African Journal of Biotechnology Vol. 9(43), pp. 7280-7288, 25 October, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Molecular characterization and similarity relationships among sunflower (*Helianthus annuus* L.) inbred lines using some mapped simple sequence repeats

R. Darvishzadeh[1,2], M. Azizi[2], H. Hatami-Maleki[3], I. Bernousi[1,2], B. Abdollahi Mandoulakani[1,2], M. Jafari[1,2] and A. Sarrafi[4]

[1]Department of Agronomy and Plant breeding, Urmia University, Iran.
[2]Institute of Biotechnology, Urmia University, Iran.
[3]Department of Agronomy and Plant breeding, University of Maragheh, Iran.
[4]Laboratoire de Biotechnologie et Amélioration des Plantes (BAP), IFR 40, INP-ENSAT, 18 Chemin de Borde Rouge, BP 32607, 31326 Castanet-Tolosan, France.

Accepted 7 September, 2010

Information about the genetic diversity and relationships among breeding lines and varieties is not only useful for germplasm conservation and inbred line identification, but also for the selection of parental lines for quantitative trait loci (QTL) mapping as well as hybrid breeding in crops, including sunflower. In order to develop mapping populations, genetic distances among twenty eight sunflower genotypes were evaluated using simple sequence repeat (SSR) markers. One hundred and two markers were generated by 38 SSR loci and the mean for the number of allele per locus was 2.32. Polymorphism information content (PIC) values ranged from 0.09 (locus ha3555) to 0.62 (locus ORS598) with an average of 0.41. Jaccard's coefficient similarity matrix for the studied sunflower genotypes varied from 0.25 to 0.9 indicating a broad genetic base. The maximum similarity (0.9) was observed between genotypes RT931 and ENSAT-R5, while the lowest similarity (0.25) was between genotypes LC1064C and LR64. Based on unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm, the studied genotypes were clustered in four groups. However, some genotypes have the same specific characters that influence their clustering, and as a result, the results of the principal coordinate analysis (PCoA) largely corresponded to those obtained through cluster analysis.

Key words: Cluster analysis, genetic diversity, principal coordinate analysis, sunflower, simple sequence repeat.

INTRODUCTION

Sunflower (*Helianthus annuus* L.), which is one of the important oilseed crops (Leclercq, 1969), is a model system for the genomic studies of the family Asteraceae (Paniego et al., 1999). Genetic analysis of sunflower is necessary because its germplasm has wide variation in characters such as yield, seed number, plant height, earliness and susceptibility to biotic and a biotic stresses (Thormann et al., 1994; Paniego et al., 1999).

A rich and diverse germplasm collection is the backbone of every successful crop improvement program. Assessing genetic diversity within a genetic pool of novel-breeding germplasm could make crop improvement more efficient by the directed accumulation of desired alleles. This is likely to speed up the breeding process and decrease the amount of plant material that needs to be screened in such experiments.

In most cases, identification of sunflower cultivars, lines and hybrids is based on morphological traits, but a number of these traits are limited, unstable and are not

*Corresponding author. E-mail: r.darvishzadeh@mail.urmia.ac.ir. Tel: + 98 441 2972399. Fax: + 98 441 2779558.

Table 1. Sunflower lines and their country of origin used in the present investigation.

Genotype no.	Sunflower line	Type[a]	Origin	Genotype no.	Sunflower line	Type[a]	Origin
G01	ENSAT-B5	BL	France	G15	SDR19	BL	USA
G02	H565R	BL	France	G16	SDB1	BL	USA
G03	ENSAT-R5	BL	France	G17	SDB3	BL	USA
G04	RHA274	BL	USA	G18	RHA266	BL	USA
G05	H543R	BL	France	G19	PAC2	BL	France
G06	RT931	BL	France	G20	C81	RIL	France
G07	AS5305	BL	France	G21	C43	RIL	France
G08	ENSAT-R4	BL	France	G22	C79	RIL	France
G09	NS-B4	BL	France	G23	AS613	BL	France
G10	LC1064C	BL	France	G24	LR64	RIL	France
G11	F651/1	BL	Hungary	G25	M6-54-1	M	France
G12	F1250/03	BL	Hungary	G26	M6-133-2	M	France
G13	B454/03	BL	Hungary	G27	M6-85-3	M	France
G14	SDR18	BL	USA	G28	M6-894-2	M	France

[a]BL, breeder's line; RIL, recombinant inbred line and M, gamma-irradiation induced mutant line.

always distinguishable between closely related accessions (Konarev, 2000). DNA markers seem to provide useful information about polymorphism, genetic relatedness and diversity (Chalmers et al., 2001). Several research results show that random amplified polymorphic DNA (RAPD) were insufficient for gaining insights into the origins of domesticated sunflowers or distinguishing between closely or distantly related germplasm accessions (Rieseberg and Seiler, 1990; Arias and Rieseberg, 1995). Restriction fragment length polymorphism (RFLP) has been used for diversity studies, but the level of revealed polymorphism via this marker is low (Hernández et al., 2001). The recent development of several hundred microsatellite markers for sunflower (Yu et al., 2002; Paniego et al., 2002) has opened the way to the analysis of molecular genetic diversity in this crop. Microsatellite markers, due to their high polymorphism, random distribution and co-dominant Mendelian inheritance, are the most reliable markers for cultivars identification and genetic diversity. Microsatellites or simple sequence repeats (SSRs) constitute the current marker system of choice for characterizing sunflower germplasm (Paniego et al., 2002; Tang et al., 2002; Yu et al., 2002; Zhang et al., 2005). They were widely applied in sunflower researches for identification of inbred lines, cultivars and wild species (Tang and Knapp, 2003; Yu et al., 2002; Liu and Burke, 2006). With the advent of high-density SSR maps for sunflower (Tang et al., 2002; Poormohammad Kiani et al., 2007a), it is now feasible to estimate genetic diversity with a large number of markers that are well distributed across the sunflower genome. The advantage of using markers with known map positions is that there is control over the coverage of the genome. It is thus possible to avoid overrepresentation of certain regions of the genetic map that can produce inaccurate estimates of genetic similarities among individuals. The aim of the current investigation is to analyze the genetic diversity in newly developed sunflower genotypes using some mapped SSR markers with enough genome coverage.

