of the major causes in the reduction of its growth and yield. The present study was designed to explore the role of sodium selenate (Na_2SeO_4) to alleviate the adverse effects of Cd on tomatoes and to determine the optimal dose of sodium selenate for reducing Cd toxicity in this crop.

Materials and Methods

The study was conducted in plastic pots during November 2019 to March 2020 and two commercial varieties of tomato having different potential against Cd toxicity i.e., Roma (susceptible) and Nagina (tolerant) were grown in wire house. Pots (20 cm hight, 55 cm width at the base and 65 cm at apex) were filled with 6 Kg washed sand. In each pot, five healthy sprouts of the same size were retained. On average, plants were grown under conditions of 24/13°C daytime temperature and an 11/13 h light/dark cycle. At the five-leaf stage, plants were given Cd treatments (0 and 20 M) in Hoagland nutritional solution using cadmium chloride as the Cd source. Treatment was applied for two weeks on alternate days. Sodium selenate treatment (0.0, 2.0, 4.0 and 6.0 µM) was carried out for one week. The experiment was conducted using a completely randomized design (CRD) using 3 replications of each group. After one week of treatment samples were collected and stored at -20℃ for subsequent analyses. Data were recorded for several growth and physiochemical characteristics.

Root and shoot length & weight: Plants were uprooted gently and given distilled water wash following blotted drying for the measurement of fresh weight and root-shoot length. These samples were dried at 65 °C for 24 hours, and the dry weight of the root and shoot was calculated using a digital balance.

Plant pigment and Gas exchange attributes: Photosynthetic pigments were determined using (Arnon, 1949) method. Leaves (0.5 g) were ground in 80% acetone. Samples were centrifuged (Eppendorf Centrifuge 5418 R) for 10 min at 1000 ×*g* and supernatant was separated in a new tube. The absorbance was recorded at 480, 645, and 663 nm using Ultraviolet - Visible spectrophotometer (Model: Hitachi U-2001, Manufacturer: Japan). The amount of total chlorophyll was calculated using the method of Yoshida & Coronel (1976).

$$\text{Chl a mg g}^{-1}) = [12.7(OD_{663})-2.69(OD_{645})] \times V/1000 \times W$$

$$\text{Chl b (mg g}^{-1}) = [22.9(OD_{645})-4.68\ (OD_{663})] \times V/1000 \times W$$

V = Plant extract sample (Volume)

W = Fresh Leaf weight

$$\text{Total chlorophyll (mg g}^{-1}) = 20.2(OD_{645}) - 8.02\ (OD_{663})] \times V/1000 \times W$$

$$\text{Carotenoids (mg g}^{-1}) = [(OD_{480}) + 0.114(OD_{663})-0.638\ (OD_{645}] \times V/1000 \times W$$

Young and completely grown top third leaf was used to calculate gas exchange parameters such as intracellular CO_2 concentration (ppm), Photosynthetic rate (µ mol m^{-2} s^{-1}), transpiration rate (E) and stomatal conductance. Measurements were made between 9:00 and 11:00 in the morning using the mobile CI-340 (Infrared Gas Analyzer) for photosynthesis. (Analytical Development Company, Hoddesdon, USA). Leaf chamber environment was controlled and different parameters like PPFD, CO_2 concentration, humidity and leaf temperature were set as required for optimal plant growth (Hosseinzadeh *et al*., 2016).

Phenolic contents: Julkunen-Tiitto (1985) procedure was used with little modification for estimation of total phenolic contents. Fresh weight 0.5 g was homogenized with 2 ml of 80% acetone. Centrifugation of homogenate at 12000 × *g* was carried out. One milliliter of Folin-reagent Ciocalteau's and two millilitres of distilled water were added after the supernatant (100 µl) had been separated. To bring the volume up to 10 ml after adding 3 ml of 20 % Na_2CO_3, distilled water was added. Shaked this concoction briskly. Uv-vis spectrophotometer (Hitachi U-2001, Japan) was used to record absorbance at 750 nm.

Total soluble proteins (TSP): For estimation of TSP using Bradford (1976) method 0.5 g fresh leaf sample was grinded into the 10 ml of 50 mM phosphate buffer. Centrifuged the sample for 10 min at 12000× *g* using temperature-controlled centrifuge machine. Using a Spectrophotometer (Hitachi U-2001, Japan), recordings for absorbance were taken at 595 nm, for the sample mixed with Bradford reagent.

***G*lycine betaine and proline contents:** Grieve & Grattan (1983) method was employed for estimation of glycine betaine contents. We homogenized 0.5 g fresh leaf in 5 ml of 0.05% toluene solution. Shacked well and filtered it using filter paper. Took 0.5 ml extract in the test tube and added HCL 1ml (2N) solution. Potassium triiodide (0.2 ml) was added in resultant solution (0.5 ml) and kept in a cold bag for 90 min. Removed it from the ice bag and 8 ml of 1-2 dichloromethane along with 2 ml distilled water were added. The first aqueous layer was removed, and the colored layer was used to record absorbance using spectrophotometer (UV-Visible) (Hitachi U-2001, Japan) under 365 nm.

According to Bates *et al*., (1973) method, 0.5 g fresh leaf sample was grinded in 10.00 ml of 3% sulphosalicylic acid. The resultant homogenate was centrifuged at 12000× *g* for 10 min and supernatant was separated. Addition of acetic acid and glacial ninhydrin in 1 ml of supernatant was followed by heating of mixture at 100 °C and subsequent cooling in icebath. After adding 5 ml of toluene, the liquid was vortexed and chilled. The absorbance was recorded at 520 nm using UV-VIS Spectrophotometer (Hitachi U-2001, Japan). The free proline content was determined based on standard curve and was expresses in µmol g^{-1} FW.

Hydrogen peroxide and malondialdehyde (MDA) contents: Hydrogen peroxide content was determined as proposed by Velikova *et al*., (2000). In this technique, 0.5 g of fresh sample leaf material was combined with 5 ml of 0.1 % trichloroacetic acid in a pre-cooled pestle and mortar. The homogenate was centrifuged at 12000 × *g*, discard the supernatant from the mixture. Added 0.5 ml of phosphate buffer to 0.5 ml of the supernatant (pH 7.00). Then, 1 ml of KI was added. A UV-VIS Spectrophotometer (Hitachi U-2001, Japan) was used to measure the absorbance at 390 nm.

Cakmak & Horst (1991) method was used to estimate MDA contents. We used 1 ml supernatant of the previous step extraction and added 4.5 ml thiobabutaric acid (0.5%) in it. The sample mixture was heated at 99°C for 20 min in a water bath. After that the solution mixture was cooled and UV-VIS Spectrophotometer (Hitachi U-2001, Japan) was used to record absorbance at 532 and 600 nm.

Enzyme extraction and assay: Antioxidant activity was measured after the homogenization of samples with 50 µM phosphate buffer (10 ml) maintaining pH of 7.8. Centrifugation at 4°C and 12000 × *g* for 20 min was carried out. The resulting supernatant was kept at -20°C and used to estimate the antioxidant contents.

Chance (1955) method was used to measure superoxide dismutase and peroxidase activities. The CAT activity was determined by reduction of hydrogen peroxide. The reaction mixture consisted of 0.1 ml enzyme extract, 1.9 ml phosphate buffer (50 mM) having pH 7 and I ml of hydrogen peroxide (50 mM) mixed in 10 ml test tube. For two minutes, at intervals of 20 s, absorbance was measured at 240 nm. To evaluate the POD activity, 1.9 ml of 50 mM phosphate buffer, 100 ml (20 mM) guaiacol, 100 ml (40 mM) H_2O_2, and 100 ml of enzyme extract were employed. An Ultraviolet-Visible spectrophotometer (Hitachi U-2001, Japan) was used to measure absorbance at 470 nm for two minutes after every 20 seconds.

Krivosheeva *et al*., (1996) method was followed to determine the ascorbate peroxidase (APX) activity monitoring reduction in absorbance of mixture having hydrogen peroxide and ascorbic acid. Solution was homogenized by mixing 1.9 ml (50 µM) phosphate, 0.5 ml H_2O_2, 0.5 ml ascorbic acid with 0.1 ml extract of plant. Absorbance was recorded at 290 nm.

Giannopolitis & Ries (1977) procedure was followed to determine superoxide dismutase (SOD) activity based upon ability to inhibit reduction of nitro blue tetrazolium chloride (NBT) at wavelength of 560 nm. Using 50 µl crude enzyme extract, 50 µl NBT, mixed with methionine (13 mM), riboflavin (1.3 µM), EDTA (75 mM) in addition with 50 mM buffer (Phosphate) of pH 7.8. Then the solution was stored in an aluminum-coated box. A 30 W fluorescent bulb was used as a light source for samples. After 15 min exposure to the light, the reaction was started, and it was stopped by turning off the light source. Using spectrophotometer (Hitachi U-2001, Japan) absorbance was recorded at 560 nm. Blank sample reading was taken without enzymatic extract. The amount of enzyme required for a 50% inhibition of NBT decrease was found to be one unit of SOD activity.

