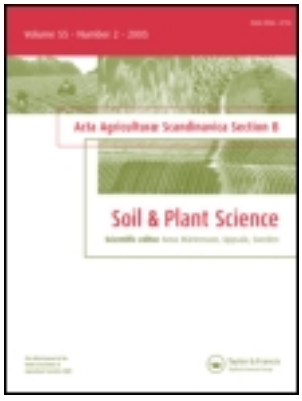

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Assessment of genomic diversity among and within Iranian confectionery sunflower (Helianthus annuus L.) populations by using simple sequence repeat markers

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ORIGINAL ARTICLE

Assessment of genomic diversity among and within Iranian confectionery sunflower (*Helianthus annuus* L.) populations by using simple sequence repeat markers

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Abstract

Genetic variation in 15 confectionery sunflower populations from different geographical regions of Iran was evaluated using microsatellites. Forty-three alleles were scored at 20 SSR loci. The average number of alleles per locus (na) and the effective allele number (ne) were 2.15 and 1.72, respectively. Observed heterozygosity (0.55) was higher than the expected (0.38) indicating lack of Hardy–Weinberg equilibrium. The genetic similarity matrix for individuals was constructed using Kosman and Leonard similarity coefficient, and Nei's Genetic Identity as well as Kosman's genetic distance was calculated among populations. Dendrograms were constructed by the unweighted pair-group method using arithmetic average (UPGMA) algorithm. The analysis of molecular variance revealed lower genetic variation among populations (14%), than within populations (86%). The average number of migrants (Nm) between populations based on AMOVA (Fst = 0.062) was 4.79. Results displayed high variation among individual plants, possibly because of the high allogamy. Genetic similarity between populations in this study could be considered as consequences of genetic equilibrium that has occurred over the long period of cultivation of confectionery sunflower in this region as well as seed exchange among provinces. The traditional assumption that selecting genotypes of different geographical origin will maximize the diversity available to a breeding project does not hold in confectionery sunflower.

Keywords: *Cluster analysis, confectionery sunflower, gene flow, genetic diversity, Kosman and Leonard similarity coefficient, simple-sequence repeats.*

Introduction

Several thousand years ago, humans domesticated virtually all the major crop species currently used in modern agriculture. During this process, cultivated plants underwent domestication bottlenecks (Tanksley and McCouch 1997) that generally reduced their gene diversity relative to their wild ancestors. After domestication, cultivated plants spread from their origin centre due to agricultural activities.

Plant genetic resources are one of the most valuable natural resources (Ramanatha and Hodgkin 2002), providing the genetic diversity necessary for both farmers and breeders to obtain new cultivars either with high yield, better quality, more adapted to abiotic stress, or more resistant to pest and pathogens (Laurentin 2009). Therefore, conservation and use of plant genetic resources plays an important role in agriculture and human food security.

Rao (2004) states morphological characterization of plant genetic resources has a limited number of traits to evaluate, which have a strong environmental influence. Furthermore, Karp et al. (1997) indicate highly heritable traits show no variation over much the material studied. DNA-based techniques for plant genetic resources characterization do not have these disadvantages, providing useful information about polymorphism, genetic relatedness and gene diversity (Chalmers et al. 2001).

Asteraceae is the largest family of the Dicotyledones class, having around 25,000 species including some of great economic importance for human nutrition, bioenergy production and flower-farming,

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for example sunflower (*Helianthus annuus* L.), which is an important oil crop.

Nowadays, two main types of sunflower are grown: for oilseed production and non-oilseed or confectionery types (Salunkhe et al. 1999). Confectionery sunflower produces large seeds with low oil content and is used in baking and snack applications (Lu and Hoeft 2007). Confectionery kernels are roasted and salted, or roasted and no salt added and marketed as edible chips. The confectionery type is one of the most popular and important crops in Iran. It is cultivated in all parts of the country especially in the northwest regions.

Because of the economic and ecological importance of wild and cultivated sunflowers, *H. annuus* has been one of the primary models for genetics and genomics research in the Compositae (Knapp 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 by this marker is low (Hernández et al. 2001). Microsatellite markers are the most reliable markers for cultivar identification and genetic diversity studies because of their high polymorphism, random distribution and co-dominant Mendelian inheritance (Hvarleva et al. 2007, Kalia et al. 2011). Microsatellites or simple sequence repeats (SSRs) constitute the current marker system for characterizing sunflower germplasm (Paniego et al. 2002, Tang et al. 2002, Yu et al. 2002, Zhang et al. 2005, Hvarleva et al. 2007, Darvishzadeh et al. 2010, Garayalde et al. 2011). They were widely applied in sunflower research for identification of inbred lines, cultivars and wild species (Yu et al. 2002, Tang and Knapp 2003, Hvarleva et al. 2007, Darvishzadeh et al. 2010, Garayalde et al. 2011). With the advent of high-density SSR maps for sunflower (Tang et al. 2002, Poormohammad Kiani et al. 2007), it is now feasible to estimate genetic diversity with a large number of markers that are well distributed across the sunflower genome.