MATERIALS AND METHODS

Plant material and DNA extraction

Twenty eight sunflower genotypes (Table 1) including recombinant inbred lines (RILs), mutant (M) and breeder lines (BL) from different regions including France, USA and Hungary, were fingerprinted through 38 microsatellite primers (Table 2). RILs coming from a cross between lines, 'PAC2' and 'RHA266', were selected for their susceptibility to the phoma black stem (Rachid Al-Chaarani et al., 2002) and basal stem rot diseases (Davar et al., 2009). Mutant lines were developed by seed irradiation of the 'AS613' genotype with gamma rays in Laboratoire de Biotechnologie et Amélioration des Plantes, Castanet-Tolosan, France, and advanced by modified single seed descent (SSD) with no prior selection (Sarrafi et al., 2000). A population of 120 mutant lines (M6) was evaluated with a French isolate of *Phoma macdonaldii* in controlled conditions (Darvishzadeh et al., 2008a, 2008b). In another study, the genetic variability of these mutant lines was evaluated for osmotic adjustment-related traits under two water treatments (Poormohammad et al., 2007b). Several mutants that consistently showed altered reactions to disease as well as drought resistance were identified and four of them were used in the present investigation. Other genotypes used in this study were inbred lines introduced from the United States Department of Agriculture (USDA), Hungary and French seed companies.

Genomic DNA of studied genotypes was extracted from the leaves of 2-weeks-old seedlings using the method described by Dellaport, (1983). Concentration of DNA was measured at 260 nm in a spectrophotometer and the quality of DNA was checked by running 5 µl of genomic DNA on 0.8% agarose gel prepared

Table 2. Primer sequences, number of alleles, polymorphic information content (PIC) and linkage groups of the 38 SSR loci applied to 28 sunflower genotypes.

Primer	Forward sequence (5'-3')	Reverse sequence (5'-3')	Number of alleles	PIC	Linkage group	Reference
ORS 149	GCTCTCTATCTCCCTTGACTCG	TGCTCTAAGATCTCAGGCGTGC	3	0.46		
ORS 160	TCCCTTCCTTTCATCGTCTGCT	TGGCAATTTGCCAAGGACC	2	0.5		
ORS 16	GAGGAAATAAATCTCCGATTCA	GCAAGGACTGCAATTTAGGG	2	0.5		
ha3878	TTTGTTTAGCATCATCATCATC	GAGACCCTAACCATAACATGA	2	0.48		
ha3513	TGACCCATTCAACTTCTTAA	TCATGGTTCCTGATGAGAAT	3	0.28	LG8	Poormohammad Kiani et al. (2007)
ha2505	GTGTCATGACTCGGT	GGACAATGTGATTGC	3	0.13		
ha1604	GCAAATGCACTAAAGGCCCC	CCCTACTCAAACCTTACCTC	3	0.2		
ORS 880	AAGTAGCTTTGCTTTCCTTCGTC	*CGAAACGCGGATTATTGTCTTAT*	2	0.41		
ORS 928	CATGGTTATTTTGGTTTGGGTTT	GCTATTATCATGTCCTTGTCCTTTT	2	0.47	LG7	Tang et al. (2002)
ha2682	CACAATCGTTTCTTTCCAAAA	ACCCATATGCCCACTCATAA	3	0.13		
ORS 920	CGTTGGACGAAGAACTTGATTT	ACTTCCGTTTGTTCCGAGCTT	2	0.5	LG16	Tang et al. (2002)
ha3555	GATATCTCTCATAAGTGCCG	GGTCTTGTGATGACGAA	3	0.09	LG12	Tang et al. (2002)
ORS 58	TGTACCAAGGGTCGTTGTCA	CGACCCCGAGTTTTGTTG	3	0.36		
ORS 154	GCACCTTTGGTGAGGAGATA	TGCATCAGTAGCTATTGTCTAT	2	0.49	LG8	Tang et al. (2002)
ORS 1068	AATTTGTCGACGGTGACGATAG	TTTTGTCATTTCATTACCCAAGG	4	0.5	LG4	Tang et al. (2002)
ORS 1265	GGGTTTAGCAAATAATAGGCACA	ACCCTTGGAGTTTAGGGATCA	2	0.22	LG9	Tang et al. (2002)
ha4142	GAGTCGACATTTTCGGAAATCG	CTTCATCTTCTGACACCCAAC	2	0.39		
ha3651	GGAATTATCCATTGTAGGTTTGG	GGATGATTGATTAATTGAGGG	2	0.48		
ha4149	CAAAAACCTCTCTCCGTTGGC	GACTCCAAAGTCCACCAAATC	2	0.5		
ha2879	CATACCGTTCTTGTTC	CAACCTCCTAGGTCA	2	0.5		
ha4057	AAACCCTTCCGACTTATCTC	TAAAGAGAGAGCCCAACAAG	2	0.46		
ha3638	GACATAATCACTAGTTGTTGGTGC	CTCCTCCCACCTCAACAATTTC	2	0.49		
ha3639	GCAACATGCAGTTCCTAATCAAAC	TCACCGAACTTCAATATCACCAC	2	0.36		
ha3691	GAATGAAGCATGTGGAAGGCGG	GTGGAGGTGATGATGGTATGAG	2	0.44		
ha4136	CCTATTCCTGATAATTCACTAAGC	GGTAGCATGCTTACATTAAGATG	3	0.35		
ORS 423	TCATATGGAGGGATCTGTTGG	AAGCAACCATAATGCATCAGAA	2	0.49	LG2	Tang et al. (2002)
ORS 718	CACTTTACGCACACCAAACC	ATGCAACACCCGAATCAAAG	2	0.27	LG3	Poormohammad Kiani et al. (2007)
ORS 844	ACGATGCAAAGAATATACTGCAC	CATGTTTAATAGGTTTTAATTCTAGGG	2	0.49	LG9	Tang et al. (2002)

in 0.5X TBE buffer.

