

## Effect of Drought Stress on Grain Yield, Quality Traits, Phyllochron and Leaf Appearance Rate of Sunflower Hybrid *Iroflor* at Different Levels of Nitrogen and Plant Population

<sup>1</sup>Esmael Gholinezhad, <sup>2</sup>Amir Aynaband, <sup>3</sup>Abdollah Hassanzade Ghorthapeh,  
<sup>4</sup>Gorban Noormohamadi and <sup>5</sup>Iraj Bernousi

<sup>1</sup>Islamic Azad University, Science and Research Branch of Khozestan, Iran

<sup>2</sup>University of Chamran Ahwaz, Iran

<sup>3</sup>Agriculture and Natural Resource Institute Research, West Azerbaijan, Iran

<sup>4</sup>Islamic Azad University, Iran

<sup>5</sup>Urmia University, Iran

**Abstract:** This work was carried out to study the effects of water stress, different levels of nitrogen application and plant population on quality traits, phyllochron and leaf appearance rate of oily sunflower. It was conducted at West-Azərbayjan's Research Center for Agriculture during 2008 and 2009. The study consisted of split-split-plot experiments using Randomized Complete Block Design with three replications. The main factor was considered to be irrigation treatments including optimum irrigation, moderate stress and severe stress in which irrigation was done after depletion of 50%, 70% and 90% of field capacity, respectively. Three nitrogen levels of 100, 160 and 220 Kg N ha<sup>-1</sup> were considered as sub plots with sub - sub plots consisting of three plant population of 5.55, 6.66 and 8.33 plant m<sup>-2</sup>. The combined variance analyses indicated that water deficiency stress, nitrogen and plant population have a considerable impact on oil and protein yield, oil and protein percentage, phyllochron and leaf appearance rate. The maximum oil yield (2213/22 kg/ha) was attributed to optimum irrigation and density 83300 plants ha<sup>-1</sup>. Severe drought stress reduced the oil yield by 62% compared to the optimum irrigation condition. Oil yield increased at higher nitrogen application rates. The response of oil yield to increase in plant population was positive. With increasing plant population, oil yield, oil percent, protein yield and phyllochron increased, but protein percent, while leaf appearance rate decreased. According to the results obtained in this study, application of 220 kg N ha<sup>-1</sup> and highest plant population under optimum or moderate drought stress conditions is recommended for suitable oil and protein yield, although an increase in nitrogen consumption and plant population has a little impact on oil and protein yield under severe drought stress conditions. Also under severe drought stress conditions leaf appearance rate was decreased significantly.

**Key words:** Sunflower • Drought Stress • Nitrogen • Plant Population • Oil and Protein Yield

### INTRODUCTION

Sunflower (*Helianthus annuus* L.) is the most important oilseed crop in the world due to its wide range of adaptability and very high seed oil content, ranging from 40-50% and 23% protein [1]. Drought stress is the most important factors of abiotic stresses that affect on growth and yield of plants [2]. Naderi *et al.* [3] showed that application drought stress decreased oil yield. Stone *et al.* [4] showed that application drought stress

decreased oil percentage from 40 to 24%. Taheriasbag *et al.* [5] showed that with increasing drought stress protein percentage was increased significantly. Nitrogen is the most important element that cause increase grain protein [6]. Namati *et al.* [7] indicated that the maximum of phyllochron obtained in condition of nitrogen disuse and with increasing nitrogen usage amount of phyllochron decreased. Hokm Ali Pour *et al.* [8] indicated that application of nitrogen fertilizer, leaf appearance rate increased but phyllochron decreased. Liyong *et al.* [9]

reported that grain protein was increased with application higher nitrogen fertilizer more than drought stress, while applying lower nitrogen decreased the grain protein under the same condition of drought stress. The researchers declared that increasing nitrogen increased grain yield, while grain oil content decrease [10]. Asgari *et al.* [11] and Hao *et al.* [12] stated that with increasing nitrogen rate grain protein percentage was increased significantly. Improving plant distances, plant population and irrigation precise control is the most ways of important evaporation reduction from soil [13]. Rahbar *et al.* [14] showed that the maximum oil yield and oil percentage were obtained from the highest plant population. Increasing of oil percentage under higher plant population is related to lower husk percent.

Therefore, the aim of this study was to evaluate the effect of different levels of nitrogen application and plant density under different moisture conditions on quality traits, phyllochron and leaf appearance rate of oily sunflower in order to achieve the optimum use of resources.

## MATERIALS AND METHODS

This experiment was conducted in order to investigate the effects of water stress, different levels of nitrogen and plant density on quality traits, phyllochron and leaf appearance rate in oily sunflower (Var: Iroflor). It was performed during 2008 and 2009 at the Research Farm of the Centre of Agriculture and Natural Resources in Orumieh, West-Azerbaijan, Iran. The experiment was implemented in a split-split-plot using randomized complete block design with three replications. The main factor was irrigation treatments including optimum irrigation, moderate stress and severe stress. Irrigation was done after depletion of 50, 70 and 90 percent of field capacity, respectively. Three nitrogen levels consisting of 100, 160 and 220 kg N ha<sup>-1</sup> were considered as sub plots with sub- sub- plots consisted of three plant densities of 5.55, 6.66 and 8.33 plant m<sup>-2</sup>. The plant distance on each row was 20, 25 and 30 cm and distance between rows was 60 cm. These spacings were randomly located in the main plots, sub plots and sub- sub plots. Each sub-plot consisted of 7 plant line. Each plant line was 4 meter long.

