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The Changes in Yield Response Factor, Water Use Efficiency, and Physiology of Sunflower Owing to Ascorbic and Citric Acids Application Under Mild Deficit Irrigation

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Abstract

Under arid and semi-arid climates, adopting the appropriate tools for alleviating water deficit impacts is a critical factor that affects the physiological characteristics and yield of sunflower. Therefore, in order to find promising field practices in sunflower cultivation, the strip plots design in randomized complete block arrangement was used to examine the effects of two irrigation regimes as 100% (FI) and 85% (DI) of crop evapotranspiration and five antioxidant treatments on physiological and agronomic traits, yield response factor, and irrigation water use efficiency (IWUE) of sunflower. The antioxidant treatments involved two rates of ascorbic acid (150 and 300 mg L^{-1}) and two rates of citric acid (250 and 500 mg L^{-1}), in addition to the check treatment (tap water). The study was conducted for two growing seasons of 2019 and 2020 at the Experimental Farm of Ain Shams University, Egypt, located in a semi-arid environment. Findings showed that exogenous application of higher rate of ascorbic acid, i.e. 300 mg L^{-1} with FI exhibited the highest increase of chlorophyll *a*, chlorophyll *b* and the lowest proline content compared to other interaction treatments. Seed yield was significantly higher with FI plus ascorbic acid 300 mg L^{-1} and DI plus ascorbic acid 300 mg L^{-1} treatments than with their counterpart check treatment in both growing seasons. Under DI, IWUE was improved with antioxidant-treated plants compared to untreated plants. Yield response factor as an indicator of crop tolerance to drought was higher than the unit (> 1) under all ascorbic acid and citric acid levels. It could be concluded that ascorbic acid and citric acids partially mitigated the reductions in growth and yield caused by low water supply. However, yield response factor demonstrated that the crop is still sensitive to drought. Thus, other applicable patterns should be adopted to increase the yield potential of sunflower for counteracting the adverse impacts of drought.

Keywords Anti-stress agents · Drought · Oil yield · Oxidative stress · Plant pigments · Sunflower productivity

Introduction

The predicted global warming and climate changes indubitably has adverse effects such as drought, salinity and nutrient deficiency on growth and productivity of economic crops (Saudy and Mubarak 2015; Abd El-Mageed et al. 2022). Such effects are most pronounced in arid and semi-arid regions, which suffer from water scarcity (Souri and Hatamian 2019). Accordingly, irrigation strategies that ensure the efficient and rational use of water and nutrients must be adopted (Saudy and El-Bagoury 2014; Saudy and El-Metwally 2019, 2022; Saudy et al. 2020a). In this respect, deficit irrigation strategy could save water and increase water use efficiency (WUE), resolving the contradiction of water needs and supply in semi-arid regions (Cui et al. 2009). Moreover, several studies proved that deficit irrigation had significant effects on water saving and increase in WUE, clarifying the priority of deficit water tactic in crop irrigation programs under water limitation in arid environment as in Egypt (El-Bially et al. 2018; El-Metwally and Saudy 2021a; El-Metwally et al. 2022a; Saudy et al. 2022b). However, using water less than normal represents abiotic stress (drought) on crop plants. Decrease in

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leaf water potential, promoting stomatal closure, decrease in leaf photosynthesis, and weakness in nutrient uptake are the most physiological phenomena associated with water deficit (Bresson et al. 2015; Yan et al. 2016; El-Metwally et al. 2021; Abd-Elrahman et al. 2022; Makhlouf et al. 2022). Decrease in sunflower yield was reported by Saudy et al. (2021a); El-Bially et al. (2022a, b) as 22.4–42.8% owing to water deficit stress.

Sunflower (*Helianthus annuus* L.) is one of the main oil crops in the world. Among oilseed crops, sunflower is the fifth most cultivated annual crop (FAO 2017). Sunflower seed has high oil (36–52%) and protein (28–32%) contents (Rosa et al. 2009) and extracted oil has low cholesterol and high unsaturated fatty acids (Qahar et al. 2010). Moreover, sunflower can adapt to different climatic and soil conditions (Kaleem et al. 2011).

Therefore, in particularly economically valuable plants, many approaches have been employed to induce stress tolerance with enhancing the crop growth and development (Saudy et al. 2018, 2021b). Ascorbic acid (ASC) is a plant nonenzymatic antioxidant and mediates biotic and abiotic stress, since it is the first line of plant defense against oxidative stress (Sharma et al. 2019). Since ASC is mostly a substrate of ascorbate peroxidase, an essential enzyme of the ascorbate-glutathione pathway, it acts as a protector against the oxidative stress by removing several free radicals (Bilska et al. 2019; Sharma et al. 2019).

Citric acid (CA) is a weak organic acid found in many fruits and is used as preservative agent in human food being an antioxidant. Inside the plant cell, citric acid plays an important role in the intermediary metabolism, being a component of the tricarboxylic acid cycle or Krebs cycle (Omar et al. 2018). In some plant species as cotton (Gebaly et al. 2013) and bean (El-Tohamy et al. 2013), CA ameliorated the adverse effect of drought.

