

Comparative Analysis of Seed Yield and Biochemical Attributes in Different Sunflower Genotypes under Different Levels of Irrigation and Salinity

Heba T. Ebeed^{(1)#}, Nemat M. Hassan⁽¹⁾, Maaty M. Keshta⁽²⁾, Olfat S. Hassanin⁽²⁾

⁽¹⁾Department of Botany and Microbiology, Faculty of Science, Damietta University, New Damietta City, PO 34517, Egypt; ⁽²⁾Agricultural Research Station at El-Sirw, Damietta Governorate, Egypt.

SUNFLOWER (*Helianthus annuus* L.) is an important oilseed crop in so many countries which suffer from seed and oil yield reduction by limited water or soil salinity. Since some responses to water and salt stress are common, other responses may vary according to the genotype and/or stress level. The role of the genetic diversity on the responses of sunflower yield, oil quality and the fatty acid composition to the different levels of irrigation and soil salinity in two field experiments were investigated. Three registered parental lines; HA 429, HA 430 and HA 20 and two hybrids; H (A9xRF6) and H (A9xRF8), in addition to one cultivar; Sakha 53 were used in this study. The results showed significant effects of genotype, level of stress and their interactions on most of the examined characteristics. Water stress caused a major reduction of protein content and oil yield than salinity stress. Different genotypes with similar oil contents had different oil yields under stress treatments. The hybrids H (A9xRF6) and H (A9xRF8) showed less reduction in oil content by both irrigation and salinity treatments, compared to the other genotypes and this candidate them for cultivation in stressed regions. The results also showed that some fatty acids, particularly stearic acid and oleic acid, were dependent on the genotype and the stress level in both experiments.

Keywords: Fatty acids, Genotypes, Irrigation, Salinity, Sunflower, Yield.

Introduction

Drought and salinity are widespread in many regions worldwide and considered the most environmental factors that limiting crop productivity (Shabala, 2013). Drought refers to a decrease in the available water than normally needed (Sheffield & Wood, 2012) while soil salinity refers to presence of electrolytic mineral solutes in high concentrations that adversely affect plants (Munns & Tester, 2008). Drought and salinity are linked in their consequences as concluded from several reports. Limited rainfall and high evaporation as well the excessive irrigation without the appropriate drainage systems contribute to the soil salinization (Qadir et al., 2014). As well as, salinity reduces the ability of plants to take up water (Malash et al., 2008), the condition identical to that caused by drought. Moreover, plant responses to drought and salinity have much in common as the reduction in the growth along with some metabolic changes as reviewed by Munns (2002). The morphological,

physiological, biochemical and molecular changes adversely affect plant productivity (Wang et al., 2000). Although, the large number of studies revealed the conserved cellular responses, such as the production of stress proteins and the accumulation of compatible solutes (Vierling & Kimpel, 1992 and Zhu et al., 1997) or the similar cell signalling pathways to drought and salinity (Hasegawa et al., 2000 and Shinozaki & Yamaguchi-Shinozaki, 2000), the information about change in oil composition in response to both conditions need to be investigated in further details.

Sunflower (*Helianthus annuus* L.), is one of the most important oil crops in the world and has occupied the second rank after soybean (FAO, 2014). The main advantage of this crop is its high oil content 42–50% of seed weight on average in addition to 15–20% protein content (Aishwarya & Anisha, 2014). Its oil composed of more than 90% unsaturated fatty acids as linoleic (18:2) and oleic (18:1) acids which have health benefits (Monotti,

#Corresponding author email: hebeed@du.edu.eg Tel: 01004091763 Fax: 0572403868

DOI: 10.21608/ejbo.2019.5043.1205

Edited by: Prof. Dr. Wedad Kasim, Faculty of Science, Tanta University, Tanta, Egypt.

©2019 National Information and Documentation Center (NIDOC)

2003). Moreover, sunflower adapts to a wide range of soil types and climates that encourages its cultivation in many semi-arid and arid regions such as western USA (Francois, 1996) and the Mediterranean (Monotti, 2003). However, its production is not sufficient due to the increase in demand and the environmental and climatic factors that adversely affect the yield (Barrón & De Mejía, 1998).

Although sunflower has been classified as moderately tolerant to salinity and tolerant to drought (Katerji et al., 2003 and Ahmad et al., 2009), some sunflower growth stages as germination, flowering and seed filling are critical stages for experiencing stress (Howell et al., 2015). Sunflower growth as well as seed and oil yield are adversely affected by drought and salinity (Hammad et al., 2002; Flagella et al., 2004; Hassan et al., 2011 and Farghaly et al., 2016). However, some contradictory results about no changes in oil content due to water stress (Mozaffari & Arshi, 1996) or salinity (Francois, 1996) which could be possibly due to the different sunflower genotypes or the level of stress. While that the quality of any seed oil is determined by its fatty acids (FAs) composition (Rahimmalek & Goli, 2013). The genotype is the most important factor that determines fatty acid composition (Abdallah et al., 1998) however, the environmental conditions were found to modify the fatty acids profile (Flagella et al., 2004; Howell et al., 2015 and Alberio et al., 2016). Some FAs as oleic acid was not stable when grown in different environment (Van der Merwe et al., 2013). Therefore, studying different genotypes for drought and salinity tolerance is very essential to better select and improve breeding strategies to enhance crop yields (Ceccarelli & Grando, 2007).

Despite a large number of studies devoted to sunflower yield or its oil composition, the comparative research for stress responses under field-grown conditions is still not available. Most of the studies were based on a single stress factor or few cultivars as done by Petcu et al. (2001), Flagella et al. (2004) and Di Caterina et al. (2007). Study of, Plaut & Grava (2000) compared sunflower's yield under both drought and salinity but without characterizing their impacts on seed biochemistry. Therefore, the present study has been carried out to evaluate the performance of different six genotypes under three different irrigation regimes and three different salinity levels; to investigate the biochemical traits tightly involved in drought/

salinity tolerance and to explore the changes in the oil content and the FAs composition to better understanding the quantitative and qualitative responses of sunflower to reduced irrigation and soil salinity and to provide some references for soil management in sunflower crop production.

Materials and Methods

Experimental design and treatments

Two field experiments were carried out separately at El-Sirw Agricultural Research Station (ESARS)-Agricultural Research Centre (ARC) (latitude: 31°14'21.86"N; longitude: 31°39'8.32"E) which is located in the northeast of the Nile delta, North Egypt during the growing season of six oil sunflower (*Helianthus annuus* L.) from March to Jun 2015 to evaluate the growth, yield, yield components and seed quality of six genotypes under water and salt stresses. Water stress was applied by reducing number of irrigations while the salinity stress was applied by the addition of sodium chloride salt to the soil. Hence, three irrigation regimes and three salinity levels were used. The genotypes used in this study were: Three pure lines; HA 429, HA 430 and HA 20 (the first two genotypes were obtained from United State Department of Agriculture (USDA) and the third was produced in the oil crops centre, ESARS, Egypt; two hybrids; H (A9xRF6) and H (A9xRF8) and one open-pollinated cultivar Sakha53; all of them were produced in ESARS. A randomized split-plot design with three replications was adopted for the reduced irrigation and salinity experiments. The main plots (70m²) were devoted to irrigation regimes or salinity treatments and the subplots were devoted to the genotypes. Plants were sown in clay soil in rows planted 0.7m apart, with the seeds placed 0.25m apart along the row. Seeds of the six genotypes were soaked in water for 4hr and then germinated in the field, March 2015. The plots were furrow-irrigated to ensure uniform growth. Super phosphate (100kg fad⁻¹) and ammonium nitrate (150kg fad⁻¹) were applied twice; the first at 10 days after emergence and at 50 days after emergence.

Irrigation experiment

All plants were irrigated every 2 weeks from emergence until flowering. After complete flowering, plots were divided into 3 groups: The first group was well-irrigated and left as control (I1) which received a total 5 irrigations throughout the experiment. The second group was subjected

to moderately reduced irrigation (I2) by omitting the pre-last irrigation (received total 4 irrigations throughout the experiment) and the third group was subjected to severely reduced irrigation (I3) by withholding irrigation completely after flowering (gained only the first three irrigations). Plants were grown under different irrigation regimes up to complete maturity of seeds which was indicated when the heads turned brown and the seed moisture content reached 10–12% of seed fresh weight. This was done by taking samples of seeds from each row and then the seeds were weighed fresh (FW) then oven-dried at 40°C for 4hr and weighed again (DW) to calculate the moisture content of the seeds.

Salinity experiment

Soil was prepared as mentioned before in the first experiment. Plots were divided into three groups which received 0, 100 or 200g NaCl was added to the soil of main plots where the first group was left as control; S0, the second S1 (moderate salinity) and the third S2 (high salinity), respectively and the soil was well mixed before sowing to secure the levels of salinity before the irrigation, then the level of salinity was kept constant all over the experimental period through keeping water holding capacity. The chosen salinity levels were measured as Electrical Conductivity (EC) and converted to ppm where S0; ECe= 2.5 and total dissolved solids (TDS)= 1514ppm, S1; ECe= 3.7 and TDS= 2400ppm and S2; ECe= 4.4 and TDS= 32600ppm. Plants were grown in the field until the end of the experiment, and the harvest was conducted after complete seed maturity (85-90 days).

Growth parameters and yield components

Five plants were randomly selected from the middle rows in each sub-plot at the end of the experiment to measure plant height (cm) and stem diameter (cm).

Yield and yield components such as head diameter, seed yield plant⁻¹, weight of 100 seeds, seed yield fad⁻¹ and oil yield fad⁻¹ were determined. Oil yield was calculated by multiplying seed oil content by seed yield. To determine the tolerance of a plant, % of seed yield decline and % of oil yield decline under the investigated treatments were calculated relative to control yield.

