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ABSCISIC ACID MEDIATED BIOCHEMICAL CHANGES IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) GROWN UNDER DROUGHT AND WELL-WATERED FIELD CONDITIONS

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ABSTRACT

Water stress is a major and ever-present threat to crop production, especially where irrigation is an inevitable aid to agriculture. Three sunflower hybrids viz. DK-4040, S-278 and SF-187 were subjected to different irrigation regimes and ABA applications. Four irrigations applied at different times with and without ABA. In control treatment i.e. four irrigations were applied 25 days after sowing (DAS), at bud initiation, flower initiation and achene formation with no ABA spray. In drought stress treatment three irrigations were applied and one irrigation skipped either at bud or at flower initiation stage with and without ABA application. The experiment laid out in RCBD with factorial arrangements of treatments. LSD test was employed to compare the means of treatment at 5% level of probability. Drought significantly decreased achene yield and oil content but increased achene protein content and leaf compatible solutes in sunflower but vice versa happened by application of ABA. Drought stress to sunflower hybrids at bud or at flower initiation increased stearic and oleic acid and decreased palmitic and linoleic acid while exogenous application of ABA under water deficit at both stages slightly decreased stearic and oleic acid but increased palmitic and linoleic acid. Sunflower crop under water deficits conditions can be grown by application of ABA which is helpful in improving the drought tolerance as well as quality of oil.

Key words: Sunflower, Abscisic acid, Drought tolerance, Compatible solutes, Fatty acids.

INTRODUCTION

Sunflower *(Helianthus annuus* L.) is one of the important oil seed crops in the world. Morphological, physiological and developmental adaptations of sunflower to the variable environmental conditions determine a unique crop.

Drought represents an extended dry period that results in crop stress and yield reduction. Water limited crop production depends on drought intensity and pattern which vary from year to year. However, in some sub-tropical countries like Pakistan, there is a high probability that crop water deficits increase in severity as the season progresses, due to lack of rainfall and to the high evaporative demand (Ashraf and Foolad, 2006).

Drought tolerance has been observed in all plant species, but its extent varies from species to species. One way to ensure future food needs of the increasing world populations should involve better use of water by the development of crop varieties those require lesser amounts of water and are more tolerant to water shortage (Jaleel *et al*., 2007). The major physiological mechanism to maintain leaf turgor pressure by decreasing osmotic potential is osmotic adjustment. Sunflower exhibits a large varietal difference for osmotic adjustment in response to water shortage (Hussain *et al*., 2014)

Compatible solutes including soluble sugars, sugar alcohols, proline and glycinebetaine are low molecular weight and highly soluble compounds, nontoxic for plant even at high cytosolic concentration. Compatible solutes protect plants from cellular dehydration through detoxification of reactive oxygen species (ROS), stabilization of membranes, structures of enzymes and proteins. Under drought, accumulation of compatible solutes occurs in the cell which lowered the osmotic potential and attracts water molecules into the cell and ultimately maintains the cell turgor (Hussain *et al*., 2010). Due to osmotic adjustment plant organelles and cytoplasmic activities take place at about normal rate which help plants to perform well in terms of growth, photosynthesis and assimilate partitioning to grain filling (Subbarao *et al*., 2000). In field crops accumulation of compatible solutes has been considered as a parameter of selection for stress tolerance. Higher amount of proline accumulation can occur in plants under stresses such as temperature, drought and starvation. Higher levels of proline would enable the plant to maintain low water

potentials (Jalil *et al*., 2007; Sankar *et al*., 2007; Hussain *et al*., 2012).

Sunflower yield is badly affected by drought stress occurred at critical stages of growth and development. Water deficits conditions reduced the head diameter, number of achenes per head, 1000-achenes weight and finally achene yield of sunflower hybrids (Hussain *et al*., 2014). Sunflower oil contains four essential fatty acids, like palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) acids (Monotti, 2003). Water scarcity at different growth stages of sunflower alter the seed composition and oil quality (Flagella *et al*., 2002) and differential drought tolerance has been noted at different growth stages of plant species (Kefale and Ranamukhaarachchi, 2006; Li-Ping *et al*., 2006). Availability of water significantly affects the fatty acids composition in sunflower genotypes particularly the change in amount of oleic and linoleic acids has been examined during water shortage at different growth stages of sunflower hybrids (Flagella *et al*., 2002; Baldini *et al.*, 2002). Oil of sunflower hybrids grown under well watered conditions had more linoleic acid content than oleic acid content (Flagella *et al*., 2002). However, oleic/linoleic acid ratio increased under stressed conditions when sunflower plants were subjected to soil water deficit during root elongation stage. This increase in oleic/linoleic acid ratio was due to increase in linoleic acid content than that of oleic acid (Santonoceto *et al*., 2003).

Compatible solutes protects the cell organelles like chloroplast, mitochondria and nucleus from detrimental effects of free radicals under drought while ABA partially closed the stomata and conserve cell water. So both are beneficial to protect the plant from cellular dehydration (Hussain *et al*., 2013).

