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Seed yield and some physiological traits of safflower as affected by water deficit stress

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Abstract

Safflower (*Carthamustinctorius* L.) is an oilseed crop adapted to drought prone arid and semi-arid environments. This study was conducted to evaluate the effects of water deficit stress on antioxidant activity, membrane stability index (MSI), leaf chlorophyll content, leaf area index (LAI) and their relationship with seed yield using 64 safflower genotypes grown under normal and water deficit stress field condition. Plants were grown under normal irrigation until branching growth stage when water deficit stress was applied to the plants. Analysis of variance showed the significant effects of genotype, water deficit and their interactions on the physiological traits that examined. Water deficit stress significantly decreased leaf area index, leaf chlorophyll content and the membrane stability index means over all 64 genotypes whereas it caused significant increase in antioxidant compounds (APX and POX). The results also revealed the positive and significant correlations between antioxidant enzyme activities with seed yield under water deficit conditions. The stress susceptibility index (SSI) identified water-deficit tolerant genotypes (Kordestan 3 and C411) that did have outstanding yield performance per se in stress environments.

Keywords: Antioxidant; Susceptibility index; Drought.

Introduction

Safflower (*Carthamustinctorius* L.) is an oilseed crop grown commercially in Iran, as one of the centers of culture in the old world

(Knowels, 1962; Pahlavani et al., 2007) and considered to be a drought-tolerant compared to other oilseed crops (Dwiedi et al., 2005).

Drought is one of the most prevalent abiotic stresses that seriously influence plant growth and crop productivity worldwide. In the light of economic and environmental significance of drought, increasing concern has been voiced regarding impacts of climate change on future drought frequency, duration and severity in various regions of the globe (Davatgar et al., 2009). Thus, development of more drought-tolerant crops will help fulfill future global food demand by enabling production in marginal lands to expand (Lichtenthaler andBuschmann, 2001). Nevertheless, the intensity and duration of water shortage and also species, development and the metabolic status of plants are important factors determining plant reaction to drought stress (Wood, 2005).

Plants are exposed to various abiotic stress during their life cycle leading to changes in normal physiological functions of the plants. An increased production of reactive oxygen species (ROS) such as superoxide anion radicals ($O_2^{\cdot\downarrow}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH) is a common consequence of drought stress. The resultant free radicals can react with key cellular structures and molecules such as photosynthetic pigments, membrane lipids, proteins and nucleic acids, and cause cellular damage (Gill and Tuteja, 2010). Different plant species adopt individual mechanisms to with the ruinous effects of drought stress. Reactive oxygen species are produced in different compartments of the plant cell, both under normal and stressful conditions. However, in order to overcome the deleterious effects of ROS, plants have developed complex antioxidant defense systems. These include both enzymatic antioxidants such as superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) and ascorbate peroxidase (APX, EC 1.11.1.11), and non-enzymatic antioxidants such as ascorbate, glutathione, carotenoids and α-tocopherol (Noctor et al., 2002). Water deficit treatments significantly increased superoxide dismutase and peroxidase activities in safflower genotypes (Hojati et al., 2011).

Membrane stability index (MSI) is a physiological index that has been widely used to evaluate drought and heat tolerance (Blum and Ebercon, 1980). Desiccation of plant cells causes cell membrane leakage of ions and electrolytes (Bandurska, 2001). The cell membrane plays an important role in maintaining cell viability, by providing both osmotic and ionic equilibrium between the cellular component and its environment and being

involved in signal transduction during water deficit conditions (Bajji et al., 2001). There is reported to be an overall decrease in MSI due to water deficit stress (Kocheva and Georgive, 2003).

The phenomenon of diminishing of water loss rate in response to water deficit is an important aspect of water deficit tolerance and can be achieved through the lowering of either leaf area expansion or transpiration per unit leaf area (Gilland Tuteja, 2010). Hence, leaf area index (LAI) is used as a selection criterion to improve drought tolerance in crop plants. Reduced plant size, leaf area and leaf area index are a major attributes for moderating water use and reducing injury under water deficit. Often crop cultivars improved for water limited environments by selection for yield under stress have constitutively reduced leaf area (Blum, 2005).

Leaf chlorophyll content is an important factor in determination of photosynthesis rates and dry mater production. Carotenoids are lipid-soluble antioxidants produced by most photosynthetic organisms not only acts as antioxidants but also acts as accessory pigments, harvesting light for photosynthesis and as photo protective agents limiting the damaging effects of high irradiance (Jonson et al., 1993).

Although water deficit tolerance is considered as a primary breeding goal for crop plants to be grown under drought stress conditions, there is a lack of information to be able to determine safflower genotypes with stabilized crop performance under such harsh conditions. Hence the present study was carried out in aiming to assess the effects of water deficit stress on antioxidant enzyme activities, membrane stability index, leaf area index and chlorophyll content of 64 safflower genotypes grown under normal and water deficit stress field conditions.

Materials and Methods

Plant materials and experimental conditions

Sixty-four safflower genotypes including commercial cultivars, landraces and breeding lines of indigenous and exotic origin were used in this study. Out of the 64 genotypes, 46 were indigenous and 18 were exotic. Field experiments were carried out at the research farm of Isfahan University of Technology located at Lavark, Najaf-Abad, Iran (40 km south west of Isfahan, 32º 32´ N, 51º 23´ E, 1630 m asl) during two growing seasons of 2011-2012.

