APPLICATION IN TWO METHODS ELEVATES DROUGHT TOLERANCE BY INCREASING ANTIOXIDANT DEFENSE SYSTEM AND REDUCING OXIDATIVE STRESS BIOMARKERS IN *SOLANUM LYCOPERSICUM* PLANT

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ABSTRACT

The current study aimed at assessing the potential effects of Se applied at three levels (0, 20, and 40 mM) in two methods (soil addition or foliar spraying) on the components of the antioxidant defense system and oxidative stress biomarkers in tomato plant growing under irrigation water deficit (from 100% to 60% of soil field capacity; SFC) during the 2017 and 2018 seasons. The results indicated that reducing irrigation water from 100% to 60% of SFC led to a marked increase in oxidative stress biomarkers (malondialdehyde; MDA, hydrogen peroxide; H_2O_2, and superoxide; $O_2^{\bullet}$), which associated with increased contents and activities of the components (enzymatic and non-enzymatic) of the antioxidant defense system in both seasons. Both 20 and 40 mM Se significantly increased contents and activities of the components of the antioxidant defense system, which were reflected in reduced oxidative stress biomarkers. Compared to foliar spray, better results were obtained with Se application to the soil. The interaction among the three factors; water deficit, Se level, and Se application method was significant. The combination of irrigation at 60% of SFC × 40 mM Se application to soil was preferable, which can be recommended for use to *support* the endogenous antioxidative defense system in tomato plant cultivated in a dry environment.

1. INTRODUCTION

Tomato is a major global vegetable crop grown both in greenhouses and in outdoor fields. During planting, tomato plants are exposed to many abiotic and biotic stresses, including water scarcity in their growing medium, especially in arid and semiarid regions such as the Mediterranean. In such regions, tomato plants should be planted under regular irrigation (Rivelli *et al*., 2013), where climate change is expected to cause frequent droughts (Nankishore and Farrell, 2016). Subsequently, water scarcity caused by drought events can have pivotal consequences for crop production, including tomatoes, where tomato yields can be reduced by up to 50% with an equivalent decrease in irrigation (Cantore *et al.*, 2016). In the Mediterranean basin, high sensitivity of tomatoes to insufficient water has prompted researchers to use quick solutions such as antioxidants or biostimulants containing antioxidants (Abd El-Mageed *et al*., 2016, 2017; Merwad *et al.*, 2018) apart from long-time breeding programs. With the application of these antioxidants, tomato plants could become drought-adapted. The term "drought-adapted" has been clarified by Verslues and Juenger (2011) to refer to high yields of drought-affected plants.

Water scarcity is the most dangerous aspect of climate change. Limited availability of irrigation water is one of the most restricting factors critically affecting various metabolic (physiological and biochemical) processes and slows down development, growth and fertility, and consequently productivity loss in crop plants under arid and semi-arid regions (Helaly *et al*. 2017; Jia *et al*., 2017; Bocchini *et al*., 2018). Sensitivity of stomata to reduced water potential can be decreased by the deficit of irrigation water, which is evidenced by limited water and turgor potential, water contents, stomatal movements, cell expansions rate, and at last poor plant growth (Cotrim *et al.,* 2011). One of the fastest processes stimulated by drought is the closure of stomata mediated by abscisic acid (ABA) (Pirasteh-Anosheh *et al*., 2016). The severity of prolonged drought stress leads to further acclimation reactions responses, including metabolic reprogramming (Zhang *et al*., 2014), and activation of the antioxidant system components; low molecular weight antioxidants and antioxidant enzymes (He *et al*., 2017; Laxa *et al*., 2019). During stress, plants have developed/adopted mechanisms (for example, antioxidants, etc.) to acclimate to water deficit stress or even to withstand periods of water deficit. However, these internal anti-drought compounds are not sufficient to enable stressful plants to withstand prolonged drought periods, so the exogenous use of certain adjuvants (e.g., selenium; Se) is important to help plants withstand water deficit stress efficiently.

As previously reported, Se induces abiotic stress alleviation, including drought stress (Hemmati *et al*., 2019; Sattar *et al.,* 2019). As found in seleno-proteins, Se contributes to antioxidative protection, improved metabolism, and regulation of redox reactions under salt and drought stresses (Kong *et al*., 2005; Sattar *et al*., 2019), protecting plants against damage caused by oxidative stress (Sieprawska *et al.,* 2015). In addition, Se enhances antioxidant enzyme activities, leading to reduced oxidative stress poisoning in terms of reducing malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and supeoxide ($O_2^{\bullet-}$) contents and consequently recovery of plant growth and production and its quality under water deficit stress (Emam *et al*., 2014; Sattar *et al.,* 2019).

Based on the above, the present study was carried out to investigate the protective role of Se application in two methods (soil addition and foliar spray) in mitigating the adverse effects of irrigation water deficit (drought stress) by improving the activity of the components of the antioxidant defense system and reducing the oxidative stress biomarkers in tomato plant, cv. Login 935. This is documented with reference to the preferred method of Se application; soil addition or foliar spray.

2. MATERIALS AND METHODS

2.1. Location, plant material, growth conditions, treatments, and experimental layout

Two pot experiments were conducted during two consecutive seasons at the experimental farm of the Faculty of Agriculture, South East Fayoum (29° 17'N; 30° 53'E), Egypt. Transplanting was performed on 7 September 2017 and 5 September 2018 using Five-week-old tomato seedlings (*Solanum lycopersicum* L.) cv. Lojain 935 F_1, Enz Zaden Company, obtained from the Ministry of Agriculture Nurseries,

Cairo, Egypt. Black colored-plastic pots (40 cm inner diameter and 42 cm in depth) were used for both experiments. Each pot was received 18 kg of air-dried soil consisting of clay and sandy soil at a ratio of 2: 1, respectively. Physical and chemical properties of the tested soil were determined according to Page et al. (1982) and Klute (1986), and the analyzed data are shown in Table 1.