Contrasting reports about the concentration of compatible solutes and fatty acid profile in sunflower genotypes under well watered and drought conditions and need to measure the effect of exogenous application of ABA under well watered and drought situation on the quantity of compatible solutes and fatty acids profile urged to design the present study. The aim of present research work was to determine whether the full or limited irrigation and exogenous application of ABA under this situation could alter the amount of compatible solutes, fatty acid profile and yield of sunflower hybrids.

MATERIALS AND METHODS

The exogenous application of ABA under drought and well water conditions was studied through field experiments executed during spring 2008 and 2009 at Agronomic Research Farm, University of Agriculture, Faisalabad, Pakistan. The soil of experimental site was sandy loam having appreciable amount of N, P, K and received good amount of rainfall in both years. Faisalabad is situated at latitude 31^0N, longitude 73^0E and altitude 186 m/610 ft. from the sea level. Three sunflower hybrids viz. DK-4040, S-278 and SF-187 were used in the current study. Hybrids seed was obtained from the Regional Office of the Pakistan Oil Development Board, Faisalabad.

The seedbed was prepared at field capacity by cultivating the field for 2 times with cultivator. Ridges were made 75 cm apart. Sunflower hybrids were sown on 17th and 12th of February 2008 and 2009 respectively. Seed was sown on ridges with the help of a dibbler by using seed rate of 8 kg ha^{-1} and plant-to-plant distance of 25 cm was maintained after thining at three leaves stage. Fertilizers were applied at the rate of 150 kg N and 100 kg P_2O_5 ha^{-1} in the form of urea and diammonium phosphate (DAP). Half dose of nitrogen and whole of phosphorus were applied at sowing, while remaining nitrogen with 1stirrigation. The first irrigation was applied at 4-6 leaf stage (25 DAS), the 2nd irrigation was applied at bud initiation stage (45 DAS) except the plots, which were subjected to water stress, the 3rd irrigation was applied at flower initiation stage (67 DAS) except the plots, which were subjected to drought stress. The 4th irrigation was given to all plots at grain formation stage (90 DAS). Field hoeing was done after 1st irrigation at field capacity. The response of application of two ABA levels (0 and 8µM) at two growth stages of sunflower hybrids (bud initiation and flower initiation) under three irrigation regimes (no drought stress, drought stress at bud initiation and drought stress at flower initiation) was assessed. Abscisic acid (+ cis-trans ABA) powder of Sigma Aldrich, Japan was applied to the crop as foliar spray. In order to make the ABA Solution weighted quantity of ABA (as per treatment) was added in a graduated cylinder and volume was made I L in volumetric flask with distilled water. Thereafter Knapsack sprayer was calibrated (250 L ha^{-1}) and use to spray solution. Distilled water was sprayed in the control plots. The crop was harvested on May 29, 2008 and June 5, 2009. The physico-chemical soil analysis and meteorological data for the growing period of the crop like average temperature, relative humidity and rainfall are presented in Table 1and 2 respectively. Data regarding compatible solutes were recorded ten days after the exogenous application of ABA. Fatty acids profile was recorded after the extraction of oil. The glycinebetaine was estimated according to the procedure mentioned by Grieve and Grattan (1983). The leaf proline was determined according to the method of Bates *et al*. (1973). The ABA concentration was determined by high performance liquid chromatography (HPLC) as described by Hansen and Dorffing (1999) and Hillman (1978). Extraction and purification for endogenous ABA was made from leaves of the control and ABA treated plants. Seven gram of plant leaves were homogenized in 80% methanol with butylated hydroxyl toluene (BHT) added

as an antioxidant. The extraction was prepared for 72h by changing of solvent after 24h. This extract was filtered by Buchner funnel. The sample was dried on rotary thin film evaporator at 35 Ċ in order to reduce it in aqueous phase. The pH of aqueous phase was adjusted to 9 and partitioned 3 times with 1/3 volume of ethyl acetate to remove basic compounds. The organic phase was removed. The aqueous phase pH readjusted to 2.50 by using 0.1 N HCl and partitioned 3 with 1/3 volume of ethyl acetate. The sample residue was dissolved in methanol and dried in oxygen free nitrogen and then again dissolved in 100% NaOH (100 µl).The ABA concentration was computed from the standard curve by using HPLC (Model 1C, A-Shimadzu Ltd. Japan, Detector SPD-6AV (Shimadzu), Colum-18, Time flow = 1ml/minute, Oven temperature = 25C°, Att. = 6, Mobile phase = Acetonytrile, wave length=254 nm).

Oil content in seeds was determined by Soxhlet Fat Extraction method (AOAC, 1990). Seeds were dried in an oven at 105° C for about 8 h. To estimate moisture content, seeds were weighed before and after drying. Two grams of achenes per thimble were ground in a coffee mill for oil content analysis. Thimbles were weighed separately, ground seeds were added and the final weight was determined. Afterwards, the thimbles were put in extractors. Six dry and clean round bottom 250 ml flasks were weighed and their weight recorded. Solvent (petroleum ether) was added to flasks, connected to the extractors and placed on heating mantles connected to with condensers.

