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Molecular characterization of *Carthamus tinctorius* and *C. oxyacanthus* germplasm using sequence related amplified polymorphism (SRAP) markers

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Abstract

Genetic diversity of 42 *Carthamus oxyacanthus* and seven *C. tinctorius* genotypes from five distinct geographical regions of Iran was evaluated using sequence related amplified polymorphism (SRAP) markers. Twelve SRAP primer combinations (PCs) amplified 293 fragments, of which 224 were polymorphic. The number of polymorphic fragments detecting per PC ranged from 13 to 24 bands with an average of 18.66. Average PIC value was 0.339 over all PCs. Cluster analysis using UPGMA method and Jaccard's similarity coefficient (r = 0.85) grouped the genotypes into five main clusters according to species and regions. Sum of the first three PCOs could represent most of (75.2%) the total variation in the original dimensions and confirm the results of cluster analysis. The obtained population genetic indexes revealed considerable diversity and heterozygous nature of *C. oxyacanthus* in comparison to *C. tinctorius*. The AMOVA results also showed significant differences in the genetic diversity among the species (Fst = 0.277; $P < 0.0001$) and regions (Fst = 0.185; $P < 0.0001$). The results revealed the existence of wide genetic base of safflower germplasm in Iran and the effectiveness of SRAP markers for studying genetic diversity and relationships among and within species and regions.

Keywords: Safflower, Iran, Genetic diversity, SRAP, AMOVA.
Abbreviations: UPGMA- Unweighted pair group method with arithmetic averages; PCR –Polymerase chain reaction; PCOA- principal coordinate analysis; PCOs- principal components; SRAP- Sequence related amplified polymorphism; AMOVA- Analysis of molecular variance.

Introduction

The genus *Carthamus* is a diverse group of plants within the Asteraceae family and is of interest due to the commercial growth of *C. tinctorius* (safflower), which can be used as an edible oil, medicinal and industrial applications and recently, as a host for the production of transgenic pharmaceutical proteins (Moloney, 2000). The different species of *Carthamus* have been classified into several different grouping systems by different taxonomists. Lopez-Gonzalez (1989) placed the 15 species into three sections (*Carthamus*, *Odonthagnathis* and *Atractylis*), based on the relationships between the species and their chromosome numbers. The section *Carthamus* contains the species with 12 sets of chromosomes including *C. tinctorius*, *C. palaestinus* and *C. oxyacanthus* (Vilatersana et al. 2005). The wild and weedy species, *C. oxyacanthus* and *C. palaestinus*, can be easily crossed with cultivated species, *C. tictorius* (Mayerhofer et al. 2011). These three species have been shown to be genetically distinct from one another based on microsatellite markers (Chapman et al. 2009), but relatively little is known about differentiation within the safflower gene pool. The wild species, *C. oxyacanthus,* is an important underutilized crop, suitable for safflower improvement and widely spread through Turkey, Western Iraq, Iran (Dittrich et al. 1979), Northwest India, Kazakhastan, Turkmenistan, and Uzbekistan (Knowles and Ashri, 1995). This species has considerable genetic diversity in Iran (Sabzalian et al. 2009) and its oil quality is comparable with cultivated safflower (Mundel and Bergman, 2009, Sabzalian et al. 2008). Evaluation on the genetic diversity among and within these two closely related species as the most likely progenitors (Ashri and Knowles, 1960; Sehgal et al. 2008), originating from different geographical regions will help to provide valuable information on the conservation and utilization of safflower germplasm. DNA markers, possessing the advantages of higher polymorphism and independent of environment and plant growth stage, have been widely employed for the assessment of genetic diversity (Li et al. 2009). Nowadays, different DNA markers have been used to determine the genetic diversity and genome mapping of safflower including EST-SSR (Chapman et al. 2009; Mayerhofer et al. 2010), AFLP (Sehgal and Raina, 2005), RFLP (Mayerhofer et al. 2010), ISSR (Ash et al. 2003; Yang et al. 2007; Sabzalian et al. 2009; Golkar et al. 2011), and RAPD (Vilatersana et al. 2005; Amini et al. 2008; Mahasi et al. 2009). Moreover, genetic purity of safflower hybrids was estimated using EST-SSR markers in safflower (Naresh et al. 2009). The sequence-related amplified polymorphism (SRAP) technique of Li and Quiros (2001) is a relatively simple and highly reproducible DNA marker that is useful for both mapping and gene tagging in plants. SRAP markers are PCR-based markers with 17 or 18 nucleotide-long primers that are used to amplify open-reading frames (ORFs). These