Microsatellite analysis

Polymerase chain reaction (PCR) amplifications were performed in a volume of 20 µl containing 2.5 µM of each primer, 0.4 U of Taq DNA polymerase (Life Technologies), 100 µM of each dNTP (Promega), 1× PCR buffer, 2.5 mM $MgCl_2$ (Promega), 0.20 µl of stabilizer (1% W-1, Life Technologies), ddH_2O and 25 ng template DNA, using a Gene Amp PCR System 9700 Thermocycler (PerkinElmer-Applied Biosystems). The touchdown PCR were used for the amplification of all investigated SSRs as: 95°C for 3 min, 1 cycle of 94°C for 30 s, 64°C for 30 s, 72°C for 45 s and was followed by 10 cycles with a decrease of annealing temperature at 1°C per cycle. This was followed by 33 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for

Table 2. Contd.

Primer	Forward sequence (5'-3')	Reverse sequence (5'-3')	Number of alleles	PIC	Linkage group	Reference
ORS 878	TGCAAGGTATCCATATTCCACAA	TATACGCACCGGAAAGAAAGTC	2	0.42	LG10	Tang et al. (2002)
ORS 613	GTAAACCCTAGGTCAATTTGCAG	ATCTCCGGAAAACATTCTCG	2	0.32	LG10	Tang et al. (2002)
ORS 988	TTGATTTGGTGAAAGTGTGAAGC	CGAACATTATTTACATCGCTTTGTC	2	0.5	LG17	Tang et al. (2002)
ORS 899	GCCACGTATAACTGACTATGACCA	CGAATACAGACTCGATAAACGACA	2	0.5	LG16	Tang et al. (2002)
ORS 996	CGGTGAGAATAACCTCGGAAGA	ATCAGTCCTTCAACGCCATTAGT	2	0.29	LG16	Tang et al. (2002)
ORS 1088	ACTATCGAACCTCCCTCCAAAC	GGATTTCTTTCATCTTTGTGGTG	2	0.5	LG10	Tang et al. (2002)
ORS 488	CCCATTCACTCCTGTTTCCA	CTCCGGTGAGGATTTGGATT	3	0.48	LG3	Tang et al. (2002)
ORS 598	CCAAATGTGAGGTGGGAGAA	ATAGTCCCTGACGTGGATGG	3	0.62	LG1	Tang et al. (2002)
ORS 822	CAATGCCATCTGTCATCAGCTAC	AAACAAACCTTTGGACGAAACTC	2	0.58	LG1	Tang et al. (2002)
ORS 331	TGAAGAAGGGTTGTTGATTACAAG	GCATTGGGTTCACCATTTCT	2	0.34	LG7	Tang et al. (2002)

45 s. However, the final extension was 20 min at 72°C (Tang et al., 2002). The reaction products were mixed with an equal volume of formamide dye (98% formamide, 10 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol) and resolved in 3% agarose gel (0.5X TBE). Then, they were stained with ethidium bromide (1 µg/ml) and photographed under UV light.

Data analysis

The amplification products were scored for the presence (1) and absence (0) of bands across the genotypes to construct a binary data matrix (Mohammadi, 2006). The genetic similarity matrix was constructed using Jaccard's similarity coefficient (Jaccard, 1908). Dendrogram was constructed by the unweighted pair-group method using arithmetic average (UPGMA) algorithm and the principal coordinate analysis (PCoA) was used to identify and resolve patterns of genetic relationships of the studied genotypes. The efficiency of clustering algorithms and their goodness of fit were determined based on the cophenetic correlation coefficient. Data analyses were performed by software NTSYS-pc version 2.11 (Rohlf, 1998). Allelic polymorphism information content (PIC) was calculated as described by Anderson et al. (1992):

$$PIC = 1 - \sum_{i=1}^{n} (P_i)^2$$

Where *Pi* is the proportion of the population carrying the i^{th} allele, calculated for each microsatellite locus.

RESULTS

Thirty-eight microsatellite primers were used to analyze genetic relationships among 28 sunflower genotypes and a total of 88 alleles were detected among studied genotypes. Number of allele per locus ranged from 2 to 4 with an average of 2.32 (Table 2). Polymorphic banding pattern of locus Ha3638 is presented in Figure 1. However, the used set of microsatellite loci showed low level of polymorphism among investigated genotypes. The discrimination power of each SSR locus was estimated by the PIC. PIC values ranged from 0.09 for locus ha3555 to 0.62 for locus ORS598 with a mean of 0.41 (Table 2). As shown in Table 3, the genetic similarity coefficients among sunflower genotypes varied from a maximum of 0.88 (between LR64 and M6-54-1 genotypes) to a minimum of 0.25 (between LC1064C and LR64 genotypes). The average of genetic similarity was 0.48, depicting a high level of genetic variation among studied sunflower genotypes. Based on UPGMA clustering method, 28 studied genotypes were grouped in four groups (Figure 2). The cophenetic correlation coefficient, a measure of the correlation between the similarity represented on the dendrograms and the actual degree of similarity, was significant ($r = 0.77$, $P<0.05$). All sunflower genotypes with exception to genotype "NS-B4" were placed in three groups of II, III and IV. In group II, the highest similarity value (*GSj* = 0.65) was observed between genotypes "C81" and "C79". Group II was further divided into three subgroups. Subgroup II consists of lines C79, C81, C43 and RHA266, where RHA266 is the parental line of these genotypes. In group III, the highest

