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Improving Performance of *Glycine max* (L.) by α-tocopherol under Deficit Irrigation in Dry Environments

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Soils in dry environments, including Egypt, suffer from irrigation water shortage. To enhance crops performance under these adverse conditions, antioxidants that proved to support plants are applied. The vitamin α-tocopherol (TOC) is an antioxidant compound that plays a crucial role in improvement of drought tolerance in plant species. Therefore, two field experiments were conducted in 2015 and 2016 seasons to assess the potential effect of foliar application with 0.5 and 1.0 mM TOC on growth, yield and its quality, concentrations of leaf photosynthetic pigments, osmoprotectants and nutrients, and water use efficiency in soybean plants grown under three irrigation levels measured as I_{100} = 100%, I_{80} = 80% and I_{60} = 60% of crop evapotranspiration (ETc). Results showed that under deficit irrigation (DI), growth, photosynthetic efficiency, concentrations of osmoprotectants and nutrients, yield and its quality, and tissue water status (relative water content and membrane stability index) were improved, while electrolyte leakage was reduced by TOC application. These results were positively reflected in plant performance and water use efficiency under water stress conditions. Therefore, TOC could be a promising material to use to mitigate the adverse effects of DI, allowing water savings of 20% – 40% without any detrimental effect on soybean performance.

Introduction

Soybean (*Glycine max* L.) is considered of the most important legume crops in many parts of the world including Egypt. It is grown primarily for oil extraction and animal feed as a protein-rich meal (Singh and Shivakumar, 2010). Its seed contains protein and oil of approximately 40% and 20%, respectively (Li-Juan and Ru-Zhen, 2010). It is among the top 10 of the most widely grown crops and is occupied more than 111 million hectares worldwide (FAO Statistical Yearbook, 2014). Water stress effects on soybean have been extensively reported, showing changes in plant morphology and seed quality, and significant reduction in seed yield from 24% to 50% (Sadeghipour *et al.*, 2012).

Continuously, plants are exposed to a variety of abiotic and biotic stresses under natural conditions of dry environments, including Egypt. These stresses, including drought, generate more of reactive oxygen species (ROS), which are potentially harmful to cell structures and components. To survive under these conditions, enzymatic and non-enzymatic detoxifications are two general protective mechanisms that plants have developed (Alscher *et al.*, 2002). To increase crop water use efficiency (WUE), particularly at a field scale, water management practices are applied such as a combined use of deficit irrigation (DI) and providing crop plants with some antioxidants.

Deficit irrigation (DI; irrigation below the optimum crop water requirements of which crop plants are subjected to a certain level of water stress either during a particular period or throughout the whole growing season) is used nowadays as a strategy to save water (Pereira *et al.*, 2002). In addition, DI is used to increase WUE, as a main goal, by reducing the amount of water applied or by reducing the number of irrigation events (Kirda, 2002).

The vitamin TOC is a low molecular weight lipophilic membrane-located antioxidant. It protects cell membranes from oxidative damage (Asada, 1999). It also improves the membrane stability and permeability, and helps to provide an optimal environment for the photosynthetic machinery (Wise & Naylor, 1987).

It has been a positive correlation between each of the shoot/root growth and TOC in two grass species; tall fescue and creeping bentgrass (Zhang and Schmidt, 2000), plant performance and TOC (Semida *et al.*, 2014 and Rady *et al.*, 2015), and antioxidant system (enzymatic and non-enzymatic antioxidants) and TOC (Orabi & Abdelhamid, 2016 andSemida *et al.,* 2016).

For dry environments, therefore, the main objective of the present study is to assess the ability of TOC to overcome the adverse effects of DI on plant performance and water use efficiency of soybean plants. Some physio-biochemical attributes were also assessed.

Materials and Methods

Two field experiments were conducted in two seasons of 2015 and 2016 at the Experimental Farm of Faculty of Agriculture, Fayoum University, Egypt. Soybean (var. Giza 111) was obtained from the Field Crop Research Institute, Agricultural Research Centre, Giza, Egypt. The experimental area was divided into plots 10.5 m^2 (3 x 3.5 m). Seeds were sown on 3and 5 June, 2015 and 2016 seasons, respectively. Seeds were drilled into one split side of ridges at the rate of 95 kg ha^{-1}. Thinning was done directly before the first irrigation to secure one plant per approximately 5 cm.

Phosphorus in the form of calcium superphosphate (15.5 % P_2O_5), at the rate of 460 Kg ha^{-1} was applied to the soil during seed bed preparation. Nitrogen in the form of ammonium nitrate (33.5 % N) at the rate of 180 kg ha^{-1} was added at two equal portions; with the first and second irrigation. Potassium in the form of potassium sulphate (48 % K_2O) at a rate of 120 kg ha^{-1} was added with the second irrigation.