The distance between two sub-plots and two main plots were 1m and 2m, respectively. Thus main plot area was 51.6 m<sup>2</sup> with total area of 2500 m<sup>2</sup>. The operations of plough and preparation of farm included a deep plough, two vertical disks, leveling, furrow, mound and plot making. The soil texture was loamy silt clay. The amount of fertilizer added to farm was determined by soil analysis. The planting was done manually after irrigation in 27 may 2008 and 2009. The grains used in this study were Hybrid Iroflor. This hybrid comes from the variety of single-cross and the group of middle ripping and has registered in 1988 in France.

The first irrigation was done in 5 June. The thinning conducted in 4-5 leaf stage. The weeding conducted in 2 stages of 20 and 40 days after planting. Nitrogen fertilizer applied in the form of surplus in 2 stages of 7-8 leaf age and flowering time. When downside of head turned to brownish yellow the final harvest was performed. In this stage seeds had 20 percent of moisture. In order to remove the edge effect, the sampling was not conducted from lateral rows. For determining soil moisture samples were taken from 2 depths of soil 0-30 and 30-60 cm in each (Table 1). Then weight moisture percentage was determined by pressure plate (armfield CAT.REF: FEL13B-1 Serial Number: 6353 A 24S98). In this experiment field capacity of soil was determined to be 26 with permanent wilting point of 14. In order to obtain the exact irrigation time, soil was sampled by auger from root development depth in each treatment 48 hours after irrigation to measure soil weight moisture. Based on the measurement, the irrigation time was determined to be at soil weight moisture of 20, 17.6 and 15.2. To implement the irrigation operation the water usage volume was calculated by the following equation 1:

$$V = \frac{(fc - \theta_m) \times \rho \times D_{root} \times A}{E_i} \quad (1)$$

- V = Irrigation water volume (m<sup>3</sup>)  
 $\theta_m$  = soil weight moisture percentage irrigation  
 A = Irrigated area (m<sup>2</sup>)  
 FC = Field capacity  
 $\rho_m$  = Soil external specific density (g cm<sup>-3</sup>)  
 D<sub>root</sub> = Root development depth (m<sup>-1</sup>)

Table 1: Chemical and physical properties of farm soil at depth of 0-30 cm

Potassium (ppm)	Phosphorus (ppm)	Nitrogen (%)	Organic Carbon (%)	Sand (%)	Silt (%)	Clay (%)	Lime (%)	Percentage of saturation (%)	pH	Electrical conductivity DS m <sup>-1</sup>
800	12	0.12	1.2	12	55	33	16	57	8	0.8

Therefore, the required water volume in each stage of irrigation in each treatment was calculated and was distributed equally based on water distribution efficiency of 90 percent by flume and chronometer. The final harvesting area was equal to  $4.8 \text{ m}^{-2}$  that was done from two middle lines of planting. Final measurements were conducted from these samples. For moisture measurement grains were located in the oven in the temperature of 72 degrees centigrade for 48 hours. Oil and protein percentages were calculated with grain analysis apparatus of oil and protein (Percon Inframatic 8620 -made in Switzerland). Oil and protein yield were calculated via multiply oil percentage  $\times$  grain yield and protein percentage  $\times$  grain yield, respectively. In order to phyllochron measurement during growth season each 3 days one time existing leaves numbers in three plants from main lines each plot was calculated when every leaf minimum 1 centimeter of length had. Meanwhile three selective plants were marked by color string and every leaf was marked by color pen after counting. Leaf appearance rate was calculated by the following equation 2:

$$\text{Leaf appearance rate} = \frac{1}{\text{phyllochron}}$$

Analysis of variance was performed using PROC ANOVA of SAS. The comparison of the means was done by Tokey's test at a probability level of 5 percent.

## RESULTS AND DISCUSSION

**Oil Yield:** The results of combined ANOVA showed that oil yield strongly affected by water stress and with intensifying stress decreased significantly (Tables 2, 4 and 5). Severe drought stress reduced oil yield by 62% compared to optimum irrigation condition. The reason for reduction of oil yield with increasing severe drought stress was reducing grain yield and oil percentage due to water deficit (Tables 4 and 5). These results confirmed results of Naderi *et al.* [3], Roshdi *et al.* [15] and Roberts [16]. Comparison of mean values for combined effect of irrigation-density showed that the highest density with optimum irrigation condition is considerably better than other cases with respect to the oil yield production whereas under severe drought stress condition change of oil yield due to increasing density was not outstanding. The reason of increase oil yield at high densities due to increase grain yield and oil percentage at optimum irrigation. These results confirmed results of Rahbar *et al.* [14] and Hejazi Dahaghani *et al.* [17]. Effect of interaction between irrigation treatments and nitrogen rates showed that oil yield was increased significantly so the maximum oil yield ( $2104.10 \text{ kg ha}^{-1}$ ) was obtained from optimum irrigation and  $220 \text{ kg nitrogen ha}^{-1}$  (Table 4). The reduction in nitrogen levels consumption from 220 to  $100 \text{ kg nitrogen ha}^{-1}$  reduced oil yield about the average of  $1975 \text{ kg ha}^{-1}$  (Table 3). This reduction was