Flasks were heated and extraction was continued for at least 6 hours, stopped extraction, removed thimbles and then reheated the flasks, so that all of the solvent could be collected in the Soxhlet extractors. The apparatus allowed to cool and flasks dried at 105 C° for 1 hr. After cooling, the flasks and oil were weighed together. Percent oil content was computed by using the following equation.

$$\% \text{ oil} = \frac{\text{Wt. of flask + oil - Wt. of flask}}{\text{Wt. of flask + seed - Wt. of flask}} \times 100$$

Nitrogen in achenes was determined according to Kjeldahl method (Bremner, 1964). One gram of each sample was transferred to the Kjeldahl flask; a digestion Tablet was added to 5 ml of concentrated H_2SO_4 and then content mixed thoroughly. The flask was placed on the digestion assembly and after that both heater and the exhaust fan were turned on. The digestion remained continue with occasional shaking of flask. When the solution became clear and all organic matter had been oxidized, then digestion was continued for another 30 minutes.

The cooled digestats was transferred to a 100 ml volumetric flask and made up its volume to 100 ml by rinsing the tubes with distilled water. Pipetted 5 ml from the volumetric flask and poured into a Markam Still Apparatus. Ten ml of NaOH (4% w/w) was added gradually through the funnel stopper (did not remove the stopper, otherwise ammonia may escape). The funnel was plugged firmly and then a few ml of the distilled water was added. Distilled it for five minutes and collected in a conical flask containing 5 ml of 2% boric acid. After 5 minutes distillation, collected the droppings from the condenser for one minute. Washed the tip of the condenser into the flask and titrated against standardized H_2SO_4. Percent crude protein was calculated using the following formula:

$$\% \text{ crude protein} = \frac{(V_1 - V_2)\, N}{100\, W} \times 14 \times 6.25 \times 100$$

Where V_1, V_2, N and W were sample titration (in ml), blank titration (in ml), normality of standardized H_2SO_4 and sample weight respectively.

Fatty acid composition in sunflower oil was determined by Gas liquid chromatography as described by Martin (1979).

The experiment was laid out in randomized complete block design (RCBD) with factorial arrangement and had three replications. Net experimental plot size was 3.0 x 5.0 m. Data about different parameters were analyzed by Fisher analysis of variance and least significant difference test at 0.05 probability level was employed to compare the differences among treatment's means (Steel *et al*., 1997)

RESULTS AND DISCUSSION

Drought stress significantly increased leaf glycinebetaine, proline, total soluble sugars and abscisic acid over the control. More increase in the same parameters was observed when drought was imposed at flower initiation than at bud initiation. Application of ABA under drought stress at bud or at flower initiation significantly increased leaf glycinebetaine, proline, total soluble sugars and abscisic acid compared to that of the control. This increase in compatible solutes and ABA content was less than drought stress employed at bud or flower initiation stage with no ABA (Table 6). Significant decrease in leaf compatible solutes and ABA content was observed when ABA applied under stress at bud initiation than in plants where no ABA was applied under the same situation (Table 6). Sunflower hybrid DK-4040 and SF-187 had statistically more leaf glycinebetaine, proline, total soluble sugars and abscisic acid than S-278. This decrease in leaf glycinebetaine content by foliar application of ABA at bud initiation under drought as compared to no ABA at same situation was 15.68% in DK-4040, 8.49% in S-278 and 14.90% in SF-187 during 2008. Similar trend was noted in 2009. Reduction in leaf proline content was 20.95% in DK-4040, 16.29% in S-278 and 20.13% in SF-187 (Table 6), in total soluble sugars was 9.31%, 15.25% and 12.02% in

DK-4040, S-278 and SF-187 (Table 6) and in abscisic acid was 54.1% in DK-4040, 59.23% in S-278 and 56.79% in SF-187 (Table 6) during 2008 and similar trend was noted during 2009. Foliar spray of ABA at flower initiation also significantly decreased leaf glycinebetaine content than no ABA application at same stage (Table 6). This reduction in leaf glycinebetaine content was 14.58% for DK-4040, 11.09% for S-278 and 9.49% for SF-187, in proline was 19.68% for DK-4040, 20.13% for S-278 and 23.89% for SF-187 (Table 6), in total soluble sugars was 12.09% for DK-4040, 17.01% for S-278 and 12.48% for SF-187 (Table 6) and in leaf abscisic acid content was 53.59% for DK-4040, 56.93% for S-278 and 55.23% for SF-187 (Table 6) during 2008. Same trend was observed during 2009.

