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Effect of Drought Stress and Nitrogen Rates on Grain Yield, Quality Traits and Physiological Indices in Sunflower Hybrid *Iroflor* at Different Plant Density

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Abstract: This experiment was carried out to study the effects of water deficiency stress, different levels of nitrogen application and plant density on quality traits, phyllochron and leaf appearance rate of oily sunflower. It was conducted at West-Azarbaijan's Research Center for Agriculture during 2008 and 2009. The study consisted of split-split-plot experiment using Randomized Complete Block Design with three replication. 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^{-1} were considered as sub plot with sub-sub plot consisting of three plant density of 5.55, 6.66 and 8.33 plant in m^2.The combined variance analysis indicated that water deficiency stress, nitrogen and plant density 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^{-1} treatment. 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 density was positive. With increasing plant density, oil yield, oil percent, protein yield and phyllochron increased, but protein percent and leaf appearance rate decreased. According to the results obtained in this research, application of 220 kg N ha^{-1} and larger plant density in optimum conditions and moderate drought stress is recommended for suitable oil and protein yield, although an increase in nitrogen consumption and plant density has a little impact on oil and protein yield in severe drought stress conditions. Also in severe drought stress conditions leaf appearance rate decreased significantly.

Key words: Drought Stress · Nitrogen · Oil Yield · Plant Density · Protein Yield · Sunflower

INTRODUCTION

Sunflower (*Helianthus annuus L.*) is the most important oilseed crop of 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 with application drought stress oil yield decreased. Stone *et al.* [4] showed that with application drought stress oil percentage decreased from 40% to 24%. Taheriasbag *et al.* [5] showed that with increasing drought stress percentage of protein increased significantly. Nitrogen is the most important element that cause increase protein of grain [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 decresed. Hokm Ali Pour *et al.* [8] indicated that with nitrogen amount, leaf appearance rate increased but phyllochron decresed. Liyong *et al.* [9] in an experiment emphasized that fertilizer of nitrogen more than drought stress grain protein

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increased and in condition of draught stress, applying lower nitrogen affected stronger than. The researchers declared that increasing nitrogen cause increase grain yield but grain oil content cause decrease [10]. Asgari *et al.* [11] stated that with increasing nitrogen grain protein percentage increased significantly. Hao *et al.* [12] reported that with increasing nitrogen rice protein content increased. Improving plant distances, plant density 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 obtained from the highest plant density. Increasing of oil percentage in higher plant density 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 in 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 deficit stress, different levels of nitrogen and plant density on quality traits, phyllochron and leaf appearance rate in 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-Azarbijan, Iran. The experiment was implemented in a split-split-plot using Randomized Complete Block design with three replication. The main plot 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^{-1} were considered as sub plot with sub-sub plot consisted of three plant densities of 5.55, 6.66 and 8.33 plant m^{-2}. The plant distance on each row was 20, 25 and 30 cm and distance between rows was 60 cm. These spacing 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^2 with total area of 2500 m^2. 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 leafage 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:

Equation (1): $$V = \frac{(fc - \theta m) \times \rho \times Droot \times A}{Ei}$$

V= Irrigation water volume (m^{-3})
Θ_m = soil weight moisture percentage irrigation
A= Irrigated area (m^{-2})
FC= field capacity
ρ_m = soil external specific density (g cm^{-3})
D_{root}= root development depth (m^{-1})

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

Potassium (ppm)	Phosphor (ppm)	Nitrogen (%)	Carbon Organic (%)	sand (%)	silt (%)	clay (%)	lime (%)	Percentage of saturation (%)	pH	Electrical conductivity DS m^{-1}
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 the water distribution efficiency of 90 percent by flume and chronometer. The final harvesting area was equal to 4.8 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 percentage was calculated with grain analysis apparatus of oil and protein (Percon Inframatic 8620-made in Switzerland). Oil and protein yield was calculated via multiply oil percentage * grain yield and protein percentage * 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:

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

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 content strongly affected by water deficit stress and with intensifying stress decreased significantly (tables 2, 4 and 5). Severe drought stress reduced the oil yield by 62% compared to the oil yield 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 case of highest density with optimum irrigation condition is considerably better than other cases with respect to the oil yield production whereas in severe drought stress condition change of oil yield due to increasing density was not outstanding. The reason of oil yield increase in high densities grain yield and oil percentage increase was in optimum irrigation. These results confirmed results of Rahbar *et al.* [14] and Hejazi Dahaghani *et al.* [17]. The examination effect of irrigation and nitrogen interaction showed that with increasing nitrogen oil yield increased significantly so the maximum oil yield (2104.10 kg ha^{-1}) obtained from optimum irrigation and 220 kg nitrogen ha^{-1} (table 4). The reduction of nitrogen levels consumption from 220 to 100 kg nitrogen ha^{-1} reduced oil yield about the average of 1975 kg ha^{-1} (table 3). This reduction was considerable (table 2). The reduction of oil yield at little

Table 2: Combined analysis of variance for traits of sunflower in different drought stress, nitrogen and plant density (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 × irrigation	2	24074.3**	1.65^{ns}	26.18**	9658.06**	3.02^{ns}	0.0147**	329.7^{ns}
Error 1	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 ×Year	2	8305.9^{ns}	0.77^{ns}	0.49^{ns}	3233.3^{ns}	0.025**	0.00037**	86.9^{ns}
Irrigation ×nitrogen	4	68824.2**	0.25^{ns}	1.08^{ns}	31992.35**	0.12**	0.0017**	71212.2**
Irrigation × nitrogen × Year	4	1961.8^{ns}	1.10^{ns}	0.52^{ns}	922.6^{ns}	0.026**	0.00049**	422.7^{ns}
Error 2	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 × Year	2	4139.4^{ns}	2.11*	2.17**	1274.2^{ns}	0.63**	0.0028**	47.4^{ns}
Irrigation ×Density	4	292286.87**	0.81*	2.01**	28450.9**	0.0051^{ns}	0.00084**	1000115.9**
Density × irrigation × Year	4	848.8^{ns}	0.49^{ns}	1.28*	951.1^{ns}	0.0078**	0.00030**	181.2^{ns}
Density × nitrogen	4	2006.6^{ns}	2.65**	0.53^{ns}	145.1^{ns}	0.00026^{ns}	0.000030**	54061^{ns}
Density × nitrogen × Year	4	564.7^{ns}	0.69^{ns}	0.13^{ns}	63.07^{ns}	0.00020^{ns}	0.000020^{ns}	16.1^{ns}
Density × nitrogen × irrigation	8	2201.1^{ns}	0.61^{ns}	0.14^{ns}	417.35^{ns}	0.00045^{ns}	0.000030*	28846^{ns}
Density × nitrogen × irrigation × Year	72	429.6^{ns}	0.16^{ns}	0.15^{ns}	149.2^{ns}	0.00037^{ns}	0.000020^{ns}	151.9^{ns}
Error 3		3681.69	0.34	0.5	1219.58	0.0022	0.00000971	20080.9
C.V	-	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: Mean comparison for traits of sunflower in different nitrogen and plant density (2008-2009)

Treatments	Protein (%)	Phyllochron (day)
Nitrogen (Kg ha^{-1})		
100	21.51b	3.17a
160	22.22a	3.10b
220	22.83a	2.93c
Density (Plant per m^{-2})		
8.33	21.30c	4.10a
6.66	22.35b	3.66b
5.55	22.91a	3.55c

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: Mean comparison for traits of sunflower in different drought stress and nitrogen (2008-2009)

	Treatments (Irrigation × Nitrogen)	Oil yield Kg ha^{-1}))	Protein yield Kg ha^{-1}))	Phyllochron (day)	Grain yield Kg ha^{-1})
Irrigation	Nitrogen (Kg ha^{-1})				
	100	1804.55c	789.72c	3.36f	3859.6c
Optimum Irrigation ×	160	1926.86v	896.98b	3.24g	4188.5b
	220	2104.10a	1024.48a	2.99h	4707.3a
	100	1025.77e	487.72ef	3.83c	2245.6e
Drought Moderate Stress ×	160	1088.41de	531.61de	3.76d	2416.05de
	220	1120.04d	574.16d	3.71e	2547.4d
	100	695.76g	350.08h	4.39a	1564.7g
Drought Severe Stress ×	160	746.08fg	398.97gh	4.33b	1715.4fg
	220	782.20f	442.56fg	4.31b	1838.5f