Determination of seed protein, sugars and proline content

Protein was extracted from a known weight of

dry ground seeds with 1N NaOH for 24hr at 4°C. The residue was removed by centrifugation 10min at 10000xg. Then 0.1ml of the supernatant was then added to 5ml Coomassie Brilliant Blue dye reagent and mixed well according to the method adopted by Bradford (1976). Optical density was measured at 595nm against water blank. Protein concentration was determined by a standard curve using bovine serum albumin (BSA) in the range of 20–100µg/ml.

Soluble sugars were extracted from about 50mg dried seeds with 80% ethanol and centrifuged at 12000xg for 10min; the extract was dried in a water bath and then resuspended in distilled water (Schortemeyer et al., 1997). An aliquot was mixed with anthrone reagent, heated for 10min, cooled in ice bath for 30min and the absorbance was recorded at 623nm (Schluter & Crawford, 2001).

Proline was extracted from a known weight of oven-dried seeds with 3% sulfosalicylic acid and centrifuged at 12000xg. An aliquot was reacted with glacial acetic acid and acidic ninhydrin for 1hr at 100°C. The reaction was terminated in an ice bath and extracted with 5ml toluene. The chromophore-containing toluene was warmed to room temperature and absorbance was measured at 520nm (Bates et al., 1973).

Determination of total oil content

Seeds were oven-dried at 40°C for 4hr, using a ventilated oven, to a moisture content of about 5%, and were then ground with a blender. Four grams of the ground seeds were used to extract the oil with petroleum ether for 16hr in a Soxhlet system according to (AOCS & Firestone, 1994). The oil extract was evaporated in a rotary evaporator at 40–60°C and the weight of oil was recorded and the oil content was determined as a percentage of the dry weight.

Fatty acids profiling

Total lipids from seeds were extracted using the modified method of Bligh & Dyer (1959). About 2–20g of dry seeds were fixed in boiling water for 5min and then ground manually with chloroform/methanol/hexane mixture (1:2:1, v/v/v). After maceration with chloroform and washing with water for 24hr, the organic phase containing total lipids was dried under a stream of nitrogen, dissolved in toluene/ethanol (4:1, v/v) mixture, and stored at -80°C for further analyses.

Preparation of fatty acid methyl ester (FAME) was carried out according to Siew et al. (1995) using 3% sodium methylate in methanol. The fatty acid composition of oils was determined using its fatty acid methyl esters and was injected into gas chromatography for analysis using a Hewlett-Packard 6890 chromatograph (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector and an electronic pressure control injector.

Statistical analysis

The data were subjected to multivariate analysis of variance (MANOVA) using SPSS 17.0 statistical package. Significant differences among the mean values were calculated using least significant difference (LSD) at $P \leq 0.05$.

Results

In this study, the investigated genotypes were able to grow, flower and set seeds and showed different performances under the investigated

conditions. All tested parameters were significantly affected by genotypes, irrigation regime/salinity, and interactions between genotype and, irrigation regimes/salinity (at $P \leq 0.05$) except for oil content that did not show genotypic differences under saline conditions.

Effect of different water regimes and salinity levels on growth and yield parameters

As indicated in Table 1, significant reduction in plant growth parameters was observed due to irrigation regimes I2 and I3 and salinity level S1 and S2. Under the three levels of irrigation I1, I2 and I3, the better growth (plant height; 158.4, 146.9 and 120.6cm and stem diameter; 3, 2.2 and 1.6cm, respectively) was recorded in Sakha53 whereas, the more reduced growth was recorded in HA 429. Under salinity, the highest values of plant height and stem diameter were observed in H (A9xRf6), H (A9xRf8) and Sakha53 genotypes under S1 and S2 while the lowest values were recorded in HA 430 due to S2.

TABLE 1. Effects of irrigation regimes (I1, I2 and I3) and salinity levels (S1 and S2) in addition to their control (S₀) on plant height and stem diameter of six sunflower genotypes.

Trait	Plant height (cm)				Stem diameter (cm)			
	I1	I2	I3	Avg.	I1	I2	I3	Avg.
Genotypes								
HA 429	102.3±2.8 ^e	74.4±2.4 ^f	51.0±2.5 ^g	75.9 ^E	1.4±0.2 ^{cd}	1.0±0.1 ^{cd}	0.7±0.0 ^d	1.1 ^D
HA 430	128.6±3.4 ^d	85.1±2.9 ^f	69.9±1.9 ^{fg}	94.5 ^D	2.4±0.1 ^b	1.6±0.1 ^c	1.1±0.1 ^{cd}	1.7 ^B
HA 20	104.0±2.9 ^e	84.6±5.6 ^f	66.5±3.0 ^g	85.0 ^D	2.1±0.1 ^b	1.7±0.1 ^{bc}	0.9±0.1 ^{cd}	1.5 ^C
H(A9xRf6)	163.6±4.2 ^b	139.6±3.6 ^{cd}	119.1±1.2 ^d	140.8 ^B	2.5±0.2 ^b	1.7±0.1 ^{bc}	1.2±0.1 ^{cd}	1.8 ^B
H(A9xRf8)	144.1±3.7 ^c	124.9±2.5 ^d	103.1±3.1 ^e	124.0 ^C	2.7±0.1 ^{ab}	1.4±0.1 ^{cd}	1.4±0.1 ^{cd}	1.8 ^B
Sakha53	185.4±6.2 ^a	146.9±4.6 ^c	120.6±2.5 ^d	151.0 ^A	3.0±0.1 ^a	2.2±0.1 ^b	1.6±0.1 ^c	2.3 ^A
Avg.	134.5 ^A	109.2 ^B	88.4 ^C		2.3 ^A	1.6 ^B	1.2 ^C	
Genotypes								
HA 429	118.6±3.4 ^{de}	107.3±1.0 ^e	77.7±1.4 ^g	101.2 ^D	1.7±0.1 ^d	1.3±0.1 ^{de}	1.0±0.1 ^{ef}	1.3 ^C
HA 430	112.3±4.1 ^{de}	87.3±2.2 ^{ef}	52.1±1.5 ^h	83.9 ^E	1.8±0.1 ^d	0.9±0.1 ^{ef}	0.6±0.1 ^f	1.1 ^D
HA 20	123.8±2.7 ^d	86.0±2.6 ^{ef}	69.0±2.0 ^g	92.9 ^D	2.0±0.1 ^{cd}	1.6±0.1 ^d	0.9±0.1 ^{ef}	1.5 ^C
H(A9xRf6)	170.7±3.5 ^b	130.8±3.4 ^d	108.7±4.5 ^{de}	136.7 ^B	3.2±0.1 ^a	2.5±0.2 ^b	1.7±0.1 ^d	2.5 ^A
H(A9xRf8)	155.3±2.4 ^c	124.8±3.1 ^d	93.7±4.5 ^{ef}	124.6 ^C	2.9±0.1 ^a	2.3±0.1 ^{bc}	2.0±0.1 ^{cd}	2.4 ^{AB}
Sakha53	194.2±5.9 ^a	138.7±7.7 ^d	110.7±2.8 ^e	147.9 ^A	3.1±0.1 ^a	2.3±0.1 ^{bc}	1.6±0.1 ^d	2.3 ^B
Avg.	145.8 ^A	112.5 ^B	85.3 ^C		2.5 ^A	1.8 ^B	1.3 ^C	

-I1, I2 and I3= 5, 4 and 3 irrigations, respectively. S0, S1 and S2= 1514, 2400 and 32600ppm NaCl, respectively.

-Means (±SE) in columns and row (interaction) for each treatment followed by the same lowercase letter(s) are not significantly different at the 5% probability level, n= 3.

-Means in each column (main effect of genotype) or row (main effect of drought or salinity levels) for each treatment followed by the same uppercase letter(s) are not significantly different at the 5% probability level.

After complete maturity of seeds, yield parameters were determined. Yield parameters were significantly declined with the decrease in number of irrigations and with the elevation of salinity level as shown in Tables 2 and 3. Under reduced irrigation I2 and I3, the hybrid H(A9xRf6) showed the highest head diameter values whereas, Sakha53 showed higher head diameter under moderate reduced irrigation I2. Moreover, H (A9xRf6) showed highest seed yield per plant and highest 100 seeds wt. under the three irrigation levels whereas, HA 430 recorded the lowest yield per plant and the lowest 100 seeds wt. On the other hand, the hybrids H (A9xRf6) and H (A9xRf8) maintained the highest seed yield per fad and the highest oil yield per fad under the three irrigation levels while the inbred line HA 429 exhibited the lowest seed yield per fad under normal irrigation and I2. Also, the inbred line HA 430 had the lowest seed yield under I3 and the lowest oil yield per fad the three regimes of irrigation. Under salinity, the highest head diameter and seed yield per plant showed in H (A9xRf8) and the highest weight of 100 seeds was found in H (A9xRf6) under both salinity levels and in H (A9xRf8) under moderate salinity. Whereas, HA 429 and HA 430 exhibited the lowest head diameter, the lowest seed yield per plant and 100 seeds wt. Similarly, the highest seed yield per fad was observed in H (A9xRf6) and the highest oil yield per fad was found in H (A9xRf6) and H (A9xRf8). However, HA 430 displayed the lowest seed yield per fad while HA 430 and HA 20 showed the lowest oil yield under salinity.