primers consist of a 14 bp-core sequence in which the first 10 bp from 5′ end are the filler sequences which are followed by CCGG in the forward-primer and AATT in the reverse-primer. In both primers, three selective nucleotides exist at the 3′-end (Li and Quiros 2001). SRAP has been successfully used to study genetic diversity and relationships in several species (Budak et al. 2004; Esposito et al. 2007; Feng et al. 2009; Ferriol et al. 2003; Fu et al. 2008; Jin et al. 2007; Martin et al. 2008; Uluturk et al. 2011; Yeboah et al. 2007). SRAP was shown to be more informative for detecting genetic diversity than other PCR-based techniques (Budak et al. 2004), since it targets the ORFs of the functional genes, so SRAPs may have a more direct relation with functional genes than other DNA markers (Li and Quiros 2001). Furthermore, it needs less time and cost to assess the relationships among different species in comparison with the other molecular markers. In this regard, in order to have better analyzes in *Carthamus*, we have chosen this marker to evaluate Iranian safflower germplasm. The objectives of the present research are (i) to assess the value of first application of SRAP marker system for its ability to distinguish *C. tinctorius* and *C. oxyacanthus* genotypes. (ii) to evaluate population genetic analysis of Iranian *C. tinctorius* and *C. oxyacanthus* genotypes according to their species and geographical origins and to study their genetic relationships.

Results

SRAP amplification

SRAP marker in safflower exhibited the remarkable characteristics of good stability and repeatability as well as clear bands. A total of 30 primer combinations (PCs) were screened and 12 appropriate PCs were selected for amplification of 49 samples from two species and six different geographical regions of Iran. The band number amplified by each PC ranged from 18 (Me4-Em5) to 30 (Me4/Em3), with the molecular weight between 80 and 1,200 bp. In total 293 bands were observed, of which 224 were polymorphic (76.4%). The average band number amplified from each PC was 24.41 bands, which included 18.67 polymorphic bands (Table 1), indicating an insignificant difference in the number of polymorphic loci amplified from the different PCs. The polymorphism information content (PIC) of SRAP PC was 0.339 with a range of variation from 0.268 to 0.425 (Table 1). Based on the results, the highest PIC value was related to Me2/Em3, which introduces it as the most informative PC for genetic diversity studies among safflower genotypes.

Genetic similarity and analysis of safflower genotypes

All the safflower genotypes were distinguished with identification of 49 different fingerprints, revealing the high power of discrimination of the used SRAP PCs and attesting the great diversity of the genotypes. The similarity matrix of the genotypes used in SRAP analysis showed the range of similarity varying between 0.33 and 0.91 with the mean of 0.51 (data not shown). The lowest similarity was showed between OXIS22 and OXKE6 genotypes of *C. oxyacanthus* (0.33), whereas the most similarity was between TINIS1420 and TINS80 genotypes of *C. tinctorius* (0.91). The data obtained from SRAP analysis was subjected to cluster analysis of genotypes. In order to distinguish the best clustering and similarity coefficient methods, the cophenetic correlation coefficient, a measure of the correlation between ultrametric similarities of tree and similarity matrix, was calculated for each method combination. Among different methods, the highest value (r =0.85) was observed for UPGMA clustering method based on Jaccard's similarity coefficient, suggesting a little distortion between the original similarity values from the similarity matrix and the values used to construct the dendrogram. The cluster analysis based on SRAP markers distinguished all genotypes and grouped them into five main clusters (Fig. 1). All of genotypes from *C. tinctorius* and some of *C. oxyacanthus* genotypes from Isfahan were placed in cluster II, whereas the remaining genotypes of *C. oxyacanthus* from different geographical regions of Iran were grouped in different clusters.