The current study hypothysized that sunflower plants have different significant response to both ASC and CA under drought conditions. Therefore, the main objective of this article is to evaluate the influence of different levels of ascorbic and citric acids on physiological and agronomic traits, yield response factor, and irrigation water use efficiency of sunflower under mild water stress compared to full irrigation.

Method and Materials

Experimental Site

The current work was conducted for two years during two growing summer seasons of 2019 and 2020, at the Experimental Farm, Faculty of Agriculture, Ain Shams University, El Nubaria region, El Behaira Governorate, Egypt (30° 30′N, 30° 20′E). According to the aridity categorization (Ponce et al. 2000), the experimental site was in a semi-arid environment with no rainfall in summer (beginning of April to late October). The averages of minimum air temperature were 21.2–20.3 °C, maximum air temperature were 33.2–32.4 °C, relative humidity were 54.3–55.8%, wind speed were 3.4–3.5 m sec^{-1}, solar radiation were 28.3–28.6 MJ m^{-2} day^{-1}, and mean class "A" pan evaporation was 3.90 and 3.92 mm d^{-1} for 2019 and 2020 seasons, respectively. The soil of the experimental site is classified as sandy-loam. In the root zone, soil water contents at the field capacity and permanent wilting point were 12.3–12.6% and 5.4–4.9% in both seasons, respectively. The properties of soil in the experimental site are shown in Table 1.

Experimentation and Procedures

This experiment was implemented in a strip-plots in randomized complete blocks design with three replicates. In the vertical plots, two levels of irrigation as 85 and 100% of full irrigation representing drought (deficit irrigation, DI) and well-watered (full irrigation, FI) treatments, respectively, were applied. The experimental unit had an area of 14 m^2 (4 m × 3.5 m) with five ridges. The distance between plots was 1.0 m to prevent the overlapping of irrigation water of the nearby treatments. Sunflower seeds (cv. Sakha 53) were planted by hand in hills at 20-cm distance

Table 1 Physico-chemical properties of soil at the experimental research station of El Nubaria, Egypt

Parameter	Unit	Value
Mechanical analysis		
Sand	%	87.3 ± 0.2
Silt	%	5.1 ± 0.1
Clay	%	7.6 ± 0.1
Bulk density	g cm^{-3}	1.62 ± 0.21
Chemical analysis		
pH (1:2.5)	–	7.8 ± 0.1
Electrical conductivity (EC)	dS m^{-1}	2.7 ± 0.2
Organic matter	%	0.83 ± 0.03
Calcium cations (Ca^{+2})	Meq L^{-1}	19.1 ± 0.6
Magnesium cations (Mg^{+2})	Meq L^{-1}	8.2 ± 0.1
Potassium cations (K^+)	Meq L^{-1}	0.83 ± 0.02
Sodium cations (Na^+)	Meq L^{-1}	1.95 ± 0.01
Chloride anions (Cl^-)	Meq L^{-1}	5.24 ± 0.03
Bicarbonate anions (HCO_3^-)	Meq L^{-1}	3.10 ± 0.05
Water status		
Saturation percentage	%	21.0 ± 0.03
Field capacity	%	12.5 ± 0.01
Wilting point	%	5.2 ± 0.03
Available water	%	7.71 ± 0.23

Values are the mean of 3 replicates ± standard errors on the ridge at the depth of 3 cm on the 19th and 21st of May in 2019 and 2020, respectively. At 15 days after sowing (DAS), plants were thinned to secure one plant per hill. The recommended doses of mineral fertilizers were applied as follows: 21.2 kg ha^{-1} P was applied during the soil preparation as calcium super phosphate 15.5% P_2O_5, while N (107.1 kg ha^{-1}) was added as ammonium nitrate (33.5% N) into five equal doses, @ 10, 20, 30, 40 and 50 DAS. Additionally, K (47.4 kg ha^{-1}) was applied as potassium sulfate (48% K_2O) in two equal doses at sowing and 50 DAS. Plants were irrigated through drip irrigation system using drippers of 2 L h^{-1} capacity.

Five treatments of antioxidant solutions occupied the horizontal plots involving two rates of ascorbic acid, ASC (150 and 300 mg L^{-1} namely: ASC150 and ASC 300, respectively) and two rates of citric acid, CA (250 and 500 mg L^{-1} namely: CA250 and CA500, respectively), in addition to the check treatment (tap water). The spray solutions were applied two twice 35 and 50 DAS, syncorinizing the plant heights of 40–50 and 90–100 cm, respectively, using a knapsack sprayer having a single nozzle with volume of 500 L ha^{-1}.

Irrigation Requirements

Reference evapotranspiration (ET_o; mm day^{-1}) was calculated from the recorded meteorological data of the study area using the FAO-56 Penman-Monteith equation (Allen et al. 1998).

The obtained reference evapotranspiration (ET_o) values during sunflower growth stages are shown in Fig. 1. Moreover, irrigation water requirement for sunflower was calculated by determining the daily crop evapotranspiration (ET_c). The depth of applied irrigation water was calculated as described by Vermeirer and Jopling (1984).

