



# Salicylic Acid as a Tolerance Inducer of Drought Stress on Sunflower Grown in Sandy Soil

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## Abstract

Agricultural water rationalization expressed in irrigating the plants below their requirements became a significant strategy in crop water management. However, reduction in crop productivity under low water supply is realized. Therefore, the current study aimed to diminish sunflower yield losses associated with deficit irrigation using salicylic acid (SA). During two seasons of 2019 and 2020 at El Nubaria region, El Behaira Governorate, Egypt, combinations of three irrigation regimes (100, 85 and 70% of crop evapotranspiration, denoted  $WR_{100\%}$ ,  $WR_{85\%}$ , and  $WR_{70\%}$ , respectively), and three levels of SA (0.0, 0.5, and 1 mM, abbreviated as  $SA_{0.0}$ ,  $SA_{0.5}$ , and  $SA_{1.0}$ , respectively) on sunflower plants performance were evaluated. Treatments were arranged in a strip-plot design with three replicates. Findings revealed that treated sunflower plants with  $WR_{100\%} \times SA_{1.0}$  contained the highest amounts of total chlorophyll and carotenoids as well as the lowest proline content. Seed yield of  $WR_{100\%} \times SA_{1.0}$  treatment was higher than that of  $WR_{70\%} \times SA_{0.0}$  by 109.7% in the first season and 125.9% in the second one. As averages of the two seasons,  $SA_{0.5}$  and  $SA_{1.0}$  lowered the reductions in seed yield from 21.0% to 15.8 and 14.4% as well as 46.2% to 40.8 and 40.1% under  $WR_{85\%}$  and  $WR_{70\%}$ , respectively, compared to the farmer common practice ( $WR_{100\%} \times SA_{0.0}$ ).  $WR_{100\%} \times SA_{1.0}$  for iodine value as well as  $WR_{100\%} \times SA_{1.0}$  and  $WR_{100\%} \times SA_{0.5}$  for seed oil % were recorded the highest. Application of  $WR_{100\%} \times SA_{1.0}$  and  $WR_{100\%} \times SA_{0.5}$  were the effective combinations for ameliorating water use efficiency. In conclusion, involving salicylic acid in irrigation programs of sunflower became a decisive action to save water and alleviate the yield losses resulting from drought stress.

**Keywords** Deficit water · Economic yield · Irrigation management · Oil seed crops · Plant hormones · Plant pigments

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## Einsatz von Salicylsäure zur Erhöhung der Trockenheitstoleranz bei Sonnenblumen in sandigen Böden

### Zusammenfassung

Die Rationierung von Wasser in der Landwirtschaft, d.h. die Bewässerung von Pflanzen unter ihrem Bedarf, wurde zu einer wichtigen Strategie im Wassermanagement. Bei geringer Wasserversorgung kommt es jedoch zu einer Verringerung der Pflanzenproduktivität. Die vorliegende Studie zielte daher darauf ab, die mit Defizitbewässerung verbundenen Ertrags-einbußen bei Sonnenblumen durch den Einsatz von Salicylsäure (SA) zu verringern. In den Jahren 2019 und 2020 wurden in der Region El Nubaria im Gouvernement El Behaira, Ägypten, Kombinationen aus drei Bewässerungsregimen (100, 85 und 70 % der Evapotranspiration der Pflanzen, abgekürzt  $WR_{100\%}$ ,  $WR_{85\%}$  bzw.  $WR_{70\%}$ ) und drei SA-Gehalten (0,0, 0,5 und 1 mM, abgekürzt  $SA_{0,0}$ ,  $SA_{0,5}$  bzw.  $SA_{1,0}$ ) auf die Leistung von Sonnenblumenpflanzen untersucht. Die Behandlungen wurden in einem Strip-Plot-Design mit drei Wiederholungen angeordnet. Die Ergebnisse zeigten, dass behandelte Sonnenblumenpflanzen mit  $WR_{100\%} \times SA_{1,0}$  die höchsten Mengen an Gesamtchlorophyll und Carotinoiden sowie den niedrigsten Prolinegehalt aufwiesen. Der Samenertrag der Behandlung  $WR_{100\%} \times SA_{1,0}$  war in der ersten Saison um 109,7 % und in der zweiten Saison um 125,9 % höher als der von  $WR_{70\%} \times SA_{0,0}$ . Im Durchschnitt der beiden Saisons verringerten  $SA_{0,5}$  und  $SA_{1,0}$  den Saatgutertrag von 21,0 % auf 15,8 bzw. 14,4 % sowie von 46,2 % auf 40,8 bzw. 40,1 % bei  $WR_{85\%}$  und  $WR_{70\%}$  im Vergleich zur üblichen Praxis der Landwirte ( $WR_{100\%} \times SA_{0,0}$ ). Bezüglich der Jodzahl wurde für  $WR_{100\%} \times SA_{1,0}$  der höchste Wert gemessen, bezüglich des Saatölanteils für  $WR_{100\%} \times SA_{1,0}$  und  $WR_{100\%} \times SA_{0,5}$ .  $WR_{100\%} \times SA_{1,0}$  und  $WR_{100\%} \times SA_{0,5}$  waren die effektivsten Kombinationen zur Verbesserung der Wassernutzungseffizienz. Zusammenfassend lässt sich sagen, dass die Einbeziehung von Salicylsäure in Bewässerungsprogramme für Sonnenblumen eine entscheidende Maßnahme ist, um Wasser zu sparen und die durch Trockenstress bedingten Ertragsverluste zu verringern.

