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MITIGATION OF CADMIUM INDUCED STRESS IN TOMATO (*SOLANUM LYCOPERSICUM* L.) BY SELENIUM

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Abstract

Pot experiments were performed to assess the role of selenium in growth regulation of *Solanum lycopersicum* and its ameliorative role under cadmium stress. Cadmium (200 µM $CdCl_2$) treatment reduced growth considerably and application of selenium (1.2µmol/L) improved growth and also mitigated the deleterious effects of cadmium. Selenium increased chlorophyll and carotenoid which were however reduced by cadmium stress. Cadmium treated plants showed higher lipid peroxidation and selenium caused improvement in membrane stability by reducing the production of hydrogen peroxide. Activities of antioxidant enzymes assayed increased with selenium treatment providing stability to cellular structures by quick removal of reactive free radicals. GSH and AsA were increased by 14.2% and 21.07% due to selenium application, however along with cadmium, it caused an increase of 40.5% and 45.7% respectively. Proline and phenol content increased with selenium providing osmotic stability to *S. lycopersicum* for cadmium tolerance. Moreover, selenium treatment declined the uptake of cadmium significantly.

Key words: Tomato, Cadmium, Antioxidants, Proline, Phenols, Lipid peroxidation, Selenium.

Introduction

The increase in concentration of cadmium is due to both natural as well as anthropogenic factors. Being toxic to living organisms and plants even at low doses, cadmium shows high mobility between soil and plants. After being taken up from soil cadmium is translocate to the upper plant parts including the edible parts, hence consumption of such cadmium containing plant products constitute a major source of Cd exposure in humans and animals (Kubo *et al*., 2016). Cadmium is continuously added to soil as a result of weathering of rock, processes like smelting and mining and overuse of phosphate fertilizer. Uptake of cadmium triggers degradation of chlorophyll pigments resulting in restricted phosynthetic rate in addition of affecting the nitrogen metabolism and enzyme activity (Khan *et al*., 2015). Cadmium shows strong affinity for sulfhydryl moiety of enzymes thereby inhibiting their activity and ultimately affecting the plant metabolism. Elevated uptake of cadmium causes enhancement in the production of reactive oxygen species (ROS) and the ROS poses damage to important metabolic processes like photosynthesis (Asgher *et al*., 2014; Khan *et al*., 2015). ROS induce oxidative stress therefore can damage metabolically important molecules like nucleic acids, proteins etc.

Several protective mechanisms are triggered to assuage the negative impact of the stress induced changes in plants (Abd_Allah *et al*., 2015; Hashem *et al*., 2015; 2016). Greater production of osmolytes like proline, glycine betaine, sugars and increased antioxidant activity directly or indirectly contribute to mitigation of stress induced damage to plant cells. In addition to this compartmentation of deleterious metal ions to less sensitive tissues and chelation of metals and metalloids by metallothioneins and phytochelatins contribute to mitigate the ill effects of stress (Liu *et al*., 2014).

From times efforts studies have been carried to lessen the cadmium induced damage by inducing several strategies like use of growth hormones, biological means like plant growth promoting bacteria or soil borne fungi and the appropriate supplementation of mineral elements (Ahanger *et al*., 2014; Abd_Allah *et al*., 2015; Iqbal *et al*., 2015; Ahmad *et al*., 2016). Addition of mineral elements promotes plant growth because of their widely accepted importance in plant growth regulation (Iqbal *et al*., 2015; Ahanger *et al*., 2015; Ahmad *et al*., 2015). Protecting different plant metabolic processes like photosynthesis, respiration and synthesis of important components like chlorophylls, enzymes and the functioning of transport proteins are the key functions of mineral elements. In addition mineral elements like nitrogen, potassium, calcium, sulphur and selenium has been reported to improve growth of plants under stress environments by modulating important protective mechanisms including osmoregulation, antioxidant metabolism and the synthesis of secondary metabolites (Hasanuzzaman *et al*., 2011; Asgher *et al*., 2014; Iqbal *et al*., 2015; Ahanger *et al*., 2015; Ahmad *et al*., 2016).

Selenium is an essential element for growth maintenance of plants and is also very much beneficial in reducing the adverse effects of stresses. Selenium has antioxidant property comparable to vitamin E, however when present at higher concentrations can cause oxidation of thiols resulting in generation of superoxide radical (Stewart *et al*., 1999). Selenium shares similarity with sulphur hence can replace it under sulphur deficient conditions thereby can help in maintaining the levels of sulphur containing aminoacids, glutathione and the coenzyme A, in addition it can also modify the structure and properties of proteins (Nowak *et al*., 2004). Selenium occurs in soil as selenite as well as selenate. Among these two forms selenate is easily leached from soil as compared to selenite which remains adsorbed to

soil and is therefore available easily for plants (Fio *et al*., 1991). Application of selenium has been reported to regulate antioxidant metabolism for mediating efficient removal of ROS and hence protecting the vital plant processes (Saidi *et al*., 2014). Selenium maintains plant functioning under cadmium stress by modulating both enzymatic and non enzymatic antioxidants thereby protecting cells from the oxidative damage (Hasanuzzaman *et al*., 2012). So it was assumed that selenium could be effective in mitigating the cadmium induced damage to *Solanum lycopersicum* L. by its active involvement in the antioxidant and osmoregulatory mechanisms.