The daily minimum and maximum air temperatures, average relative humidity, reference evapotranspiration based on the FAO Penman-Monteith equation (Allen et al., 1998) are presented in Figure 1. The soil at this site is silty clay loam, typic Haplargids of the arid tropic with pH=7.63-7.8, electrical conductivity (EC_e)=3.3-3.8 dsm^{-1} and 0.9% organic matter and mean annual precipitations and mean annual temperature were 149 mm and 15.4 ℃, respectively. A square lattice design (8×8) with two replications was used for each water deficit stress and non-stress (normal) field experiments. Each plot consisted of three 4 m long rows spaced 30 cm apart. Plants were grown under full irrigation until branching growth stage when water stress was applied. Irrigation treatments were applied based on the maximum allowable depletion (MAD) percentage of the soil available water (SAW). Plants were irrigated at 50 and 80% MAD of ASW in nonstressed and water-deficit stressed plots, respectively (Stegman, 1983). Under full irrigation (normal conditions), irrigation supply was non-limiting and corresponded to when 50% of the soil available water was depleted from the root zone. The deficit irrigation treatment (stress conditions) corresponded to when 80% of the soil available water was depleted from the root zone (Allen et al., 1998). Therefore, the number of days between two irrigations during the growing season was variable because of the evapotranspiration (ET) variation. Soil samples were collected from a soil depth of 0-20, 20-40 and 40-60 cm for both normal and water deficit conditions. The depth of irrigation was determined based on the soil water content and calculated using the following equations:

$$SAW=(\theta_{fc}-\theta_{pwp})\times D\times 100 \quad (1)$$

$$I_d=SAW\times p \quad (2)$$

$$I_g=I_d\times 100/Ea \quad (3)$$

SAW is soil available water (cm); θ_{fc} and θ_{pwp} are the volumetric soil water content (%) at field capacity (0.03 MPa) and permanent wilting point (1.5 MPa), respectively; D is the soil layer depth (cm); I_d is the irrigation depth (cm); p is the fraction of SAW (50% and 80%) that can be depleted from the root zone; I_g is the gross depth of irrigation (cm) and E is the irrigation efficiency (%) averagely assumed as 65% (Taftehand Sepaskhah, 2012). The applied irrigation water determined, based on Eq. (3), was delivered to the plots by Parshall Flume WSC type III (cumulative amount of water applied) and presented in Figure 1.

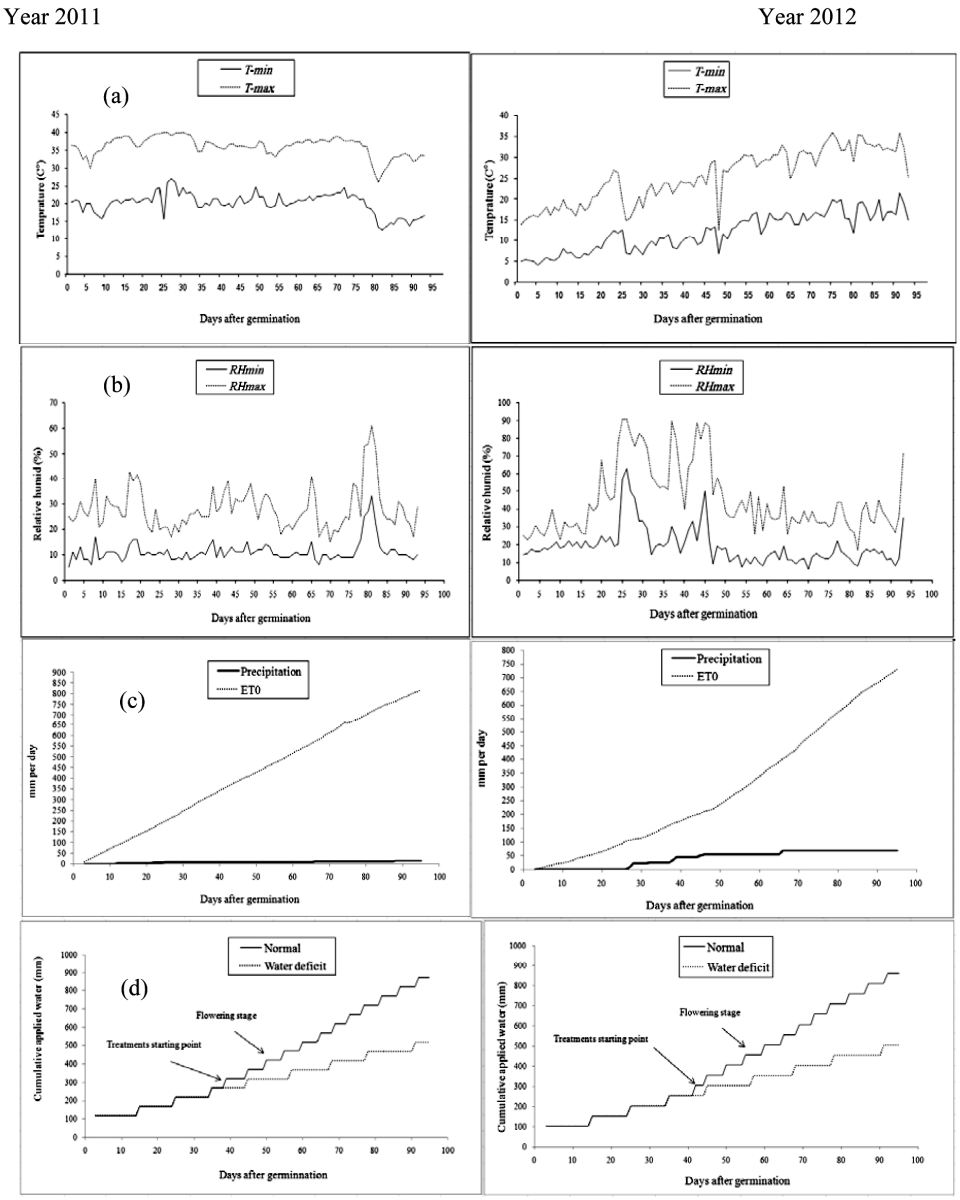

Figure 1. The daily minimum (T_{min}) and maximum (T_{max}) air temperatures (a); average relative humidity (b); reference evapotranspiration (ET_O) and precipitation (c); and cumulative amount of water applied for irrigation regimes (normal and water deficit) (d), for 2011 and 2012.