To determine stress tolerance in the investigated genotypes, the percentages of decline in seed yield and oil yield under treatments relative to control yields were calculated. Table 4 indicates that the moderate water stress reduced the seed yield by 27.4-43.5% and reduced the oil yield by 34.4-48.5% being the lowest reduction in seed and oil yield in the hybrid H (A9XRF8). Whereas, the severe water stress reduced the seed yield by 52.1-68.4% and declined the oil yield by 61-79.8% being the lowest reduction in Sakha53. On other hand, the moderate salinity lowered the seed yield by 26-64.6% and reduced the oil yield by 31.6-54.2% being the lowest reduction in the hybrids H (A9XRF8) and H (A9XRF6). However, the high salinity diminished the seed yield by 43.5-79.2% and reduced the oil yield by 56.9-71.5%. The lowest reduction by high

salinity accounted for HA 429, H (A9XRF8) and H (A9XRF6). The inbred lines HA 430 and HA20 showed lowest reduction in seed yield and oil yield than HA 429 under moderate water stress (Table 4) whereas HA 429 showed lowest reduction under moderate salinity. However, HA 429 showed lowest reduction in seed yield and oil yield than the other two inbred lines under the more reduced water I3 and the higher level of salinity S2.

Effect of different water regimes and salinity levels on biochemical characteristics

The present results showed that all the genotype had no effect on oil content under control condition except HA 430 that differed significantly compared with HA 429 and HA 20 (Fig. 1 A). Moreover, the results revealed progressive reduction in seed oil content in all genotypes with reducing irrigation number resulted in and elevation of salinity level (Fig. 1 B). The reduction in oil content due to water stress was significant for all genotypes except H (A9xRF6) that did not encounter a significant decrease in oil % under I2 and exhibited highest oil content under irrigation regime, I3 while the lowest oil content was found in HA 430. However, there was no effect of the genotype variable on oil content under salinity levels (Fig. 1 B). The protein content of sunflower seeds significantly decreased with reduced irrigation and higher salinity in all genotypes (Fig. 1 C and 1 D). Sakha53 showed highest protein content under the three irrigation regimes and the lowest protein content was found in HA 430 and HA 20 due to I3. While, the highest protein content under salinity was in HA 20 and Sakha53 and the lowest was found in HA 430 and H (A9xRF6) under S2.

Soluble sugars content of seed was accumulated with the reduction in the irrigation number as well as the elevation in the salinity level in all the genotypes (Fig. 2 A and 2 B). The highest sugar accumulation under I2 was recorded in HA 20 genotype and under I3 in HA 20 and Sakha53 (Fig. 2 A). The high salinity induced the highest sugar accumulation that was found in H (A9xRF6) and H (A9xRF8) (Fig. 2 B). Proline content was determined in mature seeds (Fig. 2 C and D). The results showed that, proline accumulated with the reduction in irrigation number in all genotypes. The highest proline accumulation under moderate drought

was recorded in Sakha53 and under severe drought in H (A9xRF8) and Sakha53 while the lowest value of proline accumulation was found in H (A9xRF6) under I3 (Fig. 2 C). However, proline accumulated greatly under the moderate salinity S1 in all genotypes then decreased slightly by S2 (Fig. 2 D). The highest proline content was found in H (A9xRF8) and Sakha53 whereas the lowest proline accumulation was found in HA 430.

Effect of different water regimes and salinity levels on fatty acids composition

As shown in Table 5, lipids extracted from sunflower seeds are dominated by C16, C18, C18:1 and C18:2 fatty acids. Analysis of fatty acid composition indicated that, oleic acid (C18:1) was the major component under control condition [49.5, 87.4, 85, 76.5, 59.3 and 54% of total fatty acids (TFAs) in HA 429, HA 430, HA 20, H (A9xRF6), H (A9xRF8) and Sakha53, respectively followed by linoleic acid (C18:2) that constituted about 40.6, 1.6, 6.1, 13.8, 31.6 and 37.2% of TFAs, in HA 429, HA 430, HA 20, H (A9xRF6), H (A9xRF8) and Sakha53, respectively then palmitic (C16) and stearic (C18) acids which represented by less than 10% of TFAs in all genotypes. Sunflower oil was characterized by the presence of a high proportion of unsaturated fatty acids than the saturated FAs (Table 5 and Fig. 3 A and B). Saturated fatty acids (SFA) represented the average of 7%-9.3% of TFAs in the well-irrigated plants (I1). As shown in Table 5, the fatty acid composition of sunflower seeds was modified by irrigation and salinity levels (Fig. 3 A and B). It was noticed that, some FAs concentration (mg/100g oil) showed decrease (Table 5) whereas their constitution % of the TFAs showed increase which points to the reduction in the TFAs and while that the quality of the oil is determined by its composition of FAs, we compared the % of the constituents of sunflower's oil and to define the change in FAs constitution % in the treated samples relative to the control, the values of the treated samples were divided by the value of control as fold change (FC) represented by coloured scale as shown in Fig. 3 C and D. $FC \leq 2$ or ≥ 2 was considered a significant change. The major four fatty acids in sunflower oil were modified by reduced irrigation, palmitic acid (C16) % of TFAs which increased by 1.7 FC in HA 20 under irrigation regime, I2. Stearic acid % (C18) diminished -1.8FC in H (A9xRF8) by

I2 and decreased by I3 to -1.7 folds in HA 430 and -2.6 folds in HA 429. Oleic acid % (C18:1) increased in HA 429 1.3 fold by I2 and 1.7FC increase by I3. Controversy, linoleic acid % was dependent on the genotype and irrigation regime as it decreased in HA 429 under I2 and I3 regimes by -1.6 and -13.4FC, respectively. However, an increase in linoleic acid % appeared in HA 430 under I2 and in HA 20 under I3. Consequently, the oleic acid % to linoleic acid % ratio (O/L) increased only in HA 429 by I2 and I3. In addition, O/L ratio diminished by the reduced irrigation I2 and I3 in HA 430 and in HA 20. Mono-unsaturated FAs (MFA) increased in HA 429 by both levels of the reduced irrigation, this increase was concomitant with oleic acid %. Poly unsaturated fatty acids (PFA) showed an increase in HA 430 and HA 20 by reduced irrigation, this increase was concomitant with the increase in linoleic acid (C16) and palmitoleic (C16:1) % (Table 5). In contrast, SFA % showed obvious change only in HA 20 as increased 1.5FC by I2.

As indicated in Table 5, analysis of the fatty acid composition of control plants of the salinity experiment (S0) was very similar to that of control plants of reduced irrigation experiment (I1). Fatty acid composition of sunflower seeds was modified by salinity level. It was observed that palmitic acid % (C16) increased by salinity in all genotypes except H (A9xRF6) under S1 and HA 429 under S2. Stearic acid % (C18) was increased in HA 429, HA 20 and Sakha53 by both salinity levels while it decreased in HA 430 and H (A9xRF8) by both levels of salinity. Oleic acid (C18:1) % showed a slight reduction by salinity in all the genotypes except in HA 430 where it greatly reduced under S2. In contrast, linoleic acid % showed obvious elevation by S1 in HA 430 and H (A9xRF6) and by S2 in HA 430 while reduced by S1 only in HA 20. Consequently, the O/L increased only in HA 20 and did not change in Sakha53 while it decreased in the remaining genotypes (Fig. 3 D). MFA decreased by both salinity levels in all genotypes except in HA 20 where it did not change. PFA did not change in HA 20 and Sakha 53 by salinity however it increased in the remaining genotypes by both salinity levels. In contrast, saturated fatty acids % (SFA) was increased by salinity in most genotypes and slightly decreased due to S1 in HA 429 and due to S2 in HA 430 and H (A9xRF6).

TABLE 2. Effects of irrigation regimes (I1, I2 and I3) and salinity levels (S1 and S2) in addition to their control (S0) on some yield parameters of six sunflower genotypes.

