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Full Length Research Paper

Genetic variation of leaf antioxidants and chlorophyll content in safflower

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An efficient antioxidant defense system represented by enzymatic and non-enzymatic forms is important in the control of oxidative stress caused by free radicals and other reactive species. Leaf antioxidant activity and chlorophyll content of 20 safflower (*Carthamus tinctoriuos* L.) genotypes comprising eight parental and 12 F_1 hybrids originated from reciprocal crosses of four parental lines were evaluated in this study. Five leaf antioxidants including ascorbate peroxidase (APX), glutathione reductase (GR), super oxide dismutase (SOD), carotenoids and lipid peroxidastioin (LP) were assessed. Chlorophyll (a, b, a+b) content was also evaluated. Analysis of variances for all traits showed the significant differences among the genotypes (P < 0.05). The highest antioxidant activity was consistently belonged to APX and GR in all genotypes. Cross 22-191×k_{21} was superior for GR and APX activity among F_1 hybrids. The highest activity of SOD and LP was observed in C_{4110} genotype. GE-62918 showed the highest content of carotenoids. The highest chl a and chl a+b was related to a F_1 hybrid (22-191×K_{21}). In the majority of F_1 hybrids the activity of antioxidant was higher and chlorophyll content was intermediate when compared to those of their parents. Significant differences were observed between reciprocal crosses for the antioxidants with the exception of the GR. This result in turn indicated that the cytoplasmic effects on the antioxidant activity in safflower. There was no significant difference between reciprocal crosses for chlorophyll content with the exception of 22-191×K_{21} cross. The heterosis effect for SOD activity was significant (143, 95 and 54.4) in three genotypes related to superior parent and in three genotypes (96.2, 40 and 31) related to mean of parents. Significant heterosis (122.86 and 156.20) was only observed for two genotypes related to mean of parents for Chl a and Chl b contents, respectively.

Key words: Safflower, *Carthamus tinctoriuos,* antioxidant, carotenoids, chlorophyll,

INTRODUCTION

A great variety of abiotic stresses including drought, salinity, UV light, air pollutants and heavy metals cause molecular damage to plants, either directly or indirectly through reactive oxygen species (ROS) formation (Alscher et al., 2002; Arnon and Sairam, 2002; Cervilla et al., 2007; Cheruth et al., 2007). Living organisms have developed complex antioxidant systems to counteract ROS and their damage (Cheruth et al., 2007; Sarowar et al., 2005). The production of ROS must be carefully regulated to avoid oxidative damage by a broad spectrum of radical scavenger systems, including antioxidative enzymes like ascorbate peroxidase (APX), glutathione reductase (GR) and superoxide dismutase (SOD), as well as non-enzymatic compounds like glutathione, carotenoids, ascorbate and α-tocopherol (Sharma and Dubey, 2005; Mittler, 2002; Yannerelli et al., 2006). In plants, glutathione and ascorbate are the major antioxidants in photosynthetic and ascorbate are the major antioxidant in photosynthetic and non-photosynthetic tissues (Laspina et al., 2005). They are able to detoxify ROS by a direct scavenging or by acting as cofactors in the enzymatic reactions that involved APX and GR enzymes (Laspina et al., 2005; Mittler, 2002). Under normal conditions, the total amount of ROS, formed in the plant is determined by the balance between the multiple ROS producing pathways and the ability of the enzymatic and non-enzymatic mechanisms to deal with them (Yannerelli et