The same amount of water was applied to all treatments until 30 DAS, the time representing elongation stage, the deficit irrigation treatment (DI) was started under each antioxidant treatment. Deficit irrigation continued till maturity stage (harvesting), therefore plants exposed to drought for about 53 and 57 days along the life cycle in the first and second seasons, respectively. Irrigation was applied at 2-day interval. Moreover, sunflower plants received irrigation water amounts of 4736.0 and 4650.8 m^3 ha^{-1} in 2019 season as well as 5312.0 and 5226.2 m^3 ha^{-1} in 2020 season, with irrigation by DI and FI, respectively.

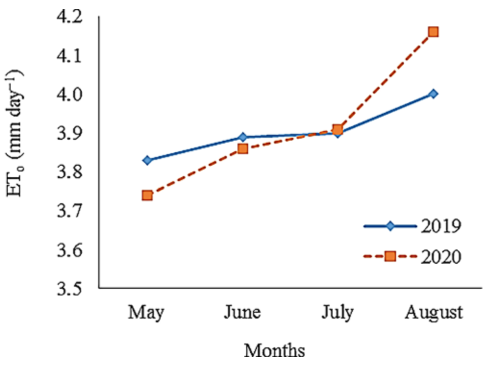


Fig. 1 Changes in reference evaporation (ET_o) during 2019 and 2020 growing seasons of sunflower at El Nubaria region, Egypt

Sampling and Assessments

Physiological Traits

The 4th leaves from the top were taken at 65 DAS and used for measuring both chlorophyll *a* and *b* (mg g^{-1} fresh wt.) according to Wettstein (1957), and free proline content (μg g^{-1} fresh wt.) according to Bates et al. (1973).

Growth Traits

At 65 DAS, five guarded plants were chosen randomly from each plot to estimate plant height, stem diameter and leaf area index (LAI) according to Beadle (1993).

Yield

At harvest dates (on the 4th and 10th August in 2019 and 2020, respectively), whole plants of each plot were harvested to estimate head weight plant^{-1}, seed yield ha^{-1}. A representative sample of seeds was obtained for estimating oil percentage using Soxhlet Apparatus with hexane as organic solvent according to AOAC (2012). Then, oil yield ha^{-1} was calculated by multiplying seed oil content by seed yield ha^{-1}.

Irrigation Water Use Efficieny (IWUE)

According to Kirda et al. (2005), IWUE (kg m^{-3}) was calculated by dividing seed yield (kg ha^{-1}) by the total amount of irrigation water applied (m^3 ha^{-1}) during each growing season.

Yield Response Factor (*Ky*)

The yield response factor (*Ky*) was computed from the seed yield for each antioxidant treatment using the pooled data across the two experimental years (Doorenbos and Kassam 1979), using Eq 1:

$$\left(1-\frac{Ya}{Yx}\right)=Ky\left(1-\frac{ETa}{ETx}\right) \tag{1}$$

where *Ya* is the seed yield (kg ha^{-1}) obtained from DI treatment, *Yx* is the seed yield (kg ha^{-1}) obtained from the FI treatment, *ETa* is the crop water consumption (m^3 ha^{-1}) under deficit irrigation (DI), and *ETx* is the crop water consumption (m^3 ha^{-1}) under full irrigation (FI).

Data Analysis

Differences in the physiological, growth, yield traits, and IWUE among treatments were statistically analyzed by two-way analysis of variance (ANOVA) for each season using Costat software program version 6.303 (2004). For comparison among means, Duncan's multiple range test was used at 0.05 probability level ($p \leq 0.05$).

Results

Physiological Traits

Results illustrated in Table 2 showed that deficit irrigation (DI) lowered chlorophyll a by 22.08 and 24.17% and chlorophyll *b* by 38.14 and 42.88% while increased proline content by 116.49 and 105.90% as comparing to full irrigation (FI) in 2019 and 2020 seasons respectively. ASC$_{300}$ gave the highest values of pigments concentration in leaves, with increases of 18.33 and 20.72% in chlorophyll *a* as well as 27.69 and 35.48% in chlorophyll *b*, while proline content reduced by 17.12 and 16.22% compared to the check treatment (Tap water) in 2019 and 2020 seasons, respectively (Table 2). In full irrigated sunflower plots, exogenous application of higher rate of ascorbic acid (FI plus ASC$_{300}$) exhibited the highest increase of chlorophyll *a*, chlorophyll *b* and the lowest proline content compared to other interaction treatments (Table 2). While, the lowest contents of chlorophyll *a* and the highest value of proline were obtained in leaves of plants grown under DI plus check treatment in both seasons. DI × CA250 or check treatment showed the minimal chlorophyll *b*.

