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Assessment of genetic diversity among sunflower genotypes using microsatellite markers

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ABSTRACT

Genetic diversity estimation of plant materials is one of the important pre-breeding activities in breeding field crops. Twenty-one microsatellite markers used to assess genetic diversity and relationship of 68 sunflower genotypes (*Helianthus annuus* L.). All of 21 pairs of SSR (Simple Sequence Repeats) markers produced a total number of 49 polymorphic bands. DNA fragments ranged from 92 to 850 bp. The highest and lowest polymorphic information content (PIC) values were determined as 0.58 and 0.10 for marker Ha806-ar and Ha494-ar. The number of alleles per locus was calculated as 2-6 with the average of 2.86. In this study, CMS (Cytoplasmic Male Sterility) lines showed the highest and Iranian hybrids showed the least polymorphism, respectively. Principal coordinates analysis revealed that Iranian hybrids were well-separated compare to the other groups. The analysis of molecular variance (AMOVA) indicated higher genetic variation within groups (90%) rather than among groups (10%). This study revealed that the SSR markers such as Ha806-ar could be a useful tool for distinguishing sunflowers genotypes. According to the study, there is a significant genetic distance among individuals. Parental lines (R26 and CMS502 lines with lowest similarity coefficient) may be useful for future sunflower crossing and hybrid breeding programs. Generally, high similarity coefficient estimation among investigated sunflower groups revealed that there was a narrow genetic base in investigated materials suggesting broadening its genetic base by introduction of new genes into existing breeding materials.

Keywords: cluster analysis; hybrid; polymorphism; principal coordinates analysis; SSR

INTRODUCTION

Sunflower is one of the most important crops in the world grown for edible oil, after soybean (*Glycine max* L.), rapeseed (*Brassica rapa* L.) and peanut (*Arachis hypogaea* L.) [1]. Since 1969, production of commercial sunflower hybrids has been utilized based on a single cytoplasmic male sterility source, PET1, discovered by leclerq. Nowadays, development of new

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CMS sources of male sterility as well as corresponding fertility restorers, is a special interest of sunflower breeders for increasing genetic diversity. The main goals of Iranian sunflower breeders are developing single and three-way cross hybrids with high oil and seed yield as well as resistance to biotic and abiotic stresses [2]. Sunflower hybrid production is based on heterosis phenomenon and there are many commercial hybrid varieties available. For commercial use of heterosis phenomenon, parental inbred lines need to be selected 6-7 generations by self-pollination. These days, there is a need for inbred parental lines with distinct genetic resources for high yield in the sunflower [3]. Evaluation of genetic diversity of such parental lines via various molecular markers is very important in sunflower hybrid breeding programs. Most of the time, identification of sunflower parental lines is based on morphologic characters but they are limited in number and unstable. DNA markers, which are available throughout the genome, are unlimited in number and environments or epistasis does not affect their presence or absence [4]. Some researchers have reported high genetic variation for sunflower [5-7]. However, some studies have also showed a narrow genetic diversity. Compared to wild sunflower, cultured sunflower has 50-60% lower genetic diversity [8].

Molecular markers, which are becoming increasingly widespread in sunflower breeding, provides great convenience in terms of cost and time [9]. Up to now, only conventional breeding methods have been used in sunflower breeding programs in Iran. However, in recent years, some studies have been reported the use of various molecular markers on Iranian sunflower germplasms such as TRAP [10], SSR [4, 11], ISSR [12], IRAP and REMAP [13]. SSR marker is more preferred than other molecular markers in genome studies because it is highly polymorphic, co-dominant inherited, highly abundant, analytically simple and readily transferable [14]. However, there are limited data available in the literature on the analysis of genomic relationships among and within cultured sunflower in Iran by using SSR markers. The aim of this study was to i) determine the genetic diversity of sunflower genotypes ii) determine high value PIC of SSR markers iii) estimation of the genetic distance between restorer and CMS inbred lines in order to be able to use them in the hybrid production program.

MATERIALS AND METHODS

Plant material: In this study, 68 sunflower genotypes (*Helianthus annuus* L.) in five different groups including 23 restorer lines, 30 CMS lines, 8 foreign hybrids, 3 Iranian hybrids and 4 foreign open pollinated cultivars were used as plant materials (Table 1). These restorers and CMS inbred lines were recently used in sunflower breeding program in Iran. 15-20 days old fresh leaves as bulk was provided for DNA isolation using fast and efficient protocol described by Zeinalzadeh Tabrizi et al. [15].

PCR components, conditions and visualization of products: Twenty one SSR primers developed by Tang et al., [16] and Paniego et al., [17] were used for SSR analysis (Table 2). PCR reaction was carried out using Multigene Gradient Thermal Cycler (TC9600-G-230V, Labnet International, Inc.). For PCR, each 20 µL reaction includes 2 µL of 10X PCR buffer, 1 µL of 25 mM $MgCl_2$, 0.5 µL of each of the 10 µM dNTPs, 0.5 µL of each of the 100 pmol primers, 1 unit of the Taq polymerase and 1 µL of template DNA.