**Schlüsselwörter** Wassermangel · Wirtschaftlicher Ertrag · Bewässerungsmanagement · Ölsaaten · Pflanzenhormone · Pflanzenpigmente

### Introduction

Global climate change has, undoubtedly, negative impact on crop growth and productivity. This is particularly acute in arid and semi-arid regions (Ahmadi and Souri 2018; Souri and Hatamian 2019), which suffer from water scarcity. Therefore, researchers are making continuous attempts to limit such adverse impacts (Saady et al. 2020; El-Bially et al. 2022). In conjunction with global warming, drought is expected to become more frequent in many regions of the earth (Pokhrel et al. 2021). Drought is one of the major global factors that reduce yield in many crops worldwide (Nawaz et al. 2015). Due to the severity of various abiotic stresses such as drought, several concerns can threaten crop production (Anderson et al. 2020; Zulfiqar and Hancock 2020). Drought is considered one of the results associated climatic changes, thus it represents a major constraint to crop production universally (Farooq et al. 2012). It has been reviewed that exposure of plants to water stress leads to serious physiological and biochemical dysfunctions (Hayat et al. 2010; Makhlof et al. 2022). Water deficit during critical growth periods of crops reduces their yield and quality, but, however, crops differ in their response to water stress at a given growth stage (El-Bially et al. 2018).

Sunflower (*Helianthus annuus* L.) is a high yielding oilseed crop and has the potential to bridge up the gap existing between consumption and domestic production

of edible oil in Egypt. Sunflower is often reported as a drought-tolerant crop given its high capacity to extract water from the subsoil (García-Vila and Fereres 2012). It has good potential for drought tolerance because of its well-developed root system and to withstand temporary wilting. However, sunflower production is greatly affected by drought (Siddique et al. 2020). It has been reported that sunflower productivity decreases with increasing drought levels (Erdem et al. 2006; Manivannan et al. 2007; Nezami et al. 2008; Siddique et al. 2020; Saady et al. 2020). Periods of water deficit at any growth stage can cause canopy senescence with subsequent reduction in seed yield (García-Vila and Fereres 2012). Sunflower productivity is mightily regulated by the availability of water and greatest yield losses manifest when water shortage occurs (Yawson et al. 2011).

Under abiotic stresses as drought, synthesizing of protectants by plants is a crucial act in inducing defense responses (Ozturk et al. 2021). In this respect, the endogenous biosynthesis of salicylic acid (SA), as a plant hormone (Smith et al. 2017), is affected by drought (La et al. 2019; Park et al. 2021). In various crops subjected to water deficit conditions, SA had the potentiality to regulate photosynthesis and antioxidant enzymes (Abdelaal 2015), as well as induce proline metabolisms, and antioxidant defense systems (Khan et al. 2014). Use of SA significantly mitigated the adverse impacts of drought and reduced lipid

peroxidation (Kang et al. 2013). Therefore, environmental stressed–plant growth was enhanced by exogenous application of SA (Chavoushi et al. 2019; Abbaszadeh et al. 2020).

The relationship between the defense mechanism against drought and the protective impact of salicylic acid on sunflower still requires more investigations. We hypothesized that there is a definite rate of SA which could have the ability to change the fate of some plant physiological events to cope the different levels of drought stress (moderate or severe). Accordingly, the present study was carried out to investigate the effect of irrigation regimes on sunflower performance and for exploring to what extend salicylic acid at different rates can improve the drought tolerance.

## Material and Methods

### Experimental Site and Crop Husbandry

The present study was conducted at the Experimental Farm, Faculty of Agriculture, Ain Shams University, El Nubaria region, El Behaira Governorate, Egypt during the two seasons of 2019 and 2020. The experimental soil was sandy in texture with organic matter of 1.23%, pH 7.85, and EC 2.4 dS m<sup>-1</sup>. According to soil taxonomy (IUSS Working Group WRB 2015), the soil is order Entisols and suborder psamments. The area of the study belongs to arid regions with no rainfall and hot–dry in summer from May to August. Table 1 shows monthly mean weather factors, i.e. maximum and minimum air temperature, relative humidity, wind speed, and solar radiation for 2019 and 2020 seasons, obtained from Central Laboratory of Meteorology, Ministry of Agriculture and Land Reclamation, Egypt. The preceding crop was wheat in the two seasons of experimentation.

Sunflower seeds, cv. Sakha 53, were sown on May 19 and 21 in the first and second seasons, respectively. Seeds were sown in hills (3–5 seeds per hill) 20 cm apart on the ridge. At 15 days after sowing (DAS), plants were thinned to one plant per hill. Plants were irrigated through drip irrigation system using drippers of 2 L h<sup>-1</sup> capacity.

### Experimental Treatments and Design

In a strip–plot design with three replicates irrigation water levels were allocated in the vertical plots, whereas the horizontal plots were devoted to salicylic acid (SA) levels. The experimental unit area was 14 m<sup>2</sup>, involving five ridges each of 4 m long and 0.7 m wide. Irrigation water levels were 100, 85 and 70% of crop evapotranspiration, representing well–watered, moderately water–stressed, and severely water–stressed conditions, and denoted WR<sub>100%</sub>, WR<sub>85%</sub>, and WR<sub>70%</sub>, respectively. The applied irrigation water had a pH of 7.53 and EC of 0.67 dS m<sup>-1</sup>. SA levels were 0.0, 0.5, and

1 mM. abbreviated as SA<sub>0.0</sub>, SA<sub>0.5</sub>, and SA<sub>1.0</sub>, respectively. SA levels were sprayed twice, at 35 and 50 DAS. Tween–20 (C<sub>58</sub>H<sub>114</sub>O<sub>26</sub>) is a trade name for polyoxyethylene (20) sorbitan monolaurate. The spray solution volume was 500 L ha<sup>-1</sup> using a knapsack sprayer with one nozzle.

### Estimation of Water Requirements

Crop evapotranspiration (ET<sub>c</sub>) was calculated by multiplying the reference crop evapotranspiration (ET<sub>o</sub>) by a crop coefficient (K<sub>c</sub>) according to Doorenbos and Pruitt (1977) using Eq. 1:

$$ET_c = K_c \times ET_o \quad (1)$$

Where:

ET<sub>c</sub>: crop evapotranspiration (mm d<sup>-1</sup>)

K<sub>c</sub>: crop coefficient (dimensionless).