Growth Traits

The irrigation regime significantly affected the plant height, stem diameter and LAI, with the FI regime resulting in 9.29, 22.95 and 35.38% increases, respectively, in the 2019 season and 9.11, 24.13 and 36.91% increases, respectively, in the 2020 season higher than the DI regime (Table 3). Compared to the check treatment ASC$_{300}$ treatment caused increases of 4.96 and 4.29%, in plant height, 7.96 and 7.77% in stem diameter, and 16.21 and 16.77% in LAI in

Table 2 Chlorophylls and proline content of sunflower response to irrigation regime and antioxidant application in 2019 and 2020 seasons

Variable		Chlorophyll, Chl (mg g^{-1} fresh wt.)				Proline content (µg g^{-1} fresh wt.)	
		Chl *a*		Chl *b*			
		2019	2020	2019	2020	2019	2020
Irrigation regime							
FI		2.368 ± 0.041a	2.238 ± 0.037a	1.232 ± 0.032a	1.033 ± 0.035a	121.34 ± 2.60b	133.30 ± 2.52b
DI		1.845 ± 0.031b	1.697 ± 0.035b	0.762 ± 0.021b	0.590 ± 0.017b	262.69 ± 5.07a	274.47 ± 4.24a
Antioxidant							
ASC150		2.223 ± 0.124b	2.087 ± 0.128b	1.063 ± 0.120b	0.867 ± 0.116b	184.99 ± 30.71c	196.53 ± 37.24c
ASC300		2.246 ± 0.118a	2.126 ± 1.21a	1.139 ± 0.117a	0.943 ± 0.115a	182.20 ± 30.59c	194.03±.35.14c
CA250		2.027 ± 0.110d	1.886 ± 0.116d	0.878 ± 0.095d	0.723 ± 0.103d	189.99 ± 30.66b	201.53 ± 30.59b
CA500		2.115 ± 0.114c	1.978 ± 0.117c	1.013 ± 0.112c	0.817 ± 0.111c	183.06 ± 30.33c	195.73 ± 41.27c
Check		1.898 ± 0.118e	1.761 ± 0.122e	0.892 ± 0.083d	0.696 ± 0.741d	219.84 ± 35.79a	231.61 ± 40.21a
Interaction							
FI	ASC150	2.502 ± 0.003a	2.373 ± 0.004a	1.330 ± 0.012b	1.131 ± 0.011b	116.32 ± 0.39f	128.03 ± 0.57f
	ASC300	2.527 ± 0.015a	2.398 ± 0.017a	1.339 ± 0.010a	1.201 ± 0.013a	113.81 ± 0.69f	125.75 ± 0.41f
	CA250	2.274 ± 0.016c	2.145 ± 0.014c	1.091 ± 0.019d	0.892 ± 0.23d	121.43 ± 0.90e	133.14 ± 1.83e
	CA500	2.369 ± 0.008b	2.239 ± 0.011b	1.264 ± 0.005c	1.065 ± 0.012c	115.29 ± 0.52f	128.48 ± 0.63f
	Check	2.164 ± 0.005d	2.034 ± 0.006d	1.076 ± 0.012d	0.878 ± 0.013d	139.85 ± 2.07d	151.11 ± 1.48d
DI	ASC150	1.946 ± 0.013f	1.800 ± 0.018f	0.796 ± 0.020f	0.603 ± 0.018f	253.66 ± 0.57c	265.03 ± 0.39c
	ASC300	2.001 ± 0.006e	1.855 ± 0.009e	0.879 ± 0.007e	0.686 ± 0.011e	250.59 ± 0.99c	262.31 ± 1.12c
	CA250	1.781 ± 0.001h	1.627 ± 0.007h	0.666 ± 0.008g	0.553 ± 0.016fg	258.55 ± 0.52b	269.92 ± 0.46b
	CA500	1.86 ± 0.005g	1.717 ± 0.012g	0.762 ± 0.009f	0.568 ± 0.011f	250.82 ± 2.62c	262.99 ± 1.67c
	Check	1.632 ± 0.004i	1.487 ± 0.008i	0.708 ± 0.028g	0.515 ± 0.016g	299.83 ± 0.71a	312.11 ± 0.90a

Values are the mean of 3 replicates ± standard errors. Different letters within columns indicate that there are significant differences at $p \leq 0.05$ based on Duncan's multiple range test

FI and DI full and deficit irrigation, representing 100 and 85 of crop evapotranspiration, respectivelu, *ASC150 and ASC 300* ascorbic acid at rates of 150 and 300 mg L^{-1}, *CA250 and CA500* citric acid at rates of 250 and 500 mg L^{-1}, respectively, *Check* tap water treatment

Table 3 Plant height, stem diammeter and leaf area index (LAI) of sunflower response to irrigation regime and antioxidant application in 2019 and 2020 seasons

