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Original scientific article

GENETIC DIVERSITY AND POPULATION STRUCTURE OF IRANIAN PISTACHIO (*Pistacia vera* L.) CULTIVARS

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Iran has a rich pistachio germplasm, thereby, the diversity and number of Iranian pistachio cultivars is unique in the world. Genetic diversity is crucial for sustainable use of genetic resources and conservation. As one of the oldest nut crops in human history, pistachio nuts have a high nutritional value and are commercially important. In the present study, the genetic variation of pistachio genotypes was investigated by nuclear ISSR markers. In this study, genetic relationships among 11 cultivars was assessed by using 12 inter simple sequence repeat (ISSR) primers. The total of 53 bands of which 44 (83%) were polymorphic were amplified by the 12 primers, an average of 4.4 bands per primer. The total number of amplified fragments was between 2 to 6 and the number of polymorphic fragments ranged from two to six. The amplified allele sizes ranged from 300 to 1600 bp. Pair-wise genetic similarity coefficients varied from 0.70 to 0.95. The UPGMA dendrogram differentiated the genotypes into two major clusters. The Mantel test showed correlation between genetic and geographical distance. AMOVA revealed a significant genetic difference among cultivars and showed that 35% of total genetic variation was due to within- cultivars diversity. The present results may be used for the conservation, core collection and future breeding of the pistachio.

Keyword: Gene flow, Genetic admixture, Network, *Pistacia vera*; genetic diversity

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INTRODUCTION

Genetic diversity is one aspect of biological diversity that is extremely important for conservation strategies, especially in rare and narrowly endemic species (MILLS and SCHWARTZ, 2005; TOMASELLO *et al.*, 2015). Preserving the genetic diversity of these plants can significantly strength their long-term survival and evolution in changing environments (FRANKHAM *et al.*, 2002). For instance, rare and endemic plants contribute to biodiversity and help preserve gene pool of local flora (FALK and HOLSINGER, 1991; OLIVIERI *et al.*, 2016).

The genus *Pistacia* is a member of the Anacardiaceae family, which comprises 11 or more species (ZOHARY, 1952). Among them, *Pistacia vera* L. is the main economically developed species, for its palatable nut seeds (ZOHARY, 1996; KAFKAS *et al*., 2002). The genus *Pistacia* is believed to have started in European and North African zones, however, as of late, the majority of scholars accept that *Pistacia* likely began in Central Asia. According to early reports, there are two primary theories concerning the broadening of *Pistacia* species: one focuses on the Mediterranean area of Europe, Northern Africa and Middle East. The alternative is the Eastern piece of the Zagros Mountains (Iran) and Caucasus areas from Crimea to the Caspian Sea (ZOHARY, 1952). Iran is the center of origin for four important *Pistacia* species: *P. vera*, *P. khinjuk* Stocks, *P. eurycarpa* Yalt. (*P. atlantica* subsp. *Kurdica* Zoh.), and *P. atlantica* Dsef. (KARIMI *et al*., 2009b). Three essential wild *Pistacia* species, including *P. vera*, *P. khinjuk*, and *P. atlantica* grow in Iran. Although Wild *P. vera* has spread to a territory of around 75,000 ha, in focal Asia, which envelopes Turkmenistan, Afghanistan, and Northeast Iran, where *P. vera* develops in the Sarakhs region, covering around 17,500 ha (BEHBOODI, 2003). Numerous studies have addressed genetic variability in *Pistacia* that were based on evaluation of morphological, physiological, and biochemical characteristics (ZOHARY, 1952; BARONE *et al*., 1993; DOLLO, 1993; TAYEFEH ALIAKBARKHANY *et al*., 2013).

Among the nut tree crops, pistachio tree ranks sixth in world production behind almond, walnut, Cashew, hazelnut and chestnut (MEHLENBACHER, 2003). Iran is the main world producer with more than 400,000 tons followed by Turkey, USA and Syria (FAOSTAT, 2004). The main cultivars grown in Iran are Ohady, Kaleh ghochi, Ahmad Aghai, Badami Zarand, Rezaii and Pust piazi (ESMAILPOUR, 2001). Nowadays, researchers are using molecular methods, i.e. genomic manipulation at the DNA level, to study genetic diversity in the crops. In this regard, many genetic studies on *Pistacia* are based on such methods (AHMAD *et al*., 2003a, b; KATSIOTIS *et al*., 2003; STRUSS *et al*., 2003; GOLAN-GOLDHIRSH *et al*., 2004; KAFKAS, 2006; KAFKAS *et al*., 2006a, b; AL-SOUSLI *et al*., 2014). Although previous studies have partially characterized pistachio diversity in Iran, they did not conduct a full analysis regarding discrimination of wild *Pistacia* and its potential breeding and implication of its conservation.

