Australian Journal of Crop Science AJCS

AJCS 5(2):197-204 (2011) ISSN:1835-2707

Effect of irrigation water deficit on antioxidant activity and yield of some sunflower hybrids

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Abstract

Sunflower (*Helianthus annuus* L.) is one of the most important oil-producing plants that it can tolerate short periods of water deficit (WD). The objective of this study was to evaluate the effects of water stress on the physiological and agronomic characteristics of four sunflower hybrids. A two-year field experiment was carried out using a randomized complete block design with four replications and a split plot arrangement. The irrigation treatments were applied based on 60 mm (full-irrigated), 120 mm (moderate water deficit) and 180 mm (extreme water deficit) of evaporation from the 'class A' pan evaporation. The results showed that water deficit, compare to full-irrigated, significantly increased the activity of all antioxidant enzymes in leaves such as Superoxide dismutase (SOD), Glutathione peroxidase (GPX), Glutathione reductase (GR) and Catalase (CAT). The correlation results showed that the seeds number per head was main yield components associated to seed yield when sunflower plants were grown under the full-irrigated and extreme water deficit. The results showed that the Euroflor and Allestar hybrids had highest seed yield in full and limited irrigation condition, respectively. The correlation results showed that there was a high, positive and significant correlation among seed yield and CAT in water irrigation deficit condition. Moreover, the GPX and CAT content can be used as a drought tolerance index to selection tolerant genotypes under moderate and extreme water deficit conditions.

Keywords: CAT, GPX, GR, *Helianthus annuus*, Number of seeds per head, SOD, 1000-Seed weight.
Abbreviations: I_1- well-irrigated; I_2- moderate water deficit; I_3- extreme water deficit; CAT- Catalase; GPX- Glutathione peroxidase; GR- Glutathione reductase; ROS- reactive oxygen species; SOD- Superoxide dismutase; WD- water deficit.

Introduction

Plants are frequently exposed to diverse stress conditions, including salt, drought, heat, low temperature, heavy metals, and oxidative stress (Munns, 2005). River intakes and aquifers are the main sources of water supply for Iranian farms. The limited water resources in the area and the cost of pumping irrigation water are the most important factors that force many farmers to reduce irrigation in many arid and semi-arid regions of the Islamic Republic of Iran. Sunflower (*Helianthus annuus* L.) is one of the most important oil-producing plants that can tolerate short periods of water deficit (Hattendorf *et al*., 1988). The various environmental stresses affects source and sink strengths by its effects on photosynthesis, growth, and general metabolism. In plants under water deficit conditions, an oxidative brush system may be observed (Larson, 1988; Burke and Mahan, 1991). Such a response is due to disturbances in the balance between production of reactive oxygen species (ROS) such as singlet oxygen, superoxide radical, hydrogen peroxide, hydroxyl ion and free hydroxyl radical ($^1O_2$, $^{\cdot}O^{-}_2$, H_2O_2, OH^- and $^{\cdot}OH$) and their scavenging by the antioxidant system (Aroca *et al*., 2003). The antioxidant system, including antioxidant enzymes such as Superoxide dismutase (SOD), Peroxidase (POX), Catalase (CAT), and small-molecule antioxidants such as glutathione, ascorbate, flavonoids, and α-tocopherol, play a key role in controlling ROS levels (Larson, 1988; Burke and Mahan, 1991). The level of damages may be limited by enzymatic and nonenzymatic scavengers of free radicals (Foyer and Noctor, 2000, Aroca *et al*., 2003). The degree to which the activities of antioxidant enzymes and the amount of antioxidants are elevated under drought stress is extremely variable among several plant species (Zhang and Kirkham, 1995) and even between the two cultivars of the same species (Bartoli *et al*., 1999). The biochemical and physiological responses of plants are a base of agronomic response to environmental stress. Characterization of the agronomic response of sunflower crop to water stress could help to stabilize production at present levels, and to identify appropriate stress tolerance mechanisms for use in future breeding efforts. Osman and Talha (1975) reported that seed yield increased as the amount of water and irrigation number increased. That limited irrigation-water can significantly decrease seed yields (Stone *et al*., 1996), especially during three growth periods: heading, flowering, and milking stages (Osman and Talha, 1975). Crop yield depends on environmental and genetic factors (Chapman, 2008) and Genetic characteristics can interact with environmental stress factors to affect crops growth and yield. The identification of attributes useful for the process of screening genotypes for drought tolerance is a major challenge to plant breeder. Thus, study of the agronomic and physiological characteristics associated to high yield potential under suboptimal environmental conditions could be used as stress tolerance indexes in future elite germplasm. The field experiments were performed to determine the agronomic and physiological responses of four current Iranian sunflower hybrids to water deficit, and to evaluate the response of antioxidant enzyme contents, GPX, GR, CAT and SOD, associated to high yield potential under water deficit conditions.