Table 2: Combined analysis of variance for traits of sunflower in different drought stress, nitrogen and plant population (2008 - 2009)

		MS						
S.O.V.	df	Oil yield	Oil percentage	Oil protein	Protein yield	Phyllochron	Leaf appearance rate	Grain yield
Year	1	151269.5**	58.32**	27.87**	22293.3**	1.151**	0.0010**	458764.9**
Irrigation	2	20829963.0**	66.19**	49.80**	3719875.6**	17.63**	0.094**	78009743.7**
Year $\times$ irrigation	2	24074.3**	1.65 <sup>ns</sup>	26.18**	9658.06**	3.02 <sup>ns</sup>	0.0147**	329.7 <sup>ns</sup>
Error a	8	11373.8	5.14	2.21	2485.9	0.0019	0.000025	44513.5
Nitrogen	2	346020.8**	46.70**	23.52**	256772.4**	0.48**	0.0040**	7444267.1**
Nitrogen $\times$ year	2	8305.9 <sup>ns</sup>	0.77 <sup>ns</sup>	0.49 <sup>ns</sup>	3233.3 <sup>ns</sup>	0.025**	0.00037**	86.9 <sup>ns</sup>
Irrigation $\times$ nitrogen	4	68824.2**	0.25 <sup>ns</sup>	1.08 <sup>ns</sup>	31992.35**	0.12**	0.0017**	71212.2**
Irrigation $\times$ nitrogen $\times$ year	4	1961.8 <sup>ns</sup>	1.10 <sup>ns</sup>	0.52 <sup>ns</sup>	922.6 <sup>ns</sup>	0.026**	0.00049**	422.7 <sup>ns</sup>
Error b	24	4931.9	1.17	2.05	2011.29	0.00057	0.00000301	32529.3
Density	2	789893.4**	152.81**	36.07**	8778.9**	4.50**	0.022**	1976288.1**
Density $\times$ year	2	4139.4 <sup>ns</sup>	2.11*	2.17**	1274.2 <sup>ns</sup>	0.63**	0.0028**	47.4 <sup>ns</sup>
Irrigation $\times$ density	4	292286.87**	0.81*	2.01**	28450.9**	0.0051 <sup>ns</sup>	0.00084**	1000115.9**
Density $\times$ irrigation $\times$ year	4	848.8 <sup>ns</sup>	0.49 <sup>ns</sup>	1.28*	951.1 <sup>ns</sup>	0.0078**	0.00030**	181.2 <sup>ns</sup>
Density $\times$ nitrogen	4	2006.6 <sup>ns</sup>	2.65**	0.53 <sup>ns</sup>	145.1 <sup>ns</sup>	0.00026 <sup>ns</sup>	0.000030**	54061 <sup>ns</sup>
Density $\times$ nitrogen $\times$ year	4	564.7 <sup>ns</sup>	0.69 <sup>ns</sup>	0.13 <sup>ns</sup>	63.07 <sup>ns</sup>	0.00020 <sup>ns</sup>	0.000020 <sup>ns</sup>	16.1 <sup>ns</sup>
Density $\times$ nitrogen $\times$ irrigation	8	2201.1 <sup>ns</sup>	0.61 <sup>ns</sup>	0.14 <sup>ns</sup>	417.35 <sup>ns</sup>	0.00045 <sup>ns</sup>	0.000030*	28846 <sup>ns</sup>
Density $\times$ nitrogen $\times$ irrigation $\times$ Year	72	429.6 <sup>ns</sup>	0.16 <sup>ns</sup>	0.15 <sup>ns</sup>	149.2 <sup>ns</sup>	0.00037 <sup>ns</sup>	0.000020 <sup>ns</sup>	151.9 <sup>ns</sup>
Error c		3681.69	0.34	0.5	1219.58	0.0022	0.00000971	20080.9
CV	-	4.83	1.30	3.18	5.71	1.25	1.14	4.83

\*\*, \* and ns significant at the 1%, 5% probability levels and non significant, respectively.

Table 3: Combined mean comparison for traits of sunflower in different drought stress, nitrogen and plant population (2008 - 2009)

Treatments	Protein (%)	Phyllochron (day)
Nitrogen (Kg ha <sup>-1</sup> )		
100	b 21.51	-
160	a 22.22	-
220	a 22.83	-
Density (Plant m <sup>-2</sup> )		
8.33	-	a 4.10
6.66	-	B 3.66
5.55	-	c 3.55

Means followed by similar letters in each column are not significantly different at the 5% level of probability according to Tokay's Test.