ET<sub>o</sub>: reference crop evapotranspiration (mm d<sup>-1</sup>).

Moreover, the depth of applied irrigation water was calculated according to the Eq. 2 given by Vermeirer and Jopling (1984) as follows:

$$AIW = \frac{ET_c \times I}{E_a (1 - LR)} \quad (2)$$

Where:

AIW: depth of applied irrigation water (mm)

ET<sub>c</sub>: crop evapotranspiration (mm day<sup>-1</sup>)

I: irrigation intervals (day)

E<sub>a</sub>: irrigation system efficiency (%; 85%)

LR: leaching requirements (since electrical conductivity of soil solution is low, LR was neglected)

The tested irrigation water levels were started after 30 DAS. The average of irrigation water amounts applied under different irrigation levels for sunflower during the two growing seasons are presented in Table 2.

### Data Recorded

#### Leaf Area and Biochemical Measurements

At 65 DAS, five sunflower plants were chosen randomly from each plot to estimate leaf area according to Beadle (1993), and, in the same time, the 4<sup>th</sup> leaves from the plant top were used for measuring each of total chlorophyll (mg g<sup>-1</sup> fresh wt.), carotenoids (mg g<sup>-1</sup> fresh wt.) according to Arnon (1949), and free proline content (μg g<sup>-1</sup> fresh wt.) using the procedure described by Bates et al. (1973). Seed oil content (by extraction using Soxhlet Apparatus with hex-

**Table 1** Means monthly of climatic parameters and reference evapotranspiration (ET<sub>o</sub>) during sunflower growth and development at El Nubaria region (2019 and 2020 seasons)

Month	Air temperature (°C)				Relative humidity (%)		Wind speed (m sec <sup>-1</sup> )		Solar radiation (MJ m <sup>-2</sup> day <sup>-1</sup> )		ET <sub>o</sub> (mm day <sup>-1</sup> )	
	Minimum		Maximum		2019	2020	2019	2020	2019	2020	2019	2020
	2019	2020	2019	2020								
May	17.31	17.06	31.42	30.34	46.09	49.72	3.40	3.63	27.52	27.58	3.83	3.74
June	21.51	19.15	32.84	31.43	56.97	54.92	3.52	3.43	28.80	29.86	3.89	3.86
July	23.06	21.80	34.17	33.45	55.90	58.84	3.49	3.52	29.42	29.48	3.90	3.91
August	23.09	23.07	34.31	34.25	58.10	59.69	3.20	3.47	27.36	27.31	4.00	4.16

Data obtained from Central Laboratory of Meteorology, Ministry of Agriculture and Land Reclamation, Egypt

**Table 2** Calculated irrigation water amounts (m<sup>3</sup> ha<sup>-1</sup>) based on irrigation levels of sunflower in 2019 and 2020 seasons

WR <sub>100%</sub>		WR <sub>85%</sub>		WR <sub>70%</sub>	
2019	2020	2019	2020	2019	2020
5311.90	5226.19	4735.99	4650.79	4160.08	4075.38

WR<sub>100%</sub>, WR<sub>85%</sub>, and WR<sub>70%</sub> applied irrigation water at 100, 85 and 70% of crop evapotranspiration, respectively

ane as organic solvent) and iodine value were determined as described by AOAC (2012).

### Crop Measurements

At harvest dates (on 4<sup>th</sup> and 10<sup>th</sup> August in 2019 and 2020 seasons, respectively), whole plants of each plot were harvested to estimate head diameter, seed yield head<sup>-1</sup>, seed index, as well as seed yield ha<sup>-1</sup>.

### Water Use Efficiency (WUE)

Based on the obtained seed yield and amount of applied irrigation water, WUE of sunflower was computed by Eq. 3, according to Jensen (1983) as follows:

$$\text{WUE} = \frac{\text{Seed yield}}{\text{Applied irrigation water}} (\text{kg m}^{-3}) \quad (3)$$

### Statistical Analysis

Data for each growing season were subjected to two-way analysis of variance (ANOVA) according to Casella (2008), using Costat software program Version 6.303 (2004). For comparing among treatment means, Duncan's multiple range test at 0.05 probability level was used.

## Results

### Physiological Traits

Data in Table 3 revealed that each shortage in water supply, less than the normal (well-watered treatment, i.e. WR<sub>100%</sub>) caused lowering in total chlorophyll and carotenoids and increasing in free proline content. Moderate water-stressed (WR<sub>85%</sub>) and severe water-stressed (WR<sub>70%</sub>) plants recorded decreases of 28.6 and 45.5% in total chlorophyll, 21.6 and 38.6% in carotenoids and increments of 119.6 and 295.2% in proline in comparison with WR<sub>100%</sub>, in the first season, respectively. The corresponding values obtained in the second season were 31.5 and 50.3; 32.8 and 43.4%, and 108.9 and 268.5%, respectively.

Foliar spraying of salicylic acid (SA) (at 0.5 or 1.0 mM, i.e. SA<sub>0.5</sub> and SA<sub>1.0</sub>, respectively) had stimulative effect on total chlorophyll and carotenoids with inhibitive impact on proline content (Table 3). These contradictory effects were pronounced with each change in the applied SA concentration; the stimulative effects of SA levels on chlorophyll and carotenoids were in the following order: SA<sub>1.0</sub> > SA<sub>0.5</sub> > SA<sub>0.0</sub>, while the opposite was the true as for proline content. SA<sub>1.0</sub> treatment achieved the highest increases amounted to 27.4 and 32.4% in total chlorophyll and 23.9 and 26.3% in carotenoids over than SA<sub>0.0</sub>, in the first and second seasons, respectively. Meanwhile, the corresponding concomitant reductions in proline content were 18.1 and 17.9%, respectively.

