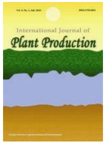

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Physiological responses of sunflower to water stress under different levels of zinc fertilizer

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Abstract

To investigate the physiological responses of sunflower (*Helianthus annuus* L., Alstar hybrid) to water stress under different levels of zinc fertilizer, an experiment was conducted at the Isfahan Agricultural Research Center, Isfahan, Iran, during 2008 and 2009 using a randomized complete block design within a split plot layout with three replications. Five irrigation treatments used in this experiment to impose water stress were IR1 (irrigation after 70 mm cumulative evaporation from class A evaporation pan (CE) during the entire growth cycle as control treatment), IR2 (irrigation after 120 mm CE during the entire growth cycle), IR3 (the same as IR1, except withholding one irrigation at initiation of peduncle elongating (R2)), IR4 (the same as IR1, except withholding one irrigation at the beginning of flowering (R5.1)) and IR5, (the same as IR1, except withholding one irrigation at 70 to 80% flowering (R5.7-8)). Irrigation treatments were allocated to main plots and three zinc fertilizer levels (0, 30 and 60 kg ha^{-1} of zinc sulfate) to subplots. Water stress reduced leaf relative water content (LRWC), chlorophyll a (CHLa) and b (CHLb), chlorophyll a/b (CHLa/b), total chlorophyll (CHLt), leaf area index (LAI), leaf dry weight (LDW) and head dry weight (HDW), but increased proline (PR) content of leaves. Sixty Kg ha^{-1} zinc sulfate fertilization could partly prevent deleterious effects of water stress at some occasions. This level of zinc sulfate application might be recommended under conditions similar to this experiment which sufficiency of soil zinc content to cope with water stress is in doubt.

Keywords: Chlorophyll; Dry weight; Leaf area index; Proline; Relative water content; Zn.

Introduction

Plants are frequently subjected to intermittent or continuous water stress during their life span. Loss of leaf turgor pressure under water stress condition suppress cell expansion and growth leading to reduction in leaf area (Gholinezhad et al., 2009; Jaleel et al., 2009; Þerbea and Petcu, 2000; Rauf and Sadaqat, 2008), dry matter accumulation and plant seed yield (Ebrahimi et al., 2011; Gholinezhad et al., 2009; Jaleel et al., 2009; Petcu et al., 2001; Oraki et al., 2012; Rauf and Sadaqat, 2008; Solimanzadeh et al., 2010).

Leaf relative water content (LRWC) is a measure of plant water status and reflects the metabolic activity of tissues and is used as a meaningful index for dehydration tolerance (Anjum et al., 2011). Drought affected leaves exhibit large reduction in LRWC (Anjum et al., 2011; Rauf and Sadaqat, 2008). Ünyayar et al. (2004) found that resistant genotypes of sunflower had higher LRWC under water stress.

The maintenance of leaf turgor under water stress might be achieved through proline accumulation in cytoplasm improving water uptake from drying soil (Anjum et al., 2011; Chaves and Oliveira, 2004; Mafakheri et al., 2010; Manivannian et al., 2007; Mattioli et al., 2009; Oraki et al., 2012; Rauf and Sadaqat, 2008), leading to leaf area expansion, increase in photosynthesis and assimilate supply for growth (Anjum et al., 2011; Ünyayar et al., 2004). Proline also protects membranes, macromolecules and sub-cellular organelles under dehydrating stress (Anjum et al., 2011; Chaves and Oliveira, 2004; Szabados and Savouré, 2010) and might be also a part of the stress signaling influencing adaptive responses (Mafakheri et al., 2010; Szabados and Savouré, 2010). Proline concentration has been shown to be higher in stress-tolerant than in stress-sensitive plants (Anjum et al., 2011; Oraki et al., 2012).

Relative chlorophyll content has a positive relation with photosynthetic rate. The decrease in chlorophyll content has been considered a typical symptom of oxidative stress and chlorophyll degradation under water stress condition (Oraki et al., 2012; Petcu et al., 2001; Pirzad et al., 2011). Both chlorophyll a and b are sensitive to soil drying (Anjum et al., 2011; Jaleel et al., 2009; Manivannian et al., 2007; Poormohammad Kiani et al., 2008; Pirzad et al., 2011). Reduction in chlorophyll content due to water stress has been shown to decrease photosynthesis, leaf area index, leaf dry weight, grain yield and biological yield of sunflower (Gholinezhad et al., 2009;

Petcu et al., 2001; Þerbea and Petcu, 2000; Solimanzadeh et al., 2010; Ünyayar et al., 2004). In the experiments of Oraki et al. (2012) with sunflower hybrids, chlorophyll a decreased, but chlorophyll b increased as water stress was intensified. No explanation for the increase in chlorophyll b was presented by the authors. Mafakheri et al. (2010) reported that chlorophyll a/b ratio in chickpea was not affected by water stress. While in the experiment of Mohammadkhani and Heidari (2007) with maize, chlorophyll a/b ratio depended on the interaction of genotype by severity of water stress. Pirzad et al. (2011) found that chlorophyll content was more sensitive to water stress than LRWC and proline content in *Matricaria chmomilla* L.

Apparently zinc is involved in the production of chlorophyll and zinc deficiency reduces chlorophyll a and b content of sunflower (Khurana and Chatterjee, 2001). Zinc is also considered an excellent protective agent against the oxidation of these vital cell components under water stress condition (Cakmak, 2000). Zinc foliar application activated enzymes involved in reactive oxygen species detoxification and increased leaf dry weight and accumulation of proline in sunflower under salt stress conditions (Ebrahimian and Bybordi, 2011). In the experiment of Siddiqui et al. (2009), addition of 15 kg ha^{-1} Zn to a clay loam soil (with 0.68 mg kg^{-1} Zn content) increased leaf area index, leaf area duration, crop growth rate, net assimilation rate and plant dry weight measured during flowering of sunflower and also increased yield.

It appears that water stress impairs plants and zinc alleviates water stress injuries. The purpose of this study was to evaluate the physiological responses of sunflower, Alstar hybrid, to intermittent and moderate water stress under various amounts of Zn fertilization.