Soil of the experimental site was analyzed in both seasons according to the procedures of Black *et al.*, (1965) and Jackson (1973), and results are shown in Table 1. Electrical conductivity (EC_e) of the tested soil was measured using a conductivity meter and values were 3.37 and 3.25 dS m^{-1} for 2015 and 2016 seasons, respectively Dahnke & Whitney (1988).

Soybean plants were irrigated every 10-d interval by different amounts of irrigation water applied. The IWA was determined as a percentage of the crop evapotranspiration (ETc), giving one of the following three DI treatments: I_{100} = 100%, I_{80} = 80% and I_{60} = 60%. The daily ETo was computed by the equation of Doorenbos &Pruitt (1992):

$$ETo = Epan \times Kpan$$

Where: ETo = reference evapotranspiration (mm d^{-1}), Epan = evaporation from the Class A pan (mm d^{-1}) and Kpan = pan evaporation coefficient. Monthly mean weather data for a 2-year study (2015 and 2016) were obtained from Itsa weather station, Fayoum Governorate. Monthly mean maximum (T_{max}) and minimum (T_{min}) temperature, relative humidity (RH), wind speed (U) and class A pan evaporation (E-pan) for Itsa weather station are shown in (Table 2). The average daily Epan at Fayoum was 7.65, 7.51, 7.07 and 6.33 mm d^{-1} for June, July, August and September, respectively.

The crop water requirements (ETc) were estimated using the crop coefficient according to the following equation:

$$ETc = ETo \times Kc$$

Where: ETc = crop water requirements, mm d^{-1} and Kc = crop coefficient. The lengths of the different crop growth stages were 20, 25, 75, and 30 days for initial, crop development, mid-season and late season stages, respectively. The crop coefficients (Kc) of initial, mid and end stages were 0.4, 1.15 and 0.50, respectively according to Allen *et al.* (1998). The plots involved irrigation treatments were isolated with 2 m fallow land to avoid the lateral movement of water from irrigation level to another. Sub-plots within each irrigation treatment were isolated by a distance of 0.5 m fallow land. The amount of IWA to each plot during the irrigation regime was determined by using this equation:

$$IWA = \frac{A \times ETc \times Ii}{Ea \times 1000} + LR$$

Where: IWA = irrigation water applied (m^3), A = plot area (m^2), ETc = crop water requirements (mm d^{-1}), Ii = irrigation intervals (d), Ea = application efficiency (65%) and LR = leaching requirements (m^3). Irrigation treatments were started beginning of the second irrigation. The amount of IWA was controlled through plastic pipe (spiles) of 50 mm diameter. One spile per plot was used to convey water for each plot. The amount of water delivered through a spile was calculated using equation of Israelsen & Hansen (1962).

$$Q = CA\sqrt{2gh} \times 10^{-3}$$

Where: Q = discharge of irrigation water (l. sec^{-1}), C = coefficient of discharge, A = cross section area of irrigation pipe (cm^2), g = gravity acceleration (cm. sec^{-2}) and h = average effective head of water (cm).

Twenty days after sowing (DAS), soybean seedlings in each plot were sprayed to run-off with tap water (control), 0.5 mM TOC and 1 mM TOC. The sprays were repeated at 35 and 50 DAS. The concentrations of TOC, and the number and timing of sprays were based on a preliminary pot trial (data not shown). To ensure optimal penetration into leaf tissues, 0.1% (v/v) Tween-20 was added to the foliar sprays as a surfactant.

Seventy-d-old soybean plants (n = 10) were carefully removed from each experimental plot and dipped in a water bucket, and were shaken gently to remove all adhering soil particles. Lengths of shoots were measured using a meter scale. Numbers of leaves and branches plant^{-1} were counted. Leaf area plant^{-1} was measured manually using a graph sheet, where the squares covered by the leaf were counted. The shoots of plants were weighed (fresh weights), and then placed in an oven at 70 °C till constant weights were taken. The dried shoots were weighed (dry weights). At the end of each experiment (27 September 2015 and 29 September 2016), all dry pods on each plant in each experimental plot were collected and counted. Dry seeds were then extracted from pods and weighed to calculate the average 100-seed weight and seed yield ha^{-1}. Oil (%) of seeds was determined in seeds according to the method described by A.O.A.C. (1995). Protein (%) of seeds was calculated by multiplying N (%) × 6.25 after determining N (%) of seeds using micro-Kjeldahl method described in the A.O.A.C. (1995).

Chlorophyll "a", chlorophyll "b", and total carotenoids concentrations (in mg g^{-1} leaf FW) were determined according to the equations shown in Lichtenthaler & Wellburn (1983). As in the method of Hayat *et al.* (2007), the RWC was then calculated using the following formula: RWC (%) = [(FM − DM) ÷ (TM − DM)] × 100. The method of Premchandra *et al.*, (1990) was used to assess the MSI and the following formula was applied: MSI (%) = [1 − (C_1 ÷ C_2)] × 100. The method of Sullivan & Ross (1979) was used to measure the total leakage of inorganic ions from leaves. The EL was calculated using the following formula: EL (%) = [(EC_2 – EC_1)/EC_3] × 100.

