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Full Length Research Paper

Effect of water deficit stress on proline contents, soluble sugars, chlorophyll and grain yield of sunflower (*Helianthus annuus* L.) hybrids

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The objective of the present work was to determine the mechanisms of tolerance of four sunflower hybrids; H_1 = Azargol, H_2 = Alstar, H_3 = Hysun 33 and H_4 = Hysun 25 to water stress under three different levels of irrigation regimes; WD_1 = irrigation after 50 mm (normal irrigation), WD_2 = 100 mm (mild stress) and WD_3 = 150 mm (intense stress) cumulative evaporation from evaporation pan class A. The results showed that water deficit stress significantly ($P \leq 0.01$) increased proline, soluble sugars and chlorophyll b but decreased chlorophyll a, total chlorophyll and grain yield in all sunflower hybrids; therefore increase of the proline, soluble sugar, chlorophyll b and decrease of the chlorophyll a, total chlorophyll and grain yield occurred when water input decreased. Although, at different level of water stress, each hybrid behaved differently according to their genetic makeup. Alstar hybrid exhibited the highest value for all the mentioned characteristics, except for soluble sugars. Under both mild and intense water deficit stress conditions, the highest value of proline, soluble sugars, chlorophyll a and chlorophyll b were recorded in Alster, Azargol, Hysun 33 and Alstar hybrids, respectively. Also, the highest value of total chlorophyll in both mild and intense water deficit stress conditions was acquired by Hysun33 and Alstar, respectively. The results also indicated that under normal irrigation, mild and intense water deficit stress, maximum grain yield was obtained in Azargol (3448 kg ha^{-1}), Alstar (2121 kg ha^{-1}) and Alstar (829 kg ha^{-1}), respectively. Therefore, among all of sunflower hybrids, Alstar hybrid under both levels of water deficit stress had the best tolerance to water deficiency stress.

Key words: Proline, soluble sugars, chlorophyll, grain yield, sunflower.

INTRODUCTION

Water shortage is the most important component of life that limits plant growth and crop productivity particularly in arid regions more than any other single environmental factor (Boyer, 1982; Soriano et al., 2004; Sinclair, 2005). Reduced precipitation together with the higher evapotranspiration is expected to subject natural and agricultural vegetation to a greater risk of drought in those areas (Samarakoon and Gifford, 1995). Even a short-term drought can cause substantial losses in crop yield (Ashraf and Mehmood, 1990). Decreasing water supply either temporarily or permanently affects morphological and physiological processes in plants adversely.

Differences in water relation characteristics reflect the differences between the species and lines, and are considered as an indicator of drought resistance or tolerance (Sobrado and Turner, 1983). Particularly, osmotic adjustment (active lowering of osmotic potential in response to drought) is a mechanism that significantly contributes towards drought resistance (Blum and Sullivan, 1986; Ludlow and Muchow, 1990).

Sunflower, with a world production of grain and oil, respectively over 28.5×10^6 Mg and 10.5×10^6 Mg and achieved on around 22.6×106 ha with a seed yield of 1.3 Mg ha^{-1} (2003–2007, means), is one of the most common grown oilseed species (FAO-STAT Agriculture, 2009). Water shortage and the increasing competition for water resources between agriculture and other sectors compel the adoption of irrigation strategies in semi arid Mediter-

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Table 1. Soil characteristics of the experimental site.

Physiochemical property	Result
Soil texture	Loam
Sand (%)	36
Silt (%)	39
Clay (%)	25
Saturation percentage	33
Organic matter (%)	1.1
NH4–N (mg/kg dry soil)	0.11
Available phosphorus (mg/kg of dry soil)	3.1
Potassium (mg/kg of dry soil)	245
Calcium (mg/kg of dry soil)	62.81
Soil pH	7.62
Electrical conductivity (dSm^{-1})	1.8

ranean regions, which may allow saving irrigation water and still maintain satisfactory levels of production (Costa et al., 2007). Water deficit effects have been extensively studied on several crops, maize (Achakzai, 2008), sorghum (Achakzai, 2007 and 2009a, b), sugar beet (Sepaskhah and Kamgar-Haghighi, 1997) and hot pepper (Dorji et al., 2005). Moreover, it is reported that dwarf sunflower lines are more drought tolerant than tall lines, showing a smaller decrease in leaf osmotic potential in response to drought stress (Angadi and Entz, 2002). The accumulation of osmolytes during stress is well documented. Recent studies demonstrated that biosynthesis of low-molecular-weight metabolites, such as proline improved plant tolerance to drought and salinity in a number of crops (Molinari et al., 2004; Zhu et al., 2005). Proline accumulation in plant cells exposed to water stress or salt is a widespread phenomenon and is often considered to be involved in stress resistance mechanisms, although its precise role continues to be controversial (Aspinall and Paleg, 1981; Yoshiba et al., 1997; Hare et al., 1999).

