SELENIUM APPLICATION IN TWO METHODS ENHANCES DROUGHT TOLERANCE BY IMPROVING PHYSIOLOGİCAL ATTRIBUTES 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 physiological attributes 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 electrolyte leakage (EL), which associated with increased contents of osmoprotectants. In contrast, relative water content (RWC), membrane stability index (MSI), photosynthetic efficiency, and Se content were decreased in both seasons. Both 20 and 40 mM Se significantly increased contents of osmoprotectants, which were reflected in reduced EL and photosynthetic efficiency, and Se content. 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 for tomato to increase its content of osmoprotectants and Se when cultivated in a dry environment.

1. INTRODUCTION

Tomato (*Solanum lycopersicum* L.) 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 can be reduced by up to 50% with an equivalent decrease in irrigation (Cantore *et al.*, 2016). Tomato plants could become drought-adapted with the application of antioxidants. The term "drought-adapted" has been clarified by Verslues and Juenger (2011) to refer to high yields of drought-affected plants. In this respect, access to the drought-adapted plant may become a concrete approach for better water use efficiency (WUE) under climate change that is expected more in the future.

Drought 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 osmotic adjustment (Blum, 2017) and metabolic reprogramming (Zhang *et al*., 2014). Many of these modulations are measurable and are utilized to clarify the seriousness of drought stress. Among these measurable traits, for instance, net photosynthesis, stomatal conductance, richness of osmoprotectants, tissue water potential, and membrane integrity (Laxa *et al.*, 2019). During stress, plants have developed/adopted mechanisms (for example, osmoprotectants, 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 (e.g., drought stress; Hemmati *et al*., 2019; Sattar *et al*., 2019). The optimum concentration of Se protects cellular chloroplasts, thus enhancing the contents of chlorophyll under water-deficit stress conditions (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).

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 physiological attributes in tomato plant, cv. Login 935. In addition, this investigation also assesses potential improvements in plant water status, photosynthetic efficiency, and osmoprotectant levels under drought stress through Se application. 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 physiological and biochemical determinations.

2.4. Determination of relative water content (RWC), membrane stability index (MSI), and electrolyte leakage (EL)

After excluding leaf midrib, 2 cm-diameter discs were taken for RWC determination (Osman and Rady, 2014). Discs were weighed for fresh mass (FM) and immersed, immediately, in deionized water in dark for 24 h. Water-saturated discs were blotted dry from adhering water drops for recording the turgid mass (TM). Discs were then dried at 70 °C for 48 h for dry mass (DM) assessment. The percentage of RWC was calculated {RWC (%) = (FM – DM) / (TM – DM) × 100}

After excluding leaf midrib, a duplicate 0.2 g leaf sample was taken in test tubes with 10 ml of deionized water to determine leaf MSI (Rady, 2011). At 40 °C, a sample was heated for 30 min with a water bath. Solution electrical conductivity (C_1) was taken. At 100 °C, the other sample was boiled for 10 min. Solution conductivity (C_2) was also measured. The percentage of MSI was calculated:

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

Total inorganic ions that leaked from leaves termed as EL were assessed with the Sullivan and Ross (1979) procedure. Twenty discs were immersed in 10 ml deionized water in a boiling tube and solution electrical conductivity (C_1) was measured. Tube content was then heated to 45 °C – 55 °C for 30 min using a water bath. Solution electrical conductivity (C_2) was scored. At 100 °C, tube content was boiled for 10 min and solution electrical conductivity (C_3) was also recorded. The percentage of EL was calculated {EL (%) = [(EC2 − EC1) / EC3] × 100}

2.5. Determination of photosynthetic efficiency

The two upper full-expanded leaves were used to determine chlorophyll content by using a chlorophyll meter (SPAD-502, Minolta, Japan). At a corresponding time in 2 sunny days, chlorophyll fluorescence as a photosynthetic efficiency was assessed by using a Handy portable PEA fluorometer (Hansatech Instruments Ltd., Kings Lynn, UK). Assessments were conducted using a corresponding leaf (the fourth from the top) on each plant. Calculations of maximum PS II Fv/Fm quantum yield were performed using the Maxwell and Johnson (2000) formulae ($Fv/Fm = (Fm - F0)/Fm$). Based on the similar absorption (PI_{ABS}), performance index of photosynthesis was calculated (Clark *et al*., 2000).