Tomato seedlings were sorted for validity and standardization. Two tomato transplants were transplanted in each pot. The pots were organized in a wire greenhouse. Tomato transplants/plants were grown under the normal climatic conditions, which were as follows: temperatures range: 24 ± 5 °C for day (12 h) and 17 ± 3 °C for night (12 h), and humidity average: 61.4 − 65.6%. Availability of sunlight inside the greenhouse was kept homogeneous. Tomato transplants were grown for 15 days with full irrigation (100% of soil field capacity; SFC) for repairing and well fixing the roots in their soil. The SFC was determined at the laboratory of soil and water analyses, Department of Soil and Water Science, Faculty of Agriculture, Fayoum University, Fayoum, Egypt. Tomato transplants were then assigned to 15 replicates (pots) of 12 treatments until harvest for applying treatments. There were three treatment factors. The first factor represented two water regimes (irrigation at 100% or 60% of SFC). The second factor represented three concentrations of selenium (Se); 0, 20, or 40 mM. The third factor represented the method of Se application; foliar spray of plants or addition to the soil with irrigation water. Both two application methods were applied two times; started 15 days after transplanting (DAT) and repeated 20 days later. Foliar sprays of Se were carried out using hand atomizer and the control plants were sprayed with distilled water. The volume of the spraying solutions was sprayed to run off, and few drops of Tween-20 were used as a surfactant. These Se concentrations and application times were selected based on a preliminary study, where they were generated best responses (data not shown).

The pots were arranged in a Split-Split design. Weight method was used to calculate the SFC of the two water treatments (100% and 60%). Daily, the pots were weighed and watered up to their corresponding target SFC, by replacing the amount of water transpired and evaporated. To avoid systematic error produced by fluctuations in the local environmental conditions, the pots were rotated every three days throughout the experiment duration.

2.2. Fertilization program

Starting from 8 DAT and for one month, fertilization was as follows: NPK fertilizer (Super f'eid 19/19/19, Technogreen Company) was added at 2 g L^{-1} for 3 times per week. Humic acid (Humutech 45%, Technogreen Company) and calcium nitrate (Calcium nitrate 15,5/0/0 + 26 Cao, Evergrow Company) were added to the soil both at a rate of 3 g L^{-1} once weekly. Amino acids (Aminoplus TG 22.5% free amino acids, Technogreen Company) at a rate of 2 cm L^{-1} and a mixture of micro-elements (Fedex, Pharmaceutica Company) at a rate of 2 g L^{-1} were sprayed once a week. Starting from 40 DAT and for another month, the fertilization rates were increased to be as follows: NPK fertilizers were added at 5 g L^{-1} for 3 times weekly. Humic acid and calcium nitrate were added to the soil both at a rate of 5 g L^{-1} once weekly. Amino acids at a rate of 5 cm L^{-1} and a mixture of micro-

elements at a rate of 5 g L^{-1} were sprayed once a week. Starting from 70 DAT, K fertilizer levels were increased to an average of 6 times a week.

2.3. Sampling

Plant samples were collected 50 days after transplanting (DAT). The upper fully-expanded leaves were used for all assays of antioxidant system components and biomarkers of oxidative stress.

2.4. Determination of ascorbate (AsA),glutathione (GSH), and α-tocopherol (α-TOC) contents

The method of Okamura (1980) was followed to determine AsA with the modification of Law *et al.* (1992). Four hundred µl chlorophyll (250–350 µg) was taken into a test tube with 200 µl trichloroacetic acid (10%) was added. The mixture was mixed in a vortex and cooled by keeping it in an ice for 5 min. To this solution, 10 µl NaOH (5 M) was added and centrifuged for 2 min in a Microfuge. Supernatant was collected. In one test tube, 200 µl supernatant was taken and 200 µl of 150 mM NaH_2PO_4 buffer, pH 7.4, also 200 µl of distilled water were added. In another test tube, 200 µl supernatant was taken to which 200 µl buffer, 100 µl of dithiothreitol (l0 mM) were added and incubated at room temperature for 15 min. After incubation, 100 µl N-ethylmaleimide (0.5%) was added. 400 µl trichloroacetic acid (10%), 400 µl H_3PO_4 (44%), 400 µl bipyridyl (4%), 70% ethanol and 200 µl $FeCl_3$ (3%) were added to both samples. Samples were incubated at 37 ◦C for 60 min and Optical density was recorded at A525. A standard curve in the range 0–40 nmol of AsA was used for calibration. The results were expressed as mmol total AsA g^{-1} FW.

The GSH content was determined according to the method of Gossett *et al.* (1994). A weight of 0.5 g leaves was homogenized in10 ml HCl (0.2 N) and centrifuged at 16,000 × g for 10 min. Supernatant solution was collected. 500 µl supernatant was taken into a test tube and neutralized with sodium phosphate buffer (0.2 M), pH 5.6. After neutralization, the extract was added to the reaction mixture consisting of sodium phosphate buffer (0.2 M), pH 7.5, EDTA (10 mM), NADPH (10 mM), DTNB (12 mM) and 20 U ml^{-1} GSH reductase enzyme. The results were expressed as mM GSH g^{-1} FW.

The content (µmol g^{-1} DW) of *α*-TOC was assessed by dissolving 20 mg of BHT (butylated hydroxytoluene) using 900 ml of extraction solvent; *n*-hexane-ethyl acetate, *n*-hexane + 100 ml of ethyl acetate as a solvent mixture. Using R-TOC, standard solutions (20–200 µg ml^{-1}) were prepared using a stock solution (50 mg/100 ml *n*-hexane). Samples were prepared and saponified (Konings *et al*., 1996). After slicing, leaf tissue was dried at 40 °C using an oven, homogenized, and suspended in water using a conical flask (1 l) with the addition of 21 g of potassium hydroxide (KOH) dissolved in 100 ml of ethanol. A weight of 0.25 g of AsA (ascorbic acid) was also added per gram test portion. At 80 °C for 40 min, saponification was done and cooling was then done immediately. The ethanol: water ratio was brought to 0.3 by using distilled water, and 9 ml of *n*-hexane: 1 ml of ethyl acetate (3 × 100 ml) was then added and followed by an extraction for the mixtures three times. Combination, water-washing, and filtration through anhydrous Na_2SO_4 into a beaker were done for the organic phases. Evaporation to

dryness for the filtrates was done. Residues obtained were then dissolved using n-hexane (HPLC grade) and were stored at −20 °C. Using HPLC system (with a Waters Bondapak C_{18} reverse-phase column), α-TOC was assessed using methanol: water (94:6) as a mobile phase (with a flow rate of 1.5 ml min^{-1}, a UV detector set at 292 nm) (Ching and Mohamed, 2001).