Some evidences have indicated that water stress deficit causes considerable decrease in grain yield of sunflower (Stone et al., 2001). Although voluminous literature is available for water stress effects on sunflower (Wise et al., 1990; Tahir and Mehdi, 2001; Angadi and Entz, 2002), information regarding the effect of normally irrigated and water deficit environment on proline, soluble sugars, chlorophyll and grain yield is scanty. Therefore this study was mainly conducted to determine whether and how water deficit conditions influence tolerance to water deficiency stress and grain yield of sunflower hybrids.

MATERIALS AND METHODS

The experimental factors were irrigation regimes consisting of three levels of irrigation after 50 mm (normal irrigation), 100 mm (mild stress) and 150 mm (intense stress) cumulative evaporation from evaporation pan class A, respectively, and genotype represented by four sunflower hybrids (Azargol, Alstar, Hysun 33 and Hysun 25). Sunflower seeds were obtained from the Plant Improvement Institute in Karaj, Iran. All combinations of the above treatments were laid out in 2009 in the field according to a split-plot randomized complete block design (RCBD) with three replicates, assigning water supply treatments to the main units and genotypes to the subunits. The soil used was loam. The soil texture was determined with the hygrometer method (Dewis and Freitas, 1970). The physiochemical characteristics of the experimental site are presented in Table 1. Electrical conductivity, pH and ions of saturation extract were determined according to Jackson (1962). The available phosphorous was determined from saturated paste extract (Olsen and Sommers, 1982). The ammonium was estimated by acid digested material (Bremner and Mulvaney, 1982) and organic matter through sulphuric acid using the Walkley-Black Method (Sahrawat, 1982).

The pre-planting irrigation was applied 15 days before sowing. As soon as the soil came into the condition of field capacity, it was well ploughed for sowing. Seeds were hand drilled on May 14, 2009 with row to row distance of 65 cm. Thinning of the plants was done 15 days after germination to keep plants at a distance of 20 cm. Water deficit treatments were applied at the vegetative stages of plant growth (Chimenti and Hall, 1993). The proline content of each hybrid was determined as done by Pquine and Lechasseur (1979) while soluble sugars were measured as described by Irigoyen et al. (1992). Chlorophyll was determined according to Wintermans and De Mots (1965) after extraction in 96% (v/v) ethanol. At maturity, yield $plant^{-1}$ was recorded. The area of 5.2 m^2 from the middle of each subplot was harvested and their seeds were separated manually from heads to determine their yield, yield components, oil and protein contents.

Analysis of variance (ANOVA) of the data from each attribute was computed using the SAS package (SAS Institute, 1988) and MSTAT Computer Program (MSTAT Development Team, 1989). The Duncan's New Multiple Range test at 5% level of probability was used to test the differences among mean values (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The analysis of variance for proline, soluble sugars, chlorophyll a, chlorophyll b, total chlorophyll and grain yield is summarized in Table 2, and it showed significant effects ($P \leq 0.01$) of water deficit stress on all the studied traits. Differences among genotypes were significant for the studied traits and the interaction between water treatments and genotypes was significant for all the studied traits (Table 2). Water deficit stress increased the levels of proline, soluble sugars and chlorophyll b (Table 3). The increase in proline, soluble sugars and chlorophyll b was more pronounced in intense water deficit stress (WD_3 = 150 mm cumulative evaporation) than that in the mild water deficit stress (WD_2 = 100 mm cumulative evaporation) (Table 3), which may be due to some physiological mechanism for reducing the adverse effect of water deficit stress on plant productivity (Blum and Sullivan, 1986; Ludlow and Muchow, 1990). Application of intense water deficit stress (WD_3) caused an increase in proline (106%), soluble sugars (342%) and chlorophyll b (49%) and a decrease in chlorophyll a (37%), total chlorophyll (14%) and grain yield (79%) as

Table 2. Mean square values from the analysis of variance of proline, soluble sugars, chlorophyll a, chlorophyll b, total chlorophyll and grain yield of sunflower hybrids (H) subjected to water deficit stress (WD).