Antioxidants, membrane stability index, chlorophyll content and leaf area index of their leaves at flowering stage were evaluated. In this experiment catalase, ascorbate peroxidase, peroxidase activities, Leaf membrane stability index, leaf area index and contents of chlorophyll and carotenoid of leaf in safflower were appraised using the following procedures.

Enzyme preparation and assay

Catalase, ascorbate peroxidase and peroxidase (CAT, APX and POX) activities were determined from the extract prepared according to the methods of (Chance and Maehly, 1955; Nakano and Asada, 1987) with some modifications. All steps of the extraction were carried out at 4 °C. Leaf samples (0.1 g fr wt) were homogenized in a cold mortar in 1 ml of 50 mM Na-phosphate buffer (pH 7) containing 2 mM α-dithiothreitol, 2 mM EDTA, 0.2% triton x-100, 50 mM Tris-HCl and 2% polyvinylpyrroli done and mixed for 15 min. The obtained extracts were immediately used to assay enzyme activities.

The assay of CAT activity was performed using a total volume of 3 ml of 50 mM Na-phosphate buffer (pH 7) containing 4.51 µl of H_2O_2 (30%) and 50 µl of enzyme extract. The decrease in absorbance at 240 nm because of degradation of H_2O_2 was monitored every 30 sec for 2 min, using a spectrophotometer U-1800 (Hitachi, Japan). CAT activity was expressed as nanomole of decomposed per milligram of protein per minute.

APX activity was determined using 3 ml of 50 mM Na-phosphate buffer (pH 7.8) containing 4.51 µl of H_2O_2 (30%), 100 µl of 5 mM ascorbate and 50 µl of enzyme extract. The decrease in absorbance at 290 nm because of degradation of H_2O_2 was monitored every 30 sec for 2 min, using a spectrophotometer U-1800 (Hitachi, Japan). APX activity was expressed as nanomole of decomposed per milligram of protein per minute.

POX activity was determined in 3 ml of 50 mM Na-phosphate buffer (pH 7.8) containing 4.51 µl of H_2O_2 (30%), 3.35 µl Guiacol and 50 µl of enzyme extract. The increase in absorbance at 470 nm because of degradation of H_2O_2 was monitored every 30 sec for 2 min, using a spectrophotometer U-1800 (Hitachi, Japan). POX activity was expressed as nanomole of decomposed per milligram of protein per minute.

Leaf protein content

Leaf protein content was determined using a Bradford reagent (Bradford, 1976) and Bovine serum albumin (Sigma) as standard. Protein content was used as denominator of the enzymatic activity fractions.

Leaf membrane stability index

Membrane stability index (MSI) was determined according to the method of (Premchandra et al., 1990) as modified by (Sairam, 1990). Leaf materials (0.1 g) were thoroughly washed in running tap water at 40 ºC for 30 min. After the expiry of the period their electrical conductivity was recorded by conductivity bridge (C_1). Subsequently the same samples were placed in boiling water bath (100 ºC) for 10 min and their electrical conductivity recorded as above (C_2).
MSI was calculated with the following equation:

$$MSI=[1-(C_1 / C_2)]\times 100 \quad (4)$$

Leaf area index

Leaves from 10 plants in each plot were cut and leaf area was measured in cm^2 by green leaf area meter (OSK-Model GA-5). Leaf area index (LAI) calculated and mean of each plot was used.

Chlorophyll and carotenoid contents

Chlorophylla (chl_a), chlorophyll b (chl_b), total chlorophyll (C_{a+b}) and carotenoids (C_{x+c}) were extracted and estimated from fresh leaves, following the standard method of (Lichtenthaler and Buschmann, 2001). The optical density measured by the absorption A at 661.6, 644.8 and 470 nm and then calculated with the following equations:

Concentration of chl_a (mg/g fw): $C_a=[(11.24\times A\ 661.6-2.04\times A\ 664.8) \times$ ml Acetone] /mg leaf tissue (5)

Concentration of chl_b (mg/g fw): $C_b=[(20.13\times A\ 644.8-4.19\times A\ 661.6) \times$ml Acetone] /mg leaf tissue (6)

Concentration of C_{a+b} (mg/g fw)$=C_a+C_b$ (7)

Concentration of carotenoids (mg/g fw): $C_{x+c}=[\{1000\times A\ 470-1.90C_a \times -63.14\ C_b / 214)\times$ ml Acetone$\}] /$ mg leaf tissue (8)

Statistical analysis

The data were examined for homogeneity and normality of residuals using Kolmogorov Smirnov and Bartlett's tests, respectively. Then combined analysis of variance (ANOVA) was carried out with data from two experimental conditions (normal and water deficit stress) using PROC GLM of SAS 9. All the effects were considered random. Mean comparisons were conducted using the Fisher's least significant difference ($LSD_{0.05}$) test.

Results and Discussion

Since there were no significant differences between two growing seasons (year) for the tested characteristics, data averaged for both years were used for ANOVA and mean comparisons. Results of combined ANOVA showed a significant effect of water deficit stress on the safflower physiological characteristics that were assessed in this study (Table 1). Analysis of combined ANOVA indicated significant differences among genotypes for all the traits under both normal and water deficit stress conditions (Table 1). As the results of this investigation with comparing genotypes under water deficit stress and non-stress conditions demonstrated a cross-over genotype× environment interaction (G×E), meaning that either genotypes or traits recognized as useful in normal environments may not be so under water deficit conditions. Combined ANOVA showed the significant genotype× year interactions for the POX activity, chlorophyll a, b, a+b, carotenoid content and seed yield (Table 1).