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: Mean comparison for quality traits of sunflower in different drought stress and plant density (2008-2009)

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

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

quantities of nitrogen consumption was reported by other researchers Singh and Patel [18]. Nitrogen consumption 220 kg nitrogen ha^{-1} in compared with 160 and 100 nitrogen ha^{-1} oil yield increased about 7 and 12 percent respectively. The reason for oil yield and grain yield increase was due to increasing food elements more often in soil and plant optimum usage from environmental factors. It was found in this study that there is a positive and significant correlation between oil yield and grain yield that agree 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 content strongly affected by water deficit stress and with intensifying stress decreased significantly (tables 2, 5 and 6). Severe drought stress reduced the oil percentage by 4% compared to the oil yield 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 cause increase grain husk thickness and reduce

Table 6: Mean comparison for Oil percentage of sunflower in different nitrogen and plant density (2008-2009)

Treatments (Nitrogen × Plant density)		
Nitrogen (Kg ha^{-1})	Plant density (Plant m^{-2})	Oil (%)
	8.33	47.40a
100 ×	6.66	45.52c
	5.55	44.20d
	8.33	46.48b
160 ×	6.66	44.62d
	5.55	43.45e
	8.33	45.48c
220 ×	6.66	43.38e
	5.55	41.66f

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: Mean comparison for traits of sunflower in different drought stress, nitrogen and plant density (2008-2009)

Treatments (Nitrogen × Plant density)		Leaf appearance rate (1/day)		
Nitrogen (Kg ha^{-1})	Plant density (Plant m^{-2})	Optimum Irrigation	Drought Moderate Stress	Drought Severe Stress
	8.33	0.271jk	0.239mno	0.214p
100 ×	6.66	0.309e	0.269k	0.234o
	5.55	0.316de	0.278hij	0.241lmn
	8.33	0.279ghi	0.244lmn	0.217p
160 ×	6.66	0.321d	0.273ijk	0.237no
	5.55	0.330c	0.282gh	0.244lmn
	8.33	0.301f	0.248l	0.218p
220 ×	6.66	0.350b	0.27hij8	0.238mno
	5.55	0.361a	0.286g	0.245lm

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

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

grain kernel percentage to grain whole consequently oil percentage decreased. These results confirmed results of Sharifi *et al.* [21] and Daneshian and Jabary [22]. Increasing nitrogen consumption oil percentage decreased (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 percent obtaind from 100*8.33 and 220*5.55 treatments 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 agree results of Alyari *et al.* [25] and Roberts [16]. 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 * plant density interaction was significant on protein percentage (Table 2). Mean comparison of two-year showed that protein percentage content strongly affected by water deficit stress and with intensifying stress increased significantly (Tables 2, 3, 5). In each irrigation level with plant density increase protein percentage decreased significantly so the maximum and minimum protein percentage were obtained in severe drought stress * 5.55 plant m^{-2} and optimum irrigation* 8.33 plant m^{-2} respectively (table 5). Severe drought stress increased the protein percentage by 8% compared to the protein percentage optimum irrigation condition. These results agree with results of Majd *et al.* [26]. The reason of protein percentage increase with intensifying drought stress was due to increasing oil percentage that it had negative and significant correlation (table 8). In this study, increasing the use of nitrogen 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 nitrogen ha^{-1} (table 3). Nitrogen consumption 220 kg nitrogen ha^{-1} in compared with 160 and 100 nitrogen ha^{-1} protein percentage increased about 2 and 5 percent respectively. The reason of protein percentage increase 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 reported by other researchers [23]. Liyong *et al.* [9] stated that fertilizer of nitrogen increase grain protein content more than drought stress and in drought stress condition lower application of nitrogen affected strongly on protein percentage. With plant density increase as a result number of more grains amount raw protein was diluted and led to grain protein content decrease. These results agree with results of Fallah and Tadayon [27]. Studding table of correlation coefficients showed that between protein percent with grain yield, oil yield and oil percentage had negative and significant correlation (table 8).