2.6. Determination of total soluble sugars (TS sugars), free proline, and selenium (Se) contents

TS sugars content was assessed as follows: 0.2 g leaves were washed with 5 ml 70% ethanol and homogenized with 5 ml 96% ethanol. The extract was centrifuged at 3500 × g for 10 min. The supernatant was collected and stored at 4◦C (Irigoyen *et al*., 1992). Freshly prepared anthrone (3 ml) was added to 0.1 ml supernatant. This mixture was incubated in hot water bath for 10 min. The absorbance was recorded at 625 nm with a Bausch and Lomb-2000 Spectronic Spectrophotometer.

Proline content in bean leaves was measured following the rapid colorimetric method of Bates *et al*. (1973). Proline was extracted from 0.5 g of dry leaf samples by grinding in 10 ml of 3% sulphosalicylic acid. The mixture was then centrifuged at 10,000 × g for10 min. Two ml of the supernatant was added into test tubes and 2 ml of freshly prepared acid-ninhydrin solution was also added. Tubes were incubated in a water bath at 90◦C for 30 min. The reaction was terminated in ice-bath. The reaction mixture was extracted with 5 ml of toluene and the vortex process was performed for 15 s. The tubes were allowed to stand at least for 20 min in the dark at room temperature to allow the toluene and aqueous phases to be separated. The toluene phase was then carefully collected into test tubes and toluene fraction was read at 520 nm using a UV-160AUV Visible Recording Spectrometer, Shimadzu, Japan. The proline content in the sample was determined from a standard curve using analytical grade proline.

A portion of 5 mL of HNO_3 were added to 0.5 g of sample in a 250 mL dry f1ask and stirred. Thus, all the material was wet. Then, 4 mL of 33% H_2O_2 were carefully added in a well-ventilated hood and slightly stirred after the addition. It was heated on a hot plate and a strong effervescence was produced. When the brown fumes were less dense (7-8 min), the solution was allowed to cool. A slightly yellow dissolution and a small white solid quantity in suspension still remained. The solution was filtered, washed with 5 mL of (1:1) HCI (density 1.18 g mL^{-1}) and diluted up to 25 mL with distilled H_2O (Pequerul *et al*., 1993). The readings of digested samples were performed using an Induction Plasma Spectrometer (ICP), Thermo Jarell Ash brand, IRIS Advantage model, following the 984.27 method in A.O.A.C. (2000). The quantification of Se was performed.

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

3.1. Effects on leaf tissue health

Leaf tissue health was determined in terms of relative water content (RWC), membrane stability index (MSI), and electrolyte leakage (EL) (Table 2, Fig. 1). For irrigation regimes, RWC and MSI were significantly reduced by 21.6 and 22.0%, and 29.4 and 30.0%, while EL was increased by 106.7 and 97.2% in the seasons of 2017 and 2018, respectively when the irrigation level decreased from 100% of SFC to 60% of SFC. For selenium (Se) level applications, both Se levels; 20 and 40 mM significantly increased RWC and MSI, and significantly decreased EL compared to the control (0 mM Se). However, the level of 40 mM Se significantly exceeded the level of 20 mM Se, elevating RWC and MSI by 22.1 and 23.2% and 35.5 and 35.7%, and decreasing EL by 47.9 and 44.9% over both seasons, respectively compared to the control. For the Se application method, there were some significant differences for MSI and EL in the season of 2017 and for MSI in the season of 2018 between the two application methods. Significant increases in RWC and MSI and significant reductions in El were obtained in favor of the Se soil addition compared to the Se foliar spraying. The increases recorded for RWC and MSI by the Se soil addition were 2.6 and 3.3%, and 5.2 and 5.4%, and the decreases recorded for EL by the Se soil addition were 6.1 and 5.5% in both seasons, respectively. 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 $Irrig_{100}$ × Se_{40} × SA or FS. For combined treatments under the stressful condition (60% of SFC), the best treatment was $Irrig_{60}$ × Se_{40} × SA, which significantly increased RWC and MSI by 54.0 and 56.8%, and 118.1 and 115.9%, and significantly decreased EL by 65.3 and 62.5% in both seasons, respectively compared to the corresponding control ($Irrig_{60}$ × Se_0 × SA) (Table 2, Fig. 1).