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et al., 2006) Increases in ROS under stresses in plant, terminated through the action of SOD, which dismutates the superoxide to hydrogen peroxide (H_2O_2) (Blokhina et al., 2003; Donahue et al., 1997). The H_2O_2 in turn is removed through the activity of the Asada-Halliwell scavenging cycle, which involves the oxidation and re-reduction of ascorbat and glutathione through the action of APX and GR. (Asada, 1992; Blokhina et al., 2003; Donahue et al., 1997). Accumulation of H_2O_2 will also lead to enhance potential for production of hydroxyl radicals, which leads to lipid peroxidatlon (Stepien and Klobus, 2005). Lipid peroxidation (LP) can be initiated enzymatically by lipoxygenases (LOX). Thiobarbituric acid reactive substances (TBARS) is measured as an index of lipid peroxidation (Cheruth et al., 2007). The higher amount of TBARS was reported under environmental stresses than control (Becana et al., 1986; Cheruth et al., 2007). Carotenoid protects cells and tissues from free radicals and singlet oxygen with an antioxidant activity (Blachburn, 1998; Johnson et al., 1993). Carotenoids are also function as light collectors (Blachburn, 1998). Carotenoids act as accessory pigments, harvesting light for photosynthesis and as photoprotective agents limiting the damaging effects of high irradiance (Johnson et al., 1993). Chlorophyll is a natural pigment that absorbs light energy for photosynthesis (Blachburn, 1998). This energy used by the plant was to synthesize glucose from carbon dioxide and water (Carter and Spiering, 2002). Differences in leaf chlorophyll content can be as an indicator of plant vigor and its capacity for photosynthesis, strongly dependent on chlorophyll content (Carter and Spiering, 2002). A greater understanding about contents of chlorophyll and carotenoid pigments, would be expected to yield improved methods of evaluating plant responses to the environmental stresses (Blachburn, 1998). Safflower (*Carthamus tinctoius* L.) is one of the oldest domesticated crops (Knowles, 1969). Historically its flowers were used to prepare fabric dyes, food coloring and for medicinal purposes because of its natural antioxidant properties (Dwiedi et al., 2005). Its major production today is for seeds used to extract its high quality edible oil (Amini et al., 2008). The research shows that there is a significant relationship between an oxidative stress caused by environmental stresses, and increase in antioxidant activity in plant (Choi et al., 2004; Sairam et al., 1998; Stepien and Klobus, 2005). In oil crops, there are some reports which shows the effect of oxidative stress on the activities of antioxidants. The effect of oxidative stress due to cadmium metal in sunflower leaves lead to a significant increase in SOD, GR and catalase activity (Laspina et al., 2005). Donahued et al. (1997) reported that resistance to fungi disease in pea was correlated with increasing in GR, SOD and APX activity in leaves.

Although antioxidant diversity has been previously reported in many plants, such as more recently in *Ficus deltoidea* accessions (Hakiman et al., 2009), saliva species (Tosun et al., 2009), persimmon (Ercisli et al., 2007), Sea buchthrone (Ercisli et al., 2007), plum (Rupasinghe et al., 2006) common beans (Cardador-Martinez et al., 2002), *Brassica* species (Kurilich et al., 1999) and potato (Spychall et al., 1990), there is a lack of information regarding safflower plant. Therefore, the objective of this study was to evaluate the antioxidant activities and chlorophyll content in safflower genotypes and their F_1 hybrids.

MATERIAL AND METHODS

Plant materials

Six breeding lines selected from various local populations of safflower along with two genotypes obtained from Germany and Mexico were used in this study (Table 1). Local lines were obtained by selfing individual plant selected from some native populations. Four parental lines were crossed in all combinations including reciprocals to produce F_1 seeds.

The genotypes were planted at Research Farm of Isfahan University of Technology located at Lavark, Najaf-Abad, Iran in spring 2008. Antioxidants and chlorophyll content of their leaves at flowering stage were evaluated. In this experiment GR, SOD, APX, LP, carotenoid and chlorophyll contents of leaf in safflower were assessed using the following procedures.