Variable		Plant height (cm)		Stem diameter (cm)		LAI	
		2019	2020	2019	2020	2019	2020
Irrigation regime							
FI		194.27 ± 0.67a	189.91 ± 0.65a	2.25 ± 0.04a	2.16 ± 0.04a	4.17 ± 0.05a	4.08 ± 0.04a
DI		177.76 ± 0.99b	174.06 ± 0.81b	1.83 ± 0.01b	1.74 ± 0.03b	3.08 ± 0.06b	2.98 ± 0.06b
Antioxidant							
ASC150		187.57 ± 3.39b	183.42 ± 4.27b	2.05 ± 0.10b	1.97 ± 013b	3.65 ± 0.23c	3.58 ± 0.51c
ASC300		190.11 ± 3.63a	185.70 ± 3.46a	2.17 ± 0.15a	2.08 ± 0.17a	3.87 ± 0.23a	3.76 ± 0.35a
CA250		184.78 ± 3.67c	180.57 ± 5.24c	1.95 ± 0.06c	1.86 ± 0.11c	3.50 ± 0.28d	3.41 ± 0.44d
CA500		186.49 ± 3.49b	182.19 ± 3.70bc	2.01 ± 0.10bc	1.93 ± 0.18 bc	3.77 ± 0.23b	3.69 ± 0.36b
Check		181.12 ± 4.33d	178.05 ± 3.80d	2.01 ± 0.10bc	1.93 ± 0.11bc	3.33 ± 0.25e	3.22 ± 0.38e
Interaction							
FI	ASC150	195.14 ± 0.37b	190.84 ± 0.41b	2.27 ± 0.03b	2.18 ± 0.04b	4.18 ± 0.01b	4.10 ± 0.3c
	ASC300	198.19 ± 0.30a	193.37 ± 0.57a	2.50 ± 0.03a	2.41 ± 0.5a	4.37 ± 0.02a	4.29 ± 0.04a
	CA250	192.95 ± 0.41c	188.47 ± 0.09 cd	2.09 ± 0.01c	2.00 ± 0.3c	4.12 ± 0.01b	4.03 ± 0.02d
	CA500	194.26 ± 0.26bc	190.38 ± 0.87bc	2.18 ± 0.01b	2.11 ± 0.3b	4.29 ± 0.01a	4.21 ± 0.01b
	Check	190.80 ± 0.50d	186.51 ± 0.58d	2.21 ± 0.06b	2.13 ± 0.07b	3.88 ± 0.04c	3.78 ± 0.01e
DI	ASC150	179.99 ± 0.33f	176.00 ± 0.31ef	1.84 ± 0.02d	1.75 ± 0.4d	3.13 ± 0.02e	3.06 ± 0.04h
	ASC300	182.03 ± 0.82e	178.04 ± 0.88e	1.84 ± 0.01d	1.75 ± 0.2d	3.36 ± 0.02d	3.24 ± 0.01f
	CA250	176.61 ± 0.50g	172.67 ± 0.30g	1.82 ± 0.02d	1.73 ± 0.3d	2.89 ± 0.01f	2.78 ± 0.01i
	CA500	178.71 ± 0.52f	174.00 ± 0.79 fg	1.83 ± 0.01d	1.75 ± 0.2d	3.26 ± 0.01d	3.17 ± 0.02g
	Check	171.45 ± 0.30h	169.59 ± 0.73h	1.81 ± 0.01d	1.73 ± 0.1d	2.77 ± 0.01g	2.66 ± 0.02j

Values are the mean of 3 replicates ± standard errors. Different letters within columns indicate that there are significant differences at $p \leq 0.05$ based on Duncan's multiple range test

FI and DI full and deficit irrigation, representing 100 and 85 of crop evapotranspiration, respectivelu, *ASC150 and ASC 300* ascorbic acid at rates of 150 and 300 mg L^{-1}, *CA250 and CA500* citric acid at rates of 250 and 500 mg L^{-1}, respectively, *Check* tap water treatment

both growing seasons, respectively. The promotive effects of CA_{500} or ASC_{150} on plant height and stem diameter as well as CA_{500} on LAI came in the second order. Overall, interaction revealed the potential of ASC_{300} to improve plant growth either with FI or DI (Table 3). Specifically for each irrigation pattern, plant height, stem diameter and LAI with ASC_{300} in both seasons as well as LAI with CA_{500} in 2019 season showed the maximum values under FI. Similar trent was also obtained under DI.

Yield

The irrigation regime significantly influenced head weight, seed yield and oil yield, with the DI regime resulting in 35.88, 22.42, and 29.95% lower values, respectively, in 2019 season and 36.76, 23.47, and 31.19% lower values, respectively, in 2020 season than the FI regime (Table 4). ASC_{300} foliar application was the most efficient treatment during both seasons, with increasing the head weight by 18.24 and 17.54%, seed yield by 6.84 and 8.84%, and oil yield by 11.83 and 14.20% in 2019 and 2020 seasons, respectively, as compared to the check treatment (Table 4).

Sunflower sprayed with ASC_{300} and irrigated with FI produced the highest values of all yield traits surpassing the other combinations. Moreover, it should be noted that the seed yield was significantly higher with FI plus ASC_{300} and DI plus ASC_{300} treatments than their counterpart check treatment achieving 13.93 and 8.89% increases in 2019 season as well as 16.23 and 11.32% increases in 2020 season, respectively.

Irrigation Water Use Efficiency

As dipeted in Fig. 2, ASC_{300} was the most effective practice for enhancing IWUE of sunflower in 2019 and 2020 seasons, surpassing the other practices either under FI or DI. With DI, IWUE was improved with antioxidant-treated plants compared to untreated plants.