Thermal Cycler program was one cycle for initial denaturation for 2 min at 96°C. Then 30 cycles of 30 sec at 96°C for denaturation, 45 sec at 50-60°C for annealing (according to the appropriate temperature of each pair of primers), 1 min at 72°C for primer extension and 15 min at 72°C for final extension. The electrophoresis of PCR products was run on 2% agarose gel in 1X SB buffer at 200 V for 120 min, stained with ethidium bromide (0.2 ug ml^{-1}) and visualized under a UV-trans illuminator. The molecular weight of bands was determined based on a DNA ladder between 50 and 1000 bp (Vivantis Product No: NM2421).

Table 1: Characteristics of sunflower genotypes used in assessment of genetic diversity by SSR marker

Genotype name	Type	Genotype name	Type
R2	Restorer Line (R)	Zaria	Foreign Open-pollinated (FO)
R3	Restorer Line (R)	Record	Foreign Open-pollinated (FO)
R5	Restorer Line (R)	CMS 16	CMS Line (CMS)
R15	Restorer Line (R)	CMS 26	CMS Line (CMS)
R19	Restorer Line (R)	CMS 28	CMS Line (CMS)
R21	Restorer Line (R)	CMS 30	CMS Line (CMS)
R22	Restorer Line (R)	CMS 32	CMS Line (CMS)
R23	Restorer Line (R)	CMS 36	CMS Line (CMS)
R24	Restorer Line (R)	CMS 38	CMS Line (CMS)
R27	Restorer Line (R)	CMS 40	CMS Line (CMS)
R29	Restorer Line (R)	CMS 42	CMS Line (CMS)
R33	Restorer Line (R)	CMS 44	CMS Line (CMS)
R34	Restorer Line (R)	CMS 46	CMS Line (CMS)
R38	Restorer Line (R)	CMS 54	CMS Line (CMS)
R41	Restorer Line (R)	CMS 58	CMS Line (CMS)
R42	Restorer Line (R)	CMS 78	CMS Line (CMS)
R43	Restorer Line (R)	CMS 82	CMS Line (CMS)
R46	Restorer Line (R)	CMS 96	CMS Line (CMS)
R50	Restorer Line (R)	CMS 110	CMS Line (CMS)
R53	Restorer Line (R)	CMS 154	CMS Line (CMS)
R55	Restorer Line (R)	CMS 212	CMS Line (CMS)
R56	Restorer Line (R)	CMS 222	CMS Line (CMS)
Armada-GL	Foreign Hybrid (FH)	CMS 234	CMS Line (CMS)
ES-Biba	Foreign Hybrid (FH)	CMS 262	CMS Line (CMS)
ES-Karamba	Foreign Hybrid (FH)	CMS 298	CMS Line (CMS)
Brocar-RM	Foreign Hybrid (FH)	CMS 310	CMS Line (CMS)
Euroflor	Foreign Hybrid (FH)	CMS 328	CMS Line (CMS)
Tekny	Foreign Hybrid (FH)	CMS 330	CMS Line (CMS)
Allstar	Foreign Hybrid (FH)	CMS 354	CMS Line (CMS)
Sor	Foreign Hybrid (FH)	CMS 358	CMS Line (CMS)
R453	Iranian Hybrid (IH)	CMS 376	CMS Line (CMS)
43/128	Iranian Hybrid	CMS 502	CMS Line (CMS)
Azargol	Iranian Hybrid		
Berezanski	Foreign Open-pollinated (FO)		
Master	Foreign Open-pollinated (FO)		

Table 2: Locus, motif, sequences and expected size range of 21 simple sequence repeat (SSR) primer pairs

