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Microsatellite Markers Variation and Seed Oil Composition of Some Safflower Genotypes Differing in Salt Tolerance

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Genetic variation of some safflower genotypes differing in salinity tolerance were analyzed using microsatellite markers, seed oil, and fatty acid composition. Molecular analysis revealed high polymorphism among the genotypes. Cluster analyses based on the Jaccard's similarity coefficient separated the safflower genotypes into two distinct groups and the physiological characters of these two groups were significantly different under salt stress conditions. Analysis of variance for seed oil content and fatty acid composition revealed significant differences among the genotypes. The salinity tolerant genotype, PI-506426, had the highest seed oil content and percentage of linoleic acid in its oil. These results indicated that high genetic variation observed among safflower genotypes could be useful for improving the salinity tolerance of safflower genotypes with high quantity and quality of seed oil. The potential of the microsatellite markers for marker assisted selection could be tested by intercrossing the genotypes from diverse groups separated by microsatellite data with different salinity tolerance ability.

Key words: *Carthamus tinctorius* L., Fatty acid composition, Genetic diversity, Safflower, Salinity, SSR markers.

Safflower ($2n = 2x = 24$) is an ancient crop commonly cultivated in the warm, dry, saline conditions of the Fertile Crescent.This crop has been traditionally used not only as a source of fabric dye, food coloring, and flavoring, but also for medicinal purposes (Weiss 1971). Safflower is currently grown as an oilseed crop (Knowles 1989) for producing cooking oil, salad oil, and margarine (Mündel and Bergman 2009, Weiss 1971). The high polyunsaturated fatty acid content of safflower oil makes it nutritionally valuable (Ascherio and Willett 1997). The high linoleic acid content (70%) is a unique characteristic of safflower oil among other oilseed crops. Additionally, some safflower genotypes with a high oleic acid content have been identified and used for food industries (Hamdan *et al.* 2009). High oleic and linoleic acid oils are valuable for food and non-food applications because they have a hypocholesterolemic effect as well as nutraceutical applications with high oxidative stability (Koyama *et al.* 2006, Moon *et al.* 2001, Nykiforuk *et al.* 2011).

Crop productivity losses due to salinity stress, therefore, it is very useful develop salt tolerant cultivars with higher yield and oil quality under saline conditions (Arzani 2008, Rahnama *et al.* 2011, Yuldasheva *et al.* 2011). To develop high yielding cultivars, the genetic diversity for salinity tolerance in the germplasm of a crop species should be evaluated and exploited as an opportunity for selecting and developing genetically salt tolerant cultivars that help to have a sustainable crop production (Ashraf and Harris 2004, Munns and Tester 2008).

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Environmental stress has a profound effect on fatty acid desaturase activity and fatty acid composition, which function as key factors in plant survival under stressful conditions. Flagella et al.(2004) reported that seed oil content of high oleic hybrid sunflower (*Helianthus annuus* L.) decreased under salt stress, and increasing salinity stress increased the oleic acid content of the oil, but decreased the linoleic acid content. Irving *et al*.(1988) found that salinity stress led to decreased oleic acid content in a high oleate safflower cultivar while the fatty acid composition of high linoleate safflower was unaltered. Yeilaghi *et al*.(2012) demonstrated that salinity stress resulted in an increase in the oleic acid content of safflower oil and a decrease in linoleic acid content, though the oil composition in the salt tolerant genotypes was less affected than that of salt sensitive genotypes. These findings indicate that seed oil content and oil composition are affected by both genetic and environmental factors.

In plant breeding programs, assessment of genetic variation is the basic step toward the selection and genetic improvement of crop species (Ramanatha and Hodgkin 2002). Morphological, biochemical, and molecular data are most commonly used for analyzing genetic variation in different crops. These markers offer the opportunity for precise evaluation of the genetic resources and genetic variation for improving economically important traits (Collard and Mackill 2008, Golkar *et al.* 2011, Khan *et al.* 2009).