Enzyme preparation and assay

GR activity was determined from the rate of nicotinamide adenine dinucleotide phosphate (NADPH) oxidation using the procedure of Donahue et al. (1997) with some modifications. Leaf tissue (0.1 g) was ground to a fine powder in liquid nitrogen and then homogenized in 0.4 ml of 50 mM phosphate buffer (pH 7.0), 1 mM ethylene diamine tetraacetic acid (EDTA), 0.05% triton 2% polyvinylpoly pyrrolidone (PVP), and 1 mM ascorbic acid. The homogenate was centrifuged at 17000×g for 20 min, and the supernatant solution that contained 0.1 M Tris buffer (pH 7.8) 2 mM EDTA, 50 µM NADPH, 0.5 mM oxidized glutathione (GSSG) and 20 µl of the extract. The assays were initiated by the addition of NADPH for 5 min at 25°C. The oxidation reaction for measuring GR activity was followed by monitoring the absorbance at 340 nm using a Beckman-DU 530 UV-Vis spectrophotometer. The concentration of GR was calculated using an extinction coefficient of 6.2 $mM^{-1}cm^{-1}$. GR activity was expressed as $µg^{-1}$ FW (Fresh weight). (One unit of GR is the amount of enzyme that oxidizes 1 mmol of NADPH min^{-1} under the assay conditions (Donahue et al., 1997).

Extracts for determination of SOD activity were prepared from 0.15 g of leaf discs homogenized under ice-cold conditions in 1.5 ml of extraction buffer, containing 25 mM phosphate buffer (pH 7.8), 0.5 mM EDTA, 0.5 g PVP and 0.025% (v/v) triton X-100 at 4°C. The homogenates were centrifuged at 10000×g for 20 min and the supernatant fraction was used for the assays (Laspina et al., 2005). Total SOD activity was assayed by the inhibition of the photochemical reduction of nitro blue tetrazolium (NBT), as described by Becana et al. (1986). The reaction mixture contained 50-150 µl of plant extract and 3.5 ml of $O_2^{\bullet-}$ generating solution, which contained 14.3 mM methionine, 82.5 µM NBT, and 2.2 µM riboflavin. Extracts were brought to a final volume of 0.3 ml with 50 µM potassium phosphate (pH 7.8) and 0.1 mM EDTA. Test tubes were shaken and placed at 30 cm from a light bank consisting of six 15 W fluorescent lamps. The reaction was allowed to run for 10 min and stopped by switching the light off. The amount of reduction in NBT was measured at 560 nm in the spectrophotometer. Blanks and controls were run in the same way but without illumination and

Table 1. Safflower parental genotypes and their crosses and reciprocals used in this study.

Entry	Parents		Origin
1	GE_{62918}	P_1	Germany
2	C_{111}	P_2	Selected from Kouseh landrace
3	C_{4110}	P_3	Selected from Kouseh landrace
4	Esf_{-14}	P_4	Selected from Isfahan landrace
5	A_2	P_5	Selected from Azarbayejan landrace
6	K_{21}	P_6	Selected from Kordestan landrace
7	II.111	P_7	Selected from Auroumieh landrace
8	22-191	P_8	Mexico
Entry		**F_1 hybrids**	**Cross type**
9		$(P_1 \times P_2)$	direct
10		$(P_2 \times P_1)$	reciprocal
11		$(P_1 \times P_6)$	direct
12		$(P_6 \times P_1)$	reciprocal
13		$(P_2 \times P_6)$	direct
14		$(P_6 \times P_2)$	reciprocal
15		$(P_2 \times P_8)$	direct
16		$(P_8 \times P_2)$	reciprocal
17		$(P_8 \times P_1)$	direct
18		$(P_1 \times P_8)$	reciprocal
19		$(P_6 \times P_8)$	direct
20		$(P_8 \times P_6)$	Reciprocal

enzyme, respectively. One unit of SOD is the amount of the enzyme that inhibits the reduction of NBT by 50% under the assay conditions.

Ascorbate peroxidase activity (APX) was measured in fresh extracts and was assayed as described by Nakano and Asada (1987). The reaction mixture contains 50 mM ascorbate and 0.1 mM EDTA. The oxidation reaction for measuring glutathione dehydrogenase (ascorbate) activity was followed by monitoring the absorbance at 290 nm using a Beckman-DU 530 UV-Vis spectrophotometer. The concentration of APX was calculated by using an extinction coefficient (ε :2.8 $mM^{-1}cm^{-1}$). One unit of APX forms 1 nmol of ascorbate oxidized per min under the assay conditions (Nakano and Asada, 1987).

