

# ENHANCEMENT IN PLANT WATER RELATIONS AND FATTY ACID PROFILE IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) THROUGH APPLICATION OF ABSCISIC ACID UNDER VARIED WATER LEVELS

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**Abstract.** Enhancement in yield and quality of sunflower by foliar spray of abscisic acid (ABA) under water deficit condition was studied during 2016 and 2017. Three irrigation treatments were applied. Four irrigations (at 4-6 leaf, at vegetative, at flowering and at grain formation stage), three irrigations (at 4-6 leaf, at pre flowering and at grain formation stage), three irrigations (at 4-6 leaf, at vegetative and at grain formation stage). Sunflower hybrid (S-278) was sprayed with different ABA concentrations (0, 10  $\mu$ m and 20  $\mu$ m) at 4-6 leaf, vegetative, flowering and grain formation stage. Foliar application of 10  $\mu$ M ABA under water stress at vegetative stage significantly enhanced plant height, head diameter, achene yield and biological yield. It also enhanced sunflower water relations by increasing water potential. ABA application and water deficit showed opposite results for yield and oil quality. Water deficit at vegetative or at flowering stage maximized stearic and oleic acid contents and minimized palmitic and linoleic acid contents, whereas foliar spray of ABA under water stress at both stages reduced stearic and oleic acid but maximized palmitic and linoleic acid.

**Keywords:** *abscisic acid, relative water contents, turgor pressure, oleic acid, palmitic acid, linoleic acid, sunflower*

## Introduction

Drought is the foremost yield-limiting feature for sunflower (*Helianthus annuus* L.) productivity in semi-arid areas (Rauf et al., 2015; Daryanto et al., 2016). In different parts of the world oil and achene yield losses due to drought stress were described (Woli et al., 2014; Yin et al., 2014). Water scarcity encourages significant alterations in physiological and biochemical processes implicated in biomass production, and alters dry matter partitioning (Fernández et al., 2012; Wu et al., 2017). Breeding for drought tolerance is thus indispensable to diminish yield losses in sunflower in drought-prone

regions (Rauf et al., 2015). In order to recognize probable sources of drought tolerance, cultivated sunflower breeding lines from different sources were assessed on the basis of comparative performance under drought stress (Hussain et al., 2017). Nevertheless, the narrow genetic base of breeding lines was one of the major restrictions for the possibility of such studies.

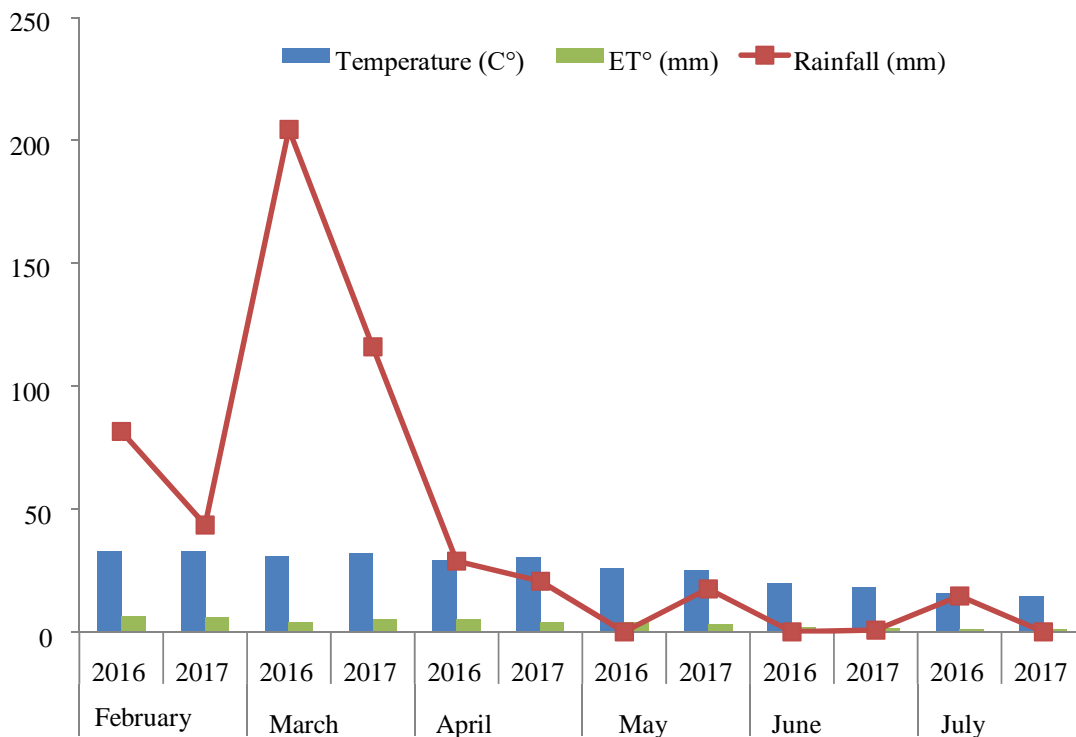
Several studies showed, abiotic stresses activate several biochemical, physiological and molecular responses that effect several plant processes at cellular level (Wang et al., 2003; Hasanuzzaman et al., 2013; Tsironi and Taoukis, 2017). To fight several environmental pressures, dynamic methods and techniques should be developed (Yin et al., 2017). Among various control measures and monitoring tools (remote sensing) to assess the impacts of drought stress on crop plants (Villegas et al., 2017; Natsagdorj et al., 2017; Song et al., 2017), agronomic measures for water conservation and chemical plant growth regulators are of great interest (Ahmad et al., 2016). Phytohormone engineering could be considered as a preferable technique to increase the production. Phytohormones are the substantial regulators in plant growth and development and also intermediaries of environmental stress responses (Sreenivasulu et al., 2012). Among several phytohormones, abscisic acid is the vital regulator of abiotic stress resistance in plants and synchronizes an arrangement of roles (Finkelstein, 2013; Wani and Kumar, 2015), allowing plants to survive with diverse stresses. In the plant, the level of abscisic acid increases through abscisic acid biosynthesis when environmental conditions are harsh. The augmented abscisic acid binds to its receptor to start signal transduction causing cellular responses to different stresses (Ng et al., 2014); thus, ABA is also termed a stress hormone (Mehrotra et al., 2014). ABA was firstly suggested to be implicated in abscission and has later been revealed to play a role in plant growth and development, comprising cell division followed by elongation, embryo maturation, seed dormancy, germination, root growth, floral initiation, and responses to both biotic and abiotic stresses, such as osmotic stress, chilling, high salinity, drought, pathogen attack and UV radiation (Finkelstein, 2013; Yoshida et al., 2014; Sah et al., 2016). ABA is significantly increased under drought or salinity stress conditions, stimulating stomatal closure, change in gene expression, and adaptive physiological responses (Kim et al., 2010; Sah et al., 2016). Abscisic acid also plays a vital role in many processes at cell level including dormancy, seed development, vegetative growth, germination etc. (Finkelstein et al., 2013; Saradadevi et al., 2017) and variation in root morphology (Harris, 2015). Meanwhile the discovery of abscisic acid, numerous struggles have been dedicated to understanding how abscisic acid is produced under stress conditions. In stressful conditions like extreme temperature, drought, and high salinity, content in plants enhances significantly, stimulating stress-tolerance effects that support plants, acclimatize, and endure under these stressful conditions (Ng et al., 2014). Under non-stress situations, abscisic acid is also mandatory for plant overall growth and development.