2.5. Determination of oxidative stress biomarkers contents

To determine lipid peroxidation in terms of malondialdehyde (MDA) content, leaf tissue (0.1 g) was homogenized with 5 ml 0.07% $NaH_2PO_4{\cdot}2H_2O$ and 1.6% $Na_2HPO_4{\cdot}12H_2O$ (50 mM) and centrifuged at 20,000 × g for 25 min. The results of MDA were expressed as $A_{532–600}$ g^{-1} FW (Heath and Packer, 1968).

To determine the content of hydrogen peroxide (H_2O_2; µmol g^{-1} FW), 250 mg of fresh leaves were homogenized using 5 ml of 5% TCA (trichloroacetic acid). Centrifugation was done at 12,000 ×*g* for 15 min at 4 °C for the homogenates. The supernatant was gathered, added to 10 mM potassium phosphate buffer (pH 7.0) + 1 M KI as a reaction medium. The absorbance was read, spectrophotometrically, at 390 nm against H_2O_2 as a standard (Velikova *et al*., 2000).

To determine the content of superoxide ($O_2^{\bullet-}$), leaf sample (100 mg) was cut into 1 mm × 1 mm fragments and immersed for 1 h at room temperature in 10 mM K-phosphate buffer, pH 7.8, 0.05% NBT and 10 mM NaN_3. Two ml of immersed solution was heated at 85 ◦C for 15 min and cooled rapidly. Optical density was measured colorimetrically at 580 nm and the $O_2^{\bullet-}$ content was expressed as A_{580} g^{-1} FW (Kubis, 2008).

2.6. Assays of enzymatic antioxidants activities

Superoxide dismutase (SOD; EC 1.15.1.1) activity was assessed by monitoring the inhibition of the photochemical reduction of nitroblue tetrazolium (NBT) (Giannopilitis and Ries, 1977; Beyer and Fridovicht, 1987; Yu *et al*., 1998). One unit of SOD activity was defined as the amount of enzyme required for the reduction of 50%NBT. SOD activity was expressed as A_{564} min^{-1} g^{-1} protein.

Catalase (CAT; EC 1.11.1.6) activity was determined by measuring the consumption of H_2O_2 (Nakano and Asada, 1981). The reaction mixture consisted of 25 mM Tris-acetate buffer, pH 7.0, 0.8 mM Na-EDTA and 20 mM H_2O_2. The enzyme assay was performed at 25 ◦C. CAT activity was expressed as A_{290} min^{-1} g^{-1} protein.

Ascorbate peroxidase (APX; EC 1.11.1.11) activity was determined following the method described by Rao *et al*. (1996) by recording the optical density at 290 nm and the activity was expressed as A_{290} min^{-1} g^{-1} protein.

Protein was estimated in crude enzyme extracts by dye binding assay (Bradford, 1976).

2.7. Experimental design and statistical analysis

The experiment was conducted as a factorial completely randomized design with two irrigation levels (100% and 60% of SFC), three Se foliar spray concentrations, and two methods of Se application in 15 replications (pots). Data are presented in terms of mean (± SE; standard error). All data were statistically analyzed using Statistica (version 9, Tulsa, OK, USA) and first subjected to

analyses of variance (ANOVA). Statistical differences between treatment means were affirmed using the Fisher LSD test at $P \leq 0.05$.

3. RESULTS

The oxidative stress biomarkers identified for this study were lipid peroxidation determined as malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and superoxide ($O_2^{\bullet-}$) contents (Table 2, Fig. 1). In addition, the antioxidant defense system components identified for this study were the osmoprotectants (total soluble sugars; TS sugars and free proline) and non-enzymatic antioxidants (free proline, selenium; Se, ascorbic acid; AsA, glutathione; GSH, and α-tocopherol; α.TOC) contents (Table 3, Figs. 2), and enzymatic antioxidants (superoxide dismutase; SOD, catalase; CAT, and ascorbate peroxidase; APX) activities (Table 4, Fig. 3). For irrigation regimes, the contents of MDA, H2O2, and O2•− were increased by 57.1 and 83.3%, 26.2 and 36.9%, and 51.0 and 74.5% in 2017 and 2018 seasons, respectively when the irrigation level decreased from 100 of SFC to 60% of SFC. These increased contents of the oxidative stress biomarkers were associated with significant increases in TS sugars, proline, AsA, GSH, and α.TOC contents, which were increased by 31.7 and 47.0%, 19.8 and 18.1%, 51.8 and 80.9%, 61.8 and 92.8%, and 144.2 and 102.6% (with reductions in Se contents by 32.3 and 26.5%) in both seasons, respectively. In addition, there were increased activities of SOD, CAT, and APX by 81.5 and 110.5%, 51.5 and 66.8%, and 65.0 and 77.4% by decreasing the irrigation level from 100% of SFC to 60% of SFC. For Se level applications, both Se levels; 20 and 40 mM significantly decreased the oxidative stress biomarkers; MDA, H_2O_2, and $O_2^{\bullet-}$ contents compared to the control (0 mM Se). These reductions in the oxidative stress biomarkers contents were accompanied with the increases in the contents of TS sugars, proline, Se, AsA, GSH, and α.TOC, as well as the activities of SOD, CAT, and APX. However, the level of 40 mM Se significantly exceeded the level of 20 mM Se. This Se level (40 mM) significantly reduced MDA, H_2O_2, and $O_2^{\bullet-}$ contents by 31.8 and 36.4%, 17.1 and 21.3%, and 24.3 and 34.2%, which were associated with increased contents/activities of antioxidant system components; TS sugars content by 44.2 and 62.2%, proline content by 28.8 and 33.9%, Se content by 140.7 and 112.9%, AsA content by 39.6 and 56.8%, GSH content by 35.7 and 50.9%, α.TOC content by 27.4 and 19.6%, SOD activity by 37.6 and 37.0%, CAT activity by 12.9 and 18.0%, and APX activity by 19.8 and 25.2% in both seasons, respectively compared to the control (0 mM Se). For the Se application method, there were significant differences for most parameters of oxidative stress biomarkers and antioxidant system components, except for MDA, H_2O_2, $O_2^{\bullet-}$, and α.TOC contents, and CAT and APX activities in the first season (2017) and H_2O_2 and α.TOC contents, and CAT and APX activities in the second season (2018) between the two application methods. Other than that, Se application for the soil significantly exceeded Se treatment through foliar application in both seasons. For the interaction of the abovementioned three factors, there were significant differences among the combined treatments, especially stressful ones. For combined treatments under 100% of SFC (normal condition), the best treatment was $Irrig_{100} \times Se_{40} \times SA$ or FS. For combined treatments under the stressful condition (60% of SFC), the