Most reports on genetic diversity have focused on the wild or oilseed sunflower, but the genetic diversity of confectionery sunflower germplasm based on molecular markers is scarcely referred to (Dong et al. 2007). The aims of the present study were to analyse intra- and inter-population variation of confectionery sunflower and indirectly detect possible gene flow among the populations using SSR markers.

Materials and methods

Plant material and DNA extraction

Fifteen confectionery sunflower populations, covering major regions cultivated with confectionery sunflower in Iran, were chosen for studying inter- and intra-population genetic diversity (Table I). As in most other studies, six plants per population were randomly chosen (He et al. 2007, Muirhead et al. 2008), and genomic DNA was extracted from the leaves of 2-week-old seedlings using the CTAB method (Ausubel et al. 1995). Genomic DNA was re-suspended in 100L TE buffer (10 mM Tris, 1 mM EDTA pH 8.0). Concentration of each DNA sample was determined spectrophotometrically at 260 nm using the BioPhotometer 6131 (Eppendorf, Germany). DNA quality was checked by running 1 l DNA in 0.8% (w/v) agarose gels in 0.5 × TBE buffer (45 mM Tris base, 45 mM boric acid, 1 mM EDTA pH 8.0). DNA samples that gave a smear in the gel were discarded.

Microsatellite analysis

Twenty microsatellite markers (Table II) out of 339 'ORS' SSR markers from the SSR database (Tang et al. 2002) and 180 'HA' SSR markers developed by INTA (Paniego et al. 2002) were used for DNA fingerprinting. The choice of SSR markers was based on clarity of produced bands and their genetic locations in order to give a uniform coverage of the

Table I. Location of confectionery sunflower populations used in present study.

Code	Location	Altitude (M)	Latitude (E)	Longitude (N)
P1	Isfahan (Dolat Abad)	5271	51°31′	32°33′
P2	Isfahan (Talkhonche)	5271	51°31′	32°33′
P3	Hamedan	5908	48°20′	34°36′
P4	Nushin Shahr (Urmia)	1311	45°3′15.39″	37°43′50.53′
P5	Salmas (Sadaghiyan)	1381	44°50′	38°13′
P6	Miyane	1091	47°42′34.95″	37°25′07.53″
P7	Mashhad	3507	59°36′	36°18′
P8	Marand	1348	45°46′10.69″	38°25′30.42″
P9	Khoy	1125	44°57′18.20″	38°35′38.34″
P10	Boukan	1353	46°12′31″	36°31′13″
P11	Shahrood 1	6912	54°58′	36°25′
P12	Shahrood 2	6912	54°58′	36°25′
P13	Sanandaj	4853	46°59′31″	35°18′31″
P14	Salmas (Ghezelje)	1384	45°06′	38°17′
P15	Khoy 6	1125	44°57′18.20″	38°35′38.34″

Table II. Primer sequences and linkage groups of 20 simple sequence repeat (SSR) loci applied to 15 population of confectionery sunflower.