The level of lipid peroxidation was measured in terms of thiobarbituric acid-reactive substances (TBARS) contents (29). Leaf samples of 0.5 g were homogenized in 10 ml of 0.1% trichloroactic acid (TCA). The homogenate was centrifuged at 15000 ×g for 5 min. 4 ml of 0.5% thiobarbituric acid in 20% TCA was added to 2 ml of aliquote of the supernatant. The mixture was heated at 95°C for 30 min and then quickly cooled in ice bath. After centrifugation at 10000 ×g for 10 min remove suspended turbidity, the absorbance of supernatant was measured at 532 nm using a Beckman-DU 530 UV-Vis spectrophotometer. The value for non- specific absorption at 600 nm was subtracted from the first value. Concentration of lipid peroxides was quantified as total TBARS in terms of (nmol g FW^{-1}) by using an extinction coefficient of 155 $m\mu^{-1}cm^{-1}$ (Sairam et al., 2002).

Chlorophyll and carotenoid contents

Chl a+b and total carotenoid contents were estimated by extraction the leaf material in 80% acetone according to Lichtenthaler et al. (1987). The optical density measured by the absorption A at 661.6, 644.8 and 470 nm and then calculated with the equations of the pigment amount in per mg per ml extract solution.

Concentration of chl a: $C_a = 11.24 \times A661.6 - 2.04 \times A664.8$
Concentration of chl b: $C_b = 20.13 \times A644.8 - 4.19 \times A661.6$
Concentration of $C_{a+b} = 4.0 \times A661.6 + 18.09 \times A644.8$
Concentration of carotenoids: $Cx+c = 1000 \times A470 - 1.90\ C_a \times -63.14\ C_b/214$

The following formulas were used for estimation of heterosis (Singh et al., 2004).

Heterosis over mid parent (H%) = ((F_1 –MP) / MP × 100)
Heterosis over superior parent (HB%) = ((F_1 –BP) /BP × 100)

STATISTICAL ANALYSES

Data were subjected to analysis of variance (ANOVA) using General linear model (GLM) of SAS program (SAS Institute. 1997). The experiment was carried out by using a completely randomized design (CRD) with three replications for chlorophyll and 2 replications for antioxidant treatments. Mean comparisons of the studied variables and heterosis were conducted using Fisher's least significant difference (LSD) test.

RESULTS

Antioxidants

Analysis of variance showed significant differences among genotypes for all type of antioxidants (Table 2).

Table 2. Analysis of variance for antioxidants in safflower genotypes and their F_1 hybrids.

		Mean square				
Source of variation	**df**	**GR**	**SOD**	**APX**	**LP(TBARS)**	**Carotenoids**
Genotypes	19	0.017*	0.304**	0.051**	1.570**	5.76*
Lines	7	0.012	0.25**	0.038**	3.52**	10.09**
Hybrids	11	0.021*	0.35**	0.055**	0.14	3.49
Lines vs. hybrids	1	0.002	0.03**	0.089**	0.03	0.3
Residual	20	0.009	0.006	0.011	.147	3.08

* and ** significant at 0.05 and 0.01 of probability levels, respectively.

Table 3. Mean comparison of antioxidant activity in parents and their crosses in safflower.