In field crops, the accumulation of compatible solutes like glycinebetaine, proline and total soluble sugars is considered a parameter of selection against drought tolerance. The current research indicated that drought stress significantly increased leaf glycinebetaine content especially at flower initiation than at bud initiation. This occurred due to initiation of osmotic adjustment, especially in DK-4040 and SF-187 via production of compatible solute (Serraj and Sinclair, 2002) by maintaining leaf turgor pressure (Hussain *et al*., 2010), protecting functional proteins, enzymes (Rubisco) and lipids of photosynthetic apparatus and sustaining electron flow through thylakoid membrane (Allakhverdiev *et al*., 2003). So, tall (DK-4040) and dwarf (SF-187) sunflower hybrids were found more drought tolerant than intermediate one (S-278). Hussain *et al*. (2010) also reported that plants with greater ability to accumulate glycinebetaine were more drought tolerant. On the other hand, exogenous application of ABA under drought reduced leaf glycinebetaine content which was an index that ABA application helped in mitigating drought stress. This mitigation in drought stress was due to improvement in water availability to plants by conserving plant moisture. Water conservation occurred due to partial closing of stomata, reduction in transpiration, increase in root penetration and inhibition of shoot growth (Alfredo and Setter, 2000; Hoad *et al*., 2001; Saleem *et al*., 2013).

Results of the present experiment depicted that drought stress significantly increased leaf proline content and more increase was observed at budding than at flowering. The increase in leaf proline content of sunflower hybrids DK-4040 and SF-187 under water deficits was due to osmotic adjustment (Serraj and Sinclair, 2002), accumulation of higher concentration of leaf proline to enable the plant to maintain its water potential (Jalil *et al*., 2007; Sankar *et al*., 2007). Because DK-4040 and SF-187 produced more leaf proline than S-278 so they were more drought tolerant. The foliar spray of ABA reduced leaf proline content and the same was reported in genotype Nantio F1 by Unyayar *et al*. (2004). The contradictory trend was observed by Unyayar *et al*. (2004) in sunflower genotype Ozdemirbey. Decrease in leaf proline content happened as a result of contained moisture due to partial closing of stomata (Hoad *et al*., 2001).

The hybrids DK-4040 and SF-187 had significantly higher leaf total soluble sugars than S-278. Drought stress significantly increased leaf total soluble sugars and a greater increase in leaf total soluble sugars content was observed when drought stress occurred at flower initiation than at bud initiation. This increase in leaf total soluble sugars of sunflower hybrids DK-4040, SF-187 and S-278 under drought stress may have been due to production of different compatible solutes as reported by Serraj and Sinclair, (2002). These findings were also related to the results of Hussain *et al*. (2013) who indicated an improvement in leaf total soluble sugars due to drought stress in maize and alfalfa. Studies further elaborated that exogenous application of ABA under water deficits statistically decreased leaf total soluble sugars which demonstrated that ABA application enhanced the drought tolerance in sunflower hybrids by mitigating the drought stress and conserving plant tissue moisture. Foliar application of ABA helped in reducing drought stress by conserving plant moisture due to partial closing of stomata, reduction in transpiration, increase in root penetration and inhibition of shoot growth Alfredo and Setter (2000).

Sunflower hybrids DK-4040 and SF-187 had significantly higher leaf ABA content than S-278. Drought stress significantly increased leaf ABA content and a greater increase in leaf ABA content was observed when drought stress occurred at flower initiation than at bud initiation. Genotypic response to foliar application of ABA in drought stress was related to the ABA concentration (Hussain *et al*., 2010; Hussain *et al*., 2014). Genotypic variation in accumulation of ABA under drought was observed in sunflower hybrids (Hussain *et al*., 2013). In water deficit leaves high concentrations of ABA came to normal level after one day of water application which recovered plant growth and development. This response of plants helped them to adapt under periodic drought and normal irrigation / rainfall. Performance of Sunflower hybrids DK-4040 and S-278 was better under drought, hence might have ability to adapt well under drought stress by maintaining ABA concentration to such a level that growth affected least and resumed after irrigation. Exogenous application of ABA had non-significant effect on leaf ABA content under well watered condition but it enhanced endogenous leaf ABA content under drought. Similar response was also noted by Borel and Simonneau, (2001), they further reported that sometimes leaf ABA content remained half of the applied ABA but it might depend upon differences in leaf size and sap volume extracted for ABA content analysis. Higher concentration of ABA applied to plants,

caused more accumulation of ABA which ultimately increased its metabolism and decreased its concentration in leaves (Jia and Zhang, 1999).

Drought stress showed significant decrease in achene oil content and this decrease was further pronounced when drought employed at flower initiation than at bud initiation. Maximum achene oil content (39.33%) was present in sunflower hybrid DK-4040 than S-278 and SF-187. Although, exogenous application of ABA at bud initiation showed increase in achene oil content but it was statistically non-significant with no ABA application at same stage. This increase was 1.54% in DK-4040, 0.81% in S-278 and 0.93% in SF-187 during 2008. Similar observations were recorded during both years of study (Table 4). Exogenous application of ABA at flower initiation also increased achene oil content but it was statistically non-significant with no ABA application at same stage. This increase was 0.69% in DK-4040, 0.46% in S-278 and 1.83% in SF-187 (Table 4) during first year of study. Same trend was noted during 2008 and 2009.