Solanum lycopersicum L. is one of the important food crops within the family *Solanaceae* and is consumed worldwide. In the present study an effort has been done to investigate the effectively of selenium application in mitigating the cadmium induced damage in tomato.

Material and Methods

Soil, plant and treatments: For the present study soil used was loamy sand soil with pH 7.5 and percent sand, clay, silt, organic carbon and total nitrogen were 87.2; 7.1; 5.7; 0.11 and 0.004% respectively. Soil was autoclaved at 121°C and 1.5 bar, thereafter cooled and distributed in polystyrene pots of 2 Kg capacity. Seeds of tomato (*Solanum lycopersicum* L. cv. Rio Grande) germinated on blotter paper and kept in dark for 3 days at 26°C. Healthy germinated seedlings were transplanted into trays which were filled with vermiculite and were grown in growth chamber maintained with photoperiod of 16/8 h light/dark, ~ 70% RH, 1800 μmol photons m^{-2} S^{-1} intense light and temperature of 25°C. Half-strength nutrient solution of Hoagland (HS) was given for two weeks and thereafter were subsequently planted in perforated polystyrene pots filled with soil. Pots were arranged in factorial completely randomized design with three replicates. Full strength of HS (50 ml) alone and supplemented with cadmium (200 μM $CdCl_2$) was given at every alternate day. Pots receiving full strength Hoagland's solution served as control. Selenium (Na_2SeO_4, 1.2 μmol/L) was sprayed directly on leaves (10 ml/plant) after every two days and control plants were sprayed with equal amount of distilled water. After completion of experiment, plants were uprooted carefully, washed with water and were soaked in EDTA (20 mM) for 15 min to remove the adsorbed metals. Fresh leaves were dried at 110°C for two successive weights. For biochemical estimations harvested leaves were immediately stored in liquid nitrogen until being analyzed.

Cadmium (Cd) and Selenium (Se) estimation: Dried plant material (0.5 g) was acid digested using nitric acid and perchloric acid (2:1) at 60°C (Jackson, 1962; Burd *et al*., 2000). Digested samples were cooled and 30% hydrogen peroxide was added followed by incubation for 2 hours. The concentrations of ions were estimated using flame atomic absorption spectrophotometer (T80 UV/VIS Spectrophotometer, PG Instruments Ltd). Standard curve (10-100 μg/ 100 ml) of each ion used as reference.

Photosynthetic pigments: Fresh leaves (100 mg) were extracted in acetone and the absorbance was read at 622, 664, and 440 nm using spectrophotometer (Lichtenthaler & Wellburn, 1983).

Determination of leaf water content (LWC)

LWC was estimated according to Smart & Bingham (1974) and calculations were done using following formula:

$$TWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}}$$

Determination of membrane stability index (MSI): Method of Sairam *et al.* (1997) was followed for determination of MSI and calculations were done using formula given below:

$$MSI = [1 - (C_1/C_2)] \times 100$$

where, C_1 = Electrical conductivity of treatment sample. C_2 = Electrical conductivity of control sample.

Estimation of proline: Known weight (0.5 g) of plant material was homogenized in sulphosalicylic acid (3%, w/v) and followed by centrifugation at 10000 for 10 minutes. Thereafter known volume of extract was mixed with acid ninhydrin and glacial acetic acid followed by incubation for 1h at 100°C, after that samples were removed kept on ice bath. Proline was separated using toluene and the absorbance was read at 520 nm (Bates *et al*., 1973).

Estimation of phenols: Phenols were estimated using Folin and Ciocalteau's phenol reagent after extracting in ethanol and optical density was read at 750 nm (Slinkard & Singleton, 1977) and pyrogallol was taken as standard.

Lipid peroxidation (MDA) and Hydrogen peroxide (H_2O_2) estimation: Lipid peroxidation was determined by monitoring the formation of malonaldehyde (MDA). Fresh sample (0.2 g) was macerated in TCA (0.1%) followed by centrifugation at 10000 × g for 5 min. Supernatant (1mL) was added to 4 mL thiobarbituric acid (5% in 20% TCA) and heated for 30 min at 100°C and thereafter cooled on ice bath and centrifuged again at 10000 ×g for 10 min and thereafter supernatant was read at 532 nm and 600 nm (Hodges *et al*., 1999). An extinction coefficient of 155 mM/cm was used for calculating lipid peroxidation.