Table 4: Combined mean comparison for traits of sunflower in different drought stress and nitrogen (2008 - 2009)

Treatments (Irrigation × Nitrogen)		Oil yield (Kg ha <sup>-1</sup> )	Protein yield (Kg ha <sup>-1</sup> )	Phyllochron (day)	Seed yield (Kg ha <sup>-1</sup> )
Ririgation treatments	Nitrogen (Kg ha <sup>-1</sup> )				
	100	c 1804.55	c 789.72	f 3.36	c 3859.6
Optimum Irrigation ×	160	b 1926.86	b 896.98	g 3.24	B 4188.5
	220	a 2104.10	a 1024.48	h 2.99	a 4707.3
	100	e 1025.77	ef 487.72	c 3.83	e 2245.6
Moderate drought stress ×	160	de 1088.41	de 531.61	d 3.76	De 2416.05
	220	d 1120.04	d 574.16	e 3.71	d 2547.4
	100	g 695.76	h 350.08	a 4.39	g 1564.7
Severe drought Stress ×	160	fg 746.08	gh 398.97	b 4.33	Fg 1715.4
	220	f 782.20	fg 442.56	b 4.31	f 1838.5

Means followed by similar letters in each column are not significantly different at the 5% level of probability according to Tokay's Test.

Table 5: Combined mean comparison for quality traits of sunflower in different drought stress and plant population (2008 - 2009)

Treatments (Irrigation × Plant density)		Oil yield (Kg ha <sup>-1</sup> )	Oil (%)	Protein (%)	Protein yield (Kg ha <sup>-1</sup> )	Seed yield (Kg ha <sup>-1</sup> )
Irrigation treatments	Plant density (Plant m <sup>-2</sup> )					
Optimum irrigation ×	8.33	a 2213.22	a 47.56	d 20.45	a 958.22	a 4679.7
	6.66	b 1932.36	c 45.22	d 21.15	b 900.16	b 4247.4
	5.55	c 1689.93	ef 43.91	c 22.22	c 852.81	c 3828.3
	8.33	d 1166.47	b 46.83	d 21.12	d 531.03	d 2511.7
Moderate drought stress ×	6.66	e 1075.39	de 44.51	bc 22.68	d 547.42	de 2412.1
	5.55	f 992.35	g 43.02	bc 22.50	d 515.03	e 2285.3
	8.33	g 755.27	cd 44.97	c 22.33	f 372.03	f 1661.3
	6.66	g 741.24	f 43.79	b 23.23	ef 398.63	f 1709.5
Severe drought stress ×	5.55	g 727.52	h 42.38	a 24.00	e 420.95	f 1747.7

Means followed by similar letters in each column are not significantly different at the 5% level of probability according to Tokay's Test.

considerable (Table 2). The reduction in oil yield at little quantities of nitrogen consumption was reported by Singh and Patel [18]. Nitrogen consumption 220 kg nitrogen ha<sup>-1</sup> in comparison with 160 or 100 nitrogen ha<sup>-1</sup>, oil yield increased about 7 and 12 %, respectively. The reason for increasing oil yield and grain yield was due to increasing the available elements more often in soil and plant optimum usage from environmental factors. In this study, there is a positive and significant correlation between oil yield and grain yield that agreement with the aforementioned results (Table 8). Golabadi *et al.* [19] showed that grain yield had the maximum positive direct effect whereas 1000-grain weight had the maximum negative direct effect on oil yield.

**Oil Percentage:** The results of combined ANOVA showed that oil percentage strongly affected by water stress and with intensifying stress decreased significantly (Tables 2, 5 and 6). Severe drought stress reduced the oil percentage by 4% compared to the optimum irrigation condition. The reason for its is that at first carbohydrates gather then this matter convert to oil and protein or other matters so whatever the length of this period is more as a result oil percentage will be top [20]. Therefore, with attention to this study can declare optimum irrigation had more time for filling grain so oil percentage was more in this treatment. Also drought stress may be increase the grain husk thickness and reduce grain kernel percentage to grain whole consequently oil percentage decreased.

Table 6: Combined mean comparison for Oil percentage of sunflower in different nitrogen and plant population (2008 - 2009)

Treatments (Nitrogen × Plant density)		
Nitrogen (Kg ha <sup>-1</sup> )	Plant density (Plant m <sup>-2</sup> )	Oil (%)
100 ×	8.33	a 47.40
	6.66	C 45.52
	5.55	d 44.20
	8.33	b 46.48
160 ×	6.66	D 44.62
	5.55	e 43.45
	8.33	c 45.48
220 ×	6.66	E 43.38
	5.55	f 41.66

Means followed by similar letters in each column are not significantly different at the 5% level of probability according to Tokay's Test.

Table 7: Combined mean comparison for traits of sunflower in different drought stress, nitrogen and plant population (2008 - 2009)

Treatments (Nitrogen × Plant density)		Leaf appearance rate (1/day)		
		Optimum irrigation	Moderate drought stress	Severe drought stress
Nitrogen (Kg ha <sup>-1</sup> )	Plant density (Plant m <sup>-2</sup> )			
100 ×	8.33	jk 0.271	mno 0.239	p 0.214
	6.66	e 0.309	k 0.269	o 0.234
	5.55	de 0.316	hij 0.278	lmno 0.241
	8.33	ghi 0.279	lmn 0.244	p 0.217
160 ×	6.66	d 0.321	ijk 0.273	no 0.237
	5.55	c 0.330	gh 0.282	lmn 0.244
	8.33	f 0.301	l 0.248	p 0.218
220 ×	6.66	b 0.350	hij 0.278	mno 0.238
	5.55	a 0.361	g 0.286	lm 0.245

Means followed by similar letters in each column are not significantly different at the 5% level of probability according to Tokay's Test.