The adverse effects of drought stress on leaf contents of chlorophyll, carotenoids, and proline were markedly diminished and less obvious when sunflower plants were sprayed with SA. Under every irrigation level, total chlorophyll and carotenoids increased while proline content decreased with

**Table 3** Leaf chlorophyll, carotenoids, and proline contents of sunflower as affected by irrigation level, salicylic acid rate in 2019 and 2020 seasons

Treatment	Carotenoids (mg g <sup>-1</sup> )		Chlorophyll (mg g <sup>-1</sup> )		Proline (mg g <sup>-1</sup> )	
	2019	2020	2019	2020	2019	2020
<i>Irrigation level, WR</i>						
WR <sub>100%</sub>	0.827 a	0.776 a	3.85 a	3.52 a	118.51 c	130.07 c
WR <sub>85%</sub>	0.648 b	0.591 b	2.75 b	2.41 b	260.22 b	271.71 b
WR <sub>70%</sub>	0.508 c	0.439 c	2.10 c	1.75 c	468.37 a	479.28 a
<i>Salicylic acid rate, SA</i>						
SA <sub>0.0</sub>	0.578 c	0.520 c	2.48 c	2.13 c	316.35 a	328.18 a
SA <sub>0.5</sub>	0.687 b	0.628 b	3.07 b	2.73 b	271.71 b	283.61 b
SA <sub>1.0</sub>	0.716 a	0.657 a	3.16 a	2.82 a	259.05 c	269.28 c
<i>WR × SA</i>						
WR <sub>100%</sub> × SA <sub>0.0</sub>	0.723 c	0.672 c	3.24 c	2.91 c	139.85 g	151.11 g
WR <sub>100%</sub> × SA <sub>0.5</sub>	0.868 b	0.817 b	4.12 b	3.79 b	111.65 h	123.36 h
WR <sub>100%</sub> × SA <sub>1.0</sub>	0.889 a	0.838 a	4.19 a	3.86 a	104.04 i	115.75 h
WR <sub>85%</sub> × SA <sub>0.0</sub>	0.551 e	0.494 e	2.34 f	2.00 f	299.83 d	312.11 d
WR <sub>85%</sub> × SA <sub>0.5</sub>	0.680 d	0.623 d	2.90 e	2.56 e	249.80 e	260.83 e
WR <sub>85%</sub> × SA <sub>1.0</sub>	0.713 c	0.656 c	3.01 d	2.67 d	231.04 f	242.18 f
WR <sub>70%</sub> × SA <sub>0.0</sub>	0.462 g	0.393 g	1.85 i	1.49 i	509.38 a	521.31 a
WR <sub>70%</sub> × SA <sub>0.5</sub>	0.514 f	0.445 f	2.19 h	1.83 h	453.66 b	466.62 b
WR <sub>70%</sub> × SA <sub>1.0</sub>	0.547 e	0.477 e	2.28 g	1.92 g	442.07 c	449.91 c

Different letters within columns indicate that there are significant differences by Duncan's multiple range test at  $p = 0.05$

WR<sub>100%</sub>, WR<sub>85%</sub>, and WR<sub>70%</sub> applied irrigation water at 100, 85 and 70% of crop evapotranspiration, respectively, SA<sub>0.0</sub>, SA<sub>0.5</sub>, and SA<sub>1.0</sub> salicylic acid level at 0.0, 0.5, and 1 mM, respectively

the involvement of SA (Table 3). In this connection, treated plants with WR<sub>100%</sub> × SA<sub>1.0</sub> contained the highest amounts of total chlorophyll and carotenoids as well as the lowest proline content. Applying WR<sub>100%</sub> × SA<sub>1.0</sub> recorded increases of 126.5 and 159.1% in total chlorophyll and 92.4 and 113.2% in carotenoids over than those recorded with applying WR<sub>70%</sub> × SA<sub>0.0</sub>, in the first and second seasons, respectively.

### Agronomic Traits

Marked influence of the applied irrigation levels was exhibited on leaf area, head diameter, seed yield/head, seed index, and seed yield ha<sup>-1</sup> of sunflower (Table 4). Each defect in the amount of irrigation water obviously decreased values of all agronomic traits. Therefore, the adverse effect was more severe when water supply lowered to 70% of ETC (WR<sub>70%</sub>). Growing sunflower plants under diminishing water requirements of 15% or 30% (from WR<sub>100%</sub> to WR<sub>85%</sub> or to WR<sub>70%</sub>) showed seed yield reductions of 24.8 and 47.8% in 2019 season and 25.3 and 48.1% in the 2020 season, respectively.

Foliar application of SA improved all agronomic traits; the highest concentration was the most effective in each case (Table 4). Application either SA<sub>0.5%</sub> or SA<sub>1.0</sub> gained increases in seed yield by about 8.8 and 10.4% in the first season and 13.5 and 15.2% in the second one, respectively.

Concerning the interaction effect, the combinations of irrigation and salicylic acid levels varied significantly in affecting all agronomic traits (Table 4). Under all irrigation treatments, foliar spraying with SA improved seed yield; more beneficial effects were provided with increasing the applied SA concentration, almost in all cases. Sunflower plants treated by WR<sub>100%</sub> × SA<sub>1.0</sub>, proved superiority over all investigated treatments except that of WR<sub>100%</sub> × SA<sub>0.5</sub> as for leaf area and seed yield ha<sup>-1</sup> in the second season. The maximum seed yield was attained by applying WR<sub>100%</sub> × SA<sub>1.0</sub> (in both growing seasons) or WR<sub>100%</sub> × SA<sub>0.5</sub> (in the second season only). Seed yield of WR<sub>100%</sub> × SA<sub>1.0</sub> treatment was higher than that of WR<sub>70%</sub> × SA<sub>0.0</sub> by 109.7% in the first season and 125.9% in the second one. Additionally, as averages of the two seasons, reductions in seed yield due to lowering water supply by 15 and 30% were 21.0, 15.8 and 14.4% as well as 46.2, 40.8 and 40.1% with SA<sub>0.0</sub>, SA<sub>0.5</sub> and SA<sub>1.0</sub>, respectively, compared to the farmer common practice (WR<sub>100%</sub> × SA<sub>0.0</sub>).