Materials and methods

Agricultural practices

The experimental design was a split plot in a randomized complete block design with four replications. Each replication was divided into three main plots, which differed in severity of imposed water shortage. The four sunflower hybrids (Azargol, Euroflor, Allestar and Karaj) were randomly distributed within the sub-plots in each of the drought stress treatments (main plots). The water deficit treatments were applied by changing in irrigation intervals. Irrigations were carried out when an amount of evaporated water (from Class ''A pan'' evaporation) reached to 60 (I_1; well-irrigated), 120 (I_2; moderate water deficit) and 180 (I_3; extreme water deficit) mm, respectively. Amount of irrigation applied identical for all WD treatments from the beginning of planting time until the complete establishment of sunflower plants (eight-leaf (V_8) stage). After this stage, the plots were irrigated according to their prescribed treatment. The Azargol, Euroflor, Allestar and Karaj hybrids are the current sunflower hybrids cultivated in many arid and semi-arid regions of Iran. The sunflowers seeds were hand-sown on 7 m × 3.6 m plots (6 rows) on 22 May 2008 and 23 May 2009, respectively. In order to prevent the lateral spread of water, plots were surrounded with dykes, and a 3-m wide strip was left bare between main plots. Seeds were sown at 3 cm depth in the middle of rows, with 0.6 m between rows and 0.20 m between seed groups. This yielded a population density of about 8.3 plants m^{-2}. Before planting, the soil surface of the cultivated area was thoroughly irrigated using a solid-set movable sprinkler system. Triple super phosphate fertilizer was applied broadcast before sowing at a rate of 100 kg ha^{-1}. The N fertilizer (150 kg ha^{-1}), in the form of urea, was applied at planting (one-third of the application) and side-dressed at the R_4 growth stage (two-thirds of the application). At the 2-3 leaf stage, plants were selected for uniformity and thinned out to the recommended plant density. Weed control was performed manually without any chemical additive.

Plant sampling and harvesting

Four representative plants per plot were sampled at the beginning of flowering (R_5 stage) (Schneiter and Miller, 1981) and brought to the laboratory, where the leaves, stems, and inflorescences were separated and the green leaf area was measured using a leaf area meter (Area Measurement System, DELTA-T, Cambridge, UK). After the sunflowers hybrids reached physiological maturity, seed yield was determined by harvesting the two central rows in the first week of September in both years. The 20 plants, as representative, were used to determine the seed numbers by measuring them per heads.