Materials and Methods

The experiment was conducted at the Kabutar Abad Agricultural Research Station, Isfahan, Iran (32˚45´ N, 51˚47´ E, elevation 1570 m above sea level) in summer of 2008 and 2009. Commonly there is no rainfall during sunflower growth cycle in this area. Table 1 shows the weather conditions during the sunflower growth period over the two years under study. A randomized complete block design within a split plot layout with 15 treatments and three replications was used in this investigation.

Table 1. Averages of some climatic parameters during growth period of sunflower in two years of the study.

climate	July		Aug.		Sept.		Oct.		Nov.	
parameters	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
maximum temperature (°C)	37.7	38.0	35.5	35.3	33.2	33.5	26.9	29.3	22.2	23.3
minimum temperature (°C)	19.7	19.9	17.1	17.6	12.3	14.0	6.9	10.0	2.6	3.5
average temperature (°C)	28.7	28.9	26.3	26.4	22.8	23.8	16.9	19.6	12.4	14.3
daily evaporation (mm)	11.9	14.0	10.6	12.3	8.1	9.9	4.2	6.1	2.5	3.6
Average humidity (%)	30.5	27.0	32.0	28.0	33.5	35.0	39.5	34.0	43.5	39.0

Five irrigation schedules were considered in this experiment: IR1, irrigation after 70 mm cumulative evaporation from class A evaporation pan (CE) during the entire growth cycle (as optimum irrigation treatment). IR2, irrigation after 120 mm CE during the entire plant growth cycle (as continuous water stress treatment). IR3, the same as IR1, except withholding one irrigation at initiation of peduncle elongating (R2), IR4, the same as IR1, except withholding one irrigation at the beginning of flowering (R5.1). IR5, the same as IR1, except withholding one irrigation at 70 to 80% flowering (R5.7-8). Growth stages were determined as described by Schneiter and Miller (1981). It has been shown that approximately 50% of soil available moisture was depleted when soybean was irrigated after 70 mm CE under our climatic-edaphic conditions (Khodambashi et al. (1988). Irrigation treatments were allocated to main plots and three zinc fertilizer levels; 0 (Zn0), 30 (Zn30) and 60 (Zn60) kg ha^{-1} of zinc sulfate (incorporated in soil before planting) to sub plots. Daily evaporation data were obtained from the nearby weather station. For determining the volume of water to be applied per irrigation, soil was sampled from 0 to 60 cm depth, the day before the anticipated irrigation time and soil moisture content was determined. Occasional soil sampling showed that there was no moisture depletion beyond 60 cm depth. The required volume of water to bring soil to field capacity was calculated on the bases of water distribution efficiency of 90% and was applied using parshall flume and chronometer.

Seeds were planted on beds. The inter-row spacing was 60 cm and inter-plant distance was 16.6 cm. Soil was silty clay. Field was under fallow during the previous year. Soil was sampled from zero to 60 cm depth before fertilizer application and was analyzed for various constituents (Table 2).

Split application of 115 kg ha^{-1} nitrogen as urea (50% at planting and the rest at 7-8 leaf stage) and 45 kg ha^{-1} P_2O_5 as treble super phosphate were mixed with soil before planting. The Alstar hybrid (a French hybrid commonly planted over the area) was planted on July 5th in both years. This date corresponds to the date of planting sunflower as the second crop in Isfahan. The land was under fallow during the previous year. Weeds were controlled by hand at 20 and 40 days after planting.

LRWC, PR, LAI, LDW and HDW were determined on IR1, IR2 and IR3 before re-irrigating IR3. Chlorophyll, LRWC, PR, LAI, LDW and HDW were measured on IR1, IR2 and IR4 before re-irrigating IR4 and on IR1, IR2 and IR5 before re-irrigating IR5. Ten leaves were randomly selected from the middle section of plants in each experimental plot for chlorophyll, LRWC and PR determination. Chla and Chlb contents were determined as described by Arnon (1949). Chlt was calculated as the sum of Chla and Chlb. LRWC was measured following the procedure described by Barrs and Weatherley (1962). PR was measured using the procedure described by Bates et al. (1973). Five plants were harvested from the middle row of each experimental plot for leaf area, LDW and HDW measurements. Leaf area meter (LP-80 Accupar PAR/LAI Ceptometer) was used for leaf area determination. Leaf area index (LAI) was calculated using the measured area of leaves and the area under sampled plants (inter-row by inter-plant distances). Sampled leaves and heads were weighted after drying at 70 ^{o}C for approximately 72 hours in a ventilated oven. Data were statistically analyzed using ANOVA procedure of SAS and the means were compared using LSD at 5 present level of probability.

Results and Discussion

Relative water content

The effect of irrigation regime on LRWC at R2 growth stage was significant in both 2008 ($P<0.05$) and 2009 ($P<0.01$) (Table 3). In 2008, LRWC was reduced about 14.5% by IR2 and IR3 decreased LRWC about 10% in comparison to IR1. In 2009, IR3 decreased LRWC around 8%. The difference between IR2 and IR1 was small and non-significant (Table 4). LRWC was not significantly affected by zinc application in the years under study (Table 3). However, application of 60 kg ha^{-1} zinc sulfate slightly increased LRWC (about 5%) in 2009 (Table 4). The interaction of irrigation by zinc treatments was significant in 2008 (Table 3). Zinc application reduced LRWC in IR1, while fertilization with 60 kg ha^{-1} zinc sulfate increased LRWC in IR3 (Figure 1).

Table 2. Chemical and physical properties of soil at 0-60 cm depth.

Year	EC ($ds\ m^{-1}$)		pH	clay	silt	sand	O.C.[1]	N	P	K	Zn	Cu	Fe	Mn
	soil	water				%					mg kg^{-1}			
2008	4.87	2.96	7.60	39.8	45.8	14.4	0.64	0.06	11.2	269	0.38	1.34	4.58	4.3
2009	5.14	4.80	7.40	41.3	44.1	14.6	0.63	0.06	27.7	288	0.33	1.76	4.26	7.3

1- Organic carbon.

