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YIELD AND QUALITY IMPROVEMENT OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) HYBRID THROUGH ABA APPLICATION UNDER WATER DEFICIT CONDITIONS

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ABSTRACT

Abscisic acid (ABA) application helps in improving sunflower oil quality and yield through ameliorating the adverse effects of limited water supply at different growth stages of sunflower hybrid. Improvement in yield and quality of sunflower hybrid by exogenous application of ABA under drought was studied through field experiment executed at the Agronomic Research Farm, University of Agriculture, Faisalabad, Pakistan. Three irrigation schedules i.e. four irrigations (25 DAS, bud initiation, flower initiation and achene formation), three irrigations (25 DAS, flower initiation and achene formation) and three irrigations (25 DAS, bud initiation, and achene formation) were used. Sunflower hybrid hysun-33 was exposed to ABA concentrations (0, 5µm and 10µm) at bud initiation or at flower initiation. ABA application to sunflower hybrids either at bud or at flower initiation under water deficit conditions improved achene oil content, yield and decreased achene protein contents. Drought stress and ABA application exhibited inverse results for oil quality and yield. Drought stress to sunflower hybrid 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. It was concluded that exogenous application of ABA to sunflower under drought improved quality and increased crop yield.

Key words: Water deficits, abscisic acid, hybrid sunflower, oil quality and yield.

INTRODUCTION

Edible oilseed crops in Pakistan can be classified as traditional (rapeseed, mustard, groundnut, sesame, cotton and non-traditional (sunflower, soybean and safflower). Rapeseed and mustard oil is not regular cooking oil due to the presence of higher concentrations of erucic acid and hence, can't be used more than 5% in oil blending for ghee manufacturing. Among the non-traditional oilseed crops sunflower can play an important role narrowing the wide gap between production of edible oils in the country and its import (Khan *et al*. 2000)

Plants are under periodic water stress due to unpredictable rainfall and limited availability of irrigation water. They have certain mechanisms which allow them to perceive the incoming stresses and rapidly regulate their physiology and metabolism to cope with these stresses. A good example of such mechanisms is the ability of plants to regulate their water loss through partial closure of stomata and reduced leaf development (Davies and Zhang. 1991).

The plant hormone ABA is produced de novo under water deficit conditions and plays a major role in response and tolerance to dehydration. ABA plays a critical role in regulating plant water status through guard cells and growth as well as by induction of genes that encode enzymes and other proteins which create cellular dehydration tolerance in field crops (Luan, 2002). It has been shown that leaf conductance is closely related to xylem ABA concentration. Closure of plant stomata depends upon the amount of xylem ABA that enters in leaves per unit time i.e. ABA flux (Jarvis and Davies., 1997). This implies that ABA is helpful to regulate stomatal movement under normal conditions due to changes in transpiration under non stressed conditions, or that ABA's role is magnified after the soil drying has led to the very low transpiration.

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 (Kafale and Ranamukhaarachchi, 2006; Li Ping *et al*., 2006; Hussain *et al*., 2012) Availability of water significantly affect 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 (Baldini *et al*., 2002). Drought has significant effect on achene oil and protein content. Achene oil content decreased (Daneshian *et al*., 2005) while protein content increased under water deficit conditions (Reddy *et al*., 2003). The major effect of irrigation was an increase of linoleic acid content and

reduction in oleic acid. However oleic/linoleic acid ratio increased under water stress, especially when plants were subjected to soil water stress during elongation stage in standard genotypes (Flagella *et al*., 2002). Santonoceto *et al*. (2003) observed higher oleic acid content of sunflower seed with increased availability of water which ultimately increased the oleic/linoleic acid ratio.

Contrasting reports about the oil quality of sunflower under well watered and drought conditions and least information about the effect of exogenous application of ABA under well watered and drought situation on protein, oil contents 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 quality and yield of sunflower hybrids.