3.2. Effects on photosynthetic efficiency

Photosynthetic efficiency was determined in terms of efficiency of PSII (Fv/Fm), performance index of PSII (PI %), and SPAD values for chlorophyll content (Table 3, Fig. 2). For irrigation regimes, irrigation of tomato pants with 60% of soil field capacity (SFC) significantly decreased Fv/Fm, PI, and SPAD values by 8.2 and 9.3%, 38.9 and 40.0%, and 24.5 and 23.2% in both seasons, respectively compared to irrigation with 100% of SFC. For selenium (Se) level applications, both 20 and 40 mM Se levels significantly increased Fv/Fm, PI, and SPAD values compared to the control (0 mM Se). The 40 mM Se level significantly exceeded the 20 mM Se level, and increased the above photosynthetic efficiency attributes by 9.0 and 7.6%, 50.5 and 51.0%, and 26.3 and 24.9% for both seasons, respectively compared to the control. For the Se application method, there were no significant differences in the three photosynthetic efficiency attributes between the two application methods, however, soil addition of Se awarded slight increases in growth traits compared to foliar spray of Se. For the interaction of the aforementioned three factors, there were significant differences among the combined treatments, especially stressful treatments. For combined treatments under 100% of SFC (normal condition), the best treatment was $Irrig_{100}$ × Se_{40} × SA. For combined treatments under 60% of SFC (stressful condition), the best treatment was $Irrig_{60}$ × Se_{40} × SA, which significantly increased Fv/Fm, PI, and SPAD values by 16.7 and 15.1%, 163.9 and 152.9%, and 51.1 and 51.1% in both 2017 and 2018 seasons, respectively compared to the corresponding control ($Irrig_{60}$ × Se_0 × SA) (Table 3, Fig. 2).

3.3. Effects on osmoprotectant contents

Osmoprotectants (total soluble sugars; TS sugars and free proline) and selenium (Se) contents are shown in Table 4, Fig. 3. For irrigation regimes, the contents of TS sugars and proline were increased by 31.7 and 47.0%, and 19.8 and 18.1%, while reductions in Se contents by 32.3 and 26.5% were observed in both seasons, respectively. For Se level applications, both Se levels; 20 and 40 mM significantly increased the contents of TS sugars, proline, and Se. However, the level of 40 mM Se significantly exceeded the level of 20 mM Se. This Se level (40 mM) significantly increased TS sugars content by 44.2 and 62.2%, proline content by 28.8 and 33.9%, and Se content by 140.7 and 112.9% in both seasons, respectively compared to the control (0 mM Se). For the Se application method, there were significant differences for the tested parameters 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 $Irrig_{100}$ × Se_{40} × SA or FS. For combined treatments under the stressful condition (60% of SFC), the best treatment was $Irrig_{60}$ × Se_{40} × SA, which significantly increased the contents of TS sugars (by 58.9 and 87.8%), proline (by 38.3 and 39.9%), and Se (by 117.5 and 104.7%) in both seasons, respectively compared to the corresponding control ($Irrig_{60}$ × Se_0 × SA) (Table 4, Fig. 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 leaf tissue health 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	Relative water content (RWC %)	Membrane stability index (MSI %)	Electrolyte leakage (EL %)	Relative water content (RWC %)	Membrane stability index (MSI	Electrolyte leakage (EL %)
	Season of 2017 (7 September)			Season of 2018 (5 September)		
Irrigation (I)	*	*	**	*	*	**
100% of SFC	89.0±6.4^{a}	74.2±5.0^{a}	10.4±0.7^{b}	89.9±6.7^{a}	75.7±5.7^{a}	10.7±0.7^{b}
60% of SFC	69.8±5.4^{b}	52.4±3.7^{b}	21.5±1.3^{a}	70.1±5.7^{b}	53.0±4.1^{b}	21.1±1.4^{a}
Se level (Se_L)	*	*	**	*	*	*
Se_0	71.4±4.9^{c}	53.3±3.8^{c}	21.7±1.4^{a}	71.6±5.4^{c}	54.1±4.2^{c}	21.4±1.0^{a}
Se_{20}	79.6±5.9^{b}	64.4±4.4^{b}	15.0±0.9^{b}	80.2±6.1^{b}	65.6±5.0^{b}	14.5±0.9^{b}
Se_{40}	87.2±6.9^{a}	72.2±5.4^{a}	11.3±0.7^{c}	88.2±7.1^{a}	73.4±5.5^{a}	11.8±0.7^{c}
Se App. (Se_{AM})	ns	*	*	ns	*	Ns
Foliar spray	78.4±5.6	61.7±4.5b	16.5±1.1a	78.7±6.1	62.7±4.8b	16.3±1.0
Soil addition	80.4±6.2	64.9±4.7a	15.5±0.9b	81.3±6.3	66.1±5.0a	15.4±1.0
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 ($P \leq 0.05$).