Determination of flavonoids content: The flavonoid contents were determined by the method of Karadeniz *et al*., (2005). The fresh plant leaf sample (0.1 g) was grinded in 80% acetone. In the pure plant leaf extract, 5% $NaNO_2$ (0.3 ml) added the 3 ml distilled water. After mixing the reaction mixture, it was allowed to reset for 5 min at room temperature. After this added the solution of 1M NaOH (2 ml) and 10% $AlCl_3$ (0.6 ml) into the reaction mixture. Added distilled water into the mixture to make the volume up to 10 ml. Absorbance was recorded at 510 nm.

Ascorbic acid content: Ascorbic acid content was measured as described previously (Mukherjee & Choudhuri, 1983). Fresh leaf material (0.1 g) was grinded in 5 ml TCA (Trichloroacetic acid). Took 4 ml in a test tube from the sample extract and added 2-3 drops of thiourea (10%) prepared in 70% Ethanol and added 2 ml of 2% dinitrophenyl hydrazine. Put the reaction mixture into the water bath for 10 min. Cooled the reaction mixture and absorbance was recorded at 530 nm by a spectrophotometer (Hitachi, U-2001 Japan).

Leaf relative water content (RWC): To measure the water contents in leaves, the method of Smart & Bingham (1974) was applied. Leaf disks of uniform size were punched out from the fresh leaf to determine the leaf RWC. To obtain the turgid weight of the leaf disc that floats on the distilled water, put the leaf disc to the distilled water after recording its fresh weight for 1 hour. The dry weight was measured after placement in oven for 24 hr. The following formula was used to measure the relative water content.

$$\text{RWC} = \frac{\text{Leaf's fresh weight} - \text{Leaf's dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Nutrient ions determination in plant tissue: The oven-dried sample of root and shoot (0.2 g) were grinded into fine powder and transferred into the digestion flasks. A 2 ml (98%) H_2SO_4 was added to each flask and put into the hot plate. The hot plate temperature rose from 50°C to 200°C till fumes were formed. Flasks were taken off the hot plate for a minute and added 1ml H_2O_2 in each flask and repeated this step until digestion was completed and the colorless solution was formed. Solution was maintained at a volume of 50 ml after the filtration process. The filtrate was used to record Cd^{2+}, K^+, Fe^{2+}, $Mg^{2+}$and Ca^{2+} contents by using a flame photometer.

Root exudates determination: The organic acids from root extract were determined by using UHPLC isocratic pump (Flexer Fx-10) (Perkin Elmer, MA, USA) with High-Performance Liquid Chromatography (HPLC). The 80% ethanol was added to the frozen root sample, took 2 ml from the prepared sample, and added to the "C-18 Column" (Brownlee Analytical C-18 (3 mm); dimensions 150 mm and 4.6 mm^2, USA). The acidified acetonitrile solution was used as mobile phase with fixed (pH 4.9) contains acetonitrile, acetic acid and H_2SO_2 in a ratio of 15:1:4. For the determination of organic acid, the flow rate was fixed at 1ml min^{-1} for 10 min. Set the temperature of the column at 45°C and took readings using a detector (UV–vis Series 200, USA) at wavelength 214 nm as described by UdDin *et al*., (2015).

Statistical Analysis

Statistical analysis was carried out to test the significance of differences among mean values using CoStat® Ver. 6.400 software. Correlation and PCA (Pearson's Correlation) among all parameters was done using R Studio® Ver. 4.2.1 for windows 10 (2022).

Results

Growth attributes: Abiotic stress is the main limiting factor for agricultural productivity all over the world and soil contamination with heavy metals is adding more to it. In this study both the varieties (Nagina, Roma) showed 43% - 34% decrease ($p \leq 0.001$) in root length after Cd stress implementation as compared to control respectively (Table 1). The Se application in both varieties increased root length significantly. The S×V×T interaction was not significant. T6 treatment performed much better than all other treatments. An increase of 61% - 49% was observed as compared to stress condition under this treatment (Table 1).

A 27% - 21% decrease in shoot length was observed in both varieties (Nagina, Roma) in comparison to control under Cd stress respectively. After the foliar application of Se, both varieties (Nagina, Roma) exhibited a 28% - 18% increase in overall shoot length of plants. Shoot fresh weight and root fresh weight exhibited a significant ($p \leq 0.001$) decrease under cadmium stress. A 44% - 40% and 33% - 30% decrease in fresh weight of root and shoot of both cultivars Nagina, Roma (Table 1) was observed respectively. After the foliar application of Selenate plant parameters increased significantly After Se application a significant ($p \leq 0.001$) increase (58% - 56%) was observed in shoot fresh weight and 49% - 39% increase in root fresh in both varieties as compared to stress. In both varieties (Nagina, Roma), dry weight of shoot and root exhibited a significant drop ($p \leq 0.001$). Foliar application of Se significantly ($p \leq 0.001$) increased the shoot dry weight (36% - 29%) and root dry weight (30% - 25%) in both varieties respectively as compared to stress (Table 1).

Photosynthetic Pigments: We noted a significant decrease ($p \leq 0.001$) in total chlorophyll contents of both varieties (Nagina, Roma) under Cd stress. A 50% - 46% decrease in total chlorophyll contents was observed under Cd stress in both varieties in contrast with control respectively (Fig. 1). The Se application resulted in a substantial increase ($p \leq 0.001$) in total chlorophyll. Chl. *a/b* ratio exhibited 28% - 23% decrease overall and its decrease was significant ($p \leq 0.001$) as compared to control in both varieties (Nagina, Roma). After the application of Se, Chl *a/b* ratio showed a significant ($p \leq 0.01$) increase, 29% - 23% in the overall production of the chlorophyll ratio as compared to stress in both varieties respectively (Fig. 1). A substantial decrease of 51- 43% was observed after the treatment of Cd stress as compared to control. Cd stressed plants showed a significant ($p \leq 0.001$) decrease in the carotenoid contents in both varieties (Nagina, Roma) respectively (Fig. 1). Foliar application of Se increased it significantly ($p \leq 0.001$) in both varieties (Nagina, Roma) respectively. An increase of 66% - 58% was observed after the foliar application of the Se respectively (Fig. 1).

Table 1. Effect of Se foliar application on growth attributes of tomato varieties under Cd stress.

		RL	SL	SFW	RFW	SDW	RDW	LA
Nagina	T0	11.43 ± 0.23	21.57 ± 0.23	2.47 ± 0.09	1.25 ± 0.08	0.74 ± 0.02	0.32 ± 0.01	24.98 ± 0.38
	T1	12.5 ± 0.29	23.27 ± 0.27	2.77 ± 0.09	1.5 ± 0.06	0.77 ± 0.03	0.35 ± 0.02	27.3 ± 0.40
	T2	15.43 ± 0.35	26.9 ± 0.21	3.27 ± 0.09	1.8 ± 0.06	0.87 ± 0.02	0.41 ± 0.01	30.03 ± 0.27
	T3	13.64 ± 0.33	24.67 ± 0.17	3.03 ± 0.09	1.60 ± 0.05	0.83 ± 0.02	0.37 ± 0.01	28.5 ± 0.25
	T4	7.1 ± 0.21	17.23 ± 0.15	1.5 ± 0.12	0.83 ± 0.07	0.64 ± 0.02	0.27 ± 0.02	21.05 ± 0.30
	T5	8.1 ± 0.32	17.97 ± 0.32	1.8 ± 0.09	1.03 ± 0.05	0.69 ± 0.01	0.29 ± 0.02	22.4 ± 0.31
	T6	10 ± 0.29	20 ± 0.29	2.23 ± 0.12	1.28 ± 0.07	0.73 ± 0.01	0.35 ± 0.02	24.6 ± 0.46
	T7	9.1 ± 0.21	19.07 ± 0.23	2.1 ± 0.10	1.15 ± 0.04	0.71 ± 0.01	0.33 ± 0.01	23 ± 0.38
Roma	T0	8.97 ± 0.26	19.5 ± 0.29	2.23 ± 0.12	0.8 ± 0.06	0.65 ± 0.03	0.3 ± 0.01	22.3 ± 0.42
	T1	10.6 ± 0.31	21.1 ± 0.21	2.53 ± 0.09	1.14 ± 0.08	0.69 ± 0.01	0.33 ± 0.01	25.17 ± 0.44
	T2	13.43 ± 0.30	25.17 ± 0.17	3.03 ± 0.09	1.63 ± 0.09	0.79 ± 0.01	0.38 ± 0.01	29.03 ± 0.37
	T3	11.93 ± 0.23	23.1 ± 0.21	2.83 ± 0.09	1.37 ± 0.09	0.72 ± 0.02	0.35 ± 0.01	27.1 ± 0.50
	T4	5.33 ± 0.24	15.57 ± 0.23	1.3 ± 0.10	0.37 ± 0.07	0.54 ± 0.02	0.24 ± 0.02	18.57 ± 0.38
	T5	6.63 ± 0.27	16.17 ± 0.17	1.63 ± 0.09	0.67± 0.07	0.59 ± 0.01	0.26 ± 0.02	20.2 ± 0.42
	T6	8.33 ± 0.24	18.77 ± 0.15	2.03 ± 0.12	1 ± 0.10	0.64 ± 0.01	0.30 ± 0.01	23.03 ± 0.55
	T7	7.47 ± 0.26	17.9 ± 0.21	1.73 ± 0.13	0.81 ± 0.06	0.61 ± 0.01	0.28 ± 0.02	21.53 ± 0.38

Treatment levels are T0 = Control, T1 = 2 µM Sodium Selenate, T2 = 4 µM Sodium Selenate, T3 = 6 µM Sodium Selenate, T4 = 20 µM Cd, T5 = 20 µM Cd + Sodium Selenate 2 µM, T6 = 20 µM Cd + 4 µM Sodium Selenate, T7 = 20 µM Cd + 6 µM Sodium Selenate. RL = Root length, SL = Shoot length, SFW = Shoot fresh weight, RFW = Root fresh weight, RDW = Root dry weight, SDW = Shoot dry weight and LA = Leaf area

Gas exchange parameters: A significant ($p \leq 0.001$) decrease (36% - 29%) was observed in the net photosynthetic attributes of both varieties Nagina and Roma (Fig. 2) under Cd stress respectively. A substantial ($p \leq 0.001$) improvement in net photosynthesis of the plants was observed after Se application under Cd stress. The CO_2 assimilation decreased significantly ($p \leq 0.001$) under Cd stress in both varieties as compared with control. A decrease of 46%-42% was exhibited by both varieties (Nagina, Roma) respectively (Fig. 2).