Table 3. Jaccard's coefficient similarity matrix for 28 sunflower genotypes based on SSR data.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	0.48																										
2	0.52	0.69																									
3	0.49	0.63	0.61																								
4	0.53	0.49	0.49	0.65																							
5	0.55	0.52	0.55	0.44	0.47																						
6	0.52	0.88	0.67	0.53	0.48	0.53																					
7	0.56	0.80	0.71	0.68	0.54	0.61	0.74																				
8	0.37	0.48	0.45	0.42	0.35	0.47	0.44	0.52																			
9	0.47	0.52	0.53	0.45	0.47	0.48	0.45	0.49	0.33																		
10	0.57	0.46	0.56	0.44	0.57	0.59	0.49	0.57	0.50	0.44																	
11	0.52	0.57	0.59	0.70	0.61	0.54	0.55	0.59	0.40	0.55	0.51																
12	0.47	0.44	0.55	0.43	0.38	0.49	0.43	0.49	0.45	0.42	0.52	0.49															
13	0.61	0.51	0.57	0.45	0.44	0.49	0.49	0.53	0.40	0.53	0.57	0.51	0.57														
14	0.61	0.54	0.62	0.54	0.56	0.44	0.48	0.56	0.42	0.54	0.52	0.57	0.47	0.61													
15	0.60	0.55	0.53	0.41	0.46	0.51	0.49	0.56	0.37	0.48	0.49	0.45	0.43	0.50	0.57												
16	0.56	0.46	0.48	0.38	0.48	0.50	0.49	0.46	0.44	0.38	0.67	0.45	0.50	0.58	0.47	0.49											
17	0.47	0.43	0.50	0.49	0.40	0.47	0.38	0.51	0.39	0.38	0.51	0.45	0.50	0.45	0.50	0.45	0.42										
18	0.46	0.47	0.51	0.47	0.39	0.50	0.45	0.51	0.42	0.53	0.51	0.49	0.56	0.44	0.56	0.45	0.49	0.51									
19	0.38	0.47	0.55	0.60	0.45	0.39	0.41	0.54	0.37	0.47	0.37	0.57	0.55	0.44	0.53	0.37	0.40	0.53	0.64								
20	0.38	0.35	0.45	0.47	0.34	0.34	0.30	0.34	0.31	0.41	0.40	0.45	0.48	0.48	0.43	0.35	0.40	0.71	0.56	0.61							
21	0.36	0.42	0.45	0.53	0.43	0.41	0.39	0.48	0.43	0.40	0.39	0.49	0.47	0.31	0.40	0.31	0.34	0.67	0.57	0.75	0.64						
22	0.43	0.37	0.39	0.35	0.38	0.48	0.39	0.40	0.38	0.54	0.48	0.38	0.44	0.42	0.41	0.30	0.43	0.36	0.65	0.45	0.41	0.40					
23	0.53	0.52	0.54	0.67	0.48	0.43	0.46	0.60	0.35	0.44	0.43	0.58	0.56	0.57	0.49	0.48	0.42	0.51	0.60	0.66	0.61	0.55	0.47				
24	0.39	0.39	0.51	0.43	0.51	0.40	0.37	0.44	0.25	0.44	0.54	0.51	0.50	0.45	0.48	0.34	0.39	0.50	0.44	0.58	0.45	0.47	0.45	0.42			
25	0.38	0.36	0.40	0.34	0.38	0.42	0.36	0.40	0.42	0.52	0.48	0.36	0.39	0.37	0.43	0.30	0.38	0.32	0.60	0.44	0.36	0.40	0.89	0.42	0.44		
26	0.35	0.37	0.43	0.34	0.32	0.41	0.36	0.42	0.35	0.41	0.52	0.36	0.43	0.39	0.33	0.30	0.30	0.54	0.52	0.42	0.57	0.51	0.63	0.46	0.50	0.63	
27	0.44	0.36	0.40	0.37	0.39	0.49	0.35	0.41	0.32	0.52	0.44	0.36	0.42	0.40	0.38	0.33	0.35	0.44	0.60	0.45	0.48	0.50	0.84	0.50	0.44	0.78	0.72

similarity value was found between genotypes AS613 and M6-133-2 (*GSj* = 0.51). However, the genotype M6-133-2 is a mutant line that was produced by gamma irradiation of AS613 seeds. In group IV, the highest similarity value was found between genotypes H565R and AS5305 (*GSj* = 0.56), as well as inbred lines with the same geographic origin (France). Comparing with group II and III, the pair-wise similarities in group IV were higher. Genotypes in group IV were clustered together at a higher similarity value. "NS-B4" was placed in a separated cluster with very low similarity to other groups.