No.	Locus	Motif	Foreword primer sequence 5′–3′	Reverse primer sequence 5′–3′	Size range (bp)
1	Ha 432	GT	CTT TAT CCC CCA CCC CCT CC	GGG TTT AGT GGC CAG TAG TTG TC	168-720
2	Ha 514	GA	GGT CAA CGG ATT TAG AGT C	GTA TTG ATT CCA ACA TCC AG	164-200
3	Ha 1327	ATT	CCG TTA GGT AGT TTA CTT GCG AC	GGT GGG GGG AAT ATT CTG AGG TG	175-550
4	Ha 1442	ATT	GCT TAT GTG CTT ACG TGT TCC TG	CTA AAC AGT TCG GCG AGT GTA GG	202-244
5	Ha 1608	ATT	GAT CTT AGG TCC GCC AC	GAT GGC ATT TGG CTA GAC	169-331
6	ORS-6	AGG	GTG GAG AGA GGT GTA GAG AGC	CAC CCC TCA CCC TGA CAC	250-260
7	ORS-5	AAC	ATC TGG AGC AGC AAA TTC AG	CTG CTG CCC ACC ATA CTG	275-350
8	HNCA-2	GT	TGA GAC AAG CAT AAG CAC	TAG ACA AGA CAA GGG ACT	208-320
9	Ha 357-ar	(GA)15	GTGGGTGTGGAAGGAAGAATC	CAGACACATGCTAGTCGTCGTG	92-132
10	Ha 360-ar	(GA)15	CAACAAGGAACCGATAACTGCT	CACCCTTCATCTCCTTC	191-199
11	Ha 494-ar	(GA)17 A (GA)2 N12 T15	GCGTTGGTTAAGGCCTGAGGTC	GAGCAGCAAACAGAGGGTACACC	193-209
12	Ha 806-ar	(GT)8 N27 (GA)6	GATGTTCCTTCCTGCAC	GGTTGGATAATGGGGCAGC	189-199
13	Ha 991-ar	(GA)4 T (GA)12	GCCCCCTTGATGCCCTTTTC	GAATCGCCATTTGAATCGCCAG	137-145
14	Ha 1167-ar	(GT)9 N2 (GT)4	CGATGTCGGCGATTCGGACTGGAG	CCCCATCTACACTTCAATACTG	155-165
15	Ha 1287-ar	(GA)26	GATATGAGCCCATCACTCATC	GAAGATATGTCAGGTCACACCC	151-171
16	ORS-31	$(AAG)_{10}$	AAT TCA TGC CCC AAG AGA TG	CAC AAT TCA TGC ATT TCT CTG G	250-300
17	ORS-53	$(T)_{30}$	GCT GGC AAT TTC TGA TAC ACG AT	CAT CTA GAC AAC GAC AGA AGA TG	450-510
18	ORS-78	$(AAG)_{10}$	GTT CGT CGA GTA CAT GTT CTG C	TTT CCC TCT GGA AAG TTG TCA	160-172
19	ORS-176	$(TG)_{16}$	CCCTAACTGGTTTTCTGACCC	AACTTTTGTTTGTTTGTCCAGG	430-480
20	ORS-204	$(GT)_{17}$	CGTCTGGCATTATGAAATCGTC	CCGCATAACAGCAATGGTCAAC	280-300
21	ORS-54	$(TACA)_{25}$	AAATCCCACTTCATACAAACGT	CCTTCAGTGCTCATGCAGTG	370-580

Statistical analysis: The clearly selectable and easily countable bands on the gel were scored as 0 (absent), 1 (present) and 9 (missing band or not amplified). The binary data matrix was analyzed using three different software packages, including NTSYS-pc version 2.11f [18], Popgen [19] and GenAlex [20]. The Polymorphic information content (PIC) values for each marker was estimated using Power Marker [21] software as described by Anderson et al. [22].

$$PIC = 1 - \sum_{i=1}^{1} p_i^2$$

where Pi is the proportion of the population carrying the ith allele, calculated for each microsatellite locus.

The genetic diversity within the groups was calculated using Nei's Gene Diversity Index [23] and Shannon's Information Index [24] using Popgen software [19]. The genetic distance between groups was measured using Nei's coefficient [23]. GenAlex program [20] was used to determine the band patterns among groups. Molecular variance analysis (AMOVA) within and among groups and principal coordinates analysis (PCoA) was examined using GenAlex [20]. Due to unbalanced genotype numbers per group and to confirm the results of the cluster analysis, principal coordinates analysis was carried out using Nei's unbiased genetic distance.

In this study, three different genetic distance (Jaccard, Dice and Simple Matching) and three different cluster method (UPGMA, Single Linkage and Complete Linkage) was tested so that the highest cophenetic coefficient was selected for genetic distance and clustering method (Dice and UPGMA, r=0.745). NTSYS-pc version 2.11f [18] was used for cluster analysis of all genotypes. The similarity matrix was calculated using SIMQUAL subprogram and Dice [2a/(2a+b+c)] similarity coefficient. Grouping was done using the SAHN subprogram and UPGMA method. The cophenetic correlation for each dendrogram was computed as a measure of goodness of fit (Mantel t-test) for the method of clustering used.

RESULTS AND DISCUSSION

In this study, all the 21 pairs of SSR markers produced a total number of 49 repeatable and scoreable polymorphic bands. DNA fragments ranged from 92 to 850 bp. The highest and lowest PIC values were determined as 0.58 and 0.10 for Ha806-ar and Ha494-ar, respectively (Table 3). Markers with high PIC value such as Ha806-ar and ORS-31 are considered suitable markers for genetic diversity differentiation among individuals. In a similar research, Sahranavard Azartamar et al., [4] found that SSR markers HA3040 and ORS-733 with higher PIC values are considered appropriate markers for studying genetic diversity in oily sunflower. In present study, the PIC values of SSR markers were closed to Lochner [25] (0.06-0.75 and average of 0.51), Erasmus [26] (0.17-0.80 and average of 0.56) and Darvishzadeh et al., [27] (0.09-0.62 and average of 0.41). The number of alleles per locus was calculated as 2 to 6 with an average of 2.86 (Table 3) suggesting the presence of high molecular genetic variability among the studied sunflower genotypes which are in agreement with the finding of Gholizadeh et al., [28]. Antonova et al., [29] used 10 SSR markers in genetic diversity of 17 sunflower inbred lines and hybrids. The average number of loci in their study was measured 2.2, which was in a range of current findings. Average number of alleles per locus was reported 3.5 in Paniego et al., [17], 3 in Solodenko and Sivolap [30] and 2.32 in Darvishzadeh et al., [27] studies.