Marker	Repeat Motifs	Forward Primer (5′→3′)	Reverse Primer (5′→3′)	Linkage group	Position (cM)[1]	Reference	Ta
ORS 331	(CT)12(AC)13	TGAAGAAGGGTTGTTGATTACAAG	GCATTGGGTTCACCATTTCT	LG7	24.2	Tang et al. (2002)	57
ORS 378	(GT)17	GTGAAACCTTCGGACCTCTG	GTACAAAACTTATAAATAAAACAATA	LG16	86.3	Tang et al. (2002)	57
ORS 488	(AC)14	CCCATTCACTCCTGTTTCCA	CTCCGGTGAGGATTTGGATT	LG3	67.2	Tang et al. (2002)	57
ORS 608	(AT)6(AG)11	CATGGAAAGCCGAGTTCTCT	CGTGCGTGATTAACATACCC	LG6	44.7	Tang et al. (2002)	57
ORS 609	(AG)20	GCGAAGGAACTGAACCGATA	GGATTTTAGTCCGCCAATCA	LG12	56.0	Tang et al. (2002)	57
ORS 621	(CT)14	CGCCTTATGCTGAGAGGAAA	CCTGAAGCGAAGAAGAATCG	LG11	1.1	Tang et al. (2002)	57
ORS 694	(AG)17	CCTGGAACTGAACCGAGAAC	GCCGTGAAACAGAGAGAGGA	LG14	35.8	Tang et al. (2002)	57
ORS 718	(CT)13	CACTTTACGCACACCAAACC	ATGCAACACCCGAATCAAAG	LG3	30.2	Tang et al. (2002)	57
ORS 728	(AG)7	CTCCATAGCAACCACCTGAAA	CCAAACTCTGAATGATACTTGTGAC	LG1	25.2	Tang et al. (2002)	57
ORS 785	(AG)17	CAAAATACCCAGGTCAAAGCA	CCTAGCTTATGGGACGTATGGA	LG4	53.8	Tang et al. (2002)	57
ORS 807	(AG)15	CCGATATTTTGACCGATATTTTGC	TCTCACCCTTCATCTCCTTCC	LG16	67.9	Tang et al. (2002)	57
ORS 844	(AC)17	ACGATGCAAAGAATATACTGCAC	CATGTTTAATAGGTTTTAATTCTAGGG	LG9	75.5	Tang et al. (2002)	59
ORS 878	(AC)11	CCAAAGGTGGGATAACCTAAAAG	TCCGTGTTCATGCATTGATT	LG10	29.9	Tang et al. (2002)	59
ORS 949	(AC)13	TGCAAGGTATCCATATTCCACAA	TATACGCACCGGAAAGAAAGTC	LG3	49.2	Tang et al. (2002)	57
ORS 996	(AG)19	CGGTGAGAATAACCTCGGAAGA	ATCAGTCCTTCAACGCCATTAGT	LG16	57.2	Tang et al. (2002)	59
ORS 1079	(CT)26	TACGACTGACGATTCCATTTCTC	AACTGGATTTCACAGGGAGTGTT	LG14	14.4	Tang et al. (2002)	59
ORS 1179	(AG)18	GATTCGGAGCTGTTAGGAGGTAG	AAACGGGAAGCAAGAATAGAACA	LG13	60.1	Tang et al. (2002)	59
ORS 1215	(CT)10	ATACTCTTCCACCCTCAAATCCA	GGTTGCGGTAGTGGTCTGTAGT	LG15	74.8	Tang et al. (2002)	59
ORS 1242	(CT)14	GCAATCGTTTCACTCTTCCATTC	TGGTCGTAGAATTGTCGGTCAT	LG15	63.3	Poormohammad Kiani et al. (2007)	59
ORS 1265	(CT)18	GGGTTTAGCAAATAATAGGCACA	ACCCTTGGAGTTTAGGGATCA	LG9	25.0	Tang et al. (2002)	59

[1]The genetic distance from the top of chromosome

Table III. Number of allele (na), effective allele number (ne), observed heterozygosity (Ho), expected heterozygosity (He) and allele frequency of the 20 simple sequence repeat (SSR) loci applied to 90 individuals of 6 confectionery sunflower populations.

					Allele frequency		
Primer	na	ne	Ho	He	A	B	C
ORS 331	2	1.72	0.47	0.41	0.60	0.40	–
ORS 378	2	1.91	0.82	0.47	0.57	0.43	–
ORS 488	3	2.27	0.97	0.56	0.30	0.52	0.187
ORS 608	2	1.76	0.57	0.42	0.34	0.66	–
ORS 609	2	1.31	0.21	0.20	0.13	0.87	–
ORS 621	2	1.55	0.46	0.32	0.24	0.76	–
ORS 694	3	2.34	1.00	0.57	0.17	0.50	0.331
ORS 718	2	1.73	0.64	0.40	0.32	0.68	–
ORS 728	2	1.52	0.47	0.28	0.77	0.23	–
ORS 785	2	1.95	0.9	0.49	0.46	0.54	–
ORS 807	3	2.07	0.49	0.49	0.22	0.22	0.554
ORS 844	2	1.60	0.22	0.35	0.33	0.67	–
ORS 878	2	1.75	0.52	0.42	0.38	0.62	–
ORS 996	2	1.83	0.65	0.45	0.49	0.51	–
ORS 949	2	1.82	0.75	0.44	0.61	0.39	–
ORS 1079	2	1.63	0.41	0.37	0.65	0.35	–
ORS 1179	2	1.24	0.18	0.17	0.14	0.86	–
ORS 1215	2	1.11	0.10	0.09	0.05	0.95	–
ORS 1242	2	1.38	0.33	0.24	0.17	0.83	–
ORS 1265	2	1.93	0.86	0.48	0.44	0.56	–
Mean	2.15	1.72	0.55	0.38			

sunflower genome (Tang et al. 2002, Poormohammad Kiani et al. 2007). Polymerase chain reaction (PCR) amplifications were performed in a volume of 15 L containing 10 μM of each primer, 0.5 Unit of Taq DNA polymerase (Life Technologies), 10 mM of each dNTP (Promega), 2 μl 10 × PCR buffer, 50 mM $MgCl_2$ (Promega), distilled water and 5 ng template DNA, using a 96-well Eppendorf Mastercycler Gradient (Type 5331, Eppendorf AG, Hamburg, Germany). Touchdown PCR was used for amplification of all SSRs as follows: 94 °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 30 cycles of 94 °C for 30 s, 54 °C for 30 s and 72 °C for 45 s. Final extension was 20 min at 72 °C (Tang et al. 2002). The reaction products were mixed with 10 μL of formamide dye (98% formamide, 10 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol) and resolved in 3% (w/v) agarose gel (0.5 × TBE), stained with ethidium bromide (1.0 μg ml^{-1}) and photographed under UV light using a Gel-Doc image analysis system (Gel Logic 212 PRO, USA).