Genotypes	Head diameter (cm)				Seed yield plant ⁻¹ (gm)				100 seeds wt. (gm)			
	I1	I2	I3	Avg.	I1	I2	I3	Avg.	I1	I2	I3	Avg.
HA 429	12.3±0.6 ^d	8.8±0.4 ^e	6.8±0.4 ^{fg}	9.3 ^C	17.8±2.3 ^{de}	12.4±0.5 ^e	9.8±0.5 ^{ef}	13.3 ^D	5.1±0.2 ^{bc}	4.3±0.1 ^{cd}	3.2±0.2 ^{de}	4.2 ^D
HA 430	14.7±0.4 ^e	8.9±0.3 ^e	5.7±0.1 ^g	9.8 ^C	16.9±1.0 ^e	9.6±1.1 ^{ef}	6.0±0.3 ^f	10.8 ^D	5.0±0.3 ^{bc}	4.3±0.2 ^{cd}	2.7±0.1 ^e	4.0 ^D
HA 20	14.2±0.3 ^{cd}	10.3±0.3 ^d	8.2±0.2 ^f	10.9 ^C	30.5±0.7 ^{cd}	17.0±0.9 ^{de}	12.5±0.4 ^e	20.0 ^C	5.5±0.2 ^b	4.5±0.1 ^{cd}	3.1±0.4 ^{de}	4.4 ^{C^D}
H(A9xRf6)	18.8±0.6 ^a	15.4±0.6 ^{bc}	11.8±0.4 ^d	15.4 ^A	58.0±1.5 ^a	46.7±1.7 ^b	35.6±2.4 ^c	46.8 ^A	7.8±0.2 ^a	6.2±0.1 ^b	5.1±0.1 ^{bc}	6.4 ^A
H(A9xRf8)	17.2±0.4 ^{ab}	11.5±0.4 ^d	10.5±0.4 ^d	13.0 ^B	54.7±4.3 ^{ab}	38.5±3.0 ^{bc}	22.2±1.3 ^d	38.5 ^B	6.9±0.2 ^{ab}	6.2±0.2 ^b	4.3±0.4 ^{cd}	5.8 ^B
Sakha53	18.2±0.2 ^a	14.5±0.8 ^{cd}	10.8±0.6 ^e	14.5 ^A	50.5±2.7 ^{ab}	34.9±1.5 ^{cd}	28.1±1.7 ^d	37.9 ^B	6.0±0.3 ^b	4.8±0.2 ^c	3.5±0.3 ^{de}	4.7 ^C
Avg.	15.9 ^A	11.6 ^B	9.0 ^C		38.1 ^A	26.5 ^B	19.0 ^C		6.1 ^A	5.0 ^B	3.7 ^C	
Genotypes	S0	S1	S2	Avg.	S0	S1	S2	Avg.	S0	S1	S2	Avg.
HA 429	12.4±0.4 ^e	10.7±0.3 ^{fg}	8.7±0.1 ^{gi}	10.6 ^D	17.8±0.8 ^{fg}	13.5±1.0 ^{gh}	11.5±0.6 ^h	14.3 ^D	4.7±0.1 ^{de}	4.1±0.1 ^e	3.4±0.1 ^{fg}	4.0 ^C
HA 430	13.1±0.5 ^{de}	8.8±0.2 ^{gi}	5.5±0.4 ^j	9.1 ^E	22.6±1.3 ^{ef}	17.4±0.8 ^{fg}	10.7±0.9 ^h	16.9 ^C	4.1±0.1 ^e	2.8±0.1 ^g	2.1±0.1 ^h	3.0 ^D
HA 20	14.1±0.2 ^{cd}	10.4±0.2 ^{fg}	7.5±0.5 ⁱ	10.7 ^D	25.5±1.1 ^{de}	17.8±0.7 ^{fg}	12.4±0.4 ^h	18.6 ^C	5.1±0.3 ^{cd}	3.8±0.1 ^{ef}	2.8±0.2 ^{gh}	3.9 ^C
H(A9xRf6)	18.2±0.6 ^b	14.4±0.6 ^{cd}	11.9±0.4 ^{ef}	14.9 ^B	45.5±1.2 ^a	30.5±1.0 ^d	17.3±0.4 ^{fg}	31.1 ^B	7.1±0.1 ^a	5.5±0.2 ^{bc}	4.4±0.2 ^d	5.7 ^A
H(A9xRf8)	20.4±0.4 ^a	15.4±0.4 ^c	13.0±0.3 ^{de}	16.3 ^A	48.2±1.7 ^a	36.8±1.9 ^c	26.4±1.4 ^{de}	37.2 ^A	6.5±0.2 ^{ab}	5.6±0.3 ^{bc}	3.9±0.2 ^{ef}	5.3 ^B
Sakha53	15.8±0.6 ^c	13.4±0.2 ^d	9.8±0.4 ^{gh}	13.0 ^C	39.3±1.2 ^{bc}	30.5±2.5 ^d	21.1±1.5 ^f	30.3 ^B	7.4±0.4 ^a	5.0±0.2 ^{cd}	4.3±0.1 ^{de}	5.6 ^A
Avg.	15.7 ^A	12.2 ^B	9.4 ^C		33.2 ^A	24.4 ^B	16.6 ^C		5.8 ^A	4.5 ^B	3.5 ^C	

-I1, I2 and I3= 5, 4 and 3 irrigations, respectively. S0, S1 and S2= 1514, 2400 and 32600ppm NaCl, respectively.

-Means (±SE) in columns and row (interaction) for each treatment followed by the same lowercase letter(s) are not significantly different at the 5 % probability level, n= 3.

-Means in each column (main effect of genotype) or row (main effect of drought or salinity levels) for each treatment followed by the same uppercase letter(s) are not significantly different at the 5 % probability level.

TABLE 3. Effects of irrigation regimes (I1, I2 and I3) and salinity levels (S1 and S2) in addition to their control (S₀) on seed yield and oil content of six sunflower genotypes.

Genotypes \ Trait	Seed yield (kg ha ⁻¹)				Oil content (kg ha ⁻¹)			
	I1	I2	I3	Avg.	I1	I2	I3	Avg.
HA 429	398.0±41.6 ^{de}	225.0±11.4 ^c	190.7±8.4 ^c	271.2 ^D	161.2±3.8 ^f	83.0±3.1 ^g	58.4±3.2 ^h	100.9 ^E
HA 430	409.7±11.7 ^d	284.7±14.3 ^{de}	129.3±12.4 ^f	274.6 ^D	169.1±5.8 ^f	89.8±4.8 ^g	34.1±3.0 ^h	97.7 ^E
HA 20	693.7±34.6 ^c	481.0±21.2 ^d	274.3±21.4 ^{d-f}	483.0 ^C	169.1±13.6 ^f	89.8±12.0 ^g	34.1±7.5 ^h	187.9 ^D
H(A9xRf6)	1739.0±32.0 ^a	1080.0±51.4 ^{bc}	782.7±58.8 ^c	1200.6 ^A	751.7±12.8 ^a	437.8±17.5 ^c	293.4±27.4 ^{de}	494.3 ^A
H(A9xRf8)	1610.7±111.5 ^a	1169.7±62.1 ^b	747.0±61.8 ^c	1175.8 ^A	675.3±45.6 ^{ab}	442.8±27.9 ^c	245.8±17.4 ^{ef}	454.6 ^B
Sakha53	1297.3±32.0 ^b	912.7±35.6 ^c	620.3±14.0 ^{cd}	943.4 ^B	559.5±36.3 ^b	356.1±16.3 ^{cd}	214.8±2.8 ^{ef}	376.8 ^C
Avg.	1024.779.7 ^A	692.2 ^B	457.4 ^C		433.2 ^A	266.1 ^B	156.8 ^C	
Genotypes	S0	S1	S2	Avg.	S0	S1	S2	Avg.
HA 429	469.3±42.4 ^h	317.7±15.7 ^{hi}	265.3±6.1 ^{hi}	350.8 ^D	205.0±19.7 ^{fg}	115.3±7.5 ^{ij}	87.0±3.2 ^{jk}	135.7 ^C
HA 430	342.3±21.5 ^h	189.7±12.1 ^{ij}	129.0±12.3 ^j	220.3 ^F	149.4±8.7 ^{ei}	68.4±4.6 ^{jk}	42.6±4.1 ^k	86.8 ^D
HA 20	653.3±28.8 ^g	231.3±27.2 ^{h-j}	136.0±4.9 ^j	340.2 ^E	153.9±11.4 ^{gi}	79.2±5.8 ^{jk}	44.5±2.4 ^k	92.5 ^D
H(A9xRf6)	1621.7±55.7 ^a	1200.3±27.8 ^{cd}	856.7±49.9 ^c	1226.2 ^A	679.9±22.6 ^a	447.3±23.6 ^c	292.8±13.7 ^{de}	473.3 ^A
H(A9xRf8)	1487.3±57.8 ^{ab}	1072.0±20.5 ^d	734.7±22.0 ^{ef}	1098.0 ^B	624.2±24.2 ^{ab}	427.1±14.3 ^c	268.7±1.7 ^{d-f}	440.0 ^A
Sakha53	1328.3±69.6 ^{bc}	862.7±40.3 ^e	636.3±30.0 ^{fg}	942.4 ^C	568.6±34.6 ^b	324.6±18.3 ^d	223.8±13.0 ^{e-g}	372.3 ^B
Avg.	933.7 ^A	645.6 ^B	459.7 ^C		396.8 ^A	243.6 ^B	159.9 ^C	

-I1, I2 and I3= 5, 4 and 3 irrigations, respectively. S0, S1 and S2= 1514, 2400 and 32600ppm NaCl, respectively.

-Means (±SE) in columns and row (interaction) for each treatment followed by the same lowercase letter(s) are not significantly different at the 5 % probability level, n= 3.

-Means in each column (main effect of genotype) or row (main effect of drought or salinity levels) for each treatment followed by the same uppercase letter(s) are not significantly different at the 5 % probability level.

TABLE 4. Percentages of reduction in the seed yield per fad and the oil yield per fad in six sunflower genotypes as affected by irrigation regimes (I2 and I3) and salinity levels (S1 and S2), compared to control values (I1 and S0).

Genotypes \ Trait	Reduction in seed yield (%)		Reduction in oil yield (%)	
	I2	I3	I2	I3
HA 429	43.5	52.1	48.5	63.8
HA 430	30.5	68.4	46.9	79.8
HA 20	30.7	60.5	46.9	79.8
H(A9xRf6)	37.9	55.0	41.8	61.0
H(A9xRf8)	27.4	53.6	34.4	63.6
Sakha53	29.6	52.2	36.4	61.6
Genotypes	S1	S2	S1	S2
HA 429	32.3	43.5	43.8	57.6
HA 430	44.6	62.3	54.2	71.5
HA 20	64.6	79.2	48.5	71.1
H(A9xRf6)	26.0	47.2	34.2	56.9
H(A9xRf8)	27.9	50.6	31.6	57.0
Sakha53	35.1	52.1	42.9	60.6

-I2 and I3= 4 and 3 irrigations, respectively, S1 and S2= 2400 and 32600ppm NaCl, respectively.