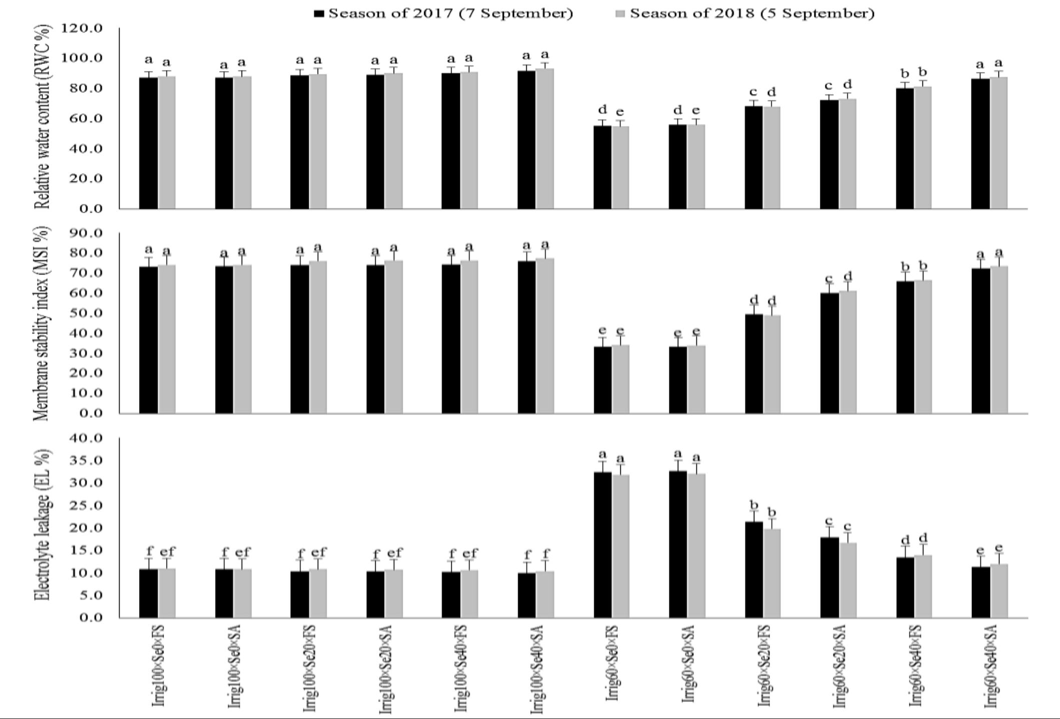

Figure 2. Interaction effects of selenium (Se) level, Se application method, and irrigation levels (100% of soil field capacity; SFC or irrigation water deficit; 60% of SFC) on leaf tissue health of tomato plants in two seasons.

Table 3. Effect of selenium (Se) levels and their application method on photosynthetic efficiency 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	Fv/Fm	Performance index (PI %)	SPAD chlorophyll	Fv/Fm	Performance index (PI %)	SPAD chlorophyll
	Season of 2017 (7 September)			Season of 2018 (5 September)		
Irrigation (I)	*	*	*	*	*	*
100% of SFC	$0.85±0.04^{a}$	$17.15±1.02^{a}$	$65.2±2.6^{a}$	$0.86±0.04^{a}$	$17.29±0.97^{a}$	$65.9±2.9^{a}$
60% of SFC	$0.78±0.03^{b}$	$10.48±0.64^{b}$	$49.2±2.0^{b}$	$0.78±0.04^{b}$	$10.38±0.61^{b}$	$50.6±2.5^{b}$
Se level (Se_L)	*	*	*	*	*	*
Se_0	$0.78±0.03^{b}$	$10.91±0.62^{c}$	$50.2±2.0^{c}$	$0.79±0.04^{c}$	$10.97±0.55^{c}$	$51.8±2.4^{c}$
Se_{20}	$0.82±0.03^{a}$	$14.11±0.83^{b}$	$58.0±2.3^{b}$	$0.82±0.04^{b}$	$13.98±0.79^{b}$	$58.3±2.7^{b}$
Se_{40}	$0.85±0.03^{a}$	$16.42±1.03^{a}$	$63.4±2.6^{a}$	$0.85±0.04^{a}$	$16.56±1.05^{a}$	$64.7±3.0^{a}$
Se App.(Se_{AM})	ns	ns	Ns	ns	ns	ns
Foliar spray	0.81±0.03	13.52±0.79	56.1±2.2	0.81±0.04	13.48±0.78	57.3±2.7
Soil addition	0.82±0.03	14.11±0.86	58.3±2.4	0.83±0.04	14.19±0.81	59.2±2.7
$I \times Se_L \times Se_{AM}$	*	*	*	*	*	*