### Seed Oil Quality

Sunflower seed oil percentage and iodine value were statistically influenced by the applied irrigation levels (Table 5). In this regard, data obviously revealed, in both seasons, enhancement effect of each addition of water supply up to the well-watered condition (WR<sub>100%</sub>). Seeds of sunflower plants

**Table 4** Leaf area, head diameter, seed yield/head, seed index, and seed yield of sunflower as affected by as affected by irrigation level, salicylic acid rate in 2019 and 2020 seasons

Treatment	Leaf area (cm <sup>2</sup> )		Head diameter (cm)		Seed weight head <sup>-1</sup> (g)		Seed index (g)		Seed yield (kg ha <sup>-1</sup> )	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
<i>Irrigation level, WR</i>										
WR <sub>100%</sub>	611.6 a	596.6 a	21.0 a	19.9 a	40.2 a	38.4 a	7.63 a	6.65 a	3023.1 a	2880.2 a
WR <sub>85%</sub>	458.9 b	445.5 b	15.3 b	14.2 b	25.3 b	23.4 b	6.58 b	5.61 b	2273.6 b	2150.2 b
WR <sub>70%</sub>	353.4 c	342.1 c	12.6 c	11.5 c	18.0 c	15.9 c	5.60 c	4.63 c	1579.3 c	1495.6 c
<i>Salicylic acid rate, SA</i>										
SA <sub>0.0</sub>	419.5 c	405.8 c	13.9 c	12.9 c	23.2 c	21.3 c	6.19 c	5.21 c	2154.2 c	1985.2 c
SA <sub>0.5</sub>	497.5 b	485.3 b	17.1 b	15.9 b	29.5 b	27.3 b	6.76 b	5.78 b	2343.3 b	2254.0 b
SA <sub>1.0</sub>	507.0 a	493.0 a	17.8 a	16.8 a	30.7 a	29.0 a	6.85 a	5.90 a	2378.5 a	2286.9 a
<i>WR × SA</i>										
WR <sub>100%</sub> × SA <sub>0.0</sub>	543.7 c	529.7 b	17.5 c	16.3 c	32.8 c	30.8 c	7.08 c	6.07 c	2754.9 c	2578.7 b
WR <sub>100%</sub> × SA <sub>0.5</sub>	640.3 b	627.7 a	22.3 b	21.3 b	42.9 b	41.2 b	7.85 b	6.86 b	3125.1 b	3017.7 a
WR <sub>100%</sub> × SA <sub>1.0</sub>	651.0 a	632.3 a	23.3 a	22.2 a	44.9 a	43.1 a	7.95 a	7.02 a	3189.1 a	3044.3 a
WR <sub>85%</sub> × SA <sub>0.0</sub>	387.3 f	372.9 e	13.6 f	12.6 f	22.1 f	20.2 e	6.16 f	5.17 e	2186.9 e	2029.1 e
WR <sub>85%</sub> × SA <sub>0.5</sub>	487.2 e	474.1 d	15.8 e	14.4 e	26.3 e	24.3 d	6.76 e	5.80 d	2303.2 d	2186.2 d
WR <sub>85%</sub> × SA <sub>1.0</sub>	502.1 d	489.5 c	16.6 d	15.5 d	27.4 d	25.7 d	6.83 d	5.86 d	2330.6 d	2235.4 c
WR <sub>70%</sub> × SA <sub>0.0</sub>	327.6 h	315.0 g	10.7 g	9.6 g	14.7 h	13.0 h	5.35 i	4.39 h	1520.7 g	1347.8 g
WR <sub>70%</sub> × SA <sub>0.5</sub>	364.9 g	354.2 f	13.3 f	12.2 f	19.3 g	16.5 g	5.67 h	4.70 g	1601.5 f	1558.2 f
WR <sub>70%</sub> × SA <sub>1.0</sub>	367.7 g	357.0 f	13.7 f	12.7 f	19.9 g	18.3 f	5.77 g	4.81 f	1615.7 f	1580.9 f

Different letters within columns indicate that there are significant differences by Duncan's multiple range test at  $p = 0.05$

WR<sub>100%</sub>, WR<sub>85%</sub>, and WR<sub>70%</sub> applied irrigation water at 100, 85 and 70% of crop evapotranspiration, respectively, SA<sub>0.0</sub>, SA<sub>0.5</sub>, and SA<sub>1.0</sub> salicylic acid level at 0.0, 0.5, and 1 mM, respectively

**Table 5** Seed oil percentage and iodine value of sunflower as affected by as affected by irrigation level, salicylic acid rate in 2019 and 2020 seasons