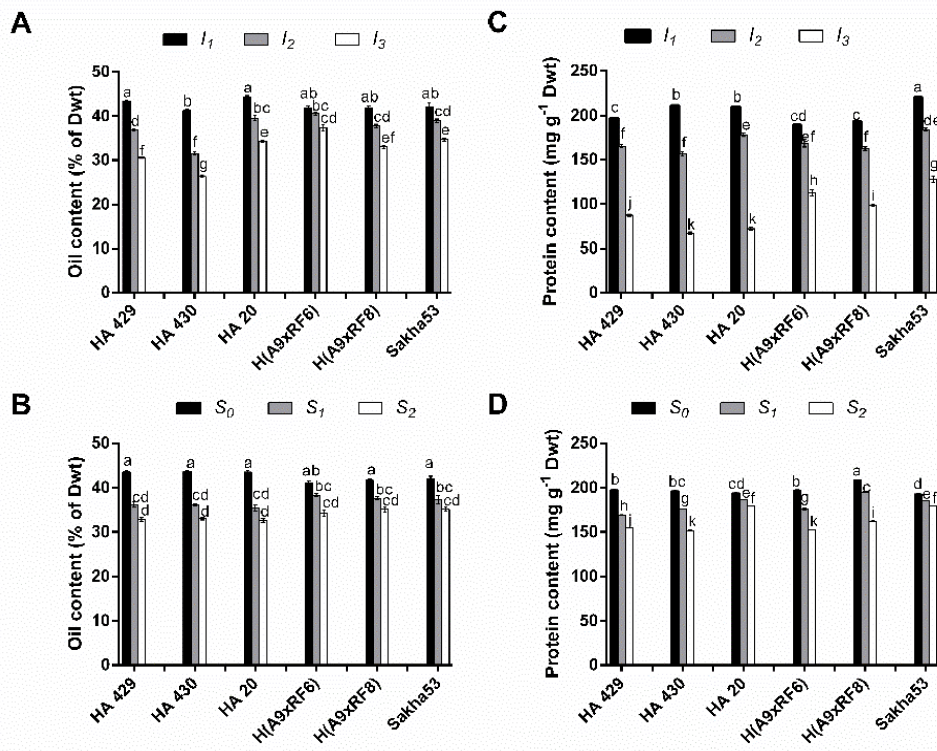


Fig. 1. Effect of irrigation regimes (I1, I2 and I3) and salinity levels (S0, S1 and S2) on seed oil content; A and B, and protein content; C and D of six sunflower genotypes [Data are means of 3 replicates± SE. Bars labelled with different letters are significantly different at P≤ 0.05. I1, I2 and I3= 5, 4 and 3 irrigations respectively. S0, S1 and S2= 1514 (control), 2400 and 32600ppm NaCl, respectively].

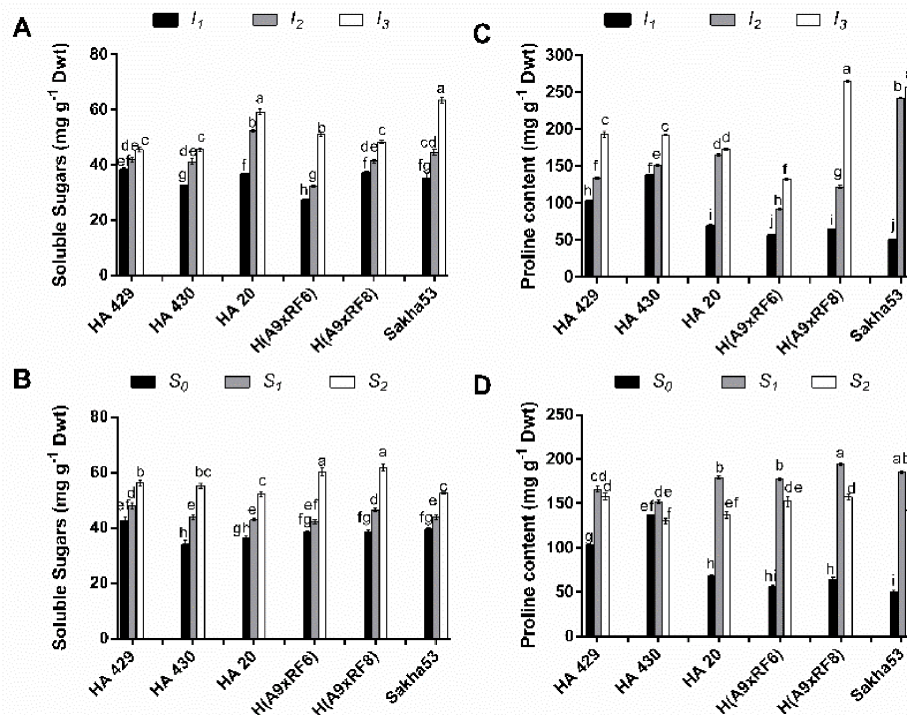


Fig. 2. Effect of irrigation regimes (I1, I2 and I3) and salinity levels (S0, S1 and S2) on seed soluble sugars; A and B, and proline content; C and D of six sunflower genotypes [Data are means of 3 replicates± SE. Bars labelled with different letters are significantly different at P≤ 0.05. I1, I2 and I3= 5, 4 and 3 irrigations respectively. S0, S1 and S2= 1514 (control), 2400 and 32600ppm NaCl, respectively].

TABLE 5. Means of fatty acids % of six sunflower genotypes as affected by irrigation regimes (I1, I2 and I3) and salinity levels (S1 and S2) in addition to their control (S0).

Genotypes	Irrigation regimes and salinity levels										
	C8:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C22:1	C22:2
I₁											
HA 429	Nd	Nd	4.5	0.1	4.2	49.5	40.6	0.4	0.2	Nd	0.5
HA 430	Nd	Nd	4.4	0.3	5.1	87.4	1.6	0.5	0.2	Nd	0.5
HA 20	Nd	Nd	4.1	0.1	3.5	85.0	6.1	0.4	0.2	Nd	0.7
H(A9xRf6)	Nd	Nd	3.8	0.2	4.5	76.5	13.8	0.5	0.2	Nd	0.5
H(A9xRf8)	Nd	Nd	5.0	0.2	3.1	59.3	31.6	0.2	0.1	Nd	0.4
Sakha53	Nd	Nd	4.9	0.1	2.8	54.0	37.2	0.3	0.2	Nd	0.5
I₂											
HA 429	Nd	Nd	5.5	Nd	4.0	65.0	24.2	0.3	0.1	Nd	1.0
HA 430	Nd	Nd	4.5	0.4	4.4	85.4	4.1	0.6	nd	Nd	0.5
HA 20	0.6	0.1	6.6	0.2	3.8	77.6	9.5	0.4	0.2	0.4	0.6
H(A9xRf6)	Nd	Nd	4.5	0.1	4.6	76.1	13.7	0.4	0.2	Nd	0.4
H(A9xRf8)	Nd	Nd	4.0	0.5	1.7	58.8	34.6	Nd	Nd	Nd	0.4
Sakha53	Nd	Nd	5.9	0.2	2.7	45.4	45.3	0.2	0.2	Nd	0.1
I₃											
HA 429	Nd	Nd	5.3	0.3	1.6	87.6	3.0	1.7	0.3	Nd	0.3
HA 430	Nd	Nd	6.2	0.9	2.9	80.7	8.9	nd	nd	Nd	0.4
HA 20	Nd	Nd	4.5	0.2	2.9	73.0	18.3	0.3	0.2	Nd	0.6
H(A9xRf6)	Nd	Nd	4.0	0.3	3.1	72.5	19.1	0.3	0.2	Nd	0.5
H(A9xRf8)	Nd	Nd	5.2	-	3.6	63.5	25.9	-	-	Nd	1.8
Sakha53	Nd	Nd	5.6	0.2	2.1	54.6	36.8	0.2	0.2	Nd	0.4
S₀											
HA 429	Nd	Nd	6.4	0.3	1.9	49.8	40.9	0.2	0.2	Nd	0.3
HA 430	Nd	Nd	5.1	0.3	3.5	86.4	3.5	0.5	0.3	Nd	0.4
HA 20	Nd	Nd	3.3	0.1	2.9	86.8	6.2	0.3	0.2	Nd	0.4
H(A9xRf6)	Nd	Nd	3.6	0.1	1.8	73.8	20.1	0.2	0.1	Nd	0.2
H(A9xRf8)	Nd	Nd	5.0	0.2	3.1	59.3	31.6	0.2	0.1	Nd	0.4
Sakha53	Nd	Nd	6.1	0.2	2.6	52.9	37.4	0.2	0.1	Nd	0.5
S₁											
HA 429	0.6	0.3	15.0	nd	4.1	42.5	36.5	0.3	0.3	Nd	0.4
HA 430	0.2	Nd	11.2	0.2	1.8	53.4	32.1	0.3	0.3	0.3	0.4
HA 20	Nd	Nd	4.1	0.1	3.8	86.8	4.0	0.4	0.2	Nd	0.6
H(A9xRf6)	Nd	Nd	3.2	1.3	0.9	55.7	38.8	Nd	Nd	Nd	0.2
H(A9xRf8)	0.1	Nd	5.2	0.4	2.9	57.7	33.0	0.3	0.1	Nd	0.4
Sakha53	1.0	0.3	9.3	0.3	3.1	50.7	33.9	0.2	0.1	0.7	0.4
S₂											
HA 429	Nd	Nd	6.2	0.1	3.3	34.6	54.8	0.4	0.2	Nd	0.4
HA 430	Nd	Nd	8.1	0.2	2.9	42.0	45.8	0.3	0.2	Nd	0.6
HA 20	0.2	0.1	5.0	0.4	3.4	84.0	5.7	0.4	0.2	0.1	0.6
H(A9xRf6)	2.1	0.3	8.1	0.6	2.9	53.6	31.2	0.3	0.2	0.5	0.3
H(A9xRf8)	Nd	Nd	5.2	0.6	2.5	44.8	47.0	Nd	Nd	Nd	Nd
Sakha53	1.3	0.4	12.1	0.4	3.5	46.4	34.3	0.2	0.1	0.9	0.3

-I1, I2 and I3 = 5, 4 and 3 irrigations respectively. S0, S1 and S2 = 1514, 2400 and 32600 ppm NaCl, respectively

-Nd: Not detected.