Randomly amplified polymorphic DNA (RAPD), inter-simple sequence repeat (ISSR), amplified fragment length polymorphism (AFLP), and simple sequence repeats (SSRs) markers have been employed to assess genetic diversity in different wild and cultivated safflower accessions (Amini *et al.* 2008, Barati and Arzani 2012, Chapman *et al.* 2009, Golkar *et al.* 2011, Johnson *et al.* 2007, Khan *et al.* 2009, Sehgal *et al.* 2009). Microsatellites (simple sequence repeats, SSRs) have better capability for discriminating individuals, varieties, and genotypes because of their polymorphism, codominant inheritance, and reproducibility (Henry 2001). Chapman *et al*.(2009) published the first set of SSR markers based on the safflower EST collection and successfully used 104 gene-based SSR markers to screen polymorphism in the genus *Carthamus*. In another study, five EST-SSR markers were generated for distinguishing safflower hybrids (Naresh *et al.* 2009). Hamdan *et al*.(2011) developed 108 genomic SSRs markers for safflower from an SSR-enriched library that are useful for the assessing the genetic diversity of this species.

Safflower is considered as a moderately salt tolerant oilseed crop. However, different studies have shown that there is considerable genetic variation for salinity tolerance among safflower genotypes at different growth stages that can be related to certain physiological and biochemical processes (Bassil and Kaffka 2002, Harrathi *et al.* 2012, Siddiqi *et al.* 2011, Yeilaghi *et al.* 2012). Although several molecular markers, as well as, agro-morphological and seed quality related traits have been used for assessing genetic diversity in safflower, (Amini *et al.* 2008, Golkar *et al.* 2011, Khan *et al.* 2009, Sabzalian *et al.* 2009) few, if any, studies have been conducted on the relationship between salinity tolerance and genetic diversity in terms of molecular markers. Therefore, the objectives of the present study were: 1) to evaluate the genetic variation based on microsatellite markers, oil content, and fatty acid composition in a subset of safflower genotypes with different levels of salinity tolerance and 2) to assess the association between salinity tolerance and molecular and biochemical markers.

MATERIALS AND METHODS

Eight genotypes (4 salt tolerant and 4 salt sensitive ones) were selected based on their seed and oil yield under saline and non-saline conditions in field experiments over two growing seasons in 2008-2009 (Yeilaghi *et al.* 2012), as well as based on their evaluation for stress tolerance (salt tolerance index), leaf Na^+ content, and leaf $K^+/Na^+$and Ca^{2+}/Na^+ ratios under extreme salt stress conditions (200mM) in a greenhouse experiment (Karimi *et al.* 2014)(Table 1).

In order to analyze seed oil and fatty acid composition of oil, a field experiment was conducted at the research farm of Isfahan University of Technology, located at Lavark, Iran (32° 32¹ N and 51° 32¹ E, 1,630 m asl) during growing season of 2012 with average annual precipitation of 149 mm and temperature of 15.4 °C. The soil type of the research farm is Typic Haplargids of the arid tropic with a texture of clay loam, pH=7.3-

7.8, electrical conductivity (EC_e) = 1.3-1.7 dsm^{-1} and 1% of organic matter. The genotypes were evaluated in a completely randomized design with three replications. Each plot consisted of three rows, 4 m long, spaced 50 cm apart. The within-row plant spacing was 10 cm. The plants were hand harvested to use the seeds for analyzing oil composition.

Genomic DNA was extracted from fresh leaves following the cetyltrimethyl-ammonium bromide (CTAB) procedure described in Murray & Thompson (1980) with some minor modifications. DNA was quantified using an agarose gel (0.7%) in 1× TAE buffer against a 100 bp DNA ladder.

Genetic diversity was determined using 104 EST-SSR primers released by Chapman *et al*. (2009) (prefixes CT1 and EL), 5 EST-SSR primers reported by Naresh *et al.* (2009) (prefix SES), and 59 polymorphic SSR primers developed by Hamdan . (2011) (prefix CAT).

Polymerase chain reactions (PCR) were performed in a total volume of 15 *µl* containing 50 ng of total genomic DNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 0.5 mM dNTPs, 0.4 µM of each primer, and 0.5 U*Taq* DNA polymerase. Touchdown PCR amplifications were performed as follows: initial denaturation for 3 min at 94°C followed by 10 cycles at 94°C for 30 s, 65°C for 30 s (annealing temperature was reduced by 1° per cycle), and 72°C for 45 s; 35 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s; and a final extension time of 20 min at 72° C.