Genotypic differences in achene oil content were observed among sunflower hybrids. Achene oil content of sunflower hybrids was reduced under drought either at budding or at flowering. Water shortage at flowering caused larger reduction in achene oil content than at budding. Drought stresses applied before reproductive stage (Daneshian *et al*., 2005; Saleem *et al*., 2013), at flowering stage (Hammadeh *et al*., 2005) and during seed filling to sunflower reduced achene oil content. Contradictory seed oil content showed stability under increasing drought stress conditions (Khan *et al*., 2000). Razi and Asad, (1999) had also observed no change in achene oil content under drought. Exogenous application of ABA under drought at budding or at flowering statistically improved achene oil content. Achene oil content might be increased by exogenous application of ABA as its application led to partial stomatal closure, decreased transpiration, inhibited shoot growth and enhanced root penetration which might have increased availability of water for oil synthesis and ultimately achene oil content increased.

Drought stress significantly increased achene protein content and more increase in achene protein content observed when stress imposed at flower initiation than at bud initiation. Sunflower hybrid DK-4040 had maximum achene protein content than S-278 and SF-187. Significant decrease in achene protein content was observed when ABA was applied under stress at bud initiation than in crop where no ABA was applied under stress at bud initiation. This decrease in achene protein content was 5.13% in DK-4040, 6.35% in S-278 and 4.47% in SF-187 (Table 4) during 2008. Similar trend was observed during both years of study. ABA spray at flower initiation also significantly decreased achene protein content than no ABA spray at same stage. This reduction in achene protein content was 4.53% for DK-4040, 3.32% for S-278 and 2.93% for SF-187 during first year of study (Table 4). Similar observations were recorded during both years of study. Giving drought stress at flower initiation and exogenous application of ABA at same stage showed statistically similar response with stress at bud initiation and no ABA application at same stage. Similar response was shown by DK-4040, S-278 and SF-187 during both years of study.

Drought at flowering stage of sunflower improved achene protein content and similar results were indicated by Reddy *et al*. (2003). This increase was due to decreased in oil content as both had negative relationship to each other (Debaek *et al*., 1998). Natali *et al*. (2003) noted that in sunflower accumulation of LEA (late embryogenesis abundant) proteins increased under water deficit conditions which improved the protein contents and also protect the plant cell from dehydration. ABA application under drought stress decreased the achene protein contents because it mitigate the adverse impact of drought. Labhilili *et al*. (1995) found that due to exogenous application of ABA under water deficit conditions dehydrin protein accumulated in the vegetative tissues of sunflower hybrids which protect the cytoplasmic structures from dehydration (Close *et al*., 1996). This discussion further highlighted the better drought tolerance capability of sunflower hybrids DK-4040 and SF-187.

Drought stress significantly decreased palmitic and linoleic acid and decrease observed when drought stress imposed at flower initiation than at bud initiation. Sunflower hybrid SF-187 had maximum palmitic acid as compared to DK-4040 and S-278 while DK-4040 had maximum linoleic acid as compared to S-278 and SF-187 (Table 4). Significant improvement in palmitic and linoleic acid was observed when ABA was applied under stress at bud initiation than in crop where no ABA was applied under stress at bud initiation. This improvement in palmitic acid was 1.53% in DK-4040, 2.47% in S-278 and 1.09% in SF-187 and in linoleic acid was 0.27%, 0.30% and 0.56% for DK-4040, S-278 and SF-187, respectively during 2008. Exogenous application of ABA at flower initiation also significantly improved palmitic acid content than no ABA application at same stage. This increase in palmitic acid was 1.57% for DK-4040, 1.09% for S-278 and 0.48% for SF-187 and linoleic acid was 0.21% for DK-4040, 0.30% for S-278 and 0.29% for SF-187 (Table 5). Similar trend was observed during both years of study. Drought stress significantly increased stearic and oleic acid and an increase was observed when drought stress was imposed at flower initiation than at bud initiation (Table 5). Stearic and oleic acid in sunflower hybrids was significantly decreased with the application of ABA under drought stress at bud initiation than no ABA under similar condition. This decrease in stearic acid was 6.66%, 3.78% and 1.34% for DK-4040,

S-278 and SF-187 respectively, while in oleic acid was 0.15%, 0.42% and 0.93% for DK-4040, S-278 and SF-187 (Table 5), respectively during 2008. Spray of ABA under drought at flower initiation also significantly decreased stearic acid than no ABA application at same stage. This reduction in stearic acid was 0.95% for DK-4040, 1.43% for S-278 and 1.09% for SF-187 and in oleic acid was 0.15%, 0.42% and 0.93% for DK-4040, S-278 and SF-187 (Table 5), respectively during 2008. Parallel trend was observed during both years of study.