After the application of Se, a 52% - 45% increase in overall assimilation took place. The CO_2 assimilation increased significantly ($p \leq 0.001$) as compared to stress after Se application. As compared to the control, both varieties indicated a 62–61% drop in stomatal conductance (Fig. 2). The stomatal conductance of the plants in both var. (Nagina and Roma) decreased significantly ($p \leq 0.001$) because of Cd treatment (Fig. 2). Foliar treatment of Se resulted in a substantial ($p \leq 0.001$) rise in stomatal conductance in both varieties under study.

As compared to control condition, a 70% – 68% drop-in transpiration rate was observed under Cd stress while foliar treatment of Se ended up in a substantial ($p \leq 0.001$) rise (93% - 79%) in transpiration rate of both varieties under study (Fig. 2).

Nutrient uptake: In the present study we observed statistically significant difference ($p \leq 0.001$) in both root and shoot Ca^{+2} content in both varieties with Se application as compared to control (Table 2). While plants treated with Cd exhibited statistically significant ($p \leq 0.001$) decrease in root and shoot Ca^{+2} of both tomato vars (Nagina, Roma). The Cd treated plants exhibited a statistically significant decrease of 28 – 19% in shoot Ca^{2+} and 29 – 22% in root Ca^{+2} as compared to untreated ones (Table 2). Foliar application of Se (4 µM) significantly increased ($p \leq 0.001$) the root and shoot Ca^{+2}.We observed 28 – 20% increase in shoot Ca^{+2} and 38 – 23% in root Ca^{+2} after the application of Se (4 µM) in comparison to control (Table 2).

Root and shoot Mg^{+2} content in present study exhibited statistically significant ($p \leq 0.001$) decrease in Cd treated plants of both varieties (Nagina, Roma). A decrease of 24 – 18% in root Mg^{+2} and 34 – 23% in shoot Mg^{+2} was observed in both varieties (Nagina, Roma) as compared to control (Table 2). Foliar application of Se (4 µM) induced statistically significant ($p \leq 0.001$) increase in both varieties as compared to Cd treated control. An increase of 29 – 18% in root Mg^{2+} and 46 – 26% in shoot Mg^{2+} of both varieties (Nagina, Roma) in comparison to the respective Cd-treated plants (Table 2).

Root and shoot K^{+} exhibited significant increase ($p \leq 0.001$) compared to the control under the administration of Se in both variants. We observed statistically substantial ($p \leq 0.001$) drop in both Root K^{+} and shoot K^{+} of both varieties (Nagina, Roma) under Cd treatment in contrast with untreated respectively (Table 2). A reduction of 31 – 29% was observed in root potassium and 30 – 25% in shoot potassium under Cd (20 µM) treatment in both varieties as compares to untreated ones respectively. The application of Se (4 µM) significantly ($p \leq 0.001$) increased root K^{+} (40 – 33%) and shoot K^{+} (35 – 22%) in both varieties under Cd treatment.

Root and shoot Fe^{+2} contents exhibited statistically significant ($p \leq 0.001$) increase (29 – 22%, 27 – 25%) in both varieties (Nagina, Roma) under Se application as compared to control (Table 2) while Cd treatment significantly decreased root and shoot Fe^{+2} contents in both varieties. An increase of 42 – 24% in root Fe^{+2} and 24 – 19% in shoot Fe^{+2} contents was observed in both varieties (Nagina, Roma) under Se application of Cd treated plants respectively (Table 2).

A statistically substantial increase ($p \leq 0.001$) in root Cd^{2+} (77 – 74%) and shoot Cd^{2+} (73 – 64%) of Cd (20 µM) treated plants as compared to control (Table 2). Foliar application of Se (4 µM) exhibited a considerable ($p \leq 0.001$) reduction in root Cd^{2+} (25 – 19%) and shoot Cd^{+2} (20 – 17% in) contents of both varieties (Nagina, Roma) under Cd treatment as compared to control respectively (Table 2).

Osmoprotectant and oxidative stress indicators: Total soluble proteins of tomato cultivars decreased significantly ($p \leq 0.001$) under treatment of Cd in both varieties. A decrease of 33 – 28% was observed in both varieties in comparison with to control (Fig. 3) while Cd treated plants exhibited a significant ($p \leq 0.001$) increase in total soluble proteins after foliar application of Se (4 µM). An increase of 33 – 29% was indicated in contrast to the Cd-treated control.

The Se foliar application significantly ($p \leq 0.001$) increased the Proline content of both varieties (Nagina, Roma) as compared to control (Fig. 3). Proline content increased drastically after the application of Cd stress. In comparison to the control plants, the Cd-treated plants demonstrated a highly significant ($p \leq 0.001$) increase in proline content (Fig. 3). An increase of 84 – 72% in both varieties under Cd stress (20 µM) was exhibited in both varieties (Nagina, Roma) respectively. Foliar application of Se increased 31% - 14% increase was observed in both varieties as compared to Cd treated control.

We noted a statistically significant increase in total phenolics and flavonoid contents in both varieties (Nagina, Roma) under Se application as compared to control. Foliar application of Se (4 µM) increased the total phenolics content significantly ($p \leq 0.001$) in both varieties as compared to Cd stressed control. An increase of 27% - 19% in total phenolics content of both varieties was observed as compared to Cd treated ones under foliar application of Se.

Flavonoid contents of both varieties presented a significant ($p \leq 0.001$) decrease under Cd stress. Cd stress (20 µM) resulted in 60 – 57% decrease in flavonoids content of both varieties (Nagina, Roma) as compared to control respectively. Foliar application of Se (4 µM) increased the flavonoid content in both plants significantly ($p \leq 0.001$) as compared to Cd stress. Nagina exhibited a significant ($p \leq 0.001$) increase i.e., 75% and Roma also exhibited a significant ($p \leq 0.001$) increase i.e., 64% under Se (4 µM) application as compared to Cd treated control.

MDA and H_2O_2 presented a significant increase after foliar application of Se as compared to untreated plants. Cd stress significantly ($p \leq 0.001$) increased the MDA and H_2O_2 as compared to control. Foliar application of Se increases 81 – 50% and 72 – 35% in MDA and H_2O_2 respectively to stress treated plants (Fig. 4).