Table 8: Matrix of simple correlation coefficient among different traits

	Oil yield	Oil percentage	Protein percentage	Protein yield	Phyllochron	Leaf appearance rate	Seed yield
Oil yield	1						
Oil percentage	0.49**	1					
Protein percentage	0.61**	0.90**	1				
Protein yield	0.97**	0.30ns	0.43*	1			
Phyllochron	0.69**	-0.10ns	0.10ns	0.80**	1		
Leaf appearance rate	0.69**	0.11ns	0.07ns	0.81**	0.98**	1	
Grain yield	0.99**	0.40*	0.54**	0.99**	0.75**	0.75**	1

\*\*, \* and ns significant at the 1%, 5% probability levels and non significant respectively.

These results confirmed results of Sharifi *et al.* [21] and Daneshian and Jabary [22]. Increasing nitrogen fertilizer rate decreased oil percentage (Table 6). In each level of nitrogen with increasing plant density oil percentage increased considerably. So that the maximum and minimum oil percentage about 47.40 and 41.66 % was obtained from 100 N x 8.33 plant m<sup>-2</sup> and 220 N x 5.55 plant m<sup>-2</sup>, respectively (Table 6). The reduction of oil percentage with increasing chemical fertilizers application has been reported by Abbdel-Sabour and Abo-El-Seoud

[23] and Nandhagopal *et al.* [24]. In low densities more light radiate to farm so some light reflex it cause temperature of internal canopy increase consequently it cause obtained oil in plant decompose and oil percentage decrease but in high densities lower light radiate to farm and vast part of light in top parts of canopy is take and spend for photosynthesis besides rate of evaporation from canopy area is low as a result internal canopy space is almost cool and oil percentage increase. These results are in agreement with the findings of Alyari *et al.* [25] and

Roberts [26]. Oil percentage had positive and significant correlation with grain yield and oil yield but with protein percentage had negative and significant correlation (-90\*\*) (Table 8).

**Protein Percentage:** The results of combined ANOVA showed that the effect of drought stress, nitrogen, plant density and irrigation x plant density interaction was significant on protein percentage (Table 2). Mean comparison of two-year showed that protein percentage strongly affected by water stress and with intensifying stress increased significantly (Tables 2, 3 and 5). In each increase of irrigation level with plant density protein percentage decreased significantly so the maximum and minimum protein percentage were obtained at severe drought stress x 5.55 plant m<sup>-2</sup> and optimum irrigation x 8.33 plant m<sup>-2</sup>, respectively (Table 5). Severe drought stress increased protein percentage by 8% compared to the optimum irrigation condition. These results are in agreement with those obtained by Majd *et al.* [27]. The reason of increase protein percentage with intensifying drought stress was due to increasing oil percentage that it had negative and significant correlation (Table 8). In this study, increasing nitrogen fertilizer rates increased protein percentage (Table 3). This is because of ability of the plant to access to more nitrogen and increasing reproductive and productive parts.

There is not any significant difference between fertilizer of 160 and 220 kg N ha<sup>-1</sup> (Table 3). Application of 220 N kg ha<sup>-1</sup> in comparison with 160 and 100 N ha<sup>-1</sup> resulted in an increase of protein percentage by 2 and 5 %, respectively. The increase of protein percentage with increasing nitrogen was due to absorbing more nitrogen from soil, photosynthesis increase, increasing nitrogen content in upwards organs and transfer more nitrogen to grain. Increasing grain protein percentage with application of nitrogen fertilizer also was reported by Abbdel-Sabour and Abo-El-Seoud [23]. Liyong *et al.* [9] stated that nitrogen fertilizer increased grain protein content more than drought stress while, at drought stress condition lower application of nitrogen affected strongly on protein percentage. These results are in agreement with those obtained by Fallah and Tadayon [28]. Table 8 illustrated the correlation coefficients showed that between protein percent and grain yield, oil yield and oil percentage had negative and significant correlation.

**Protein Yield:** The results of combined ANOVA showed that the effect of drought stress, nitrogen, plant density, irrigation x plant density and irrigation x nitrogen

interaction was significant on protein yield (Table 2). Mean comparison of two-year showed that protein yield strongly affected by water stress and with intensifying stress decreased significantly (Tables 2, 4 and 5). In each irrigation level with increasing application of nitrogen protein yield was increased significantly so the maximum and minimum protein yield were obtained at optimum irrigation x 220 kg N ha<sup>-1</sup> and severe drought stress x 100 kg N ha<sup>-1</sup>, respectively (Table 4). Severe drought stress decreased the protein yield by 51% compared to the optimum irrigation condition. The decrease of protein yield with increasing severe drought stress was due to the reduction in seed yield.