PCR amplicons were separated using 12% non-denaturing polyacrylamide gels (w/v) (Atto, Tokyo, Japan) and stained with $AgNO_3$ ($AgNO_3$ 1% + 10 mL 1.5× formaldehyde 37%) for visual detection (Bassam *et al.* 1991).

The seeds were dried at 60°C for 5h, using ventilated oven and then were ground with blender. Ten grams of ground seed were used to extract the oil, using petroleum ether for 6 h in a Soxhelet system according to the AOCS method (AOCS 1993).

The fatty acid composition of eight seed samples was determined and their average was used for each plot of the genotypes. The analysis of fatty acid composition was performed based on simultaneous extraction and methylation of the fatty acids (Garcés and Mancha 1993, Velasco and Fernandez-Martinez 2001) followed by gas–liquid chromatography (GLC) using a Perkin-Elmer Autosystem gas–liquid chromatograph (Perkin-Elmer Corporation, Norwalk, CT, USA). A 2-m-long column packed with 3% SP-2310/2% SP-2300 on Chromosorb WAW (Supelco Inc., Bellefonte, PA, USA) was used. The oven, injector, and flame ionization detector were held at 198°, 275°, and 250°C, respectively.

Statistical analysis was carried out using SAS V.9 (SAS Institute Inc., Cary, USA). Analysis of variance (ANOVA) was performed using PROC GLM (General Linear Model) based on a completely randomized design with 3 replications for oil content and fatty acid composition data. Mean comparisons were conducted using the Fisher's least significant difference (LSD) test at the 0.05 level of probability.

Allele number, polymorphism information content (PIC), gene diversity (or expected heterozygosity), and observed heterozygosity were calculated using the Powermarker 3.25 software (Liu and Muse 2005). Presence(1) or absence (0) of an SSR allele for each microsatellite was recorded visually, and the data were converted to a similarity matrix using the Jaccard's coefficient of similarity(Anderberg 1973). Average seed oil content and fatty acid composition were used to compute a dissimilarity matrix of the genotypes based on Euclidean distances. The similarity and dissimilarity matrices were used for cluster analysis of the genotypes and dendrograms were constructed according to the unweighted pair group method with arithmetic means (UPGMA), using the NTSYS-pc V2.1 statistical package (Rohlf 2002). To explain more precisely the variance observed among the data, principal coordinate analysis (PCoA) was carried out to transform the multidimensional genetic distances between the genotypes into a two-dimensional representation. The degree of association between matrices was calculated using the Mantel test (Mantel 1967).

RESULTS AND DISCUSSION

In this study, eight safflower genotypes were screened with 168 microsatellite markers; 80.9% of the 345 bands generated were polymorphic (Table 2). Gene diversity (expected heterozygosity) of individual microsatellites varied from 0.12 (for EL-383136 and CAT-103) to 0.78 (for EL-377435). The number of alleles identified for

each microsatellite varied from 2 (28% of the microsatellite markers) to 5 (for EL-383136, a gene-based marker) with a mean value of 2.05 over all the microsatellite loci. The average value of polymorphism information content (PIC) of the polymorphic microsatellite markers was 0.25 and it ranged from 0.11 for genomic marker CAT-102 to 0.75 for gene-based marker of EL-383136. Mean gene diversity of microsatellite markers was 0.29 and the mean value of the observed heterozygosity was 0.03. Chapman *et al*. (2009) observed a gene diversity of 0.54 for *Carthamus* species, and Barati & Arzani (2012) reported a gene diversity value of 0.37 for their collection of cultivated and wild genotypes of safflower. The overall level of genetic diversity observed in this study was lower than the other estimates of microsatellite diversity reported for *Carthamus*, which is not unexpected as exotic safflower genotype is constantly introduced to Iran in breeding programs may have led to reduced genetic diversity. Despite the relatively high molecular polymorphism, a low level of heterozygosity was observed, which is probably due to the high degree of self-fertility in safflower and artificial selection by breeders.