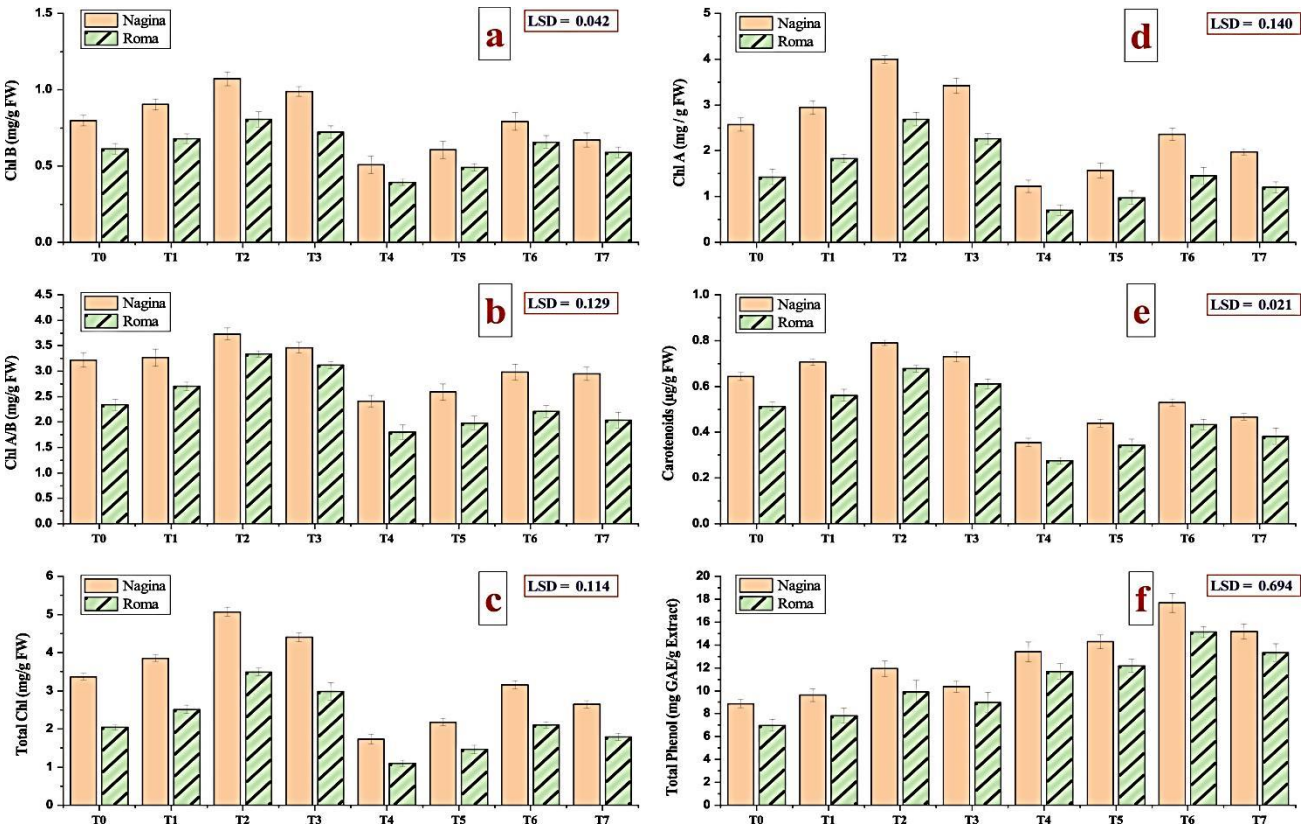

Fig. 1. Effect of foliar application of Se on Chlorophyll b (a), Chlorophyll A/B (b), Total Chlorophyll (c), Chlorophyll a (d), Carotenoids (e), Total Phenol (f) of two varieties of Tomato (*Lycoperscicum esculantum* mill.) (Roma, Nagina) under Cd stress conditions. Treatment levels are T0 = Control, T1 = 2 µM Sodium Selenate, T2 = 4 µM Sodium Selenate, T3 = 6 µM Sodium Selenate, T4 = 20 µM Cd, T5 = 20 µM Cd + Sodium Selenate 2 µM, T6 = 20 µM Cd + 4 µM Sodium Selenate, T7 = 20 µM Cd + 6 µM Sodium Selenate.

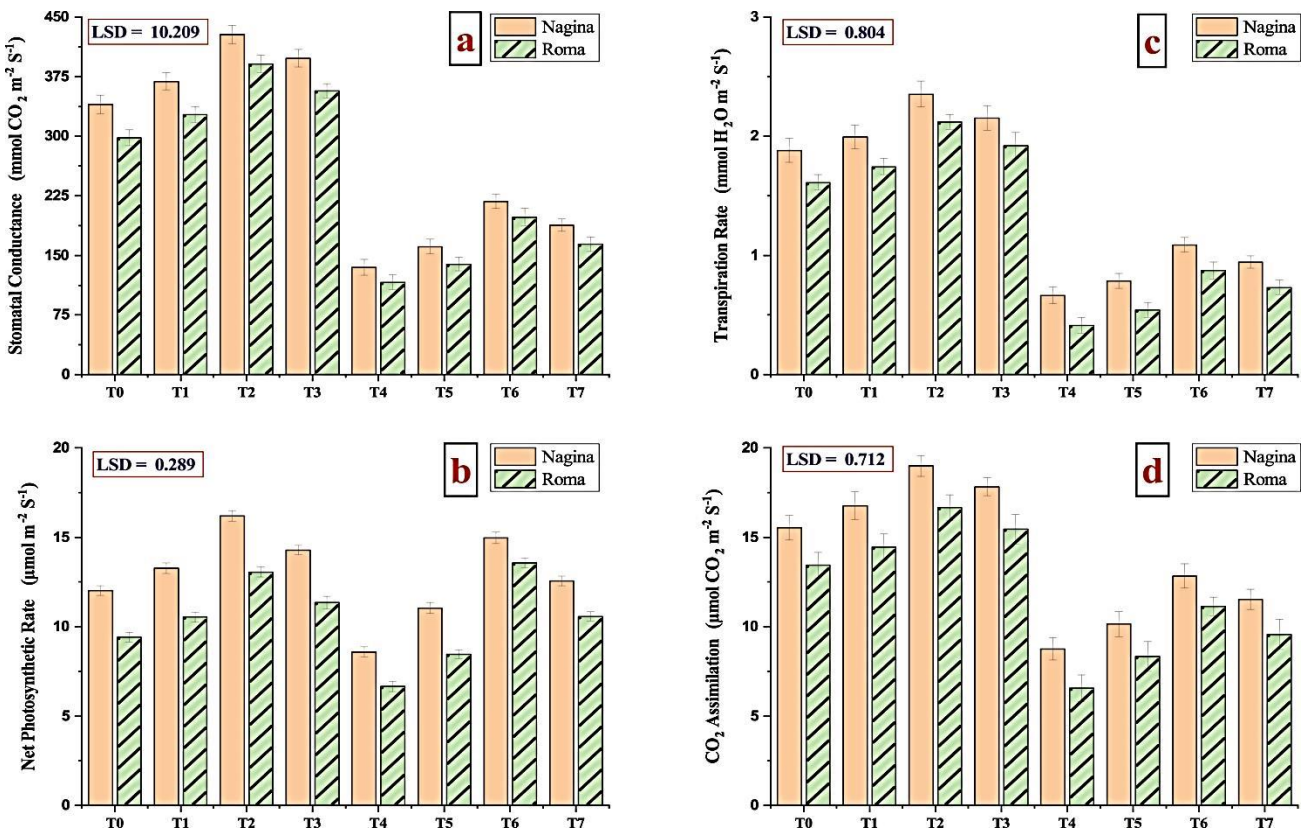

Fig. 2. Effect of foliar application of Se on Stomatal conductance (a), Net Photosynthesis (b), Transpiration rate (c), CO_2 Assimilation (d) of two Tomato (*Lycoperscicum esculantum* mill.) varieties (Roma, Nagina) under Cd stress. Treatment levels are T0 = Control, T1 = 2 µM Sodium Selenate, T2 = 4 µM Sodium Selenate, T3 = 6 µM Sodium Selenate, T4 = 20 µM Cd, T5 = 20 µM Cd + Sodium Selenate 2 µM, T6 = 20 µM Cd + 4 µM Sodium Selenate, T7 = 20 µM Cd + 6 µM Sodium Selenate.

Table 2. Effect of foliar application of Se on Shoot Ca^{2+}, Root Ca^{2+}, Shoot Mg^{2+}, Root Mg^{2+}, Shoot K^{+}, Root K^{+}, Shoot Fe^{2+}, Root Fe^{2+}, Shoot Cd^{2+}, Root Cd^{2+} for different varieties of tomato under heavy metal stress (Cadmium).