Induction of diversity in *Pistacia* species are based on morphological characteristics which usually can be achieved by budding or grafting selected scions onto seedling rootstocks of the same species or other *Pistacia* species. *Pistacia* species have a high genetic diversity due to their dioecious character, pollination mechanism. Because of these factors high selectivity in rootstocks breeding is required, and therefore knowledge of the genetic relationships among *Pistacia* species would be very useful in pistachio rootstock breeding.

ISSR is a dominant marker like RAPD (scored using presence or absence of band at a locus) but with greater robustness in repeatability and extremely high variability. These features

make ISSR better than other readily available marker systems in investigating the genetic variation among very closely related individuals and in crop cultivar classification (FANG and ROOSE, 1997; NAGAOKA and OGIHARA, 1997). Recently, this marker technique has been used to detect DNA polymorphism and genetic diversity in a wide pistachio germplasm originating from seven countries accompanied with AFLP and RAPD markers (KAFKAS *et al.*, 2006). The objectives of the study were 1) to assess genetic diversity and relationships among some of Irainian pistachio cultivars 2) to set up and use first inter simple sequence repeats (ISSR) technique in pistachio cultivar identification in Iran.

MATERIALS AND METHODS

Plant materials

In this study, fruit samples of 11 pistachio genotypes (11 females) were collected from the Semnan Pistachio Germplasm Collection located in Semnan, Damghan city, Iran (Table 1, Fig 1, Fig2). Details of sampling sites are mentioned (Table 1). Vouchers were deposited at the herbarium of Islamic Azad University, Science and Research Branch, Tehran, Iran (IAUH).

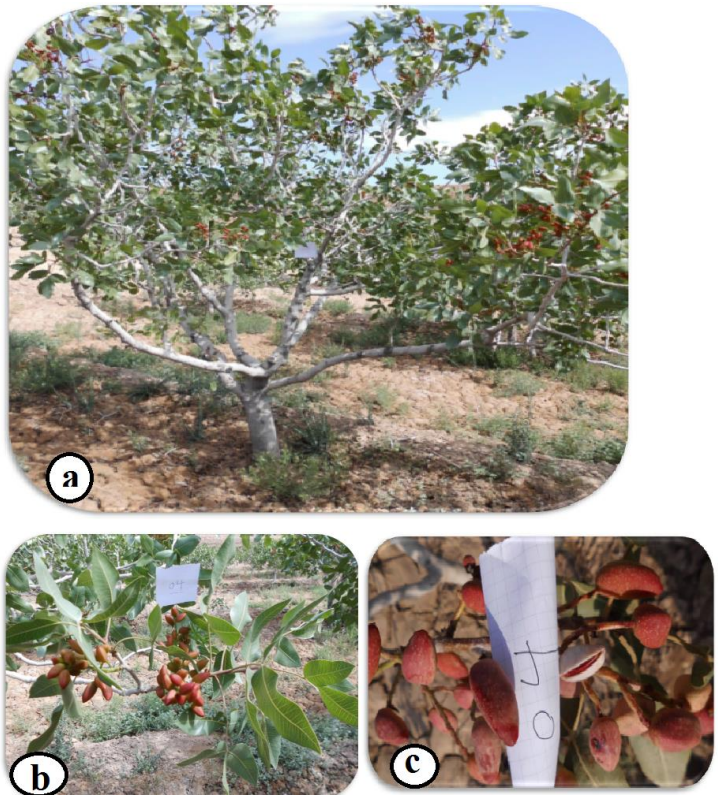

Fig 1. *Pistacia vera* general shape, a) Habit; b) Leaf and fruit; c) Fruit.