Principal coordinate analysis (PCoA) based on genetic similarity matrices were used to visualize the genetic relationships among genotypes. The first three eigenvectors accounted for 75.2% of the total molecular variation, with 50.6% explained by the first, 20.2% by the second and 4.4 by the third at the DNA sequence (Fig. 2).

Genetic structure of C. tinctorius and C. oxyacanthus species

Population genetic studies revealed some valuable information about populations. The obtained effective number of alleles (*Ne*), Nei's gene diversity index or expected heterozygosity (*He*) and Shannon's diversity index (*I*) within *C. oxyacanthus* species were more than *C. tinctorius* (Table 2, Analysis 1), indicating heterozygous nature and considerable diversity of *C. oxyacanthus* in Iran. Analysis of molecular variance (AMOVA) was performed to study two groups of species differentiation and to estimate the percentage of intra and inter-species genetic variation which revealed a significant variation among the studied species (Fst = 0.277; P < 0.0001). The AMOVA analysis results showed that 27.72 and 72.28% variation were accounted for among and within species, respectively (Table 3, Analysis 1).

Geographical diversity of C. oxyacanthus genotypes in Iran

In order to study *C. oxyacanthus* genotypes more accurately, these genotypes were grouped into five populations according to their geographical origins (Table 4). Estimates of the genetic diversity and population structures are summarized in Table 2, Analysis 2. The observed number of alleles (*Na*) within geographical regions in each locus varied from 1.48 (Arak and Azarbayejan) to 1.75 (Isfahan), whereas effective number of alleles ranged from 1.31 (Arak) to1.45 (Isfahan). Expected heterozygosity (*He*) and Shannon's information index (*I*) were used as two useful intra-population gene diversity indices. Mean value of *He* and *I* ranged from 0.1828 to 0.2676 and 0.2718 to 0.4007, respectively. As a whole, among the five regions, the highest Nei's genetic diversity or expected heterozygosity (*He*), Shannon's information index (*I*), the observed and effective number of alleles (*Na* and *Ne*) and the lowest *Fst* were observed in Isfahan population (Table 2, Analysis 2). Analysis of molecular variance (AMOVA) was also performed to study differentiation in five populations of genotypes and to estimate the percentage of intra and inter-region genetic variation (Table 3, Analysis 2). Significant variation was observed among the studied populations (Fst = 0.185; P < 0.0001). The results of the AMOVA analysis revealed that 81.42% of total genetic variation occurred within regions and only 18.58% was attributed among regions.

Table 1. Polymorphism number and rate for 12 SRAP primer pairs used to amplify 49 genomic DNA templates of safflower genotypes.

Primer combination	No. total bands	No. polymorphic bands	% of polymorphism	PIC value
Me1-Em2	29	23	79.3	0.320
Me3-Em2	23	13	56.5	0.268
Me3-Em6	22	16	72.7	0.268
Me4-Em1	26	18	69.2	0.384
Me4-Em3	30	22	73.3	0.325
Me4-Em5	18	13	72.2	0.369
Me4-Em6	21	16	76.2	0.366
Me5-Em1	24	19	79.2	0.421
Me5-Em2	20	16	80	0.425
Me5-Em4	28	24	85.7	0.303
Me5-Em5	27	23	85.2	0.349
Me5-Em6	25	21	84	0.276
Total	293	224	-	-
Means	24.41	18.67	76.4	0.339