Free proline concentration (mg g^{-1} DW) in dried soybean leaves was measured using the Bates *et al.* (1973) method. Total soluble sugars concentration (mg g^{-1} DW) was determined according to Irigoyen *et al.* (1992). Total free amino acids (mg g^{-1} DW) was extracted and measured according to Dubey & Rani (1989). Leaf nitrogen and phosphorus (mg g^{-1} DW) were determined according to Hafez & Mikkelsen (1981) and Jackson (1967), respectively. Leaf potassium (mg g^{-1} DW) was assessed using a Perkin-Elmer, Flame Photometer (Page *et al.*, 1982). Leaf Fe, Mn and Zn (mg kg^{-1} DW) were determined using a Perkin-Elmer, Model 3300, Atomic Absorption Spectrophotometer (Chapman & Pratt, 1961).

WUE values as kg seed yield per m^{-3} of applied water were calculated for different treatments after harvest according to the equation of (Jensen, 1983):

$$\mathrm{WUE} = \frac{\text{seed yield (Kg ha}^{-1})}{\text{water applied (m}^{3}\text{ ha}^{-1})}$$

The experiments were conducted in a split plot arrangement in randomized complete blocks design with three replications. Three DI treatments were randomly distributed in the main plots whilst, three TOC levels were randomly allocated to the sub-plots. All data were subjected to analysis of variance (ANOVA), after testing for homogeneity of error variances according to the procedure outlined by Steel *et al.*, (1997).Combined analysis of data of the two seasons was conducted and significant differences between treatments were compared at $P \leq 0.05$ by Duncan's multiple range test.

Results and Discussion

Growth characteristics

Results in Table 3 show that increase in DI from 20% (I_{80} treatment) to 40% (I_{60} treatment) exhibited significant decreases in growth traits (i.e., shoot length, number of leaves $plant^{-1}$, number of branches $plant^{-1}$, leaf area $plant^{-1}$, shoot FW and shoot DW) of soybean plants. The reductions were 9.0% – 19.4%, 7.0% – 16.8%, 25.9% – 32.8%, 14.4% – 28.8%, 11.8% – 25.9% and 11.0% – 23.2%, respectively compared to growth characteristics of plants grown under full irrigation (I_{100} treatment). However, exogenously-applied 0.5 mM TOC was more effective, compared to 1.0 mM TOC, at increasing the above growth characteristics of soybean plants grown under DI compared to controls (have no TOC application).The increases were 46.1%, 26.4%, 51.5%, 11.2%, 29.0% and 28.6%, respectively for I_{80} treatment, and were 68.5%, 33.5%, 74.1%, 15.1%, 32.4% and 36.5%, respectively for I_{60} treatment, compared to their controls.

Photosynthetic pigment concentrations

Data in Table 4 show that DI significantly reduced the concentrations of chlorophyll "a", chlorophyll "b" and carotenoids. These reductions were gradual with a gradual increase of DI from 20% (I_{80} treatment) to 40% (I_{60} treatment) and were 10.6% – 19.1%, 14.3% – 21.4% and 22.7% – 31.8%, respectively compared to full irrigation (I_{100} treatment). On the other side, exogenously-applied 0.5 mM TOC wasmore effective, compared to 1.0 mM TOC, at alleviating the harmful effects of DI stress. This treatment increased the concentrations of chlorophyll "a", chlorophyll "b" and carotenoids by 16.9%, 15.2% and 26.7%, respectively for I_{80} treatment, and by 22.4%, 24.1% and 41.7%, respectively for I_{60} treatment compared to control plants.

Membrane stability index, relative water content and electrolyte leakage

Data of membrane stability/health in terms of MSI%, RWC% and EL% of soybean plants grown under DI are shown in Table 5. A gradual increase in DI from 20% (I_{80} treatment) to 40% (I_{60} treatment) showed significant decreases in RWC% and MSI% by 5.0% – 11.2 % and 4.3% – 9.8%, respectively, while revealed significant increases in EL% by 18.0% – 30.3% compared to plants grown under full irrigation (I_{100} treatment). However, exogenously-applied TOC was observed to alleviate the detrimental effects of DI stress and significantly increased RWC% and MSI%, and significantly decreased EL% compared to controls (have no TOC application). The TOC applied at a rate of 0.5 mM was more effective, compared to 1.0 mM TOC, at increasing RWC% and MSI% by 12.5% and 14.2%, and 13.5% and 15.5%, and decreasing EL% by 14.7% and 20.1% for I_{80} treatment and I_{60} treatment, respectively compared to controls.