Fig 2. Fruit morphology of the studied genotypes of *Pistacia vera*; A: Kaleghochi (Pust Sefid); B: Shahpasand (Pust Sefid); C: Akbari (Pust Ghermez); D: Khanjari Damghan; E: Kaleghochi (Pust Ghermez); F: Shahpasand (Pust Ghermez); G: Fakhri; H: Akbari (Pust Sefid); I: Abbas-Ali; J: Ahmad Agaei; K: Menghar Kalaghi.

Table 1. List of pistachio cultivars examined for genetic relatedness using ISSR marker system in this study

Code	Genotypes	Voucher no.
1	Kaleghochi (Pust Sefid)	IAUH-000014968
2	Shahpasand (Pust Sefid)	IAUH-000014969
3	Akbari (Pust Ghermez)	IAUH-000014920
4	Khanjari Damghan	IAUH-000014921
5	Kaleghochi (Pust Ghermez)	IAUH-000014922
6	Shahpasand (Pust Ghermez)	IAUH-000014923
7	Fakhri	IAUH-000014924
8	Akbari (Pust Sefid)	IAUH-000014925
9	Abbas-Ali	IAUH-000014926
10	Ahmad Agaei	IAUH-000014927
11	Menghar Kalaghi	IAUH-000014928

Morphological studies

In total 28 morphological (28 quantitative) characters were studied`. Five to ten plant specimens were randomly studied or morphological analyses. These are: Number of buds; Density of buds; Length of multiple buds; Width of multiple buds; Thickness of multiple buds; Number of leaflets; Length of leaves; Width of leaves; Length of petioles; Length of the terminal leaf; Width of the terminal leaf; Length of inflorescence; Number of racemules per flower; Percentage of fruit formed; Fruit length; Fruit width; Fruit thickness; Number of fruit per inflorescence; Kernel infestation; Weight of 100 pistachios; Weight of 100 dried pistachios; Weight of 100 dried pistachio kernels; Ratio of the weight of 100 dried pistachio; kernels to the weight of 100 pistachios; The ratio of the pistachio kernel to the testa; Length of the pistachio kernel; Width of the pistachio kernel; Thickness of the pistachio kernel.

DNA extraction and ISSR assay

Fruit were used randomly from 5-10 plants in each of the studied cultivars. These were dried by silica gel powder. CTAB activated charcoal protocol was used to extract genomic DNA (ESFANDANI- BOZCHALOYI *et al*., 2019). The quality of extracted DNA was examined by running on 0.8% agarose gel. For the ISSR analysis, 18 primers from UBC (University of British Columbia) series were tested for DNA amplification. 12 primers were chosen for ISSR analysis of genetic variability, based on band reproducibly (Table 2).

Table 2. Details about the banding pattern revealed by ISSR primers

Primer name	Primer sequence (5'-3')	TNB	NPB	PPB	PIC	PI	EMR	MI
ISSR-1	(CA)8AG	6	6	100.00%	0.36	5.34	8.55	3.44
ISSR-2	(AC)8YG	6	4	63.00%	0.43	4.88	8.56	3.65
ISSR-3	(AG)8G	2	1	50.00%	0.25	5.23	7.23	7.47
ISSR-4	(GA)8RC	4	3	72.00%	0.55	3.66	7.56	2.67
ISSR-5	(AG)8YT	3	3	100.00%	0.44	3.21	10.60	5.55
ISSR-6	(GA)8T	6	5	86.00%	0.35	4.66	8.56	8.67
ISSR-7	(CA)8T	5	5	100.00%	0.44	3.21	9.60	4.55
ISSR-8	(GA)8A	4	3	75.00%	0.43	6.56	9.34	2.17
ISSR-9	(AG)8T	3	3	100.00%	0.34	4.21	6.60	5.59
ISSR-10	(AC)8YT	4	3	66.00%	0.47	3.37	9.55	3.45
ISSR-11	(GACA)5	4	2	50.00%	0.53	6.56	8.34	6.11
ISSR-12	BDB(TCC)7	6	5	86.00%	0.59	4.22	10.11	4.33
Average		5.3	4.4	83.00%	0.42	4.39	8.66	5.9

TNB - the number of total bands, NPB: the number of polymorphic bands, PPB (%): the percentage of polymorphic bands, PI: polymorphism index, EMR, effective multiplex ratio; MI, marker index; PIC, polymorphism information content for each of CBDP primers