Around the globe enormous research has been done on various crop plants to enhance water use efficiency (WUE) by sowing better varieties and improvement of drought tolerance by foliar application of abscisic acid (ABA). But little or no data is existing on the interaction of abscisic acid and use of diverse cultivars of *Helianthus annuus* to alleviate the impacts of water stress in different agro-ecological conditions of Pakistan. Therefore the current study has been planned to adjust ABA application stage for attaining greater yields of sunflower under water stress and to associate the performance of different *Helianthus annuus* hybrids under water stress environments.

## Materials and methods

### *Study area, soil and weather condition*

Field experiments were conducted to study the response of spring planted sunflower hybrids to different irrigation schedules and foliar spray of abscisic acid. Trials were conducted in 2016 and 2017 at the Agronomic Research Farm, University of Agriculture, Peshawar, Pakistan. The climate of Peshawar region is semi-arid (34.01°N, 71.35°E) at an altitude of 350 meters above sea level. Peshawar is situated about 1600 km north of the Indian Ocean. All the analytical work associated to research was conducted in the Stress Physiology Laboratory, Nuclear Institute for Food and Agriculture, NIFA, Peshawar. Soil analysis of the experimental soil was given in *Table 1*. The climate of the area is semiarid where the mean annual rainfall is very low (300 to 500 mm), 60–70% rainfall occurs in summer, whereas the remaining 30–40% rainfall occurs in winter (*Fig. 1*).



**Figure 1.** Monthly meteorological data for experimental years 2016 and 2017 of Agronomic Research Farm, University of Agriculture, Peshawar, Pakistan

### *Experimental design and treatments*

The layout of experiment was randomized complete block design (RCBD) with factorial arrangement with three replications. Net plot size was 3.0 m × 6.0 m. Three irrigation schedules (I) were imposed which were (I<sub>1</sub>) four irrigations (at 4-6 leaf, at vegetative, at flowering and at grain formation stage), (I<sub>2</sub>) three irrigations (at 4-6 leaf, at pre flowering and at grain formation stage), (I<sub>3</sub>) three irrigations (at 4-6 leaf, at vegetative and at grain formation stage) and five ABA concentration (C<sub>1</sub>: control, C<sub>2</sub>: 10 μM ABA at vegetative stage; C<sub>3</sub>: 10 μM ABA at flowering stage, C<sub>4</sub>: 20 μM ABA at

vegetative stage and C<sub>5</sub>: 20 µM ABA at flowering stage. The first irrigation was applied when the plant had 4-6 leaves (25 DAS), the 2nd irrigation was applied at vegetative stage (45 DAS) excluding the plots, which were exposed to water deficit at this stage, the 3rd irrigation was applied at flowering stage (67 DAS) excluding the plots, which were exposed to water deficit at this stage. The 4th irrigation was given to all plots at grain formation stage (90 DAS). ABA was weighed according to per treatment and was added in a graduated cylinder and volume was made 1 L in volumetric flask with distilled water. Afterward Knapsack sprayer was calibrated (250 L ha<sup>-1</sup>) and used to spray solution. While distilled water was sprayed on the control plots.

**Table 1.** Physicochemical analysis of soil during 2016-17

Characteristic	Unit	Value	
		2016	2017
Sand	%	54	56
Silt	%	24.2	20
Clay	%	21.8	22
Textural class	-	Sandy clay loam	Sandy clay loam
Saturation percentage	%	32	30
Ec	dS/m	1.44	1.56
pH	-	7.6	7.9
Organic matter	%	0.71	0.68
Organic carbon	%	0.44	0.39
Total nitrogen	%	0.041	0.038
Available P	ppm	6.1	6.6
Available K	ppm	135	131

### Crop husbandry

The seedbed was prepared by pre-soaking up to 10 cm irrigation then cultivating the field for 2-3 times with tractor-mounted cultivator. *Helianthus annuus* hybrids were sown on 19th and 13th of February 2016 and 2017, on ridges using dibbler with seed rate of 8 kg ha<sup>-1</sup>. Ridges were made 75 cm distant and plant to plant distance of 25 cm was kept. Seed of sunflower hybrid S-278 were obtained from Syngenta Company. Fertilizers were applied at the rate of 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> in the form of triple super phosphate (TSP) and 150 kg N in the form of urea. Half nitrogen and full phosphorus were applied at sowing, whereas remaining nitrogen was applied with 1st irrigation. Irrigation was done as per treatment by flooding. To keep field free from weeds field hoeing was done. Plant protection measures were conducted to keep crop free of insect pests, diseases and parrots. For control of whitefly and head rot Polo and Radomil Gold were applied, respectively. The crop was harvested on May 30, 2016 and June 6, 2017.

### Measurements

#### Agronomic traits

From each plot, ten plants were selected randomly. The plant height was determined with measuring tape and then averaged. In order to find head diameter, 10 heads were

randomly selected from each plot and their diameter was measured with the help of a measuring tape and then averaged. For calculation of achene yield plants were harvested at maturity, sunflower' heads were detached after drying then threshed manually to find the achene yield plot<sup>-1</sup>. The moisture contents were calculated from random achene samples. The achene yield was attuned to 10% moisture content and expressed in kg ha<sup>-1</sup>. Weight of air-dried plants (excluding achenes) was noted on plot basis and then converted into kg ha<sup>-1</sup>. To compute total biological yield, noted weight was added to the already calculated achene yield (kg ha<sup>-1</sup>).