Table 3. Analysis of variance (F value)1 for traits2 of sunflower in different water stress and zinc fertilization at R2 growth stage.

Source of variation	LRWC		PR		LAI		LDW		HDW	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Replication	2.74	0.36	0.15	1.62	2.92	1.26	1.43	0.62	2.97	0.48
Irrigation	7.56*	21.11**	54.36**	37.35**	7.77*	18.87**	8.90*	2.60	9.56*	68.17**
Zinc	0.85	1.09	1.24	2.13	1.04	19.71**	0.16	4.63*	8.32**	0.22
Irrigation×Zinc	3.34*	0.37	1.36	1.37	2.76	1.36	1.81	0.86	1.36	2.87
CV	9.97	6.55	2.97	3.31	14.28	10.90	12.57	14.18	10.41	14.16

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

2- LRWC= relative water content, PR= proline, LAI= leaf area index, LDW= leaf dry weight, HDW= head dry weight.

Table 4. Mean comparison1 for traits2 of sunflower in different water stress and zinc fertilization at R2 growth stage.

Treatments	LRWC		PR (μmol gr^{-1})		LAI		LDW (kg ha^{-1})		HDW (kg ha^{-1})	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
					Irrigation					
IR1	0.491^{a}	0.627^{a}	113^{b}	109^{c}	2.78^{a}	2.46^{a}	2229^{a}	1909^{a}	1557^{a}	1253^{a}
IR2	0.420^{b}	0.611^{a}	127^{a}	117^{b}	1.84^{b}	1.80^{b}	1533^{b}	1533^{a}	1253^{b}	865^{b}
IR3	0.443^{ab}	0.579^{b}	126^{a}	125^{a}	1.96^{b}	2.00^{b}	1906^{ab}	1711^{a}	1382^{ab}	875^{b}
					Zinc					
Zn0	0.462^{a}	0.594^{a}	123^{a}	115^{a}	2.14^{a}	1.72^{b}	1833^{a}	1619^{b}	1310^{b}	972^{a}
Zn30	0.436^{a}	0.601^{a}	123^{a}	119^{a}	2.28^{a}	1.94^{b}	1905^{a}	1896^{a}	1559^{a}	999^{a}
Zn60	0.456^{a}	0.621^{a}	121^{a}	117^{a}	2.14^{a}	2.58^{a}	1930^{a}	1637^{b}	1324^{b}	1023^{a}

1- Within each column and for each factor, means followed by the same letter are not significantly different at the 5% level of probability according to LSD test.
2- LRWC= relative water content, PR= proline, LAI= leaf area index, LDW= leaf dry weight, HDW= head dry weight.

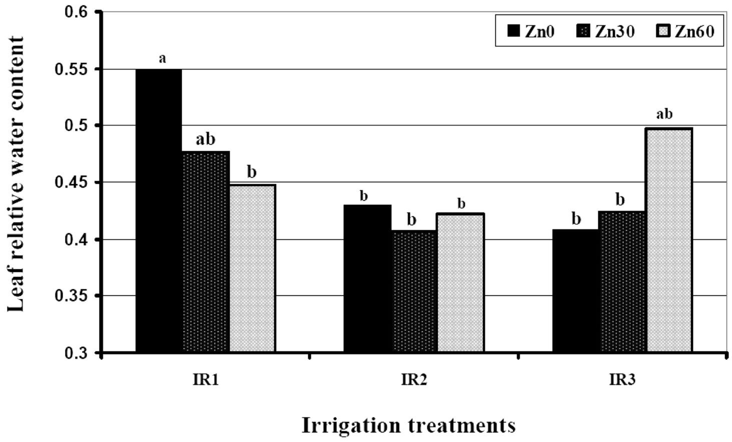

Figure 1. Interaction effects of irrigation with zinc on LRWC at R2 growth stage in 2008. See the text for definition of irrigation treatments. Columns with the same letter are not significantly different at 5% level of probability according to LSD test.

Irrigation treatment at R5.1 growth stage significantly ($P<0.05$) affected LRWC in both years (Table 5). In 2008, the difference between IR1 and IR2 for LRWC was not statistically significant. But around 10% reduction in LRWC was observed for IR4 treatment. In 2009, IR2 and IR4 caused about 10 and 13% reduction in LRWC, respectively as compared to IR1 (Table 6). In both years, LRWC was not significantly affected by zinc fertilization at R5.1 growth stage (Table 6) and no increase in LRWC was found due to zinc application (Table 5). The interaction of irrigation with zinc treatments on LRWC was not significant in both years (Table 5).

At R5.8-7 growth stage, the effect of irrigation regime on LRWC was significant ($P<0.05$) in 2008 and in 2009 (Table 7). In 2008, the reduction in LRWC brought about by IR2 was about 10% and by IR5 was about 6% as compared to IR1. In 2009, IR2 decreased LRWC around 11% and IR5 reduced LRWC about 6% (Table 8). LRWC was not significantly affected with zinc fertilization at R5.7-8 growth stage (Table 7) and no increase in LRWC was found due to zinc application (Table 8). The interaction of irrigation with zinc treatments on LRWC was not significant in none of the years under study (Table 7).

LRWC may indicate plant water status and metabolic activity of tissues (Anjum et al., 2011). Reduction in LRWC due to water stress has been shown in many sunflower genotypes (Gholinezhad et al., 2009; Rauf and Sadaqat, 2008; Ünyayar et al., 2004). Although the sunflower hybrid under study (Alstar) has been shown to be the most drought resistant genotype among the several hybrids commonly planted over the country (Oraki et al., 2012), the significant decreases in LRWC due to water stress at all growth stages found in the present experiment implicates that Alstar hybrid is also sensitive to water stress. Reductions in LRWC were much higher at R2 and R5.1 growth stages than at R5.7-8 growth stage. The differences between R5.1 and R5.7-8 growth stages could be attributed to diminution in water demand of plants due to aging of leaves. No literature reference was found for documenting. The differential responses of sunflower growth stages to water stress in term of WRC deserves further evaluation.