Enzyme extractions and assays

For extracting antioxidant enzymes, Frozen leaf tissues (500 mg fresh weight) were ground in a mortar under chilled conditions in omogenization buffer (2 ml) containing phosphate buffer (0.1 M, pH 7.5), and ethylene diamine tetraacetic acid (EDTA, 0.5 mM). The homogenate, filtered through four layers of muslin cloth, was centrifuged at 12,000g for 10 min at 4°C. The resulting supernatant was used for the assay of different enzymes. Proteins content were determined by the method of Bradford (1976) using bovine serum albumin (BSA) as standards. For estimation of SOD (EC 1.15.1.1) activity, method of Beyer and Fridovich (1987) was followed. SOD activity was assayed by measuring the inhibition of photo-reduction of nitroblue tetrazolium (NBT) at 560 nm using UV–Vis spectrophotometer. A unit of SOD is defined as that being present in the volume of extract that caused inhibition of the photoreduction of NBT by 50%, and was expressed in enzyme units (mg^{-1} protein). Catalase (CAT) activity was determined by monitoring the disappearance of H_2O_2, measuring a decrease in the absorbance at 240 nm (Aebi, 1984). The reaction was carried in a reaction mixture containing 1.0 ml of the 0.5 M (pH 7.2) phosphate buffer, 3 mM EDTA, 0.1 ml of the enzyme extract and 0.3% H_2O_2, and allowed to run for 3 min. The enzyme activity was calculated using the extinction coefficient 0.036 mM^{-1} cm^{-1}. One enzyme unit (U) determines the amount of enzyme necessary to decompose 1 μmol of H_2O_2 per mg protein per min at 25°C and expressed as U mg^{-1} protein. Activity of Glutathione reductase (GR) (EC 1.6.4.2) was determined by the method of Foyer and Halliwell (1976) and modified by Rao (1992). The supernatant was immediately used to assay GR activity through glutathione-dependent oxidation of NADPH at 340 nm. About 1 ml reaction mixture, containing 0.2 mM NADPH, 0.5 mM GSSG and 50 μl of enzyme extract, was run for 5 min at 25°C by using UV–vis spectrophotometer. The activity was calculated by using extinction coefficient 6.2 mM^{-1} cm^{-1} and expressed in enzyme unit (mg $protein)^{-1}$. One unit of enzyme is the amount necessary to decompose 1 μmol of NADPH per min at 25°C. Activity of GPX (EC 1.11.1.9) was determined as described by Rotruck *et al*. (1973). The leaf sample was homogenized in 0.4 m Tris–HCl buffer (pH 7.0), and the reaction mixture contained 0.2 ml of tissue homogenate, 0.2 ml of 0.4 M Tris–HCl buffer (pH 7.0), 0.1 ml of 10 mM sodium azide, 0.2 ml of glutathione and 0.1 ml of 0.2 mm hydrogen peroxide. The contents were incubated at 37 °C for 10 min. The reaction was stopped by the addition of 0.4 ml of 10% trichloroacetic acid (TCA), and centrifuged. The supernatant was assayed for glutathione content by using Ellmans reagent. One unit of enzyme activity is the amount of glutathione consumed per minute at 37 °C.

Weather and soil conditions

The experiment was carried out at an experimental farm of Islamic Azad University with an altitude of 1303 m, Karaj province, Iran during the 2008 and 2009 growing seasons. This location is a semi arid area characterized by warm and dry summers; long-term (30 years) mean annual rainfall and temperature are 243 mm and 23.36 ◦C, respectively. The meteorological data recorded during the trial period in each growing season are given in Table 1.

Table 1. Mean monthly temperature and total monthly rainfall during the sunflower growing season and the long term period (1975–2005) recorded at a weather station near the experimental site.

	Temperature (°C)			Rainfall (mm)		
	2008	2009	1975-2005	2008	2009	1975-2005
May	19.1	18.9	20.3	19.9	19.8	20.8
June	24.7	24.5	26.2	0.3	0.1	2.3
July	27.1	27.2	28.4	4.6	5.2	3.1
August	27.4	27.3	28.2	1.8	1.5	1.4
September	23.5	23.7	24.1	0.4	0.2	0.6

Table 2. Physico-chemical Characteristics of soil in the experimental field.

Soil Characteristics	Values			
	2008		2009	
	0-30 cm	30-60	0-30 cm	30-60
EC (dSm^{-1})	1.45	2.25	1.31	2.09
pH	7.87	7.62	7.26	7.3
Organic carbon (%)	0.72	0.49	0.64	0.51
Total N (%)	0.07	0.05	0.04	0.03
Available P (mg kg^{-1})	6.1	5.7	8.3	6.5
Available K (mg kg^{-1})	182	186	191	173

Table 3. The antioxidants content and statistical groupings in some sunflower hybrids under different irrigation regimes and ANOVA significance levels.