		Shoot Ca2+	Root Ca2+	Shoot Mg2+	Root Mg2+	Shoot K+	Root K+	Shoot Fe2+	Root Fe2+	Shoot Cd2+	Root Cd2+
Nagina	T0	6.47 ± 0.23	9.23 ± 0.18	6.63 ± 0.30	2.8 ± 0.12	35.33 ± 0.44	22.33 ± 0.73	0.79 ± 0.03	0.52 ± 0.02	1.94 ± 0.06	7.35 ± 0.12
	T1	6.9 ± 0.17	9.6 ± 0.21	7.01 ± 0.27	2.9 ± 0.16	36.4 ± 0.49	23.73 ± 0.30	0.82 ± 0.02	0.54 ± 0.02	1.81 ± 0.09	6.88 ± 0.11
	T2	7.6 ± 0.26	11.52 ± 0.25	8.03 ± 0.39	3.47 ± 0.15	39.7 ± 0.47	27.2 ± 0.23	0.89 ± 0.01	0.61 ± 0.02	1.61 ± 0.08	6.49 ± 0.20
	T3	7.15 ± 0.19	10.17 ± 0.18	7.37 ± 0.26	3.07 ± 0.15	37.6 ± 0.44	24.83 ± 0.41	0.85 ± 0.02	0.57 ± 0.01	1.72 ± 0.40	6.66 ± 0.13
	T4	5.17 ± 0.18	7.6 ± 0.20	5.03 ± 0.23	2.37 ± 0.12	28 ± 0.29	17.73 ± 0.48	0.65 ± 0.02	0.45 ± 0.02	5.34 ± 0.12	12.02 ± 0.12
	T5	5.53 ± 0.15	7.93 ± 0.15	5.33 ± 0.32	2.48 ± 0.14	29.07 ± 0.23	18.8 ± 0.75	0.67 ± 0.02	0.47 ± 0.01	5.22 ± 0.12	11.13 ± 0.24
	T6	6.24 ± 0.14	9.03 ± 0.18	6.37 ± 0.47	2.75 ± 0.12	33.4 ± 0.47	21.43 ± 0.46	0.73 ± 0.02	0.53 ± 0.03	5.03 ± 0.10	9.8 ± 0.17
	T7	5.93 ± 0.26	8.47 ± 0.15	5.68 ± 0.25	2.6 ± 0.15	31.37 ± 0.46	19.8 ± 0.36	0.71 ± 0.01	0.49 ± 0.02	5.11 ± 0.11	10.19 ± 0.17
Roma	T0	5.5 ± 0.23	8.2 ± 0.17	5.07 ± 0.22	2.22 ± 0.10	29.6 ± 0.46	18.4 ± 0.72	0.72 ± 0.01	0.43 ± 0.01	1.74 ± 0.12	5.33 ± 0.11
	T1	5.8 ± 0.29	8.7 ± 0.12	5.47 ± 0.32	2.34 ± 0.09	30.77 ± 0.41	19.27 ± 0.50	0.75 ± 0.02	0.46 ± 0.02	1.62 ± 0.15	4.54 ± 0.14
	T2	6.5 ± 0.23	10.5 ± 0.17	6.63 ± 0.26	2.77 ± 0.15	34.87 ± 0.45	23.5 ± 0.29	0.82 ± 0.02	0.51 ± 0.01	1.42 ± 0.10	4.01 ± 0.11
	T3	6.13 ± 0.26	9.34 ± 0.11	5.87 ± 0.35	2.5 ± 0.17	32.15 ± 0.36	21.07 ± 0.26	0.78 ± 0.01	0.48 ± 0.01	1.51 ± 0.04	4.27 ± 0.15
	T4	4.43 ± 0.24	6.37 ± 0.23	3.9 ± 0.23	1.82 ± 0.12	22.13 ± 0.24	13.1 ± 0.38	0.58 ± 0.01	0.34 ± 0.01	4.41 ± 0.13	10.36 ± 0.18
	T5	4.67 ± 0.27	6.6 ± 0.17	4.27 ± 0.26	1.99 ± 0.08	23.19 ± 0.40	14.5 ± 0.62	0.61 ± 0.01	0.36 ± 0.02	4.22 ± 0.11	9.59 ± 0.17
	T6	5.33 ± 0.30	7.83 ± 0.18	4.9 ± 0.40	2.14 ± 0.08	27.1 ± 0.21	17.43 ± 0.48	0.66 ± 0.01	0.42 ± 0.02	3.6 ± 0.19	7.55 ± 0.24
	T7	5.03 ± 0.12	7.1 ± 0.12	4.57 ± 0.18	2.04 ± 0.08	25.17 ± 0.40	15.7 ± 0.53	0.63 ± 0.02	0.39 ± 0.01	3.86 ± 0.23	8.49 ± 0.09

Treatment levels are as T0 = Control, T1 = 2 µM Sodium Selenate, T2 = 4 µM Sodium Selenate, T3 = 6 µM Sodium Selenate, T4 = 20 µM Cd, T5 = 20 µM Cd + Sodium Selenate 2 µM, T6 = 20 µM Cd + 4 µM Sodium Selenate, T7 = 20 µM Cd + 6 µM Sodium Selenate

Table 3. Foliar application effect of Se on root organic acids of tomato varieties under Cd stress.

		Cit. A	Ace. A	Mal. A	Tart. A	Oxa. A
Nagina	T0	5.06 ± 0.15	25.93 ± 0.81	2.09 ± 0.09	243 ± 0.88	0.68 ± 0.02
	T1	5.20 ± 0.15	27.47 ± 0.78	2.16 ± 0.05	250 ± 1.24	0.70 ± 0.03
	T2	5.44 ± 0.18	31.60 ± 0.72	2.32 ± 0.08	261 ± 1.20	0.76 ± 0.03
	T3	5.32 ± 0.17	29.10 ± 0.84	2.22 ± 0.06	255 ± 1.45	0.72 ± 0.02
	T4	7.44 ± 0.20	36.17 ± 0.75	3.27 ± 0.07	290 ± 0.96	0.98 ± 0.02
	T5	6.26 ± 0.15	31.47 ± 0.96	2.81 ± 0.06	284 ± 1.20	0.87 ± 0.03
	T6	5.75 ± 0.16	28.90 ± 0.67	2.59 ± 0.09	271 ± 0.86	0.79 ± 0.03
	T7	5.99 ± 0.21	30.17 ± 0.69	2.70 ± 0.06	278 ± 1.07	0.84 ± 0.03
Roma	T0	4.16 ± 0.17	26.27 ± 0.72	2.02 ± 0.07	239 ± 0.93	0.66 ± 0.02
	T1	5.08 ± 0.29	27.03 ± 0.80	2.06 ± 0.06	240 ± 1.18	0.68 ± 0.02
	T2	5.41 ± 0.14	30.27 ± 0.64	2.13 ± 0.09	246 ± 1.45	0.73 ± 0.03
	T3	5.17 ± 0.18	28.53 ± 0.87	2.09 ± 0.05	242 ± 1.15	0.70 ± 0.02
	T4	6.74 ± 0.47	32.23 ± 0.93	2.72 ± 0.03	256 ± 1.01	0.90 ± .02
	T5	6.05 ± 0.19	29.33 ± 1.03	2.61 ± 0.08	253 ± 1.27	0.85 ± 0.02
	T6	5.54 ± 0.19	26.43 ± 0.82	2.30 ± 0.09	250 ± 1.02	0.74 ± 0.02
	T7	5.79 ± 0.20	28.27 ± 0.75	2.51 ± 0.07	252 ± 1.21	0.81 ± 0.02

Treatment levels are as T0 = Control, T1 = 2 µM Sodium Selenate, T2 = 4 µM Sodium Selenate, T3 = 6 µM Sodium Selenate, T4 = 20 µM Cd, T5 = 20 µM Cd + Sodium Selenate 2 µM, T6 = 20 µM Cd + 4 µM Sodium Selenate, T7 = 20 µM Cd + 6 µM sodium selenate. Cit. A= Citric acid, Ace. A= Acetic acid, Mal. A= Maleic acid, Tart. A= Tartaric acid, Oxa. A= Oxalic acid

Ascorbic acid exhibited a substantial ($p \leq 0.001$) rise in both varieties under Cd stress application (Fig. 4). After the application of Se an increase of 18% (Nagina) and 17% (Roma) was observed as compared to stress condition. In both varieties (Nagina, Roma), MDA and H_2O_2 exhibited a significant increase ($p \leq 0.001$). An increase of 81 – 50% and 72 – 35% in MDA and H_2O_2 was observed as compared to the control when exposed to Cd, respectively (Fig. 3). The foliar application of Se significantly ($p \leq 0.001$) decreased both parameters.

Enzymatic Antioxidant: SOD and POD demonstrated a significant ($p \leq 0.001$) rise in both varieties (Nagina, Roma). Under Cd stress, SOD and POD levels increased by 44–30% and 75–57%, respectively as compared to control (Fig. 4). Compared to Cd stress, foliar application of Se boosted the activity of superoxide dismutase (10% - 6%) and peroxidase (14% - 13%) in Nagina and Roma varieties (Fig. 4). Under Cd stress, CAT and APX showed a substantial ($p \leq 0.001$) rise (63% - 49%) and (68% - 58%) in both varieties (Nagina, Roma), respectively. When compared to stress treatment, the foliar application of Se considerably ($p \leq 0.001$) lowered these parameters. Glutathione Reductase increased in both varieties (Nagina and Roma) by 90 - 81% in contrast with respective untreated plants.

Organic acids (root exudates): Citric acid showed significant improvements of 47 and 36% in both verities under Cd stress in comparison with the control (Table 3), but foliar application of Se considerably reduced it ($p \leq 0.001$) in both cultivars (Nagina and Roma). A decrease of 23 – 18% was observed in citric acid after application of Se. Under Cd stress conditions, acetic acid showed a rise of 39 to 23% in both varieties in comparison with control, however foliar application of Se reduced it significantly ($p \leq 0.001$) in both varieties (Table 3).

In both varieties (Nagina and Roma) maleic acid increased up to 56 and 35%, respectively as compared to the control, although foliar application of Se considerably reduced it ($p \leq 0.001$). Under Cd stress tartaric acid in both tomato cultivars under study increased by 19 – 7% as compared to control. Foliar treatment of Se considerably ($p \leq 0.001$) reduced it in both varieties (Nagina and Roma) (Table 3). Under stress, oxalic acid also showed a 45 – 36% increase in both Vars. as compared to the control, but foliar Se application significantly ($p \leq 0.001$) reduced it.