Yield Response Factor

For providing an indication of the tolerance level of sunflower crop to water deficit stress, *K*y was estimated for antioxidant treatment with pooled data from the two growing seasons (Fig. 3). *K*y factor values were >1 under any

Table 4 Head weight, seed yield and oil yield of sunflower response to irrigation regime and antioxidant application in 2019 and 2020 seasons

Variable		Head weight (g)		Seed yield (kg ha^{-1})		Oil yield (kg ha^{-1})	
		2019	2020	2019	2020	2019	2020
Irrigation regime							
FI		65.63 ± 1.2a	63.07 ± 1.26a	2885.5 ± 22.4a	2731.7 ± 26.1a	1099.2 ± 12.6a	1008.8 ± 13.5a
DI		42.08 ± 0.4b	39.88 ± 1.44b	2238.5 ± 8.0b	2090.4 ± 11.5b	769.9 ± 5.7b	694.1 ± 10.6b
Antioxidant							
ASC150		55.40 ± 6.0b	53.15 ± 5.9b	2572.2 ± 143.2b	2413.6 ± 134.1c	942.2 ± 73.1b	857.0 ± 67.5c
ASC300		58.63 ± 6.2a	55.93 ± 4.2a	2640.0 ± 162.7a	2507.5 ± 158.9a	977.2 ± 81.1a	898.6 ± 77.0a
CA250		51.73 ± 4.7d	49.44 ± 5.6d	2542.3 ± 138.2c	2384.8 ± 143.2d	930.3 ± 71.7c	845.1 ± 70.5d
CA500		53.92 ± 5.0c	51.26 ± 4.8c	2584.6 ± 152.2b	2445.5 ± 158.2b	949.4 ± 78.1b	869.6 ± 76.8b
Check		49.58 ± 4.3e	47.58 ± 5.3e	2470.9 ± 127.7d	2303.9 ± 123.1e	873.8 ± 64.0d	786.9 ± 60.0e
Interaction							
FI	ASC150	68.90 ± 0.8b	66.43 ± 0.6b	2892.6 ± 5.5c	2713.4 ± 2.5c	1105.8 ± 0.6c	1007.9 ± 2.6c
	ASC300	72.48 ± 0.4a	69.88 ± 0.6a	3003.8 ± 8.3a	2862.6 ± 13.7a	1158.6 ± 4.3a	1070.8 ± 4.8a
	CA250	62.39 ± 0.4d	60.11 ± 0.6d	2851.2 ± 9.8d	2704.5 ± 19.8c	1090.6 ± 4.0d	1002.7 ± 6.5c
	CA500	65.18 ± 0.4c	62.19 ± 04c	2925.1 ± 3.7b	2799.3 ± 7.1b	1124.2 ± 1.3b	1041.3 ± 2.7b
	Check	59.18 ± 0.5e	56.72 ± 0.3e	2754.9 ± 22.1e	2578.7 ± 13.6d	1016.9 ± 7.2e	921.2 ± 2.3d
DI	ASC150	41.91 ± 0.2gh	39.87 ± 0.7gh	2251.9 ± 2.8 fg	2113.7 ± 4.1f	778.5 ± 1.8g	706.0 ± 2.1f
	ASC300	44.77 ± 0.3f	41.97 ± 0.4f	2276.2 ± 2.1f	2152.3 ± 6.4e	795.8 ± 1.0f	726.5 ± 3.1e
	CA250	41.06 ± 0.2hi	38.76 ± 0.2 hi	2233.4 ± 3.5g	2065.2 ± 6.0g	770.0 ± 1.6g	687.5 ± 3.2g
	CA500	42.67 ± 0.2g	40.33 ± 0.3g	2244.2 ± 1.6g	2091.7 ± 3.8h	774.6 ± 0.4g	697.8 ± 1.3 fg
	Check	39.98 ± 0.2i	38.44 ± 0.3i	2186.9 ± 7.7h	2029.1 ± 11.6h	730.8 ± 3.19h	652.6 ± 2.3h

Values are the mean of 3 replicates ± standard errors. Different letters within columns indicate that there are significant differences at $p \leq 0.05$ based on Duncan's multiple range test

FI and DI full and deficit irrigation, representing 100 and 85 of crop evapotranspiration, respectivelu, *ASC150 and ASC 300* ascorbic acid at rates of 150 and 300 mg L^{-1}, *CA250 and CA500* citric acid at rates of 250 and 500 mg L^{-1}, respectively, *Check* tap water treatment

antioxidant treatment. The values of *K*y factor were noted as follows: ASC$_{300}$ > CA$_{500}$ > CA$_{250}$ > ASC$_{150}$ > check treatment.