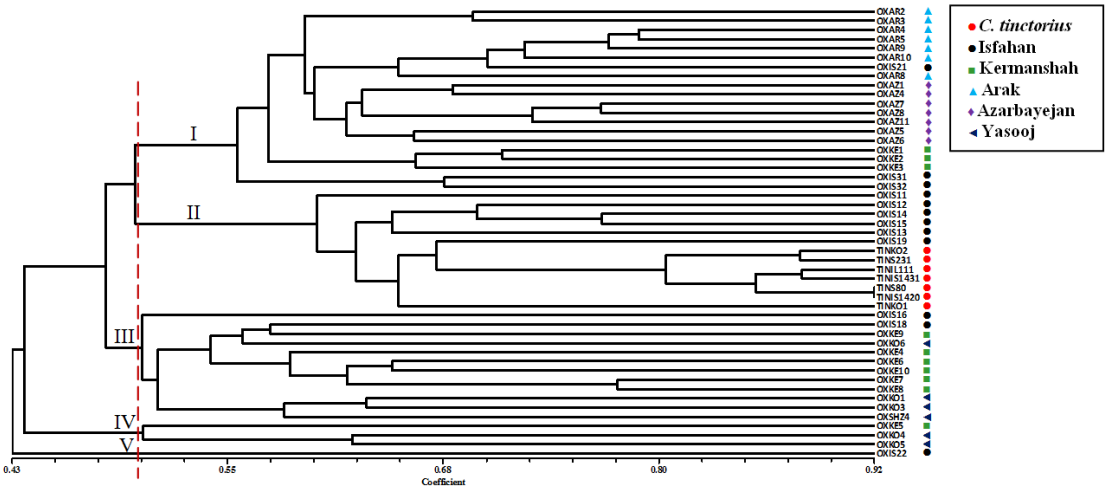

Fig 1. UPGMA dendrogram of seven *C. tinctorius* from Isfahan, Iran (red color) and 42 *C. oxyacanthus* genotypes from different geographic regions of Iran as indicated by symbols based on SRAP genetic similarities.

Discussion

The present study is the first attempt to use SRAPs as a basis molecular marker for genetic variability among and within *C. tinctorius* and *C. oxyacanthus* species in Iran. Our data indicated that this technology can detect considerable polymorphisms (76.4%) in our genotypes, suggesting that it will be useful in safflower germplasm characterization and fingerprinting purposes. This study provides fundamental evidence that SRAP marker is a simple, informative, reproducible and suitable approach to evaluation of molecular diversity and phylogenetic relationships in safflower.

The PIC value and the polymorphism rate were used to measure the genetic diversity. High, medium or low polymorphism is in accordance with PIC > 0.5, 0.5 > PIC > 0.25 and PIC < 0.25, respectively (Xie et al. 2010). Moreover, the mean value of the PIC obtained in this study was 0.339, indicating that the markers could develop medium polymorphism which is useful for genetic variation of safflower genotypes, and possibly in other *Carthamus* species. The cluster analysis based on SRAP data showed that there was a considerable agreement between geographic origin and their genomic similarities. Similar results were obtained in the study of genetic diversity among *C. tinctorius* genotypes from different geographical regions of Iran using ISSR markers (Golkar et al. 2011). Similarities in genotypes grouped in the same cluster could also appear because of participating a common lineage, convergent evolution and selection of superior genotypes by farmers. According to the dendrogram (Fig. 2), all of *C. oxyacanthus* genotypes from Arak and Azarbayejan were grouped in a same cluster with three genotypes from Isfahan and three of them from Kermanshah (Cluster I, Fig. 1). All of *C. tinctorius* genotypes were placed in cluster II with most of *C. oxyacanthus* genotypes from Isfahan. This could show the close relationship of these two species and confirmed the results of Ashri and Knowles (1960) and Sehgal et al. (2008), who proposed that *C. oxyacanthus* is a progenitor of *C. tinctorius*. The remaining genotypes from Isfahan, Kermanshah and Yasooj were clustered in different groups as clusters III, IV and V. This could be due to the exchange of plant materials across the regions during the safflower cultivation. Some aspects of interrelation among materials studied that were not

Table 2. Summary of genetic variation statistics for all loci in different populations of safflower grouped based on species (Analysis 1) and geographical distribution of *C. oxyacanthus* species in Iran (Analysis 2).

Analysis	Population name	No. Of genotypes	*Na*	*Ne*	*He*	*I*	F_{ST}	*Nm*
1	*C. oxyacanthus*	42	1.9118	1.4975	0.2935	0.4437	0.27134	1.5965
	C. tinctorius	7	1.3319	1.1691	0.1069	0.1653	0.31246	
2	Arak	7	1.4874	1.3115	0.1828	0.2718	0.20636	1.4564
	Isfahan	12	1.7563	1.4569	0.2676	0.4007	0.16610	
	Azarbayejan	7	1.4874	1.3274	0.1885	0.2779	0.20026	
	Kermanshah	10	1.6849	1.4054	0.2405	0.3617	0.18089	
	Yasooj	6	1.5504	1.3667	0.2131	0.3148	0.19238	
	Total means	49	1.9160	1.5079	0.2979	0.4488	---	

Note: *Na* = Observed number of alleles; *Ne* = Effective number of alleles; *He*= Nei's gene diversity; *I* = Shannon's Information index; F_{ST} = Fixation Index; *Nm* = estimate of gene flow.