Free proline, total soluble sugars and total free amino acids

Data shown in Table 6 reveal that it has been a significantly increase in the concentrations of free proline, total soluble sugars and total free amino acids in soybean leaves as a result of DI. A gradual increase in DI from 20% (I_{80} treatment) to 40% (I_{60} treatment) gradually increased significantly the concentrations of these attributes by 32.2% – 54.2%, 11.6% – 23.6% and 19.3% – 39.1%, respectively compared to full irrigation (I_{100} treatment). However, exogenously-applied 0.5 mM TOC was more effective, compared to 1.0 mM TOC, at increasing the concentrations of free proline, total soluble sugars and total free aminoby 21.7% and 23.8%, 14.6% and 16.5%, and 11.5% and 13.2% for I_{80} treatment and I_{60} treatment, respectively compared to control plants (have no TOC application).

Macro- and micro-nutrients

Data recorded in Table 7 show that a gradual increase in DI from 20% (I_{80} treatment) to 40% (I_{60} treatment) significantly decreased macro- and micro-nutrients such as N, P, K, Fe, Mn and Zn by 8.9% – 18.4%, 23.1% – 28.2%, 14.7% – 17.6%, 10.3% – 10.7%, 9.6% – 15.6% and 8.3% – 18.1%, respectively compared to full irrigation (I_{100} treatment). However, exogenously-applied 0.5 mM TOC was more effective, compared to 1.0 mM TOC, at increasing N, P, K, Fe, Mn and Zn compared controls (have no TOC application).The increases were 17.8%, 52.2%, 21.0%, 16.5%, 19.6% and 18.0%, respectively for I_{80} treatment and were 27.8%, 57.1%, 22.8%, 17.8%, 21.8% and 24.5%, respectively for I_{60} treatment compared to control plants.

Yield and yield quality

Number of pods plant^{-1}, seed yield plant^{-1}, weight of 100-seed, seed yield ha^{-1}, oil% and protein% were negatively affected by DI (Table 8). A gradual increase in DI from 20% (I_{80} treatment) to 40% (I_{60} treatment) revealed significant reductions in yield and its quality by 6.8% – 14.8%, 14.4% – 24.8%, 6.8% – 13.6%, 15.8% – 31.6%, 10.2% – 17.5% and 8.3% – 14.3%, respectively compared to full irrigation (I_{100} treatment). However, exogenously-applied 0.5 mM TOC, compared to 1.0 mM TOC, was more effective at increasing the aforementioned yield characteristics compared to controls (have no TOC application). These increases were 38.9%, 23.9%, 30.3%, 24.1%, 16.0% and 17.3%, respectively for I_{80} treatment and were 44.2%, 27.7%, 35.1%, 26.1%, 20.5% and 22.5%, respectively for I_{60} treatment compared to control plants.

Water use efficiency (WUE)

Data in Table 8 demonstrate that WUE was significantly affected by irrigation treatments under study. In fact, this parameter significantly increased with increasing the DI from 20% (I_{80} treatment) to 40% (I_{60} treatment) by 7.1% – 14.3% compared to full irrigation (I_{100} treatment). In addition, exogenously-applied TOC was observed to increase WUE compared to controls (have no TOC application). Applied 0.5 mM TOC was more effective, compared to 1.0 mM TOC, at increasing WUE by 25.0% and 29.3% for I_{80} treatment and I_{60} treatment, respectively compared to controls.

Dry environments (arid and semi-arid regions) characterize by low water availability (drought) that causes a great problem for agricultural sector (Mekdad & Rady 2016). This study attempts to follow deficit irrigation (DI) strategy to qualify soybean crop to resist/tolerate drought with supporting plant by antioxidants application such as α-tocopherol (TOC).

Many reports concluded that plant response to drought stress depends on species and genotype, length and severity of water deficit (WD) stress, and age and development stage of plant (Zhang & Oweis1999).The vitamin TOC is a potential signaling molecule. The action mechanisms of TOC in improving drought stress tolerance in crop plants are not fully interpreted. This effect depends not only on the applied concentration and the application method but also on the overall plant status (i.e., developmental stage, oxidative balance of the cells and acclimation by biotic or abiotic stresses).

The present investigation suggests that the exogenous application of TOC may help to alleviate the harmful effects of stress initiated in various crops subjected to WD stress; DI. It was observed, in the present study, that the stress generated from DI, reduced growth attributes of soybean plants (Table 3). The inhibiting effects of WD stress on plant growth have previously been reported for soybean plants (Abd El-Mageed *et al.*, 2016a& b).A decline in plant growth and response to DI might be due to decrease in cell elongation caused by the inhibiting effect of WD on growth-promoting hormones which, in turn, lead to decreases in cell turgor, volume and eventually growth. However, foliar-applied TOC treatments have shown to increase growth characteristics, particularly shoot fresh and dry weights in soybean plants. These positive results with TOC were similarly observed earlier for some crops such as soybean(Rady *et al.*, 2015),flax(Sadak & Dawood, 2014),onion (Semida *et al.*, 2016), faba beans (Semida *et al.*, 2014 and Orabi & Abdelhamid 2016). The vitamin TOC, as an antioxidant, deactivates photosynthesis-derived reactive oxygen species (ROS), and prevents the increase in lipid peroxidation by scavenging lipid peroxyl radicals in thylakoid membranes. Levels of TOC change differentially in response to environmental restrictions, depending on the magnitude of the stress and species-sensitivity to stress(Semida *et al.*, 2016).This agrees with the results of this study that foliar-applied TOC significantly increased soybean yields under DI stress (Table 8).