PCR reactions were carried in a 25µl volume containing 10 mM Tris-HCl buffer at pH 8; 50 mM KCl; 1.5 mM MgCl2; 0.2 mM of each dNTP (Bioron, Germany); 0.2 µM of a single primer; 20 ng genomic DNA and 3 U of *Taq* DNA polymerase (Bioron, Germany). The amplifications' reactions were performed in Techne thermocycler (Germany) with the following program: 3Min initial denaturation step 95°C, followed by 35 cycles of 30 s at 95°C; 1:30 min at 43-59°C and 2 min at 72°C. The reaction was completed by final extension step of 10 min at 72°C. The amplification products were observed by running on 1% agarose gel, followed by the ethidium bromide staining. The fragment size was estimated by using a 100 bp molecular size ladder (Fermentas, Germany).

Data analyses

Morphological studies

In total 28 morphological (28 quantitative) characters were studied. Five plant specimens were randomly studied for morphological analyses. Morphological characters were first standardized (Mean = 0, Variance = 1) and used to establish Euclidean distance among pairs of taxa (PODANI, 2000). For grouping of the plant specimens, The UPGMA (Unweighted paired group using average) and Ward (Minimum spherical characters) as well as ordination methods of MDS (Multidimensional scaling) and PCoA (Principal coordinate analysis) were used (PODANI, 2000). ANOVA (Analysis of variance) were performed to show morphological difference among the populations while, PCA (Principal components analysis) biplot was used to identify the most variable morphological characters among the studied populations (PODANI, 2000). PAST version 2.17 (HAMMER *et al.*, 2012) was used for multivariate statistical analyses of morphological data.

Molecular analyses

ISSR bands obtained were coded as binary characters (presence = 1, absence = 0) and used for genetic diversity analysis. Discriminatory ability of the used primers was evaluated by means of two parameters, polymorphism information content (PIC) and marker index (MI) to characterize the capacity of each primer to detect polymorphic loci among the genotypes (POWELL *et al.*, 1996). MI is calculated for each primer as MI = PIC × EMR, where EMR is the product of the number of polymorphic loci per primer (*n*) and the fraction of polymorphic fragments (β) (WEISING *et al.*, 2005). The number of polymorphic bands (NPB) and the effective multiplex ratio (EMR) were calculated for each primer. Parameter like Nei's gene diversity (H), Shannon information index (I), number of effective alleles, and percentage of polymorphism (P% =number of polymorphic loci/number of total loci) were determined (WEISING *et al.*, 2005; FREELAND *et al.*, 2011). Shannon's index was calculated by the formula: H' = -Σpiln pi. Rp is defined per primer as: Rp = ∑ Ib, were "Ib" is the band informativeness, that takes the values of 1-(2x [0.5-p]), being "p" the proportion of each genotype containing the band. The percentage of polymorphic loci, the mean loci by accession and by population, UHe, H' and PCA were calculated by GenAlEx 6.4 software (PEAKALL and SMOUSE, 2006).

Nei's genetic distance among populations was used for Neighbor Joining (NJ) clustering and Neighbor-Net networking (FREELAND *et al*., 2011; HUSON and BRYANT, 2006). Mantel test checked the correlation between geographical and genetic distance of the studied populations (PODANI, 2000). These analyses were done by PAST ver. 2.17 (HAMER *et al*., 2012),

DARwin ver. 5 (2012) and SplitsTree4 V4.13.1 (2013) software.

AMOVA (Analysis of molecular variance) test (with 1000 permutations) as implemented in GenAlex 6.4 (PEAKALL and SMOUSE, 2006), and Nei's Gst analysis as implemented in GenoDive ver.2 (2013) (MEIRMANS and VAN TIENDEREN, 2004) were used to show genetic difference of the populations. Moreover, populations' genetic differentiation was studied by G'ST est = standardized measure of genetic differentiation (HEDRICK, 2005), and D_est = Jost measure of differentiation (JOST, 2008).