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. Several indices such as mean number of alleles per locus (n_a), effective allele number (A_e), allele frequency, observed heterozygosity (H_o) and expected heterozigosity (H_e) were estimated using GenAlEx 6.41 software (Peakall and Smouse 2006) according to the following equations:

$$n_a = \sum_{i=1}^{n} n_{ai}/n$$

where n_a is the number of alleles at i^{th} locus and n is the number of loci;

$$A_e = \frac{1}{\sum_{i=1}^{n} p_i^2}$$

where A_e is the effective allele number at a locus, and p_i is the frequency of the i^{th} allele in a locus (Hartl and Clark 1997);

$$\text{Allele frequency} = \frac{2N_{XX} + N_{XY}}{2N}$$

which was calculated locus by locus, where N_{XX} is the number of homozygotes for allele $X(XX)$, and N_{XY} is the number of heterozygotes containing the allele $X(Y$can be any other allele). N = the number of samples (Hartl and Clark 1997);

$$H_o = \sum_{i=1}^{n} H_{oi}/n = \sum_{i=1}^{n} (1 - \sum_{j=1}^{mi} q^2{}_{ij})/n$$

where H_{oi} represents the observed heterozygosity of the i^{th} allele, and q_{ij} is the frequency of the j^{th} homozygous allele at i^{th} locus (Hartl and Clark 1997);

$$H_e = \sum_{i=1}^{n} Hi/n = \sum_{i=1}^{n} (1 - \sum_{j=1}^{mi} q^2{}_{ij})/n$$

where H_i is the expected heterozygosity of the i^{th} allele**,** and q_{ij} is the frequency of the j^{th} homozygous allele at i^{th} locus (Lynch and Milligan 1994).

Genetic similarity among individuals was calculated based on the Kosman and Leonard dissimilarity coefficient (KL coefficient) (Kosman and Leonard 2007). This method has many advantages to others such as Jaccard (Jaccard 1908), Dic (Nei and Li 1979), and simple matching (Sneath and Sokal 1973) in diploid organisms evaluated with co-dominant markers (Kosman and Leonard 2007). The Kosman and Leonard similarity coefficient

calculation was performed using software under development by Dr. Evsey Kosman (personal communication). These dissimilarity coefficients were transformed in similarity coefficient by 1 – KL coefficient for each pair of individuals. Genetic structure at population level, within and between populations, was estimated by analysis of molecular variance (AMOVA) using GenAlEx software version 6.41. Analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was conducted to calculate the variance components and significance levels of variation within a population and among populations. AMOVA derived genetic differentiation (Fst) (analogous to traditional F statistics) and gene flow values between pairs of populations were calculated according to the following equations:

$$F_{ST} = \frac{V_{AP}}{(V_{AP} + V_{WP})}$$

where V_{AP} is the variance among populations and V_{WP} is the variance within populations;

$$Nm = [(1/F_{ST}) - 1]/4$$

where Nm is the gene flow and F_{ST} represents the degree of population genetic differentiation (Frankham et al. 2004). Additionally, Nei's distance (Nei 1978) was calculated between pairs of populations. Kosman and Leonard genetic distance among and within populations was also calculated. Dendrograms were constructed by the unweighted pair group method using arithmetic average (UPGMA) algorithm.

Results and discussion

In this study 20 microsatellite loci were used to analyse genetic relationships among and within 15 confectionery sunflower populations and a total of 43 alleles were detected. Number of alleles per locus ranged from 2 to 3 alleles with an average of 2.15 (Table III). A similar result was obtained by Darvishzadeh et al. (2010) who observed 2.32 alleles per locus among plant introductions from American and European countries analysed at 38 SSR loci, but it was lower than the mean number of alleles per locus reported in other studies among inbred lines and hybrids (Yu et al. 2002, Tang and Knapp, 2003). Differences in results reported by various studies could be because of differences in number of genotypes studied, their genetic background, and number of markers used as well as techniques applied to detect polymorphism. For example, Tang and Knapp (2003) used 122 microsatellite marker loci for genotyping nine elite confectionery and oilseed sunflower inbred lines and 3.5 alleles per locus were reported. A possible cause of the difference in results could be the matrix used for resolve PCR products. The present study used agarose instead of polyacrylamide, therefore sensitivity in resolving bands could be less.