** 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 ($P \leq 0.05$).

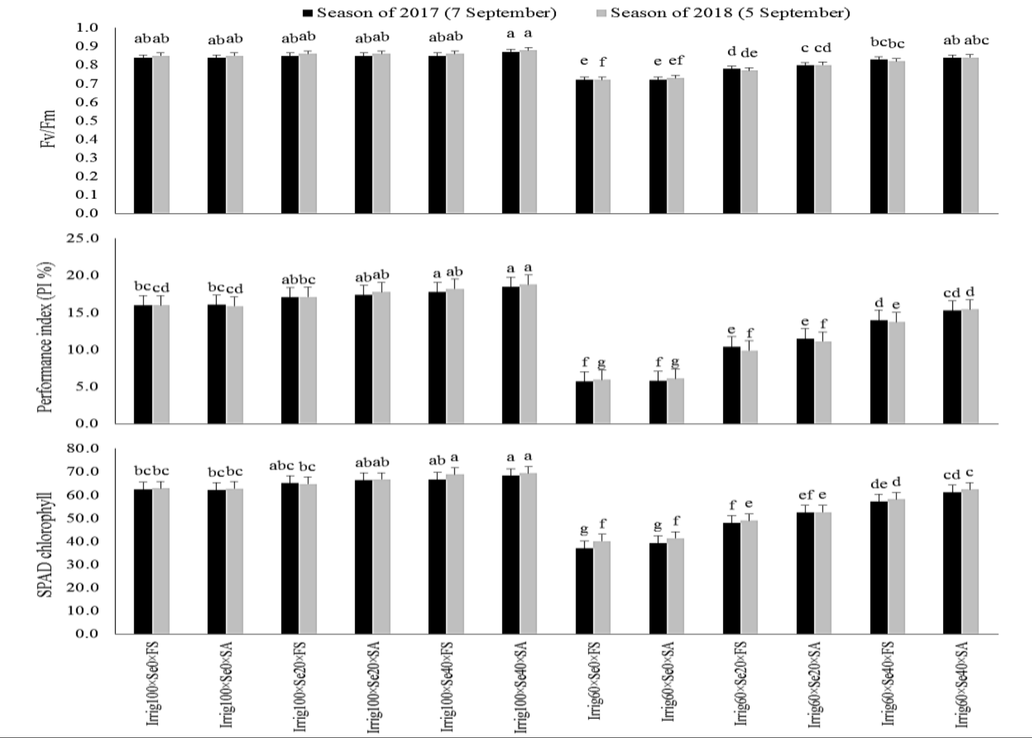

Figure 2. Interaction effects of selenium (Se) level, Se application method, and irrigation levels (100% of soil field capacity; SFC or irrigation water deficit; 60% of SFC) on photosynthetic efficiency of tomato plants in two seasons.