Salinity is shown to decreases LRWC in sunflower (Ebrahimian and Bybordi, 2011). Soil and water salinity was higher in 2009 than in 2008 (Table 2). In spite of this, LRWC was lower in 2008 than in 2009 at R2 and R5.1 growth stages. In contrast to the unexpected responses of LRWC to salinity at R2 and R5.1 growth stages, LRWC was higher in 2008 than in 2009 at R5.7-8 growth stage which is in agreement with the Ebrahimian and Bybordi (2011) results. The differential responses of LRWC to salinity at various growth stages could not be explained here.

Although zinc is considered to protect vital cell components under water stress condition (Cakmak, 2000), but it is not known to increase water absorption potential of plants and effect on LRWC. Consequently, lack of significant effect of zinc fertilization on LRWC in the present experiment might be acceptable. The reduction in LRWC due to zinc fertilization under no water stress condition and increase in LRWC by 60 kg ha^{-1} zinc sulfate application in IR3 (irrigation after 140 mm CE) which was found at R2 growth stage (Figure 1) could not be interpreted here and further investigation may be needed to clarify this interaction.

Table 5. Analysis of variance (F value)1 for traits2 of sunflower in different water stress and zinc fertilization at R5.1 growth.

Source of variation	LRWC		CHLa		CHLb		CHLa/b		CHLt		PR		LAI		LDW		HDW	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Replication	0.04	1.41	0.51	0.58	0.47	1.41	0.16	3.80	0.74	1.97	2.95	3.45	1.51	0.74	0.25	0.14	1.56	0.79
Irrigation	12.96*	15.55*	31.56**	113.27**	28.63**	17.48*	0.17	11.82*	102.96**	62.62**	49.36**	21.41**	13.53*	14.30*	6.67	0.42	1.62	8.05*
Zinc	3.90*	0.09	12.64**	13.34**	2.79	0.85	3.02	1.57	19.20**	9.34**	1.53	0.27	0.36	6.07*	0.19	0.21	0.60	0.66
Irrigation × Zinc	1.36	0.15	0.54	2.08	0.72	0.18	0.32	0.53	1.48	1.43	0.42	1.95	1.64	0.54	1.35	0.84	0.66	0.34
CV	6.16	6.89	7.54	6.11	5.11	8.95	11.44	10.65	4.08	5.05	2.69	2.25	14.89	13.72	13.16	14.68	14.53	13.62

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

2- LRWC= relative water content, CHLa= chlorophyll a, CHLb= chlorophyll b, CHLa/b= chlorophyll a/ chlorophyll b, CHLt= total chlorophyll, PR= proline, LAI= leaf area index, HDW= head dry weight, LDW= leaf dry weight.

Table 6. Mean comparison1 for traits2 of sunflower in different water stress and zinc at R5.1 growth stage.

Treatments	LRWC		CHLa		CHLb		CHLa/b		CHLt		PR		LAI		LDW		HDW	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
			(mg gr^{-1})		(mg gr^{-1})				(mg gr^{-1})		(μmol gr^{-1})				(kg ha^{-1})		(kg ha^{-1})	
Irrigation																		
IR1	0.520^{a}	0.580^{a}	0.747^{a}	0.678^{a}	0.745^{a}	0.625^{a}	1.02^{a}	1.10^{b}	1.49^{a}	1.30^{a}	93^{b}	103^{b}	2.78^{a}	2.51^{a}	2688^{a}	2087^{a}	4571^{a}	2769^{a}
IR2	0.500^{a}	0.524^{b}	0.612^{b}	0.673^{a}	0.591^{b}	0.519^{b}	1.03^{a}	1.29^{a}	1.21^{c}	1.19^{b}	101^{a}	106^{b}	1.94^{b}	1.84^{b}	2129^{a}	1847^{a}	3752^{a}	2227^{b}
IR4	0.470^{b}	0.511^{b}	0.642^{b}	0.550^{b}	0.639^{b}	0.500^{b}	1.00^{a}	1.11^{b}	1.28^{b}	1.05^{c}	104^{a}	112^{a}	2.42^{a}	2.05^{b}	1958^{a}	2051^{a}	4106^{a}	2231^{b}
Zinc																		
Zn0	0.522^{a}	0.538^{a}	0.601^{a}	0.583^{a}	0.637^{b}	0.537^{a}	0.94^{b}	1.11^{a}	1.23^{b}	1.12^{b}	98^{a}	107^{a}	2.07^{a}	2.22^{b}	2215^{a}	1946^{a}	3962^{a}	2312^{a}
Zn30	0.496^{ab}	0.542^{a}	0.682^{a}	0.642^{a}	0.664^{ab}	0.542^{a}	1.05^{ab}	1.20^{a}	1.35^{a}	1.18^{a}	100^{a}	107^{a}	2.08^{a}	2.24^{b}	2259^{a}	2006^{a}	4152^{a}	2431^{a}
Zn60	0.492^{b}	0.535^{a}	0.718^{a}	0.676^{a}	0.674^{a}	0.565^{a}	1.07^{a}	1.20^{a}	1.39^{a}	1.24^{a}	99^{a}	106^{a}	2.25^{a}	2.65^{a}	2302^{a}	2034^{a}	4316^{a}	2485^{a}

1- Within each column and for each factor, means followed by the same letter are not significantly different at the 5% level of probability according to LSD Test.

2-LRWC= relative water content, CHLa= chlorophyll a, CHLb= chlorophyll b, CHLa/b= chlorophyll a/ chlorophyll b, CHLt= total chlorophyll, PR= proline, LAI= leaf area index, HDW= head dry weight, LDW= leaf dry weight.

Table 7. Analysis of variance (F value)1 for traits2 of sunflower in different water stress and zinc fertilization at R5.7-8 growth stage.