best treatment was $Irrig_{60}$ × Se_{40} × SA, which significantly decreased MDA, H_2O_2, and $O_2^{\bullet-}$ contents by 50.0 and 54.8%, 28.9 and 36.3%, and 40.6 and 50.9% in both seasons, respectively compared to the corresponding control ($Irrig_{60}$ × Se_0 × SA). These reductions in the oxidative stress biomarkers contents were associated with the increases in the contents of TS sugars (by 58.9 and 87.8%), proline (by 38.3 and 39.9%), Se (by 117.5 and 104.7%), AsA (by 88.2 and 130.6%), GSH (92.1 and 107.5%), and α.TOC (by 43.7 and 35.6%), as well as the activities of SOD (by 74.6 and 55.7%), CAT (by 26.3 and 27.3%), and APX (37.7 and 44.4%) in both seasons, respectively compared to the corresponding control ($Irrig_{60}$ × Se_0 × SA) (Tables 3– 4, Figs. 2– 3).

Table 1. Some initial physic-chemical properties of the experimental soil

Properties	Value
Clay (%)	63.0
Silt (%)	20.0
Sand (%)	17.0
Texture class	Clay
Soil field capacity (SFC)	33.3
pH [at a soil: water(w/v) ratio of 1:2.5]	7.78
ECe ($dS.m^{-1}$; soil – paste extract)	2.57
$CaCO_3$ (%)	4.78
Organic matter (%)	1.03
Available N (mg kg^{-1} soil)	495
Available P (mg kg^{-1} soil)	72.9
Available K (mg kg^{-1} soil)	574

Table 2. Effect of selenium (Se) levels and their application method on the contents of oxidative stress biomarkers of tomato plants grown under well watering (100% of soil field capacity; SFC) or irrigation water deficit (60% of SFC) in two seasons

Source of variation	MDA ($A_{532-600}$ g^{-1} FW)	H_2O_2 (µmole g^{-1} FW)	$O_2^{\bullet-}$ (A_{580} g^{-1} FW)	MDA ($A_{532-600}$ g^{-1} FW)	H_2O_2 (µmole g^{-1} FW)	$O_2^{\bullet-}$ (A_{580} g^{-1} FW)
	Season of 2017 (7 September)			Season of 2018 (5 September)		
Irrigation (I)	**	*	**	**	*	**
100% of SFC	0.14±0.002[b]	1.30±0.02[b]	0.51±0.01[b]	0.12±0.003[b]	1.22±0.03[b]	0.47±0.01[b]
60% of SFC	0.22±0.004[a]	1.64±0.03[a]	0.77±0.02[a]	0.22±0.006[a]	1.67±0.05[a]	0.82±0.02[a]
Se level (Se_L)	*	*	*	*	*	*
Se_0	0.22±0.004[a]	1.64±0.03[a]	0.74±0.02[a]	0.22±0.006[a]	1.64±0.05[a]	0.79±0.02[a]
Se_{20}	0.16±0.003[b]	1.41±0.03[b]	0.62±0.01[b]	0.15±0.004[b]	1.40±0.04[b]	0.62±0.02[b]
Se_{40}	0.15±0.002[c]	1.36±0.02[b]	0.56±0.01[c]	0.14±0.003[b]	1.29±0.03[b]	0.52±0.01[c]
Se App. (Se_{AM})	Ns	ns	Ns	*	ns	*
Foliar spray	0.18±0.003	1.49±0.03	0.66±0.01	0.18±0.004[a]	1.47±0.04	0.67±0.02[a]
Soil addition	0.17±0.003	1.45±0.03	0.62±0.01	0.16±0.004[b]	1.42±0.04	0.61±0.01[b]
I × Se_L × Se_{AM}	*	*	*	*	*	*

** and * indicate respectively differences at $P \leq 0.05$ and $P \leq 0.01$probability level, and "ns" indicates not significant difference. Means followed by the same letter in each column are not significantly different according to the LSD test ($P \leq 0.05$).