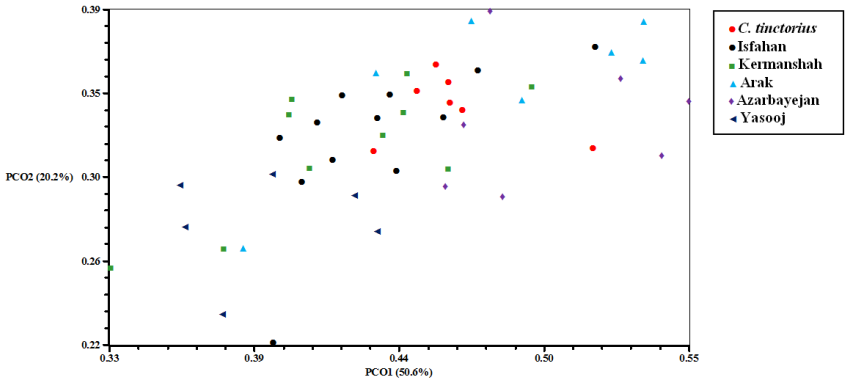

Fig 2. Principal coordinate analysis (PCoA) of seven *C. tinctorius* (red color) and 42 *C. oxyacanthus* genotypes from different geographic regions of Iran as indicated by symbols based on 224 SRAP markers.

Table 3. Analysis of molecular variance (AMOVA) in 49 safflower genotypes grouped according to species (analysis 1) and geographical regions (analysis 2) based on 238 SRAP markers.

Analysis	Source of variation	Degree of freedom	Sum of squares	Variance components	% of variance	P- value
1	Among species	1	143.969	9.856	27.72	< 0.0001
	Within species	47	1207.786	25.697	72.28	
2	Among regions	4	272.338	5.38954	18.58	< 0.0001
	Within regions	37	873.971	23.62085	81.42	

recognizable by cluster, revealed by the principal coordinate analysis (PCOA). Sum of the first three PCOs could be represented most of (75.2%) the total variation in the original dimensions. Therefore, this result demonstrates proper distribution of SRAP markers through entire genome and confirms the results of cluster analysis; in agreement with the results of Uzun et al. (2010). The AMOVA analysis results revealed the high and low level of genetic diversity within and among genotypes from different geographical regions, respectively (Table 3). This suggests that these regions have a high genetic overlap as a result of extensive gene flow. Gene differentiation and gene flow are important indexes to evaluate the population genetic structure. The gene flow estimated in this study (Table 2) for two species were moderate as described by Nm = 1.5965 and it is higher than the gene flow among regions (1.4564) that may be due to geographic distance by limited pollen and seed dispersal. The gene flow is negatively correlated with the gene differentiation. Therefore, we could refer to the wide genetic base of safflower and the existence of some common ancestors among the genotypes in Iran.

Materials and Methods

Plant materials and DNA extraction

Young leaf samples of 49 genotypes from two *Carthamus* species, which include 42 *C. oxyacanthus* and seven *C. tinctorius*, were used as starting material to carry out a SRAP marker analysis (Table 4). The genotypes used in this study

Table 4. Iranian safflower genotypes used in this study based on species and geographical distribution.