Field water deficit stress caused significant increases in mean of activities of antioxidant enzymes with the exception of CAT. Increased POX and CAT activities was reported in water deficit stressed soybean when compared to control plants (Zhang et al., 2008). The discrepancy in CAT activity under drought stress may be explained by the intensity and duration of water shortage, and the metabolic status of plants. In fact, further research to resolve this discrepancy is needed to understand the influences of water deficit stress on activity of CAT of safflower genotypes. Genotypes varied significantly for antioxidant activities under both environmental conditions (Table 1). APX activity had significant

positive relationship (r=0.53**) (data not shown). This correlation mentioned influence of APX on safflower genotypes under water deficit condition was more than other antioxidants that were evaluated in present investigation. The greater activities of antioxidant enzymes were observed in Kordestan 3 accompanied with its higher seed yield under deficit water stress. On the other hand, CW-4440 had the lowest POX and APX activities and produced the lowest seed yield (Tables 2 and 4). The overall behavior of the antioxidant enzymes in this present study suggests an increase of cellular H_2O_2 that would contribute to the oxidative stress of safflower plants.

The 64 safflower genotypes used in the present study showed reduced membrane stability means with water deficit (Table 3). Significant variations were observed among genotypes in respect to MSI under both environmental conditions. MSI of safflower genotypes ranged from 51.2 for Kino-76 to 73.5 for PI-258417 under normal and 41.4 for Dincer to 68.9 for C_{444} under water deficit field conditions. The results also show an inverse and significant relationship between seed yield loss due to water deficit with MSI under water deficit stress (r= -0.52**) (data not shown). Moreover, lower MSI of Dincer accompanied with the greatest seed yield loss of this genotype. While C_{444} had highest value of MSI with lower seed yield, higher yield loss as well as lower APX and CAT activity. This result may indicate the underlying physiological mechanisms that contribute in water deficit tolerance. Modifications of lipid composition of plasma membranes are vital in sustaining membrane fluidity, integrity and functionality when confronting external perturbations (Yeilaghi et al., 2012).

Safflower genotypes differed significantly in respect to LAI under both normal and water deficit stress conditions. Means of LAI were significantly reduced by water deficit in most of the genotype sand ranged from 6.24 of IL to 2.7 of PI-405985 and 5.75 of IL to 1.88 of S6-697-324 under normal and water deficit stress conditions, respectively (Table 3). Often, crop cultivars improved for water limited environments by selection for yield under drought stress have constitutively reduced leaf area (Blum, 1996). Likewise, Diepenbrock (2000) reported that LAI genotypes of *Brassica napus* genotypes reduced due to drought stress. Blum (2005) suggested that small leaf area is beneficial under drought stress because

causes dehydration-avoidant. LAI had positive and significant relationship with seed yield under normal ($r=0.58^{**}$) and water deficit stress ($r=0.46^{**}$) conditions (data not shown).

Water deficit stress decreased total chlorophyll and carotenoids contents means (Table 4). The reduction in photosynthesis under water deficit stress can also be attributed to a decrease in chlorophyll content. Water deficit reduced the chlorophyll content in water deficit susceptible genotypes and increased in water deficit tolerant genotypes. These results are in agreement with those of other oilseed crop (*Sesamumindicum*) (Abraham et al., 2008). Mean of chl_{a+b} ranged from 0.83 for Kino-76 to 1.55 for PI-506426 under non stressed experiment and from 0.68 for Hamedan 38 to 1.32 mg g^{-1} fw for Syrian under stress condition. Chlorophyll loss is associated to environmental stress and the variation in total chlorophyll/carotenoids ratio may be a good indicator of stress in plants (Hendry and Price, 1993). Lower values for the ratio of $chl_{(a+b)/(x+c)}$ are an indicator of senescence and stress to the plant and the photosynthetic apparatus. In present study the lowest seed yield and greater seed yield loss of Kermanshah genotype accompanied with the lowest value for the ratio of $chl_{(a+b)/(x+c)}$ (data not shown) of this genotype provided further evidence supporting this fact. Chl_a had the positive and significant relationship with the Chl_b, carotenoid and ratio of $chl_{(a+b)/(x+c)}$ in both field conditions (data not shown). The assessment of photosynthetic pigments and consequently their relationships is an important indicator of senescence (Hendry and Price, 1993). The results also show the positive and significant relationship between seed yield with chl_a ($r=0.52^{**}$) and $chl_{(a+b)/(x+c)}$ ($r=0.59^{**}$) (data not shown) in water deficit stress condition. Greater seed yield of Syrian may be for high level of chl_{a+b} and carotenoid of this genotype. Thus selection of genotypes with higher chlorophyll value under non-stress condition leads to high seed yield under both environmental conditions. Differences in leaf chlorophyll content can be as an indicator of plant vigor and its capacity for photosynthesis, strongly dependent on chlorophyll content (Carter andSpiering, 2002). The significant variation for Chl_a, Chl_b and carotenoid content has also been reported by other researchers (Jonson et al., 1993).

Table 1. Combined analysis of variance for seed yield and physiological traits in safflower genotypes grown under two environmental conditions (normal and water deficit-stress) in two growing seasons of 2011-2012.