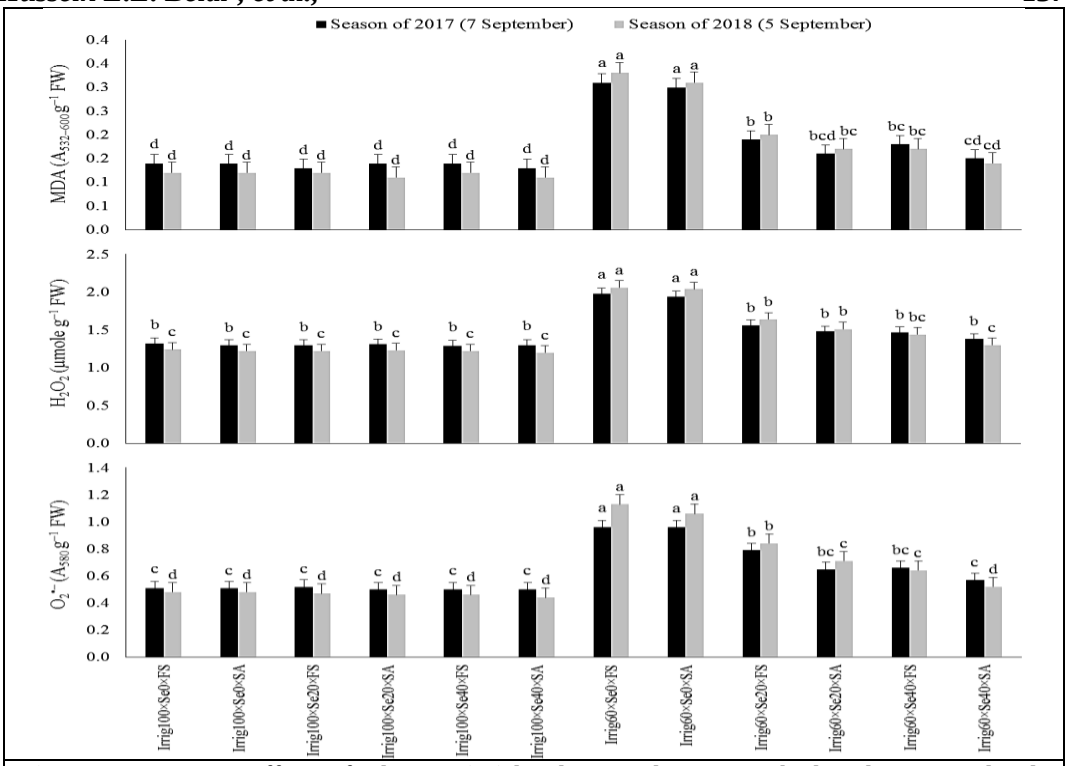

Figure 1. Interaction effects of selenium (Se) level, Se application method, and irrigation levels (100% of soil field capacity; SFC or irrigation water deficit; 60% of SFC) on the oxidative stress biomarkers of tomato plants in two seasons.

Table 3. Effect of selenium (Se) levels and their application method on the contents of non-enzymatic antioxidants of tomato plants grown under well watering (100% of soil field capacity; SFC) or irrigation water deficit (60% of SFC) in two seasons

Source of variation	AsA (µmol g^{-1} FW)	GSH (µmol g^{-1} FW)	α-tocopherol (µM g^{-1}DW)	AsA (µmol g^{-1} FW)	GSH (µmol g^{-1} FW)	α-tocopherol (µM g^{-1} DW)
	Season of 2017 (7 September)			Season of 2018 (5 September)		
Irrigation (I)	**	**	**	**	**	**
100% of SFC	0.785±0.001^{b}	0.531±0.000^{b}	2.17±0.05^{b}	0.807±0.002^{b}	0.583±0.001^{b}	2.27±0.04^{b}
60% of SFC	1.192±0.003^{a}	0.859±0.002^{a}	5.30±0.11^{a}	1.460±0.003^{a}	1.124±0.003^{a}	4.60±0.09^{a}
Se level (Se_L)	*	*	*	*	*	*
Se_0	0.831±0.001^{c}	0.596±0.001^{c}	3.21±0.06^{b}	0.880±0.002^{c}	0.676±0.002^{c}	3.11±0.05^{b}
Se_{20}	0.975±0.002^{b}	0.682±0.001^{b}	3.91±0.08^{a}	1.141±0.003^{b}	0.864±0.002^{b}	3.47±0.06^{a}
Se_{40}	1.160±0.002^{a}	0.809±0.002^{a}	4.09±0.09^{a}	1.380±0.003^{a}	1.020±0.002^{a}	3.72±0.07^{a}
Se Ap. (Se_{AM})	*	*	ns	*	*	ns
Foliar spray	0.938±0.001^{b}	0.651±0.001^{b}	3.65±0.07	1.063±0.002^{b}	0.800±0.002^{b}	3.37±0.06
Soil addition	1.039±0.002^{a}	0.740±0.001^{a}	3.82±0.08	1.205±0.003^{a}	0.907±0.002^{a}	3.50±0.07
I×Se ×Se_{AM}	*	*	*	*	*	*

** and * indicate respectively differences at $P \leq 0.05$ and $P \leq 0.01$probability level, and "ns" indicates not significant difference. Means followed by the same letter in each column are not significantly different according to the LSD test ($P \leq 0.05$).