#### *Plant water relations*

Water potential, relative leaf water content, turgor pressure and osmotic potential were recorded 10 days after the ABA foliar application. To find the relative leaf water content (RLWC), third leaf from the top (fully expanded) of two different plants from different treatment was taken. Leaves were cut at base of lamina and wrapped in plastic bags then transported to laboratory immediately. Fresh weight (FW) was calculated after two hours. After that turgid weight (TW) was found by saturating leaves in distilled water for time period of 16-18 h at room temperature. After saturation, leaves were prudently and rapidly blotted dry to find turgid weight. Dry weight (DW) was calculated after drying of leaf samples in oven for time period 72 h at 70 °C. Relative leaf water content (RLWC) was calculated through the procedure of Schonfeld et al., 1988 and then averaged (Eq. 1).

$$RLWC (\%) = (FW - DW) / (TW - DW) \times 100 \quad (\text{Eq.1})$$

where, FW= fresh weight of leaf; DW= dry weight of leaf; and TW = turgid weight of leaf.

Leaf water potential was ranged from 8.00 to 10.00 A.M. It was computed through Scholander pressure chamber by following the method of Scholander et al. (1965). In order to compute, water potential, leaf was kept in a freezer at -20 °C for 7 days. After that frozen leaf was defrosted in order to extract cell sap through a disposable syringe. Then osmotic potential was computed from extracted sap via an osmometer (Wescor 5500). Turgor pressure was calculated from the difference of water potential ( $\Psi_w$ ) and osmotic potential ( $\Psi_s$ ) (Eq. 2).

$$(\Psi_p) = (\Psi_w) - (\Psi_s) \quad (\text{Eq.2})$$

#### *Quality parameters*

##### *Oil content*

Oil content in seeds was calculated using Soxhlet Fat Extraction method (AOAC, 1990). For about 10 h, seeds were dried in an oven at 105 °C. Seeds were weighed before and after drying for determination of moisture content. For oil content determination, two grams of achenes per thimble were crushed in a coffee mill. Thimbles were weighed individually, crushed seeds were added and the final weight was estimated. Later, the thimbles were placed in extractors. Six dry and clean round bottom 250 ml flasks were weighed and their weight noted. Solvent (petroleum ether) was added to flasks, attached to the extractors and placed on heating mantles attached to with condensers. Flasks were heated and extraction was continued for at least 6 h,

stopped extraction, removed thimbles and then reheated the flasks, so that all of the solvent might be collected in the Soxhlet extractors. The apparatus permitted to cool and flasks dried at 105 °C for 1 h. After cooling, the flasks and oil were weighed together. Percent oil content was calculated via the following equation (Eq. 3).

$$\text{Oil contents (\%)} = \frac{(\text{flask weight} + \text{oil weight}) - \text{flask weight}}{(\text{flask weight} + \text{seed weight}) - \text{flask weight}} \times 100 \quad (\text{Eq.3})$$

### *Fatty acid profile*

Gas liquid chromatography was used to determine fatty acid composition in sunflower oil by following Martin (1979). Oil was extracted from seed of *Helianthus annuus* hybrids by using Rancy oil seed crusher. In a loop of oil, 0.5 ml petroleum and 1 ml methylating solution ether was added in test tube. Solution was swirled to disperse loop and petroleum ether was also added to rinse loop during sampling. After addition of 1 ml distilled water; it was kept for 10 min. One µl solution was taken from upper layer and injected into the gas chromatograph. The total peak area and area of each fatty acid peak was computed by an electronic integrator and expressed as percentage of the total area of the peak.

### *Statistical analysis*

Collected data was analyzed by using Fisher's analysis of variance technique in MSTAT-C. The differences among treatments, means etc. was compared through least significant difference (LSD) test at 5% probability (Steel et al., 1997). Factorial experiment under Randomized Complete Block Design was used for analysis over years. Microsoft Excel Program was used for contrast study (Microsoft, version 2013).

## **Results**

### ***Response of agronomic traits of sunflower to irrigation schedules and ABA application***

Effect of year on plant height was statistically found non-significant during 2016 and 2017 but taller plants were observed in 2017 over 2016 (Table 2). Irrigation levels and ABA application exhibited significant impact on plant height during 2016-17 while their interactions were found non-significant. Under no water stress, plant height was significantly enhanced. Water deficit significantly reduced plant height with a larger reduction in plant height when stress was enforced at vegetative over at flowering stage. Negative impact of water deficit at both stages might be mitigated by spraying of ABA. In different ABA concentrations and its application stages, 10 µM ABA spray under water deficit at vegetative or at flowering stage considerably maximized plant height (Table 2). Orthogonal contrasts showed that 4 irrigations produced taller plants over three irrigations; ABA applied at vegetative stage produced taller plants over when applied at flowering stage. The contrast between 10 µM and 20 µM ABA was non-significant (Table 2).

Effect of year on head diameter was statistically not-significant during 2016 and 2017 but larger head diameter was observed in 2017 over 2016. Head diameter was significantly influenced by irrigation schedules and foliar spray of ABA at diverse growth stages while interactive effect had non-significant impact during both years of

study (Table 2). Water deficit reduced head diameter over no stress. Water stress imposed at flowering stage caused significant reduction in head diameter and this reduction was more prominent in those treatments where stress was imposed at vegetative stage. Negative impact of water stress at both stages might be declined by foliar spray of ABA (Table 2). Orthogonal contrasts showed that 4 irrigations vs. 3 irrigations, ABA application at vegetative stage vs. ABA application at flowering and 3 irrigations (miss at vegetative) vs. 3 irrigations (miss at flowering) were significant but orthogonal contrast 10  $\mu$ M ABA vs. 20  $\mu$ M ABA was statistically non-significant during 2016-17.

**Table 2.** Agronomic traits of sunflower as affected by irrigation levels and ABA application

Irrigation levels (I)	Plant height (cm)		Head diameter (cm)		Achene yield (kg ha <sup>-1</sup> )		Biological yield (kg ha <sup>-1</sup> )	
	2016	2017	2016	2017	2016	2017	2016	2017
I <sub>1</sub>	190.99a	193.31a	16.87a	17.57a	2696.98a	2859.33a	12133.37a	12322.65a
I <sub>2</sub>	170.71c	171.02c	12.60b	12.73b	2043.77b	2192.47b	11744.09b	11890.12b
I <sub>3</sub>	180.90b	182.81b	10.41c	10.78c	1951.31c	2002.18c	11240.79c	11128.35c
LSD (0.05)	1.09	1.2	0.28	0.37	39.77	41.49	25.48	24.98
ABA levels and its application stages (C)								
C <sub>1</sub>	182.78a	183.47a	13.93a	14.15a	2252.87b	2387.68a	12028.15a	11990.02c
C <sub>2</sub>	180.60b	183.20a	13.90a	14.57a	2359.91a	2483.96a	11900.68b	12010.88b
C <sub>3</sub>	183.49a	183.43a	13.89a	14.24a	2359.89a	2457.96a	12023.25a	12193.92a
C <sub>4</sub>	178.04bc	179.61b	12.62b	12.94b	2111.47c	2259.34b	11289.99c	11467.38d
C <sub>5</sub>	179.40b	181.11b	12.10c	12.56b	2069.23d	2169.17c	11288.34c	11239.66e
LSD (0.05)	1.41	1.55	0.37	0.48	51.34	53.63	32.89	32.25
Interaction	ns	ns	ns	ns	ns	ns	ns	ns