Genotype	GR ($ug^{-1}FW$)	SOD ($ug^{-1}FW$)	APX ($umg^{-1}protein$)	LP ($nmolg^{-1}FW$)	Carotenoids (mg/ml)
1	0.46^{ab}	0.41^{b}	2.00^{a-d}	1.50^{bc}	6.82^{a}
2	0.46^{ab}	0.37^{bcd}	2.19^{ab}	1.72^{bc}	2.90^{bcd}
3	0.42^{ab}	1.04^{a}	2.17^{abc}	5.06^{a}	1.67^{cd}
4	0.44^{ab}	0.44^{b}	2.09^{a-d}	1.00^{cd}	4.43^{a-d}
5	0.32^{abcd}	0.95^{a}	1.85^{de}	0.40^{d}	1.39^{d}
6	0.45^{ab}	0.90^{a}	1.90^{b-e}	1.62^{bc}	4.43^{a-d}
7	0.29^{bcd}	0.90^{a}	1.90^{b-e}	1.04^{cd}	4.79^{a-d}
8	0.53^{a}	0.17^{de}	2.29^{a}	1.27^{bcd}	5.15^{abc}
9	0.40^{ab}	0.13^{e}	2.00^{a-d}	1.53^{bc}	5.74^{ab}
10	0.38^{ab}	0.90^{a}	1.80^{de}	1.67^{bc}	3.92^{a-d}
11	0.47^{ab}	0.91^{a}	2.22^{a}	1.44^{bcd}	3.87^{a-d}
12	0.39^{ab}	0.16^{de}	1.66^{e}	1.30^{bcd}	2.77^{bcd}
13	0.53^{ab}	0.92^{a}	1.9^{b-e}	1.85^{bc}	2.72^{bcd}
14	0.40^{ab}	0.12^{e}	2.06^{a-d}	1.90^{bc}	4.19^{a-d}
15	0.35^{ab}	0.14^{e}	1.9^{b-e}	1.27^{bcd}	4.79^{a-d}
16	0.43^{ab}	0.90^{a}	1.90^{b-e}	1.47^{bcd}	2.85^{bcd}
17	0.48^{ab}	0.80^{a}	2.20^{a}	2.17^{b}	3.72^{a-d}
18	0.39^{ab}	0.39^{bc}	2.04^{a-d}	1.55^{bc}	4.03^{a-d}
19	0.30^{ab}	1.04^{a}	1.89^{cde}	1.41^{bcd}	3.08^{bcd}
20	0.59^{a}	0.11^{e}	2.00^{a-d}	1.41^{bcd}	5.93^{ab}

Means with a common letter within each column not significantly differ at LSD 1%.

The variance of genotypes was partitioned to lines, hybrids and lines versus hybrids (Table 2). For GR activity, hybrids were differed significantly. Parental lines and their F_1 hybrids showed significant differences for SOD and APX (Table 2).

Lines differed significantly ($P < 0.01$) for antioxidant activity with exception of GR. Lines varied from their hybrids only for SOD and APX. Heterosis effect was significant for 10 (54.40**), 16 (143.00**) and 17 (95.00**) genotypes related to the superior parent and for 11 (40.00**), 13 (31.00**), and 19 (15.50**) genotypes related to the mean of parents for SOD activity, while their reciprocal crosses did not show any significant heterosis (Table 7). There was a significant difference in reciprocal crosses for, SOD, APX and carotenoid activity that could be related to cytoplasmic effects (Table 2).The results of mean comparisons of antioxidant activity in parents and their crosses were presented in Table 3. Means of GR activity for genotypes varied between 0.29 and 0.59 ug^{-1} FW, respectively. GR means grouped the genotypes into four different groups. Among parental lines, 22-191 had the highest GR activity. The SOD activity varied between 0.11 ($P_8 \times P_6$) to 1.04 (C_{4110}) $ug^{-1}FW$. The SOD means grouped the genotypes into six different groups (Table 3). APX and LP had higher enzymatic activity than GR and SOD. The highest and lowest amount of APX activity was 2.29 and 1.66 (umg^{-1}) ascorbate oxidized, respectively. The APX mean grouped the genotypes into eight different groups and showed the highest genetic variation among the antioxidants. The highest APX content belonged to

Table 4. Analysis of variance of chlorophyll content in safflower.

Source of variation	df	Mean square		
		Chl a	Chl b	Chl a+b
Genotypes	19	262.93**	94.431**	368.43**
Lines	7	209.40	93.93	424.35**
Lines vs. hybrids	1	664.78**	11.23	32.8
Hybrids	11	260.45**	102.30**	363.35*
Residual	40	125.181	43.349	176.64

* and ** significant at 0.05 and 0.01 of probability levels, respectively

Table 5. Mean comparisons of safflower chlorophyll (mg/ml).