Discussion

Drought as abiotic stress is considered as one of the most important consequences of climate change, which is an obstacle to crop productivity. Drought stress has tremendous impacts on plants, which could involve imbalance, stress damage, growth delay, and low availability of nutrients (Mubarak et al. 2021). Several chemical compounds are exogenously applied to improve grwoth and health of plants under both normal or stress conditions (Noureldin et al. 2013; Saudy et al. 2020b, 2022a; El-Metwally and Saudy 2021b; Elgala et al. 2022; El-Metwally et al. 2022b). The current study investigated some drought response mechanisms in sunflower under application of ASC and CA. As presented in Table 2, decreasing irrigation water by 15% than normal led to reduction in chlorophyll content and increase in proline content. Such findings are in accordance with those found by El-Bially et al. (2018) and Mostafa (2020) who reported that photosynthetic pigments of sunflower leaf (chlorophyll *a* and *b*) had large decline, but proline content increased due to drought stress. It has been reported that reactive oxygen species (ROS) overproduced in plant cells exposed to environmental stresses (Hatamian et al. 2020; Souri et al. 2019) can damage membrane and other essential macromolecules like photosynthetic pigments, proteins, DNA and lipids. Biochemical damage was measured due to production of ROS which eventually led to poor growth and metabolic damage of the plant (Zafar et al. 2015). High ROS levels cause serious dysfunction in many processes such as hormonal equilibrium, gene expression, pathways of signaling, photosynthetic efficiency, protein inactivation, inhibit the action of multiple enzymes involved in metabolic pathways and decrease grain yield, resulting in lipid and DNA oxidation (Huang et al. 2012; Choudhury et al. 2017). It has been proved that plant growth regulators and osmoprotectants like free amino acids, sugars, and polyamines have protective roles against drought (Chan et al. 2013). In this regrad, proline accumulation in stressed plants has been well established to play a key role as osmoregulation defense mechanism, leading to prevent the cell osmotic pressure and survive in the extreme conditions (Souri and Bakhtiarizade 2019; Souri and Tohidloo 2019). In this connection, Manivannan et al. (2007) reported that water stress caused reduction in activity

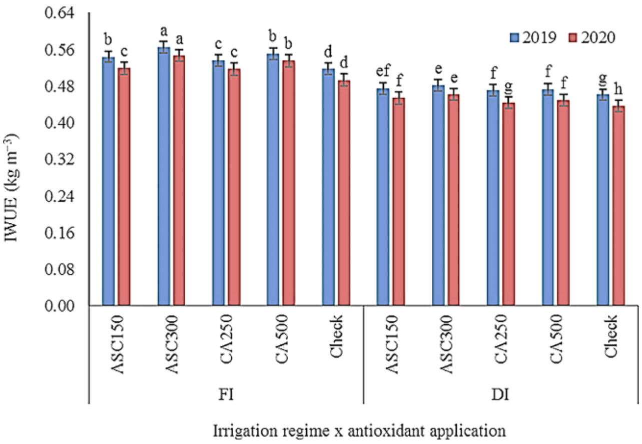


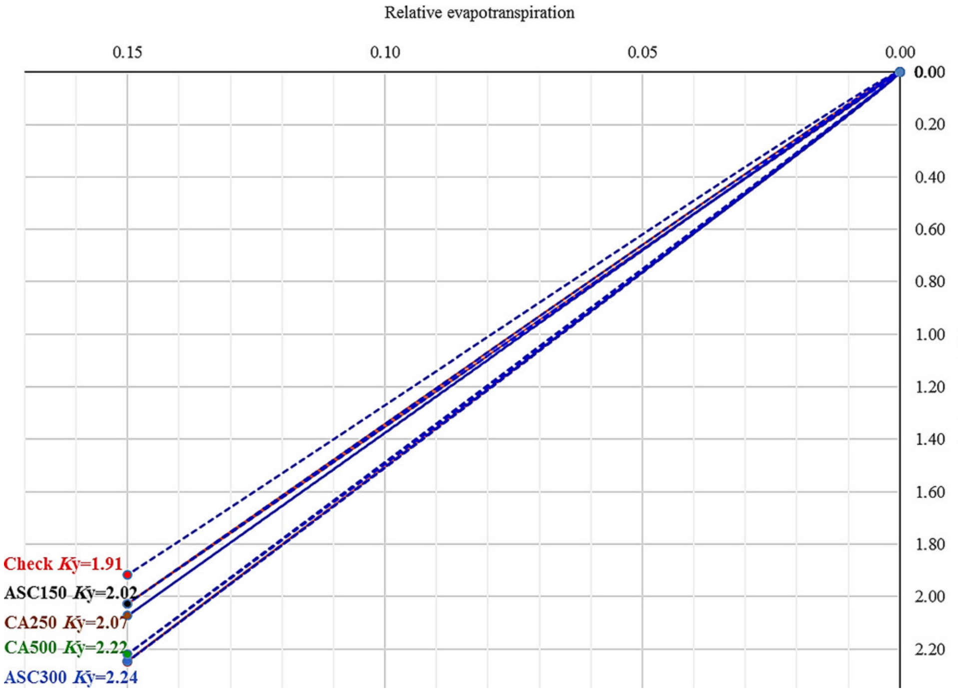
Fig. 2 Irrigation water use efficiency (IWUE) of sunflower response to irrigation regime and antioxidant applications in 2019 and 2020 seasons. (*FI and DI* full and deficit irrigation, representing 100 and 85 of crop evapotranspiration, respectively, *ASC150 and ASC300* ascorbic acid at rates of 150 and 300 mg L^{-1}, *CA250 and CA500* citric acid at rates of 250 and 500 mg L^{-1}, respectively, *Check* tap water treatment). (Values are the mean of 3 replicates ± standard errors. Different letters between bars indicate that there are significant differences at $p \leq 0.05$ based on Duncan's multiple range test)

of proline oxidase so, proline content increased. Moreover, a large proportion of the drought-responsive proteins are involved in photosynthesis. Rubisco (Ribulose bisphosphate carboxylase) is a vital enzyme associated with carbon fixation (Feller et al. 2008). Rubisco similarly showed decreasing abundance under drought stress, indicating that drought negatively affects the key protein of the photosynthetic apparatus (Wang et al. 2017).