Mean values in column carrying different letters are statistically significant at 5% probability level. I<sub>1</sub>: No stress (4 irrigations), I<sub>2</sub>: Stress at vegetative stage (3 irrigations), I<sub>3</sub>: Stress at flowering stage (3 irrigations), C<sub>1</sub>: Control, C<sub>2</sub>: 10  $\mu$ M ABA at vegetative stage, C<sub>3</sub>: 10  $\mu$ M ABA at flowering stage, C<sub>4</sub>: 20  $\mu$ M ABA at vegetative stage, C<sub>5</sub>: 20  $\mu$ M ABA at flowering stage, ns: non-significant

**Table 2a.** Orthogonal contrasts of agronomic traits

Orthogonal contrasts	Plant height (cm)		Head diameter (cm)		Achene yield (kg ha <sup>-1</sup> )		Biological yield (kg ha <sup>-1</sup> )	
	2016	2017	2016	2017	2016	2017	2016	2017
4 irrigations vs. 3 irrigations	*	*	*	*	*	*	*	*
10 m ABA vs. 20 m ABA	ns	ns	ns	ns	ns	ns	ns	ns
ABA spray at vegetative vs. ABA spray at flowering stage	*	*	*	*	*	*	*	*
3 irrigations (missed at vegetative stage) vs. 3 irrigations (missed at flowering stage)	*	*	*	*	*	*	*	*

\*: Significant, ns: non-significant

Effect of year was statistically significant for achene yield during both years and significantly maximum achene yield was observed in 2017 over 2016 (Table 2).

Analysis of the data exhibited that achene yield was significantly influenced by irrigation levels and spray of ABA at diverse growth stages while interactive impact had not-significant impact during both years (*Table 2*). Water stress reduced achene yield over no stress. When drought was enforced at flowering stage achene yield was significantly reduced and this reduction was more over stress imposed at vegetative stage. Damaging impacts of water stress at both stages might be decreased by foliar ABA spray (*Table 2*). Orthogonal contrasts for 4 irrigations vs. 3 irrigations, ABA application at vegetative stage vs. ABA application at flowering stage and 3 irrigations (miss at vegetative) vs. 3 irrigations (miss at flowering) were significant but orthogonal contrast 10  $\mu\text{M}$  ABA vs. 20  $\mu\text{M}$  ABA was statistically non-significant during 2016-17.

Year impact on biological yield was statistically significant during 2016-17 and significantly maximum biological yield was observed in 2017 over 2016. Irrigation levels and ABA spray significantly affected biological yield while their interaction was found insignificant. Biological yield was significantly increased in control treatments (no water stress) over stress imposed at vegetative or flowering stage (*Table 2*). Under various irrigation schedules influence of ABA application was deviating (*Table 2*). When the crop encountered no water stress, foliar spray of 10 or 20  $\mu\text{M}$  ABA at vegetative or flowering stage considerably reduced biological yield with respect to that of the control during 2016-17. Orthogonal contrasts between 4 irrigations vs. 3 irrigations, ABA application at vegetative vs. ABA application at flowering 3 irrigations (miss at vegetative) vs. 3 irrigations (miss at flowering) were significant for biological yield whereas orthogonal contrast 10  $\mu\text{M}$  ABA vs. 20  $\mu\text{M}$  ABA was statistically non-significant during both years.

### ***Response of plant water relations to irrigation schedules and ABA application***

Effect of year on leaf water potential was non-significant during 2016 and 2017 and leaf water potential was maximum in 2017 over 2016. Levels of irrigation and foliar ABA spray effect was prominent on leaf water potential during both years. Water deficit significantly reduced leaf water potential over control. Leaf water potential was significantly reduced when water stress imposed at flowering over vegetative stage. Mean values clearly showed that foliar ABA application significantly influenced the leaf water potential (*Table 3*). When crop encountered water deficit at vegetative stage, the application of 10  $\mu\text{M}$  ABA significantly improved leaf water potential with respect to control but it was statistically comparable with 20  $\mu\text{M}$  ABA spray at vegetative stage (*Table 3*). Orthogonal contrasts for 4 irrigations vs. 3 irrigations, ABA application at vegetative vs. ABA application at flowering and 3 irrigations (miss at vegetative stage) vs. 3 irrigations (miss at flowering) were significant but the contrast 10  $\mu\text{M}$  ABA vs. 20  $\mu\text{M}$  ABA was statistically non-significant during 2016-17.

Year impact on leaf osmotic potential was statistically significant during 2016-17 and significantly maximum leaf osmotic potential was observed in 2016 over 2017. Irrigation schedules and ABA application significantly influenced leaf osmotic potential while their interactions during both years were found insignificant. Water stress at both vegetative and flowering stage significantly reduced leaf osmotic potential over control. Leaf osmotic potential was reduced when water stress executed at flowering over vegetative stage. Mean values showed that foliar application of ABA significantly influenced the leaf osmotic potential. Under diverse irrigation schedules, performance of foliar application of ABA was divergent. When crop encountered no water stress at vegetative or flowering stage, foliar ABA spray reduced leaf osmotic potential during



2016-17. Orthogonal contrast between 4 irrigations vs. 3 irrigations and 3 irrigations (miss at vegetative stage) vs. 3 irrigations (miss at flowering stage) for leaf osmotic potential were significant during 2016 and 2017 but orthogonal contrast for ABA application at vegetative stage vs. ABA application at flowering stage was non-significant in 2016 and statistically significant in 2017. Likewise contrast 10  $\mu\text{M}$  ABA vs. 20  $\mu\text{M}$  ABA was statistically non-significant during 2016-17.

Effect of year on leaf turgor pressure was statistically significant during 2016 and 2017 and statistically higher leaf turgor pressure was observed in 2017 over 2016. Leaf turgor pressure was significantly influenced by irrigation schedules and foliar application of ABA at various growth stages while their interaction had non-significant impact during 2016-17. Water deficit reduced leaf turgor pressure over no stress (Table 3). Turgor pressure was significantly reduced when water stress was enforced at flowering stage and this reduction was higher over when stress was enforced at vegetative stage (Table 2).