S.O.V	df	Proline (mg l^{-1})	Soluble sugars (mg g^{-1} FW)	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Grain yield (kg ha^{-1})
Rep	2	1.26 ns	7787.03 ns	26.65*	17.48**	87.21**	147177.33**
Water deficit (WD)	2	1309.04**	215431.73**	239.02**	51.46**	69.50**	12825106.54**
Error a	4	2.91	1253.95	2.47	0.19	2.08	16140.67
hybrid (H)	3	1295.73**	114709.91**	155.70**	26.44**	308.63**	1130703.75**
WD × H	6	340.44**	21007.53**	119.32**	9.45**	98.56**	840906.04**
Error b	18	14.20	4603.622	5.36	0.66	4.26	48939.56
CV	---	12.31	35.87	12.30	7.78	7.05	15.01

* = $p < 0.05$; ** = $p < 0.01$; NS = non-significant.

Table 3. Effect of irrigation treatments and hybrids on studied traits.

Treatment†		Proline (mg l^{-1})	Soluble sugars (mg g^{-1} FW)	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Seed yield (kg ha^{-1})
Water deficit stress (WD)[1]	WD_1	19.08^{c}	76.31^{c}	23.50^{a}	8.38^{c}	31.89^{a}	2591^{a}
	WD_2	33.26^{b}	153.79^{b}	18.32^{b}	10.47^{b}	28.80^{b}	1274^{b}
	WD_3	39.45^{a}	337.21^{a}	14.62^{c}	12.52^{a}	27.15^{c}	552^{c}
Hybrid (H)[2]	H_1	19.69^{c}	352.31^{a}	14.85^{c}	8.61^{c}	23.46^{d}	1585^{b}
	H_2	45.43^{a}	176.05^{b}	24.34^{a}	12.71^{a}	37.06^{a}	1914^{a}
	H_3	22.10^{c}	105.16^{c}	19.52^{b}	10.59^{b}	30.11^{b}	1284^{c}
	H_4	35.15^{b}	122.91^{bc}	16.55^{c}	9.92^{b}	26.47^{c}	1107^{c}

† WD_1= Normal irrigation; WD_2= Mild water deficit stress; WD_3= Intense water deficit stress; H_1= Azargol; H_2= Alstar; H_3= Hysun 33; H_4= Hysun 25. $^{a, b, c, d}$ Within columns, means followed by the same letters are not significantly different ($P < 0.05$).

compared with the normally irrigated ones (Table 3). Based on the comparison among sunflower hybrids, the sunflower hybrid Alstar showed the highest amounts of proline (45.43 mg l^{-1}), chlorophyll a (24.34 mg g^{-1} FW), chlorophyll b (12.71 mg g^{-1} FW), total chlorophyll (37.06 mg g^{-1} FW) and grain yield (1914 kg ha^{-1}), while sunflower hybrid Azargol had the highest value of soluble sugar (176.05 mg g^{-1} FW) (Table 3). The means comparison for the water treatment-hybrid interaction is summarized in Table 4. The results of this study indicated that in well-watered conditions, dwarf sunflower hybrid Hysun 25 had the highest proline content (23.69 mg l^{-1}) while the tall sunflower hybrid Azargol had the lowest level of proline (16.54 mg l^{-1}) (Table 4). Based on the results shown in Table 4, under mild and intense water deficit stress, the amount of proline in all sunflower hybrids increased. In mild and intense water deficit stress conditions, sunflower hybrid Alstar with values 50.68 and 67.95 mg l^{-1}, respectively had the highest proline and showed statistical significant in comparison with other sunflower hybrids (Table 4). Also, under mild and intense water deficit stress conditions, sunflower Azargol produced the lowest amount of proline (Table 4). Table 4 shows that in all water treatments, sunflower hybrid Azargol had the highest amount of soluble sugars, while lower levels of soluble sugars were accumulated in Hysun 33, Hysun 25 and Hysun 33 under normal irrigation, mild and intense water deficit stress condition, respectively (Table 4). Water deficit stress had significant adverse effect on chlorophyll a content in all the sunflower hybrids (Table 4). In normal irrigation, the dwarf sunflower hybrid Alstar and in both mild and intense water deficit stress conditions, the sunflower hybrid Hysun 33 had the highest chlorophyll a content (Table 4). Generally, comparison among all the water treatments and sunflower hybrids indicated that the maximum chlorophyll content was obtained in normal irrigation by sunflower hybrid Azargon (Table 4).