Hydrogen peroxide (H_2O_2) levels were determined by homogenizing leaf tissue (0.5 g) in 0.1% (w/v) TCA followed by centrifugation at 12000 × g for15 min. 0.5 mL supernatant was added to 0.5 mL potassium phosphate buffer (10 mM, pH 7.0) and 1 mL potassium iodide (1 M). The absorbance of the supernatant was measured at 390 nm and H_2O_2 concentration was determined from standard curve (Sergiev *et al*., 1997). Results were expressed as nmol g^{-1}FW.

Assay of antioxidant enzymes: Fresh leaf tissues (0.4 g) were extracted using pre-chilled mortar and pestle in 4 mL cold buffer (50 mM potassium phosphate, pH 7.0) containing 4% PVP. Homogenate was centrifuged at 14000 ×g at 4 °C for 30 min and supernatant was used as enzyme source for assay (Malik & Singh, 1980). For estimation of superoxide dismutase (SOD, EC1.15.1.1) assay mixture (1 mL) contained 50 mM phosphate buffer (pH 7.4), methionine (13 mM), nitroblue tetrazolium (NBT, 75 µM), EDTA (0.1mM), riboflavin (2 µM) and 100 µL enzyme extract. Activity was assayed by measuring the photochemical reduction of NBT at 560 nm after incubating samples for 15 min under fluorescent tubes and the amount of protein that caused 50% inhibition was considered as one unit of SOD activity (Beauchamp & Fridovich, 1971).

Catalase (CAT, EC1.11.1.6) was assayed by monitoring the disappearance of hydrogen peroxide (H_2O_2) at 240nm. Assay mixture was 100 mM phosphate buffer (pH7.0), 0.1 mM EDTA, 0.1% H_2O_2 and 100 µL enzyme extract in a final volume of 1ml. Molar extinction coefficient (ε = 39.4 $mM^{-1}cm^{-1}$) was used for calculation of CAT activity and was expressed as Unit/ mg protein (Aebi, 1984).

Ascorbate peroxidase (APX, EC1.11.1.1) activity was determined by monitoring the decomposition of H_2O_2 and decrease in absorbance was observed at 290nm. 2mL reaction mixture contained, phosphate buffer (50 mM, pH 7.8), 0.1 mM EDTA, 0.3 mM ascorbate and 100 µL enzyme extract and H_2O_2 (Nakano & Asada, 1981). Molar extinction coefficient of 2.8 $mM^{-1} cm^{-1}$ for AsA was used for calculating APX activity.

Glutathione reductase (GR, EC1.6.4.2) was determined by observing the NADPH dependent reduction of GSSG at 412nm (Smith *et al*., 1988).

Determination of non enzymtic antioxidants: For estimation of GSH, 500mg fresh plant tissue was extracted in phosphate buffer and followed by centrifugation at 3000 × g for 15 min. Thereafter 500µL supernatant was mixed with 5, 5-dithiobis-2-nitrobenzoic acid and left for 10 minutes. Thereafter absorbance was measured at 412nm and GSH concentrations were determined using standard curve of GSH (Ellman, 1959).

For estimation of ascorbic acid leaf tissue was extracted in 6% trichloroacetic acid and homogenate was mixed with 2% (w/v) dinitrophenyl-hydrazine (prepared in 50% H_2SO_4) and 10% (w/v) thiourea (prepared in 70% ethanol) and mixture was boiled in a water bath for 15 min thereafter was cooled at room temperature and centrifuged at 1000 × g for 10 min at 4°C. The resulting pellet was dissolved in 80% H_2SO_4 and its absorbance was read at 530 nm (Mukherjee & Choudhuri, 1983). Calculation were done using a calibration curve of ascorbic acid and expressed as µmol / g fresh weight.

Statistical analysis: Data presented is mean of three replicates and data was statistically analyzed using and least significant difference (LSD) was calculated using analysis of variance for a completely randomized design.

Results

Cadmium stress reduced the height of shoot and length of root by 68.2% and 110.6% respectively (Table 1). However, selenium (1.2 µmol/L) supplementation enhnaced shoot height and root length by 15.01% and 5.9% respectively (Table 1). Dry weight of shoot and root was reduced by 42.5% and 55.07% due to cadmium stress while as selenium treated cadmium stressed plants showed only 19.07% and 24.6% reduction (Table 1).

Leaf area, number of leaves per plant and leaf water content was increased by 44.1%, 20.9% and 14.2% due to selenium supplementation (Table 2). Cadmium treatment reduced leaf area, number of leaves per plant and leaf water content by 67.03%, 20.5% and 21.2% respectively (Table 2). Selenium treatment to cadmium stressed plants resulted in an increase of 29.5% in leaf area (Table 2).

Selenium (1.2 µmol/L) reduced uptake of cadmium by 72%, however, cadmium (200 µM) treatment reduced selenium uptake by 34.8% (Table 3). In root accumulation of cadmium was reduced by 64.4% due to selenium (Table 3).