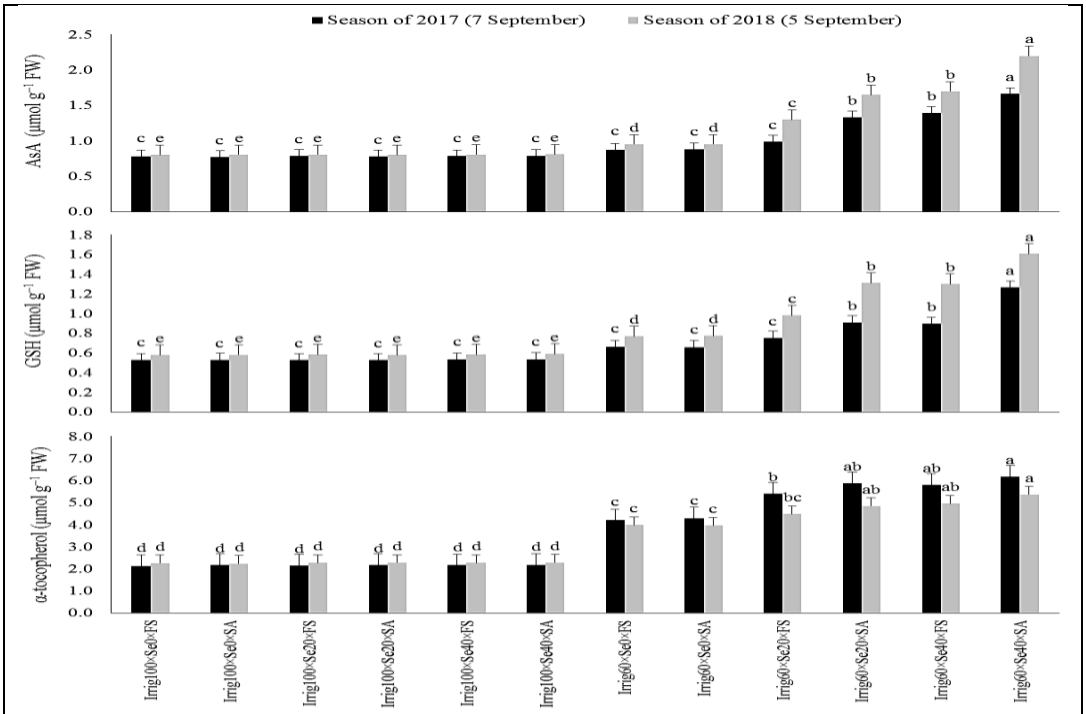

Figure 2. Interaction effects of selenium (Se) level, Se application method, and irrigation levels (100% of soil field capacity; SFC or irrigation water deficit; 60% of SFC) on the contents of non-enzymatic antioxidants of tomato plants in two seasons.

Table 4. Effect of selenium (Se) levels and their application method on the activities of antioxidant enzymes of tomato plants grown under well watering (100% of soil field capacity; SFC) or irrigation water deficit (60% of SFC) in two seasons

Source of variation	SOD (A_{564} min^{-1} g^{-1} protein)	CAT (A_{290} min^{-1} g^{-1} protein)	APX (A_{290} min^{-1} g^{-1} protein)	SOD (A_{564} min^{-1} g^{-1} protein)	CAT (A_{290} min^{-1} g^{-1} protein)	APX (A_{290} min^{-1} g^{-1} protein)
	Season of 2017 (7 September)			Season of 2018 (5 September)		
Irrigation (I)	**	**	**	**	**	**
100% of SFC	13.5±0.1^{b}	52.2±0.3^{b}	80±0.2^{b}	12.4±0.1^{b}	51.5±0.3^{b}	84±0.2^{b}
60% of SFC	24.5±0.3^{a}	79.1±0.4^{a}	132±0.4^{a}	26.1±0.3^{a}	85.9±0.6^{a}	149±0.5^{a}
Se level (Se_L)	*	*	*	*	*	*
Se_0	15.7±0.2^{c}	61.4±0.4^{b}	96±0.2^{c}	16.2±0.2^{c}	62.9±0.4^{b}	103±0.3^{c}
Se_{20}	19.8±0.3^{b}	66.1±0.4^{a}	106±0.3^{b}	19.4±0.2^{b}	69.0±0.4^{a}	118±0.4^{b}
Se_{40}	21.6±0.3^{a}	69.3±0.4^{a}	115±0.3^{a}	22.2±0.2^{a}	74.2±0.5^{a}	129±0.4^{a}
Se App. (Se_{AM})	*	ns	ns	*	ns	ns
Foliar spray	18.4±0.2^{b}	64.5±0.4	103±0.2	18.1±0.2^{b}	66.7±0.4	114±0.3
Soil addition	19.6±0.2^{a}	66.8±0.4	108±0.2	20.4±0.2^{a}	70.7±0.4	119±0.4
$I \times Se_L \times Se_{AM}$	*	*	*	*	*	*

** and * indicate respectively differences at $P \leq 0.05$ and $P \leq 0.01$probability level, and "ns" indicates not significant difference. Means followed by the same letter in each column are not significantly different according to the LSD test ($P \leq 0.05$).

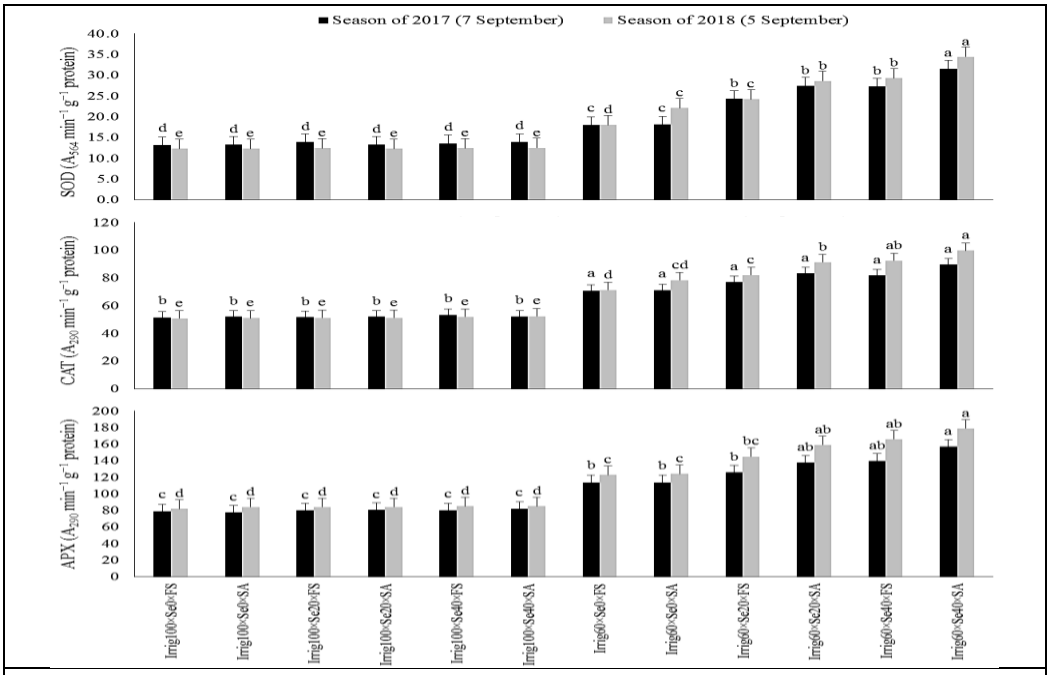

Figure 3. Interaction effects of selenium (Se) level, Se application method, and irrigation levels (100% of soil field capacity; SFC or irrigation water deficit; 60% of SFC) on the activities of antioxidant enzymes of tomato plants in two seasons.