The genetic structure of populations was studied by Bayesian based model STRUCTURE analysis (PRITCHARD *et al.*, 2000), and maximum likelihood-based method of K-Means clustering of GenoDive ver. 2. (2013). For STRUCTURE analysis, data were scored as dominant markers (FALUSH *et al.*, 2007). The Evanno test was performed on STRUCTURE result to determine proper number of *K* by using delta *K* value (EVANNO *et al.*, 2005). In K-Means clustering, two summary statistics, pseudo-F, and Bayesian Information Criterion (BIC), provide the best fit for k (MEIRMANS, 2012). Gene flow was determined by (i) Calculating Nm an estimate of gene flow from Gst by PopGene ver. 1.32 (1997) as: Nm = 0.5(1 - Gst)/Gst. This approach considers equal amount of gene flow among all populations. (ii) Population assignment test based on maximum likelihood as performed in Genodive ver. in GenoDive ver. 2. (2013). The presence of shared alleles was determined by drawing the reticulogram network based on the least square method by DARwin ver 5. (2012).

RESULTS

Morphometry

In present study 11 female genotypes were collected. ANOVA test revealed significant difference in quantitative morphological characters among the studied populations ($P < 0.05$). Clustering and PCoA plot of *Pistacia vera* cultivars based on morphological characters produced similar results therefore only PCoA plot is presented and discussed (Fig. 3).

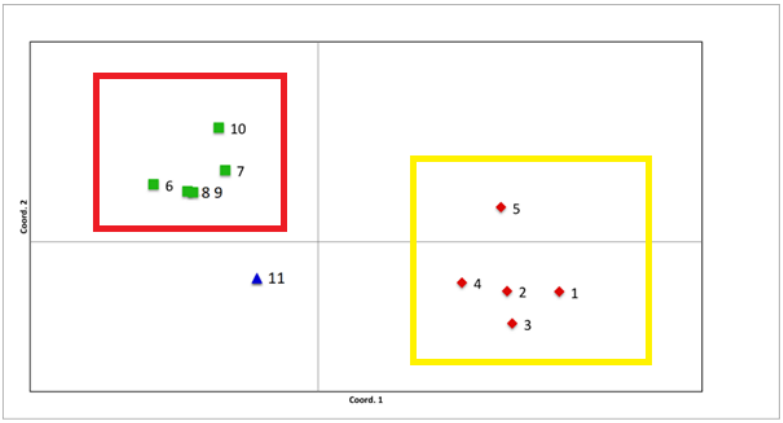

Fig. 3. Principal coordinate analysis (PCoA) for the studied genotypes of *P. vera* based on morphological character. Note: Population numbers are according to Table 1.

Based on PCoA analysis, the 11 female genotypes were divided into three major groups (Fig. 3). Group I consists of five genotypes: Kaleghochi (Pust Sefid); Shahpasand (Pust Sefid); Akbari (Pust Ghermez); Khanjari Damghan and Kaleghochi (Pust Ghermez) genotypes. The leaves of these genotypes were larger than other groups; also pistachio nuts were smaller and lighter than most of the genotypes. The second group included Shahpasand (Pust Ghermez); Fakhri; Akbari (Pust Sefid); Abbas-Ali and Ahmad Agaei. Leaf size of these genotypes was the smallest among all genotypes; Group III consists of Menghar Kalaghi genotypes. They had large leaf surface areas and high percentages of half-crackedness; they were early maturing as well. Of course, weather conditions are one of the factors that have to be taken into account because the yield and fruit bearing of pistachio trees vary under different weather conditions. However, cold winters and hot summers are favorable in pistachio production.

Populations' genetic diversity

12 ISSR primers were screened to study genetic relationships among the studied genotypes*;* all the primers produced reproducible polymorphic bands in all 11 *Pistacia vera* cultivars. An image of the ISSR amplification generated by ISSR-3 primer is shown in Figure 4. A total of 44 amplified polymorphic bands were generated across 11 *Pistacia vera* cultivars. The size of the amplified fragments ranged from 300 to 1600 bp. The highest and lowest number of polymorphic bands was 6 for ISSR-1 and 1 for ISSR-3, on an average of 4.4 polymorphic bands per primer. The PIC of the 12 ISSR primers ranged from 0.25 (ISSR-3) to 0.59 (ISSR-12) with an average of 0.42 per primer. MI of the primers ranged from 2.17 (ISSR-8) to 8.67 (ISSR-6) with an average of 5.9 per primer. EMR of the ISSR primers ranged from 6.60 (ISSR-9) to 10.60 (ISSR-5) with an average of 8.66 per primer (Table 2). The primers with the high EMR values were considered to be more informative in distinguishing the genotypes.