Since drought affects plant physiology as well as nutrient availability and accumulation (Mubarak et al. 2021; Salem et al. 2021, 2022), crop traits and its potentiality to expliot irrigation water were influenced (Tables 2 and 3). Drought stress caused significant reduction in growth, yield traits as well as IWUE because irrigation is the key factor for obtaining high yield (FAO 2010). Herein, deficit water significantly reduced root length, stem length, total leaf area, fresh and dry weight of sunflower plants (Manivannan et al. 2007). Thus, drought stress has been shown to significantly decrease plant height (Sincik et al. 2013), stem diameter (Saeed et al. 2015), leaf area index (Furtado et al. 2016), head weight (Ibrahim et al. 2016), seed yield (Soleimanzadeh 2012), oil yield (Kassab et al. 2012) and water use efficiency (El-Bially et al. 2018). These reductions could be due to lowering content of chlorophyll in leaves (Table 2), which leads to a decrease in photosynthesis rate and the dry matter accumulation.

On the other site, plant cells have many effective defense mechanisms to remove the harmful effect of ROS (Birben et al. 2012). ASC and CA are non-enzymatic antioxidants that play an important role to protect plants from oxidative damage by scavenging and sweep of ROS (Prasad and Upadhyay 2011; Tahjib-ul-Arif et al. 2021). Therefore, enhancements in leaves content of chlorophylls *a* and *b* were obtained owing to the application of antioxidants (Table 2). Previous studies have shown that foliar application of ASC or CA significantly increased leaf pigmints of stressed plants (Amin and Ismail 2015; El-Mantawy 2017; Farid et al. 2017, 2019). On the other hand, proline content in leaves was reduced when ASC and CA were sprayed under

Fig. 3 The change in yield response factor (*Ky*) of sunflowr with application of ascorbic and citric acids. (*ASC150 and ASC300* ascorbic acid at rates of 150 and 300 mg L^{-1}, *CA250 and CA500* citric acid at rates of 250 and 500 mg L^{-1}, respectively, *Check* tap water treatment)



drought stress as compared to control (see Table 3),. Proline stabilizes subcellular structures and molecules experiencing osmotic stress conditions by working as a molecular chaperone, maintaining the integrity of proteins. Previous studied have shown that foliar application of ASC or CA significantly decreased proline content in leaves (El-Bially et al. 2018; Mostafa 2020). Since antioxidants alleviate the effects of stress, ASC and CA significantly decrease leaves proline content as compared to control treatment (Mostafa 2020). CA foliar application improved the germination rate and root weight of sunflower plant by improving the activities of several antioxidants enzymes including superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase (Ondrasek et al. 2019). Using ASC and CA as foliar applications, especiallly with higher rates (ASC_{300} and CA_{500}), counteraceted the harmful effect of drought stress and significantly increased growth and yield attributes. These results are in consistance with the findings of El-Mantawy (2017). Since ASC is a preventive agent and the important compound working nonenzymatic system reinforcement against ROS in plant (Qian et al. 2014), foliar application of ASC enhanced physiological and biochemical traits, productivity and water use efficiency of sunflower under abiotic stress (Saudy et al. 2021a).

Despite ASC and CA alleviated partially the adverse impacts of deficit water, sunflower plants still sensetive to low water supply. In this regard, Fig. 3 showed that *K*y values exceeded the unit (higher than 1.0) clarifing the sensetivity of sunflower to drought. *K*y indicates the relationship between relative yield and relative crop water consumption (Lovelli et al. 2007; Singh et al. 2010), with values >1 indicating that the crop is very sensitive to water stress, values <1 indicating that the crop is more tolerant to water stress, and values of 1 indicating that the relative yield reduction is equal to the relative water use reduction (Steduto et al. 2012). Accordingly, our findings refer to that sunflower plants require additional practices to increase their tolerance to deficit water stress. The value of *K*y was affected by climate and soil conditions, irrigation method and applied amount of irrigation water (Aydinsakir et al. 2021). Candogan and Yazgan (2016) reported that *K*y value was greater than 1 (1.21) and stated that soybean was sensitive to water stress.

Coclussion

It could be concluded that reduction in the economic product of sunflower (seed yield) could not completely be compensated by application of antioxidants, i.e. ascorbic and citric. However, the adverse effects of drought were partially alleviated with antioxidant especially ascorbic acid. Also, since sunflower markedly responded to increasing the antioxidant level up to 300 mg L^{-1} for ascorbic acid and 500 mg L^{-1} for citric acid, this opens the field to further studies to examine higher rates of each under drought stress in sunflower. Moreover, other agronomic practices should be tested and adopted along application of antioxidant since sunflower still sensetive to drought as proved from measuing of yield response factor.

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Conflict of interest H.S. Saudy, M.E. El-Bially, F.A. Hashem, M.G. Shahin and Y.A. El-Gabry declare that they have no competing interests.

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