Irrigation	Hybrid	GPX (U mg^{-1}protein)	CAT (U mg^{-1}protein)	GR (U mg^{-1}protein)	SOD (U mg^{-1}protein)
I_1	Azargol	42.18b	18.86a	1.81b	76.49ab
	Euroflor	46.06a	19.24a	2.19a	76.92ab
	Allestar	44.80a	19.00a	1.93b	79.38a
	Karaj	40.76b	18.07b	1.75b	71.70b
I_2	Azargol	67.09b	23.60b	3.11a	113.38b
	Euroflor	59.69c	22.16c	2.87b	135.10a
	Allestar	80.92a	26.70a	3.25a	143.05a
	Karaj	56.16d	21.48c	2.51c	103.31b
I_3	Azargol	49.43b	20.82b	2.26ab	93.12ab
	Euroflor	49.99b	20.14c	2.26ab	88.19bc
	Allestar	56.14a	21.63a	2.41a	95.48a
	Karaj	45.07c	20.34c	2.04b	87.23c
Source of variation	df				
Year (Y)	1	ns	ns	ns	ns
Water deficit (WD)	2	***	***	***	***
Y×WD	2	ns	ns	ns	ns
Hybrid (H)	3	**	***	***	***
WD×H	6	**	***	***	***
Y×H	3	ns	ns	ns	ns
Y×WD×H	6	ns	ns	ns	ns

Irrigation: well-irrigated (I_1), moderate water deficit (I_2) and extreme water deficit (I_3) treatments. Antioxidant enzymes include: Superoxide dismutase (SOD), Glutathione peroxidase (GPX), Glutathione reductase (GR) and Catalase (CAT). Means within each column followed by the same letter are not statistically different at α= 0.05 by LSD test. ***, ** and * indicate, respectively, significance at P level of 0.001, 0.01 and 0.05.

One week before sowing, soil samples were taken to determine soil characteristics. Two composite soil samples were collected at depths of 0–30 and 30-60 cm. They were air dried, crushed and tested for pH, electrical conductivity (EC), organic carbon, total N, available P and K.

Statistical analyses

All data were analyzed with an analysis of variance (ANOVA) using the general liner model procedure in SAS (SAS Institute, 2002). The assumptions of variance analysis were tested by ensuring that the residuals were random and homogenous, with a normal distribution about a mean of zero. Means were separated using Fisher's protected least significance difference (LSD) test at the 95% level of probability. Correlation analyses using PROC CORR in SAS were conducted to determine the relationship between measured parameters and seed yield.

Results

Weather condition

The mean monthly temperature was below normal (long-term temperature); Furthermore, the mean monthly rainfall often had the same trend in both years during the growth season. The mean monthly temperature and rainfall between the first and second year were approximately similar during the sunflower growing season (Table 2). This negligible variation between two years could be explained non significant interaction of the year and treatments in most traits.

Antioxidant enzymes

Glutathione peroxidase

The GPX content was affected by water deficit and hybrid as well as the interaction of WD×H (Table 3). The GPX was increased by drought stress and the highest level of it produced under I_2 treatment (Fig 1). The GPX content difference between Azargol and Karaj hybrids, as well as between Euroflor and Allestar hybrids was not significant under the well-irrigated conditions. In moderate irrigation deficit, the GPX content in hybrids had a decline order of Allestar›Azargol›Euroflor›Karaj. In high-irrigation deficit, the highest and lowest GPX were content in Allestar and Karaj hybrid. While, the differences in GPX content between Azargol and Euroflor hybrids was not significant (Table 3). There was a positive and significant relationship between seed yield and GPX under the full-irrigated (0.78) and moderate water deficit (0.91) (Table 5 and 6).

Glutathione reductase

The main effect of water deficit, hybrid and the interaction of WD×H were significant (Table 3). The drought stress increased the GR level compare to well-irrigated condition. Moreover, the GR level in I_2 treatment was more than I_3 treatment (Fig 1). In well-irrigated condition, the highest level of GR obtained from Euroflor hybrid. Furthermore, the differences in GR among Azargol, Allestar and Karaj hybrid were not significant. In moderate irrigation deficit, the GR content in hybrids had a decline order of Allestar›Azargol› Euroflor›Karaj. In extreme irrigation deficit, the highest and lowest GR levels were obtained from Allestar and Karaj hybrid. Although, the differences among Azargol, Euroflor and Allestar hybrids as well as among Azargol, Euroflor and Karaj were not significant (Table 3). There was a positive and significant relationship between seed yield and GR under the full-irrigated (0.62) and moderate water deficit (0.73) (Table 5 and 6).

Superoxide dismutase

The SOD content was affected by water deficit and Hybrid as well as the interaction of WD×H (Table 3). The means comparison showed that the SOD content had a decline order of I_2›I_3›I_1 (Fig 1). In well-irrigated condition, the highest and lowest SOD obtained from Allestar and Karaj hybrid, respectively. The same trend was observed in water irrigation deficit treatments (Table 3). The correlation results showed that a negative and significant relationship between LAI and SOD under deficit irrigation conditions (Table 6 and 7).