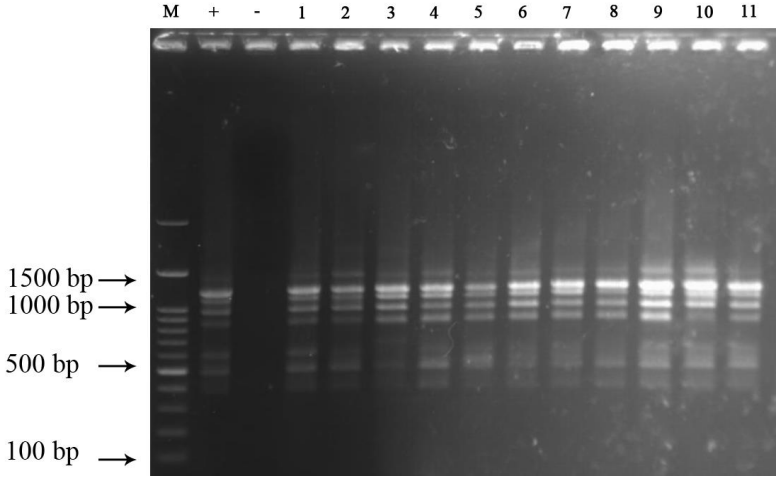

Figure 4. Results of amplification with primer ISSR3 (AG)8G on agarose 1.8% with 14 lanes gel tray. M= molecular weight; 1-11 individuals of *P.vera.*

Genetic diversity parameters determined in 11 pistachio genotypes are presented in Table 3. The highest value of percentage polymorphism (59.26%) was observed in Akbari (Pust Sefid) (population No.8) which shows high value for gene diversity (0.27). and Shanon' information index (0.38). Population Akbari (Pust Ghermez) (No.3) has the lowest value for percentage of polymorphism (21.34%) and the lowest value for Shanon, information index (0.12), and He (0.099).

Table 3. Genetic diversity parameters in the studied populations of pistachio cultivars (N = number of samples, Ne = number of effective alleles, I= Shannon's information index, He = gene diversity, UHe = unbiased gene diversity, P%= percentage of polymorphism, populations).

Pop	N	Na	Ne	I	He	uHe	%P
pop1	9.000	0.805	1.166	0.151	0.098	0.104	39.33%
pop2	10.000	0.943	1.239	0.209	0.139	0.146	42.53%
pop3	9.000	0.517	1.143	0.128	0.099	0.084	21.34%
pop4	11.000	1.379	1.359	0.229	0.217	0.227	35.52%
pop5	10.000	1.115	1.295	0.257	0.171	0.180	50.57%
pop6	9.000	0.805	1.166	0.151	0.098	0.104	33.33%
pop7	10.000	0.724	1.167	0.143	0.095	0.100	28.74%
pop8	8.000	0.499	1.067	0.38	0.271	0.34	59.26%
pop9	9.000	0.261	1.024	0.192	0.23	0.23	43.15%
pop10	6.000	0.555	1.021	0.29	0.25	0.28	43.53%
pop11	10.000	0.431	1.088	0.23	0.22	0.23	57.53%

Population genetic differentiation

AMOVA (PhiPT = 0.81, P = 0.001), and Gst analysis (0.229, p = 0.001) revealed significant difference among the studied genotypes. It also revealed that, 35% of total genetic variability was due to within genotypes diversity and 65% was due to among genotypes genetic differentiation. Pairwise AMOVA produced significant difference among the studied genotype. Moreover, we got high values for Hedrick standardized fixation index after 999 permutation (G'st = 0.583, P = 0.001) and Jost, differentiation index (D-est = 0.255, P = 0.001). These results indicate that the cultivars of *P. vera* are genetically differentiated from each other.

Mantel test after 5000 permutations produced significant correlation between genetic distance and geographical distance in these genotypes (r = 0.77, P = 0.0001). Therefore, the genotypes that are geographically more distant have less amount of gene flow, and we have isolation by distance (IBD) in cultivars of *P. vera*.