The effective allele number (A_e) was 1.72 (Table III). This parameter takes into account both the number of alleles and their frequencies. It allows comparing populations where the number and distributions of alleles differ drastically. For any given number of alleles, the expected heterozygosity is highest when all the allele frequencies are equal, therefore the highest effective number of alleles will be obtained when heterozygosity is high. For this case, in average, the effective allele number indicates allelic frequencies close to similar values within each locus. However, SSR ORS1215 has a value of 1.11; it means frequencies of its two alleles are quite different, which is identifying rare alleles for this microsatellite.

The power of each SSR locus for studying genetic diversity was estimated by the expected heterozygosity values, which ranged from 0.09 for locus ORS1215 to 0.57 for locus ORS694, with a mean of 0.38 (Table III). Markers with high heterozygosity values such as ORS694 and ORS488 could be effectively used in genetic diversity studies in sunflower. H_e in this research is in agreement with the findings of Hvarleva et al. (2007) but it was lower than the values obtained in other studies, where it ranged from 0.49 (Paniego et al. 2002) to 0.55 (Yu et al. 2002). Average observed heterozygosity (Ho) ranged from 0.18 to 0.97 with a mean value of 0.55 (Table III). There is a considerable difference between observed and expected heterozygosity for several SSR analysed, indicating lack of Hardy-Weinberg equilibrium.

The genetic structure of plant populations reflects the interactions of various factors, including the long-term evolutionary history of the species (shifts in distribution, habitat fragmentation and population isolation), genetic drift, mating system, gene flow and selection pressure (Schaal et al. 1998).

All pair-wise Fst between populations, obtained with AMOVA, varied from 0.018 (P14–P15) to 0.127 (P1–P9) with an average of 0.062 (Table IV) showing the low differentiation among populations, because the low value of Fst indicates little differentiation among populations. The non-hierarchical AMOVA analyses revealed that 14% of the total variation was attributed to the variability among the populations whereas 86% was accumulated within the populations (data are not presented). The average number of migrants (Nm) between populations based on AMOVA (Fst = 0.062) was 4.79 (Table IV) representing the high number of migrations from one population to another. Generally, if $Nm < 1$, then local differentiation of populations

Table IV. F_{st} and N_m value among confectionery sunflower populations.

Population X	Population Y	Fst	Nm	Population X	Population Y	Fst	Nm
P1	P2	0.071	3.269	P9	P11	0.059	3.962
P1	P3	0.067	3.473	P10	P11	0.080	2.884
P2	P3	0.075	3.067	P1	P12	0.071	3.257
P1	P4	0.094	2.420	P2	P12	0.078	2.966
P2	P4	0.114	1.938	P3	P12	0.051	4.680
P3	P4	0.053	4.498	P4	P12	0.055	4.256
P1	P5	0.065	3.596	P5	P12	0.038	6.406
P2	P5	0.066	3.528	P6	P12	0.051	4.696
P3	P5	0.053	4.447	P7	P12	0.048	5.009
P4	P5	0.065	3.587	P8	P12	0.028	8.690
P1	P6	0.100	2.238	P9	P12	0.065	3.575
P2	P6	0.076	3.055	P10	P12	0.075	3.099
P3	P6	0.057	4.161	P11	P12	0.024	10.161
P4	P6	0.054	4.363	P1	P13	0.068	3.446
P5	P6	0.041	5.789	P2	P13	0.086	2.662
P1	P7	0.096	2.361	P3	P13	0.047	5.017
P2	P7	0.104	2.145	P4	P13	0.052	4.530
P3	P7	0.045	5.284	P5	P13	0.029	8.290
P4	P7	0.083	2.752	P6	P13	0.039	6.146
P5	P7	0.064	3.626	P7	P13	0.055	4.320
P6	P7	0.087	2.623	P8	P13	0.022	10.973
P1	P8	0.080	2.874	P9	P13	0.058	4.070
P2	P8	0.088	2.599	P10	P13	0.073	3.177
P3	P8	0.073	3.161	P11	P13	0.017	14.659
P4	P8	0.092	2.467	P12	P13	0.008	31.185
P5	P8	0.029	8.449	P1	P14	0.094	2.397
P6	P8	0.065	3.597	P2	P14	0.116	1.903
P7	P8	0.078	2.949	P3	P14	0.057	4.159
P1	P9	0.127	1.712	P4	P14	0.057	4.156
P2	P9	0.122	1.807	P5	P14	0.040	5.988
P3	P9	0.070	3.305	P6	P14	0.073	3.198
P4	P9	0.063	3.688	P7	P14	0.051	4.689
P5	P9	0.030	8.125	P8	P14	0.051	4.674
P6	P9	0.060	3.935	P9	P14	0.050	4.723
P7	P9	0.069	3.381	P10	P14	0.036	6.613
P8	P9	0.071	3.265	P11	P14	0.048	4.948
P1	P10	0.122	1.807	P12	P14	0.033	7.242
P2	P10	0.116	1.904	P13	P14	0.032	7.587
P3	P10	0.059	4.004	P1	P15	0.097	2.337
P4	P10	0.057	4.162	P2	P15	0.112	1.978
P5	P10	0.055	4.305	P3	P15	0.057	4.126
P6	P10	0.069	3.389	P4	P15	0.063	3.732
P7	P10	0.063	3.690	P5	P15	0.033	7.416
P8	P10	0.102	2.212	P6	P15	0.065	3.602
P9	P10	0.040	6.070	P7	P15	0.048	4.982
P1	P11	0.082	2.812	P8	P15	0.045	5.282
P2	P11	0.089	2.570	P9	P15	0.044	5.423
P3	P11	0.060	3.921	P10	P15	0.050	4.720
P4	P11	0.067	3.497	P11	P15	0.035	6.832
P5	P11	0.027	8.903	P12	P15	0.031	7.836
P6	P11	0.039	6.200	P13	P15	0.033	7.233
P7	P11	0.065	3.569	P14	P15	0.018	13.277
P8	P11	0.026	9.458				
Mean						0.062	4.79