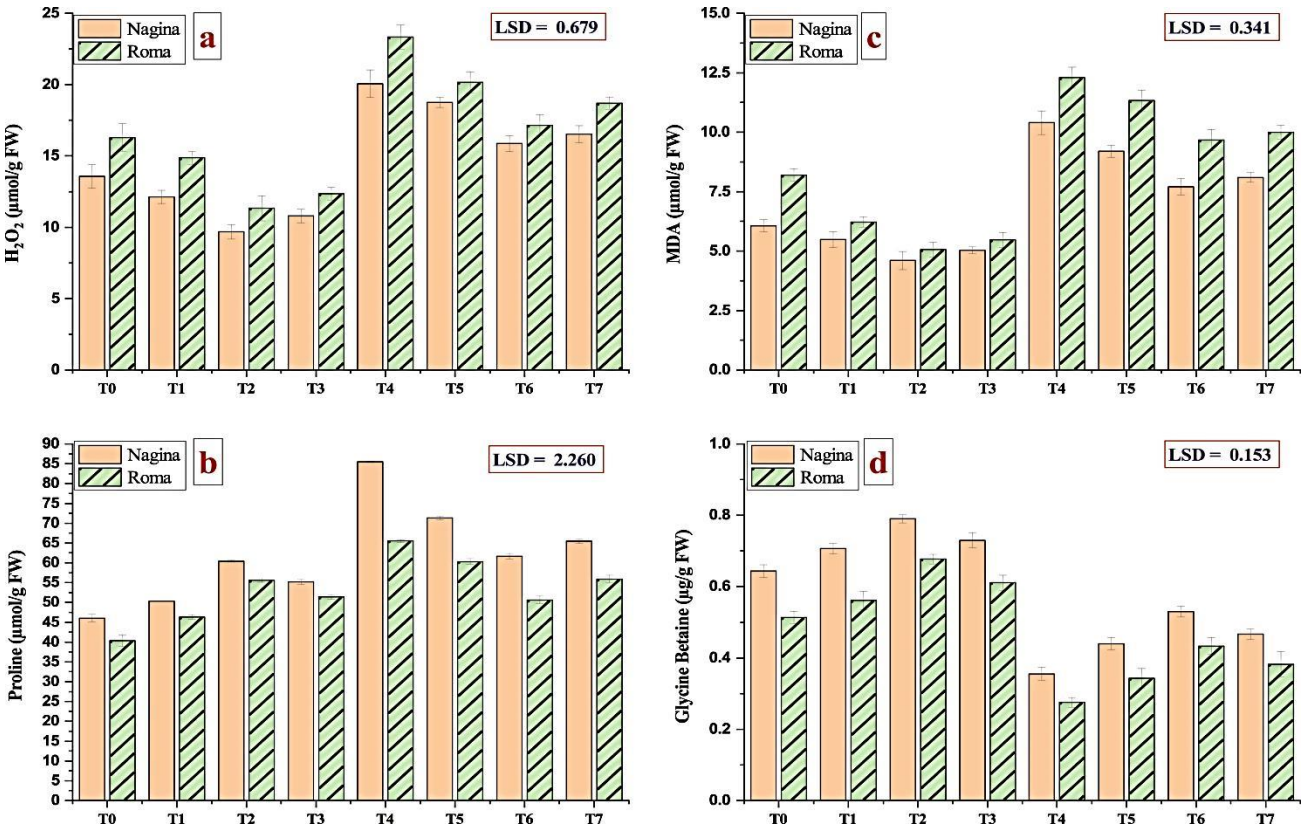

Fig. 3. Effect of foliar treatment of Se on the H_2O_2 (hydrogen peroxide) (a), Proline (b), MDA (Malondialdehyde) (c), Glycine betaine (GB) (d) of two varieties of Tomato (*Lycoperscicum esculantum* mill.) (Roma, Nagina) under Cd stress. Treatment levels are T0 = Control, T1 = 2 µM Sodium Selenate, T2 = 4 µM Sodium Selenate, T3 = 6 µM Sodium Selenate, T4 = 20 µM Cd, T5 = 20 µM Cd + Sodium Selenate 2 µM, T6 = 20 µM Cd + 4 µM Sodium Selenate, T7 = 20 µM Cd + 6 µM Sodium Selenate.

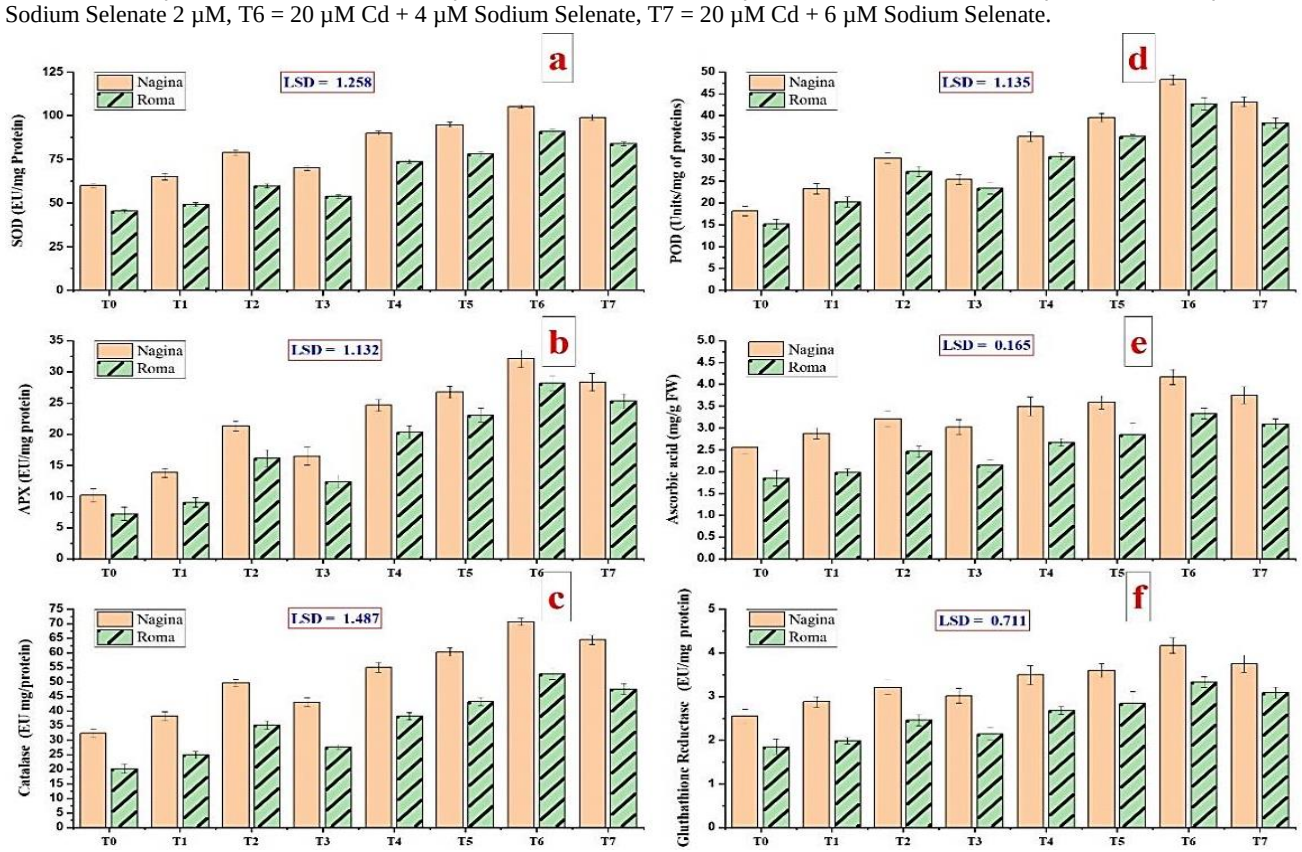

Fig. 4. Effect of foliar application of Se on SOD (Superoxide dismutase) (a), APX (Ascorbate peroxidase) (b), Catalase (CAT) (c), POD (peroxidase) (d), Ascorbic acid (e), Glutathione reductase (GR) (f) of two varieties of Tomato (*Lycoperscicum esculantum* mill.) (Roma, Nagina) under Cd stress. Treatment levels are T0 = Control, T1 = 2 µM Sodium Selenate, T2 = 4 µM Sodium Selenate, T3 = 6 µM Sodium Selenate, T4 = 20 µM Cd, T5 = 20 µM Cd + Sodium Selenate 2 µM, T6 = 20 µM Cd + 4 µM Sodium Selenate, T7 = 20 µM Cd + 6 µM Sodium Selenate.