Population's grouping based on genetic data

The comparison between genetic identity and genetic distance (Table 4) showed a genetic similarity (0.95) between populations Khanjari Damghan and Kaleghochi (Pust Ghermez) (nos.4 and 5), while the lowest genetic similarity value (0.70) occurs between Kaleghochi (Pust Sefid) and Akbari (Pust Sefid) (pop. nos. 1 and 8).

Table 4. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among the studied cultivars of P. vera based on ISSR

Pop ID	1	2	3	4	5	6	7	8	9	10	11
1	****	0.8368	0.8583	0.8316	0.8494	0.7198	0.7520	0.7046	0.7848	0.7559	0.8642
2	0.1781	****	0.9105	0.8758	0.8892	0.7961	0.8139	0.8098	0.8643	0.8476	0.8741
3	0.1528	0.0937	****	0.9195	0.9356	0.8539	0.8709	0.8522	0.8662	0.8691	0.9451
4	0.1843	0.1327	0.0839	****	0.9576	0.8116	0.8173	0.8293	0.8259	0.8794	0.8098
5	0.1632	0.1175	0.0666	0.0434	****	0.8044	0.8411	0.8258	0.8397	0.8644	0.8522
6	0.3288	0.2280	0.1579	0.2087	0.2176	****	0.8993	0.8553	0.8642	0.7733	0.8293
7	0.2851	0.2059	0.1383	0.2018	0.1731	0.1061	****	0.8703	0.8741	0.8152	0.8258
8	0.2816	0.2110	0.1599	0.1872	0.1915	0.1563	0.1389	****	0.9451	0.8329	0.7546
9	0.2424	0.1458	0.1437	0.1912	0.1747	0.1459	0.1346	0.0565	****	0.8479	0.8098
10	0.2799	0.1653	0.1403	0.1285	0.1457	0.2571	0.2044	0.1828	0.1649	****	0.8522
11	0.1383	0.2018	0.1731	0.3288	0.2280	0.1579	0.2087	0.2176	0.1915	0.1563	****

Populations' genetic affinity

The dendrogram obtained from the ISSR markers by the UPGMA method revealed high genetic variation between the studied cultivars and grouped them into two main clusters (Figure 5).

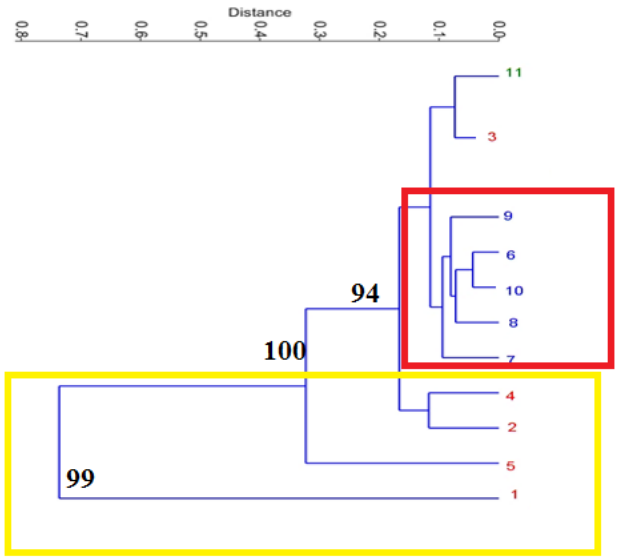

Fig 5. UPGMA dendrogram produced using Dice's coefficient based on ISSR markers in the studied genotypes of *P. vera.* Note: genotypes numbers are according to Table 1.

The first main cluster contained 5 cultivars including Kaleghochi (Pust Sefid); Shahpasand (Pust Sefid); Akbari (Pust Ghermez); Khanjari Damghan and Kaleghochi (Pust Ghermez) genotypes have high similarities. Also, they have high similarities in morphological traits such as nut shape, kernel color and kernel size. The second cluster consisted of 5 genotypes including Shahpasand (Pust Ghermez); Fakhri; Akbari (Pust Sefid); Abbas-Ali and Ahmad Agaei. Also, they have high similarities in traits related to fruit (nut and kernel size and colour) and leaf.

Populations genetic structure

Evanno test performed on STRUCTURE analysis produced the best number of k = 3. This genetic grouping is in agreement with UPGMA tree and NeighborNet diagram result presented before.