MATERIALS AND METHODS

Improvement in yield and quality of sunflower hybrid by exogenous application of ABA under drought was studied through field experiment executed at the Agronomic Research Farm, University of Agriculture, Faisalabad, Pakistan. The meteorological data were collected from the Agro-meteorological cell, Department of Crop Physiology, University of Agriculture. Faisalabad. Pakistan. The experiment was laid out in randomized complete block design (RCBD) with factorial arrangement and replicated thrice. Sunflower hybrid Hysun-33 was sown on 17th and 12th of February, 2008 and 2009 respectively. Hybrid seed was planted on ridges with the help of dibbler by using seed rate of 8 kg ha $^{-1}$. Ridges were made at 75 cm apart and after thining25 cm plant-to-plant distance was maintained. Urea and diammonium phosphate (DAP) were used to get 150 kg N and 100 kg P_2O_5 ha^{-1}. Half nitrogen and full dose of phosphorus were given at sowing; remaining nitrogen was applied with 1st irrigation. Weighted quantity of ABA (-cis-trans ABA) Sigma Aldrich, Japan was added in a graduated cylinder and I L volume was made in volumetric flask by adding 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.

First irrigation was applied at 4-6 leaf stage [25 days after sowing (DAS)], 2nd irrigation at bud initiation stage (45 DAS) except the plots which were subjected to water stress at this stage, 3rd irrigation at flower initiation stage (67 DAS) except the plots subjected to water stress at this stage and 4th irrigation to all plots at achene formation stage (90 DAS). The effect of application of three ABA levels (0, 5µM, 10µM) at two growth stages (bud initiation and flower initiation) under three irrigation regimes (no drought stress, drought stress at bud initiation and drought stress at flower initiation) was studied.

Oil content in seeds was measured by Soxhlet Fat Extraction method (AOAC, 1990). Seeds were dried in an oven at 105^{o} C for about 8 hours. For estimation of moisture content, achenes weighted before and after drying. Two gram of achenes per thimble was ground in a coffee mill. 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. Solvent (petroleum ether) was added to flasks, connected to the extractors and placed on heating mantles connected with condensers. Flasks were heated and extraction was continued for 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^{o} for 1 hr. After cooling, the flasks and oil were weighed together. Percent oil content was calculated by putting the values in the following equation.

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

Achenes protein content was determined according to Kjeldahl method (Bremner, 1964). 1g 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 was mixed thoroughly. The flask was placed on the digestion assembly and 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. Cooled digestats was transferred to a 100 ml volumetric flask and its volume was made to 100 ml by rinsing the tubes with distilled water. Pipetted 5 ml from the volumetric flask and poured into a Markam Still Apparatus. 10 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 few ml of the distilled water was added. Distilled it for 5 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 = Sample titration (in ml)
V_2 = Blank titration (in ml)
N = Normality of standardized H_2SO_4
W = Sample weigh

Fatty acid composition in sunflower oil was determined by Gas liquid chromatography as described by Martin (1979). A Rancy oil seed crusher was used for preparation of oil. One ml methylating solution, 0.5 ml petroleum ether and a loop of oil was added in 10 x 15 mm test tube. Loop was swirled in the solution for dispersing and also rinsed loop with petroleum ether between sampling. The test tube was capped and the solution mixed for 30 minutes at room temperature. 1 ml distill water was added to the tube; it was mixed and waited for 10 minutes. One µl of the upper layer solution was injected into the gas chromatograph. An electronic integrator was used to compute the total area of the peaks and area of each fatty acid peak was expressed as percentage of the total area of the peak.

The data regarding different plant parameters were pooled over years and analyzed statically with Fisher Analysis of Variance Technique and LSD test at 0.05 probability was used to measure the difference among means of treatments (Steel *et al*., 1997).