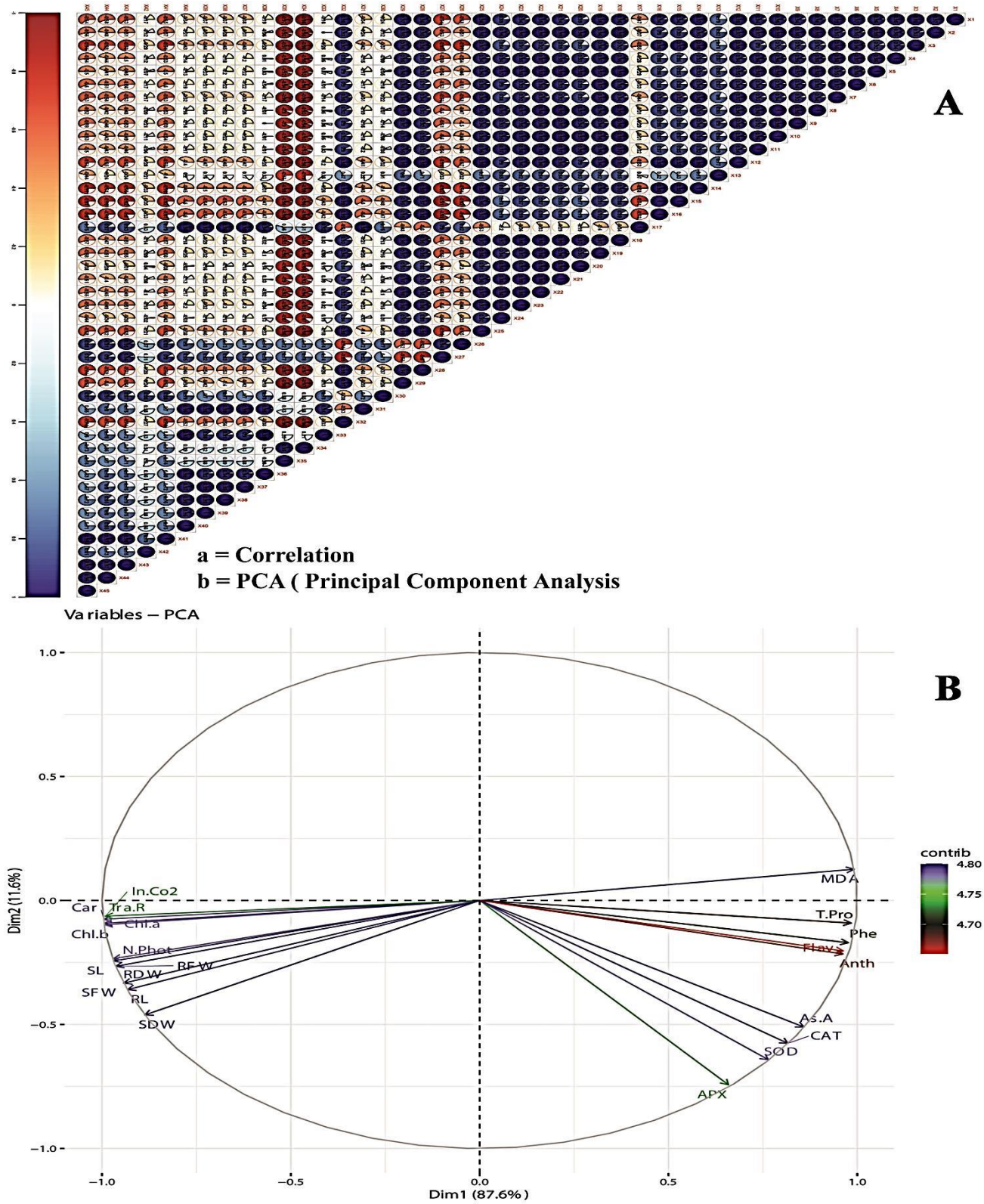

Fig. 5. (a) Correlation between all parameters of Tomato (*Lycoperscicum esculantum* mill. Abbreviations used are as X14 = CO_2 assimilation, X28 = Relative water content, X32 = Flavonoids, X12 = Carotenoids, X16 = Transpiration Rate, X15 = Stomatal conductance, X25 = Shoot's iron content, X03 = Shoot's fresh weight, X29 = Total soluble protein, X01 = Root's Length, X07 = Leaf area, X19 = Root's calcium content, X02 = Shoot's Length, X18= Shoot's Calcium content, X23 = Shoot's potassium content, X22 = Root's potassium content, X06 = Root's dry weight, X10 = Total chlorophyl, X08 = Chlorophyl *a*, X09 = Chlorophyl *b,* X05 = Shoot's dry weight, X04 = Root's fresh weight, X21 = Root's Magnesium content, X11 = Chlorophyl *a/b,* X24 = Root's iron content, X20 = Shoot's magnesium content, X13 = Net photosynthesis rate, X33 = Ascorbic acid, X42 = Acetic acid, X36 = Catalase, X30 = Proline, X40 = Superoxide dismutase, X31 = Total phenols, X38 = Ascorbate peroxidase, X39 = Peroxidase, X37 = Glutathione reductase, X17 = Glycine betaine, X44 = tartaric acid, X26 = Root's cadmium content, X43 = Malic acid, X45 = Oxalic acid, X41 = Citric acid, X27 = Shoot's Cadmium content, X35 = Malondialdehyde.

The correlations among different studied attributes are illustrated in Fig. 5 (a). The CO_2 assimilation has positive correlation with RWC, Flav, Car, Trans.R, S.Con, Shoot Fe^{2+}, SFW, TSP, RL, L.Are, Root Ca^{2+}, SL, Shoot Ca^{2+}, Shoot K^{+}, Root K^{+}, RDW, T. Chl, Chl.a, Chl.b, SDW, RFW, Root Mg^{2+}, Chl a/b, Root Fe^{2+}, Shoot Mg^{2+}, N.PR. CO_2 assimilation has highly negative correlation with SOD, T. Phe, APX, POD, GR, Gly.Be, Ta.A, Root Cd^{2+}, Ma.A, Ox.A, C.A, Shoot Cd^{2+}, MDA, H_2O_2. RWC is in highly positive correlation with Flav, Car, Trans.R, S.Con, Shoot Fe^{2+}, SFW, TSP, RL, L.Are, Root Ca^{2+}, SL, Shoot Ca^{2+}, Shoot K^{+}, Root K^{+}, RDW, T. Chl, Chl.a, Chl.b, SDW, RFW, Root Mg^{2+}, ChlA/B, Root Fe^{2+}, Shoot Mg^{2+}, N.PR.

RWC has a highly negative correlation with T. Phe, APX, POD, GR, Gly.Be, Ta. A, Root Cd^{2+}, Ma.A, Ox.A, C.A, Shoot Cd^{2+}, MDA, H_2O_2. Flav is in highly positive correlation with Car, Trans.R, S.Con, Shoot Fe^{2+}, SFW, TSP, RL, L.Are, Root Ca^{2+}, SL, Shoot Ca^{2+}, Shoot K^{+}, Root K^{+}, RDW, T. Chl, Chl.a, Chl.b, SDW, RFW, Root Mg^{2+}, ChlA/B, Root Fe^{2+}, Shoot Mg^{2+}, N.PR.

Tra. R is in highly positive correlation with S.Con, Shoot Fe^{2+}, SFW, TSP, RL, L.Are, Root Ca^{2+}, SL, Shoot Ca^{2+}, Shoot K^{+}, Root K^{+}, RDW, T. Chl, Chl.a, Chl.b, SDW, RFW, Root Mg^{2+}, ChlA/B, Root Fe^{2+}, Shoot Mg^{2+}, N.PR. Tra. R has highly negative correlation with CAT, Pro, T. Phe, APX, POD, GR, Gly.Be, Ta. A, Root Cd^{2+}, Ma. A, Ox. A, C.A, Shoot Cd^{2+}, MDA, H_2O_2. Fig. 5 (b) presents the Principal Component analysis between plant growth, photosynthetic parameters, biochemical, enzymes, root exudates and ions. S. Con, Trans. R, Flav are negatively correlated with CO_2.Ass, RWC, SFW, Car, Shoot Fe^{2+}, TSP, RL, L.Are, Root Ca^{2+}, Shoot K^{+}, RDW, SL, Root Mg^{2+}, SDW, Root Fe^{2+}, Shoot Mg^{2+}, N.Pr. S.Con, Trans.R, Flav are positively correlated with each other. CO_2.Ass, RWC, SFW, Car, Shoot Fe^{2+}, TSP, RL, L.Are, Root Ca^{2+}, Shoot K+, RDW, SL, Root Mg^{2+}, SDW, Root Fe^{2+}, Shoot Mg^{2+}, N.Pr are also positively correlated with each other. Among the extracted components contribution is 70.1% by Dim 1, and 23.4% by Dim 2, which sums up to 93.5%.

Discussion

Plants are exposed to diverse kinds of stresses worldwide and heavy metal accumulation drastically reduces plant growth and yield. It has been confirmed that tomato and date palm experienced a decrease in biomass and biochemical characteristics (Rahmatizadeh *et al*., 2019; Abass, 2018) due to hazardous effects of heavy metals. The Se was considered toxic for plants in the past but current studies revealed the positive effects of selenate to improve plant growth and development under stress (Silva *et al*., 2018). The absorption of Cd in plants drastically affects the overall growth of the plant (Abass, 2018; Alves *et al*., 2020). Different particles like metals and metalloids at specific concentration tend to reduce heavy metal toxicity (Konate *et al*., 2017) so, it was confirmed that particles like Se help to mitigate heavy metal stress by modulating different response systems in crops (Kumar *et al*., 2012; Ahmad *et al*., 2016). The Cd toxicity inhibits different plant mechanisms by binding to active sites, resulting in poor growth of plants (Sobkowiak & Deckert, 2004).

Plant dry weight and leaf area decreased significantly in the present study. The decrease in plant fresh weight might be attributed to the decrease in RWC. Decrease in RWC reduces rate of cell mitosis and cell elongation (Hassan *et al*., 2016) thus decreasing the biomass and leaf area of plants under study. The Cd stress reduced the plant dry weight in pepper reported by Huang *et al*., (2015) and Hassan *et al*., (2016) as compared with control. These outcomes are aligned with what we observed in our study. Decrease in these parameters might be attributed to more uptake of Cd as compared to other nutrients. The Cd and Ca have a competition for binding with receptors due to which decrease in uptake of one or both can occur. In stressful conditions, excessive Cd uptake destroys the plant metabolic machinery which results in reduced plant vegetative growth. After application of Se the plant growth improved but 2 µM Se with 20 µM Cd had no significant effect as compared to control while 4 µM Se with 20 µM Cd improved plant growth significantly. The higher concentration of Se has negative feedback, as it decreased plant growth as compared to 4 µM Se with Cd treatment. Our results are in alignment with previous studies (Abass, 2018).