Table 4. Effect of selenium (Se) levels and their application method on the contents of osmoprotectants and 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	Soluble sugars (mg g^{-1} DW)	Free proline (mg g^{-1} DW)	Se content (mg kg^{-1} DW)	Soluble sugars (mg g^{-1} DW)	Free proline (mg g^{-1} DW)	Se content (mg kg^{-1} DW)
	Season of 2017 (7 September)			Season of 2018 (5 September)		
Irrigation (I)	**	*	**	**	*	**
100% of SFC	13.9±0.2^{b}	70.7±1.1^{b}	23.2±0.2^{a}	16.8±0.1^{b}	69.8±1.1^{b}	21.1±0.2^{a}
60% of SFC	18.3±0.3^{a}	84.7±1.5^{a}	15.7±0.1^{b}	24.7±0.2^{a}	82.4±1.5^{a}	15.5±0.1^{b}
Se level (Se_L)	*	*	**	*	*	**
Se_0	12.9±0.2^{c}	66.9±1.0^{c}	11.3±0.1^{c}	15.6±0.1^{c}	64.3±1.0^{c}	11.6±0.1^{c}
Se_{20}	16.7±0.3^{b}	80.2±1.3^{b}	19.8±0.2^{b}	21.3±0.2^{b}	77.7±1.4^{b}	18.8±0.1^{b}
Se_{40}	18.6±0.4^{a}	86.2±1.5^{a}	27.2±0.3^{a}	25.3±0.2^{a}	86.1±1.5^{a}	24.7±0.2^{a}
Se App. (Se_{AM})	*	*	*	*	*	*
Foliar spray	15.2±0.2^{b}	74.6±1.2^{b}	17.4±0.2^{b}	19.5±0.2^{b}	72.7±1.2^{b}	16.5±0.1^{b}
Soil addition	16.9±0.3^{a}	80.8±1.4^{a}	21.4±0.2^{a}	22.0±0.2^{a}	79.4±1.4^{a}	20.2±0.2^{a}
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 ($P \leq 0.05$).

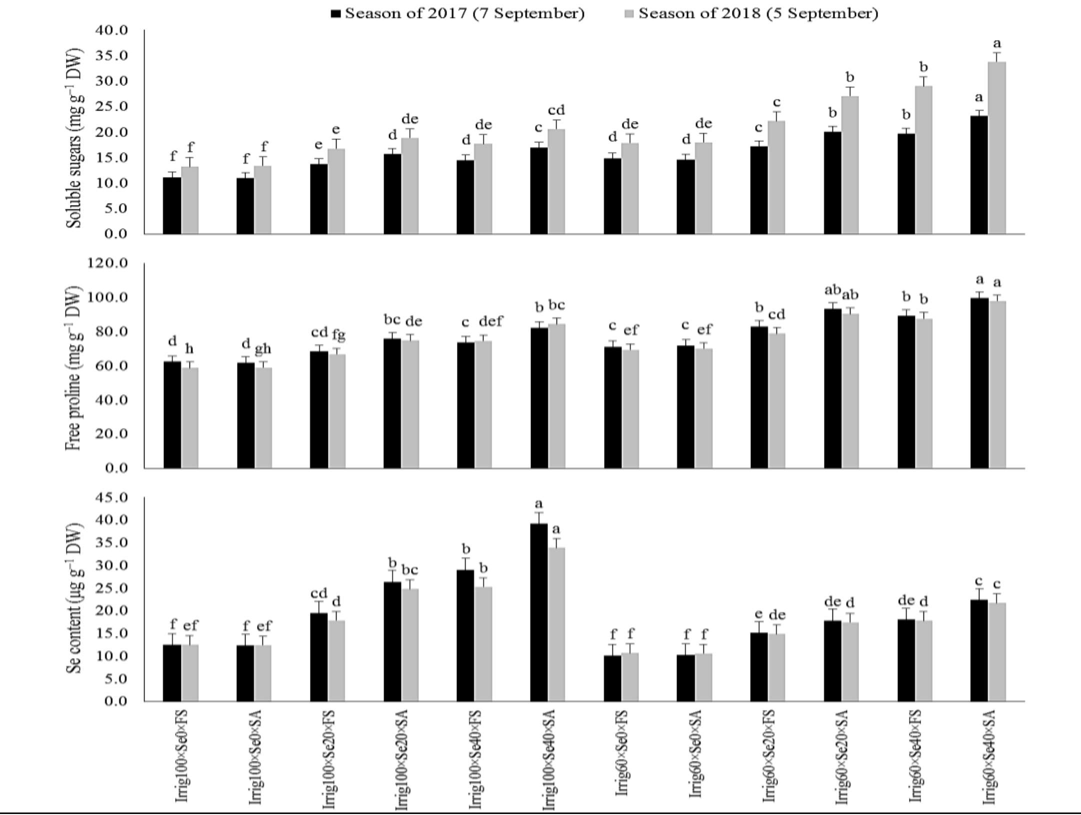

Figure 3. Interaction effects of selenium (Se) level, Se application method, and irrigation levels (100% of soil field capacity; SFC or irrigation water deficit; 60% of SFC) on the contents of osmoprotectants and non-enzymatic antioxidants of tomato plants in two seasons.