RESULTS AND DISCUSSION

Achene oil content (%): Impact of exogenous application of ABA was divergent under different irrigation schedules (Table 2). Providing no drought stress at either growth stage, exogenous application of ABA decreased achene oil content during both years. Under drought spraying of 5µM ABA at bud initiation statistically increased achene oil content compared to the control. Giving drought stress at bud initiation and spraying 10µM ABA also improved achene oil content over that of the control but this improvement was statistically similar with application of 5µM ABA at bud initiation. When crop faced drought stress at bud initiation but ABA (5 or 10µM) was applied at flower initiation, it significantly decreased achene oil content compared to the control. Similar observation was recorded during both years of study. By imposing drought stress at flowering and applying 5µM ABA at the same stage statistically improved achene oil content over that of the control. Although exogenous application of 10µM ABA under drought at flower initiation significantly increased achene oil content but it was statistically similar with control and the application of 5µM ABA under same situation during both years. During 2009 almost similar results were found (Table 2). Drought stresses implied before reproductive stage (Daneshian *et al*., 2005), at flowering stage (Hammadeh *et al*., 2005) and during seed filling of sunflower deteriorate oil content. Contradictory seed oil content showed stability under increasing drought stress conditions (Khan *et al*., 2000). Achene oil content might be increased by exogenous application of ABA as its application led to inhibit shoot growth and enhanced root penetration (Alfredo and Setter, 2000) which ultimately increased moisture availability for oil synthesis in achene and achene oil content increased.

Achene protein content (%): The role of ABA application was different under different irrigation schedules (Table 2). Under no drought exogenous application of ABA at bud or flower initiation stage statistically increased achene protein content over that of the control during both years of study. When crop faced drought stress at bud initiation, application of 10µM ABA at flowering resulted in maximum achene protein content increased but it was statistically similar with exogenous application of 5µM ABA at flower initiation under same situation during both years. When crop faced drought stress at flower initiation stage, control and exogenous application of 5 or 10µM ABA at either stage showed statistically non significant effect on achene protein content during both years of study (Table 2). Reddy *et al*. (2003) pointed out that imposition of drought stress at flowering drastically reduced achene yield but improved achene protein content. This increase in protein content was due to negative relationship between oil and protein content (Debaeke *et al*., 1998) and accumulation of LEA (late embryogenesis abundant) protein like dehydrin in white spruce (*Picea glauca*) (Richard *et al*., 2000) and in sunflower (Natali *et al*., 2003). These proteins protected plant cells from damage under drought stress. This inverse relationship is due to more production of proteins under drought stress and less transformation of metabolites to oil which ultimately decreased oil yield of sunflower hybrid. Exogenous application of ABA decreased achene protein content; ABA might have helped in reducing drought stress by conserving plant moisture due to partial closing of stomata, reduction in transpiration, inhibition of shoot growth and enhancement of root penetration in soil (Hoad *et al*., 2001).

Fatty Acid Profile

Stearic and Oleic acid: Role of exogenous application of ABA was different under different irrigation schedules (Table 2). Under no drought stress, exogenous application of ABA significantly increased stearic and oleic acid during both years of study and maximum content were recorded with the spray of 10µM ABA at flower initiation stage. Exogenous application of 10µM ABA at flower initiation by imposing stress at bud initiation significantly improved stearic and oleic acid than all other treatments. While applying 5 or 10µM ABA at bud initiation under drought stress at bud initiation decreased stearic and oleic acid over that of the control. Same trend was observed during both years. Exogenous application of 10µM ABA at bud initiation after employing drought at flower initiation significantly increased stearic and oleic acid than all other treatments during 2008 and similar response was also shown in

2009. Exogenous application of 5µM ABA under same situation also increased stearic and oleic acid but lesser than application of 10µM ABA. Giving drought stress at flower initiation and applying ABA (5 or 10µM) at same stage significantly decreased stearic and oleic acid over that of the control. Similar trend was noted during both years of study (Table 2).

Palmitic and Linoleic acid: Role of exogenous application of ABA was divergent under different irrigation schedules (Table 3). Under no drought stress exogenous application of ABA decreased palmitic and linoleic acid during both years of study. By spraying of 5µM ABA under drought at bud initiation statistically increased palmitic and linoleic acid compared to the control. Giving drought stress at bud initiation and spraying 10µM ABA also improved palmitic and linoleic acid over that of the control but this improvement was lesser than application of 5µM ABA at bud initiation. When crop faced drought stress at bud initiation but ABA (5 or 10µM) was applied at flower initiation, it significantly decreased palmitic and linoleic acid compared to the control. Similar observation was recorded during both years of study.