Drastic decrease in the pigments might be attributed due to the collapse of chlorophyll in tomato leaves under Cd stress. Heavy metal stress destroys antenna complexes resulting in reduced fluorescence of chlorophyll ultimately disturbing electron transfer from PS-II to PS-I. The Cd might increase production of chlorophyllase which degrades chlorophyll resulting in decreased plant growth. It is obvious that Cd toxicity causes significant increase in ROS generation, which damage cell membrane systems and macromolecules. Many researchers have observed similar results (Parmar *et al*., 2013; Elloumi *et al*., 2014). The Cd toxicity plays an active role by disturbing the enzymatic machinery, which results in decreased production of chlorophyll biosynthesis enzymes like aminolevulinic acid. There was a decrease in Mg uptake. (Zouari *et al*., 2016) in date palm under Cd stress. The mg^{+2} being major component of chlorophyll synthesis (Cenkci *et al*., 2010), its decreased uptake affects the plant growth negatively. The Se applications improve root shoot Mg^{+2} contents thus increasing biosynthesis of photosynthetic pigments. It also increased the uptake of elements like iron, manganese, and zinc. These elements work as cofactors for synthesis of pigments (Feng *et al*., 2013; Feng *et al*., 2021) and play a role in reducing Cd toxicity (Abass, 2018). The Se uptake increased the overall pigments in plant which ultimately increased the total chlorophyll components in both tomato varieties under investigation and produced more assimilates to increase dry mass of plant components under Cd stress. Stress elevation response may be species/genotype specific. Results of Hédiji *et al*., (2015) and Hediji *et al*., (2010) in tomato plants are in alignment with our results.

Relative water content (RWC) was decreased significantly as compared to control under Cd stress. Because minerals are necessary for photosynthesis and metal toxicity harmed the plant's photosystems, thus lowering the rate of photosynthesis which also resulted in decrease of water content. Disturbance in respiration and photosynthetic balance leads to decreased RWC in plants. The Se increased the RWC by ameliorating the Cd toxic effects. Reduction in Cd uptake leads to protection of photosystems. Another attribute for higher RWC might be due to the role of Se in enhancing ROS scavenging activities of plants which improves plant growth (Wu *et al*., 2016; Xu *et al*., 2022). Different types of enzymatic and non-enzymatic antioxidants are produced to encounter stress conditions (Abass, 2018; Feng *et al*., 2021) as it is evident in the present study.

MDA and H_2O_2 are indicators of oxidative damage (Ofoe *et al*., 2022) to the cell components. We found that during Cd stress as ROS increases, the level of MDA and H_2O_2 increase significantly (Abass, 2018; Ofoe *et al*., 2022). Plants exhibited decreased growth, increased MDA and H_2O_2 levels under Cd application. Increased ROS in plants might be result of ROS chain burst. Oxidative stress increased the above parameters overall. Differential production of MDA and H_2O_2 in both varieties is due to change in DNA makeup of both varieties (Rizwan *et al*., 2017). Less levels of MDA and H_2O_2 represents that variety is Cd stress tolerant to some extent. After the application of Se, levels of MDA and H_2O_2 decreased significantly. It might be attributed due to better production of ROS scavenging molecules. Reduction in Cd uptake might be attributed to uptake of Se instead of Cd from roots and binding of Se with active sites for production of ROS scavenging species (Filek *et al*., 2008; Qing *et al*., 2015). According to reports, Se can enhance plant growth which were exposed to heavy metal stress, such as Cd stress (Zembala *et al*., 2010; Wu *et al*., 2016; Riaz *et al*., 2021).

SOD, POD, and CAT are enzymatic antioxidants and help in scavenging ROS species (Xu *et al*., 2022). Due to stress, the increased level of ROS results in activation of plant defense system (Ahmad *et al*., 2017). The defense mechanism of plants in return produce scavengers like SOD and POD, which help in improvement of plant metabolism (Ahmad *et al*., 2015). In stressful conditions these scavengers produce in larger quantity as compared to control but not enough to mitigate the stress condition. This effects the plant photosystems PS-I and PS-II, resulting in low plant growth (Ci *et al*., 2010; EF *et al*., 2015; EF *et al*., 2016). After the application of Se, SOD, POD, GR, and CAT increased significantly. This indicates plant recovery from stress condition and better production of these enzymatic antioxidants helps in the removal of ROS (Hernández-Baranda *et al*., 2019). Decreased levels of ROS can be indicated by low levels of MDA and H_2O_2 (Rahmatizadeh *et al*., 2019). The recovery mechanism behind it might be attributed to the role of Se in protection of chlorophyll machinery. In addition, it might be due to the role of Se in the activation of genes producing antioxidant enzymes (Rizwan *et al*., 2017).

Proline, Phenol, and glycine betaine are basically osmolytes. These all help in amelioration of heavy metal stress (Alyemeni *et al*., 2018). After Cd stress, plant internal mechanism increases these molecules, because they help in mitigating the heavy metal stress. Proline increment during stress indicates the plant recovery, but it's not enough to mitigate the Cd stress. Proline and phenol help in balancing osmolytes in cells. Proline and glycine betaine intend to protect the membrane structure and other parts of the plant cells like amino acids and proteins (Hayat *et al*., 2012; Ahanger *et al*., 2014). By adjusting ROS scavenging machinery, glycine betaine aids in reducing oxidative stress in plants. Proline has been reported for helping metabolism and replacement of water required for metabolic processes. Improved RWC and osmolyte concentration help in amelioration of toxic effects of Cd. Excess of these substances has not been reported as toxic or having negative impact. In contrast excess of these molecules make cellular environment stable by stabilizing cellular structures and cellular functions (Ahanger *et al*., 2017). APX and GR (Glutathione Reductase) are involved in formation of different components for the glutathione and ascorbate cycle-ROS detoxification pathway (Ahmad *et al*., 2018). After the implementation of selenate these parameters increased significantly. Improvement in plant metabolism and better production of these attributes might be due to Se induced upregulation and prevention of cellular metabolism from ROS and oxidative damage (Ahanger *et al*., 2015). 2 µM Se with 20 µM Cd had no significant effect as compared to control. 4 µM Se with 20 µM Cd improve plant growth significantly. 6 µM Se with 20 µM Cd has negative feedback, as it decreases osmolytes as compared to 4 µM Se with Cd.

Stomatal conductance, Net photosynthesis, Transpiration rate and CO_2 assimilation were severely hindered by Cd stress. Gas Exchange parameters were reduced significantly resulting in reduced water uptake. Cd uptake in roots results in less uptake of water and other useful components. Due to Cd stress, antenna complex of photosystems was destroyed (Elloumi *et al*., 2014). Due to which photosynthetic rate was severely disturbed. Due to disturbance of net photosynthetic rate, uptake of water and mineral transport for photosynthesis decreased significantly (Khan *et al*., 2015; Zouari *et al*., 2016). CO_2 assimilation also decreased significantly. Reduced photosynthesis results in decreased stomatal conductivity. Se application improved the gas exchange parameters by improving photosynthesis. Se helps reducing oxidative stress results in better performance of photosynthetic machinery. It then improves stomatal conductance, transpiration rate, CO_2 assimilation. In alignment with our results, (Haghighi *et al*., 2016) reported increased Transpiration rate, CO2 accumulation, Net photosynthetic activity, and Stomatal conductance in *Cucumis sativus*. Differential rates might be attributed due to changes in genetic make-up of different species.

Cd stress significantly decreased the ions uptake like Ca^{2+}, Mg^{2+}, Fe^{2+} and K^{+}. The Cd as a divalent ion can compete with different ions Ca^{2+}, Mg^{2+} and Fe^{2+} while moving across the membrane. Uptake of Cd^{2+} is hindered by Ca^{2+} presence and vice versa. These both compete for

Ca^{2+} uptake channels. So, concentration of one can or can be affected by the uptake of others. (Sarwar *et al*., 2010) reported the reason for decrease in decreased Cd toxicity. Phytoclatin formed by the distribution of Cd in different partitions of plant. Fe also helped in decreased uptake of Cd and its transportation. Iron is part of photosynthetic machinery of plants that take part in enhancing photosynthesis during high light phase (Nazar *et al*., 2012). Cd stress increased the root exudates significantly. Due to presence of tri-carboxyl group, citrate and malate form stable compounds with metal cations (Qin *et al*., 2007; Qing *et al*., 2015). Increase in organic acid concentration as root exudates might be a counter mechanism for amelioration of metal stress.

Acknowledgments

The first author is thankful to the Department of Botany GCUF for providing resource to complete this work. The findings of this work are a part of Doctorate's studies of Muhammad Bedar Bekhat Naseem.

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(Received for publication 22 April 2022)