4. DISCUSSION

Drought, as one of the most important abiotic stress problems, limits agricultural production globally. Approximately 45% of the world's agricultural land is constantly under drought stress (Bot *et al*., 2000). If the stress conditions caused by lack of irrigation water, which cause a regression of plant growth and productivity, continue for a long time and/or increase in severity, it may cause irreversible regression and eventually plant death. Drought stress leads to the generation of oxidative stress in terms of increased contents of malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and superoxide ($O_2^{\bullet-}$) (Table 2, Fig. 1). This higher generation of oxidative stress is associated with increased production of enzymatic and non-enzymatic antioxidants (Tables 3–4, Figs. 2–3), which cope with the components of oxidative stress under reduced water conditions (Grzesiak *et al.* 2013; Filek *et al.* 2015; Sattar *et al*., 2019). These results suggest that the deficiency of Se in soil may be one of the reasons leading to poor drought tolerance in most plant species. Many investigations have explained the importance of Se to raise drought tolerance in plants (Feng *et al.*, 2013; Emam *et al*., 2014; Nawaz *et al*., 2015, 2016; Sieprawska *et al*., 2015; Bocchini *et al*., 2018; Hemmati *et al*., 2019; Sattar *et al*., 2019). The results of all these reports are consistent with the results of the current study that the application of Se to plants grown under water deficits either through foliar spraying or through soil addition significantly reduces oxidative stress components (Table 3, Fig. 2). These positive results can be obtained due to the increased contents of low molecular weight antioxidants

(Tables 2 and 3, Figs. 1 and 2), and activities of enzymatic antioxidants (Table 4, Fig. 3). In this regard, Proietti *et al*. (2013) reported that Se application increases the plant's tolerance to oxidative damage caused by drought stress by improving the components of the plant's antioxidant defense system. The effect of Se on plants depends on its concentration (Hartikainen *et al.,* 2000). The favorable results gathered in this study display the effectiveness of Se application at a suitable level, especially through soil addition, in elevating drought stress tolerance in tomato plants.

The increased activities of the protective parameters; low molecular weight antioxidants and enzymatic antioxidants (Tables 3–4, Figs. 2–3) elevated in the current study by Se application may be protected cellular plasma membranes from lipid peroxidation in terms of reduced MDA, as well as reduced contents of H_2O_2 and $O_2^{\bullet-}$ (Table 2, Fig. 1). This leads to decrease of EL and photo-oxidation (Seppänen *et al*., 2003), increase of MSI, and maintain leaf tissues in health status, membrane integrity (Proietti *et al*., 2013), and water relations (Nawaz *et al*., 2013).

The regulative effect of Se can be optimized by improving the contents of low molecular weight antioxidants and the activities of antioxidant enzymes. The improvements in these plants' defense system components (Table 3–4, Figs. 2–3) can antagonize the oxidative stress; increased contents of MDA, H_2O_2 and $O_2^{\bullet-}$. Undoubtedly, Se plays some pivotal roles in discouraging excessive production of ROS induced by drought stress as observed in the current study (Table 2, Fig. 1). To overcome ROS overproduced under drought stress (Table 2, Fig. 1), tomato plant needs to increase its endogenous components of the antioxidant defense system, and this was achieved through the application of Se. Tomato plants in this study produced more proline and soluble sugars with the application of Se under normal and water deficit stress conditions (Table 2, Fig. 1).

Drought stress significantly increased the contents of low molecular weight antioxidants; ascorbate (AsA), glutathione (GSH), and α-tocopherol (α-TOC) in tomato plants and the application of Se stimulated further increases (Table3, Fig. 2). AsA and GSH play protective roles against oxidative stress and lipid peroxidation induced by stress due to their antioxidative activities (Rady and Hemida, 2016; Rady *et al.,* 2019). AsA is an extremely powerful scavenger of ROS because of its ability to donate electrons in different (enzymatic and non-enzymatic) reactions. Under stress, AsA protects cellular membranes by directly scavenging of $O_2^{\bullet-}$ and OH^- (Semida and Rady, 2014). In this study, activities of GSH and AsA were elevated (through AsA-GSH cycle) under stress to contribute to lowering H_2O_2 and MDA levels with Se treatment. Thus, the balance of the pool of AsA and GSH should be rigorously controlled with an appropriate APX activity, which also improved in this study by Se (Table 4, Fig. 3), to enhance the cellular antioxidative capacity to avoid damages of oxidative stress (Foyer and Noctor, 2011). The effective improvement in the contents of low molecular weight antioxidant by Se is a good indicator of the mitigation of drought-induced ROS in drought-stressed tomato plants (Table 2, Fig. 1). The increasing contents of AsA and GSH by Se application indicate an improvement in the AsA-GSH cycle, which functions against overproduction of ROS. This cycle controls H_2O_2 level in plant

cells. Originally, glutathione reductase (GR), DHAR, and MDHAR provide APX by substrates through forming GSH and AsA (Hasanuzzaman *et al*., 2017). Moreover, Rady *et al*., (2019) indicated that α-TOC, as a non-enzymatic lipophilic antioxidant, is capable of scavenging many ROS (including H_2O_2) and free radicals under stress. The higher α-TOC content obtained with Se application (Table 3, Fig. 2) was met with reduced oxidative stress biomarkers; MDA, $O_2^{\bullet-}$, and H_2O_2 contents (Table 2, Fig. 1) for plasma membranes integrity. Phospholipids of plasma membranes are a distinctive target of different oxidants, but α-TOC effectively inhibits lipid peroxidation and possibly promote membrane repair by inhibiting the formation of oxidized phospholipids that may theoretically interfere with the events of membrane fusion (Howard *et al.*, 2011).