Genetic diversity of the groups was shown according to the Nei's Gene Diversity Index [31] and the Shannon's Information Index [32]. According to these indices, CMS group had the highest (0.307 and 0.443, respectively) and the Iranian hybrid group had the lowest genetic diversity (0.166 and 0.197, respectively).

The mean genetic variation within groups was calculated as 0.245 and 0.332, respectively (Table 4). In this study, CMS group showed the highest percentage of polymorphic loci (81.63%) with 26.714 polymorphic bands and the Iranian hybrid group consisted of 2.408

polymorphic bands with the least percentage of polymorphic loci (32.65%) (Table 4). The mean number of polymorphic bands and percentage of polymorphic loci in the five groups varied by 11.98% and 59.18%. In recent years, use of SSR markers considered as the easiest method to predict the level of polymorphism.

Table 3: Characteristics of investigated SSR loci

No.	Locus	Allele No.	Polymorphic Allel No.	Allele Frequency	Gene Diversity	Heterozygosity	PIC	Polymorphism Percentage
1	Ha 432	6	4	0.58	0.49	0.64	0.37	66.7%
2	Ha 514	2	2	0.72	0.40	0.14	0.32	100%
3	Ha 1327	5	5	0.59	0.48	0.11	0.37	100%
4	Ha 1442	2	1	0.73	0.40	0.55	0.32	50%
5	Ha 1608	4	4	0.56	0.49	0.11	0.37	100%
6	ORS-6	2	2	0.65	0.45	0.70	0.35	100%
7	ORS-5	3	3	0.66	0.45	0.12	0.35	100%
8	HNCA-2	3	2	0.66	0.45	0.68	0.35	66.7%
9	Ha 357-ar	2	2	0.60	0.48	0.15	0.37	100%
10	Ha 360-ar	2	2	0.56	0.49	0.09	0.37	100%
11	Ha 494-ar	3	3	0.95	0.10	0.04	0.10	100%
12	Ha 806-ar	3	3	0.39	0.66	0.79	0.58	100%
13	Ha 991-ar	2	2	0.74	0.38	0.00	0.31	100%
14	Ha 1167-ar	2	2	0.62	0.47	0.00	0.36	100%
15	Ha 1287-ar	5	5	0.79	0.35	0.05	0.34	100%
16	ORS-31	3	3	0.47	0.59	0.03	0.51	100%
17	ORS-53	3	3	0.63	0.48	0.10	0.38	100%
18	ORS-78	2	2	0.63	0.47	0.01	0.36	100%
19	ORS-176	2	2	0.70	0.42	0.18	0.33	100%
20	ORS-204	2	2	0.51	0.50	0.66	0.37	100%
21	ORS-54	3	2	0.55	0.50	0.02	0.37	66.7%

Table 4: Band number, number of different alleles, number of effective alleles, Shannon's information index, expected heterozygosity, unbiased expected heterozygosity and percentage of polymorphic loci

Groups		N	Na	Ne	I	He	UHe	PO
R	Mean	20.592	1.592	1.492	0.412	0.280	0.287	73.47%
FH	Mean	6.776	1.449	1.374	0.318	0.214	0.230	59.18%
IH	Mean	2.408	1.061	1.243	0.197	0.136	0.166	32.65%
FO	Mean	3.449	1.286	1.362	0.291	0.200	0.236	48.98%
CMS	Mean	26.714	1.694	1.527	0.443	0.301	0.307	81.63%
Total	Mean	11.988	1.416	1.400	0.332	0.226	0.245	59.18%

R: Restorer Line; FH: Foreign Hybrid; IH: Iranian Hybrid; FO: Foreign Open-pollinated; CMS: Cytoplasmic Male Sterility

According to SSR markers, the total band patterns across groups are given in Table 5. CMS group with 43 bands had the heights different bands compared to the Iranian hybrids with 36 bands. Two private bands (referring to unique bands found only within one population) of CMS group and one private band of Iranian hybrid group could be effective in identification of such heterotic groups.