No.	Code	Species	Geographical region	Latitude	Longitude
1	OXAR2	*C. oxyacanthus*	Arak, Markazi, Iran	34° 4' N	49° 42' E
2	OXAR3	*C. oxyacanthus*	Arak, Markazi, Iran	34° 4' N	49° 42' E
3	OXAR4	*C. oxyacanthus*	Arak, Markazi, Iran	34° 4' N	49° 42' E
4	OXAR5	*C. oxyacanthus*	Arak, Markazi, Iran	34° 4' N	49° 42' E
5	OXAR8	*C. oxyacanthus*	Arak, Markazi, Iran	34° 4' N	49° 42' E
6	OXAR9	*C. oxyacanthus*	Arak, Markazi, Iran	34° 4' N	49° 42' E
7	OXAR10	*C. oxyacanthus*	Arak, Markazi, Iran	34° 4' N	49° 42' E
8	OXIS11	*C. oxyacanthus*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
9	OXIS12	*C. oxyacanthus*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
10	OXIS13	*C. oxyacanthus*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
11	OXIS14	*C. oxyacanthus*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
12	OXIS15	*C. oxyacanthus*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
13	OXIS16	*C. oxyacanthus*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
14	OXIS18	*C. oxyacanthus*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
15	OXIS19	*C. oxyacanthus*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
16	OXIS21	*C. oxyacanthus*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
17	OXIS22	*C. oxyacanthus*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
18	OXIS31	*C. oxyacanthus*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
19	OXIS32	*C. oxyacanthus*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
20	OXAZ1	*C. oxyacanthus*	Azarbayejan, Iran	35° 41' N	51° 22' E
21	OXAZ4	*C. oxyacanthus*	Azarbayejan, Iran	35° 41' N	51° 22' E
22	OXAZ5	*C. oxyacanthus*	Azarbayejan, Iran	35° 41' N	51° 22' E
23	OXAZ6	*C. oxyacanthus*	Azarbayejan, Iran	35° 41' N	51° 22' E
24	OXAZ7	*C. oxyacanthus*	Azarbayejan, Iran	35° 41' N	51° 22' E
25	OXAZ8	*C. oxyacanthus*	Azarbayejan, Iran	35° 41' N	51° 22' E
26	OXAZ11	*C. oxyacanthus*	Azarbayejan, Iran	35° 41' N	51° 22' E
27	OXKE1	*C. oxyacanthus*	Kermanshah, Iran	34° 18' N	47° 3' E
28	OXKE2	*C. oxyacanthus*	Kermanshah, Iran	34° 18' N	47° 3' E
29	OXKE3	*C. oxyacanthus*	Kermanshah, Iran	34° 18' N	47° 3' E
30	OXKE4	*C. oxyacanthus*	Kermanshah, Iran	34° 18' N	47° 3' E
31	OXKE5	*C. oxyacanthus*	Kermanshah, Iran	34° 18' N	47° 3' E
32	OXKE6	*C. oxyacanthus*	Kermanshah, Iran	34° 18' N	47° 3' E
33	OXKE7	*C. oxyacanthus*	Kermanshah, Iran	34° 18' N	47° 3' E
34	OXKE8	*C. oxyacanthus*	Kermanshah, Iran	34° 18' N	47° 3' E
35	OXKE9	*C. oxyacanthus*	Kermanshah, Iran	34° 18' N	47° 3' E
36	OXKE10	*C. oxyacanthus*	Kermanshah, Iran	34° 18' N	47° 3' E
37	OXKO1	*C. oxyacanthus*	Yasooj, Kohkiloie, Iran	31° 3' N	51° 12' E
38	OXKO3	*C. oxyacanthus*	Yasooj, Kohkiloie, Iran	31° 3' N	51° 12' E
39	OXKO4	*C. oxyacanthus*	Yasooj, Kohkiloie, Iran	31° 3' N	51° 12' E
40	OXKO5	*C. oxyacanthus*	Yasooj, Kohkiloie, Iran	31° 3' N	51° 12' E
41	OXKO6	*C. oxyacanthus*	Yasooj, Kohkiloie, Iran	31° 3' N	51° 12' E
42	OXSHZ4	*C. oxyacanthus*	Yasooj, Kohkiloie, Iran	31° 3' N	51° 12' E
43	TINS231	*C. tinctorius*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
44	TINIS1431	*C. tinctorius*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
45	TINIS1420	*C. tinctorius*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
46	TINKO1	*C. tinctorius*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
47	TINKO2	*C. tinctorius*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
48	TINIL111	*C. tinctorius*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E
49	TINS80	*C. tinctorius*	Isfahan, Isfahan, Iran	32° 39' N	51° 40' E

Table 5. Sequence of forward and reverse SRAP primers used in this study.