will result, and if $Nm > 1$, then there will be little differentiation among populations (Wright 1951). The high level of gene flow may be a result of seed movement due to human influences. In accordance with gene flow item, Fst confirms the lack of inter-population diversity.

Kosman and Leonard diversity coefficient within populations ranged from 0.207 (population Hamedan) to 0.328 (population Sadaghiyan) (Table V). Most variation was found within populations, as expected for an allogamous, self-incompatible and insect-pollinated species (Hamrick and Godt 1989).

Table V. Genetic distance among 15 confectionery sunflower population.

Population	Location	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15	KW
P1	Isfahan (Dolat Abad)	0	0.165	0.195	0.257	0.197	0.235	0.250	0.214	0.284	0.280	0.221	0.244	0.215	0.247	0.250	0.240
P2	Isfahan (Talkhonche)	0.09	0	0.203	0.281	0.237	0.217	0.266	0.239	0.299	0.276	0.244	0.255	0.248	0.288	0.262	0.225
P3	Hamedan	0.084	0.087	0	0.205	0.194	0.199	0.182	0.218	0.235	0.207	0.228	0.243	0.188	0.199	0.188	0.207
P4	Nushin Shahr (Urmia)	0.134	0.161	0.076	0	0.226	0.172	0.234	0.230	0.221	0.233	0.228	0.232	0.208	0.184	0.194	0.246
P5	Salmas (Sadaghiyan)	0.096	0.092	0.075	0.098	0	0.205	0.239	0.187	0.192	0.223	0.183	0.233	0.183	0.180	0.189	0.328
P6	Miyane	0.148	0.098	0.072	0.069	0.057	0	0.223	0.194	0.206	0.187	0.179	0.181	0.145	0.218	0.199	0.216
P7	Mashhad	0.128	0.133	0.063	0.12	0.089	0.116	0	0.224	0.244	0.219	0.220	0.217	0.220	0.205	0.182	0.278
P8	Marand	0.115	0.124	0.094	0.125	0.038	0.084	0.096	0	0.241	0.262	0.147	0.170	0.152	0.175	0.159	0.218
P9	Khoy	0.198	0.184	0.106	0.09	0.037	0.085	0.099	0.099	0	0.215	0.230	0.260	0.225	0.215	0.198	0.279
P10	Boukan	0.187	0.163	0.089	0.069	0.074	0.099	0.088	0.141	0.048	0	0.266	0.273	0.244	0.191	0.207	0.309
P11	Shahrood 1	0.111	0.115	0.074	0.089	0.037	0.047	0.079	0.024	0.081	0.111	0	0.177	0.172	0.200	0.171	0.279
P12	Shahrood 2	0.105	0.112	0.078	0.077	0.063	0.066	0.065	0.03	0.108	0.117	0.025	0	0.158	0.203	0.193	0.295
P13	Sanandaj	0.101	0.128	0.068	0.075	0.05	0.052	0.072	0.024	0.09	0.112	0.018	0.011	0	0.183	0.176	0.279
P14	Salmas (Ghezelje)	0.144	0.177	0.085	0.072	0.059	0.099	0.068	0.065	0.063	0.044	0.061	0.048	0.046	0	0.157	0.268
P15	Khoy 6	0.138	0.16	0.08	0.082	0.043	0.082	0.06	0.054	0.056	0.065	0.039	0.042	0.044	0.022	0	0.223

Kosman's genetic distance between populations has been shown above diagonal and Nei's genetic distance has been shown below diagonal. KW shows Kosman's genetic diversity within populations.