Principal coordinate analysis showed that the first three eigenvalues explained 34.05% of the cumulative variation, which were then plotted to identify the diversity of the genotypes (Figure 3). It showed the close genetic relationship between AS613, M6-133-2, M6-85-3 and M6-894-2 as well as between C43, C79, LR64, M6-54-1, RHA266, C81, PAC2 and B454/03 that had also been

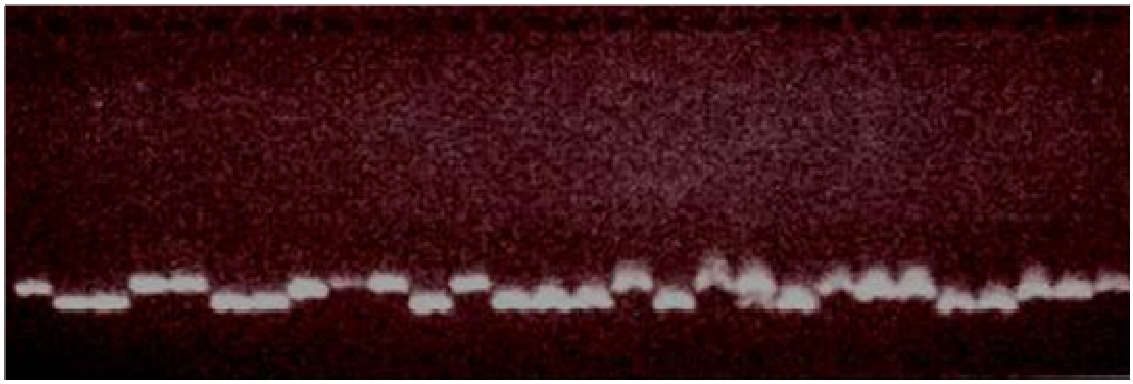

Figure 1. Polymorphism detected by SSR primer Ha3638. Lanes from left to right: M6-85-3, C43, RHA266, F651/1, NS-B4, B454/03, C79, M6-894-2, ENSAT-B5, ENSAT-R4, C81, RT931, LR64, F1250/03, RHA274, SDR18, H543R, LC1064C, PAC2, ENSAT-R5, SDB1, M6-133-2, H565R, M6-54-1, SDR19, AS613, AS5305 and SDB3.

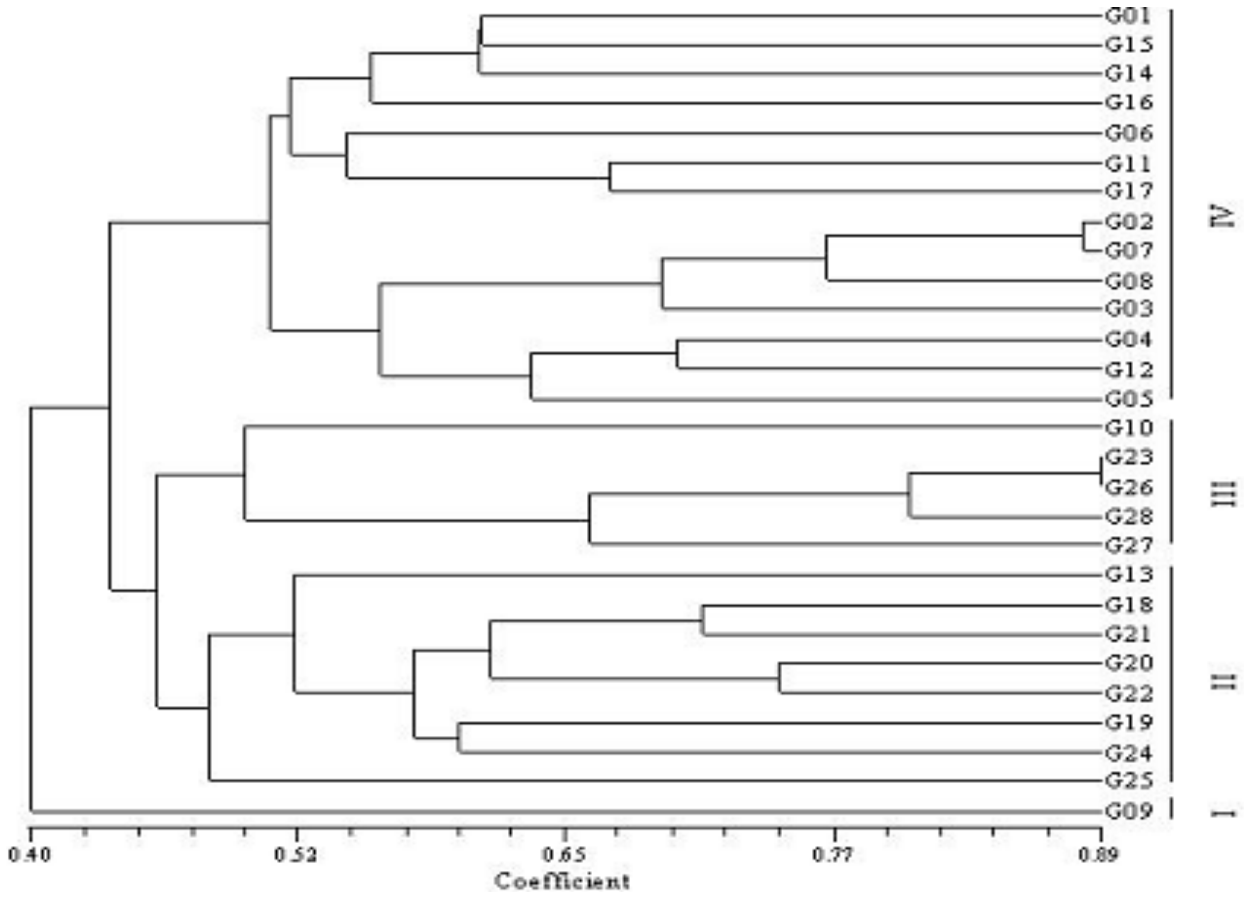

Figure 2. UPGMA dendrogram of 28 sunflower genotypes that used 38 SSR loci and Jaccard's similarity coefficient.

observed through UPGMA dendrogram (Figure 2).