Table 5: Total band patterns for binary data across groups by SSR markers

	Groups				
	R	FH	IH	FO	CMS
No. Bands	42	42	36	39	43
No. Bands Freq. >= 5%	40	42	36	39	42
No. Private Bands	0	0	1	0	2
No. LComm Bands (<=25%)	0	0	0	0	0
No. LComm Bands (<=50%)	0	4	1	1	2

No. Bands: No. of Different Bands; No. Bands Freq.>=5%: No. of Different Bands with a Frequency>=5%; No. Private Bands: No. of Bands Unique to a Single Population; No. LComm Bands (<=25%): No. of Locally Common Bands (Freq.>=5%) Found in 25% or Fewer Populations; No. LComm Bands (<=50%): No. of Locally Common Bands (Freq.>= 5%) Found in 50% or Fewer Populations

Table 6 provides the genetic diversity among the groups according to Nei's gene diversity index [23]. The highest genetic variation was found between the Iranian hybrids and Restorer lines (0.282) while the lowest genetic distance was determined between Restorer and CMS lines (0.070). Cluster analysis among the five groups using Nei's genetic distance and UPGMA method could help better understanding the relationship of the groups. Two heterotic groups (Iranian hybrids and the others) were determined by cutting the dendrogram (Fig. 1).

Table 6: Pairwise groups matrix of Nei's unbiased genetic distance using SSR markers

Groups	R	FH	IH	FO	CMS
R	0				
FH	0.110	0			
IH	0.282	0.258	0		
FO	0.140	0.176	0.206	0	
CMS	0.070	0.171	0.265	0.183	0

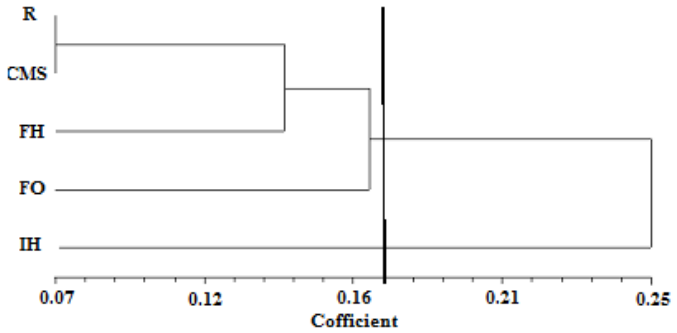

Figure 1: Dendrogram obtained by cluster analysis of sunflower groups using UPGMA method

Principal coordinates analysis (PCoA) revealed that the first two components determined 71% of total variance. Biplot of first and second components showed that Iranian hybrids were well-separated compare to the other groups (Fig. 2). In Dong et al., [33] study, principal component analysis showed that the 43.05% of total variance was contributed by the first two principal components. Hongtrakul et al., [34] used the principal coordinate analysis to genetic diversity of 23 sunflower restorer and CMS lines using AFLP markers. Results of their analysis revealed that the genotypes are divided into two main groups on a 2D biplot: a maintainer (B line) group and a restorer (R line) group. The first three components explained 34% of total variance. Darvishzadeh et al., [27] used principal coordinate analysis to determine the genetic diversity of 28 sunflower genotypes using SSR marker and to confirm the results of their cluster analysis. The first three components determined 34.05% of their total variance.

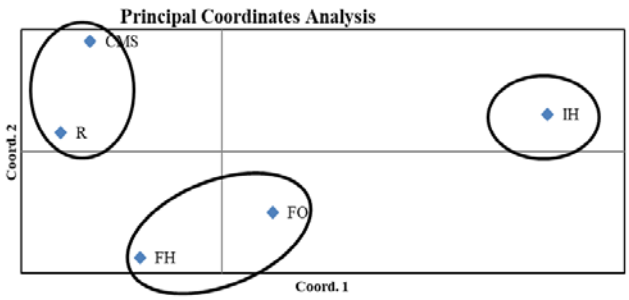

Figure 2: 2D biplot of sunflower groups obtained from principal coordinates analysis

Figure 3 provides cluster analysis among all genotypes to find farthest inbred lines from the aspect of genetic distance to may use in future sunflower hybrid production program. Minimum

similarity coefficient (maximum genetic distance) was calculated between R26 and CMS502 lines (0.364) and the average of 0.673 over 49 SSR polymorphic bands using Dice genetic similarity coefficient. Dice coefficient is one of the most commonly used similarity to estimate genetic distances. In addition, the calculated cophenetic correlation coefficient (r=0.745) in this study indicated that how similar the final hierarchical pattern and initial similarity (or distance) matrix are. Higher cophenetic correlation coefficient indicates a better goodness of fit of clustering method. Gholizadeh et al. [28] reported that among the different methods, the highest cophenetic value (r=0.764) was observed for the UPGMA created based on Jaccard's similarity coefficients. Darvishzadeh et al., [27] also used UPGMA method and Jaccard's similarity coefficients for genetic diversity of sunflower inbred lines. Yue et al., [7] reported that the boundaries between the groups based on the Simple Matching coefficient matrix were sharper than those based on the Dice coefficient matrix. Thus, the Simple Matching coefficient matrix was chosen for dendrogram construction in their study. Lower similarity coefficient was measured for CMS group (0.661) rather the other groups (Table 7). This result reflects that the genetic diversity of CMS lines is higher than the other groups and largely corresponded to those obtained through other analysis. The analysis of molecular variance (AMOVA) indicated higher genetic variation within groups (90%), than among groups (10%). The average number of migrants (Nm) among groups based on AMOVA was measured 4.57 (Table 8).