Treatment	Oil %		Iodine value	
	2019	2020	2019	2020
<i>Irrigation level, WR</i>				
WR <sub>100%</sub>	38.18 a	37.02 a	131.92 a	130.14 a
WR <sub>85%</sub>	34.92 b	33.76 b	124.37 b	122.72 b
WR <sub>70%</sub>	31.46 c	30.32 c	117.82 c	116.19 c
<i>Salicylic acid rate, SA</i>				
SA <sub>0.0</sub>	33.35 c	32.13 c	121.59 c	119.87 c
SA <sub>0.5</sub>	35.51 b	34.36 b	125.89 b	124.24 b
SA <sub>1.0</sub>	35.70 a	34.61 a	126.62 a	124.93 a
<i>WR × SA</i>				
WR <sub>100%</sub> × SA <sub>0.0</sub>	36.91 b	35.73 b	129.98 c	128.08 c
WR <sub>100%</sub> × SA <sub>0.5</sub>	38.75 a	37.58 a	132.57 b	130.85 b
WR <sub>100%</sub> × SA <sub>1.0</sub>	38.87 a	37.75 a	133.20 a	131.49 a
WR <sub>85%</sub> × SA <sub>0.0</sub>	33.42 d	32.17 d	121.41 f	119.79 f
WR <sub>85%</sub> × SA <sub>0.5</sub>	35.62 c	34.53 c	125.37 e	123.71 e
WR <sub>85%</sub> × SA <sub>1.0</sub>	35.72 c	34.59 c	126.31 d	124.66 d
WR <sub>70%</sub> × SA <sub>0.0</sub>	29.71 g	28.49 g	113.38 i	111.73 i
WR <sub>70%</sub> × SA <sub>0.5</sub>	32.16 f	30.98 f	119.74 h	118.18 h
WR <sub>70%</sub> × SA <sub>1.0</sub>	32.53 e	31.49 e	120.34 g	118.65 g

Different letters within columns indicate that there are significant differences by Duncan's multiple range test at  $p = 0.05$

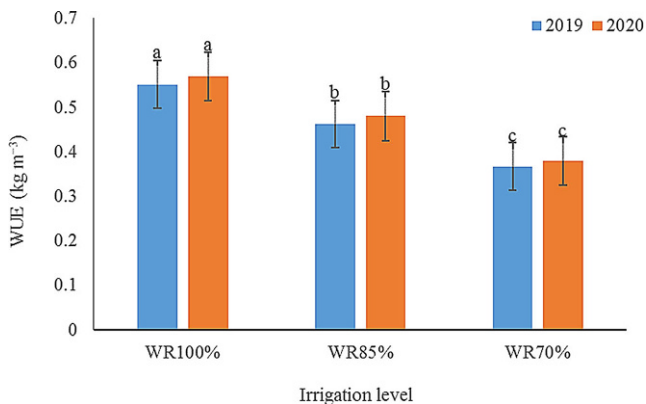
WR<sub>100%</sub>, WR<sub>85%</sub>, and WR<sub>70%</sub> applied irrigation water at 100, 85 and 70% of crop evapotranspiration, respectively, SA<sub>0.0</sub>, SA<sub>0.5</sub>, and SA<sub>1.0</sub> salicylic acid level at 0.0, 0.5, and 1 mM, respectively

grown under water requirement reduction of 15% or 30% (from  $WR_{100\%}$  to  $WR_{85\%}$  or to  $WR_{70\%}$ ) had 8.5 and 17.6% less in seed oil% and 5.7 and 10.7% less in iodine value in 2019 season, respectively, comparing well-watered plants. The corresponding reductions in the 2020 season were 8.8 and 18.1% for seed oil% and 5.7 and 10.7% for iodine value, respectively. On the other hand, it could be obviously noted that foliar application of SA improved seed oil percentage and iodine value as well. The advantages of SA achieved in seed oil percentage and iodine value were almost more with  $SA_{1.0}$ . In both seasons,  $WR_{100\%} \times SA_{1.0}$  for iodine value as well as  $WR_{100\%} \times SA_{1.0}$  and  $WR_{100\%} \times SA_{0.5}$  for seed oil % recorded the highest. Data clearly illustrated that implicating SA spray, in the applied combination treatments, had beneficial effects on seed oil % and iodine value. Meanwhile, the lowest values of both traits were detected in oil of seeds produced by plants grown under severely water-stress condition without receiving SA ( $WR_{70\%} \times SA_{0.0}$ ).

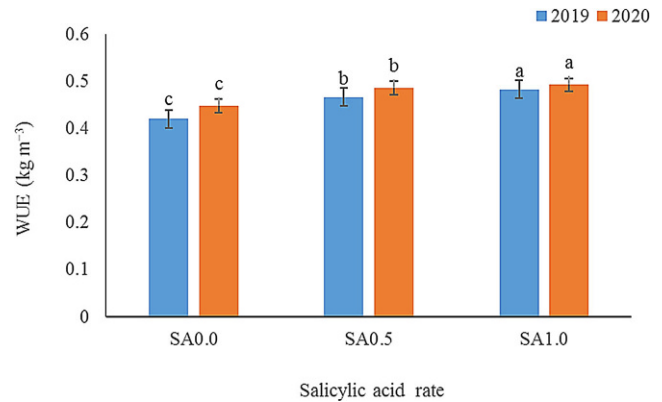
### Water Use Efficiency

As depicted in Figs. 1 and 2, the more the irrigation amount and SA rate increase, the more the WUE increases. Accordingly, the increases in WUE due to  $WR_{100\%}$  and  $WR_{85\%}$  were 49.9 and 26.5% in the first season and 50.2 and 26.0% in the second one, respectively compared to  $WR_{70\%}$  (Fig. 1). Also, WUE showed increases of 10.0 and 8.4% as well as 15.1 and 13.4% with application of  $SA_{1.0}$  and  $SA_{0.5}$  in the first and second season, respectively, compared to  $SA_{0.0}$  (Fig. 2).