The results of this study showed that the water deficit stress increased the chlorophyll b content in all the sunflower hybrids; therefore, the highest and the least chlorophyll b content was obtained in intense water deficit stress by sunflower hybrid Alstar (17.63 mg g^{-1} FW) and in normal irrigation by sunflower hybrid Azargol (6.19 mg g^{-1} FW), respectively (Table 4). The highest total chlorophyll content in normal irrigation and intense water deficit stress was obtained by Alstar with values 49.02 and 32.02 mg g^{-1} FW respectively, but in the mild water deficit stress, Hysun 33 had the highest amount of this trait (Table 4). Total imposition of water deficit stress

Table 4. Effect of irrigation treatment-hybrid interaction on proline, soluble sugars, chlorophyll a, chlorophyll b, total chlorophyll and grain yield.

Treatment†		Proline (mg l^{-1})	Soluble sugars (mg g^{-1})	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Seed yield (kg ha^{-1})
WD1[1]	H_1	16.54[g]	151.80[def]	17.69[bcd]	6.19[f]	23.88[ef]	3448[a]
	H_2	17.68[fg]	58.27[fg]	39.60[a]	9.42[d]	49.02[a]	2793[ab]
	H_3	18.40[efg]	33.44[g]	20.09[bc]	9.87[cd]	29.96[c]	2437[b]
	H_4	23.69[de]	61.70[fg]	16.63[cde]	8.05[e]	24.69[e]	1688[cd]
WD2[1]	H_1	20.95[defg]	272.70[bc]	13.53[e]	9.34[de]	22.78[f]	893[ef]
	H_2	50.68[b]	142.70[def]	19.04[bc]	11.09[bc]	30.13[c]	2121[bc]
	H_3	23.28[def]	105.10[defg]	21.40[b]	10.96[bc]	32.35[b]	1154[de]
	H_4	38.14[c]	94.71[efg]	19.32[bc]	10.51[cd]	29.84[c]	929[ef]
WD3[1]	H_1	21.6d[efg]	632.40[a]	13.32[e]	10.31[cd]	23.64[ef]	413[ef]
	H_2	67.95[a]	327.20[b]	14.38[de]	17.63[a]	32.02[b]	829[ef]
	H_3	24.62[d]	177.00[cde]	17.07[cde]	10.96[bc]	28.03[d]	263[f]
	H_4	43.63[c]	212.30[cd]	13.70[e]	11.91[b]	24.90[e]	704[ef]

† WD_1= Normal irrigation; WD_2= Mild water deficit stress; WD_3= Intense water deficit stress; H_1= Azargol; H_2= Alstar; H_3= Hysun 33; H_4= Hysun 25. [a, b, c, d, e, f, g] Within columns, means followed by the same letters are not significantly different ($P < 0.05$).

caused 1, 32 and 6% decrease in total chlorophyll content of sunflower hybrids Azargol, Alstar and Hysun 33, respectively, and 0.8% increase in Hysun 25 (Table 4). The highest and least grain yield in normal irrigation was found in Azargol (3448 kg ha^{-1}) and Hysun 25 (1688 kg ha^{-1}), respectively (Table 4). However, in the mild and intense water deficit stress conditions, the grain yield with cultivar Alstar had the highest value (2121 and 829 kg ha^{-1} respectively) and Azargol and Hysun 33 had the lowest value (893 and 263 kg ha^{-1}, respectively) (Table 4). The means comparison between the three irrigation treatments for the studied traits showed that water deficit stress had significant adverse effect on grain yield but dwarf sunflower hybrids especially Alstar due to its high adaptability yielded an economically grain yield in water deficit stress conditions (Table 3). Ashraf and Mehmood (1990) reported that even a short term water deficit stress can cause substantial losses in crop yield and that is in agreement with our results.

Out work is in agreement with previous studies that showed that water deficit stress has adverse effects on sunflower productivity. A large genetic variation was observed for proline, soluble sugars, chlorophyll a and b and total chlorophyll accumulation and grain yield in well watered and water deficit stress conditions. In our study, dwarf cultivars especially sunflower hybrids Alstar under water deficit stress conditions maintained the highest grain yield. Angadi and Entz (2002) reported that dwarf sunflower lines are more drought tolerant than tall lines. From the results in this work, it seems that proline and soluble sugars might confer drought stress tolerance to sunflower hybrids by increasing some mechanism such as osmotic adjustment.

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