Source of variation	df	CAT	APX	POX	MSI	Mean square		Chl_{a+b}	$Chl_{a/b}$	Carotenoid	LAI	Seed yield
						Chl_a	Chl_b					
Environment (E)	1	14.4^{**}	207^{**}	142.9^{**}	5910^{**}	2.03^{**}	0.25^{**}	29.24^{**}	0.67^{ns}	0.05^{**}	78.81^{**}	45908372^{**}
Year (Y)	1	0.17^{ns}	0.12^{ns}	3.29^{ns}	0.61^{ns}	0.04^{ns}	0.01^{ns}	22.8^{**}	2.46^{ns}	0.02^{*}	0.7^{ns}	28203^{ns}
E×Y	1	0.00001^{ns}	0.00001^{ns}	0.04^{**}	0.72^{ns}	0.00001^{ns}	0.00001^{ns}	24.1^{**}	0.27^{ns}	0.00001^{ns}	0.00001^{ns}	0.00001^{ns}
Block/ (E×Y)	4	0.28^{ns}	0.05^{ns}	1.74^{**}	164.1^{**}	0.03^{**}	0.003^{ns}	0.01^{**}	1.98^{ns}	0.002^{**}	2.08^{**}	942701^{**}
Genotype (G)	63	3.54^{**}	0.15^{**}	8.23^{**}	98.84^{**}	0.04^{**}	0.01^{**}	0.02^{**}	2.14^{**}	0.002^{**}	2.93^{**}	933564^{**}
G×E	63	3.22^{**}	0.16^{**}	8.19^{**}	116.5^{**}	0.02^{**}	0.01^{**}	0.01^{**}	1.12^{ns}	0.003^{**}	1.28^{**}	310340^{**}
G×Y	63	0.006^{ns}	0.001^{ns}	0.004^{**}	31.76^{ns}	0.007^{*}	0.005^{**}	0.01^{**}	0.8^{ns}	0.005^{**}	0.01^{ns}	124552^{*}
G×E×Y	63	0.00001^{ns}	0.00001^{ns}	0.02^{**}	21.2^{ns}	0.00001^{ns}	0.00001^{ns}	0.03^{ns}	0.17^{ns}	0.00001^{ns}	0.00001^{ns}	0.00001^{ns}
Residual	252	0.2	3.71	0.00005	37.81	0.005	0.003	0.003	0.88	0.0007	0.37	89601

APX-ascorbate peroxidase, CAT-catalase, Chl_a-chlorophyll a, Chl_b-chlorophyll b, LAI-leaf area index, MSI-membrane stability index, POX-peroxidase, ns: Non-significant.

* Significantat $P<0.05$.

** Significantat $P<0.01$.

Table 2. Mean of antioxidants in 64 saff lower genotypes grown under normal and water deficit stress field conditions.

Genotypes	Origin	Catalase (nanomole min^{-1} $mgprotein^{-1}$)		Ascorbate peroxidase (nanomole min^{-1} mg $protein^{-1}$)		Peroxidase (nanomole min^{-1} $mgprotein^{-1}$)	
		normal	stress	normal	stress	normal	stress
C111	Iran	1.99	2.49	0.40	1.82	0.22	3.30
C116	Iran	1.52	1.85	027	1.66	3.92	7.11
C411	Iran	3.10	1.63	0.42	1.75	0.51	11.10
C444	Iran	1.40	0.63	0.45	1.47	3.05	6.48
C4110	Iran	1.69	2.84	0.41	1.88	1.59	2.67
S6-58/41-168	Iran	2.96	2.203	0.31	1.57	0.85	6.54
S6-697-307	Iran	1.62	3.20	0.39	1.80	1.19	5.10
S6-697-324	Iran	1.85	4.48	0.69	1.74	6.72	3.55
IL	Iran	1.44	2.64	0.31	1.75	0.85	5.86
N/27	Iran	2.84	0.26	0.47	1.78	1.21	4.21
73-14-34	Iran	3.38	2.86	0.42	1.78	1.95	2.94
PI-405985	Iran	2.56	0.77	0.39	1.76	1.19	3.17
LRV-51-51	Iran	1.41	1.41	0.25	1.76	0.95	7.97
LRV-55-295	Iran	2.61	0.94	0.52	1.58	3.20	3.08
Hamedan17	Iran	1.68	2.86	0.35	1.76	1.90	3.70
Hamedan21	Iran	2.42	1.71	0.45	1.55	1.11	6.04
Hamedan38	Iran	1.55	0.35	0.36	2.04	2.33	9.13
Hamedan40	Iran	1.72	1.34	0.60	1.61	5.10	6.55
Kordestan1	Iran	1.80	2.79	0.62	1.62	6.83	5.35
Kordestan2	Iran	2.25	0.26	0.90	1.76	4.31	6.41
Kordestan3	Iran	1.68	4.48	0.47	2.62	6.20	8.14
Kordestan4	Iran	3.27	1.12	0.31	2.05	0.58	7.33
Kordestan5	Iran	2.78	1.67	0.98	2.11	1.56	5.68
Kordestan6	Iran	1.84	2.08	0.36	1.71	4.42	2.63
Kordestan7	Iran	2.63	3.29	0.40	1.93	1.36	4.09
Kordestan8	Iran	1.08	1.00	0.48	1.68	3.12	3.68
Kordestan9	Iran	1.05	2.19	0.29	1.82	3.42	4.44
Darab1	Iran	3.04	2.8	0.54	1.62	1.76	3.63
Darab2	Iran	3.08	2.75	0.61	1.15	0.90	3.17
Darab4	Iran	3.97	3.35	0.54	1.78	4.16	2.40
Darab9	Iran	1.34	2.96	0.51	1.62	3.96	2.06
Khorasan62	Iran	1.88	2.40	0.61	1.76	5.58	4.96
Khorasan330	Iran	2.04	2.10	0.37	1.98	0.39	4.44
Khorasan376	Iran	2.19	1.88	0.52	1.58	3.91	3.04
Khorasan508	Iran	2.72	2.68	0.65	2.11	1.91	3.54
Kermanshah	Iran	2.10	1.56	0.45	1.65	1.11	2.27
Kermanshah44	Iran	3.37	1.58	0.44	1.87	1.48	3.00
Kermanshah46	Iran	3.23	0.76	0.43	1.51	0.44	3.31
Kermanshah47	Iran	3.66	3.66	0.34	2.07	2.56	4.10
Kemanshah60	Iran	3.07	1.93	0.60	2.07	1.93	3.26
Esfahan4	Iran	2.55	1.54	0.59	1.98	5.00	7.85
Esfahan kuse	Iran	2.72	1.49	0.29	1.60	3.40	3.23
Marand	Iran	2.52	1.94	0.88	1.62	5.91	3.73

Continue Table 2.