Imposing drought stress at flower initiation and applying 5µM ABA at the same stage statistically increased palmitic and linoleic acid over that of the control. Although exogenous application of 10µM ABA under drought at flower initiation significantly increased palmitic and linoleic acid over that of the control but this increase was lesser than with the application of 5µM ABA under same situation during both years. Drought stress at flower initiation and application of 5 or 10 µM ABA at bud initiation significantly reduced palmitic and linoleic acid compared to that of the control during 2008. The decrease in linoleic acid was statistically similar with control when 5µM ABA applied at bud initiation during 2009 (Table 3). Fatty acid profile was also affected by drought and exogenous application of ABA under drought or no drought stress. Flagella *et al*. (2002) had also reported 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 drought stress. 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 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 (Hoad *et al*., 2001).

Achene yield (kg ha $^{-1}$): Drought stress statistically decreased achene yield compared to no stress. Achene yield was significantly decreased when drought stress was imposed at flower initiation and this decrease was more than when stress was employed at bud initiation. ABA helped in improving achene yield under water deficits conditions. Providing no drought stress at either growth stage, exogenous application of ABA decreased achene yield during both years (Table 3). Drought stress at bud initiation and spraying by 5µM ABA to crop at same stage statistically increased achene yield over that of the control. Imposition of drought stress at bud initiation and spraying 10µM ABA also improved achene yield over that of the control but this improvement was lesser than an application of 5µM ABA at bud initiation. When the crop faced drought stress at bud initiation but ABA (5 or 10µM) was applied at flower initiation, it significantly decreased achene yield compared to the control. Similar observation was recorded during both years of study (Table 3). By imposing drought stress at flowering and applying 5µM ABA at the same stage statistically improved achene yield over that of the control. 10µM ABA application under drought at flower initiation significantly increased achene yield over that of the control but this increase was lesser than that of application of 5µM ABA under same situation during both years. Drought at flower initiation and exogenous application of 5 or 10µM ABA at bud initiation significantly decreased achene yield over that of the control. Same trend was observed during both years of study (Table 3). Physiological and biochemical features of plant changed under drought (Keyvan, 2010). Present research highpoint that drought stress at budding and flowering statistically decreased achene yield and its components in sunflower (Ardakani *et al*., 2005). Drastic effects of water shortage were more pronounced at flowering than at budding (Demir *et al*., 2006; Din *et al*., 2011; Hussain *et al*., 2012). Foliar application of ABA under control (no water stress) significantly reduced the achene yield while its application under drought stress at bud and flower initiation stage improved achene yield. Stress hormone ABA played important role in dehydration tolerance by facilitating penetration of roots in soil of maize (Hartung *et al*., 1994) which ultimately increased assimilates production and yield through improving water relation and osmotic adjustment in plants (Hussain *et al*., 2010). Contradictory Ayub *et al*. (2000) reported that exogenous application of ABA under drought to *Vigna radiate* L. showed non-significant response to seed yield.

It was observed that three irrigations (25 DAS, at flower initiation and at achene formation),when 5µM ABA was applied at bud initiation under drought resulted in more profitable as compared to exogenous application of same concentration of ABA at flower initiation. So, it is suggested that three irrigations (25 DAS, at flower

initiation and at achene formation), with foliar spray of 5µM ABA should be applied at bud initiation under drought. The Dominance and Marginal analysis revealed that under drought stress application of 5µM ABA at bud initiation increased percent MRR (marginal rate of return) as compared to exogenous application of same concentration of ABA at flower initiation. Sunflower production under water deficits conditions due to limited water availability could be more benefited when farmer applied 5µM ABA at bud initiation. The increase in net benefits and MRR under drought and application of 5µM ABA at bud initiation was due to increase in achene yield.