In this study, the increased activities of antioxidant enzymes (e.g., SOD, CAT, and APX; Table 4, Fig. 3) indicate excessive production of ROS under drought stress (Hasanuzzaman and Fujita, 2011). In addition, the activities of these enzymes were further increased by Se addition. This result is attributed to the increased antagonistic effect of Se in response to ROS overproduction as noted in this study (Tables 2 and 4, Figs. 1 and 3). These enzymes function as a highly efficient detoxification mechanism of $O_2^{\bullet-}$ and H_2O_2 and help prevent the formation of highly toxic HO^- (Mittler *et al*., 2004). In this study, the abundant elevation in SOD, CAT, and APX activities stimulated by Se application provides additional evidence that Se regulates the dismutation of $O_2^{\bullet-}$ to H_2O_2 (Cartes *et al*., 2010) or may be directly implicated in quenching of $O_2^{\bullet-}$ and H_2O_2 in plant cells (Xu *et al*., 2007). Some previous investigations have also shown an elevation in the activity of antioxidative machinery in Se-treated plants under different abiotic stresses (Habibi, 2013; Nawaz *et al*., 2015; Balal *et al*., 2016). It is necessary to maintain a balance between SOD and other ROS-scavenging enzymes to assess the steady-state level of $O_2^{\bullet-}$ and H_2O_2 in plant cells (Mittler *et al*., 2004). This behavior results in keeping the ROS level under control in plant tissues, improving plant growth and its performance under drought stress conditions. Thus, the exogenous Se application at the appropriate level is implicated in the reactivation of ROS quenchers such as enzymes tested in this study to help minimize the levels of oxidative stress biomarkers in drought-stressed tomato plants. This helps prevent lipid peroxidation for effective photosynthesis activity and alteration of chlorophyll biosynthetic pathway to increase pigments for higher yield and its quality in plants under drought stress (Djanaguiraman *et al*., 2005; Habibi, 2013). The positive alteration in the metabolism of antioxidant system components by the application of Se under water deficit had been evidenced. For example, application of Se altered the metabolism of AsA- GSH system in plants (Wang *et al.*, 2011).

Results of this study display that Se application through soil addition was more effective to produce to some extent better results than its application through foliar spray (Tables 1− 3, Fig. 2).

5. CONCLUSIONS

From the results obtained in this study, it is concluded that soil supplementation with Se through irrigation water was more effective than foliar spray of Se in mitigating the adverse effects of irrigation water deficit stress

conditions. High activities of the components (enzymatic and non-enzymatic compunds) of antioxidative defense system were obtained. In addition, the high contents of lipid peroxidation and other oxidative stress biomarkers produced under drought stress. This indicates that the effect of Se on one parameter under stress directly affect others due to the regulatory role of Se in stressful plants. Therefore, the supplementation of soil with Se may be used as a useful strategy to minimize the adverse impacts of irrigation water deficit stress for sustainable tomatoes productions under the scenario of growing climate change.

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استخدام السيلينيوم بطريقتين يزيد تحمل الجفاف بزيادة نظام مضادات الأكسده الدفاعية وإنخفاض الدلائل الحيوية للإجهاد التأكسدي في نبات الطماطم

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الملخص:

تهدف الدراسة الحالية إلى معرفة تأثيرات استخدام السيلينيوم بثلاث تركيزات (صفر ، ٢٠ ، ٤٠ ملليمول) بطريقتي إضافة (إضافة أرضية، رش ورقي) وذلك على مكونات مضادات الأكسدة الدفاعية والدلائل الحيوية للإجهاد التأكسدي في نبات الطماطم النامي تحت ظروف الإجهاد المائي (نقص مياه الرى من ١٠٠% إلى ٦٠% من السعة الحقلية للتربة) وذلك خلال موسمي ٢٠١٧ ، ٢٠١٨ م. وقد أظهرت النتائج المتحصل عليها من هذه الدراسة أن إنخفاض مياه الري من ١٠٠% الى ٦٠% من السعه الحقلية أدى الى زيادة ملحوظة في الدلائل الحيوية للإجهاد التأكسدي

(malondialdehyde; MDA, hydrogen peroxide, H_2O_2, and superoxide; $O_2^{\bullet-}$)

والتي تكون مرتبطة بزيادة في محتوى ونشاط المركبات الإنزيمية وغير الإنزيمية للنظام الدفاعي التأكسدي في كلا الموسمين. وقد تلاحظ أن كلا التركزين أدى الى حدوث زيادة معنوية في محتوى ونشاط مكونات النظام الدفاعي التأكسدي. ولقد وجد أن الإضافة الأرضية للسيلينيوم قد أعطت نتائج أفضل مقارنة بالرش الورقي. أظهر التفاعل بين العوامل الثلاثة المستخدمة في الدراسة (الإجهاد المائي، مستوى السيلينيوم، طريقة الرش) تأثيراً معنوياً. ولقد وجد أن استخدام الري بمستوى ٦٠% من السعه الحقلية الإضافة الأرضية بتركيز ٤٠ ملليمول قد أعطى أفضل النتائج والذي يمكن التوصية باستخدام هذه المعاملات لتدعيم كفاءة نظام الدفاع التأكسدي داخل نبات الطماطم المنزرع فى البيئة الجافة.

الكلمات الدالة: نقص الماء – السيلينيوم – مضادات الأكسدة – نطام الدفاع التأكسدي