Yatabe et al. (2007) reported similar results in the centre of origin, finding most genetic diversity (approximately 93%) within populations. Also, Garayalde et al. (2011) analysed 10 populations of wild sunflower with SSR and ISSR markers and reported a high level of genetic diversity (approximately 80%) within populations. Intra-population improvement programmes should, therefore, target selection of individual plants with desirable traits, since the populations are more diverse.

Inter-population genetic distance based on Kosman and Leonard distance ranged from 0.145 to 0.299 and based on Nei's genetic distance ranged from 0.011 to 0.198 (Table V). Based on Kosman's genetic distance, samples from 'Talkhonche' were distantly related to samples of 'Khoy' (0.299) and there was the least genetic distance between 'Miyane' and 'Sanandaj' populations (0.145). Based on Nei's genetic distance, samples from 'Dolat Abad' were distantly related to samples of 'Khoy' (0.198) and there was the least genetic distance between 'Shahrood 2' and 'Sanandaj' populations (0.011). Despite differences between both methodologies, both coincide in separating Dolat Abad and Talkhonche from the rest. The genetic distance between populations is a valuable parameter for germplasm conservation and plant breeding programs. Hybridization/crossing between any distantly related populations is expected to yield more heterosis and vigorous plants constituting many of the different traits contained in the two parental lines. Therefore, hybridization or crossing between distantly related populations of the present study could be an appropriate strategy for inter-population landrace improvement programmes, for example Dolat Abad or Talkhonche crossed to another one of the analysed populations.

The UPGMA dendrograms representing relationships between individuals and population are shown in Figures 1 and 2, respectively. The UPGMA dendrogram of individuals (Figure 1) did not divide