The greatest genetic similarity coefficient was observed between C411 and Arak (0.62), while the lowest similarity (0.46) was detected between the paired genotypes of PI-506426 and Kurdistan 6, PI-506426 and 307-S6-697, and PI-405985 and C411 (Table 3).

The dendrogram based on genetic similarities did not completely agree with the reported geographical origins of each accession (Table 1). Chapman *et al* (2010) proposed that safflower has a single origin somewhere west of the Fertile Crescent followed by subsequent expansion into Europe, Asia and Africa. Thus the reasons for the poor agreement might be the genetic overlap among the safflower genotypes from different regions and the exchange of germplasm among regions.

The microsatellite markers used in this study classified the selected salt tolerant and salt sensitive genotypes into two clusters, C1 and C2(Fig. 1). Cluster C1 included four salt tolerant genotypes and one salt sensitive genotype, C411. Means of the physiological and biochemical characters were calculated for the clusters separated by microsatellite markers (Table 4). Leaf Na^+ content and Ca^{2+}/Na^+ ratio were significantly different (P=0.05) between C1 and C2 clusters. Higher Na^+ content and lower Ca^{2+}/Na^+ ratio in genotypes of cluster C2 were consistent with their salt sensitivity. Pakniyat *et al.* (1997) found that AFLP markers were significantly associated with

Table 1. Safflower genotypes and their geographical origin, salinity tolerance index (STI), leaf Na^+ content, leaf K^+/Na^+ and Ca^{2+}/Na^+ based on Karimi *et al.* (2014).

No	Genotype[a]	Origin	STI[b]	Leaf Na^+ (mg/g dw)	Leaf K^+/Na^+	Leaf Ca^{2+}/Na^+	Seed oil content (%)	Fatty acids composition (%)[c]			
								C16:0	C18:0	C18:1	C18:2
1	Kurdistan6(T)	Iran	1.19	7.93	0.41	1.80	30.40	6.37	2.21	18.04	73.91
2	Arak(T)	Iran	1.21	8.7	0.42	1.26	30.51	6.92	2.25	17.84	72.54
3	PI-301055(T)	Turkey	0.71	7.8	0.59	1.24	31.10	6.89	2.05	17.77	73.29
4	PI-506426(T)	China	0.76	8.52	0.78	1.56	32.73	5.93	2.21	14.69	77.72
5	PI-198844(S)	France	0.46	21.66	0.18	0.67	25.28	6.72	2.07	16.33	74.57
6	PI-405985(S)	Iran	0.62	15.97	0.25	0.85	27.43	7.07	1.99	19.50	72.29
7	307-S6-697(S)	Iran	0.66	13.24	0.37	1.08	29.95	6.67	2.18	18.69	72.98
8	C411(S)	Iran	0.50	15.19	0.30	1.02	26.08	6.55	1.70	17.16	75.23
	LSD(0.05)		0.24	2.51	0.08	0.15	3.03	0.32	0.16	1.95	1.96
Orthogonal contrasts[d]											
Tolerant vs. Sensitive			0.33^{*}	137.0^{**}	0.15^{*}	0.63^{**}	32.0^{**}	0.10^{ns}	0.076^{ns}	1.39^{ns}	0.71^{ns}

[a]T and S represent salt tolerant and salt sensitive genotypes, respectively.
[b] STI: salinity tolerance index
[c] Fatty acids: palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2)
[d] Means of two groups being contrasted
[ns] non-significant, * and ** significant at 5 and 1% level of probability, respectively

shoot Na^+ content and $\delta^{13}C$ in barley. Zeng *et al.* (2004) indicated that rice genotypes with diverse genetic background had different adaption to saline soils. They showed that there was a highly significant correlation between matrixes of genetic similarity based on microsatellite markers and taxonomic distances based on ion data. In another research on rice, SSR markers showed significant association with several traits under different salt stress conditions (Sakina *et al.* 2015).

Table 2. Mean values of the characteristics for the microsatellite primer pairs used in this study.