Genotypic variation in fatty acid profile was observed among sunflower hybrids. Results depicted that sunflower hybrid SF-187 had more amount of palmitic acid, stearic acid and oleic acid as compared to sunflower hybrids DK-4040 and S-278 while hybrid DK-4040 had more linoleic acid. Drought stress at budding and at flowering slightly increased stearic acid and oleic acid while palmitic acid and linoleic acid decreased. More increase was observed in stearic acid when drought stress occurred at flowering while similar response was noted in oleic acid in crop that faced drought at budding. Palmitic and linoleic acids decreased when water deficits occurred at budding or at flowering but more reduction in both fatty acids was observed when stress was given at flowering. Flagella *et al*. (2002) had also reported a decrease in stearic acid and oleic acid and increase in palmitic acid and linoleic acid in sunflower under irrigation. Contradictory, in sunflower oleic acid reduced and palmitic acid increased under water deficits. Exogenous application of ABA either at budding or at flowering under water deficits slightly decreased stearic acid and oleic acid while its application slightly improved palmitic acid and linoleic acid. This reduction in stearic and oleic acid and improvement in palmitic and linoleic acid reflected that ABA was helpful in mitigating the adverse effects of drought by improving water availability to plants. Water availability might be improved by conserving plant moisture due to partial closing of stomata, reduction in transpiration, increase in root penetration and inhibition of shoot growth (Alfredo and Setter, 2000; Hoad *et al*., 2001; Saleem *et al*., 2013).

Drought stress significantly decreased achene yield and decrease in achene yield observed when stress imposed at flower initiation than at bud initiation. Achene yield in sunflower hybrids was significantly increased with the application of ABA under drought stress at bud initiation than no ABA under similar condition (Table 5). This increase was 48.12% in DK-4040, 46.03% in S-278 and 41.55% in SF-187 during 2008 and 48.09% for DK-4040, 46.07% for S-278 and 40.78% for SF-187 during 2009 (Table 5). Foliar application of ABA at flower initiation also significantly increased achene yield than no ABA application at same stage. This improvement in achene yield was 102.75% for DK-4040, 61.36% for S-278 and 66.68% for SF-187 during 2008 and 102.79% in DK-4040, 61.41% in S-278 and 63.97% in SF-187 (Table 5) during 2009.

Meaningful orthogonal contrasts 4 irrigations vs 3 irrigations and ABA vs no ABA for leaf glycinebetaine**,** proline, total soluble sugars, abscisic acid, achene oil content, protein content, stearic acid and achene yield were significant while palmitic, oleic and linoleic acid were non-significant (Table 3). These meaningful orthogonal contrasts compared the combination of treatments like 4 irrigations vs 3 irrigations and ABA application vs no ABA application and their impacts on biochemical parameters and achene yield. Application of 4 or 3 irrigations as well as ABA or no ABA did not significantly affect the concentration of palmitic, oleic and linoleic acid. So water shortage only affect the leaf contents of leaf glycinebetaine**,** proline, total soluble sugars, abscisic acid, achene oil content, protein content, stearic acid and achene yield. Meaningful orthogonal contrasts provided information which were helpful in achieving focus objective of current study to determine whether the full or limited irrigation and exogenous application of ABA under this situation could alter the amount of compatible solutes, fatty acid profile and yield of sunflower hybrids.

Achene yield of sunflower hybrids was significantly different under drought and normal irrigation. Maximum achene yield (3551.17 $kgha^{-1}$) was noted in DK-4040 compared to SF-187 (3443.23 $kgha^{-1}$) and S-278 (3021.55 $kgha^{-1}$). Bakht *et al*. (2006) also noted more achene yield in sunflower hybrid DK-4040 than SF-187. Divergent achene yield of sunflower hybrids was also observed by Bakht *et al*. (2006). Water deficits to sunflower hybrids at flowering reduced achene yield than at budding. Timing of drought stress was more crucial than its intensity. Limited water application to sunflower at heading, flowering and milking reduced achene yield and reduction in yield occurred when drought occurred at flowering in sunflower (Daneshian *et al*., 2005), in rape seed and mustard (Rad *et al*., 2005) and in soybean (Brevedan and Egli, 2003). This reduction in achene yield of sunflower hybrids under drought might be occurred due to decrease in head diameter (Daneshian *et al*., 2005; Hussain *et al*., 2000), number of achenes per head (Liu *et al*., 2004) and 1000 achene weight (Daneshian *et al*., 2005) which finally decreased yield. Foliar application of ABA to sunflower hybrids under drought at budding produced more achene yield than ABA applied at flowering. This improvement in achene yield may have been due to the ability of ABA to regulate water loss in the plant through partial closure of stomata, reduction in leaf development (Hussain *et al*., 2014), enhance plant tolerance against cellular dehydration increasing root penetration and increased water use efficiency (Heschel and Hausmann, 2001) which might finally increase translocation of assimilates to achenes. In jewelweed exogenous application of ABA affected water

use efficiency and more increase in water use efficiency was observed in drought plants than that of well-watered plants (Heschel and Hausmann, 2001).