Catalase

The CAT content was affected by water deficit and Hybrid as well as the interaction of WD×H (Table 3). Water deficit increased CAT content in sunflower plants. Although, CAT level decreased with the increase of water deficit severity (Fig 1).

Among the hybrids, Azargol, Euroflor and Allestar had no significant differences in catalase content under the full-irrigated conditions. The highest CAT content was observed in Allestar hybrid under the moderate and extreme water deficit. The lowest CAT content was observed in Karaj and Euroflor hybrids under the moderate and extreme water deficit, respectively. Although, there was no significant difference in CAT content between Euroflor and Karaj hybrid under the water deficit conditions (Table 3). There was a high and positive correlation between CAT level and seed yield in moderate (0.92) and extreme water deficit (0.62) (Table 6 and 7).

Agronomic features

LAI

The water deficit and hybrid as well as the interaction of WD×H had the significant effect on the leaf area index (Table 4). The means comparison showed that the water stress reduced LAI and the most reduction effect obtained from I_3 treatment (Fig 2). In well-irrigated treatment, the Euroflor and Allestar hybrid had the highest and lowest LAI, respectively. While, the difference in LAI was not significant among Euroflor and Karaj hybrid. In moderate water deficit, the LAI among hybrids had a decline order of Euroflor› Karaj› Azargol› Allestar. In extreme irrigation deficit, the highest and lowest LAI was observed in Euroflor and Allestar hybrids. Although, the difference in LAI was not significant among Euroflor and Karaj hybrids (Table 4). There was a high and negative relationship between LAI and seed yield under water deficit conditions (Table 6 and 7).

1000-seed weight

The analysis of variance showed that the WD and H treatments as well as the interaction of WD×H had the significant effect on 1000-seed weight (Table 4). The seed

Table 4. Sunflower seed yield, number of seeds per head, 1000-seed weight and LAI in some sunflower hybrids under different irrigation regimes and ANOVA significance levels.

Irrigation	Hybrid	Seed yield (kg ha^{-1})	LAI	Number of seeds per head	1000-seed weight (g)
I_1	Azargol	3045.02c	3.75b	494.50d	74.12a
	Euroflor	3878.97a	4.07a	763.88a	61.25c
	Allestar	3556.67b	3.52c	603.63c	71.10b
	Karaj	2957.81c	4.01a	668.50b	53.87d
I_2	Azargol	1267.97b	3.47c	261.54c	58.20a
	Euroflor	1185 .17b	3.79a	330.50b	43.39c
	Allestar	1882.53a	3.26d	421.51a	53.57b
	Karaj	926.17c	3.64b	334.75b	33.88d
I_3	Azargol	721.53b	3.06b	202.75b	43.03b
	Euroflor	430.28d	3.28a	139.38c	37.19c
	Allestar	782.21a	2.99c	214.63a	44.13a
	Karaj	582.18c	3.23a	224.13a	31.47d
Source of variation	df				
Year (Y)	1	ns	ns	ns	*
Water deficit (WD)	2	***	***	**	**
Y×WD	2	ns	ns	ns	ns
Hybrid (H)	3	***	**	**	***
WD×H	6	**	***	**	***
Y×H	3	ns	ns	ns	ns
Y×WD×H	6	ns	ns	ns	ns

Irrigation: well-irrigated (I_1), moderate water deficit (I_2) and extreme water deficit (I_3) treatments. Means within each column followed by the same letter are not statistically different at α= 0.05 by LSD test. ***, ** and * indicate, respectively, significance at P level of 0.001, 0.01 and 0.05.

Table 5. Correlation coefficients between antioxidant content, LAI, 1000-seed weight, Number of seeds per head and seed yield under the full-irrigated condition.