Markers Indexes	Microsatellite
Number of polymorphic assay units	100
Number of polymorphic bands	279
Number of monomorphic bands	66
Percent of polymorphism	80.9
Major allele frequency	0.78
Number of alleles	2.05
Expected heterozygosity (Gene diversity, H_e)	0.29
Observed heterozygosity (H_o)	0.03
Polymorphic information content (PIC)	0.25

The results of ANOVA revealed that the genotypes were significantly different for seed oil content and oil fatty acid composition (Table1). Seed oil content ranged from 25.28% in PI-198844 to 32.73% in PI-506426. Similar to previous reports (Johnson *et al.* 1999, Velasco and Fernandez-Martinez 2001), oleic and linoleic acids were the predominant fatty acids of the oil and each ranging from 14.69-19.50% and 72.29-77.72%, respectively. These results were in agreement with those reported by Yeilaghi *et al.* (2012). Linoleic acid content in seed oil of PI-506426 was the highest amount (77.72%) compared to the other genotypes. Palmitic, stearic, and oleic acids were the highest in PI-405985 (7.07%), Arak (2.25%), and PI-405985 (19.50%), respectively.

Cluster analysis for oil and fatty acid content classified the safflower genotypes into distinct groups with the Chinese accession (PI-506426) grouped farthest away from the rest of the genotypes (Fig.2). Clustering patterns based on oil and fatty acid composition did not correspond to classifications based on salinity tolerance or geographic origin. The largest Euclidean distance

Table 3. Genetic distances based on Euclidean distance of seed oil content and fatty acid composition (below diagonal), and Jaccard's similarity coefficient based on SSR data (above diagonal).

Genotype	Kurdistan6	Arak	PI-301055	PI-506426	PI-198844	PI-405985	307-S6-697	C411
Kurdistan6		0.52	0.54	0.46	0.54	0.52	0.49	0.47
Arak	1.50		0.57	0.55	0.51	0.49	0.50	0.62
PI-301055	1.12	0.97		0.58	0.51	0.50	0.49	0.61
PI-506426	5.60	6.53	5.72		0.51	0.49	0.46	0.56
PI-198844	5.45	5.82	6.13	8.30		0.55	0.51	0.48
PI-405985	3.75	3.52	4.18	9.06	4.48		0.50	0.46
307-S6-697	1.26	1.14	1.52	6.84	5.48	2.77		0.51
C411	4.63	5.27	5.44	7.56	1.39	4.04	4.76	

Table 4. Means of the clusters of the genotypes for leaf ion content under salt stress, seed oil content and oil fatty acid composition.

Cluster	Genotypes /cluster	STI	Leaf Na^+	Leaf K^+/Na^+	Leaf Ca^{2+}/Na^+	Seed oil content	Fatty acids composition (%) [c]			
							C16:0	C18:0	C18:1	C18:2
C1	4	0.87[a]	9.63[b]	0.50[a]	1.38[a]	30.16[a]	6.53[a]	2.08[a]	17.10[a]	74.54[a]
C2	3	0.58[a]	16.96[a]	0.27[a]	0.87[b]	27.55[a]	6.82[a]	2.08[a]	18.17[a]	73.28[a]

In each column, means followed by the same superscript were not significantly different at 5% level of probability, using the LSD test.

Table 5. Correlation coefficients among the biochemical and physiological traits of safflower genotypes.

	Oil content	Palmetic acid	Stearic acid	Oleic acid	Linoleic acid	STI	leaf Na^+	K^+/Na^+
Palmetic acid	-0.42	1						
Stearic acid	0.68	-0.20	1					
Oleic acid	-0.20	0.76*	-0.09	1				
Linoleic acid	0.18	-0.89**	-0.08	-0.92**	1			
STI	0.60	-0.08	0.66	0.15	-0.24	1		
leaf Na^+	-0.90**	0.30	-0.47	0.05	-0.06	-0.74*	1	
K^+/Na^+	0.89**	-0.60	0.41	-0.53	0.53	0.30	-0.81**	1
Ca^{2+}/Na^+	0.79*	-0.61	0.49	-0.22	0.30	0.74*	-0.87**	0.84**