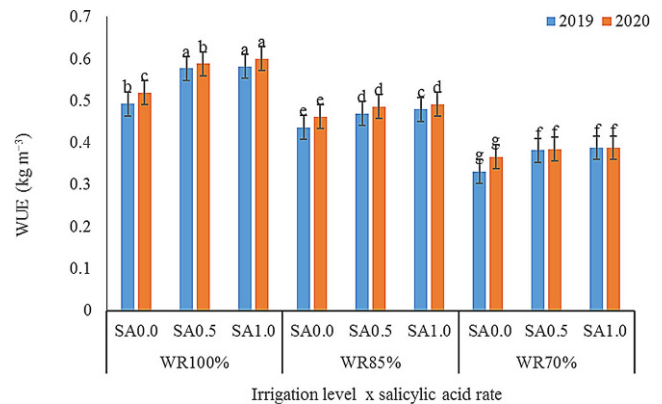
The beneficial effect of SA implication on WUE was pronounced under well-watered condition, in both seasons. The differences in WUE between  $WR_{100\%} \times SA_{1.0}$  and  $WR_{100\%} \times SA_{0.5}$  didn't reach the  $P < 0.05$  level of significance in 2020 season, with the superiority of  $WR_{100\%} \times SA_{1.0}$



**Fig. 1** Water use efficiency (WUE) of sunflower as affected by irrigation level in 2019 and 2020 seasons. (Different letters of the bars indicate that there are significant differences by Duncan's multiple range test at  $p = 0.05$ .  $WR_{100\%}$ ,  $WR_{85\%}$ , and  $WR_{70\%}$  applied irrigation water at 100, 85 and 70% of crop evapotranspiration, respectively)



**Fig. 2** Water use efficiency (WUE) of sunflower as affected by salicylic acid rate in 2019 and 2020 seasons. (Different letters of the bars indicate that there are significant differences by Duncan's multiple range test at  $p = 0.05$ .  $SA_{0.0}$ ,  $SA_{0.5}$ , and  $SA_{1.0}$  salicylic acid level at 0.0, 0.5, and 1 mM, respectively)



**Fig. 3** Water use efficiency (WUE) of sunflower as affected by the interaction of irrigation level and salicylic acid rate in 2019 and 2020 seasons. (Different letters of the bars indicate that there are significant differences by Duncan's multiple range test at  $p = 0.05$ .  $WR_{100\%}$ ,  $WR_{85\%}$ , and  $WR_{70\%}$  applied irrigation water at 100, 85 and 70% of crop evapotranspiration, respectively,  $SA_{0.0}$ ,  $SA_{0.5}$ , and  $SA_{1.0}$  salicylic acid level at 0.0, 0.5, and 1 mM, respectively)

in 2019 season. On the contrary, severely water-stressed plants without receiving foliar SA ( $WR_{70\%} \times SA_{0.0}$ ) recorded the lowest WUE (Fig. 3).

### Discussion

Since drought causes changes in molecular, biochemical, physiological, and morphological aspects resulting in negative effects on plant growth and development (Nam et al. 2020; Taha et al. 2020; Babaei et al. 2021; El-Metwally and Saady 2021; El-Metwally et al. 2021; Salem et al. 2022), injurious impacts of water deficit on different sunflower traits were occurred (Table 3, 4 and 5). The present study confirmed the negative impact of water deficit/on

plant physiological status. Owing to the relationship between the plant biochemical compounds and deficit water, drought stress led to degradation of photosynthesis pigments (chlorophylls and carotenoids), as well as accumulation of proline (Table 3). Such findings are in accordance with those recorded by Manivannan et al. (2007) and Saudy et al. (2021) who reported that subjecting sunflower plants to water deficit caused decreases in total chlorophyll and carotenoids and increases proline content in leaves. During drought, reactive oxygen species (ROS) accumulate, which are toxic at elevated levels, due to reduced electron transport chain activity (Hasanuzzaman et al. 2020). Overproduction of ROS is concomitant to various abiotic stresses (Gill and Tuteja 2010; Hossain et al. 2014; Soury et al. 2019; Hatamian et al. 2020). ROS react with and deteriorate nucleic acids (DNA), proteins, photosynthetic pigments, and membrane lipids (Zulfiqar and Ashraf 2021). Elevated ROS levels lead to inactivation of proteins and inhibit the activity of multiple enzymes involved in metabolic pathways, and result in oxidation of lipids and DNA (Hossain et al. 2014). Consequently, ROS can damage membrane and other essential macromolecules (such as photosynthetic pigments, proteins, DNA and lipids), so reducing contents of chlorophylls and carotenoids as shown under drought stress (Table 3). However, plants have natural defense systems involving non-enzymatic and enzymatic antioxidants that efficiently ameliorate the negative impacts of excessive ROS (Zulfiqar and Ashraf 2021). Moreover, plants acclimate to ROS-induced stress by producing various beneficial compatible solutes, such as proline and glycine betaine (Shemi et al. 2021). Subjecting sunflower plants to drought stress increases proline content while reduced the activity of proline oxidase in leaves (Manivannan et al. 2007). Content of chlorophyll, which is one of the major chloroplast components for photosynthesis, has a positive relationship with photosynthetic rate (Anjum et al. 2011). Degradation in photosynthetic pigments can directly limit photosynthetic potential which adversely affects plant productivity. The decrease in chlorophyll content under drought stress may be the result of pigment photo-oxidation and chlorophyll degradation. Photosynthetic pigments of plants are important for harvesting light and production of reducing powers (Anjum et al. 2011). Accordingly, marked reductions were exhibited in leaf area, head diameter, seed yield/head, seed index, seed yield ha<sup>-1</sup>, seed oil content and iodine value (Table 4) as well as water use efficiency (Fig. 1) of sunflower subjected to drought stress. Many researchers have reported decreasing performance of sunflower growth under water stress conditions (Erdem et al. 2006; Nezami et al. 2008). Water deficit reduces sunflower yield and quality (El-Bially et al. 2018; El-Metwally et al. 2022). Following drought, stomata close progressively with a parallel decline in net photosynthesis and water-use efficiency (Anjum

et al. 2011). The closure of stomata significantly decreases the photosynthetic and transpiration rate (Jaleel et al. 2007) and negatively influences the crop growth and productivity (Du et al. 2010). Drought stress significantly decreased all the photosynthetic parameters, i.e. net photosynthetic rate, stomatal conductance, internal carbon dioxide concentration, as well as water use efficiency (WUE) and transpiration rate (Hayat et al. 2008). The lack of photosynthetic pigment contents in the leaves (Table 3), under stresses, leads to a decrease in the efficiency of photosynthesis, which in turn affects the productivity and decrease seeds oil content of sunflower plants (Saudy et al. 2021). Moreover, the harmful effect of drought could be attributed to reduce the availability of nutrients in soil with disturbance in plant nutritional status associated low water supply (Saudy and El-Metwally 2019; Mubarak et al. 2021; Salem et al. 2021; Abd-Elrahman et al. 2022).