Application of 10  $\mu\text{M}$  ABA under water deficit at vegetative stage improved leaf turgor pressure with respect to control. Water deficit at vegetative stage and application of 20  $\mu\text{M}$  ABA enhanced turgor pressure than control and this enhancement was statistically comparable with 10  $\mu\text{M}$  ABA application at vegetative stage. When crop confronted water deficit at vegetative stage but ABA (10 or 20  $\mu\text{M}$ ) was sprayed at flowering stage, it considerably reduced turgor pressure over control. Comparable observation was noted during 2016-17. Orthogonal contrasts between 4 irrigations vs. 3 irrigations, ABA application at vegetative stage vs. ABA application at flowering stage and 3 irrigations (miss at vegetative) vs. 3 irrigations (miss at flowering stage) were significant for turgor pressure but contrast 10  $\mu\text{M}$  ABA vs. 20  $\mu\text{M}$  ABA was statistically non-significant during both 2016-17 (Table 3).

**Table 3.** Plant water relations of sunflower as affected by irrigation levels and ABA application

Irrigation levels	Water potential (-MPa)		Osmotic potential (-MPa)		Turgor potential (MPa)		Relative water content (%)	
	2016	2017	2016	2017	2016	2017	2016	2017
I <sub>1</sub>	0.674a	0.681a	1.545a	1.561a	0.972a	0.881a	85.57a	86.36a
I <sub>2</sub>	0.939b	0.949b	1.678b	1.696b	0.741b	0.748b	79.23b	79.76b
I <sub>3</sub>	1.042c	1.053c	1.704c	1.723c	0.664c	0.671c	75.82c	76.81c
LSD (0.05)	0.015	0.015	0.019	0.021	0.11	0.014	0.28	0.43
ABA levels and its application stages								
C <sub>1</sub>	0.829a	0.838a	1.608b	1.625b	0.779a	0.798a	80.33b	81.25a
C <sub>2</sub>	0.831a	0.840a	1.596a	1.613a	0.766b	0.774b	80.92a	81.68a
C <sub>3</sub>	0.847a	0.856a	1.626c	1.643c	0.780a	0.788a	80.45b	81.13a
C <sub>4</sub>	0.965b	0.975b	1.694d	1.712d	0.726c	0.737c	79.99c	80.91ab
C <sub>5</sub>	0.951b	0.961b	1.687d	1.705d	0.737c	0.744c	79.30d	80.19b
LSD (0.05)	0.019	0.019	0.012	0.012	0.014	0.014	0.36	0.563
Interaction	ns	ns	ns	ns	ns	ns	ns	ns

Mean values in column carrying different letters are statistically significant at 5% probability level. I<sub>1</sub>: No stress (4 irrigations), I<sub>2</sub>: Stress at vegetative stage (3 irrigations), I<sub>3</sub>: Stress at flowering stage (3 irrigations), C<sub>1</sub>: Control, C<sub>2</sub>: 10  $\mu\text{M}$  ABA at vegetative stage, C<sub>3</sub>: 10  $\mu\text{M}$  ABA at flowering stage, C<sub>4</sub>: 20  $\mu\text{M}$  ABA at vegetative stage, C<sub>5</sub>: 20  $\mu\text{M}$  ABA at flowering stage, ns: non-significant

**Table 3a.** Orthogonal contrasts of plant water relations

Orthogonal contrasts	Water potential (-MPa)		Osmotic potential (-MPa)		Turgor potential (MPa)		Relative water content (%)	
	2016	2017	2016	2017	2016	2017	2016	2017
4 irrigations vs. 3 irrigations	*	*	*	*	*	*	*	*
10 m ABA vs. 20 m ABA	ns	ns	ns	ns	ns	ns	ns	ns
ABA spray at vegetative vs. ABA spray at flowering stage	*	*	ns	*	*	*	*	*
3 irrigations (missed at vegetative stage) vs. 3 irrigations (missed at flowering stage)	*	*	*	*	*	*	*	*

\*: Significant, ns: non-significant

Effect of year on relative leaf water content was statistically not-significant during 2016-17 but relative leaf water content was slightly higher during 2017 over 2016. Analysis of the data revealed that relative leaf water content was significantly influenced by irrigation schedules and foliar application of ABA at diverse growth stages while their interaction had non-significant impact during both years (*Table 3*). Water deficit reduced relative leaf water content over no stress. When water deficit was imposed at flowering stage relative leaf water content was significantly reduced and this reduction was higher over where stress was enforced at vegetative stage. Negative impact of water deficit at both stages might be decreased by foliar spray of ABA (*Table 3*). Influence of foliarly applied ABA was different under diverse irrigation levels (*Table 3*). No water deficit at both growth stages, foliar application of ABA reduced relative leaf water content during 2016-17. Orthogonal contrasts between 4 irrigations vs. 3 irrigations, ABA application at vegetative stage vs. ABA application at flowering stage and 3 irrigations (miss at vegetative stage) vs. 3 irrigations (miss at flowering stage) were significant for relative leaf water content but orthogonal contrast for 10  $\mu$ M ABA vs. 20  $\mu$ M ABA was statistically no-significant during 2016-17.

#### ***Response of quality parameters of sunflower to irrigation schedules and ABA application***

Effect of year on achene oil content was statistically non-significant but oil content was higher in 2017 with respect to 2016 (*Table 4*). Mean values exhibited that oil content was significant during irrigation treatments and foliar application of ABA at various growth stages while their interactions was found non-significant during both years (*Table 4*). Water stress reduced oil content compared to no stress. Oil content significantly reduced when water deficit was executed at flowering stage and this reduction was higher over when deficit was enforced at vegetative stage (*Table 4*). Under water deficit condition foliar spray of 10  $\mu$ M ABA at vegetative stage improved oil content over control. Water deficit at vegetative stage and 20  $\mu$ M ABA application also enhanced oil content than control but this enhancement was statistically comparable with 10  $\mu$ M ABA application at vegetative stage. When crop confronted water deficit at vegetative stage but ABA (10 or 20  $\mu$ M) was sprayed at flowering stage, it significantly reduced oil content than control. Comparable observation was observed during 2016-17.