-Caprylic acid (C8:0), Myristic acid (C14:0), Palmitic (C16:0), Palmitoleic acid (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3), Arachidic (C20:0), Erucic (C22:1) and Eicosapentaenoic (C22:2) acids.

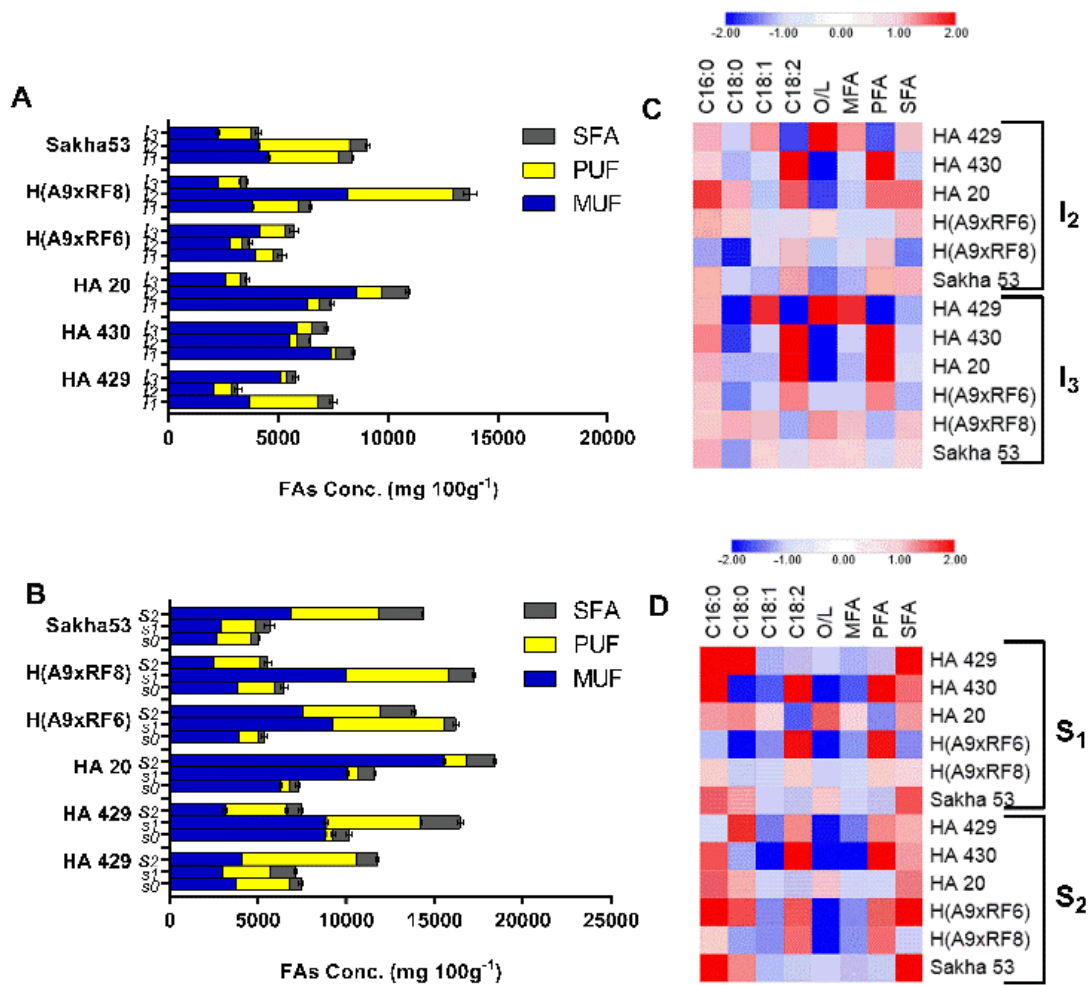


Fig. 3. Effect of irrigation regimes (I₁, I₂ and I₃) and salinity levels (S₀, S₁ and S₂) on changes of fatty acids in six sunflower genotypes. A and B: Effect of irrigation and salinity on fatty acid concentration in six sunflower genotypes (mg 100g⁻¹ oil). [Data are means of 3 replicates ± SE. MFA: Mono-unsaturated fatty acids (C16:1, C18:1, C22:1), PFA: Poly-unsaturated fatty acids (C18:2, C18:3, C22:2) and SFA: Saturated fatty acids (C8:0, C14:0, C16:0, C18:0, C20:0)]. C and D: Heat map of relative major fatty acids content (%) as affected by reduced irrigation I₂ and I₃ relative to control I₁ and by salinity levels S₁ and S₂ relative to S₀. Data are expressed as fold change (FC). Blue colour indicates decrease in FAs constitution relative to the control and red colour indicates an increase in FAs constitution relative to control. Significant increase= FC ≤ -2 and significant decrease= FC ≥ 2. C16:0; palmitic, C18:0; stearic, C18:1; oleic, C18:2; linoleic, O/L; oleic/linoleic].

Finally, reduced irrigation and soil salinity did not significantly affect the other investigated minor FAs that did not detected by the gas chromatography in all analysed samples or that detected and constituted less than 1.0 % of TFAs. Otherwise, the short chains fatty acid caprylic acid (C8:0) that constituted about 1.0 % and 1.3 % of TFAs in Sakha53 under S₁ and S₂, respectively and constituted about 2.1% in H(A9xRF6) under S₂.

Discussion

Sunflower (*Helianthus annuus* L.) is an important oilseed crop in so many countries in which seed and oil yield decrease by limited water or soil salinity. While some responses to water and salt stresses are common, other responses may vary according to the genotype and/or stress level. To test this hypothesis and compare responses this study was carried out to examine the effect of different levels of irrigation and salinity on seed

and oil yields and the biochemical composition of sunflower seeds.

It was indicated from the results that the growth and the yield parameters were significantly affected by genotypes, stress levels and their interactions. Growth (plant height and stem diameter) and yield components (head diameter, seed yield per plant and 100 seeds wt.) as well as seed and oil yields per fed were adversely affected by reducing irrigation (I2 and I3) and salinity stress (S1 and S2). Yield reduction in sunflower by drought and salinity stress was observed also by Hassan et al. (2011) and Farghaly et al. (2016). Our results revealed that the genotypes which showed the better growth under stress levels showed better yield and vis-versa. H (A9xRf6) and H (A9xRf8) showed the highest seed yield and oil yield per fad in both experiments under stress conditions I2, I3, S1 and S2 and they showed the highest growth and yield component parameters under the same conditions. Similarly, the lower yield per fad and the lower oil yield per fad were recorded in the inbred lines HA 429 and HA 430 under drought and in HA 430 and HA 20 under salinity which showed reduced growth and low yield per plant under salinity treatment. These results suggest that, the reduced yield by limited irrigation or soil salinity was attributed to decrease in 100 seed weight and head diameter which were basically due to reduced growth (Flagella et al., 2004). The strong correlation between growth and yield was reported earlier by Hammad et al. (2002) and Rafiei et al. (2013) who referred the reduction in the yield to the reduction in the photosynthesis and assimilates available for grain filling.

The present findings revealed changes in the level of the primary seed reserves, oil, proteins, soluble sugars and proline in addition to variation of the fatty acid composition within the six studied genotypes and in response to levels of the treatment. This suggested that the variation of the genotype contributed to metabolic variation amongst seeds. Oil content of seeds progressively reduced by progressive reduction in irrigation I2 and I3 in all genotypes except H (A9xRF6) that showed a significant reduction in oil content only under I3, compared to well-irrigated I1. The most affected genotypes by reduced irrigation were the pure lines HA 429 and HA 430. On other hand, there was no effect of genotype variable on oil content under soil salinity. These results

enforce the suggestion that the reduction in oil yield was due to reduction in oil content which attributed to the reduced irrigation. The reduction in sunflower's oil content by water stress has also been reported (Iqbal et al., 2005 and Hassan et al., 2011) and by salinity (Di Caterina et al., 2007). The reduction in oil content could be due to the inhibition of lipid biosynthesis and/or induction of the activities of lipolytic and peroxidative enzymes (Gigon et al., 2004). The inhibition of lipid biosynthesis was validated earlier under water and salinity stress (Ali et al., 2009 and Farghaly et al., 2016). The hybrids H (A9xRF6) and H (A9xRF8) showed less reduction in oil content by both irrigation and salinity treatments and this candidate them for cultivation in stressed regions.

Similarly, protein content of seeds markedly decreased by progressive deficit irrigation and elevated salinity level in all genotypes. Although, the reduced irrigation particularly I3 resulted in bigger drop in protein content rather than salinity. This may be attributed to protein degradation caused by proteolytic activities or reduction in protein synthesis under water stress than salinity stress. Reduction in sunflower seed protein in response to reduced irrigation was also reported previously by Hassan et al. (2011). Sakha53 was the least affected genotype in terms of seed protein content under reduced irrigation and salinity which enforce the assumption of the role of genotype variable in seed quality under stress.