Genotypes	Origin	Catalase (nanomole min^{-1} $mgprotein^{-1}$)		Ascorbate peroxidase (nanomole min^{-1} $mg\ protein^{-1}$)		Peroxidase (nanomole min^{-1} $mgprotein^{-1}$)	
		normal	stress	normal	stress	normal	stress
Zarghan	Iran	2.48	2.13	0.40	1.86	0.41	7.48
Sina	Iran	1.33	3.61	0.70	2.05	7.91	5.10
Arak	Iran	1.40	2.24	0.50	1.59	4.66	4.93
Dincer	Turkey	4.45	3.53	0.57	1.82	1.51	4.99
Yinice	Turkey	2.24	1.70	0.62	1.96	2.93	2.63
C1055	Turkey	2.57	0.57	1.13	1.54	1.21	4.71
PI-198844	France	2.54	1.62	0.36	1.74	2.51	7.98
PI-253384	Palestine	3.32	2.32	0.57	1.78	7.41	5.51
PI-250190	Pakistan	2.42	3.40	0.44	1.78	1.09	2.75
PI-250537	Egypt	2.87	4.06	0.32	1.76	4.13	4.40
PI-506426	China	2.88	2.44	0.54	1.86	1.70	4.17
Cyprus Bregon	Cyprus	3.3	1.83	0.53	1.58	3.54	4.62
Syrian	Syria	4.18	1.88	0.36	1.65	1.38	6.68
PI-258417	Portugal	3.94	1.58	0.37	1.65	7.61	6.50
Hartman	USA	1.56	2.92	0.34	1.84	1.60	2.78
Gila	USA	2.03	0.43	0.53	1.83	3.44	6.33
CW-4440	USA	2.76	0.78	0.65	1.12	1.50	1.97
S-541	USA	2.14	3.31	0.51	1.86	4.54	6.10
PI-537636-S	USA	2.62	0.94	0.44	1.60	3.23	4.21
PI-537636	USA	3.40	1.44	0.34	1.89	1.07	7.62
Kino-76	Mexico	0.93	2.04	0.44	1.53	3.31	9.26
LSD 0.05		0.33	1.06	0.12	0.29	0.67	1.32

Table 3. Mean of physiological traits in 64 saff lower genotypes grown under normal and water deficit stress field conditions.

Genotypes	Membrane stability index (%)		Leaf area index		Chlorophyll a ($mg\ g^{-1}fw$)		Chlorophyll b ($mg\ g^{-1}fw$)	
	normal	stress	normal	stress	normal	stress	normal	stress
C111	70.56	55.25	3.98	2.91	0.89	0.82	0.27	0.19
C116	56.11	62.8	4.00	3.72	0.90	0.81	0.27	0.29
C411	68.16	49.53	4.26	3.50	0.87	0.57	0.26	0.23
C444	61.63	68.90	4.23	2.83	0.95	0.78	0.40	0.28
C4110	65.37	54.29	2.90	3.35	0.83	0.73	0.29	0.26
S6-58/41-168	59.58	55.99	4.57	2.68	1.01	0.76	0.41	0.22
S6-697-307	56.98	58.24	3.69	3.03	0.95	0.86	0.29	0.39
S6-697-324	58.18	53.44	3.64	1.88	0.92	0.73	0.32	0.27
IL	55.98	65.75	6.24	5.75	1.01	0.87	0.35	0.23
N/27	66.55	48.05	4.31	2.37	0.76	0.79	0.36	0.31
73-14-34	67.67	59.22	3.74	2.27	0.96	0.82	0.35	0.22
PI-405985	63.28	60.42	2.70	2.78	0.74	0.59	0.32	0.23
LRV-51-51	59.18	45.78	3.41	2.81	0.91	0.89	0.29	0.25
LRV-55-295	58.79	62.04	3.60	2.68	0.94	0.75	0.31	0.18
Hamedan17	60.90	56.87	4.94	4.74	0.76	0.80	0.22	0.25
Hamedan21	60.78	58.40	3.99	2.96	0.93	0.83	0.26	0.26
Hamedan38	68.14	64.32	4.15	5.08	0.80	0.47	0.20	0.21
Hamedan40	54.14	56.54	3.63	2.65	0.89	0.86	0.30	0.30

Continue Table 3.