Impact of year on stearic acid content was statistically significant during 2016-17 and maximum stearic acid content was observed during 2016 over 2017. Analysis of the data revealed that irrigation levels and ABA application exhibited significant impact on stearic acid content during 2016-17. Water deficit significantly improved stearic acid content and maximum increment in stearic acid content was noted when water deficit was executed at flowering stage over at vegetative stage (*Table 4*). Foliar spray of 20  $\mu\text{M}$  ABA at flowering stage by enforcing stress at vegetative stage significantly enhanced stearic acid content over all treatments. Whereas spraying 10 or 20  $\mu\text{M}$  ABA at vegetative stage under water stress at vegetative stage reduced stearic acid content than control. Similar trend was noticed during 2016-17. Orthogonal contrasts between 4 irrigations vs. 3 irrigations and 3 irrigations (miss at vegetative stage) vs. 3 irrigations (miss at flowering stage) were significant for stearic acid content but orthogonal contrasts 10  $\mu\text{M}$  ABA vs. 20  $\mu\text{M}$  ABA, and ABA application at vegetative stage vs. ABA application at flowering stage were statistically non-significant during 2016-17.

Orthogonal contrasts for 4 irrigations vs. 3 irrigations, ABA application at vegetative stage vs. ABA application at flowering stage and 3 irrigations (miss at vegetative stage) vs. 3 irrigations (miss at flowering stage) were significant for oil content but orthogonal contrast 10  $\mu\text{M}$  ABA vs. 20  $\mu\text{M}$  ABA was statistically non-significant during 2016-17.

Effect of year on oleic acid content was statistically significant during 2016 and 2017 and statistically higher oleic acid was observed in 2016 over 2017. Oleic acid content was statistically influenced by irrigation levels and ABA spray while their interaction was found insignificant. Water stress significantly improved oleic acid content and maximum increment in oleic acid content was noted when water deficit was executed at vegetative stage over at flowering stage (*Table 4*). Water stress at vegetative stage and spraying of 20  $\mu\text{M}$  ABA at flowering stage had significantly improved oleic acid content over all treatments. Enforcing water deficit at vegetative stage and spraying 10  $\mu\text{M}$  ABA at flowering stage also significantly improved oleic acid content with respect to control but this increment was lower with respect to spraying of 20  $\mu\text{M}$  ABA at the similar stage. Whereas spraying 10 or 20  $\mu\text{M}$  ABA at vegetative stage under water deficit at vegetative stage reduced oleic acid content than control. Same trend was observed during 2016-17. Orthogonal contrasts for 4 irrigations vs. 3 irrigations was significant but orthogonal contrasts 10  $\mu\text{M}$  ABA vs. 20  $\mu\text{M}$  ABA and ABA application at vegetative stage vs. ABA application at flowering stage and 3 irrigations (miss at vegetative stage) vs. 3 irrigations (miss at flowering stage) were statistically non-significant during both years.

Palmitic acid content was significantly affected during 2016 and 2017 and statistically higher palmitic acid content was observed in 2017 over 2016. Different irrigation levels and foliar spray of ABA at diverse growth stages significantly affected palmitic acid content while their interaction was found insignificant. Water stress reduced palmitic acid content over no stress. Palmitic acid content was considerably minimized when water stress was enforced at flowering stage and this reduction was maximum than where water deficit was enforced at vegetative stage (*Table 4*). Spray of 10  $\mu\text{M}$  ABA under water stress at vegetative stage statistically increased palmitic acid content over all treatments. Water stress at vegetative stage and application of 20  $\mu\text{M}$  ABA also enhanced palmitic acid content than control but this enhancement was lower than spraying of 10  $\mu\text{M}$  ABA at vegetative stage. When crop confronted water stress at vegetative stage but ABA (10 or 20  $\mu\text{M}$ ) was sprayed at flowering stage, it significantly reduced palmitic acid content over control. Same results was noted during both years.

Orthogonal contrasts between 4 irrigations vs. 3 irrigations was significant for palmitic acid content but orthogonal contrasts 10  $\mu$ M ABA vs. 20  $\mu$ M ABA, 3 irrigations (miss at vegetative stage) vs. 3 irrigations (miss at flowering stage) and ABA application at vegetative vs. ABA application at flowering stage were statistically non-significant during 2016-17.

**Table 4.** Oil content and fatty acid profile of sunflower as affected by irrigation levels and ABA application

Irrigation levels (I)	Oil content (%)		Stearic acid (%)		Oleic acid (%)		Palmitic acid (%)		Linoleic acid (%)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
I <sub>1</sub>	41.42a	41.61a	3.49c	3.40c	11.63c	11.52c	6.78a	6.83a	78.31a	78.37a
I <sub>2</sub>	39.96b	40.34b	3.65b	3.55b	11.79a	11.68a	5.61b	5.769b	76.91b	76.85b
I <sub>3</sub>	39.28c	39.46c	3.79a	3.66a	11.72b	11.67b	5.36c	5.50c	76.55c	76.65c
LSD (0.05)	0.23	0.26	0.081	0.07	0.01	0.01	0.012	0.014	0.013	0.015
ABA levels and its application stages (C)										
C <sub>1</sub>	40.49a	40.88a	3.61c	3.51c	11.88c	11.61c	5.97a	6.06a	77.27b	77.26c
C <sub>2</sub>	40.46a	40.70a	3.61c	3.47d	11.72b	11.65b	5.94b	6.04b	77.34a	77.45a
C <sub>3</sub>	40.18b	40.37b	3.62c	3.52bc	11.63d	11.53d	5.97a	6.05ab	77.22d	77.30b
C <sub>4</sub>	40.07b	40.25b	3.64b	3.53b	11.82a	11.68a	5.87c	5.96c	77.24c	77.20b
C <sub>5</sub>	39.88b	40.13b	3.74a	3.62a	11.73b	11.65b	5.81d	5.92d	77.18e	77.23b
LSD (0.05)	0.3	0.34	0.02	0.01	0.01	0.012	0.013	0.012	0.011	0.01
Interaction	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Mean values in column carrying different letters are statistically significant at 5% probability level. I<sub>1</sub>: No stress (4 irrigations), I<sub>2</sub>: Stress at vegetative stage (3 irrigations), I<sub>3</sub>: Stress at flowering stage (3 irrigations), C<sub>1</sub>: Control, C<sub>2</sub>: 10  $\mu$ M ABA at vegetative stage, C<sub>3</sub>: 10  $\mu$ M ABA at flowering stage, C<sub>4</sub>: 20  $\mu$ M ABA at vegetative stage, C<sub>5</sub>: 20  $\mu$ M ABA at flowering stage, ns: non-significant