Forward (5'-3')	Reverse (5'-3')
Me1:TGAGTCCAAACCGGATA	Em1:GACTGCGTACGAATTAAT
Me2:TGAGTCCAAACCGGAGC	Em2:GACTGCGTACGAATTTGC
Me3:TGAGTCCAAACCGGAAT	Em3:GACTGCGTACGAATTGAC
Me4:TGAGTCCAAACCGGACC	Em4: GACTGCGTACGAATTTG
Me5: TGAGTCCAAACCGGAA	Em5:GACTGCGTACGAATTAAC
	Em6:GACTGCGTACGAATTGCA

were obtained from different geographical regions of Iran as mentioned in Table 4. The plant materials were treated with liquid nitrogen and stored at −80◦C until being used. Genomic DNA was extracted from frozen young leaves of each genotype according to the modified method of Murray and Thompson (1980). The quality and concentration of DNA samples were determined using 0.7% agarose gel electrophoresis in TAE buffer against known concentrations of unrestricted lambda DNA and were verified by spectrophotometric measurement. DNA samples were diluted to 25ng/µl and stored at -20 ºC.

PCR amplification and gel electrophoresis

The SRAP analysis was performed as described by Li and Quiros (2001). The PCR reaction was performed in a total volume of 15 µl reaction mixtures containing 50 ng templates DNA, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.3 µM of each forward and reverse primers (Table 5), 1.5 µl of 10x PCR buffer, 1 unit of *Taq* DNA polymerase and sterile doubled distilled water. Amplification were carried out in a Techne thermal cycler (Germany) with the following PCR program: 3 min of initial denaturing at 94 °C, five cycles of three steps: 1 min of denaturing at 94 ºC, 1 min of annealing at 35 ºC and 1 min of elongation at 72 ºC, 35 cycles of 94 ºC for 1 min, 50 ºC for 1 min, and 72 ºC for 1 min; followed by a final extension 10 min at 72 ºC. The PCR products were mixed with 10 µl of formamide loading buffer (95% formamide, 20 mM EDTA, pH 8.0, Xylene cyanol and Bromophenol blue) analyzed on 8% non-denatured polyacrylamide gels in 1x TBE buffer along with 100 bp DNA ladder (Fermenthas Co.) as size marker. PCR products were visualized by silver staining (Bassam et al. 1991).

Data analysis

All clearly detectable and reproducible SRAP amplicons were scored as band presence (1) and absence (0) and the matrix of SRAPs data was constructed. A marker index was calculated for the SRAP markers to characterize the capacity of each primer to detect polymorphic loci among and within the populations. As such, the marker index was the sum of the polymorphism information content (PIC) values for all the selected markers amplified by a particular primer pair. The PIC value was calculated using the formula $PIC=1-\Sigma Pi^2$, where *Pi* is the frequency of the *i*th allele (Smith et al. 1997). The data obtained from the SRAP profiles with different individual primers, as well as collective ones, were subjected to the construction of a similarity matrix using Jaccard's (Jaccard 1908) coefficients of similarity. The matrices were then used for a cluster analysis using the unweighted pair group method with arithmetic averages (UPGMA), and the results were summarized as dendrograms using NTSYSpc software 2.02 (Rohlf, 1998). The cophenetic correlation coefficient was calculated to measure the goodness-of-fit between the cophenetic matrix and original similarity matrix. The frequency of occurrence of each marker in each plant was computed, to render a matrix of 49 individual plants by SRAP markers. These matrices were subjected to principal coordinate analysis (PCOA) using NTSYSpc software 2.02. Analysis of molecular variance (AMOVA) was performed to estimate variance components for SRAP data, partitioning the variation into within and among populations, using Arlequin 3.1 software (Excoffier et al. 2005). POPGENE32 software (Yeh and Young 1999) was used to compute the number of effective loci, the percentage of polymorphic loci, Shannon's information index (*I*), observed number of alleles (*Na*), expected heterozygosity (*He*) and effective number of alleles (*Ne*).

Conclusion

The results of the present study demonstrated that the SRAP markers could be powerful tools and effective marker system for detecting genetic diversity among safflower genotypes and provide useful information about the relationships of species and geographical regions. Moreover, it revealed a wide genetic base of safflower germplasm and considerable diversity and heterozygous nature of *C. oxyacanthus*. The use of the genotypes from other *Carthamus* species would provide more useful information to assist the classification and relationships of species.

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