Not only SA had a beneficial effect on plant growth and development under water stress conditions but also under normal water supply. In this respect, SA is one of the main plant growth regulators that play a vital role in controlling and modulating photosynthesis under both normal and stressful condition (Arif et al. 2020). SA has key role in enhancing photosynthesis by upregulating photosynthetic enzyme and carbohydrate metabolism (Khodary 2004). SA application increased photosynthetic activity, chlorophyll content, and enzyme activity under both normal and stress conditions (Li et al. 2014). Therefore, total chlorophyll and carotenoids increased while proline content decreased with SA supply under every irrigation level (Table 3). Such findings may be reflected and interpreted, partially at least, the enhancements achieved in sunflower growth and productivity due to exogenous application of SA. The enhancement in sunflower growth with application of SA could be attributed to the role of SA in maintenance the photosynthetic machinery and activity, then promoting the plant growth. SA, which is a plant hormone (Davies 2010) is a multifaceted plant growth regulator which participates in a wide range of growth, metabolism and defense systems; it acts a typical plant hormone modulating all plant responses and providing plant immunity against various stresses (Arif et al. 2020). SA enhances various physiological processes like photosynthesis, chlorophyll and other pigment, plant growth and development, and flowering (Arif et al. 2020). SA plays a key role in providing tolerance to the plants exposed to water stress, i.e. drought or flooding (Hayat et al. 2010). Exogenous application of SA facilitates growth; and flowering; up-regulates photosynthesis; increases the activity of enzymatic and non-enzymatic antioxidants (Arif et al. 2020). Additionally, the generation of ROS during drought stress requires up-regulation of detoxification systems such as super oxide dismutase (SOD) and catalase enzymes and biosynthesis of ROS scavengers (You and Chan



2015). Herein, SA enhances the activities of antioxidants enzyme system i.e. ascorbate peroxidase (APX) and superoxide dismutase (SOD) with a concomitant decline in the activity of catalase (CAT), and can protect and enhance the enzymes of nitrate metabolism under stressful environments (reviewed by Hayat et al. 2010). Also, SA enhances the activity of ROS scavenger enzymes, participates in eliciting abiotic stress responses such as drought (Arif et al. 2020), Application of SA in drought-stressed plants resulted in growth recovery, increased photosynthesis, and reduced oxidative stress (Zulfiqar et al. 2021). Therefore, foliar application of SA improved leaf area, head diameter, seed yield/head, seed index, seed yield ha<sup>-1</sup> (Table 4), oil seed content and iodine value (Table 5) as well as WUE (Fig. 2) of sunflower. Also, WUE increases with alleviating water stress by applying SA (Fig. 3). Thus, the maximal values of WUE were recorded with WR<sub>100%</sub> × SA<sub>1.0</sub>, followed by that of WR<sub>100%</sub> × SA<sub>0.5</sub>. These findings could be owing to producing seed yields under such conditions more than under other ones, resulting in higher WUE values. At low WUE, photosynthetic carbon assimilation is decreased due to decreasing flow of CO<sub>2</sub> into mesophyll tissue and the closure of stomata (Chaves et al. 2003; Flexas et al. 2004). Following drought, stomata close progressively with a parallel decline in net photosynthesis and WUE (Anjum et al. 2011). Moreover, different concentrations of SA increased total leaf chlorophyll content, photosynthesis and stomatal conductance under normal and stress conditions (Bastam et al. 2012; Ghasemzadeh and Jaafar 2013). SA stimulated CO<sub>2</sub> fixation and efficiency of photosynthetic quantum (Poór et al. 2011). SA also helped to ignite photosynthetic process by closing stomata and by suppressing or slowing electron transport metabolism of PSII (Janda et al. 2012). Photosynthesis, stomatal conductance, gaseous exchange, CO<sub>2</sub> assimilation rate, and chlorophyll content were enhanced after applying SA (Babar et al. 2014). SA elevated photosynthetic rate by facilitating carboxylation rate, chlorophyll amount that is SPAD values and by increasing turgor (Tahjib-Ul-Arif et al. 2018). Additionally, SA increased the chlorophyll, carotenoid, nitrogen, potassium and phosphorus level and nitrate reductase activity (Hashmi et al. 2012). It also maintains membrane integrity of chloroplast membrane (Huang et al. 2016).

## Conclusions

It is interesting to conclude that salicylic acid has a substantial role for ameliorating growth and yield of sunflower. Such effect was more pronounced under water deficit, where salicylic acid partially alleviated the detrimental impact of low irrigation level. The current study proved that salicylic acid could be regarded as an inducer for drought

tolerance. In this respect, compared to the farmer common practice (WR<sub>100%</sub> × SA<sub>0.0</sub>), SA<sub>0.5</sub> and SA<sub>1.0</sub> alleviated the yield losses from 21.0% to 15.8 and 14.4% as well as 46.2% to 40.8 and 40.1% under lowering water supply by 15 and 30%, respectively. The global climatic changes and water scarcity, as in arid and semi-arid regions, often obligate farmers to irrigate the crops using water amounts less than required or optimum. This means subjecting crop plants to drought stress, leading to yield losses. Therefore, inserting the plant growth regulator salicylic acid as a save and cheap practice in drought-imposed sunflower cultivation has become important to relatively alleviate the associated deleterious of water deficit.

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

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