Features	Seed yield	CAT	GPX	GR	SOD	1000-seed weight	LAI	Number of seeds per head
Seed yield	1.00							
CAT	0.53**	1.00						
GPX	0.78***	0.56**	1.00					
GR	0.62***	0.26	0.39*	1.00				
SOD	0.51**	0.08	-0.47**	0.67**	1.00			
1000-seed weight	0.08	0.41*	-0.23	0.01	0.01	1.00		
LAI	0.07	-0.13	-0.15	0.14	0.01	-0.73**	1.00	
Number of seeds per head	0.62**	0.03	0.36*	0.44*	0.37*	-0.72**	-0.59**	1.00

Antioxidant enzymes include: Superoxide dismutase (SOD), Glutathione peroxidase (GPX), Glutathione reductase (GR) and Catalase (CAT).
***, ** and * indicate, respectively, significance at P level of 0.001, 0.01 and 0.05.

weight was decreased significantly by the water deficit (Fig 2). The differences in seed weight among hybrids were significant in full irrigation and mid-irrigation deficit as well as the highest and lowest seed weight obtained from Azargol and Karaj hybrid, respectively (Table 4). The seed weight in Allestar was more than that in Euroflor hybrid under the well-irrigated condition. While, seed weight in Allestar was more than Euroflor hybrid under I_2 treatment. In I_3 treatment, the seed weight in hybrids had a decline order of Allestar› Azargol› Euroflor›Karaj (Table 4). There was a positive and significant correlation between seed weight and seed yield under the water irrigation deficit conditions (Table 6 and 7). Although, the relationship between seed weight and seed yield was poor under the full-irrigated conditions (Table 5).

The number of seeds per head

The number of seeds per head of sunflower plant was affected by WD and H as well as the interaction of WD×H (Table 4). The largest of seeds per head obtained from full

Table 6. Correlation coefficients between antioxidant content, LAI, 1000-seed weight, Number of seeds per head and seed yield under the moderate water irrigation deficit.

Features	Seed yield	CAT	GPX	GR	SOD	1000-seed weight	LAI	Number of seeds per head
Seed yield	1.00							
CAT	0.92**	1.00						
GPX	0.91***	0.95**	1.00					
GR	0.73***	0.66***	0.65***	1.00				
SOD	0.45**	0.46**	-0.57**	0.31	1.00			
1000-seed weight	0.69***	0.68***	0.71***	0.73***	0.35*	1.00		
LAI	-0.74***	-0.82***	-0.81***	-0.53**	-0.42*	-0.61**	1.00	
Number of seeds per head	0.45*	0.52**	0.52**	0.27	0.23	-0.08	-0.39*	1.00

Antioxidant enzymes include: Superoxide dismutase (SOD), Glutathione peroxidase (GPX), Glutathione reductase (GR) and Catalase (CAT).***, ** and * indicate, respectively, significance at P level of 0.001, 0.01 and 0.05.

Table 7. Correlation coefficients between antioxidant content, LAI, 1000-seed weight, Number of seeds per head and seed yield under the extreme water irrigation deficit.

Features	Seed yield	CAT	GPX	GR	SOD	1000-seed weight	LAI	Number of seeds per head
Seed yield	1.00							
CAT	0.62**	1.00						
GPX	0.43*	0.56**	1.00					
GR	0.29	0.44*	0.46**	1.00				
SOD	0.26	0.49**	0.17	-0.13	1.00			
1000-seed weight	0.66***	0.57**	0.72***	0.45**	0.26	1.00		
LAI	-0.82***	-0.69***	-0.59**	-0.36*	-0.42*	-0.76***	1.00	
Number of seeds per head	0.76***	0.34	-0.04	0.01	0.11	0.03	-0.45**	1.00

Antioxidant enzymes include: Superoxide dismutase (SOD), Glutathione peroxidase (GPX), Glutathione reductase (GR) and Catalase (CAT).
***, ** and * indicate, respectively, significance at P level of 0.001, 0.01 and 0.05.

irrigation condition and water stress reduced the seed numbers (Fig 2). Among the hybrids, the Euroflor and Azargol hybrids had the highest and lowest seed numbers under full-irrigated treatments, respectively (Table 4). Among the hybrids, the Allestar and Azargol hybrids had the highest and lowest seed numbers under the moderate deficit, respectively (Table 4). But the highest and lowest seed numbers under extreme water deficit obtained from Karaj and Euroflor hybrids, respectively. Moreover, the differences in the seed numbers were not significant between Karaj and Allestar hybrids. There was a significant and high positive correlation between seeds number and yield in I_1 and I_3 treatments (Table 5 and 7). However, this relationship was not strong under the moderate water deficit (Table 6).