Protein Yield: The results of combined ANOVA showed that the effect of drought stress, nitrogen, plant density, irrigation * plant density and irrigation * nitrogen interaction was significant on protein yield (table 2). Mean comparison of two-year showed that protein yield strongly affected by water deficit stress and with intensifying stress decreased significantly (tables 2, 4 and 5). In each irrigation level with increasing application of nitrogen protein yield increased significantly so the maximum and minimum protein yield were obtained in optimum irrigation * 220 kg nitrogen ha-1 and severe drought stress * 100 kg nitrogen ha-1 respectively (table 4). Severe drought stress decreased the protein yield by 51% compared to the protein yield optimum irrigation condition. The reason of protein yield decrease with increasing severe drought stress was due to reduction of grain yield.

Increasing protein yield resulting use of high nitrogen is related to grain yield and protein percentage increase. Because with increasing food elements in soil theirs absorbing from soil increase and this affair led to grain yield and protein yield increase. Similar these results reported by other researchers [27, 28]. Researchers mentioned genotype, environmental factors and agronomy managements in growth and development of sunflower important on grain protein yield [29]. Mean comparison of two-year showed that in each irrigation level with plant density increase protein yield increased significantly so the maximum and minimum protein percentage were obtained in optimum irrigation* 8.33 plant m^{-2} and severe drought stress * 8.33 plant m^{-2} respectively (table 5). These results agree with results of Fallah and Tadayon [27]. Studding table of 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 (table 8).

Phyllochron: The results of combined ANOVA showed that the effect of drought stress, nitrogen, plant density and irrigation * nitrogen interaction was significant on phyllochron (table 2). Mean comparison of two-year showed that phyllochron strongly affected by water deficit 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 in severe drought stress * 100 kg nitrogen ha^{-1} and optimum irrigation * 220 kg nitrogen ha^{-1} respectively (table 4). Severe drought stress increased phyllochron compared to the phyllochron optimum irrigation condition. The reason of protein yield increase with increasing severe drought stress was due to reduction of leaf appearance rate resulting water deficit. Increasing plant density levels had significant effect on phyllochron and leaf appearance rate so that with plant density increase, leaf appearance rate decreased but phyllochron increased (table 3). These results agree with results of Longnecker *et al.* [30]. Albert and Carberry [31] and Mc Cullough [32] in oneself experiments phyllochron increase attributed to moisture decrease in soil. Hokm Ali pour *et al.* [8] showed that with increasing plant density, phyllochron increased but with increasing nitrogen, phyllochron decreased. Studding table of correlation coefficients showed that among phyllochron with grain yield, protein percentage and oil yield negative and significant correlation (table 8).

Leaf Appearance Rate: The results of combined ANOVA showed that the effect of drought stress, nitrogen, plant density and irrigation * nitrogen, irrigation * plant density, nitrogen * plant density and irrigation * nitrogen * 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 deficit 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 the leaf appearance rate by 30% compared to the leaf appearance rate optimum irrigation condition. In each level of irrigation with increasing application of nitrogen leaf appearance rate increased significantly (table 4). In each level application of nitrogen with plant density increase, leaf appearance rate decreased significantly (table 6). In each level of irrigation the maximum and minimum leaf appearance rate were obtained from 220 kg nitrogen ha^{-1} *5.55 plant m^{-2} and 100 kg nitrogen ha^{-1} *8.33 plant m^{-2} respectively (table 7). Ellis *et al.* [33] reported that if photosynthesis active radiation become twice, leaf appearance rate increase 17%. Hokm Ali Pour *et al.* [8] showed that with increasing nitrogen, leaf appearance rate increased but phyllochron decreased. Studding table of 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 (table 8).

Grain 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, grain yield decreased. Increasing nitrogen application caused grain yield to increase. The consumption of more quantities of fertilizer at optimum irrigation condition caused considerable increase in grain yield whereas at severe drought stress condition using more quantities of nitrogen did not increased grain 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 grain yield (table 3). Many researchers have pointed to grain yield increase due to increasing plant density [35, 36]. The most grain yield of about 4679.7 kg ha^{-1} obtained from the highest density at optimum irrigation treatment but in moderate and severe drought stress conditions yield increase due to the increase of density was not significant. These results are showing that the use of high densities can be useful at optimum condition. Liang *et al.* [37] reported that maximum grain yield in maize need high plant density, more irrigation, use of plenty of fertilizer and meeting temperature requirements. In low densities grain yield was low 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. By plant density increase, grain yield per area unit became more due to the increase in the number of plants per area.