Source of variation	LRWC		CHLa		CHLb		CHLa/b		CHLt		PR		LAI		LDW		HDW	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
Replication	2.98	1.21	0.31	0.08	0.14	0.72	0.45	0.73	0.04	0.41	0.29	0.77	1.11	3.16	3.62	3.08	0.06	0.12
Irrigation	9.48^{*}	14.30^{*}	25.78^{**}	6.05	14.06^{*}	14.51^{*}	7.48^{*}	8.45^{*}	33.48^{**}	9.88^{*}	11.68^{*}	14.45^{*}	8.79^{*}	23.21^{**}	30.00^{**}	0.53	1.36	35.31^{**}
Zinc	1.66	0.99	0.06	4.16^{*}	0.09	0.30	0.32	0.46	0.05	2.13	0.10	0.20	7.81^{**}	0.81	1.03	0.15	1.20	0.55
Irrigation×Zinc	1.59	0.60	0.74	0.91	0.45	0.99	0.36	1.02	0.68	1.01	0.18	0.32	0.85	0.61	1.41	0.27	0.66	0.20
C.V	4.39	4.10	6.24	6.64	11.20	14.49	9.33	12.73	6.92	7.35	8.10	6.30	12.43	14.25	13.00	14.74	10.39	14.21

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

2- LRWC= relative water content, CHLa= chlorophyll a, CHLb= chlorophyll b, CHLa/b= chlorophyll a/ chlorophyll b, CHLt= total chlorophyll, PR= proline, LAI=leaf area index, HDW= head dry weight, LDW= leaf dry weight.

'able 8. Mean comparison1 for traits2 of sunflower in different water stress and zinc at R5.7-8 growth stage.

Treatments	LRWC		CHLa		CHLb		CHLa/b		CHLt		PR		LAI		LDW		HDW	
	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009	2008	2009
			(mg gr^{-1})		(mg gr^{-1})				(mg gr^{-1})		(µmol gr^{-1})				(kg ha^{-1})		(kg ha^{-1})	
Irrigation																		
IR1	0.685^{a}	0.627^{a}	0.671^{a}	0.609^{a}	0.407^{a}	0.407^{a}	1.67^{ab}	1.56^{b}	1.07^{a}	1.01^{a}	88^{b}	102^{b}	3.10^{a}	2.14^{a}	2164^{a}	1452^{a}	6133^{a}	5101^{a}
IR2	0.614^{b}	0.589^{b}	0.603^{b}	0.607^{a}	0.334^{b}	0.355^{a}	1.81^{a}	1.70^{ab}	0.93^{c}	0.96^{a}	96^{a}	103^{b}	1.60^{b}	1.57^{b}	1603^{b}	1043^{a}	5064^{a}	3985^{b}
IR5	0.642^{ab}	0.556^{b}	0.619^{b}	0.556^{b}	0.387^{a}	0.297^{b}	1.60^{b}	1.87^{a}	1.00^{b}	0.85^{b}	96^{a}	108^{a}	1.86^{b}	1.35^{b}	1342^{b}	1253^{a}	5987^{a}	3928^{b}
Zinc																		
Zn0	0.656^{a}	0.588^{a}	0.630^{a}	0.561^{b}	0.371^{a}	0.344^{a}	1.72^{a}	1.66^{a}	1.00^{a}	0.90^{a}	93^{a}	105^{a}	1.67^{b}	1.62^{a}	1680^{a}	1119^{a}	5507^{a}	4163^{a}
Zn30	0.652^{a}	0.585^{a}	0.628^{a}	0.598^{ab}	0.378^{a}	0.364^{a}	1.67^{a}	1.68^{a}	1.00^{a}	0.96^{a}	93^{a}	103^{a}	1.76^{b}	1.68^{a}	1689^{a}	1299^{a}	5735^{a}	4416^{a}
Zn60	0.633^{a}	0.600^{a}	0.635^{a}	0.613^{a}	0.379^{a}	0.352^{a}	1.68^{a}	1.79^{a}	1.01^{a}	0.96^{a}	93^{a}	105^{a}	3.12^{a}	1.77^{a}	1740^{a}	1330^{a}	5942^{a}	4435^{a}

- Within each column and for each factor, means followed by the same letter are not significantly different at the 5% level of probability according to LSD Test.

;- LRWC= relative water content, CHLa= chlorophyll a, CHLb= chlorophyll b, CHLa/b= chlorophyll a/ chlorophyll b, CHLt= total chlorophyll, IR= proline, LAI=leaf area index, HDW= head dry weight, LDW= leaf dry weight.

Chlorophyll

The effect of irrigation treatment on CHLa content of leaves measured at R5.1 growth stage was significant ($P<0.01$) in the years under study (Table 5). In 2008, IR2 and IR4 reduced CHLa approximately about 18 and 10%, respectively as compared to IR1. In 2009, IR4 decreased CHLa about 19%, but IR2 reduced CHLa less than 1% and it was not significantly different from IR1 (Table 6). The effect of zinc treatment on CHLa content of leaves measured at R5.1 growth stage was significant in both years (Table 5). Application of 30 and 60 kg ha^{-1} zinc sulfate resulted in approximately 13.5 and 19.5% increase in CHLa content of leaves measured at R5.1 in 2008, respectively and 10 and 16% in 2009, respectively (Table 6). The interaction of irrigation with zinc treatments on CHLa content of leaves was not significant in the years under study (Table 5).

At R5.7-8 growth stage, the effect of irrigation treatment on CHLa content of leaves was significant at one percent level of probability in 2008 and non-significant in 2009 (Table 7). In 2008, IR2 and IR5 decreased CHLa approximately 10 and 8%, respectively as compared to IR1. The difference between IR2 and IR5 was not statistically significant. Although non-significant, around 9% reduction in CHLa content was observed with IR5 treatment (Table 8). CHLa content of leaves was significantly ($P<0.05$) affected by zinc fertilization at R5.7-8 growth stage in 2009, but not in 2008 (Table 7). The increases in CHLa content due to 30 and 60 kg ha^{-1} zinc sulfate application were 7 and 9%, respectively in 2009 (Table 8). The interaction of irrigation with zinc treatments on CHLa content of leaves was not significant either in 2008 or in 2009 (Table 7).

At R5.1 growth stage, the effect of irrigation regime on CHLb content of leaves was significant in 2008 ($P<0.01$) and in 2009 ($P<0.05$) (Table 5). In 2008, the reduction in CHLb content brought about by IR2 was 21% and by IR4 was 14% as compared to IR1. In 2009, IR2 decreased CHLb content around 17% and IR4 reduced CHLb content about 20% (Table 6). CHLb content of leaves was not significantly affected by zinc fertilization at R5.1 growth stage (Table 5) and no considerable increase in CHLb content was found due to zinc application (Table 6). The interaction of irrigation with zinc treatments on CHLb content was not significant either in 2008 or in 2009 (Table 7).