Seed yield

The sunflower seed yield was affected by WD and H as well as the interaction of WD×H treatments (Table 4). The water deficit reduced seed yield in sunflower plant and the less yield was obtained from I_3 treatment (Fig 2). The Euroflor and Allestar hybrids had highest seed yield in full and limited irrigation condition, respectively. Among the hybrids, the Karaj hybrid had less seed yield under the well-irrigated and moderate irrigation deficit. However, among the current hybrids, the Euroflor hybrid had less seed yield under extreme irrigation deficit condition (Table 4).

Discussion

Our results showed that the activities of antioxidant enzymes increased under the mid-irrigation deficit in comparison to full-irrigated condition. Although, the activities of antioxidant enzymes decreased when sunflower plants suffered the extreme drought stress. The previous researches showed that enzymatic antioxidant systems, including CAT, GPX, GR and SOD played an important role in scavenging harmful oxygen species (Larson, 1988; Burke and Mahan, 1991); and the activities of these antioxidant enzymes were altered when plants were subjected to stress. In full-irrigated condition, the highest and lowest antioxidant enzymes content often were in Euroflor and Karaj hybrid, respectively. However, often were in Euroflor and Karaj hybrid, respectively. However, the highest antioxidant enzymes content were obtained from Allestar hybrid under water deficit conditions. The correlation results showed that there was a high, positive and significant correlation among yield, CAT and GPX in mid-irrigation deficit. There was a high relationship between GPX content and sunflower seed yield under full-irrigated and moderate water deficit conditions. Thus, GPX content can be used as a drought tolerance index to selection tolerant Hybrids under moderate water deficit. Moreover, the highest significant relationship between yield and antioxidant enzymes content was related to CAT in extreme water deficit condition. Singh *et al*. (2010) found that a significant positive correlation of peroxidase with

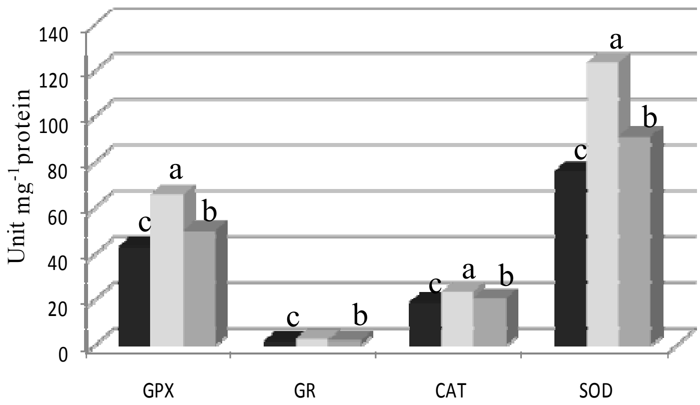

Fig 1. The response of sunflower antioxidants content to different irrigation regimes. Irrigation: well-irrigated (I_1), moderate water deficit (I_2) and extreme water deficit (I_3) treatments. Antioxidant enzymes include: Superoxide dismutase (SOD), Glutathione peroxidase (GPX), Glutathione reductase (GR) and Catalaz (CAT).
Statistically different values ($P < 0.05$) are indicated with different letters above the columns.

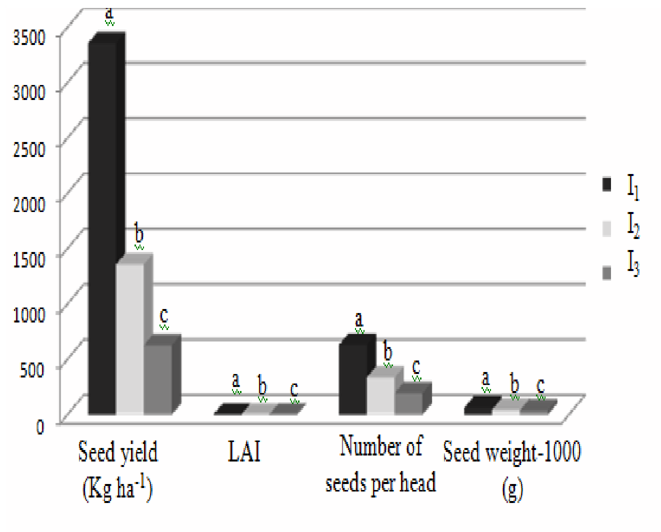

Fig 2. The response of sunflower seed yield, number of seeds per head, 1000-seed weight and LAI means to different irrigation regimes. Irrigation: well-irrigated (I_1), moderate water deficit (I_2) and extreme water deficit (I_3) treatments. Statistically different values ($P < 0.05$) are indicated with different letters above the columns.