Increasing protein yield may be due to high nitrogen application which reflected to increase seed yield and protein percentage. This may be due to the plants absorbs more available elements from soils led to increase grain yield and protein yield. Similar results were reported by Fallah and Tadayyon [28] and Gooding *et al.* [29]. Researchers mentioned genotype, environmental factors and agronomy managements in growth and development of sunflower important on grain protein yield [30]. Mean comparison of two-year showed that in each increase in irrigation level with plant density increased protein yield significantly so the maximum and minimum protein percentage were obtained in optimum irrigation x 8.33 plant m<sup>-2</sup> and severe drought stress x 8.33 plant m<sup>-2</sup>, respectively (Table 5). These results are in agreement with the findings of Fallah and Tadayon [28]. Table 8 illustrated the correlation coefficients showed that among protein yield with grain yield and oil yield positive and significant correlation and with traits such as protein percentage and phyllochron had negative and significant correlation.

**Phyllochron:** The results of combined ANOVA showed that the effect of drought stress, nitrogen, plant density and irrigation x nitrogen interaction was significant on phyllochron (Table 2). Mean comparison of two-year showed that phyllochron strongly affected by water stress and with intensifying stress increased significantly (Tables 2, 3 and 4). In each irrigation level with increasing application of nitrogen phyllochron decreased significantly so the maximum and minimum protein yield were obtained at severe drought stress x 100 kgN ha<sup>-1</sup> and optimum irrigation x 220 kg N ha<sup>-1</sup>, respectively (Table 4). Severe drought stress increased phyllochron compared to the optimum irrigation condition. The increase of protein yield with increasing severe drought stress was due to the reduction in leaf appearance rate

resulting water deficit. Increasing plant density levels had significant effect on phyllochron and leaf appearance rate so that with increasing plant density, leaf appearance rate was decreased while , phyllochron increased (Table 3). These results are in agreement with the findings of Longnecker *et al.* [31]. Albert and Carberry [32] and Mc Cullough [33] reported that the phyllochron was increase by decreasing soil moisture . Hokm Ali pour *et al.* [8] showed that with increasing plant density, phyllochron increased, while with increasing nitrogen, phyllochron was decreased. Table 8 illustrated the correlation coefficients showed that among phyllochron with seed yield, protein percentage and oil yield negative and significant correlation.

**Leaf Appearance Rate:** The results of combined ANOVA showed that the effect of drought stress, nitrogen, plant density and irrigation x nitrogen, irrigation x plant density, nitrogen x plant density and irrigation x nitrogen x plant density interactions was significant on leaf appearance rate (Table 2). Mean comparison of two-year showed that leaf appearance rate strongly affected by water stress and with intensifying stress decreased significantly (Tables 2, 4, 5, 6 and 7). The maximum and minimum leaf appearance rate was obtained in optimum irrigation and severe drought stress, respectively (Table 7). Severe drought stress reduced leaf appearance rate by 30% compared to the optimum irrigation condition. In each level of irrigation with increasing application of nitrogen leaf appearance rate increased significantly (Table 4). In each level of nitrogen application with increasing plant density, leaf appearance rate was decreased significantly (Table 6). In each level of irrigation the maximum and minimum leaf appearance rate were obtained from 220 kg N ha<sup>-1</sup> x 5.55 plant m<sup>-2</sup> and 100 kg nitrogen ha<sup>-1</sup> x 8.33 plant m<sup>-2</sup>, respectively (Table 7). Ellis *et al.* [34] reported that if photosynthesis active radiation become twice, leaf appearance rate increase by 17%. Hokm Ali Pour *et al.* [8] showed that with increasing nitrogen, leaf appearance rate increased ,while phyllochron decreased. Table 8 illustrated the correlation coefficients showed that among leaf appearance rate with grain yield, protein yield and oil yield had positive and significant correlation but it had negative and significant correlation with phyllochron.

**Seed yield:** Results of combined ANOVA showed that the effect of drought stress, nitrogen and plant density was significant on grain yield (Table 2). With increasing severe drought stress, seed yield decreased. Increasing nitrogen application increase seed yield . Application of high N fertilizer rates up to .....kg ha<sup>-1</sup> at optimum

irrigation condition increased seed yield, whereas application of .....Kg N ha<sup>-1</sup> at severe drought stress condition did not increased seed yield. It seems that this situation results from absorption reduction and increasing nitrogen waste due to water deficit in soil. The results of Martin *et al.* [34] confirm obtained results in this survey on nitrogen use efficiency at drought stress condition. Increasing number of plants per area was accompanied with considerable increase in seed yield (Table 3). Many researchers have pointed to seed yield was increased with increasing plant density [35, 36]. The most seed yield of about 4679.7 kg ha<sup>-1</sup> obtained from the highest density at optimum irrigation treatment ,while at moderate and severe drought stress conditions yield was increased with increasing of plant density. These results showed that using of high densities can be useful at optimum condition. Liang *et al.* [37] reported that the maximum grain yield in maize need high plant density, more irrigation, use of plenty of fertilizer and meeting temperature requirements. In low densities seed yield was decreased due to decreasing the number of plants per area and increasing nitrogen was effective to some extent due to the limitation in capacity of each plant to use nitrogen and therefore extra nitrogen was not used and remained out of plant access.