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, caused by irrigation water deficit in this study, causes water deficiency in cellular cytoplasm and desiccation that negatively affects osmotic conditions and plant performance. Drought stress not only reduces plant growth and deactivates the health of leaf tissues (Tables 2 and 3, Figs. 1 and 2), but also disrupts the efficiency of photosystem II (Table 4, Fig. 3) due to damages occurred to photochemical proteins such as PsbQ, Lhcb5, and Lhcb6 proteins (Chen *et al.*, 2016). Along with these negative results due to drought stress, selenium (Se) content was found to decrease significantly in tomato plants. Further, Se concentration found in the soil used in this study was 0.05– 0.07 mg kg^{-1} soil (the control). This very low concentration classifies the soil as Se-deficient (Table 4, Fig. 3; Tan *et al.*, 1989; Saha *et al.*, 2017). 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 improved leaf tissue health (Table 2, Fig. 1) and photosynthetic efficiency (Table 3, Fig. 2). These positive results can be obtained due to the increased contents of osmoprotectants (Table 4, Fig. 3) and increased plant Se content (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). Therefore, 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 maintenance of favorable water status in leaf tissue cells is considered a prime defense mechanism in drought-stressed plants (Kaldenhoff *et al*., 2008). Furthermore, plant growth promoted by Se results from an increased starch accumulation in chloroplasts (Pennanen *et al*., 2002) and stimulating water uptake through improved root activity under drought condition (Proiettia *et al*., 2013). The growth-promoting response is also reported in many works with different plant species (soybean– Djanaguiraman *et al*., 2004; potato– Turakainen *et al*., 2004; forage crops– Dhillona *et al*., 2007; and rice– Wang *et al*., 2013).

Reducing irrigation regime from 100% of SFC to 60% of SFC significantly decreased leaf tissue health in terms of reduced relative water content (RWC) and membrane stability index (MSI), and increased electrolyte leakage (EL). This negative result was observed in water deficit-stressed leaf tissue due to higher transpiration rate than water uptake. However, Se application in two methods (foliar spray or soil addition, especially at 40 mM) recovered drought-stressed leaf tissues and significantly increased RWC and MSI, and significantly reduced EL (Table 2, Fig. 1). This positive result regarding leaf tissue health may be due to that Se regulates water status (Nawaz *et al*., 2013) and increases osmoprotectants; soluble sugars and proline contents (Table 4, Fig. 3) under drought stress. The increased contents of the protective parameters such as osmoprotectants (Tables 3-4, Figs. 2–3) elevated in the current study by Se application may be protected cellular plasma membranes from lipid peroxidation, leading 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). It has been reported that Se application significantly increases water retention in plant tissues by elevating the uptake of water by dense and activated root system under water deficit conditions (Yao *et al*., 2009) through the increase in organic and nonorganic osmoprotectants without reducing the transpiration rate (Kuznetsov *et al*., 2003; Emam *et al*., 2014).

Photosynthesis efficiency, in terms of Fv/Fm, performance index (PI), and SPAD chlorophyll in the current study, was significantly decreased with reducing irrigation water to 60% of SFC. However, photosynthesis efficiency was improved by the application of Se under drought stress (Feng *et al*., 2013). Presence of Se in plants with appropriate content reduced the effect of drought stress by reducing the production of reactive oxygen species (ROS) such as H_2O_2 and $O_2^{\bullet-}$ (Feng *et al*. 2013), which could be responsible for quenching photosynthetic pigments. With exposing plants to drought stress, chloroplasts are damaged, leading to impairment in photosynthesis. However, the optimal application of Se can reduce the deterioration of chloroplasts (Malik *et al*., 2012), resulting in increased chlorophyll contents in plant leaves even under conditions of excessive production of ROS. Application of Se can activate photosynthesis process by regulation of PSII and/or plants' defense system components against the damage caused by ROS (Habibi, 2013; Proietti *et al*., 2013). The positive effects of Se associate with Se-mediated regulation of physiological and biochemical processes, such as increased Fv/Fm and chlorophylls content, and activation of antioxidant machinery in water deficit-stressed plants (Nawaz *et al*., 2016), positively reflecting in photosynthesis efficiency.