Genotype	Chl a	Chl b	Chl a+b
1	22.10^{bcd}	13.06^{a-e}	35.26_{abc}
2	6.11_{de}	2.27^{df}	8.42_{def}
3	19.31^{b-e}	16.35^{abc}	35.75^{abc}
4	24.07^{bcd}	7.23^{c-f}	22.17^{a-f}
5	3.54^{e}	0.72^{f}	2.73^{f}
6	13.37^{-b}	13.37^{a-d}	29.26^{a-d}
7	8.49^{b-f}	8.49^{def}	25.83^{a-e}
8	26.77^{abc}	4.981^{def}	20.09^{b-f}
9	17.10^{b-e}	6.84^{c-f}	23.89^{a-f}
10	20.26^{b-e}	19.63^{a}	40.37^{ab}
11	19.75^{b-e}	3.63^{def}	14.77^{c-f}
12	15.81^{b-e}	3.62^{def}	12.57^{def}
13	21.14^{b-e}	4.03^{def}	15.97^{c-f}
14	26.83^{abc}	7.43^{c-f}	20.68^{b-f}
15	23.10^{bcd}	4.98^{def}	18.03^{c-f}
16	12.69^{b-e}	3.64^{def}	12.91^{def}
17	$13. 60^{b-e}$	9.68^{a-f}	23.34^{a-f}
18	12.99^{b-e}	5.42^{def}	18.47^{b-f}
19	9.05^{cde}	1.63^{f}	6.75^{ef}
20	44.73^{a}	18.49^{ab}	43.9^{a}

Means with a common letter within each column not significantly differ at LSD 5%.

22-191 line. The maximum activity of LP was related to C_{4110} genotype, and the lowest one belonged for A_2line. The LP means grouped the genotypes into six different groups. Carotenoids had a high genetic variation among tested antioxidants. The mean grouped in to seven different groups. Carotenoid content varied from 6.821 mg/ml in GE-62918 to 1.390 mg/ml in A_2 genotype. A_2 line possessed the lowest mean of carotenoids (Table 3). According to Table 3, F_1 ($P_8 \times P_6$) showed a significant difference in reciprocal crosses for GR, that it may represents a significant cytoplasmic effect in this cross for this antioxidant. A positive and significant correlation ($r=0.55^*$) was observed between GR and APX (Table 6).

Chlorophyll

Analysis of variance showed significant differences among genotypes for chlorophyll content (Table 4). Parental lines significantly differed for only Chl a+b, while F_1 hybrids varied significantly for all type of chlorophylls (Table 4). The result of parental lines compared to their F_1 hybrids indicated a significant difference for Chla content. For chlorophyll content, the heterosis effect was only significant in 20 ($122.86.00^*$) and 10 (156.20^*) genotypes for Chl a and Chl b, respectively in safflower genotypes (Table 7). Mean comparisons of genotypes for Chl a and Chl a+b indicated that ($P_8 \times P_6$) F_1 hybrids and A_2 (P_5) had the highest and lowest of both chl a and chl a+b contents, respectively (Table 5). The content of chl b was lower than chl a in all genotypes. There was more variation among genotypes for chl b than chl a (Table 5). Chlorophyll a+b content had a similar trend as Chl a in the genotypes. For all type of chlorophyll content only a reciprocal cross ($P_8 \times P_6$) showed a significant difference

Table 6. Correlation coefficients of antioxidants and chlorophyll contents.

Trait	GR	SOD	APX	LP (TBARS)	Carotenoids	Chl a	Chl b	Chl a+b
GR	1.00	-0.07	0.55**	0.03	0.09	0.40	0.03	-0.41
SOD	-0.07	1.00	0.08	0.07	0.03	0.43	0.23	-0.19
APX	0.55**	0.08	1.00	0.28	0.04	0.43	0.24	-0.24
LP(TBARS)	-0.03	0.07	0.28	1.00	0.13	0.13	0.26	-0.25
Carotenoids	-0.09	-0.03	0.04	0.13	1.00	0.54*	0.37.	0.54*
Chl a	-0.40	-0.43	-0.43	-0.13	0.03	1.00	0.52*	0.62**
Chl b	0.03	0.23	0.24	0.26	0.07	0.52*	1.00	0.96**
Chl a+b	-0.41	-0.19	-0.24	-0.25	0.28	0.62*	0.96**	1.00

* and ** significant at 0.05 and 0.01 of probability levels, respectively

Table 7. Estimation of heterosis (H) and heterobeltiosis (HB) for antioxidants and chlorophyll content in safflower genotypes.