## CONCLUSION

Among the most important results obtained about application of water stress, significant reduction of oil yield, oil percentage, protein yield, leaf appearance rate and seed yield at severe and moderate stress in comparison with optimum irrigation can be pointed out. By increasing nitrogen fertilizer from 100 to 220 kg N ha<sup>-1</sup> most of the aforementioned traits increased significantly. With plant density increase traits of oil yield, oil percentage, phyllochron and grain yield increased too increasing plant density from 5.55 to 8.33 plants m<sup>-2</sup>, protein percentage and leaf appearance rate were significantly decreased. Combined effects of irrigation x nitrogen showed that with increasing nitrogen, oil yield, protein yield, leaf appearance rate and seed yield were increased. The interaction between irrigation and plant density showed that at optimum irrigation with t increasing plant density, oil yield, oil percentage, protein yield and grain yield were increased ,while at severe drought stress the highest seed yield was obtained from the lowest density. The interaction between nitrogen and plant density showed that the highest oil percentage was obtained from the lowest N fertilizer application and highest plant density.

The most protein percentage obtained from the highest fertilizer level. In the Interaction between of plant density\*irrigation\*nitrogen the most leaf appearance rate obtained from optimum irrigation and the highest fertilizer level and the lowest plant density level.

Therefore, at optimum irrigation condition for planting sunflower var. Iroflor fertilizer treatment of 220 kg nitrogen ha<sup>-1</sup> and plant density of 8.33 plants per m<sup>-2</sup> and at drought stress condition fertilizer treatment of 160 kg nitrogen ha<sup>-1</sup> and density of 5.55 plants per m<sup>-2</sup> is recommended.

### REFERENCES

- Hatim, M. and G.Q. Abbasi (Ed.), 1994. Oilseed crops. In Crop Production. National Book Foundation, Islamabad, Pakistan.
- Jaleel, C.A., P. Manivannan, A. Wahid, M. Farooq, H.J. Al-Juburi, R. Somasundaram and R. Panneersel Vam, 2009. Drought Stress in Plants: A Review on Morphological Characteristics and Pigments Composition. Int. J. Agric. Biol., 11(1): 100-105.
- Naderi, M.R., G. Nour-mohammadi, I. Majidi, F. Darvish, A.H. Shirani-rad and H. Madani, 2005. Evolution of summer safflower reaction to different intensities of drought stress at Isfahan region. J. Agronomy Sciences of Iran. 7(3): 212-225.
- Stone, L.R., D.E. Goodrum. M.N. Jaffar and A.H. Khan, 2001. Rooting front and water depletion Depths in grain sorghum and sunflower. Agron. J., pp: 1105-1110.
- Taheriasbagh, F., A. Fayazmoghaddam and A. Hassanzadeh GHortapeh, 2008. Evaluation of effects of plant date and irrigation management on growth and development and yield of sunflower Var. Iroflor in Orumieh. The Theses of M. SC. University of Urmia. Agriculture Faculty. pp: 128.
- Regina, H., 2008. Influence of Macro - and Micro nutrient fertilization on fungal contamination and fumonisin production in corn grains. Food Control., 19: 36-43.
- Namati, A.R., R. Sayad Sharifi, A. Gholipori and A. Vafaye, 2010. The effect of nitrogen use amount and time on phyllochron and leaf appearance rate of Maize. The 11<sup>th</sup> Congress of .
- Hokm Ali Pour, S., R. Seyed Sharifi, M. Ghadim Zade and S.H. Chamaati Somarin, 2007. Investigation plant density and nitrogen levels on phylochron and leaf appearance rate of Corn. J. Soil and Water Sci., 21(2): 159-169.
- Liyong, H., C., Hao, Z. Guangsheng and F. Tingong, 2007. The influence of drought on Brassica napus L. development under different nitrogenous level. P. 235-236. Proceeding of the 12th International Rapeseed Congress Sustainable Development in Cruciferous Oilseed Crops Production. 26-30 March Wuhan, China. Science Press USA Inc.,
- Chkerol hosseini, M.R., 2006. The effects of N, P on safflower quality and quantity yield in dry conditions. Iranian Journal of Soil and Water Sci., 2(1): 17- 24.
- Asghari, A., K.H. Razmjo and M. Mazaheri, 2006. The effect of nitrogen content on yield and grain protein percentage of 4 var. from grain sorghum. Agric. Science and Natural Resources J., 13: 2.
- Hao, H.L., Y.Z. Wei, X. Yang, Y. Feng and C.Y. Wu, 2007. Effects of different nitrogen fertilizer levels on Fe Mn, Cu and Zn concentration in shoot and quality in rice (*Oryza sativa*). Rice. Sci., 14(4): 289- 294.
- Pirzad, A., 2007. Effects of irrigation and plant density on some physiological characters and compositions of German chamomile *Matricaria chamomilla* L". Ph. D. thesis, Tabriz University, pp: 195.
- Rahbar, S., S. Mohammadi, L. Hoshmand and T. Shams Borhan, 2008. Evaluation of effect of plant density on yield and yield components of three var. sunflower. The 10<sup>th</sup> Congress Agronomy and Breeding of Iran. Karaj. 19-20 Jul. pp: 246.
- Roshdi, M., H. Heydari Sharifabad, M. Karimi, G.H. Nourmohammadi and F. Darvish, 2006. A Syrvey on the impact of water deficiency over the yield of sunflower seed cultivar and its components. J. Agri. Sci., 12(1): 109-121.
- Hajazi Dahaghani, S.M.R., A. Rezaee and B. Majd Nasiri, 2009. Evaluation of the most suitable plant density and arrangement of sunflower varieties in Isfahan. National Congress Oily Seeds. University of Sanati Isfahan. 1 and 2 October. pp: 53.
- Singh, J. and A.L. Patel, 1996. Dry matter distribution different parts of wheat under water stress at various growth stage. Field Crop Abstracts. 49(11): 10-16.
- Golabadi, M., M.R. Shahsavari and Z. Abasi, 2009. Study of related traits with grain oil yield in promising hybrids of Sunflower via analyses rout coefficients. National congress oily seeds. University of Sanati Isfahan. 23 and 24 October. pp: 139.
- Anonymous. 1993. The part of oily seeds researches. Report of Sunflower investigation. Institution of breeding and preparing seed and seedling of Karaj. pp: 60.