Drought stress partially inhibited photosynthesis by the reduction in the concentrations of chlorophyll "a", chlorophyll "b" and carotenoids (Table 4). The decrease in chlorophyll concentrations under drought stress is a commonly observed phenomenon (Mohamed & Akladious, 2014). However, foliar-applied TOC increased chlorophyll "a", chlorophyll "b" and carotenoids under DI conditions (Table 4). The increase in carotenoids concentration by TOC might play a key role as a free radical scavenger (Sakr & El-Metwally, 2009) and enhancing plant capacity to reduce the damage caused by ROS (Orabi & Abdelhamid, 2016).

Table.1 Physical and chemical properties* of the experimental site in both 2015and 2016 seasons

Properties	2015	2016
Physical properties:		
Sand %	79.5	79.2
Silt %	7.8	8.2
Clay %	12.7	12.6
Texture class	Loamy sand	Loamy sand
Bulk density g cm^{-3}	1.63	1.65
Field capacity (%)	25.27	24.43
Wilting point (%)	9.75	9.75
Available water (%)	15.63	15.63
Chemical properties:		
EC_e dS m^{-1}	3.37	3.25
pH	7.85	7.83
Organic matter (%)	1.05	1.13
Exchangeable cations (mg kg^{-1} soil):		
Ca^{++}	11.53	10.05
Mg^{++}	8.3	6.9
Na^{+}	16.03	17.25
K^{+}	0.59	0.61
Soluble anions (mg kg^{-1} soil):		
CO_3^{-}	0.00	0.00
H CO_3^{-}	2.53	3.41
Cl^{-}	13.03	12.43
SO_4^{-}	21.07	17.93
Available nutrients:		
N %	0.005	0.003
P (mg kg^{-1} soil)	531.21	529.23
K (mg kg^{-1} soil)	73.21	71.13
Fe (mg kg^{-1} soil)	3.61	5.53
Mn (mg kg^{-1} soil)	10.59	10.43
Zn (mg kg^{-1} soil)	0.73	0.55

*All analyses were done in Central Laboratory of Soil, Water and Plant Analysis (Iso-17025), Faculty of Agriculture, Fayoum University, Egypt.

Table.2 Monthly mean maximum and minimum temperature, relative humidity, wind speed and class A pan evaporation for Itsa Station across the 2015 and 2016 seasons

Months	T_{max} (°C)	T_{min} (°C)	RH (%)	U (m^{s-1})	E-pan ($mm\ d^{-1}$)
June	37.63	21.05	54.09	5.54	7.65
July	39.11	22.31	54.99	5.16	7.51
August	38.78	22.85	57.04	4.84	7.07
September	35.98	21.40	57.93	4.84	6.33

Table.3 Effect of exogenously-applied of α-tocopherol (TOC; mM) on growth characteristics of Glycine max (L.) plants grown under deficit irrigation

Treatments		Shoot length (cm)	No. of leaves $plant^{-1}$	No. of branches $plant^{-1}$	Leaf area $plant^{-1}$ (dm^2)	Shoot FW (g)	Shoot DW (g)
Irrigation	TOC						
I_{100}	0	37.8 ± 1.5c	21.9 ± 0.9c	4.9 ± 0.5b	10.4 ± 1.2b	35.0 ± 1.3c	7.3 ± 0.7b
	0.5	50.1 ± 2.9a	26.7 ± 1.7a	6.3 ± 0.7a	11.6 ± 1.3a	44.7 ± 2.6a	9.0 ± 1.1a
	1	47.9 ± 2.1b	24.5 ± 1.3b	6.1 ± 0.6a	11.3 ± 1.3a	42.1 ± 1.8b	8.4 ± 0.9a
	Mean	**45.3A**	**24.4A**	**5.8A**	**11.1A**	**40.6A**	**8.2A**
I_{80}	0	32.3 ± 1.2c	20.1 ± 0.7c	3.3 ± 0.3b	8.9 ± 0.9b	30.7 ± 1.5c	6.3 ± 0.5b
	0.5	47.2 ± 2.7a	25.4 ± 1.5a	5.0 ± 0.5a	9.9 ± 1.2a	39.6 ± 2.3a	8.1 ± 0.9a
	1	44.2 ± 1.7b	22.5 ± 1.2b	4.7 ± 0.4a	9.7 ± 1.1a	37.3 ± 1.9b	7.6 ± 0.8a
	Mean	**41.2B**	**22.7B**	**4.3B**	**9.5B**	**35.8B**	**7.3B**
I_{60}	0	25.7 ± 0.9c	17.3 ± 0.6c	2.7 ± 0.1b	7.3 ± 0.7b	25.3 ± 0.7c	5.2 ± 0.3b
	0.5	43.3 ± 2.3a	23.1 ± 1.1a	4.7 ± 0.3a	8.4 ± 0.9a	33.5 ± 1.9a	7.1 ± 0.6a
	1	40.5 ± 1.1b	20.5 ± 0.9b	4.3 ± 0.2a	8.1 ± 0.8a	31.4 ± 1.2b	6.7 ± 0.5a
	Mean	**36.5C**	**20.3C**	**3.9B**	**7.9C**	**30.1C**	**6.3C**