Table 1. Pre-sowing analysis of research field

	Determination	Unit	Mean Value	
			2008	2009
A	Physical Analysis			
	Sand	%	66.6 ±0.999	64.5 ±0.866
	Silt	%	16.6 ±0.624	18.5 ±0.693
	Clay	%	16.8 ±0.608	17 ±0.520
	Textural class		Sandy loam	
B	Chemical Analysis			
	pH		8.2 ±0.265	8.00 ±0.265
	EC	dS m^{-1}	1.37 ±0.026	1.42 ±0.040
	Organic matter	%	0.74 ±0.030	0.70 ±0.026
	Total Nitrogen	%	0.046 ±0.003	0.047 ±0.002
	Available Phosphorus	mg kg^{-1}	6.52 ±0.082	6.58 ±0.130
	Available Potassium	mg kg^{-1}	171 ±3.606	170 ±5.568

+ Means and standard deviation values from triplicate samples

Table 2. Metrological data during cropping period

Month	Minimum Temperature (Ċ)		Maximum Temperature (Ċ)		Relative Humidity (%)		Rainfall (mm)	
	2008	**2009**	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**
February	13.14	16.02	17.23	18.54	37.34	64.14	6.8	18.2
March	22.72	20.77	26.85	23.99	37.84	53.52	0	14
April	26.03	26.25	30.52	31.11	33.57	41.67	16	22.9
May	30.76	32.43	36.89	35.98	30.16	31.42	75.5	9.11
June	32.92	33.84	39.21	38.67	48	33.6	41.7	9.6

Table 3. Meaningful orthogonal contrasts

	4 I vs 3 I		ABA vs no ABA	
Parameters	2008	2009	2008	2009
Glycinebetaine	*	*	*	*
Proline	*	*	*	*
Total soluble sugars	*	*	*	*
Abscisic acid	*	*	*	*
Achene oil content	*	*	*	*
Protein content	*	*	*	*
Stearic acid	*	*	*	*
Palmitic acid	ns	Ns	ns	Ns
Oleic acid	ns	ns	ns	Ns
linoleic acid	ns	ns	ns	ns
Achene yield	*	*	*	*

4 I, four irrigations; 3 I, three irrigations; AB, abscisic acid spray at bud initiation; vs, versus; *, significant; ns, non-significant; ABA, abscisic acid

Table 4. Role of exogenous application of abscisic acid on quality of sunflower hybrids

Hybrids	Treatments	Achene oil content (%)		Achene protein content (%)		Palmitic acid (%)		Linoleic acid (%)	
DK-4040		2008	2009	2008	2009	2008	2009	2008	2009
	C	39.33a	39.91a	22.67d	22.99d	6.12a	6.19a	77.62a	77.67a
	SBNA	37.58b	38.12b	24.95b	25.30b	5.78c	5.85c	75.73c	75.94b
	SBA	38.16b	38.71b	23.67c	24.01c	5.87b	5.92b	75.85b	75.93b
	SFNA	35.94c	36.46c	26.00a	26.370a	5.63e	5.69e	75.59d	75.67c
	SFA	36.19c	36.71c	24.82b	25.17b	5.72d	5.81d	75.68c	75.74d
S-278	C	37.32a	37.86a	20.57d	20.86d	5.88a	5.97a	74.15a	74.26a
	SBNA	35.68b	36.20b	22.81b	23.13b	5.51c	5.58c	73.67c	73.79c
	SBA	35.97b	36.49b	21.36c	21.66c	5.65b	5.74b	73.79b	73.86b
	SFNA	34.29c	34.79c	24.04a	24.37a	5.43d	5.49d	73.49e	73.58e
	SFA	34.45c	34.94c	23.24b	23.57b	5.49c	5.56c	73.61d	73.68d
SF-187	C	38.90a	39.47a	21.53d	21.83d	6.43a	6.49a	76.12a	76.21a
	SBNA	37.36b	37.90b	23.26b	23.59b	6.08e	6.15d	74.78c	74.83c
	SBA	37.71b	38.26b	22.22c	22.53c	6.25b	6.28b	75.01b	75.08b
	SFNA	34.86c	35.36c	24.19a	24.53a	6.13cd	6.19c	74.41e	74.43e
	SFA	35.51c	36.03c	23.48b	23.81b	6.16c	6.28b	74.53d	74.55d
LSD (5%)		0.696	0.708	0.624	0.634	0.038	0.026	0.057	0.031

C, control (no abscisic acid application); SBNA, stress at bud initiation and no abscisic acid application; SBA, stress at bud initiation and abscisic acid application; SFNA, stress at flower initiation and no abscisic acid application; SFA, stress at flower initiation and abscisic acid application; LSD, least significant differences; Mean values sharing the same letter in a column do not differ significantly at P= 0.05.