Controversy, the results revealed high accumulation of sugars and proline in all genotypes by drought and salinity that could be due to their utilization as a source of energy or to sustain metabolism (Osório et al., 1998 and Khalid, 2006). The level of proline accumulation was found be dependent on the species and the plant organs (Verbruggen & Hermans, 2008) moreover, the genotypic differences in the proline concentration have been previously reported in sunflower (Canavar et al., 2014 and Oraki et al., 2012). The highly accumulated proline genotypes were considered to be more tolerant to abiotic stress by several authors as (Abbas et al., 2014 and Vendruscolo et al., 2007). Proline accumulation in response to stress could be utilized in biosynthesis of proline-rich proteins. These proteins have specific properties and specific functions under stress conditions (Roshandel & Flowers, 2009). Differences in proline accumulation between the

seeds of different genotypes were investigated. The results showed higher proline contents in inbred lines HA 429 and HA 430 than the hybrid H (A9xRF6) and this probably linked to tolerance mechanism. This contradicts with findings of Khalid (2006) found higher proline content and higher capacity to accumulate proline in response to osmotic stress in hybrids than inbred lines. HA 429 and HA 430 had been released as salt tolerant parental oilseed maintainer lines (Jan & Seiler, 2007).

Plants synthesize fatty acids and store them as triacylglycerols (TAG) in seeds to be utilized during seed germination (Zhao et al., 2018). In this study; reduced irrigation and soil salinity not only reduced seeds oil content but also modified the oil composition of FAs. The modification of the FAs composition by the environmental conditions was also reported by Heuer et al. (2005), Ali et al. (2009) and Echarte et al. (2010). Palmitic acid in most of the genotypes increased by both water and salinity stress, however palmitic acid in H (A9xRF8) unchanged by salinity. The increase in palmitic acid in sunflower by water stress was also reported by (Flagella et al., 2002). However, stearic acid was dependent on the genotype and level of stress, as it increased in some genotypes and decreased or unchanged in some others according to stress level. It was reported earlier that stearic acid increased in sunflower by drought (Ali et al., 2009).

Oleic acid was found to be dependent on the genotype and the stress level in both experiments. Under sever reduced irrigation, only HA 429 showed an increase in oleic acid while all the remaining genotypes slightly decreased its constitution, however the main effect of salt stress on the oleic acid was a decrease by both salinity levels in all the genotypes and the most affected genotype was HA 430. Similarly, Kim et al. (2006) showed that drought stress increased the oleic acid content of 14 cultivars and that of 4 cultivars decreased. The negative effect of water stress on the oleic acid content has been reported in canola (Triboi-Blondel & Renard, 1999). Oleic and linoleic acids showed a reverse relationship under both the reduced irrigation and the soil salinity. These results totally agree with the results of Petcu et al. (2001) who reported an increase in the linoleic acid content and decrease in the oleic acid content of sunflower under water stress and with those of Baldini et al. (2002) who

reported an increase in oleic/linoleic acid ratio by water stress in sunflower.

MFA in most genotypes unchanged by the reduced-irrigation while it decreased by salinity. Whereas the PFA elevated by reduced irrigation as well as salinity levels in most genotypes. However, SFA unchanged in the most genotypes under limited irrigation while it was dependent on the stress level and the genotype under salinity in most of the genotypes. In accordance with our results, Farghaly et al. (2016) indicated that salinity also increased SFA and PFA while decreased MFA in sunflower.

Conclusion

In conclusion, this study has extended our knowledge on the interaction between genotype and stress levels and their effects on the biochemical composition of sunflower seeds. The results revealed that the long-term of withholding irrigation and the high level of soil salinity sharply dropped the seed yield and the oil yield in all the genotypes and altered the biochemical composition of the seeds and the genotype variable contributed to metabolic variation amongst seeds. Reduced irrigation decreased the seed's protein content sharply more than salinity and Sakha53 was the less affected genotype. The results also showed that some fatty acids particularly stearic acid, oleic acid was dependent on the genotype and the stress level in both experiments. Based on the reduction in the oil content and the oil yield, H (A9xRF8) had the best performance under the moderate levels of water stress and salinity and H (AxRF6) was the most tolerant genotype for the higher levels of withholding water and soil salinity. The parental line HA 429 showed lowest reduction in the seed yield reduction under both experimental conditions which candidates it for using in production of salt and drought tolerant breeding programmes. Finally, the controlled withholding irrigation regime (I2)/ the lower salinity level (S1) rather than long-term withholding of water after anthesis/ the higher soil salinity, with selection of a genotype may be used for production of specific fatty acids. So, further work is needed to investigate the role of the environmental stress levels on the FAs biosynthetic enzymes in different genotypes for better understanding the mechanism by which FAs accumulate in the seeds.

Acknowledgments: This work is part of O.S.H. PhD Thesis at Faculty of Science, Damietta University, Egypt. This research was supported by ESARS, at which the field experiments were done. Damietta University provided the analysis and research facilities.

References

- AOC and Firestone, D. (1994) Official Methods and Recommended Practices of the American Oil Chemists' Society. AOCS Press.
- Abbas, S., Ahmad, S., Sabir, S. and Shah, A. (2014) Detection of drought tolerant sugarcane genotypes (*Saccharum officinarum*) using lipid peroxidation, antioxidant activity, glycine-betaine and proline contents. *J. Soil Sci Plant Nutr.* **14**(1). Doi.org/10.4067/S0718-95162014005000019.
- Abdallah, A., Ahumada, M.H. and Gradziel, T.M. (1998) Oil content and fatty acid composition of almond kernels from different genotypes and California production regions. *J. Am. Soc. Hortic. Sci.* **123**, 1029-1033.
- Ahmad, S., Ahmad, R., Ashraf, M.Y., Ashraf, M. and Waraich, E.A. (2009) Sunflower (*Helianthus annuus* L.) response to drought stress at germination and seedling growth stages. *Pak. J. Bot.* **41**, 647-654.
- Aishwarya, S. and Anisha, V. (2014) Nutritional composition of sunflower seeds flour and nutritive value of products prepared by incorporating sunflower seeds flour. *Int. J. Pharm. Res. Allied Sci.* **3**, 45-49.
- Alberio, C., Izquierdo, N.G., Galella, T., Zuil, S., Reid, R., Zambelli, A. and Aguirrezábal, L.A. (2016) A new sunflower high oleic mutation confers stable oil grain fatty acid composition across environments. *Eur. J. Agron.* **73**, 25-33.
- Ali, Q., Ashraf, M. and Anwar, F. (2009) Physico-chemical attributes of seed oil from drought stressed sunflower (*Helianthus annuus* L.) plants. *Grasasy Aceites*, **60**, 475-481.
- Baldini, M., Vegetake, P. and Udine, U. (2002) Effects of different water availability on fatty acid composition of the oil in standard and high oleic sunflower hybrids. *Ital. J. Agron.* **6**, 119-126.
- Barrón, C.M. and De Mejía, G.E. (1998) Comparative study of enzymes related to proline metabolism in tepary bean (*Phaseolus acutifolius*) and common bean (*Phaseolus vulgaris*) under drought and irrigated conditions, and various urea concentrations. *Plant Foods Hum. Nutr.* **52**, 119-132.
- Bates, L.S., Waldren, R.P. and Teare, I.D. (1973) Rapid determination of free proline for water-stress studies. *Plant Soil*, **39**, 205-207.
- Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**, 911-917.
- Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248-254.
- Canavar, Ö., Götz, K.P., Ellmer, F., Chmielewski, F.M. and Kaynak, M.A. (2014) Determination of the relationship between water use efficiency, carbon isotope discrimination and proline in sunflower genotypes under drought stress. *Aust. J. Crop Sci.* **8**, 232-242.
- Ceccarelli, S. and Grando, S. (2007) Decentralized-participatory plant breeding: An example of demand driven research. *Euphytica*, **155**(3), 349-360.
- Di Caterina, R., Giuliani, M.M., Rotunno, T., De Caro, A. and Flagella, Z. (2007) Influence of salt stress on seed yield and oil quality of two sunflower hybrids. *Ann. Appl. Biol.* **151**, 145-154.
- Echarte, M.M., Angeloni, P., Jaimes, F., Tognetti, J., Izquierdo, N.G., Valentinuz, O. and Aguirrezábal, L.A. (2010) Night temperature and intercepted solar radiation additively contribute to oleic acid percentage in sunflower oil. *F Crop Res.* **119**, 27-35.
- FAO U (2014) FAOstat. Retrieved Feb 2014.
- Farghaly, F.A., Radi, A.A., Abdel-Wahab, D.A. and Hamada, A.M. (2016) Effect of salinity and sodicity stresses on physiological response and productivity in *Helianthus annuus*. *Acta Biol. Hung.* **67**, 184-194.
- Flagella, Z., Rotunno, T. and Tarantino, E., et al (2002) Changes in seed yield and oil fatty acid composition of high oleic sunflower (*Helianthus annuus* L.) hybrids in relation to the sowing date and the water