* , ** Significant at the 0.05 and 0.01 level of probability, respectively

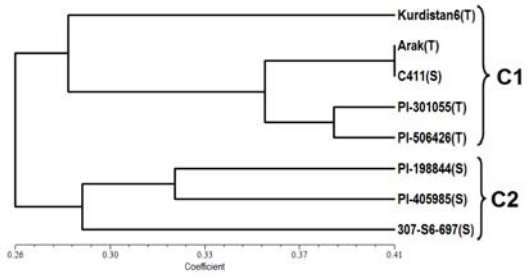

Fig. 1. Variation among eight safflower genotypes characterized by using a UPGMA dendrogram based on Jaccard's similarity coefficients calculated from microsatellite marker data. The letters of T and S represent salt tolerant and sensitive genotypes, respectively, based on the results of Karimi *et al.* (2014).

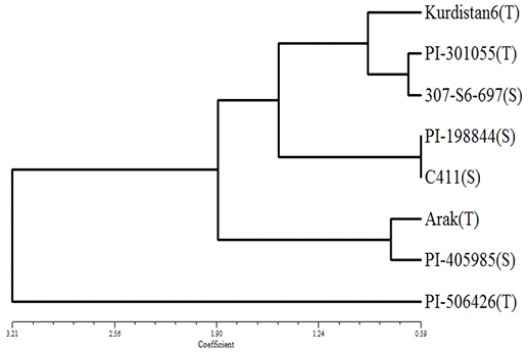

Fig. 2. The UPGMA dendrogram based on seed oil content and oil fatty acid composition by using the Euclidean distance coefficients. The letters of T and S represent salt tolerant and sensitive genotypes, respectively, based on the results of Karimi *et al.* (2014).

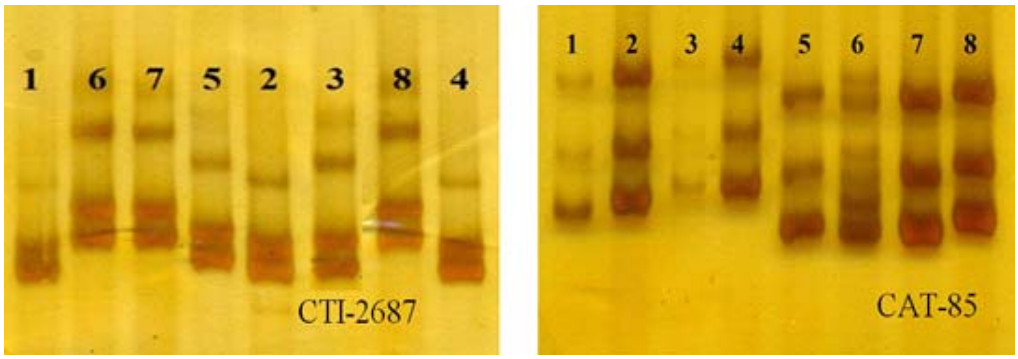

Fig. 3. The PCR amplification profile of microsatellite markers CTI-2687 and CAT-85 for eight safflower genotypes; the numbers correspond to the genotypes listed in Table 1

of 9.06 was found between PI-506426 and PI-405985 (Table 3). These results were in agreement with those of Khan et al. (2009) who reported that neither morphological diversity nor genetic diversity show associations with geographic origin. Previous phylogenic studies of different *Carthamus* species indicated that European and Mediterranean accessions were grouped together, while East Asian accessions were clusteredat the greatest distance (Chapman *et al.* 2010, Jaradat and Shahid 2006, Johnson *et al.* 1999, Khan *et al.* 2009).

Means of seed oil and fatty acid composition were calculated for two groups of salt tolerant and salt sensitive genotypes. Seed oil content in salt tolerant genotypes were significantly (P = 0.05) different from that of sensitive genotypes. The highest seed oil content, STI, leaf K^+/Na^+ and Ca^{2+}/Na^+ ratio and the lowest Na^+ content were observed in tolerant genotypes (Table 1).