Table 1. Pre-sowing analysis of research field

	Determination	Unit	Value	
			2008	2009
A	Physical Analysis			
	Sand	%	65.6	65.3
	Silt	%	16.8	17.5
	Clay	%	17.6	17.2
	Textural class		Sandy clay loam	
B	Chemical Analysis			
	pH		7.94	8.41
	EC	dS m^{-1}	1.45	1.38
	Organic matter	%	0.69	0.73
	Total Nitrogen	%	0.051	0.049
	Available Phosphorus	mg kg^{-1}	5.93	6.28
	Available Potassium	mg kg^{-1}	173	171

Table 2. Interactive effects of irrigation schedules and exogenous application of ABA on achene yield and quality of sunflower

	Achene oil content (%)		Achene protein content (%)		Stearic acid (%)		Oleic acid (%)	
Treatments	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**
				No water stress				
CL	41.24a	41.97a	21.86d	21.25c	3.39e	3.31e	11.53de	11.43c
AB1	40.49b	40.55b	22.62c	21.61bc	3.49c	3.38c	11.67b	11.61a
AF1	40.20b	40.38c	22.99bc	21.99b	3.53b	3.46b	11.56d	11.42c
AB2	40.04b	40.18c	23.42ab	22.01b	3.44d	3.32d	11.73a	11.61a
AF2	40.11b	39.95d	23.95a	22.95a	3.58a	3.48a	11.62c	11.51b
				Water stress at bud initiation				
CL	39.00b	39.39b	24.59c	23.52bc	3.64c	3.51c	11.78c	11.72b
AB1	39.79a	40.15a	24.14c	23.08c	3.45e	3.29e	11.69e	11.52d
AF1	38.48c	38.68c	24.98ab	23.89ab	3.71b	3.68d	11.82b	11.71b
AB2	39.41ab	39.65ab	24.29bc	23.19c	3.52d	3.46d	11.74d	11.64c
AF2	38.12c	38.82c	25.30a	24.21a	3.91a	3.75a	11.91a	11.77a
				Water stress at flower initiation				
CL	38.25b	38.28bc	25.64a	24.61a	3.78c	3.69c	11.71c	11.67b
AB1	38.10bc	38.39b	25.25a	24.85a	3.87b	3.71b	11.78b	11.79a
AF1	38.86a	39.06a	24.98a	24.62a	3.59e	3.45e	11.48e	11.42d
AB2	37.78c	37.94c	25.60a	25.19a	3.94a	3.78a	11.96a	11.79a
AF2	38.42ab	38.62b	25.17a	24.77a	3.71d	3.62d	11.63d	11.64c
LSD at 0.05	0.524	0.597	0.695	0.581	0.017	0.015	0.023	0.021

CL, control (no ABA application); AB1, exogenous application of 5µM abscisic acid at bud initiation; AF1, exogenous application of 5µM abscisic acid at flower initiation; AB2, exogenous application of 10µM abscisic acid at bud initiation; AF2, exogenous application of 10µM abscisic acid at flower initiation; LSD, least significant difference; ns, non-significant. Mean values sharing the same letter in a column do not differ significantly at P= 0.05; 4I, four irrigations; 3I, three irrigations; AB, abscisic acid spray at bud initiation; AF, abscisic acid spray at flower initiation.

Table 3. Interactive effects of irrigation schedules and exogenous application of ABA on quality of sunflower