The effect of irrigation regime on CHLb content of leaves measured at R5.7-8 growth stage was significant ($P<0.05$) in both years (Table 7). CHLb content of IR5 was slightly and non-significantly lower than CHLb content of IR1, but IR2 caused 18% reduction of CHLb content as compared to IR1 in 2008. In 2009, IR2 decreased CHLb content around 13% and IR5 reduced CHLb content about 27% (Table 8). CHLb content of leaves was not significantly affected by zinc fertilization at R5.7-8 growth stage in none of the years under study (Table 7) and the differences between treatments were small (Table 8). CHLb content was not significantly affected by the interaction of irrigation with zinc treatments in both years (Table 7).

CHLa/b was not significantly affected by irrigation treatment at R5.1 growth stage in 2008, but the effect of irrigation on CHLa/b was significant ($P<0.05$) in 2009 (Table 5). In that year, there was no significant difference between IR1 and IR4 treatments, but about 17% increase in CHLa/b was brought about by IR2 (Table 6). In both years, CHLa/b of leaves was not significantly affected by zinc fertilization at R5.1 growth stage (Table 5). However, slight increase in CHLa/b was found due to 30 kg ha^{-1} zinc sulfate application in both years (Table 6). The interaction of irrigation with zinc treatments on CHLa/b of leaves was not significant in both years (Table 5).

At R5.7-8 growth stage, the effect of irrigation regime on CHLa/b of leaves was significant ($P<0.05$) in both years (Table 7). IR2 increased CHLa/b around 8% in 2008 and about 9% in 2009 as compared to IR1. The differences between IR1 and IR4 treatments were small in 2008. CHLa/b was increased about 20% by IR4 in 2009 (Table 8). In both years, CHLa/b was not significantly affected by zinc fertilization at R5.7-8 growth stage (Table 7) and no increase in CHLa/b was found due to zinc application (Table 8). The interaction of irrigation with zinc treatments on CHLa/b was not significant in both years (Table 7).

CHLt was significantly ($P<0.01$) affected by irrigation treatment at R5.1 growth stage in both years (Table 5). CHLt was reduced in IR2 around 19% and in IR4 approximately 14% in comparison to IR1 in 2008. These reductions were 8 and 19%, respectively in 2009 (Table 6). The effect of zinc treatment on CHLt of leaves measured at R5.1 growth stage was significant ($P<0.01$) in both 2008 and 2009 (Table 5). Application of 30 kg ha^{-1} zinc sulfate caused 13.5 and 10% increase in CHLt content of leaves measured at R5.1 in 2008 and 2009, respectively. The increases in CHLt content due to 60 kg ha^{-1} zinc sulfate were about 13% in 2008 and 11% in 2009 (Table 6). The interaction effect of irrigation with zinc treatments on CHLt content of leaves was not significant in both years (Table 5).

The effect of irrigation regime on CHLt content of leaves measured at R5.7-8 growth stage was significant in 2008 ($P<0.01$) and in 2009 ($P<0.05$)

(Table 7). In 2008, IR2 and IR5 decreased CHLt approximately 7 and 13%, respectively as compared to IR1. CHLt was reduced in IR2 around 5% and in IR5 about 16% in comparison to IR1 in 2009 (Table 8). CHLt content of leaves was not significantly affected by zinc fertilization at R5.7-8 growth stage in both years (Table 7). However, about 7% increase in CHLt content was observed due to zinc fertilization in 2009 (Table 8). In both years CHLt content was not significantly affected by the interaction of irrigation with zinc treatments (Table 7).

Reduction in net photosynthesis under water stress (Anjum et al., 2011; Þerbea and Petcu, 2000) has been attributed to reduction in chlorophyll content of plants (Jaleel et al., 2009; Mafakheri et al., 2010; Manivannian et al., 2007; Mohammadkhani and Heidari, 2007; Oraki et al., 2012; Petcu et al., 2001). In our experiment, both components of CHLt (CHLa and CHLb) were reduced due to water stress. Generally reduction in CHLb was higher than CHLa leading to higher CHLa/b indicating that CHLb is more sensitive to water stress than CHLa. Our results are in contrast to the experiments of Oraki et al. (2012) with sunflower hybrids in which chlorophyll a decreased, but chlorophyll b increased as water stress was intensified. Observations of Manivannian et al. (2007) with sunflower and Mafakheri et al. (2010) with chickpea indicated that CHLb was not more drought sensitive than CHLa. In the experiments of Mohammadkhani and Heidari (2007) with maize CHLb increased at moderate water stress. The results of these later authors indicated that CHLa/b ratio depended on the interaction of genotype with severity of water stress.

Zinc is known to protect chlorophyll against free radicals which are produced under water stress (Cakmak, 2000). Reduction in CHLa and CHLb as the result of zinc deficiency has been reported in sunflower (Khurana and Chatterjee, 2001). In our experiment, CHLa but not CHLb, was increased by zinc fertilization leading to increase in CHLa/b. However, the increase in CHLa did not increase CHLt. The zinc content of the soil was 0.38 and 0.33 mg kg^{-1} of soil in 2008 and 2009, respectively (Table 2). Apparently, this level of zinc is not sufficient to prevent water stress injuries to sunflower under our edaphic condition. Zinc application to sunflower has been shown to alleviate some deleterious effects of salinity, but zinc foliar application could not increase chlorophyll content of sunflower in the experiments of Ebrahimian and Bybordi (2011). This is consistent with our study in which zinc fertilization did not improve chlorophyll content of leaves in 2009 (with higher soil and water salinity) as compared to 2008.