**Table 4a.** Orthogonal contrasts of oil content and fatty acid profile of sunflower

Orthogonal contrasts	Oil content (%)		Stearic acid (%)		Oleic acid (%)		Palmitic acid (%)		Linoleic acid (%)	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
4 irrigations vs. 3 irrigations	*	*	*	*	*	*	*	*	*	*
10 m ABA vs. 20 m ABA	ns	ns	ns	ns	ns	ns	ns	ns	ns	Ns
ABA spray at vegetative vs. ABA spray at flowering stage	*	*	ns	ns	ns	ns	ns	ns	ns	Ns
3 irrigations (missed at vegetative stage) vs. 3 irrigations (missed at flowering stage)	*	*	*	*	ns	ns	ns	ns	ns	Ns

\*: Significant, ns: non-significant

Effect of year on linoleic acid content was significant and statistically higher linoleic acid was observed in 2017 over 2016. Linoleic acid content was significantly influenced by irrigation levels and foliar spray of ABA at various growth stages while their interaction was found non-significant effect on linoleic acid content. Water stress

reduced linoleic acid content over no stress. Considerably lesser linoleic acid was noted when water stress was enforced at flowering stage than where water deficit was enforced at vegetative stage (*Table 4*). Spray of 10  $\mu\text{M}$  ABA under water stress at vegetative stage statistically augmented linoleic acid content over control. Water stress at vegetative stage and application of 20  $\mu\text{M}$  ABA also enhanced linoleic acid content than control but this enhancement was lower than spraying of 10  $\mu\text{M}$  ABA at vegetative stage. When crop confronted water deficit at vegetative stage but ABA (10 or 20  $\mu\text{M}$ ) was sprayed at flowering stage, it considerably reduced linoleic acid content over control. Same observation was recorded during 2016-17. Orthogonal contrasts for 4 irrigations vs. 3 irrigations was significant for linoleic acid content but orthogonal contrasts 10  $\mu\text{M}$  ABA vs. 20  $\mu\text{M}$  ABA and ABA application at vegetative stage vs. ABA application at flowering stage and 3 irrigations (miss at vegetative) vs. 3 irrigations (miss at flowering stage) were statistically non-significant during both years.

## Discussion

### *Agronomic and yield related traits*

Water stress is one of the severe threats to crop production in semi-arid and arid areas of Pakistan. In present trial, foliar spraying of abscisic acid to sunflower under water deficit either at vegetative or flowering stage has been noticed to considerably enhanced crop growth, yield, osmotic adjustment, development, water relations, achene quality and fatty acid profile over no spray of ABA under water stress.

Water deficit both at vegetative or flowering stage declined sunflower plant height and maximum decrease in plant height was recorded when water deficit happened at bud initiation (Unger, 1983). Exogenous ABA application under no water stress condition reduced plant height. Under no water stress condition, shoot growth reduced by ABA application in maize seedling (Zhang et al., 2012) which finally reduced plant height. ABA foliar application under water stress enhanced plant height since it perform as shoot growth regulator under water scarce environments (Zhang et al., 2012). In the current trial less concentration (10  $\mu\text{M}$  ABA) supported vegetative growth under water stress whereas the greater concentration (20  $\mu\text{M}$  ABA) could have facilitated in survival of plant by regulating processes like plant size growth and stomatal opening (Sah et al., 2016). Controversially foliar spray of ABA under water stress and no stress condition decreased height of shoot in *Populus davidiana* which was owed to lesser accumulation of dry matter and its maximum distribution to *Populus davidiana* roots (Chunyang et al., 2004).

Water stress significantly reduced head diameter of sunflower and maximum reduction in head diameter happened when crop encountered water deficit at flowering over vegetative stage (Hussain et al., 2015, 2017). This decrease was due to reduction in photosynthates production (Dong et al., 2017; Mila et al., 2017) and their minimum distribution to the floral parts (Dong et al., 2017). Spraying of 10  $\mu\text{M}$  ABA under water deficit at flowering stage considerably enhanced head diameter because abscisic acid useful in water maintenance response of plants and may have enhanced plant growth through improvement in water use efficiency (WUE) (Wei et al., 2015; Dong et al., 2017).

For better production of crops, normal irrigations are essential but under water scarcity situation, it is significant to recognize important growth stage where irrigation can be missed without significantly reducing crop production (Wang et al. 2017). Water

deficit both at vegetative or flower initiation stage reduced achene yield along with its attributes in *Helianthus* (Hussain et al., 2014, 2015). Harsh water scarcity effects were more apparent at flower initiation stage over at vegetative stage (Hussain et al., 2015). ABA foliar application during normal irrigation also considerably declined the yield, as it only enhanced yield during water deficit both at vegetative or flower initiation stage. From tolerance to dehydration, the plant hormone ABA played imperative (Wani et al., 2016) by helping dispersion of roots of maize in soil (Zhang et al., 2012) which finally augmented production of assimilates and yield through enhancing osmotic adjustment and water relation in plants like sunflower (Hussain et al., 2014, 2015; Wani et al., 2016; Dong et al., 2017). Abscisic acid sprayed under water deficit altered the sorghum physiology and growth and its application on seed enhanced grain yield (Traore and Sullivan, 1990). Contrary Ayub et al. (2000) described that foliar spray of abscisic acid during water stress to mung bean exhibited non-significant response to grain yield.

In current study biological yield of *Helianthus* was maximally reduced when water stress was enforced at vegetative over at flowering stage. Foliar application of 10 or 20  $\mu\text{M}$  ABA under water stress at vegetative stage enhanced biomass but maximum increment in biomass was observed when 10  $\mu\text{M}$  abscisic acid sprayed during this stage. This enhancement in biomass was due to decrease in shoot' water loss, roots deeper dispersion and increment in WUE which eventually augmented production of crop. Same findings were reported in *Helianthus* (Zhang et al., 2012; Hussain et al., 2015; Dong et al., 2017) and maize (Hartung et al., 1994). Contradictory ABA foliar application under water deficit and well-watered condition decreased biomass production in *Populus* which is owed to lesser dry matter accumulation and maximum distribution to roots of plant (Chunyang et al., 2004) ultimately may reduce above ground vegetation.

### ***Plant water relations***

Results of current trial showed that water potential became more adverse under water scarcity both at vegetative or at flowering stage and the exogenous application of abscisic acid considerably averted the negative impact of water stress by making the water potential in sunflower plants less negative. When ABA sprayed at vegetative stage over at flowering stage water potential became less negative. This enhancement in water potential indicate improvement of water shortage tolerance in *helianthus* by foliar application of abscisic acid, which eventually retained plant moisture through restricted and steady stomatal closure and by increasing root penetration and lastly help in plant survival through dehydrin protein production under cellular desiccation. Alfredo and Setter (2000) described that Cassava has capacity of partial stomatal closure under water stress due to abscisic acid accumulation in vegetative parts specifically in leaves which decreased leaf development and lastly continuation of growth happened after watering the crop again. Foliar application of abscisic acid responds to water stress condition by storing dehydrin protein in sunflower vegetative tissues (Aguado et al., 2014), poplar and wheat (Pelah et al., 1997). Dehydrin protein saves plants from cellular desiccation (Baker et al., 1988; Dure et al., 1989; Close, 1996). Jones and Corlett (1992) noticed contrary plant response as plant metabolic processes were more prone to cell size and turgor pressure compared to absolute water potential.