CONCULUTION

Different levels of Water deficit stress had different effects on plant. Among the most important results obtained about application of water deficit stress, significant reduction of, oil yield, oil percentage, protein yield, leaf appearance rate and grain yield at severe and moderate stress in comparison with optimum irrigation can be pointed out. By increasing nitrogen fertilizer from 100 to 220 kg nitrogen ha^{-1} most of the aforementioned traits increased significantly. With plant density increase traits of oil yield, oil percentage, phyllochron and grain yield increased too but with plant density increase from 5.55 to 8.33 plants per hectare, protein percentage and leaf appearance rate found out significant reduction. Combined effects of irrigation*nitrogen showed that with increasing nitrogen, oil yield, protein yield, leaf appearance rate and grain yield increased. Interaction between of irrigation*plant density showed that at optimum irrigation with the increase in plant density, oil yield, oil percentage, protein yield and grain yield increased but at severe drought stress the highest grain yield obtained from lowest density. Interaction between of nitrogen*plant density showed that the highest oil percentage obtained from the lowest level of fertilizer application and the 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^{-1} and plant density of 8.33 plants per m^{-2} and at drought stress condition fertilizer treatment of 160 kg nitrogen ha^{-1} and density of 5.55 plants per m^{-} is^{2} recommended.

REFERENCES

1. Hatim, M. and G.Q. Abbasi (Ed.), 1994. Oilseed crops. In Crop Production. National Book Foundation, Islamabad, Pakistan.
2. 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.
3. 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. Journal of Agronomy Sciences of Iran, 7(3): 212-225.
4. 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.
5. Taheriasbagh, F., A. Fayazmoghaddam and A. Hassanzadeh GHorttapeh, 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.
6. Regina, H., 2008. Influence of Macro-and Micro nutrient fertilization on fungal contamination and fumonisin production in corn grains. Food control, 19: 36-43.
7. 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 11th congress of.
8. Hokm Ali Pour, S., R. Seyed Sharifi, M. Ghadim Zade and Sh. 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.
9. Liyong, H., C. Hao, Z. Guangsheng and F. Tingong, 2007. The influence of drought on Brassica napus L. development under different nitrogenous level. pp: 235-236. Proceeding of the 12th International Rapeseed Congress Sustaible Development in Cruciferous Oilseed Crops Production. 26-30 March Wuhan, China. Science Press USA Inc.
10. 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 sciences, 2(1): 17-24.
11. 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. Agri science and natural resources J., 13: 2.
12. 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 Science, 14(4): 289-294.
13. 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.
14. 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 10th congress agronomy and breeding of Iran. Karaj. 19-20 Jul., pp: 246.
15. 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.
16. Roberts, E.H., 1998. Quantifying seed deterioration. ln: M.B. Mcdonald, J.r. and C.J. Nelcon(eds), Physiology of seed deterioration, Cssa special publication, Madison, Wl, U. S. A, pp: 101-123.
17. 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.
18. 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.

19. 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.
20. Anonymous, 1993. The part of oily seeds researches. Report of Sunflower investigation. Institution of breeding and preparing seed and seedling of Karaj, pp: 60.
21. 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 10th congress agronomy and breeding of Iran. Karaj. 19-20 Jul., pp: 247.
22. 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.
23. 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 Environment, 60: 157-164.
24. Nandhagopal, A., K.S. Subramanian and A. Gopalan, 1995. Respnse of sunflower hybrids to nitrogen and phosphorus under irrigated condition. Madras Agricultural Journal, 82: 80-83.
25. Aliyari, H., F. Shekari and F. Shekari, 2000. Oily Seeds, Agronomy and Physiology. Tabriz. Publications of Abdi Tabriz, pp: 182.
26. Majd, A., P. Jenobi, J. Daneshian and M. Zaynipor, 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. Daries 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 Science, 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 Research, 31: 55-59.
32. Mc Cuullovgh, D.E., P.H. Girardin, M. Mihajlovic, A. Agvilera 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 Science, 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 pheotypes. 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.