No	Cross	Chl a+b		Chl b		Chl a		Crotenoids		LP (TBARS)		APX		SOD		GR	
		HB	H	HB	H	HB	H	HB	H	HB	H	HB	H	HB	H	HB	H
9	$P_2 \times P_1$	-32.2	9.38	-47.6	-10.7	-22.6	21.2	-15.8	18.1	-11.0	-7.4	1.3	5.7	-68.2	-66.6	-13.0	-9.0
10	$P_1 \times P_2$	14.4	84.8	50.3	156.2*	-8.32	1.13	-15.8	18.1	-2.9	3.7	-17.8	-13.8	54.4**	56.6**	-0.13	-7.0
11	$P_6 \times P_1$	-41.0	-93.2	-74.5	-72.5	28.8	11.3	-43.2	-31.1	-11.1	-7.6	-5	13.8	1.00	40.0**	2.1	27.0
12	$P_1 \times P_6$	-64.3	-104.9	-72.9	-72.5	-28.4	-10.8	-59.3	-50.7	-19.7	-16.6	-5	-2.56	-82.2	-75.3	-15.21	5.4
13	$P_6 \times P_2$	-45.4	-15.2	-69.8	-49.0	58.1	117.0	-10.5	-25.6	7.55	10.7	-5.93	0.98	2.2	31.0**	26.1	48.5
14	$P_2 \times P_6$	-29.3	9.7	-44.4	-4.9	100	75.4	-5.4	14.2	10.4	13.7	-13.2	-6.86	-13.3	-80.9	-4.7	14.2
15	$P_8 \times P_2$	-10.2	26.4	0.0	37.5	-13.7	40.5	-6.9	18.9	-26.8	-14.7	-17.0	-15.17	-61.1	-48.1	-33.9	-25.5
16	$P_2 \times P_8$	-35.7	-9.4	-26.9	.55	-52.6	-22.8	-44.6	-29.1	-14.5	-1.16	-3.93	-1.78	143**	233**	-18.8	-8.5
17	$P_8 \times P_1$	-33.8	-12.1	-25.8	7.7	-49.1	-44.3	-4.54	-37.7	48.5	56.5	-3.93	2.33	95**	175.8**	-9.4	-2.0
18	$P_1 \times P_8$	47.6	-33.2	-58.4	-39.3	-51.4	-46.8	-40.9	-32.6	3.3	11.5	-10.9	-4.67	-.04	34.4	-26.4	-20.4
19	$P_6 \times P_8$	-7.0	-6.9	-87.8	-82.2	-66.1	-54.9	-40.1	-35.6	-12.9	-2.43	-17.4	-9.56	15.5	96.2**	-43.3	-38.7
20	$P_8 \times P_6$	2.6	4.6	38.2	101.5	67.0	122.8*	15.4	23.7	-2.4	-2.43	-12.6	-4.30	-87.7	-79.24	11.3	-20.4

including the significant effects of cytoplasm in the inheritance of chlorophyll contents. A highly significant and positive correlation (r = 0.629**) was found between Chl a and Chl a+b content and Chl a and carotenoid content (r = 0.547*) (Table 6). Also a highly significant positive correlation was found between chlorophyll a and Chl b content (0.524*) and between Chl b and Chl a+b (r = 0.967**) content (Table 6).