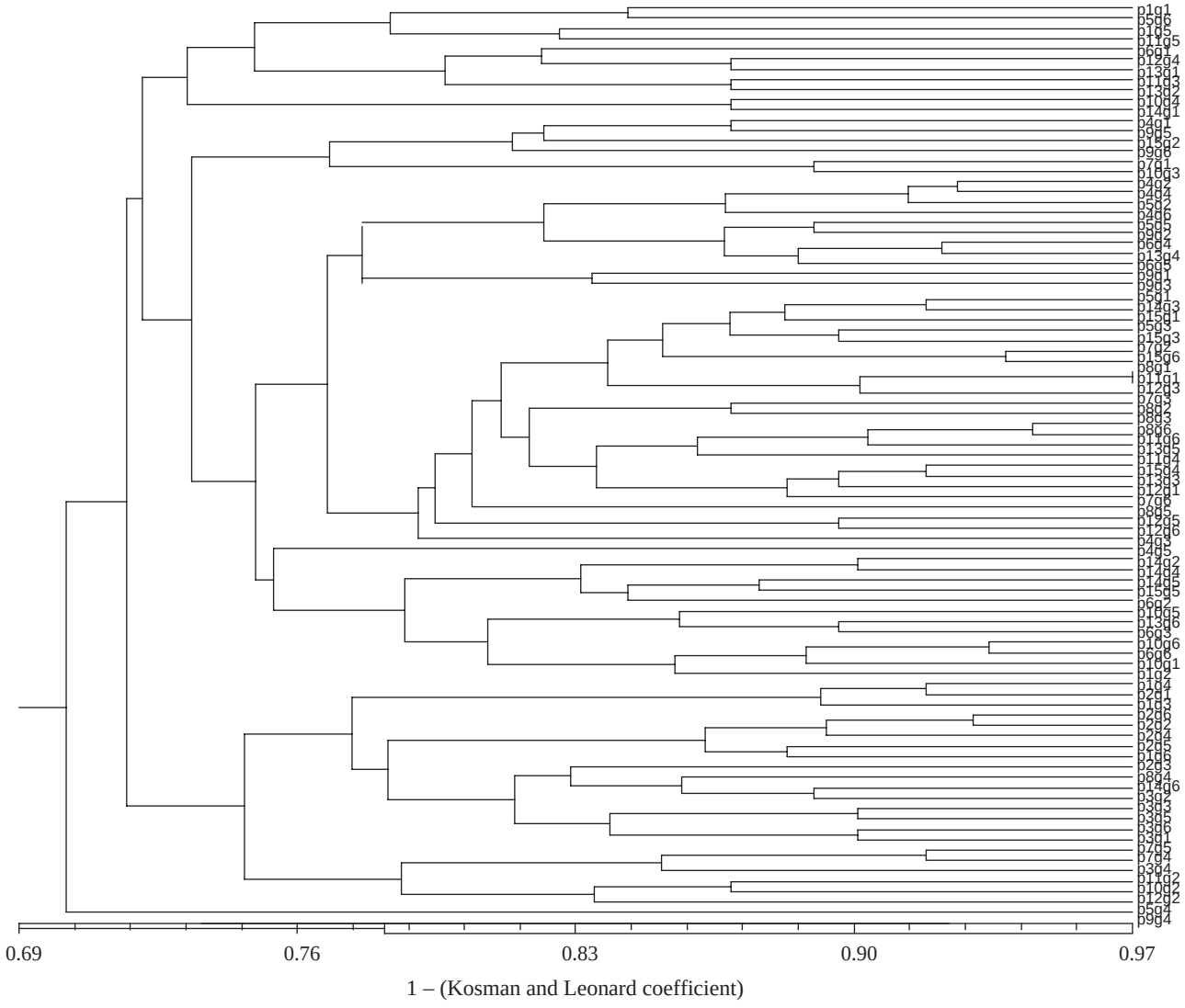

Figure 1. UPGMA clustering of 90 confectionery sunflower genotypes based on Kosman and Leonard dissimilarity index.

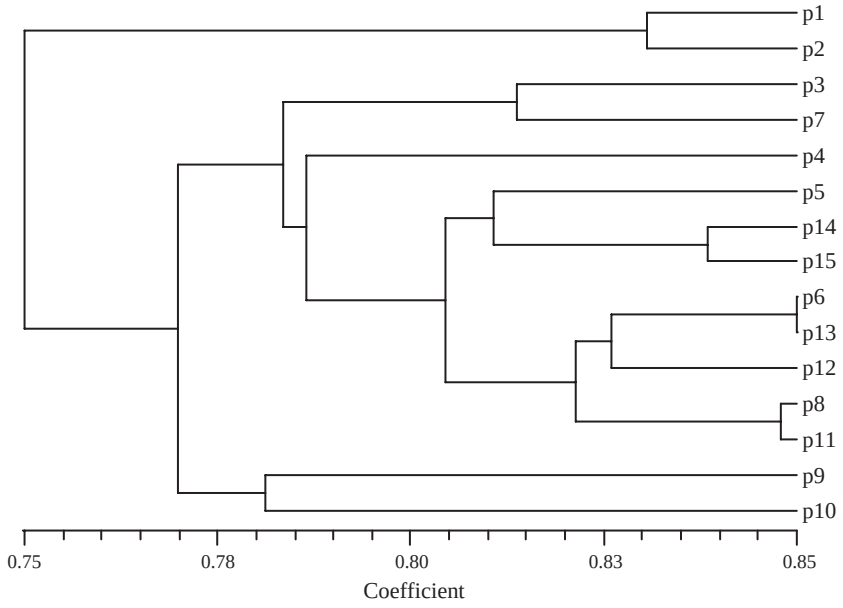

Figure 2. UPGMA clustering of 15 confectionery sunflower populations based on Kosman and Leonard genetic distance.

the individuals into distinct groups resembling the different populations. Generally genotypes were evenly distributed along the dendrogram, confirming high intra-population diversity. Similar effects were observed for AFLP analyses of wild and cultivated sunflower in research by Quagliaro et al. (2001). The UPGMA dendrogram of population revealed three major groups of confectionery sunflower populations (Figure 2). In the first group were situated two populations from 'Isfahan'. Group III contained two populations and other studied populations grouped in the second cluster. Cluster II contained populations from different regions. Differentiation between 'Isfahan' populations and other populations indicated that they were genetically different from other populations which is due to lack of transmission to this population from other populations. Several possible reasons could be given for the genetic similarity among populations from different regions. Most of the materials might have originally been introduced from the same source. There could also be a tendency, particularly among resource-poor farmers in marginal areas, of selecting for the same traits of interest like yield stability, resistance to diseases, insects and abiotic calamities and low dependence on external inputs (de Boef et al. 1996). Although the original sources might vary, the crop might have also been forced to evolve in the same direction by this kind of local breeding for the same targets which may emanate from similar economic, social, cultural and ecological reasons in the area. Our result indicates there was no association between geographical origin and SSR patterns. Using both genetic diversity and distance parameters simultaneously in plant improvement programmes would be the best approach. In this case, the best individuals selected from the diverse population(s) and crossed with individuals selected from population(s) that is/are comparatively distant from the former population could yield good results. In conclusion, high genetic variability (86%) was found within populations of confectionery sunflower and there was no association between geographical origin and SSR patterns. Therefore it is suggested that selection of parents for hybridization does not need to be based on geographic diversity. Our result also revealed that there was considerable gene flow among populations.

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