Mean values (n = 10) in the same column for each trait with the same lower- or upper-case letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$. mM = millimole; cm = centimeter; dm = decimeter; FW = fresh weight; DW = dry weight; g = gram; I_{100}, I_{80} and I_{60} = irrigation with 100%, 80% and 60% of ETc, respectively.

Table.4 Effect of exogenously-applied of α-tocopherol (TOC; mM) on the concentrations of leaf photosynthetic pigments of Glycine max (L.) plants grown under deficit irrigation

Treatments		Chlorophyll"a" ($mg\ g^{-1}$ FW)	Chlorophyll "b" ($mg\ g^{-1}$ FW)	Carotenoids ($mg\ g^{-1}$ FW)
Irrigation	TOC			
I_{100}	0	0.88 ± 0.08b	0.39 ± 0.04b	0.18 ± 0.03b
	0.5	0.99 ± 0.09a	0.45 ± 0.06a	0.25 ± 0.04a
	1	0.95 ± 0.08a	0.43 ± 0.05a	0.23 ± 0.04a
	Mean	**0.94A**	**0.42A**	**0.22A**
I_{80}	0	0.77 ± 0.06b	0.33 ± 0.04b	0.15 ± 0.02b
	0.5	0.90 ± 0.07a	0.38 ± 0.05a	0.19 ± 0.03a
	1	0.86 ± 0.07a	0.36 ± 0.05a	0.17 ± 0.02a
	Mean	**0.84B**	**0.36B**	**0.17B**
I_{60}	0	0.67 ± 0.04b	0.29 ± 0.03b	0.12 ± 0.01b
	0.5	0.82 ± 0.06a	0.36 ± 0.04a	0.17 ± 0.03a
	1	0.79 ± 0.05a	0.35 ± 0.03a	0.15 ± 0.02a
	Mean	**0.76B**	**0.33B**	**0.15B**

Mean values (n = 10) in the same column for each trait with the same lower- or upper-case letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$. mM = millimole; mg = milligram**;** g = gram; FW = fresh weight; I_{100}, I_{80} and I_{60} = irrigation with 100%, 80% and 60% of ETc, respectively.

Table.5 Effect of exogenously-applied of α-tocopherol (TOC; mM) on leaf relative water content (RWC%), membrane stability index (MSI%) and electrolyte leakage (EL%) of Glycine max (L.) plants grown under deficit irrigation

Treatments		RWC (%)	MSI (%)	EL (%)
Irrigation	TOC			
I_{100}	0	77.5 ± 3.1c	64.3 ± 2.9c	13.2 ± 1.5a
	0.5	87.5 ± 4.3a	72.0 ± 3.9a	11.3 ± 1.4b
	1	79.3 ± 3.8b	67.9 ± 3.2b	12.1 ± 1.3b
	Mean	**81.4A**	**68.1A**	**12.2C**
I_{80}	0	73.6 ± 2.8c	61.4 ± 2.4c	15.6 ± 1.7a
	0.5	82.8 ± 4.1a	69.7 ± 3.6a	13.3 ± 1.6b
	1	75.4 ± 3.7b	64.6 ± 3.1b	14.3 ± 1.6b
	Mean	**77.3B**	**65.2B**	**14.4B**
I_{60}	0	67.7 ± 2.7c	57.5 ± 2.1c	17.9 ±1.8a
	0.5	77.3 ± 3.7a	66.4 ± 3.3a	14.3 ±1.7b
	1	71.9 ± 3.3b	60.2 ± 2.5b	15.5 ±1.8b
	Mean	**72.3C**	**61.4C**	**15.9A**

Mean values (n = 10) in the same column for each trait with the same lower- or upper-case letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$. mM = millimole; I_{100}, I_{80} and I_{60} = irrigation with 100%, 80% and 60% of ETc, respectively.