Genotypes	Membrane stability index (%)		Leaf area index		Chlorophyll a (mg g^{-1}fw)		Chlorophyll b (mg g^{-1}fw)	
	normal	stress	normal	stress	normal	stress	normal	stress
Kordestan1	56.46	57.40	4.25	3.11	0.82	0.70	0.22	0.22
Kordestan2	56.44	46.93	4.00	3.51	0.90	0.79	0.27	0.26
Kordestan3	60.79	48.92	4.05	3.31	1.01	0.89	0.36	0.27
Kordestan4	62.57	56.68	4.06	2.97	0.88	0.64	0.35	0.22
Kordestan5	59.30	55.24	4.26	3.15	0.83	0.70	0.18	0.16
Kordestan6	61.19	54.35	5.13	5.57	0.88	0.64	0.29	0.18
Kordestan7	63.13	46.61	3.52	2.60	0.77	0.64	0.22	0.25
Kordestan8	64.28	45.50	4.43	3.59	0.87	0.76	0.30	0.18
Kordestan9	65.28	42.91	3.46	2.45	0.91	0.78	0.29	0.23
Darab1	60.87	48.53	4.3	4.32	0.80	0.66	0.21	0.17
Darab2	60.93	49.66	4.00	3.06	0.93	0.92	0.28	0.29
Darab4	58.73	60.98	4.74	3.13	0.87	0.85	0.25	0.23
Darab9	63.18	55.21	3.56	3.25	0.90	0.78	0.23	0.30
Khorasan62	53.86	54.03	4.70	3.98	0.97	0.63	0.27	0.20
Khorasan330	67.05	54.97	4.61	3.65	0.92	0.70	0.27	0.25
Khorasan376	57.42	46.71	3.41	2.47	0.94	0.85	0.29	0.27
Khorasan508	59.16	56.85	3.52	2.54	0.90	0.86	0.28	0.27
Kermanshah	56.23	59.59	4.56	3.71	0.89	0.75	0.30	0.27
Kermanshah44	63.95	56.38	3.91	2.65	0.96	0.64	0.34	0.32
Kermanshah46	60.28	53.00	4.91	3.16	0.84	0.88	0.27	0.33
Kermanshah47	60.96	43.89	5.02	5.02	1.07	0.82	0.32	0.37
Kemanshah60	58.94	45.18	3.44	4.84	0.78	0.68	0.31	0.17
Esfahan4	61.68	55.17	4.39	3.76	0.88	0.78	0.30	0.23
Esfahan kuse	51.79	54.98	3.60	3.03	0.80	0.57	0.30	0.31
Marand	58.70	61.66	3.76	2.86	0.92	0.65	0.30	0.23
Zarghan	63.01	56.38	2.77	2.22	0.88	0.80	0.29	0.23
Sina	59.93	57.60	3.70	3.85	0.87	0.86	0.29	0.27
Arak	52.67	53.01	4.02	3.27	0.75	0.72	0.21	0.18
Dincer	56.16	41.38	4.76	2.54	0.93	0.82	0.24	0.24
Yinice	61.79	51.45	3.79	2.41	0.89	0.84	0.27	0.23
C1055	62.39	53.81	4.43	3.62	0.97	0.73	0.29	0.25
PI-198844	59.75	50.16	4.67	2.44	0.94	0.77	0.35	0.24
PI-253384	64.85	56.15	4.20	2.78	0.82	0.64	0.35	0.21
PI-250190	63.01	49.46	4.29	3.76	0.72	0.84	0.36	0.32
PI-250537	52.75	48.68	4.73	3.32	0.88	0.75	0.23	0.21
PI-506426	65.35	56.62	3.79	3.54	0.96	0.71	0.59	0.22
Cyprus Bregon	56.70	56.40	4.29	3.89	0.86	0.81	0.32	0.34
Syrian	67.38	54.59	5.26	2.96	0.95	0.98	0.31	0.34
PI-258417	73.49	42.01	3.91	2.85	0.93	0.75	0.32	0.23
Hartman	58.91	43.15	4.88	2.90	0.92	0.86	0.30	0.26
Gila	63.52	67.92	3.19	4.16	0.83	0.75	0.27	0.19
CW-4440	57.99	50.35	3.22	3.19	0.80	0.76	0.23	0.21
S-541	56.66	51.50	4.04	2.50	0.77	0.69	0.24	0.19
PI-537636-S	54.06	58.66	3.89	3.86	0.81	0.48	0.27	0.24
PI-537636	69.61	64.13	4.43	3.15	0.80	0.66	0.23	0.21
Kino-76	51.20	45.64	3.31	3.98	0.64	0.60	0.19	0.18
LSD 0.05	9.80	13.70	1.2	1.3	0.13	0.14	0.10	0.09

Table 4. Mean of physiological traits in 64 safflower genotypes grown under normal and water deficit stress field conditions.