	Palmitic acid (%)		Linoleic acid (%)		Achene yield (kg ha^{-1})	
Treatments	**2008**	**2009**	**2008**	**2009**	**2008**	**2009**
No water stress						
CL	6.86a	6.91a	77.38a	77.46a	2909.30a	3115.18a
AB1	6.74c	6.82b	77.29c	77.36c	2817.35b	2988.53b
AF1	6.79b	6.81b	77.35b	77.39b	2745.75bc	2824.59cd
AB2	6.69d	6.75c	77.25c	77.29d	2689.10c	2877.74c
AF2	6.78b	6.82b	77.27cd	77.36c	2579.54d	2740.76d
Water stress at bud initiation						
CL	5.65c	5.76c	75.87c	75.69c	2042.13c	2194.71c
AB1	5.79a	5.84a	76.29a	76.38a	2551.84a	2730.92a
AF1	5.59d	5.67d	75.53d	75.62d	1937.81d	2083.36d
AB2	5.74b	5.79b	75.25b	75.98b	2305.10b	2457.42b
AF2	5.23e	5.34e	75.62e	75.59e	1636.69e	17652.28e
Water stress at flower initiation						
CL	5.37c	5.49c	75.57c	75.63c	1960.62c	2006.39c
AB1	5.28d	5.44d	75.49d	75.62c	1863.49d	1885.51d
AF1	5.52a	5.65a	75.79a	75.91a	2548.69a	2618.82a
AB2	5.16e	5.31e	75.24e	75.34d	1494.10e	1587.21e
AF2	5.44b	5.59b	75.67b	75.78b	2144.35b	2168.63b
LSD at 0.05	0.031	0.023	0.018	0.019	91.756	95.472

CL, control (no ABA application); AB1, exogenous application of 5µM abscisic acid at bud initiation; AF1, exogenous application of 5µM abscisic acid at flower initiation; AB2, exogenous application of 10µM abscisic acid at bud initiation; AF2, exogenous application of 10µM abscisic acid at flower initiation; LSD, least significant difference; ns, non-significant. Mean values sharing the same letter in a column do not differ significantly at P= 0.05; 4I, four irrigations; 3I, three irrigations; AB, abscisic acid spray at bud initiation; AF, abscisic acid spray at flower initiation.

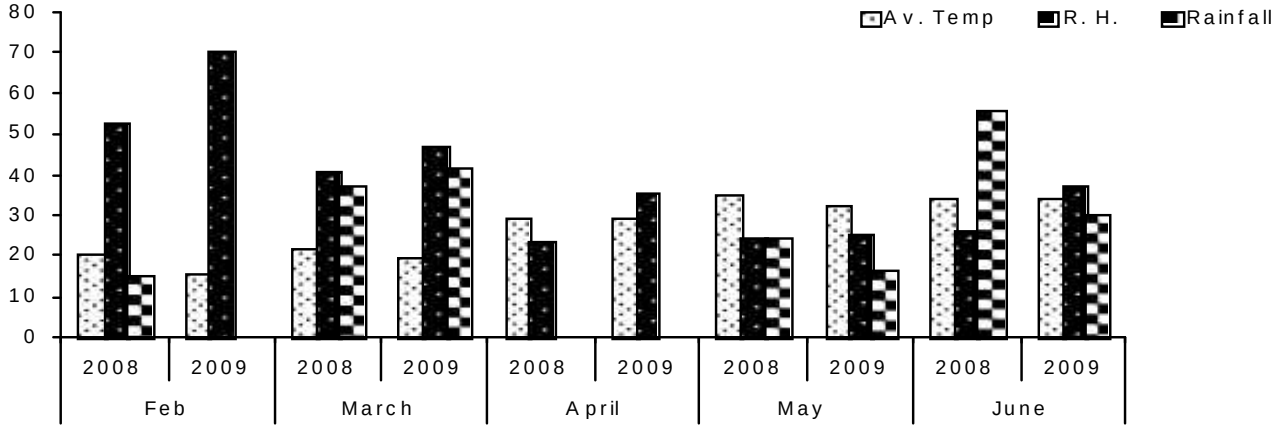

Fig.1 Mean temperature (ċ), relative humidity (%) and rainfall (mm) during the duration of experiment (February-June)

Conclusion: Drought stress and exogenous application of ABA under no water deficit conditions significantly decreased achene oil content and improved achene protein content. ABA application either at bud or at flower initiation under drought improved achene oil but decreased achene protein content. Drought stress at bud or at flower initiation and foliar application of ABA under no water deficits slightly increased stearic and oleic acid but decreased palmitic and linoleic acid and vice versa occurred by exogenous application of ABA under drought either at bud or at flower initiation stages.

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