Exposure of tomato to cadmium stress declined chlorophyll a, chlorophyll b, total pigments and carotenoids by 45.4%, 27.9%, 44.4% and 66.4% respectively (Table 4). However supplementation of selenium (1.2 µmol/L) enhanced chlorophyll a, chlorophyll b, total pigments and carotenoids by 17.02%, 28.1%, 19.4% and 11.05% respectively (Table 4). Relative to cadmium stressed ones, plants supplemented with selenium along with cadmium showed 27.7%, 15.1%, 27.8% and 52.07% increase in chlorophyll a, chlorophyll b, total pigments and carotenoids (Table 4).

Table 1. Effect of cadmium (200 µM) stress on shoot height (cm), shoot dry weight (g), root depth (cm), root dry weight (g) and shoot/root (%) of tomato (*Solanum lycopersicum* L.) plant grown with and without selenium (1.2 µmol/L).

Treatment	Morphological criteria			
	Shoot		Root	
	Height (cm)	Dry weight (g)	Depth (cm)	Dry weight (cm)
Control	42.57	3.67	18.96	2.07
Cadmium	13.52	2.11	7.92	0.93
Selenium	48.96	4.87	20.15	2.42
Cadmium + Selenium	27.34	2.97	13.85	1.56
LSD at 0.05:	4.76	0.71	1.34	0.26

Data presented are the means ± SE (n = 3)

Table 2. Effect of cadmium (200 µM) stress on leaf area, number of leaf per plant and leaf water content (LWC %) in *Solanum lycopersicum* L. grown with and without selenium (1.2 µmol/L).

Treatment	Leaf characters		
	Leaf area (cm^2/ plant)	Leaf number (leaf/ plant)	LWC (%)
Control	9.13	7.34	78.93
Cadmium	3.01	5.86	62.13
Selenium	16.34	9.28	92.04
Cadmium + Selenium	12.96	6.13	70.34
LSD at 0.05:	2.94	0.74	6.31

Data presented are the means ± SE (n = 3)

Table 3. Cadmium (mg/g dry wt) content and effect of cadmium (200 µM) stress on selenium (ug/g DW) accumulation in *Solanum lycopersicum* L. grown with and without selenium (1.2 µmol/L).

Treatment	Accumulation of ions (mg/ g dry weight)			
	Cadmium		Selenium	
	Leaf	Root	Leaf	Root
Control	ND	ND	ND	ND
Cadmium	0.076	2.983	ND	ND
Selenium	ND	ND	37.12	12.93
Cadmium + Selenium	0.021	1.034	24.18	5.37
LSD at 0.05:	0.025	0.78	9.23	6.01

Data presented are the means ± SE (n = 3)
ND: Not detected under the experimental conditions

Table 4. Effect of cadmium (200 µM) stress on cholorphyll a, cholorphyll b, carotenoid and total photosynthetic pigments content in *Solanum lycopersicum* L. grown with and without selenium (1.2 µmol/L).

Treatment	Photosynthetic pigments (mg/ g fresh weight)					
	Chl A	Chl B	Chl A/B	Chl A+B	Carotenoids	Total pigments
Control	1.243	0.591	2.103	1.834	0.378	2.212
Cadmium	0.675	0.426	1.584	1.101	0.127	1.228
Selenium	1.498	0.823	1.820	2.321	0.425	2.746
Cadmium + Selenium	0.934	0.502	1.860	1.436	0.265	1.701
LSD at 0.05:	0.23	0.12	0.34	0.51	0.07	0.48

Data presented are the means ± SE (n = 3)

Selenium (1.2 µmol/L) supplementation enhanced membrane stability index, hydrogen peroxide and lipid peroxidation by 3.03%, 36.9% and 9.3% respectively (Fig. 1A-C). Relative to control, cadmium treatment increased hydrogen peroxide and lipid peroxidation by 65.2% and 48.2% respectively (Fig. 1A-C). Membrane stability index was reduced by 31.6% in cadmium stressed plants while as cadmium stressed selenium treated plants showed only 10.2% reduction (Fig. 1A).

Cadmium treatment alone increased proline and total phenol by 59.9% and 59.6% respectively (Fig. 2A, B). Selenium treatment caused an increase of 29.4% and 25.8% in proline and total phenol content while as in combination with cadmium, selenium (200 µM Cd + 1.2 µmol /L Se) increased proline and total phenol by 46.8% and 40.5% respectively (Fig. 2A, B).

Activity of antioxidant enzymes including superoxide dismutase, catalase, glutathione reductase and ascorbate peroxidase increased by 52.1%, 71.3%, 45.5% and 45.5% in cadmium stressed plants (Fig. 3A-D). Selenium supplemented plants maintained 16.03%, 21.7%, 16.7% and 16.7% increased activity of superoxide dismutase, catalase, glutathione reductase and ascorbate peroxidase respectively (Fig. 3A-D). However selenium supplementation to cadmium stressed plants caused an increase of 35.1%, 56.0%, 34.04% and 34.04% in activity of superoxide dismutase, catalase, glutathione reductase and ascorbate peroxidase respectively (Fig. 3A-D).