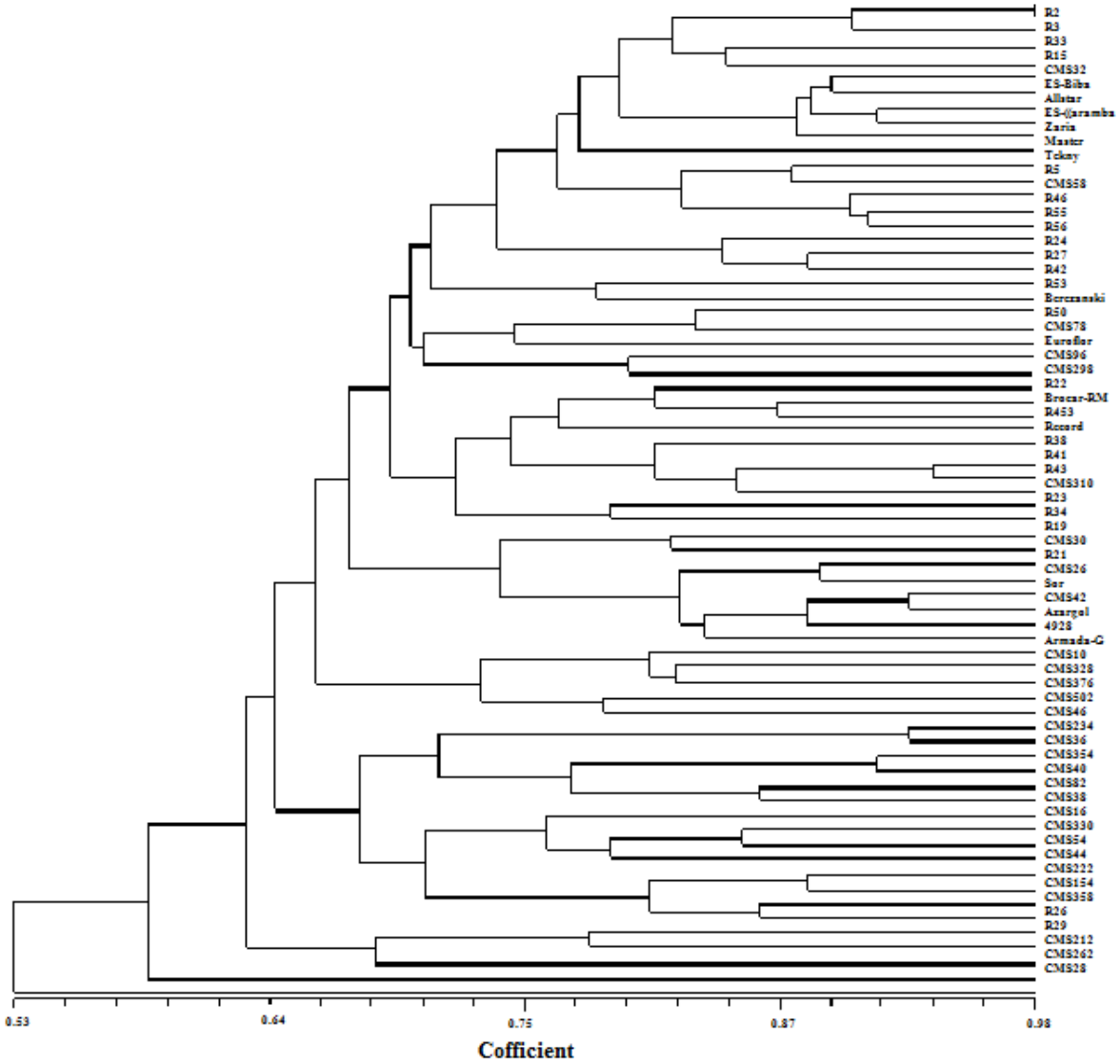

Figure 3: Dendrogram of 68 sunflower genotypes using Dice coefficient and UPGMA method

Higher genetic variability within population is a result of the mixed mating system [35] or may be due to high allogamy nature of the sunflower plant [36] or variations in selection, adaptation, migration, environment and human activities [37] or small size of the groups studied and insufficient number of markers studied for complete genome coverage [4]. In our previous research, higher genetic variation estimated within groups (87%), than among groups (13%) by using TRAP marker [10]. Same results displayed high variation within populations than among populations in Jannatdoust et al., [36], Sahranavard Azartamar et al., [4], Kholghi et al., [38] and Basirnia et al. [13] studies.