Proline

The effect of irrigation regime on PR content of leaves at R2 growth stage was significant ($P<0.01$) in both years (Table 3). IR2 and IR3 treatments increased PR content around 12% as compared to IR1 in 2008. PR was increased in IR2 about 8% and in IR3 approximately 16% in comparison to IR1 in 2009 (Table 4). In both years, PR was not significantly affected by zinc fertilization at R2 growth stage (Table 3) and no increase in PR was found due to zinc application (Table 4). The interaction of irrigation by zinc treatments on PR was not significant in both years (Table 3).

At R5.1 growth stage, the effect of irrigation regime on PR of leaves was significant ($P<0.01$) in both years (Table 5). Nine percent increase by IR2 and 12% increase by IR4 treatments in PR content were observed in 2008. In 2009, IR2 could not significantly increase PR. But about 9% increase in PR was found in IR4 treatment as compared to IR1 (Table 6). PR was neither statistically affected with zinc fertilization nor by the interaction of irrigation with zinc treatments at R5.1 growth stage in 2008 and 2009 (Table 5) and no increase in PR was found due to zinc application (Table 6).

The effect of irrigation regime on PR content of leaves measured at R5.7-8 growth stage was significant ($P<0.05$) in both years (Table 7). About 9% increases in PR was brought about by IR2 and IR5 in 2008 and around 6% by IR5 in 2009 (Table 8). In both years, PR content of leaves was not significantly affected by zinc fertilization and by the interaction of irrigation with zinc treatments at R5.7-8 growth stage (Table 8).

The purpose of PR accumulation in response to water stress is to maintain leaf turgor and improve water uptake from drying soil (Anjum et al., 2011; Chaves and Oliveira, 2004; Mafakheri et al., 2010; Manivannian et al., 2007; Mattioli et al., 2009; Oraki et al., 2012; Rauf and Sadaqat, 2008). PR accumulation was found to be the most important characteristics of Alstar hybrid among the sunflower genotypes commonly cultivated in Iran (Oraki et al., 2012). In the present experiment, the amount of PR accumulated in leaves of Alstar was generally higher at R2 growth stage than R5.1 and R5.7-8 growth stages. It seems that the ability of sunflower leaves to accumulate PR to cope against water stress declines as they mature.

Zinc application did not increase PR at any growth stage in our investigation. Both zinc (Cakmak, 2000) and PR (Anjum et al., 2011; Chaves and Oliveira, 2004; Mattioli et al., 2009; Szabados and Savouré, 2010) are reported to protect membranes, macromolecules and sub-cellular organelles under dehydrating stress. Our results show that Zn and PR act independently without any interaction.

Leaf Area index

LAI was significantly affected by irrigation treatments at R2 growth stage in 2008 ($P<0.05$) and 2009 ($P<0.01$) (Table 3). LAI was reduced in IR2 around 34% and in IR3 approximately 29% in comparison to IR1 in 2008. These reductions were 27 and 19%, respectively in 2009 (Table 4). The effect of zinc treatment on LAI measured at R2 growth stage was only significant ($P<0.01$) in 2009 (Table 3). Application of 30 and 60 kg ha^{-1} zinc sulfate caused around 13 and 50% increase in LAI measured at R2 in 2008, respectively. Although the effect of zinc on LAI was not significant in 2008, but application of 30 kg ha^{-1} zinc sulfate resulted in 7% increase in LAI (Table 4). The interaction of irrigation with zinc treatments on LAI was not significant in none of the years under study (Table 3).

The effect of irrigation treatment on LAI measured at R5.1 growth stage was significant ($P<0.05$) in 2008 and 2009 (Table 5). In 2008, IR4 resulted in 13% reduction in LAI, which was not statistically different from IR1. The reduction due to IR2 was 30%. In 2009, about 27 and 18% reduction in LAI was observed in IR2 and in IR4 treatments, respectively in comparison to IR1 (Table 6). The effect of zinc treatment on LAI measured at R5.1 growth stage was only significant ($P<0.05$) in 2009 (Table 5). Application of 60 kg ha^{-1} zinc sulfate resulted in around 38% increase in LAI in 2009. No considerable differences in LAI were found between zinc treatments in 2009 (Table 6). Interaction of irrigation with zinc treatments on LAI was not significant in both years (Table 5).

At R5.7-8 growth stage, the effect of irrigation regime on LAI was significant in both 2008 ($P<0.05$) and in 2009 ($P<0.01$) (Table 7). Forty nine percent decreases by IR2 and 40% decrease by IR5 treatments in LAI were observed in 2008. In 2009, the decreases in LAI due to these treatments were 29 and 39%, respectively (Table 8). LAI was significantly ($P<0.01$) affected by zinc fertilization at R5.7-8 growth stage in 2008 (Table 7). Application of 60 kg ha^{-1} zinc sulfate resulted in 87% increase in LAI in this year (Table 8). In both years, LAI was not significantly affected by the interaction of irrigation with zinc treatments (Table 7).

Reduction in leaf turgor and photosynthesis under water stress condition suppress cell expansion and growth leading to diminution of leaf area

(Anjum et al., 2011; Jaleel et al., 2009). In agreement with other researches (Gholinezhad et al., 2009; Manivannian et al., 2007; Petcu et al., 2001; Þerbea and Petcu, 2000; Ünyayar et al., 2004) water stress severely decreased LAI at all growth stages in the present study. The rate of LAI reduction at R5.7-8 growth stage was greater than earlier growth stages. Apparently, water stress strongly enhances senescence of matured leaves of sunflower. Our result also suggests that changes of LAI could be used as a very important indicator of sunflower response to water stress.

Considerable increase in LAI at all growth stages as the result of 60 kg ha^{-1} zinc sulfate application under our condition may indicate that 0.38 mg kg^{-1} soil zinc content may not be sufficient for achieving high LAI in sunflower. LAI was also increased in the experiment of Siddiqui et al. (2009), as the result of 15 kg ha^{-1} Zn addition of to a clay loam soil with 0.68 mg kg^{-1} Zn content. Higher response of LAI to Zn fertilization at R2 and R5.1 growth stages in 2009 is in agreement with the findings of Ebrahimian and Bybordi (2011) about beneficial effects of zinc under salinity conditions.