with SOD and CAT. They reported that they could serve as a reliable biochemical marker to identify the genotypes having higher activity of other antioxidants such as SOD and CAT. Bowler *et al*. (1992) reported an enhanced stress tolerance due to higher activity of SOD. Luhova *et al*. (2003) observed increased tolerance to pathogens by higher activity of peroxidase and lignification. Toivonen and Sweeney (1998) reported that higher activity of SOD and peroxidase increases the shelf life of broccoli heads. She *et al*. (2003) observed that higher SOD and CAT activities in Chinese cabbage delayed the leaf senescence during post-harvest. The LAI, seed yield and some yield components reduced when sunflower plants were imposed to drought stress (Fig 2). The number of seed per head was the main yield component affected seed yield when sunflower plants grown under the well-irrigated and extreme water deficit conditions (Table 5 and 7). While, seed weight was the main yield component determined seed yield when sunflower plants endured moderate water stress (Table 6). The decrease of LAI in some filed crops exposed to water stress stage has been reported previously (Connor and Jones, 1985; Pandey *et al*., 2000; Mansouri-Far *et al*., 2010). It found that reducing radiation capture due to loss in LAI under water shortage conditions resulted in assimilation reduction (Shangguan *et al*., 2000: Saneoka *et al*., 2004). The previous studies showed that limited irrigation-water can significantly decrease sunflower seed yields (Stone *et al*., 1996), especially during three growth periods: heading, flowering, and milking stages (Osman and Talha, 1975). A decrease in the number of seeds per head and seed weight after the irrigation deficit at different growth periods was also reported (Göksoy *et al*., 2004; Beheshti and Behboodi fard, 2010). The number of seeds per head is positively associated with seed yield in sunflower (Punia and Gill, 1994; Doddamani *et al*., 1997). The results showed that the Euroflor and Allestar hybrids had the highest seed yield under full and limited irrigation conditions, respectively (Table 4). The results of correlation analysis showed that a significant, high and negative relationship between seed yield and LAI when sunflower plants grown under water irrigation deficit conditions (Table 6 and 7). The Allester hybrid with the less LAI had the highest seed yield when it was grown under water irrigation deficit condition (Table 4). Thus, the hybrids with less LAI could be more benefit in water stress conditions. It was found that there was a diversity between cultivars to response drought stress, and these variations involve in resistant reactions (Tollenaar and Lee, 2002; Casadebaig *et al*. 2008; Mansouri-Far *et al*., 2010). Among drought adaptation strategies, the minimization of water loss can be achieved through the lowering of either leaf area, stomatal conductance or a reduction of the energy load of the plant (Sadras *et al*., 1993; Rauf and Sadaqat, 2008). Casadebaig *et al*. (2008) found that a robust relationship between leaf expansion or daily transpiration and available soil water content. Pandy *et al*. (2000) found that reducing LAI to decrease transpiration and a deeper root system to increase water extraction are the adaptive strategies in maize plant under water stress.

Conclusion

Plants respond to drought stress through alteration in physiological and biochemical processes. Our results showed that the activities of antioxidant enzymes increased under the WD in comparison to full-irrigated condition. However, the activities of antioxidant enzymes were less in the extreme water deficit than the moderate water deficit. Moreover, the water deficit reduced seed yield and some yield components. The advantage of high seed yield in Euroflor hybrid under adequate water irrigation condition gave up when sunflower plants grown in water deficit condition. The sunflower hybrids with less LAI were produced more seed yield when the sunflower plants endured water stress. The findings of this research also showed that the GPX and CAT content can be used as a drought tolerance index to selection tolerant genotypes under moderate and extreme water deficit conditions.

Acknowledgements

The authors are grateful to Dr. S. F. Saberali for some scientific assistance and statistical advisory.

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