Genotypes	Chl_{a+b}		$Chl_{a/b}$		Carotenoid (mg g^{-1}fw)		Seed yield (Kg h^{-1})	
	normal	stress	normal	stress	normal	stress	normal	stress
C111	1.15	0.99	3.28	4.33	0.20	0.20	1986.2	1026.9
C116	1.17	1.13	3.35	2.83	0.23	0.22	1781.3	1349.1
C411	1.13	0.79	3.45	2.61	0.20	0.17	2555.5	2530.7
C444	1.35	1.04	2.40	3.07	0.22	0.22	1795.1	783.3
C4110	1.12	0.97	3.00	2.97	0.21	0.19	2089.2	1149.6
S6-58/41-168	1.42	1.00	2.59	3.37	0.20	0.21	2172.5	1269.6
S6-697-307	1.24	1.25	3.33	2.33	0.26	0.22	1785.2	1128.5
S6-697-324	1.25	0.99	2.90	2.71	0.24	0.13	1972	1640.3
IL	1.36	1.13	3.03	3.53	0.25	0.22	3025.5	2308.4
N/27	1.12	1.08	2.16	2.71	0.23	0.21	1686.6	875.4
73-14-34	1.31	1.06	2.76	3.62	0.23	0.19	1790.2	1360.2
PI-405985	1.05	0.80	2.34	2.75	0.22	0.18	1432.3	806.1
LRV-51-51	1.2	1.16	3.13	3.34	0.23	0.20	1829.4	1505.5
LRV-55-295	1.25	0.93	3.07	4.08	0.22	0.17	2138.2	1641.7
Hamedan17	0.97	1.05	3.76	3.19	0.20	0.21	2050	1451.9
Hamedan21	1.19	1.10	3.56	3.16	0.24	0.21	2473.5	1793.2
Hamedan38	1.00	0.68	4.04	2.40	0.19	0.12	2520.5	2128
Hamedan40	1.18	1.17	3.19	2.85	0.24	0.26	1496	1062.6
Kordestan1	1.04	0.91	3.76	3.16	0.18	0.16	2417.9	1977.9
Kordestan2	1.17	1.05	3.31	3.04	0.24	0.21	1834.3	1505.2
Kordestan3	1.37	1.21	2.94	3.22	0.25	0.23	1822.5	2161.0
Kordestan4	1.23	0.84	2.53	3.10	0.24	0.19	1613.7	945.4
Kordestan5	1.01	0.87	4.52	5.26	0.19	0.16	2494.1	1959.8
Kordestan6	1.17	0.82	3.45	3.61	0.23	0.13	2240.	1391.6
Kordestan7	0.99	0.89	3.58	2.65	0.18	0.17	1901.9	1275
Kordestan8	1.17	0.96	2.98	4.31	0.21	0.22	1990.6	1787.7
Kordestan9	1.19	0.99	3.50	3.45	0.20	0.21	2103.9	1485.5
Darab1	1.01	0.84	3.78	4.16	0.21	0.17	1677.4	1025.3
Darab2	1.21	1.17	3.33	3.11	0.20	0.23	2099	1460.2
Darab4	1.12	1.10	3.49	3.48	0.22	0.21	2092.1	1160.2
Darab9	1.13	1.07	4.00	2.75	0.23	0.20	1839.2	1085.7
Khorasan62	1.24	0.84	3.65	3.22	0.24	0.17	3100	1729.7
Khorasan330	1.18	0.93	3.42	2.98	0.23	0.20	2323.5	1258.8
Khorasan376	1.23	1.11	3.26	3.21	0.21	0.20	2321.5	1187.8
Khorasan508	1.18	1.12	3.24	3.14	0.23	0.21	1574.2	1508.4
Kermanshah	1.19	1.01	2.99	2.90	0.24	0.21	1676.4	1069.6
Kermanshah44	1.30	0.96	2.82	2.04	0.21	0.23	1813.7	767.2
Kermanshah46	1.12	1.20	3.03	2.77	0.24	0.22	1749	940.1
Kermanshah47	1.39	1.18	3.30	2.33	0.21	0.22	3574.5	1792.6
Kemanshah60	1.08	0.88	2.49	3.71	0.26	0.19	1476.4	1117.8
Esfahan4	1.18	0.99	2.93	3.51	0.22	0.20	1878.4	1748.2
Esfahan kuse	1.10	0.87	2.82	1.93	0.25	0.15	1805.6	1310
Marand	1.21	0.86	3.22	2.9	0.24	0.19	1564.7	1060
Zarghan	1.16	1.04	3.08	3.36	0.21	0.19	2133.3	1539.9

Continue Table 4.

Genotypes	Chl_{a+b}		$Chl_{a/b}$		Carotenoid (mg g^{-1}fw)		Seed yield (Kg h^{-1})	
	normal	stress	normal	stress	normal	stress	normal	stress
Sina	1.16	1.14	3.14	3.08	0.21	0.17	1858.8	1716.1
Arak	0.96	0.89	3.56	4.32	0.20	0.20	1580.3	1458.4
Dincer	1.16	1.07	4.09	3.37	0.18	0.20	2694.1	1197.7
Yinice	1.16	1.07	3.34	3.69	0.23	0.22	2499	1470.5
C1055	1.27	0.99	3.36	2.91	0.26	0.17	2285.2	1482.8
PI-198844	1.30	0.99	2.68	3.4	0.24	0.19	2311.7	1388.2
PI-253384	1.17	0.87	2.36	3.01	0.19	0.18	2018.6	1877.9
PI-250190	1.08	1.15	1.99	2.72	0.13	0.21	1811.7	1541.7
PI-250537	1.11	0.97	4.02	3.53	0.18	0.19	2211.7	1606.8
PI-506426	1.55	0.93	1.66	3.30	0.16	0.20	2103.9	1402.9
Cyprus Bregon	1.18	1.13	2.66	2.88	0.19	0.20	1804.9	1227.2
Syrian	1.25	1.32	3.07	2.92	0.23	0.23	2394.1	2060.4
PI-258417	1.26	0.97	3.00	3.26	0.22	0.20	1804.9	1507.9
Hartman	1.22	1.12	3.09	3.46	0.21	0.20	2476.4	1629.8
Gila	1.10	0.94	3.11	3.84	0.21	0.18	1648	1274.1
CW-4440	1.02	1.01	3.52	3.52	0.20	0.21	1741.1	1125
S-541	1.00	0.87	3.30	3.80	0.18	0.21	2392.1	1083.9
PI-537636-S	1.08	0.69	2.97	2.18	0.23	0.15	1790.2	925.8
PI-537636	1.04	0.88	3.38	3.01	0.19	0.17	1922.5	1013
Kino-76	0.83	0.75	3.40	3.68	0.18	0.19	1643.8	1431.6
LSD 0.05	0.15	0.15	1.31	1.60	0.04	0.04	595.2	574.1

Conclusion

The results of present study showed that genotypes varied significantly for the tested physiological traits, with the exception of $chl_{a/b}$ under both normal and water-deficit conditions.

Among the safflower genotypes, Hamedan 38 possessed the greatest values of APX and CAT, MSI and LAI which these physiological features accompanied by a high seed yield under water-deficit stress conditions. However, based on stress susceptibility index (SSI) (Fischer and Maurer 1978) Kordestan3 and C411 genotypes were identified as being the most water-deficit stress tolerant genotypes. The lowest value of SSI of these two genotypes accompanied by the highest activities of antioxidant enzymes (CAT, APX and POX). It is interesting to note that the above-mentioned 3 genotypes were all member of the superior ranked group for seed yield under water-deficit conditions. Therefore, these data support the role of antioxidant enzymes (CAT, APX and POX), MSI and LAI involved in water deficit tolerant mechanisms in safflower.

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