GSH and AsA was increased by 64.1% and 56.08% respectively due to cadmium stress (Fig. 4A, B). As compared to the control, selenium supplemented plants showed 14.2% and 21.07% increase in content of GSH and AsA respectively (Fig. 4A, B). However, cadmium stressed and selenium supplemented plants showed an increase of 40.5% and 45.7% in GSH and AsA (Fig. 4A, B).

Discussion

Agricultural soils that are contaminated with cadmium does not support proper growth and development of the plants, the plants have the potential to avoid toxicity of toxic metals by making selective absorption of such metals and metalloids (Ahmad *et al*., 2011; Hashem *et al*., 2016). Similarly in our present study plants that were treated with cadmium showed significant growth reductions concomitant with the accumulation of cadmium, however, selenium application provided tomato plants with the strength to avoid accumulation of cadmium. This enhanced cadmium uptake and accumulation by tomato plants reduced their length and weight of shoot as well as root to a considerable extent which may have caused because of its irreversible effects on the activity of proton pumps mediating the cell elongation (Liu *et al*., 2004). Cadmium stress affects cell cycle progression and thereby causes restrictions in growth patterns of cells (Sobkowiak & Deckert, 2004). Reduced growth rate in cadmium stressed tomato plants can be because of reduced leaf area that may have contributed to decline in the production of photosynthates however, selenium supplemented plants maintained relatively higher leaf area therefore conferring role of selenium in ameliorating the ill effects of cadmium on photosynthesis (Khan *et al*., 2015). Moreover selenium treated plants showed less cadmium content as compared to untreated plants which may have contributed to efficient allocation of important mineral ions like nitrogen, iron, magnesium to photosynthetic apparatus for maintaining the optimal activity of photosynthesis (Hasanuzzaman *et al*., 2011; 2012). Such inhibition of growth due to cadmium stress has been observed earlier by other workers also (Ahmad *et al*., 2011; Asgher *et al*., 2014; Abd_Allah *et al*., 2015; Hashem *et al*., 2015). Recently in *Cassia italica*, Hashem *et al*. (2016) have demonstrated that cadmium stress causes drastic decline in the growth by altering the

normal metabolism. Plants supplemented with selenium maintained higher water content as compared to untreated controls as well as cadmium stressed plants. Cadmium reduces chlorophyll synthesis and the subsequent biosynthesis of photosynthetic protein components by inhibiting the uptake of essential mineral elements thereby affecting the normal homeostasis and growth of plants (Nazar *et al*., 2012; Abd_Allah *et al*., 2015). Results pertaining to reduction in chlorophyll contents observed in our present study due to cadmium stress are in confirmation with the results of Ahmad *et al*. (2011) and Asgher *et al*. (2014) for *Brassica juncea*, Abd_Allah *et al*. (2015) for sunflower. In tomato plants, similar reduction of chlorophyll pigments have been reported by Hashem *et al*. (2015). Improvement in growth and growth associated attributes like leaf area in selenium treated plants could have been as a result of enhanced synthesis of pigment that contributed for improved production of photoassimilates (Khan *et al*., 2015). Supplementing optimal concentration of selenium results in maintained growth by causing significant improvement in the growth and chlorophyll synthesis (Saffaryazdi *et al*., 2012).