Table.6 Effect of exogenously-applied of α-tocopherol (TOC; mM) on leaf concentrations of free proline, total soluble sugars and total free amino acids of Glycine max (L.) plants grown under deficit irrigation

Treatments		Free proline ($mg\ g^{-1}$ DW)	Total soluble sugars ($mg\ g^{-1}$ DW)	Total free amino acids ($mg\ g^{-1}$DW)
Irrigation	TOC			
I_{100}	0	0.53 ± 0.06b	21.5 ± 1.1c	61.7 ± 2.6c
	0.5	0.64 ± 0.07a	23.7 ± 1.4a	71.5 ± 3.5a
	1	0.61 ± 0.07a	22.3 ± 1.2b	66.9 ± 2.9b
	Mean	**0.59C**	**22.5C**	**66.7C**
I_{80}	0	0.69 ± 0.07b	23.3 ± 1.2c	74.7 ± 3.1c
	0.5	0.84 ± 0.08a	26.7 ± 1.7a	83.3 ± 4.2a
	1	0.81 ± 0.08a	25.4 ± 1.6b	80.9 ± 3.7b
	Mean	**0.78B**	**25.1B**	**79.6B**
I_{60}	0	0.80 ± 0.07b	25.5 ± 1.5c	86.1 ± 3.6c
	0.5	0.99 ± 0.09a	29.7 ± 1.9a	97.5 ± 4.7a
	1	0.95 ± 0.08a	28.3 ± 1.8b	94.7 ± 4.1b
	Mean	**0.91A**	**27.8A**	**92.8A**

Mean values (n = 10) in the same column for each trait with the same lower- or upper-case letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$. mM = millimole; mg = milligram**;** DW = dry weight; g = gram; I_{100}, I_{80} and I_{60} = irrigation with 100%, 80% and 60% of ETc, respectively.

Table.7 Effect of exogenously-applied of α-tocopherol (TOC; mM) on shoot concentration of macronutrients (mg g-1 DW) and micronutrients (mg kg-1 DW) of Glycine max (L.) plants grown under deficit irrigation

Treatments: Irrigation	Treatments: TOC	N	P	K	Fe	Mn	Zn
I_{100}	0	14.7 ± 1.6c	3.1 ± 0.3b	12.5 ± 1.6c	217 ± 7.5c	127 ± 5.1c	63 ± 3.1c
	0.5	17.1 ± 1.9a	4.5 ± 0.4a	14.9 ± 1.8a	249 ± 13.3a	145 ± 8.7a	81 ± 4.1a
	1	15.4 ± 1.7b	4.2 ± 0.4a	13.3 ± 1.6b	233 ± 10.4b	132 ± 7.7b	72 ± 3.6b
	Mean	**15.7A**	**3.9A**	**13.6A**	**233A**	**135A**	**72A**
I_{80}	0	13.5 ± 1.5b	2.3 ± 0.2b	10.5 ± 1.4c	194 ± 6.5c	112 ± 3.4c	61 ± 3.1c
	0.5	15.9 ± 1.8a	3.5 ± 0.3a	12.7 ± 1.6a	226 ± 10.1a	134 ± 7.9a	72 ± 3.7a
	1	13.6 ± 1.6b	3.2 ± 0.3a	11.5 ± 1.5b	208 ± 8.3b	120 ± 4.7b	65 ± 3.3b
	Mean	**14.3B**	**3.0B**	**11.6B**	**209B**	**122B**	**66B**
I_{60}	0	11.5 ± 1.3c	2.1 ± 0.2b	10.1 ± 1.4c	191 ± 6.4c	101 ± 3.3c	53 ± 2.5c
	0.5	14.7 ± 1.5a	3.3 ± 0.3a	12.4 ± 1.5a	225 ± 9.9a	123 ± 4.9a	66 ± 3.5a
	1	12.3 ± 1.4b	3.1 ± 0.3a	11.2 ± 1.5b	207 ± 8.1b	117 ± 4.3b	57 ± 2.9b
	Mean	**12.8C**	**2.8B**	**11.2B**	**208C**	**114C**	**59C**

Mean values (n = 10) in the same column for each trait with the same lower small or upper bold-case letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$. mM = millimole; mg = milligram; g = gram; kg = kilogram; macronutrients [(i.e. nitrogen (N), phosphorus (P) and potassium (K)]; micronutrients [(i.e. iron (Fe), manganese (Mn) and zinc (Zn)]; I_{100}, I_{80} and I_{60} = irrigation with 100%, 80% and 60% of ETc, respectively.