Table 7: Dice coefficient between groups of sunflower using SSR markers

Groups	Dice coefficient
R	0.721
FH	0.757
IH	0.750
FO	0.753
CMS	0.661
Mean	0.728

Table 8: AMOVA for 68 sunflower genotypes based on 21 SSR polymorphic loci

Source	Degrees of freedom (df)	Sum of squares (SS)	Mean squares (MS)	Estimated variance	Variance percentage	Stat	Value	P(rand >= data)
Among groups	4	105.072	26.268	1.277	10%	PhiPT	0.099	0.010
Within groups	63	736.001	11.683	11.683	90%			
Total	67	841.074		12.960	100%			

Genetic diversity of cultivated sunflower has narrowed due to cultural activities and breeding programs [3, 39]. Gentzbittel et al., [8] in a study of 17 sunflower lines using RFLP markers found lower genetic variability than the other field crops. They expressed the transfer of new genes from the wild sunflower species to cultivated sunflower. In Ronicke [40] study, genetic similarity among 25 inbred lines ranged from 0.58 to 0.98 with an average of 0.70. In Hongtrakul et al. [34] study using AFLP marker in 24 sunflower inbred lines, genetic similarity varied between 0.70 and 0.91. Yue et al., [7] showed that genetic similarity in 177 sunflower inbred lines ranged from 0.30 to 0.97 and an average of 0.58. The genetic similarity using SSR markers was determined an average of 0.728 (Table 7). The results showed that investigated sunflower genotypes had a narrow genetic base in general.

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Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Hu J, Seiler G, Kole C. Genetics, Genomics and Breeding of sunflower: CRC Press; 2010.
2. Zeinalzadeh Tabrizi H, Ghaffari M. Production of sunflower hybrids based on new cytoplasmic male sterility sources. 3rd International Symposium on Plant Protection and Plant Health in Europe; 2009; Berlin, Germany.
3. Liu A, Burke JM. Patterns of nucleotide diversity in wild and cultivated sunflower. Genetics 2005;173:321-330.

4. Sahranavard Azartamar F, Darvishzadeh R, Ghadimzadeh M, Azizi H, Aboulghasemi Z. Identification of SSR loci related to some important agro morphological traits in different oily sunflower (*Helianthus annuus* L.) lines using association mapping. J Plant Gene Res 2015;2:15-32. [In Persian]
5. Lawson W, Henry R, Kochman J, Kong G. Genetic diversity in sunflower (*Helianthus annuus* L.) as revealed by random amplified polymorphic DNA analysis. Crop Pasture Sci 1994;45:1319-1327.
6. Liu J, Liu G-S, Jan C-C. Comparison of genetic diversity of the germplasm resources of confectionary sunflower (*Helianthus annuus*) in China based on RAPDs and AFLPs. Acta Bot Sin 2003;45:352-358.
7. Yue B, Cai X, Vick BA, Hu J. Genetic diversity and relationships among 177 public sunflower inbred lines assessed by TRAP markers. Crop Sci 2009;49:1242-1249.
8. Gentzbittel L, Zhang Y-X, Vear F, Griveau B, Nicolas P. RFLP studies of genetic relationships among inbred lines of the cultivated sunflower, *Helianthus annuus* L.: evidence for distinct restorer and maintainer germplasm pools. Theor Appl Genet 1994;89:419-425.
9. Eryiğit T, Kumlay AM, Sancaktaroğlu S. Ayçiçeği (*Helianthus annuus* L.) çeşit teşhisinde morfolojik, biyokimyasal ve moleküler tekniklerin kullanımı. 9 Tarla Bitkileri Kongresi 2011; Bursa/Turkey. [In Turkish]
10. Zeinalzadeh-Tabrizi H, Haliloglu K, Razban-Haghighi A. Genetic diversity of sunflower genotypes (*Helianthus annuus* L.) using TRAP markers. Crop Biotechnol 2015;12:39-53. [In Persian]
11. Jannatdoust M, Darvishzadeh R, Azizi H, Ebrahimi M, Ziaefard R, Gholinezhad E. Identification of retrotransposon markers associated with agromorphological traits in confectionary sunflower (*Helianthus annuus* L.) under normal and drought stress conditions. J Crop Breed 2017;8:183-197.
12. Hatami MH, Darvishzadeh R, Mohseni Z. Evaluation of genetic diversity and classification of advanced sunflower lines using ISSR markers. J Agr Biotechnol 2014;3:33-44. [In Persian]
13. Basirnia A, Darvishzadeh R, Mandoulakani BA. Retrotransposon insertional polymorphism in sunflower (*Helianthus annuus* L.) lines revealed by IRAP and REMAP markers. Plant Biosyst 2014;1-12.
14. ZeinalzadehTabrizi H. Genetic diversity of sunflower genotypes using SSR and TRAP markers. Ph.D. Thesis. Graduate School of Natural and Applied Sciences. Ataturk University, Erzurum, Turkey; 2014 [In Turkish]
15. ZeinalzadehTabrizi H, Hosseinpour A, Aydin M, Haliloglu K. A modified genomic DNA extraction method from leaves of sunflower for PCR based analyzes. J Biol Env Sci 2015; 7:222-225.
16. Tang S, Yu J-K, Slabaugh M, Shintani D, Knapp S. Simple sequence repeat map of the sunflower genome. Theoretical and Applied Genetics. 2002;105:1124-1136.
17. Paniego N, Echaide M, Muñoz M, Fernández L, Torales S, Faccio P, Fuxan I, Carrera M, Zandomeni R, Suárez, EY. Microsatellite isolation and characterization in sunflower (*Helianthus annuus* L.). Genome 2002;45:34-43.
18. Rohlf FJ. NTSYS-pc: numerical taxonomy and multivariate analysis system: Applied Biostatistics; 1992.
19. Yeh F, Yang R, Boyle T, Ye Z, Mao J. POPGEN Ver. 1.32. The user-friendly software for population genetic analysis. Molecular Biology and Biotechnology Center, University of Alberta, Alberta, Canada. 1997.
20. Peakall R, Smouse PE. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 2006;6:288-295.
21. Liu K, Muse SV. PowerMarker: an integrated analysis environment for genetic marker analysis. Bioinformatics 2005;21:2128-2129.
22. Anderson JA, Churchill G, Autrique J, Tanksley S, Sorrells M. Optimizing parental selection for genetic linkage maps. Genome 1993;36:181-186.