DISCUSSION

Plants have evolved a complex antioxidant system to avoid the harmful effects of ROS (Arnon and Sairam, 2002; Sharma and Dubey, 2005; Gambarova and Gins, 2008). Antioxidants like SOD, APX and GR have a protective role in plant cells (Arnon and Sairam, 2002; Asada, 1992; Blokhina et al., 2003). It seems that the activities of these antioxidants in the safflower genotypes

have not been appeared in the literature, yet. The results of present study for genetic diversity for antioxidant activities in safflower are in agreement with those of previous workers in other plants (Tosun et al., 2009; Hakiman et al., 2009; Ercisli et al., 2007; Ercisli et al., 2007; Rupasinghe et al., 2006; Cardador-Martinez et al., 2002; Kurilich et al., 1999; Spychall et al., 1990). This variation was more significant for APX and LP content rather than GR and SOD (Table 3). The least variation among genotypes was observed for GR activity. It may represent that these genotypes have similar content of GR activity. The highest mean for GR activity was related to a F_1 hybrid (22-191×K_{21}) (Table 3). SOD activity showed more variation than GR. APX and GR with GR considering a positive significant correlation between GR and APX (0.55**). This may be due to the similar intra-cellular pathways for these two antioxidants. Accordingly, Kurilich et al. (1999) reported that the significant and positive correlation between ß- carotene and α -tocopherol was the result of similar pathways in their synthesis. Furthermore, positive cooperation between GR and APX activity in Asada-Halliwell scavenging cycles could be as a result of the positive correlation between these anti-oxidants contents (Asada, 1992; Blokhina et al., 2003). The genotypic variation for APX was higher than GR and SOD. The highest and lowest content of LP (TBARS) was related to c_{4110} and K_{21} genotypes, respectively. The majority of F_1 hybrids have a higher GR and APX activities than their parents. The trend of contents of SOD, APX and GR was in agreement with that of Choi et al., (2004). The results of genetic variation in safflower for antioxidant activities was consistent with that of Hakiman et al. (2009) who found genetic variation for ascorbate oxidase, ascorbate peroxidase, and non- enzymatic antioxidants in *F. deltoidea* accessions undernormal conditions. Also, Rupasinghe et al. (2006) found higher content of phenols and antioxidant content in some crosses of plum genotypes, rather than their parental lines. In the most cases the F_1 hybrids possessed the intermediate activity for SOD, carotenoids and LP when compared to those of their parents. This result could be attributed to the additive genetic effects that governing these antioxidants. In a comparison among parental lines, Iranian genotypes have a higher activity of LP and SOD, while the highest activity GR and APX was related to 22-191, a spiny Mexican line (Table 3). This result could be described to a positive relation between the presence of spine and antioxidant activity in safflower. As anticipated, this line is also highly resistance to environ-mental stresses and diseases. The highest genetic variation was observed for carotenoid content and highest content of carotenoids was related to GE-62918 genotypes possessing three different colors of flowers in a plant (Table 3). Similar to GR and APX activity, the highest content for Chl a and Chl a+b was related to F_1 hybrid of 22-191×k_{21} (Table 5). The highest and the least genetic variation was related to Chl a+b and Chl a, respectively (Table 5). According to the results, most of the hybrid mean were intermediate of their parents mean for Chl and carotenoid contents. The significant variation for Chl a, Chl b and carotenoid content has also been reported by other researchers (Johnson et al., 1993; Kurilich et al., 1999). The positive relationship between Chl a and Chl b contents was in agreement with previous report in another species (Johnson et al. 1993). Except for 22-191×k21, other reciprocal crosses have not significantly differed for Chl a, Chl b and Chl a+b. A_2 parental line having the lowest mean of Chl and carotenoid contents, had the lowest yield as well (Table 5). This result was in agreement with that of Carter and Knapp (2001) who reported a positive relationship between Chl and carotenoid contents and plant yield (9). In agreement with the report of Johnson et al. (1993) report, there was a significant correlation between chloro-phyll content and caroteniod content in safflower. In conclusion, employing superior crosses for higher activity of antioxidants and Chl may be suggested in safflower breeding programs. This study was the first step to exploiting the breeding ways to enhance the potential contents of antioxidants and chlorophyll, via crossing between suitable parents and using superior F_1 crosses.

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