Water deficit both at vegetative or flowering stage depressed the water potential which also triggered a comparable reduction in osmotic potential. Ultimate decrease in osmotic potential was result of solutes accumulation in various plant parts through

osmotic adjustments (Serraj and Sinclair, 2002; Hussain et al., 2015). ABA foliar application to helianthus caused osmotic potential less adverse. When ABA was sprayed at vegetative stage over at flowering stage, osmotic potential became less negative. This enhancement in osmotic potential was the result of compatible solutes accumulation like glycinebetaine (Serraj and Sinclair, 2002), proline (Hussain et al., 2015) and total soluble sugars. Such compounds are supportive in delay of desiccation through ROS detoxification, maintenance of membranes in addition to enzymes arrangement (Ludlow and Muchow, 1990; Subbarao et al., 2000). Hussain et al. (2015) exhibited that under water stress condition, key physiological mechanism was decrease in osmotic potential to keep leaf turgor pressure. Contrarily ABA foliar application may increment the action of definite enzymes such as ribonuclease and  $\alpha$ -amylase. These enzymes may be involved in breakdown of starches plus other related materials thus making osmotic potential more adverse (Dong et al., 2017; Mila et al., 2017).

Water stress at vegetative or flower initiation stage considerably decreased turgor pressure of leaf. Decrease in turgor pressure of leaf was owed to reduction in water potential and already reported in wheat (Blum, 1997) and Helianthus (Hussain et al., 2015). ABA foliar application during water stress considerably enhanced leaf turgor pressure which was maximum at vegetative over at flower initiation stage. This enhancement in leaf turgor potential was result of plant efforts to maintain moisture. These types of findings were also reported in maize (Zhang et al., 2012) and in poplar (Pelah et al., 1997).

Results of the current study exposed that water stress at vegetative or flowering stage severely decreased sunflower relative water content of leaf. Decline in relative leaf water content in Helianthus was also noticed by Hussain et al. (2015). Further reduction in relative water content of leaf was noticed when water deficit imposed at vegetative over flower initiation stage. ABA foliar application considerably improved relative leaf water content under water deficit situations. These enhancements in relative water content of leaf was owed to plant moisture preservation through limited and steady stomata closure, root proliferation for water abstraction and dehydrin protein buildup which ultimately rescued plant from cellular desiccation. The same result was noticed in poplar, wheat and cassava (Pelah et al., 1997; Alfredo and Setter, 2000; Aguado et al., 2014). Contrary to this, ABA foliar application to maize during water stress did not increment relative water content of leaf (Unyayar et al., 2004).

### ***Oil contents and fatty acid profile***

Water stress both at vegetative and flower initiation stage decreased achene oil content. Oil content was observed to increase when water deficit was applied at flower initiation stage over vegetative stage. Water deficit applied prior to reproductive stage (Saleem et al., 2013), at flower initiation stage (Hammadeh et al., 2005) and for the duration of seed filling stage (Mekki et al., 1999; Hussain et al., 2015) of Helianthus declined oil content. In contrast the seed oil content exhibited constancy during incrementing water scarcity (Khan et al., 2000). Hussain et al. (2015) also reported no significant reduction in oil content under water deficit. ABA spraying under water deficit at vegetative or at flower initiation stage statistically enhanced oil content. Seed oil content may be improved by abscisic acid foliar spray because its spray result in limited stomata closure, reduced transpiration rate, repressed shoot development and improved root proliferations (Alfredo and Setter, 2000; Saleem et al., 2013) which eventually improved water accessibility for oil production in achene yield.

Water deficit and ABA foliar application under water deficit or well-watered conditions influenced fatty acid profile. Results of present trial emphasized that oleic acid and stearic acid reduced whereas linoleic acid and palmitic acid improved during well-watered and ABA foliar application improved oleic acid and stearic acid whereas reduced linoleic acid and palmitic acid. Water deficit at vegetative and at flower initiation stage slightly improved oleic acid and stearic acid whereas linoleic acid and palmitic acid reduced. Further increment was noticed in stearic acid when water deficit imposed at flower initiation stage whereas same response was noticed in oleic acid when sunflower encountered water deficit at vegetative stage. Linoleic acid and Palmitic acid were reduced under water shortage situation imposed at vegetative or at flower initiation stage but further decrease in both fatty acids was noted in crop that confronted water deficit at flower initiation stage. Flagella et al. (2002) also described reduction in oleic acid and stearic acid and increment in linoleic acid and palmitic acid in *Helianthus* under irrigation. Contrary, in *Helianthus*, oleic acid decreased and palmitic acid improved under water shortages (Saleem et al., 2013; Hussain et al., 2015). ABA foliar application both at vegetative and at flower initiation stage under water shortage somewhat reduced oleic acid and stearic acid, whereas its application to some extent enhanced palmitic acid and linoleic acid. This decrease in stearic and oleic acid and enhancement in linoleic and palmitic acid revealed that abscisic acid was useful in extenuating the negative impacts of water stress through enhancing water availability to plants. Water availability may be enhanced through maintaining moisture content in plant by restricted stomatal closure, decrease in transpiration rate, increment in root proliferation and inhibiting shoot growth (Alfredo and Setter, 2000; Hoad et al., 2001; Saleem et al., 2013; Hussain et al., 2015, 2017; Dong et al., 2017;).

## Conclusion

It is concluded from this experiment that foliar spray of 10  $\mu\text{M}$  ABA under water scarcity at vegetative stage significantly enhanced plant height, head diameter, achene yield and biological yield with respect to no abscisic acid. It also enhanced water relations by enhancing turgor pressure and water potential which specifies that ABA foliar application was useful in improving sunflower drought tolerance.

Foliar application of 10  $\mu\text{M}$  abscisic acid at flowering stage by enforcing water stress at this stage also enhanced plant height, head diameter, achene yield and biological yield as compared to no ABA but this increment was lower than with the 10  $\mu\text{M}$  ABA foliar application at vegetative stage after enforcement of water deficit at this stage.

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