- regime. *Eur. J. Agron.* **17**, 221-230.
- Flagella, Z., Giuliani, M.M., Rotunno, T., Di Caterina, R. and De Caro, A. (2004) Effect of saline water on oil yield and quality of a high oleic sunflower (*Helianthus annuus* L.) hybrid. *Eur. J. Agron.* **21**, 267-272.
- Francois, L.E. (1996) Salinity effects on four sunflower hybrids. *Agron. J.* **88**, 215-219.
- Gigon, A., Matos, A.R., Laffray, D., Zuily-Fodil, Y. and Pham-Thi, A.T. (2004) Effect of drought stress on lipid metabolism in the leaves of *Arabidopsis thaliana* (Ecotype Columbia). *Ann. Bot.* **94**, 345-351.
- Hammad, M., Tahir, N., Imran, M. and Hussain, M.K. (2002) Evaluation of Sunflower (*Helianthus annuus* L.) Inbred Lines for Drought Tolerance. *Int. J. Agric. Biol.* **3**.
- Hasegawa, P.M., Bressan, R.A., Zhu, J.K. and Bohnert, H.J. (2000) Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Biol.* **51**, 463-499.
- Hassan, N.M., El-Sayed, A.K.A., Ebeid, H.T. and Nemat Alla, M.M. (2011) Molecular aspects in elevation of sunflower tolerance to drought by boron and calcium foliar sprays. *Acta Physiol. Plant.* **33**.
- Heuer, B., Ravina, I. and Davidov, S. (2005) Seed yield, oil content, and fatty acid composition of stock (*Matthiola incana*) under saline irrigation. *Aust. J. Agric. Res.* **56**, 45-47.
- Howell, T.A., Evett, S.R., Tolk, J.A., Copeland, K.S. and Marek, T.H. (2015) Evapotranspiration, water productivity and crop coefficients for irrigated sunflower in the U.S. Southern High Plains. *Agric. Water Manag.* **162**, 33-46.
- Iqbal, N., Ashraf, M.Y. and Ashraf, M. (2005) Influence of water stress and exogenous glycinebetaine on sunflower achene weight and oil percentage. *Int. J. Environ. Sci. Technol.* **2**, 155-160.
- Jan, C.C. and Seiler, G.J. (2007) Sunflower. Genet Resour Chromosom. *Eng. Crop Improv.* **4**, 103-165.
- Katerji, N., Van Hoorn, J.W., Hamdy, A. and Mastrorilli, M. (2003) Salinity effect on crop development and yield, analysis of salt tolerance according to several classification methods. *Agric. Water Manag.* **62**, 37-66.
- Khalid, K.A. (2006) Influence of water stress on growth, essential oil, and chemical composition of herbs (*Ocimum* sp.). *Int. Agrophysics*, **20**, 289-296.
- Kim, K.S., Ryu, S.N. and Chung, H.G. (2006) Influence of drought stress on chemical composition of sesame seed. *Korean Journal of Crop Science*, **51**(1), 73-80.
- Malash, N.M., Flowers, T.J. and Ragab, R. (2008) Effect of irrigation methods, management and salinity of irrigation water on tomato yield, soil moisture and salinity distribution. *Irrig. Sci.* **26**, 313-323.
- Monotti, M. (2003) Growing non-food sunflower in dryland conditions. *Ital. J. Agron.* **8**, 3-8.
- Mozaffari, R. and Arshi, Y. (1996) Research on the effects of water stress on some morphological traits and yield components of sunflower (*Helianthus annuus*). *Seed Plant J. Agric. Res. Seed Plant Improv. Inst.* (Iran Islam Republic).
- Munns, R. (2002) Comparative physiology of salt and water stress. *Plant Cell Environ.* **25**, 239-250.
- Munns, R. and Tester, M. (2008) Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* **59**, 651-681.
- Oraki, H., Khajani, F.P. and Aghaalikhana, M. (2012) Effect of water deficit stress on proline contents, soluble sugars, chlorophyll and grain yield of sunflower (*Helianthus annuus* L.) hybrids. *African J. Biotechnol.* **11**, 164-168.
- Osório, J., Osório, M.L., Chaves, M.M. and Pereira, J.S. (1998) Water deficits are more important in delaying growth than in changing patterns of carbon allocation in *Eucalyptus globulus*. *Tree Physiol.* **18**, 363-373.
- Petcu, E., Arsintescu, A. and Stanciu, D. (2001) The effect of drought stress on fatty acid composition in some Romanian sunflower hybrids. *Rom. Agric. Res.* **15**, 39-43.
- Plaut, Z. and Grava, A. (2000) Response of sunflowers to quantities of irrigation water, irrigation regimes and salinities in the water and soil. *Journal of Crop Production*, **2**(2), 299-315.

- Qadir, M., Quill rou, E., Nangia, V., Murtaza, G., Singh, M., Thomas, R.J., Drechsel, P. and Noble, A.D. (2014) Economics of salt-induced land degradation and restoration. *Nat. Resour. Forum*, **38**, 282-295.
- Rafiei, F., Reza, M., Darbaghshahi, N., Reza, M., Rezai, A. and Majd Nasiri, B. (2013) Survey of yield and yield components of sunflower cultivars under drought stress. *Int. J. Adv. Biol. Biomed. Res.* **1**, 1628-1638.
- Rahimmalek, M. and Goli, S.A.H. (2013) Evaluation of six drying treatments with respect to essential oil yield, composition and color characteristics of *Thymys daenensis* subsp. *daenensis*. Celak leaves. *Ind. Crops Prod.* **42**, 613-619.
- Roshandel, P. and Flowers, T. (2009) The ionic effects of NaCl on physiology and gene expression in rice genotypes differing in salt tolerance. *Plant Soil*, **315**, 135-147.
- Schluter, U. and Crawford, R.M.M. (2001) Long-term anoxia tolerance in leaves of *Acorus calamus* L. and *Iris pseudacorus* L. *J. Exp. Bot.* **52**, 2213-2225.
- Schortemeyer, M., Stamp, P. and Feil, B. (1997) Ammonium tolerance and carbohydrate status in maize cultivars. *Ann Bot.* **79**, 25-30.
- Shabala, S. (2013) Learning from halophytes: Physiological basis and strategies to improve abiotic stress tolerance in crops. *Ann. Bot.* **112**, 1209-1221.
- Sheffield, J. and Wood, E.F. (2012) "Drought: Past Problems and Future Scenarios". Routledge
- Shinozaki, K. and Yamaguchi-Shinozaki, K. (2000) Molecular responses to dehydration and low temperature: Differences and cross-talk between two stress signaling pathways. *Curr. Opin. Plant Biol.* **3**, 217-223.
- Siew, W., Chong, C. and Tan, Y. (1995) Composition of the oil in palm kernel from *Eiaeis*. *Guineensis*, **72**, 1587-1589.
- Triboi-Blondel, A.M. and Renard, M. (1999) Effects of temperature and water stress on fatty acid composition of rapeseed oil. *10th International Rapeseed Congress*, pp. 26-29.
- Van der Merwe, R., Labuschagne, M.T., Herselman, L. and Hugo, A. (2013) Stability of seed oil quality traits in high and mid-oleic acid sunflower hybrids. *Euphytica*, **193**, 157-168.
- Vendruscolo, E.C.G., Schuster, I., Pileggi, M., Vendruscolo, E.C., Schuster, I., Pileggi, M., Scapim, C.A., Molinari, H.B., Marur, C.J. and Vieira, L.G. (2007) Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *J. Plant Physiol.* **164**, 1367-1376.
- Verbruggen, N. and Hermans, C. (2008) Proline accumulation in plants: A review. *Amino Acids*, **35**, 753-759.
- Vierling, E. and Kimpel, J.A. (1992) Plant responses to environmental stress. *Curr. Opin. Biotechnol.* **3**, 164-170.
- Wang, W.X., Vinocur, B., Shoseyov, O. and Altman, A. (2000) Biotechnology of plant osmotic stress tolerance physiological and molecular considerations. In: *IV International Symposium on In vitro Culture and Horticultural Breeding*, pp. 285-292.
- Zhao, M., Zhang, H., Yan, H., Qiu, L. and Baskin, C.C. (2018) Mobilization and role of starch, protein, and fat reserves during seed germination of six wild grassland species. *Frontiers in Plant Science*, **27**(9), 234.
- Zhu, J-K., Hasegawa, P.M., Bressan, R.A. and Bohnert, H.J. (1997) Molecular aspects of osmotic stress in plants. *CRC Crit. Rev. Plant Sci.* **16**, 253-277.

(Received 5/9/2018;
accepted 19/1/2019)

تحليل مقارن لمحصول البذرة و السمات البيوكيميائية في أنماط وراثية مختلفة من عباد الشمس تحت مستويات مختلفة من الري و الملوحة

هبة طلعت عبيد⁽¹⁾، نعمت محمد حسن⁽¹⁾، معاطي قشطة⁽²⁾، ألفت سعد حسنين⁽²⁾
⁽¹⁾قسم النبات و الميكروبيولوجي - كلية العلوم - جامعة دمياط - مدينة دمياط الجديدة - مصر، ⁽²⁾محطة البحوث الزراعية بالسرو - محافظة دمياط - مصر.

عباد الشمس (*Helianthus annus L.*) هو محصول هام من البذور الزيتية في العديد من البلدان التي تنخفض فيها محاصيل البذور والزيت بسبب قلة المياه أو ملوحة التربة. في حين أن بعض الاستجابات للإجهاد المائي والملحي شائعة، قد تختلف الاستجابات الأخرى وفقا للنمط الوراثي أو مستوى الإجهاد. هدفت هذه الدراسة إلى مقارنة دور التنوع الوراثي في استجابة محصول دوار الشمس ونوعية الأحماض الدهنية بالزيت إلى مستويات مختلفة من الري وملوحة التربة في تجربتين حقليتين وللتحقق من تأثير مستويات الإجهاد المختلفة على تكوين الأحماض الدهنية في الزيت تم استخدام ستة تراكيب وراثية من عباد الشمس في هذه الدراسة. أظهرت النتائج تأثيرات مرتبطة بالتركيب الوراثي كذلك مستوى الإجهاد وتفاعلاتها على معظم الصفات المدروسة. أدى الإجهاد المائي إلى نقص في محتوى البروتين وإنتاجية الزيت بشكل أكبر من ملوحة التربة. أظهرت الهجن H (A9xRF6) و H (A9xRF8) أقل انخفاض في محتوى الزيت نتيجة معاملة كل من الري والملوحة مقارنة بالأنماط الوراثية الأخرى ولهذا يرشح زراعتهما في المناطق المجهدة. أظهرت النتائج أيضا أن بعض الأحماض الدهنية خاصة حامض الإستياريك وحمض الأوليك كانت تعتمد على النمط الجيني ومستوى الإجهاد في كلا التجربتين.