Leaf dry weight

The effect of irrigation treatment on LDW measured at R2 growth stage was only significant ($P<0.05$) in 2008 (Table 3). In this year, the reduction of LDW due to IR2 was around 31% and due to IR3 was approximately 14% in comparison to IR1. In spite of the non-significant effect of irrigation treatments on LDW in 2009 (Table 5), about 20% reduction in LDW was observed in IR2 treatment and around 10% in IR3 treatment as compared to IR1 (Table 4). In 2009, LDW was significantly ($P<0.05$) affected by zinc fertilization at R2 growth stage (Table 3). In this year, around 17% increase in LDW was found due to 30 kg ha^{-1} zinc sulfate application (Table 4). LDW was not significantly affected by the interaction of irrigation with zinc treatments in both years (Table 3).

At R5.1 growth stage, LDW was not significantly affected by irrigation regime in both years (Table 5). In spite of this, LDW was reduced in IR2 around 21% and in IR4 approximately 27% in comparison to IR1 in 2008 (Table 6). LDW was not significantly affected by zinc fertilization at R5.1 growth stage in both 2008 and 2009 (Table 5) and no considerable increase in LDW was found due to zinc application (Table 6). In both years, LDW was not significantly affected by the interaction of irrigation with zinc treatments (Table 5).

The effect of irrigation treatment on LDW measured at R5.7-8 growth stage was only significant (P<0.01) in 2008 (Table 7). In this year, IR2 resulted in 26% and IR5 in 38% reduction in LAI as compared to IR1 (Table 8). In spite of non-significant effect of irrigation treatment on LDW in 2009 (Table 7), about 28 and 14% reductions in LDW was observed in IR2 and IR5 treatments, respectively (Table 8). The effect of zinc treatment on LDW measured at R5.7-8 growth stage was non-significant in both 2008 and 2009 (Table 7). However, application of 30 and 60 kg ha^{-1} zinc sulfate caused 16 and 19% increase in LDW, respectively in 2009 (Table 8). The interaction of irrigation with zinc treatments on LDW was not significant in both years (Table 7).

Reduction in leaf area and damage to chlorophyll due to water stress can decrease LDW (Anjum et al., 2011; Jaleel et al., 2009; Manivannian et al., 2007; Þerbea and Petcu, 2000). In agreement with these findings, LDW decrease in the present study at all growth stages as the consequences of water stress was in conformity with the reduction of LAI. However, the diminution of LDW showed less severity than the depression of LAI.

Increase in LDW due to zinc application was more pronounced in 2009 which was associated with higher soil and irrigation water salinity. The beneficial effect of zinc fertilization on LDW under salinity stress conditions has been shown by Ebrahimian and Bybordi (2011).

Head dry weight

HDW was significantly affected by irrigation regimes at R2 growth stage in 2008 (P<0.05) and in 2009 (P<0.01) (Table 3). HDW was reduced in IR2 around 20% and in IR3 approximately 11% in comparison to IR1 in 2008. These reductions were 31 and 30%, respectively in 2009 (Table 4). The effect of zinc treatments on HDW measured at R2 growth stage was only significant (P<0.01) in 2008 (Table 3). Application of 60 kg ha^{-1} zinc sulfate resulted in around 19% increase in HDW in this year. No considerable differences in HDW were found between zinc treatments in 2009 (Table 4). In both years, HDW was not significantly affected by the interaction of irrigation with zinc treatments (Table 3).

The effect of irrigation treatment on HDW measured at R5.1 growth stage was significant in 2009 at 5% level of probability (Table 5). In this year, about 20% reduction in HDW was observed in IR2 and 19% in IR4 treatment. Although irrigation treatment on HDW was non-significant in

2008, IR2 resulted in 18% and IR4 in 10% reduction in HDW as compared to IR1 (Table 6). The effect of zinc treatment on HDW measured at R5.1 growth stage was non-significant in both 2008 and 2009 (Table 5), but application of 60 kg ha^{-1} zinc sulfate resulted in 8% increase in HDW in 2008 (Table 6). The interaction of irrigation with zinc treatments on HDW was not significant either in 2008 or in 2009 (Table 5).

At R5.7-8 growth stage, HDW was significantly ($P<0.01$) affected by irrigation treatments only in 2009 (Table 7). In this year, IR2 decreased HDW around 22% and IR5 approximately 23% as compared to IR1 (Table 8). In spite of the non-significant effect of irrigation regime on LDW in 2008 (Table 7), LDW was reduced in IR2 around 17% in comparison to IR1 (Table 8). Contrary to the non-significant effect of zinc treatment on HDW at R5.7-8 growth stage in both years (Table 7), about 9 and 7% increase in HDW was observed in 2008 and 2009, respectively due to 60 kg ha^{-1} zinc sulfate application (Table 8). In both years, HDW was not significantly affected by the interaction of irrigation with zinc treatments (Table 7).

Water stress during sunflower head development and anthesis can decrease number of flowers and seeds per head (Ebrahimi et al., 2011; Gholinezhad et al., 2009; Jaleel et al., 2009; Rauf and Sadaqat, 2008; Solimanzadeh et al., 2010). This in conjunction with diminution in assimilates available for growth under water stress (Anjum et al., 2011; Jaleel et al., 2009) can lead to reduction in HDW. In agreement with these findings, HDW decreased due to water stress in the present experiment. The reductions were more pronounced at R2 growth stage when head development accelerates and in 2009 which was associated with higher soil and irrigation water salinity. The effect of zinc fertilization on HDW was erratic. In general, most increases were the result of 60 kg ha^{-1}zinc sulfate application.

Conclusions

Although PR increases under water stress condition to enhance water absorption and protect cell macromolecules and organelles; however this cannot completely prevent the injuries of water stress on LRWC, chlorophyll and leaf and head growth of sunflower. Smaller levels of leaf chlorophyll content, LAI, LDW and HDW were obtained in 2009 when soil and irrigation water salinity were higher. In many cases zinc fertilization did not alleviate the adverse effects of water stress. However, 60 kg ha^{-1} zinc sulfate application could be beneficial when soil zinc content may not be sufficient. The deleterious effects of water stress were found to depend on the interaction of plant growth stage and other environmental factors, especially on soil and water salinity.

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