DISCUSSION

Information about the genetic diversity and relationships among breeding lines and varieties is not only useful for germplasm conservation and inbred line identification, but also for the selection of parental lines for hybrid breeding in crops, including sunlfower (Senior et al., 1998; Sun et al., 2001). Microsatellites or SSRs constitute the current marker system of choice for characterizing sunflower

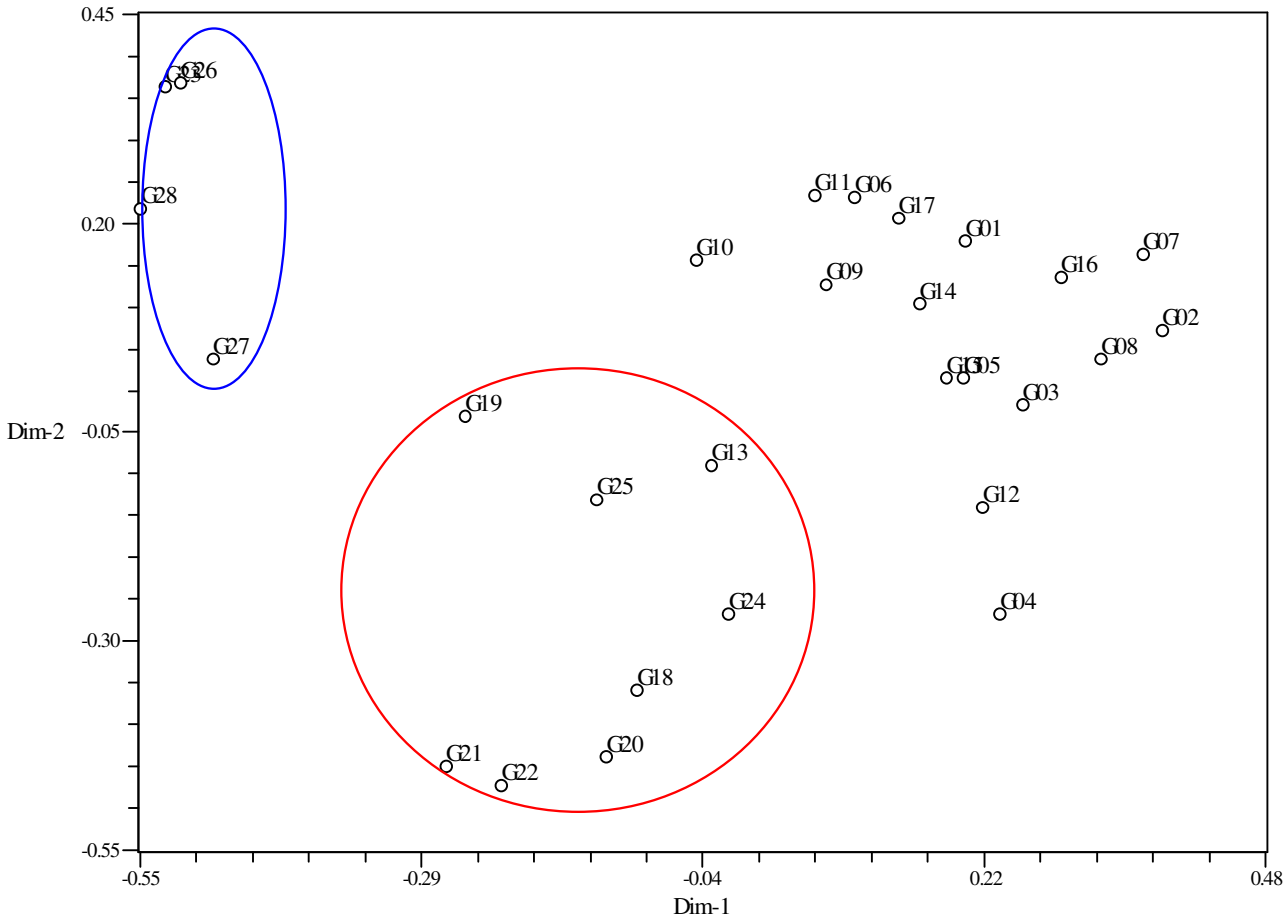

Figure 3. Two dimensional plot of the genetic relationship among 28 sunflower genotypes as revealed by PCoA.

germplasm (Yu et al., 2002; Paniego et al., 2002; Tang et al., 2002; Tang and Knapp, 2003; Zhang et al., 2005; Hvarleva et al., 2007).

In the current study, the mean number of allele per locus is 2.32, which is close to that obtained in Hvarleva et al. (2007) (Table 2). It is much lower than the mean number of allele per locus reported in other studies for inbred lines and hybrids (Tang and Knapp, 2003 and Yu et al., 2002). The lower value obtained in the study's research may be due to the low number of markers analyzed in comparison with previous studies. For example, Tang and Knapp (2003) used 122 microsatellite marker loci for genotyping 9 elite confectionery and oilseed sunflower inbred lines and 3.5 allele per locus averagely reported.

In the study's research work, agarose-gel electrophoresis was used for the screening of the microsatellites, compared to polyacryl-amide-gel electrophoresis or automated analysis. This is the most-appropriate technology for routine analysis of these kinds of markers. However, it is possible that an automated detection system would be able to resolve allelic variation at a finer scale than gel-electrophoresis analysis, and consequently, the number of alleles obtained would even be higher than that reported in this work. The automated detection system utilized in some study such as Hokanson et al. (1998) was capable of resolving allelic variation at a finer scale.

PIC values estimate the discriminatory power of a marker and is defined as the probability that a given marker genotype of an affected parent's offspring will permit the deduction of the parental genotype at the marker locus (Botstein et al., 1980). The mean of PIC values for markers used in this study was 0.41, and among 28 sunflower genotypes, it ranged from 0.09 for locus ha3555 to 0.62 for ORS598 loci (Table 2). Markers with high PIC values such as ORS160, ORS16, ORS920, ORS1068, Ha4149, Ha2879, ORS988, ORS899, ORS1088, ORS598 and ORS822 could be effectively used in genetic diversity studies of sunflower.