23. Nei M, Tajima F, Tateno Y. Accuracy of estimated phylogenetic trees from molecular data. J Mol Evol 1983;19:153-170.
24. Lewontin RC. The Apportionment of Human Diversity. Evolutionary Biology: Springer; 1995.p.381-398.
25. Lochner TC, Agriculturae MS. Prediction of heterotic groups and hybrid performance in south african sunflower (*Helianthus annuus* L.) germplasm using SSR analysis. Msc Thesis, University of the Free State, South Africa. 2011.
26. Erasmus TE. Genetic diversity of proprietary inbred lines of sunflower, determined by mapped SSR markers and total protein analysis. Ph.D. Thesis, University of KwaZulu-Natal, Pietermaritzburg, South Africa. 2010.
27. Darvishzadeh R, Azizi M, Hatami-Maleki H, Bernousi I, Abdollahimandoulakani B, Jafari M, Sarrafi A. Molecular characterization and similarity relationships among sunflower (*Helianthus annuus* L.) inbred lines using some mapped simple sequence repeats. Afr J Biotechnol 2010;9:7280-7288.
28. Gholizadeh S, Darvishzadeh R, Mandoulakani BA, Bernousi I, Alavi SR, Masouleh AK. Molecular characterization and similarity relationships among flue-cured tobacco (*Nicotiana tabacum* L.) genotypes using simple sequence repeat markers. Not Bot Horti Agrobot Cluj Napoca 2012;40:247-253.
29. Antonova T, Guchetl S, Tchelustnikova T, Ramasanova S. Development of marker system for identification and certification of sunflower lines and hybrids on the basis of SSR-analysis. Helia 2006;29:63-72.
30. Solodenko A, Sivolap Y. Genotyping of *Helianthus* based on microsatellite sequences. Helia 2005;28:19-26.
31. Nei M. Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 1973;70:3321-3323.
32. Shannon CE, Weaver W. The mathematical theory of communication (Urbana, IL. University of Illinois Press IL; 1949.
33. Dong G, Liu G, Li K. Studying genetic diversity in the core germplasm of confectionary sunflower (*Helianthus annuus* L.) in China based on AFLP and morphological analysis. Russ J Genet 2007;43:627-635.
34. Hongtrakul V, Huestis GM, Knapp SJ. Amplified fragment length polymorphisms as a tool for DNA fingerprinting sunflower germplasm: genetic diversity among oilseed inbred lines. Theor Appl Genet 1997;95:400-407.
35. Colombo C, Nucci S, Priolli R, Zucchi M, Carhalho C, Chrfl B. Genetic diversity of Macaw Palm (*Acrocomia aculeata*) by microsatellite markers. Proceedings of the International Plant and Animal Genome Conference, San Diego, CA, USA; 2013.
36. Jannatdoust M, Darvishzadeh R, Ziaeifard R, Ebrahimi MA, Maleki HH, Gholinezhad E. Analysis of genetic diversity and population structure of confectionery sunflower (*Helianthus annuus* L.) native to Iran. J Crop Sci Biotechnol 2016;19:37-44.
37. Solouki M, Mehdikhani H, Zeinali H, Emamjomeh A. Study of genetic diversity in Chamomile (*Matricaria chamomilla*) based on morphological traits and molecular markers. Sci Hortic 2008;117:281-287.
38. Kholghi M, Darvishzadeh R, Bernousi I, Pirzad A, Laurentin H. Assessment of genomic diversity among and within Iranian confectionery sunflower (*Helianthus annuus* L.) populations by using simple sequence repeat markers. Acta Agr Scand B Sil Plant Sci 2012;62:488-498.
39. Tang S, Knapp SJ. Microsatellites uncover extraordinary diversity in native American land races and wild populations of cultivated sunflower. Theor Appl Genet 2003; 106:990-1003.
40. Ronicke S, Hahn V, Friedt W. Resistance to *Sclerotinia sclerotiorum* of 'high oleic' sunflower inbred lines. Plant Breed 2005;124:376-381.