Table.8 Effect of exogenously-applied of α-tocopherol (TOC; mM) on yield, yield quality and water use efficiency (WUE) of Glycine max (L.) plants grown under deficit irrigation

Treatments: Irrigation	Treatments: TOC	No. of pods plant^{-1}	Seed yield plant^{-1} (g)	100-seed weight (g)	Seed yield (ton h^{-1})	Oil (%)	Seed-protein (%)	WUE (Kg m^{-3})
I_{100}	0	20.1 ± 2.2c	13.6 ± 1.7b	15.9 ± 1.7b	3.4 ± 0.4c	19.3 ± 2.5b	38.7 ± 2.9b	0.37 ± 0.04c
	0.5	27.7 ± 2.8a	16.7 ± 2.1a	19.5 ± 2.2a	4.2 ± 0.6a	22.5 ± 2.7a	43.9 ± 3.3a	0.46 ± 0.07a
	1	23.3 ± 2.4b	15.7 ± 1.8a	17.6 ± 1.9b	3.8 ± 0.5b	19.9 ± 2.5b	41.1 ± 3.2a	0.42 ± 0.05b
	Mean	**23.7A**	**15.3A**	**17.7A**	**3.8A**	**20.6A**	**41.2A**	**0.42C**
I_{80}	0	18.5 ± 2.1c	11.7 ± 1.6b	14.2 ± 1.4b	2.9 ± 0.3c	17.5 ± 2.2b	34.7 ± 2.7b	0.40 ± 0.06c
	0.5	25.7 ± 2.5a	14.5 ± 1.7a	18.5 ± 1.9a	3.6 ± 0.4a	20.3 ± 2.5a	40.7 ± 3.1a	0.50 ± 0.09a
	1	22.1 ± 2.3b	13.2 ± 1.7a	16.7 ± 1.5b	3.2 ± 0.4b	17.7 ± 2.3b	38.0 ± 3.1a	0.44 ± 0.07b
	Mean	**22.1B**	**13.1B**	**16.5B**	**3.2B**	**18.5B**	**37.8B**	**0.45B**
I_{60}	0	16.3 ± 1.9c	10.1 ± 1.3b	13.1 ± 1.3b	2.3 ± 0.2c	15.6 ± 1.7b	31.1 ± 2.5b	0.41 ± 0.07c
	0.5	23.5 ± 2.6a	12.9 ± 1.5a	17.7 ± 1.5a	2.9 ± 0.3a	18.8 ± 2.1a	38.0 ± 2.9a	0.53 ± 0.09a
	1	20.7 ± 2.3b	11.6 ± 1.4a	15.0 ± 1.4b	2.5 ± 0.2b	16.7 ± 1.9b	36.4 ± 2.8a	0.46 ± 0.08b
	Mean	**20.2C**	**11.5C**	**15.3C**	**2.6B**	**17.0C**	**35.3C**	**0.48A**

Mean values (n = 10) in the same column for each trait with the same lower- or upper-case letters are not significantly different by Duncan's multiple range test at $P \leq 0.05$. mM = millimole; g = gram; h = hectare; kg = kilogram; m = meter; I_{100}, I_{80} and I_{60} = irrigation with 100%, 80% and 60% of ETc, respectively.

Soybean plants subjected to DI and exogenously-applied with TOC, particularly at 0.5 mM, exhibited increases in nutrient concentrations relative to their corresponding control plants (Table 7), showing mitigation of the adverse effect of DI stress on mineral composition in soybean leaves. Increasing the osmotolerance and/or regulating the various processes including absorption of nutrients from soil solution

(Sadak and Dawood, 2014) due, directly or indirectly, to application of TOC are of mechanisms of TOC to restore mineral nutrients in soybean plants. In addition, Rady *et al.* (2015) reported some roles for TOC in plants such as improving membrane permeability and increasing soluble protein concentrations to protect the membranes and membrane-bound enzymes. Thus, TOC protect plants against stress toxicity through its roles in maintaining the structural integrity of the plasma membrane and increasing the uptake of mineral nutrients.

Water status of the soybean leaves was expressed by RWC% and water use efficiency (WUE) parameters (Tables 5 and 8). The WUE increased under DI and further increased by TOC application. In this study, the RWC and MSI were increased as EL was decreased under DI stress by the application of TOC, suggesting maintaining the healthy metabolic processes in soybean leaves. Semida *et al.,* (2014) and Rady *et al.,* (2015) concluded that there is a relationship between the RWC and the biomass (fresh and dry weights) in soybean under the interactive effect of DI stress and TOC application. This indicates that the water status of soybean leaves is basically dependent on the respective shoot biomass. This also suggests that soybean plants with greater biomass can maintain higher water content in their leaves and thus plants can be more tolerant to drought.

In conclusion, exposure of soybean plants to DI resulted in decreases in plant growth, RWC, MSI, leaf photosynthetic pigments, nutrient concentrations and yields, and increases in the concentrations of osmoprotectants (i.e., free proline, total soluble sugars and total free amino acids), electrolyte leakage and WUE. Therefore, this study suggests spraying soybean plants with 0.5 mM TOC to overcome the adverse effects of DI stress by increased concentrations of osmoprotectants and increased RWC acting as osmotic and metabolic regulators or substrates and in a part as cell component stabilizers. Accordingly, our study considered TOC as a beneficial foliar application at a rate of 0.50 mM to improve growth, physio-biochemical attributes, yield and WUE of soybean plants under DI with saving waters by 20% - 40% to use in other needed purposes under the conditions of WD worldwide caused by climatic changes.

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