The relationships among investigated traits showed significant and negative correlation (r = -0.90) between seed oil content and leaf Na^+ content (Table 5). Seed oil content were also highly correlated with both K^+/Na^+ and Ca^{2+}/Na^+ ratios in leaf tissues under salt stress.These results were in agreement with the findings of Yeilaghi *et al.* (2012). They found that there were strong and positive relationship between seed oil content and oil yield of safflower genotypes produced under non-saline and saline field conditions. They also concluded that seed oil content and its fatty acid composition of salt tolerant genotypes of safflower were less affected by salinity stress than salt sensitive ones. Siddiqi *et al.* (2011) also reported that salt tolerant line of safflower had higher leaf and root K^+,$K^+/Ca^{2+}$and $Ca^{2+}/Na^+$and seed yield, seed oil content, seed oil α-tocopherol and palmitic acid than the salt sensitive line.

Genetic improvement of salt tolerance via indirect selection and the use of molecular markers linked to the traits associated with salinity tolerance is a useful approach for minimizing the efforts of screening, for either direct selection in traditional breeding or indirect selection through QTLs (Yamaguchi and Blumwald 2005). Although microsatellite markers used in this study were not directly associated with salinity tolerance traits, they partially discriminate the salt tolerant genotypes from salt sensitive ones. Microsatellite markers CTI-2687, EL-380570, CAT-3, CAT-6, CAT-35, CAT-43, and CAT-85 separated salt tolerant from salt sensitive genotypes (Fig.3).

Relationship between Jaccard similarity index based on SSR markers and Euclidian distance based on ion contents and seed oil and fatty acid data were calculated by comparison between matrices. There was no significant correlation between SSR markers and biochemical parameters (r = 0.29). This finding was in agreement with those of previous studies conducted in safflower showing that non-significant correlation was found between the results of genotypic classifications using RAPD markers (Khan *et al.* 2009) and ISSR markers (Golkar *et al.* 2011), and biochemical traits. This discrepancy may be explained by the fact that biochemical traits such as oil content and fatty acid composition are affected by both genetic and environmental factors as well as their interactions (Yeilaghi *et al.* 2012).

The genotypes that were used in this study were characterized as salt tolerant or salt sensitive based on the results of a preliminary study in which salinity tolerance index (STI) and leaf ion content were measured at the seedling stage; however, the results of this study successfully demonstrated that the genotypes of these two groups exhibited the lowest genetic similarity and indicating that they can be used as parents in genetic studies and breeding programs for improving salinity tolerance in safflower.

The results of this study pointed out a possible relationship between salt tolerance and higher oil content in safflower. For example, the salt tolerant genotype PI-506426 had the highest seed oil content (32.15%), the highest linoleic acid (77.72%), and the lowest oleic acid content (14.69%) in oil under these experimental conditions. This genotype also showed the highest K^+/Na^+ ratio (0.78) in previous study conducted by Karimi *et al.* (2014). These results were in agreement with those of Yeilaghi *et al*.(2012)who showed that salt tolerant genotypes of safflower had higher seed oil content and oil yield and were less affected by salinity stress with respect to these traits. Based on the report of Zhang *et al*.(2009, 2012) that fatty acid unsaturation was possibly involved in the regulation of Na^+ and other ion homeostatic activities under salt stress, it can be speculated that the genotype PI-506426 may possess a lipid

poly-unsaturation mechanism that can affect on its salt tolerance. However, further study is required to address the physiological details of salt tolerance in safflower. Identifying different salt tolerance mechanisms and pyramiding them into a cultivar is a possible approach of improving salt tolerance in safflower.

Although the Mantel test for finding correlations between microsatellite markers and salinity tolerant traits showed no significant correlation ($r = 0.29$), but the microsatellite markers used in this study could classify the genotypes into two distinct groups differing in terms of salinity tolerant traits (Table 4). The results of this study revealed that these markers can be useful in screening of safflower germplasm for salt tolerance. However, the confirmation of any association between these markers with the QTL's of the traits related to salt tolerance is necessary to find out their usefulness for marker assisted selection.

Among the genotypes investigated,the salt tolerant genotype PI-506426 showed the highest seed oil content and its oil was rich in linoleic acid. Also, this genotype was the most genetically distant from the salt-sensitive genotype 307-S6-697. Therefore, PI-506426 can be used as a good parent in future genetic studies and breeding programs to develop salt-tolerant safflower cultivars with higher oil yield and oil quality to grown under saline environmental conditions.

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