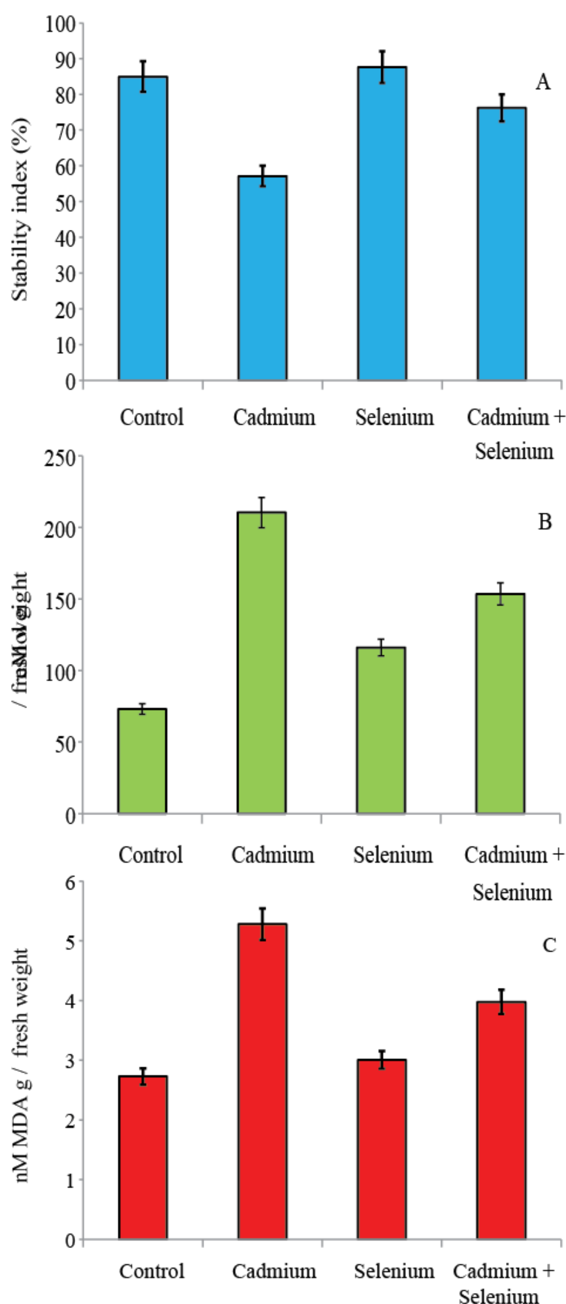

Fig. 1. Effect of cadmium (200 µM) on membrane stability index (**A**), hydrogen peroxide (**B**) and lipid peroxidation (**C**) in *Solanum lycopersicum* L. grown with and without selenium (1.2 µmol/L). Data presented are the means ± SE (n = 3).

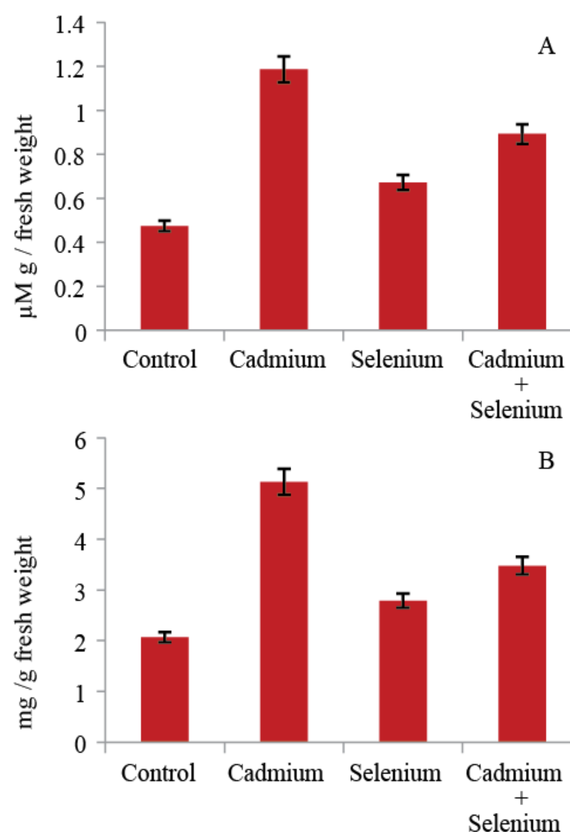

Fig. 2. Effect of cadmium (200 µM) on Proline (**A**) and Total phenols (**B**) in *Solanum lycopersicum* L. grown with and without selenium (1.2 µmol/L). Data presented are the means ± SE (n = 3).

The findings of present study pertaining to impact of cadmium stress on membrane stability index clearly justify the protective role of selenium. Though selenium treated plants were found to maintain slightly higher contents of hydrogen peroxide and the lipid peroxidation rates as compared to control plants but were found to reverse this effect when applied in combination with cadmium (Fig. 1A-C). Results of enhanced lipid peroxidation and hydrogen peroxide concomitant with reduced membrane stability in present study support the findings of John *et al*. (2009), Ahmad *et al*. (2011) and Asgher *et al*. (2014) for mustard; Hasanuzzaman *et al*. (2012) for rapeseed and Hashem *et al*. (2016) for *Cassia italica*. Cadmium stress causes unsaturation of fatty acids and increased lipid peroxidation hence affecting the membrane functioning (Filek *et al*., 2008;

Abd_Allah *et al*., 2015). Plants growing under stress exhibit higher activity of lipid degrading enzymes like lipoxygenase, hence bringing down the membrane stability and also alters their functioning which ultimately results in reduced growth (Djebali *et al*., 2005). Selenium treated plants maintained higher membrane stability reflecting in improved activity of membranes by maintaining higher content of unsaturated fatty acids (Filek *et al*., 2008). Selenium supplementation reduced the production and accumulation of free radicals and thereby providing protection to cells and the major metabolic processes like photosynthesis (Khan *et al*., 2015). Increased ROS production in cadmium stressed plants has resulted in enhanced lipid peroxidation which was however allayed by selenium supplementation significantly. In *Cucumis melo* L., KeLing *et al*. (2013) have also demonstrated that application of optimal concentration of selenium reduced the radical induced damage to membranes resulting in enhanced growth and metabolism.