Studied genotypes were clustered in four groups based on UPGMA clustering method (Figure 2). Some studied genotypes have specifically, the same characters or coancestory relations that influence their clustering. Genotype RHA266 was the parent of genotypes C81, C43, C79 and all located in cluster II. AS613 was the

original line of mutant genotypes M6-133-2, M6-85-3 and M6-894-2. All these lines were clustered together in group III. In group IV, genotypes SDR18, SDR19, SDB1 and SDB3 are resistant to phomopsis disease (data not published) and are developed in sunflower breeding programs of South Dakota University. Highly diverse sunflower genotypes derived from the current study could be used in further breeding programs. The results of PCoA largely corresponded to those obtained through cluster analysis. Genotypes AS613, M6-133-2, M6-85-3 and M6-894-2 or genotypes C43, C79, LR64, M6-54-1, RHA266, C81 and B454/03 were found to be closer to each other (Figure 3). However, this is in agreement with other findings (Kumar et al., 2009; Sorkhe et al., 2007).

Information about genetic diversity also permits the classification of germ-plasm into heterotic groups, which is particularly important to hybrid breeding. Even though the genetic mechanisms that explain heterosis are not fully understood, it is well documented that crosses between unrelated, and consequently genetically distant parents, show greater hybrid vigor than crosses between closely related parents (Stuber, 1994). Estimates of molecular marker-based genetic distance have proven to be a useful way in describing existing heterotic groups, identifying new heterotic groups and assigning inbreeds of unknown genetic origin to established heterotic groups (Dubreuil et al., 1996; Saghai Maroof et al., 1997; Hongtrakul et al., 1997; Pejic et al., 1998; Casa et al., 2002). In the next step, some genotypes showing high genetic distance based on SSR markers will be chosen and crossed in a diallel mating design in order to produce the F1 hybrids. Parental genotypes and their F1 hybrids will be evaluated for yield and yield components in greenhouse as well as field experiments in a randomized complete block design with three replications. Moreover, relationship between heterosis and genetic distance based on SSR markers will be investigated. This experiment also encourages us to identify the most promising combination (F1) in order to produce a mapping population for identifying the QTLs controlling agronomic traits.

ACKNOWLEDGEMENT

The authors are grateful for the support provided by the Institute of Biotechnology, Urmia University, Iran.

Abbreviations

SSR, Simple sequence repeat; **QTL,** quantitative trait loci; **PIC,** polymorphism information content; PCoA, principal coordinate analysis; **RAPD,** random amplified polymorphic DNA; **RFLP,** restriction fragment length polymorphism; **RILs,** recombinant inbred lines; **M,** mutant; **BL,** breeder lines; **SSD,** single seed descent; **PCR,** polymerase chain reaction.

REFERENCES

Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrells ME (1992). Optimizing parental selection for genetic linkage maps. Genome, 36: 181-186.

Arias DM, Rieseberg LH (1995). Genetic relationships among domesticated and wild sunflowers (*Helianthus annuus* L.). Econ. Bot. 49: 239-248.

Botstein D, White RL, Skolnick M, Davis RE (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32: 314-331.

Casa AM, Mitchell SE, Smith OS, Register JC, Wessler SR, Kresovich S (2002). Evaluation of Hbr (MITE) markers for assessment of genetic relationships among maize (*Zea mays* L.) inbred lines. Theor. Appl. Genet. 104: 104-110.

Chalmers KJ, Campbell AW, Kretschmer J, Karakousis A (2001). Construction of three linkage maps in bread wheat, *Triticum aestivum* L. Aust. J. Agric. Res. 52: 1089-1119.

Darvishzadeh R, Hewezi T, Gentzbittel L, Sarrafi A (2008a). Quantitative real-time RT-PCR based gene expression profiling in compatible and partial compatible sunflower-*Phoma macdonaldii* interactions. Crop Prot. 27: 740-746.

Darvishzadeh R, Poormohammad Kiani S, Huguet T, Sarrafi A (2008b). Genetic variation and identification of molecular markers associated with partial resistance to black stem in gamma-irradiation induced mutants in sunflower (*Heliantus annuus* L.). Can. J. Plant Pathol. 30: 106-114.

Davar R, Darvishzadeh R, Majd A, Ghosta Y, Sarrafi A (2009). QTL mapping of partial resistance to basal stem rot in sunflower (*Helianthus annuus* L.) using recombinant inbred line population. 6th National Biotechnology Congress of Iran; Aug 13-15; Tehran: Milad Tower Conference Hall.

Dellaporta SL (1983). A plant minipreparation. Plant Mol. Biol. Rep. 1: 19-21.

Dubreuil P, Dufour P, Krejci E, Causse M, De Vienne D, Gallais A, Charcosset A (1996). Organization of RFLP diversity among inbred lines of maize representing the most significant heterotic groups. Crop Sci. 36: 790-799.

Hernández P, De la Rosa R, Rallo L, Dorado G (2001). Development of SCAR markers in olive (*Olea europaea*) by direct sequencing of RAPD products: applications in olive germplasm evaluation and mapping. Theor. Appl. Genet. 103: 788-791.

Hokanson SC, Szewc-McFadden AK, Lamboy WF, McFerson JR (1998). Microsatellite (SSR) markers reveal genetic identities, genetic diversity and relationships in a *Malus domestica* borkh core subset collection. Theor. Appl. Genet. 97: 671-683.

Hongtrakul V, Huestis GM, Knapp SJ (1997). Amplified fragment length polymorphisms as a tool for DNA fingerprinting sunflower germplasm: Genetic diversity among oilseed inbred lines. Theor. Appl. Genet. 95: 400-407.