20. Sharifi, A., H. Madani, A. Mehreban, H.R. Mobaser, D. Mazaheri and R. Norani, 2008. The effect of irrigation cut in different stages on physiologic indices of Sunflower. The 10<sup>th</sup> congress agronomy and breeding of Iran. Karaj. 19-20 Jul. pp: 247.
21. Daneshian, J. and H. Jabbari, 2008. Effect of limited irrigation and plant density on morphological characteristics and grain yield in a dwarf Sunflower hybrid (CMS26\* R103) as second crop. Journal of Agronomy Sciences of Iran. 10(4): 377-388.
22. Abbdel-Sabour, M.F. and M.A. Abo-El-Seoud, 1996. Effects of organic-waste compost addition on sesame growth, yield and chemical composition. Agriculture, Ecosystems and Environ., 60: 157-164.
23. Nandhagopal, A.K., S. Subramanian and A. Gopalan, 1995. Response of sunflower hybrids to nitrogen and phosphorus under irrigated condition. Madras Agricultural J., 82: 80-83.
24. Aliyari, H., F. Shekari and F. Shekari, 2000. Oily Seeds, Agronomy and Physiology. Tabriz. Publications of Abdi Tabriz. pp: 182.
25. Roberts, E.H., 1998. Quantifying seed deterioration. In: M. B. McDougal, J. r. and C. J. Nelcon(eds), Physiology of seed deterioration ,Cssa special publication, Madison, WI, U. S. A, pp: 101-123.
26. Majd, A., P. Jenobi, J. Daneshian and M. Zaynipoor, 2009. Evaluation of drought stress effects on protein changes in Sunflower. National congress oily seeds. University of Sanati Isfahan. 23 and 24 October. pp: 143.
27. Fallah, S. and A. Tadayyon, 2009. Effects of plant density and nitrogen rates on yield, nitrate and protein of silage Maize. EJCP. 2(2): 105-121.
28. Gooding, M.J., G. Smith, W.P. Davies and P.S. Kettlewell. 1997. The use of residual maximum likelihood to model grain quality characters of wheat with variety, climatic and nitrogen fertilizer effects. J. Agric. Sci. Cambridge, 128:135-142.
29. Nimbal, V.R. and V.S. Dodamani, 1993. Water use efficiency and oil content of sunflower under different irrigation and nutrient levels. Journal of Maharashtra Agricultural Universities. 18: 459-460.
30. Longnecker, N.E.G., M. Kirby and A. Robson, 1993. Leaf emergence, tiller growth and apical development of nitrogen-deficient spring wheat. Crop Sci., 33:154-160.
31. Albert, D.G. and P.S. Carberry, 1993. The influence of water deficit prior to tassel initiation on maize growth, development and yield. Field Crop Res., 31: 55-59.
32. McCullough, D.E., P.H. Girardin, M. Mihajlovic, A. Aguilera and M. Tollenaar, 1994. Influence of Nitrogen supply on development and dry matter accumulation of an old new maize hybrid. Can. J. Plant and Soil. Sci., 74: 461-466.
33. Ellis, R.H., R.J. Summer, G.O. Edmeades and E.D. Roberts, 1992. Photoperiod, temperature and the interval from sowing to tassel initiation in diverse cultivars of maize. Crop Sci., 32: 1225-1232.
34. Martin, D.L., D.G. Watts, L.N. Mielke, K.D. Frank and D.E. Eisen-Hauer, 1992. Evolution of nitrogen and irrigation management for corn production using water high in nitrate. Soil Sci. Soc. Amer. J., 49: 1056-1062.
35. Sadras, V.O., D.J. Cannor and D.M. Whitfield, 1998. Yield, yield components and source-sink relationships in water- stressed sunflower. Field Crop Res., 31: 27-39.
36. Schneiter, A.A., B.L.L. Janson and T.L.H. Enerson, 1992. Rooting depth and water use of different sunflower phenotypes. Pro. 13. Lnt. Sunf. Pisa. Italy.
37. Liang, B.C., M.R. Millard and A.F. Mackenzie, 1992. Effects of hybrid, population densities, fertilization and irrigation on grain corn (*Zea mays* U) in Quebec. Can. J. Plant Sci., 72: 1163-1170.