Accumulation of osmolytes like proline was observed in plants subjected to cadmium treatment and our results are in support of the results of Ahmad *et al*. (2011) for mustard, Abd_Allah *et al*. (2015) for sunflower and Hashem *et al*. (2016) for *Cassia italica*. Selenium application also improved accumulation of proline alone as well as in combination with cadmium and this increased proline accumulation due selenium resulted in enhancement in leaf water content. Accumulation of proline, sugars and betaines is one of the protective mechanisms which plants adapt on exposure to stress and proline accumulation due to selenium supplementation provides a strong justification for positive association of selenium with growth and cadmium stress tolerance. In addition of the osmoregulatory role, proline has several other important roles in plants including ROS scavenging, maintaining the structural property of proteins and membranes (Hayat *et al*., 2012). During stressed conditions proline synthesis is upregulated with a simultaneous reduction in its catabolism (Iqbal *et al*., 2015). Khan *et al*. (2015) have demonstrated that application of selenium upregulates proline synthesis by increasing activity of gamma-glutamyl kinase. Similar to our results, corroborate the results of Hasanuzzaman *et al*. (2012) and Khan *et al*. (2015) who have also reported increased proline accumulation due to selenium supplementation and thereby the better growth performance under stressed conditions. Proline replaces water in the metabolic processes and does not interfere with the metabolism, in addition also brings stability to several important structures (Zhifang & Loescher, 2003). Other important growth stimulatory response of selenium application observed was increase in accumulation of phenols. Present study reveals enhanced phenol synthesis due to cadmium treatment and results observed are in corroboration with the results of Abd_Allah *et al*. (2015) and Hashem *et al*. (2016) for sunflower and *Cassia italica*, respectively. Phenol and the phenol derivatives have growth promotory roles in plants under different biotic and abiotic stresses. Increased synthesis of phenols imparts higher antioxidant activity and in addition contribute to the cell wall formation thereby protecting plants from stress outbreaks (Ahanger *et al*., 2015; Hashem *et al*., 2016). Our results of increased phenol content due to selenium supplementation corroborate with Robbins *et al*. (2005) and Gasecka *et al*. (2015). Lachman *et al*. (2011) and Saffaryazdi *et al*. (2012) have demonstrated that plants maintaining relatively higher selenium uptake show increased phenol synthesis and hence exhibit higher antioxidant activity for better growth adaptation. Protective compounds like phenols and proline both can contribute to quick removal of ROS and therefore mediating protection to membranes and increased accumulation of phenols and proline due to selenium application provide an extra protection to tomato seedlings exposed to cadmium stress (Saffaryazdi *et al*., 2012).

Present study revealed that cadmium treatment upregulated the activity of antioxidant enzymes studied and these observations support the results of Ahmad *et al*. (2011) for mustard, Alqarawi *et al*. (2014) for *Ephedra aphylla*, Hashem *et al*. (2016) for *Cassia italica*. Antioxidant defence system has enzymatic as well as non enzymatic components which work in close coordination to bring down the levels of ROS for protecting plant functioning. In the present study selenium treated plants maintained optimal content of non enzymatic components, ascorbic acid and reduced glutathione, thereby supporting the functioning of antioxidant enzymes by acting as reductants (Hasanuzzaman *et al*., 2012; Abd_Allah *et al*., 2015). Increase in activity of antioxidant enzymes due to selenium supplementation has been earlier demonstrated by other workers also e.g., Filek *et al*. (2008) and Hasanuzzaman *et al*. (2012) in rapeseed and Khan *et al*. (2015) in mustard. Kaklewski *et al*. (2008) have demonstrated that supplementing optimal concentration of selenium to rapeseed seedlings improved growth by upregulating the activity of ascorbate-glutathione pathway enzymes. Similarly in our present study selenium application enhanced the activity of ascorbate peroxidase and glutathione reductase which was reflected in reduced hydrogen peroxide production and enhanced membrane stability in them. Increased activity of antioxidant enzymes work for quick removal of toxic radicals hence prevent oxidative damage to important cellular structures like membrane lipids, proteins (Hashem *et al*., 2016). Increased glutathione reductase activity in selenium supplemented plants can contribute to protection of photosynthetic apparatus by maintain the optimal electron transport and restricting the formation of superoxide radicals (Khan *et al*., 2015). Increase in activities of antioxidant enzymes in selenium supplemented cadmium stressed plants mediated the growth by maintaining photosynthesis, cellular redox potential and maintaining membrane integrity by eliminating the free radicals (Abd_Allah *et al*., 2015; Ahanger *et al*., 2015).