Hvarleva TZ, Bakalova A, Chepinski I, Hristova-Cherbadji M, Hristov M, Atanasov A (2007). Characterization of bulgarian sunflower cultivars and inbred lines with microsatellite markers. Biotechnol. Biotechnol. Equip. (Bulgaria), 21(4): 408-412.

Jaccard P (1908). Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44: 223-270.

Konarev VG (2000). Cultivar identification and gene pool registration by seed proteins in cultivated plants. St. Petersburg: Vses. Inst. Rastenievod, Russia.

Kumar V, Sharma S, Sharma AK, Sharma S, Bhat KV (2009). Comparative analysis of diversity based on morpho-agronomic traits and microsatellite markers in common bean. Euphytica, 173: 249-262.

Leclercq P (1969). Une stérilité male cytoplasmique chez le tournesol. Ann. Amelior Plant, 19: 99-106.

Liu A, Burke JM (2006). Patterns of nucleotide diversity in wild and cultivated sunflower. Genetics, 173: 321-330.

Mohammadi SA (2006). Analysis of molecular data: Genetic diversity and population structure aspects (Key paper). 9th Iranian Crop Production and Breeding Science Congress, Aboureyhan campus-University of Tehran, Iran, Aug. 27-29 (Farsi).

Paniego N, Muñoz M, Echaide M, Fernandez L, Faccio P, Zandomeni R, Suarez E, Hopp E (1999). Microsatellite development for

sunflower. Plant and Animal Genome VII Conf., San Diego: Starford Univ. Press.
Paniego N, Echaide M, Munoz M, Fernandez L, Torales S, Faccio P, Fuxan I, Crrera M, Zandomeni R, Syarez EY, Hopp E (2002). Microsatellite isolation and characterization in sunflower (*Helianthus annuus* L.). Genome, 45: 34-43.
Pejic I, Ajmone-Marsan P, Morgante M, Kozumplick V, Castiglioni P, Taramino G, Motto M (1998). Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSRs, and AFLPs. Theor. Appl. Genet. 97: 1248-1255.
Poormohammad Kiani S, Talia P, Maury P, Grieu, P, Heinz R, Perrault A, Nishinakamasu V, Hopp E, Gentzbittel L, Paniego N, Sarrafi A (2007a). Genetic analysis of plant water status and osmotic adjustment in recombinant inbred lines of sunflower under two water treatments. Plant Sci. 172: 773-787.
Poormohammad Kiani S, Maury P, Darvishzadeh R, Nouri L, Grieu P, Sarrafi A (2007b). Genetic variation and identification of molecular markers associated with osmotic adjustment-related traits in gamma irradiation-induced mutants of sunflower (*Helianthus annuus* L.). J. Genet. Breed. (in press).
Rachid Al-Chaarani G, Roustaee A, Gentzbittel L, Mokrani L, Barrault G, Dechamp-Guillaume G, Sarrafi A (2002). A QTL analysis of sunflower partial resistance to downy mildew (*Plasmopara halstedii*) and black stem (*Phoma macdonaldii*) by the use of recombinant inbred lines (RILs). Theor. Appl. Genet. 104: 490-496.
Rieseberg LH, Seiler GJ (1990). Molecular evidence and the origin and development of the domesticated sunflower (*Helianthus annuus* L.). Econ. Bot. 44: 79-91.
Rohlf FJ (1998). NTSYS-pc: Numerical taxonomy and multivariate analysis system, version 2.02. Exter Software, Setauket, New York.
Saghai-Maroof MA, Yang GP, Zhang Q, Gravois KA (1997). Correlation between molecular marker distance and hybrid performance in U.S. Southern long grain rice. Crop Sci. 37: 145-150.
Sarrafi A, Kayyal H, Rachid Al-Chaarani G, Cantin F, Chaline AS, Durielle AS (2000). Inheritance of organogenesis parameters in cotyledons of sunflower (*Heliantus annuus* L). J. Genet. Breed. 54: 227-231.
Senior ML, Murphy JP, Goodman MM, Stuber CW (1998). Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. Crop Sci. 38: 1088-1098.
Sorkhe K, Shiran B, Gradziel TM, Epperson BK, Martinez-Gomez P, Asadi A (2007). Amplified fragment length polymorphism as a tool for molecular characterization of almond germplasm: genetic diversity among cultivated genotypes and related wild species of almond, and its relationships with agronomic traits. Euphytica, 156: 327-344.
Stuber CW (1994). Heterosis in plant breeding. Plant Breed Rev. 12: 227-251.
Sun GL, William M, Liu J, Kasha KJ, Pauls KP (2001). Microsatellite and RAPD polymorphisms in Ontario corn hybrids are related to the commercial sources and maturity ratings. Mol. Breed. 7: 13-24.
Tang S, Knapp SJ (2003). Microsatellites uncover extraordinary diversity in Native American land races and wild populations of cultivated sunflower. Theor. Appl. Genet. 106: 990-1003.
Tang S, Yu JK, Slabaugh MB, Shintani DK, Knapp SJ (2002). Simple sequence repeat map of the sunflower genome. Theor. Appl. Genet. 105: 1124-1136.
Thormann CE, Ferreira ME, Camargo LEA, Tivang JG, Osborn TC (1994). Comparison of RFLP and RAPD markers to estimating genetic relationships within and among cruciferous species. Theor. Appl. Genet. 88: 973-980.
Yu JK, Mangor J, Thompson L, Edwards KJ, Slabaugh MB, Knapp SJ (2002). Allelic diversity of simple sequence repeats among elite inbred lines of cultivated sunflower. Genome, 45: 652-660.
Zhang LS, Le Clerc V, Li S, Zhang D (2005). Establishment of an effective set of simple sequence repeat markers for sunflower variety identification and diversity assessment. Can. J. Bot. 83: 66-72.