Table 5. Role of exogenous application of abscisic acid on quality and achene yield of sunflower hybrids

Hybrids	Treatments	Stearic acid (%)		Oleic acid (%)		Achene Yield (Kgha^{-1})	
DK-4040		**2008**	**2009**	**2008**	**2009**	**2008**	**2009**
	C	3.83e	3.87e	12.11e	12.09d	3325.64a	3551.17a
	SBNA	4.18c	4.07c	14.23c	14.11c	1923.34c	2052.08d
	SBA	3.92d	3.98d	14.16d	14.09c	2801.35b	2992.87b
	SFNA	4.25a	4.18a	14.36a	14.28a	1309.67d	1395.12e
	SFA	4.21b	4.13ab	14.29b	14.21b	2553.65b	2727.59c
S-278	C	3.87e	3.81e	11.13e	11.98e	2827.97a	3021.55a
	SBNA	4.11c	4.06c	11.48c	11.39c	1684.96d	1796.69d
	SBA	3.96d	3.87d	11.29d	11.22d	2415.08b	2578.96b
	SFNA	4.25a	4.15a	11.68a	11.62a	1223.25e	1302.66e
	SFA	4.19ab	4.12b	11.58b	11.49b	1913.19c	2041.92c
SF-187	C	4.21e	4.14d	13.13e	13.02e	3221.58a	3443.23a
	SBNA	4.52c	4.42b	14.61c	14.60c	2038.34d	2175.73d
	SBA	4.46d	4.38c	14.16d	14.12d	2844.32b	3022.76b
	SFNA	4.63a	4.52a	14.82a	14.76a	1432.07e	1632.75e
	SFA	4.58b	4.51a	14.71b	14.65b	2488.98c	2613.89c
LSD (5%)		0.029	0.027	0.022	0.040	49.051	63.369

C, control (no abscisic acid application); SBNA, stress at bud initiation and no abscisic acid application; SBA, stress at bud initiation and abscisic acid application; SFNA, stress at flower initiation and no abscisic acid application; SFA, stress at flower initiation and abscisic acid application; LSD, least significant differences; Mean values sharing the same letter in a column do not differ significantly at P= 0.05.

Table 6. Role of exogenous application of abscisic acid on compatible solutes and ABA of sunflower hybrids

Hybrids	Treatments	GB (umol g^{-1} d. wt.)		Pro (umol g^{-1} f. wt.)		TSS (mg g^{-1} d. wt.)		ABA (µmol g^{-1} f. wt.)	
DK-4040		2008	2009	2008	2009	2008	2009	2008	2009
	C	14.32e	10.92d	4.34d	4.25d	76.59e	74.99e	0.16e	0.16e
	SBNA	20.02b	16.45a	7.30a	7.61a	97.17b	96.95b	0.78d	0.75d
	SBA	16.88d	14.31c	5.77c	5.57c	88.12d	85.36d	1.70b	1.69b
	SFNA	21.25a	17.24a	6.91b	6.35b	102.25a	98.39a	0.84c	0.83c
	SFA	18.15c	15.25b	5.55c	5.25c	89.87c	87.96c	1.81a	1.79a
S-278	C	11.40d	7.96c	4.03e	3.92e	61.81d	59.28e	0.10d	0.10d
	SBNA	17.53b	14.23a	6.20a	6.10b	84.18b	81.50b	0.53c	0.53c
	SBA	16.04c	12.62b	5.19c	4.81c	71.34c	67.25c	1.30b	1.29b
	SFNA	18.65a	14.42a	5.96b	6.47a	86.16a	85.05a	0.59c	0.59c
	SFA	16.58bc	13.27b	4.76d	4.37d	71.50c	73.24d	1.37a	1.36a
SF-187	C	12.57d	9.70d	4.35e	4.16e	73.06e	69.32e	0.14e	0.14e
	SBNA	19.73ab	16.45ab	7.06a	6.78a	94.23b	92.14b	0.70d	0.69d
	SBA	16.79c	13.70c	5.59c	5.29c	82.92d	80.47d	1.62b	1.61b
	SFNA	20.74a	17.24a	6.78b	6.22b	97.93a	95.45a	0.77c	0.76c
	SFA	18.77b	15.64b	5.16d	4.98d	85.35c	82.92c	1.72a	1.71a
LSD (5%)		1.048	0.925	0.278	0.348	1.253	1.170	0.065	0.064

GB: glycinebetaine, PRO: proline, TSS: total soluble sugars, ABA: abscisic acid, C: control (no abscisic acid application), SBNA: stress at bud initiation and no abscisic acid application, SBA: stress at bud initiation and abscisic acid application, SFNA: stress at flower initiation and no abscisic acid application, SFA: stress at flower initiation and abscisic acid application, LSD: least significant differences: Mean values sharing the same letter in a column do not differ significantly at P= 0.05.

Conclusion: Water deficit at critical growth stages has pronounced effects on biochemical parameters of sunflower. Drought stress to sunflower hybrids at bud or at flower initiation increased protein contents, compatible solutes, stearic and oleic acid and decreased achene yield, oil contents, palmitic and linoleic acid while exogenous application of ABA under water deficit at both stages improved oil quality and yield of drought tolerant sunflower hybrids DK-4040 and SF-187.

It was advised that ABA application is of assistance to tolerate adverse effects induced by drought and improves the achene oil quality, leaf compatible solutes and yield of sunflower.

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