With exposing to drought stress, plants react by accumulating more metabolites such as free proline and soluble sugars to contribute, in association with endogenous Se, to drought stress tolerance by maintaining cellular turgor due to the osmotic balance, leading to the stability and integrity of plasma membranes, thus preventing or reducing EL and poto-oxidation, and preventing oxidative damage in plants (Hayat *et al*., 2012; Nawaz *et al*., 2016). Nawaz *et al.* (2015) reported that Se regulates net osmolyte accumulations or simple passive solute concentrations to help maintain water status in drought-stressed plants, increasing RWC as described also by Hajiboland *et al*. (2014). Nawaz *et al.* (2016) concluded that Se application stimulates the activity of amylase to hydrolyze starch to increase simple soluble sugars under water deficit conditions. This positive response may also relate to the increased activity of fructose 1, 6-bisphosphatase (F1,6-BPase); a key enzyme in the metabolism of carbohydrates (Owusu-Sekyere *et al*., 2013). In addition, the breakdown of structural proteins has shown to occur to improve biosynthesis of amino acids and their accumulation to modify cellular osmotic adjustment under drought stress conditions (Hsu *et al*., 2003; Nawaz *et al*., 2016). Djanaguiraman *et al.* (2004) suggested that the application of Se disturbs the metabolism of amino acids, which increases the contents of soluble proteins and the activity of nitrate reductase in water deficit-stressed 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).

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 2– 3, Figs. 1– 2). This result may be due to that Se added to irrigation water is easily absorbed by the root system and transolcated to plant shoot more effectively than the diffusion of Se ions from the surface of leaves to epidermal cells through foliar spray.

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 contents of osmoprotectants with the increase of Se content in plant tissues were associated with high cellular relative water content and membrane stability index against electrolyte leakage 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 بتحسين الصفات الفسيولوجية
في نبات الطماطم المجهد بالجفاف

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الملخص:

تهدف هذه الدراسة إلي إلقاء الضوء على تأثيرات السيلينيوم المستخدم بتركيزات (صفر، ٢٠، ٤٠ ملليمول) بطريقتين من الإضافة (إضافة أرضية، رش ورقي) على الصفات الفسيولوجية لنباتات الطماطم النامية تحت ظروف الإجهاد المائي (نقص مياه الرى من ١٠٠% إلى ٦٠% من السعة الحقلية للتربة). قد تم إجراء التجربة خلال موسمي ٢٠١٧، ٢٠١٨م، وقد بينت النتائج المتحصل عليها أن إنخفاض مستوى السعة الحقلية للتربة من ١٠٠% الى ٦٠% أدى إلى حدوث زيادة ملحوظة في نسبة المتسربات الإلكتروليتيه والتى أظهرت إرتباطاً مع زياده المحتوى من الواقيات الإسموزية على العكس من ذلك أظهر المحتوى النسبي للماء وثبات الغشاء وكفاءة البناء الضوئي ومحتوى النبات من السيلينيوم إنخفاضاً في كلا الموسمين. أدى استخدام كلا التركزين إلى حدوث زيادة معنوية في محتوى الواقيات الإسموزية والتى انعكست بدورها في إنخفاض المتسربات الإلكتروليتيه وكفاءة البناء الضوئي ومحتوى النبات من السيلينيوم. ومقارنة بالرش الورقي للسيلينيوم فقد أظهرت الإضافة الأرضية نتائج أفضل وأظهرت معاملة التفاعل بين العوامل الثلاثة المستخدمة في الدراسة (الإجهاد المائي، مستوى السيلينيوم، طريقة الإضافة). من ناحية أخرى فإن معاملة الري بمقدار٦٠% من السعة الحقلية من التربة والاضافة الأرضية بتركيز ٤٠ ملليمول من السيلينيوم كانت أفضل المعاملات تأثيراً والتى يمكن التوصية باستخدامها لزيادة محتوى الواقيات الإسموزية والسيلينيوم في نباتات الطماطم وذلك عند زراعتها في البيئه الجافة.

الكلمات الدالة:
السيلينيوم – الإجهاد المائي– نبات الطماطم – الصفات الفسيولوجية