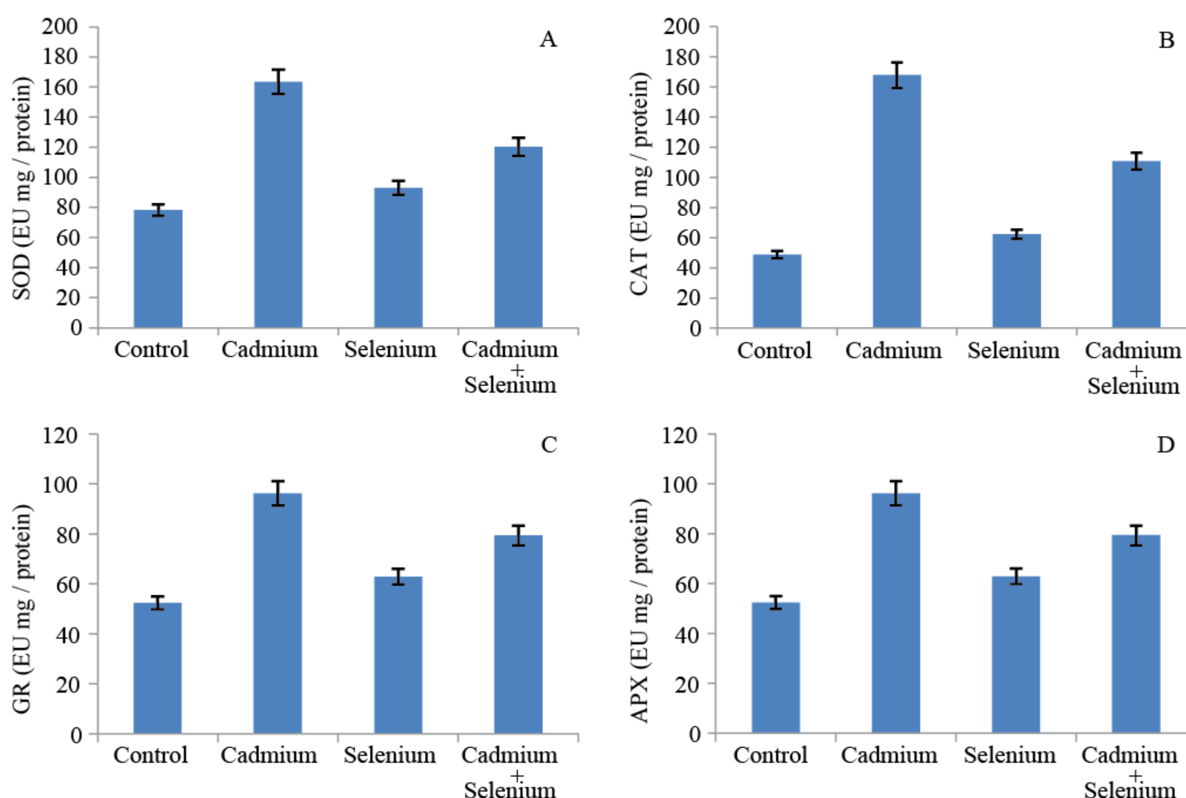

Fig. 3. Effect of cadmium (200 µM) on activity of superoxide dismutase (**A**), Catalase (**B**), Glutathione reductase (**C**) and Ascorbate peroxidase (**D**) in *Solanum lycopersicum* L. grown with and without selenium (1.2 µmol/L). Data presented are the means ± SE (n = 3).

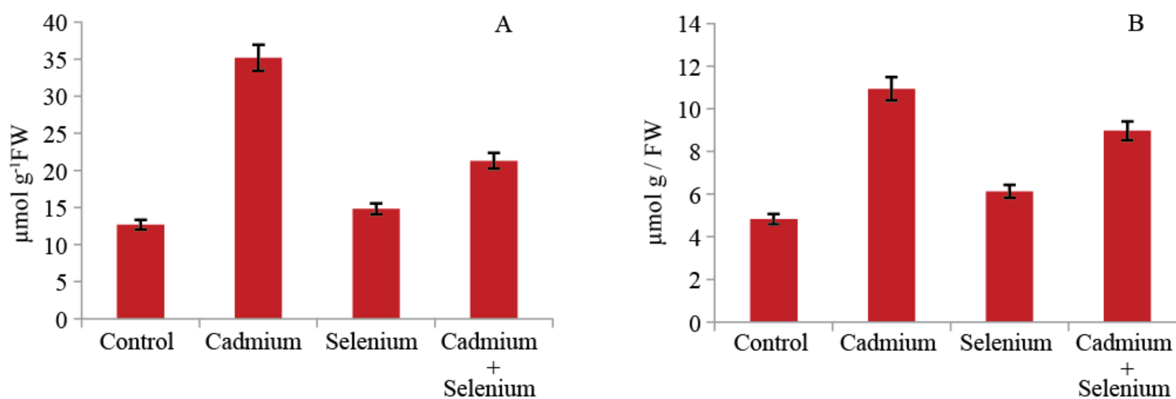

Fig. 4. Effect of cadmium (200 µM) on reduced glutathione (**A**) and ascorbic acid (**B**) content of *Solanum lycopersicum* L. grown with and without selenium (1.2 µmol/L). Data presented are the means ± SE (n = 3).

Conclusion

Conclusively cadmium stress reduced growth of tomato plants drastically and supplementation of selenium ameliorated the cadmium induced deleterious changes to considerable extent by upregulating the antioxidant metabolism and also increasing the synthesis of osmotic constituents. Reduced lipid peroxidation and hydrogen peroxide production in selenium treated plants justifies the role of selenium in preventing plants from the cadmium stress induced damage. Results of present study support the role of selenium for improvement of tomato under cadmium stress.

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