Both these analyses revealed that cultivars of *P. vera.* show genetic stratification. STRUCTURE plot based on k =3, revealed genetic difference of populations 1-5 (differently colored), also it showed genetic affinity between populations 6-10 (similarly colored), (Fig. 6).

As evidenced by STRUCTURE plot based on admixture model, these shared alleles comprise very limited part of the genomes in these populations and all these results are in agreement in showing high degree of genetic stratification within cultivars of *Pistacia vera*.

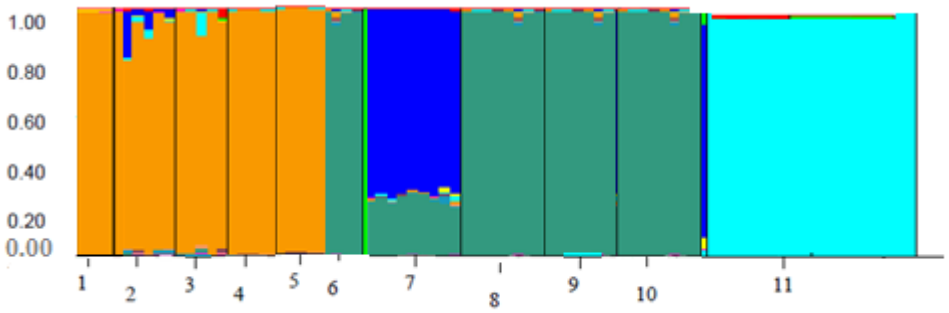

Fig. 6. Bayesian clustering analysis (K= 2) based on ISSR markers for the studied genotypes of *P. vera* (11 samples) performed using STRUCTURE. Vertical black lines separate genotypes

DISCUSSION

Population genetic study provides valuable information about genetic structure of plants, the stratification versus gene flow among the species populations, genetic divergence of the populations, etc. (ESFANDANI- BOZCHALOYI, 2017a; 2017b; 2017c; 2017d). These information have different applications, and from pure understanding of biology of the species to conservation of endangered species, choosing of proper parents for hybridization and breeding and phylogeography and mechanism of invasion (FREELAND *et al*., 2011).

Pistachio has important socio-economic and ecological impacts in the arid and semi-arid agricultural regions of Iran (KAFKAS *et al*., 2006). In addition, Iran hosts a wide genetic diversity of *Pistacia* spp. and more than 300 pistachio genotypes have been collected across the

country. Iran therefore possesses valuable germplasm for pistachio improvement and conservation programs. Assessing genetic diversity and relationships among cultivars of Iranian pistachio, using discriminative and robust markers, is therefore important.

This study was aimed at evaluating the genetic diversity of Iranian pistachio in order to aid the conservation of its germplasm. The obtained information about the genetic variation between and within different populations will prepare the ground for the formulation of appropriate conservation strategies. The present analysis revealed that Iranian-cultivated pistachio germplasm is highly variable, presumably due to specific local genetic backgrounds, breeding pressure and/or limited interchange of genetic material. The unique nature of the Iranian pistachio germplasm revealed by our results, supports the case for the implementation of more intense characterization, conservation and breeding strategies. Also, the ISSR markers used were useful for determination of genetic diversity among pistachio cultivars in Iran.

The results of this molecular assay in fingerprinting of the 11 pistachio genotypes are presented in table 3. In ISSR, according to the reported results of (KAFKAS *et al*., 2006), first six primers were used and after initial screening three out of them primers eventually selected for the final analysis. A total of 28 bands were amplified by the three primers, an average of 9.3 bands per primer of which 13 (46/42%) were polymorphic. The total number of amplified fragments was between seven to 12 and the number of polymorphic fragments ranged from three to five.

During the ISSR screening in this study, good amplification products were obtained from primers based on GA, CA and GAA repeats. But primers based on CT, GT and CAA repeats produced few large separate bands which finally were eliminated for the final analysis. KAFKAS *et al*., 2006) using 20 primers obtained a total of 156 bands, an average of 7.7 bands per primer, of which 73(46.2%) were polymorphic which is similar to our results in this study.

MIRZAEI *et al*. (2005) reported 80% polymorphism among 22 Iranian cultivars and wild pistachio species. The difference in polymorphism reported in the current study and that of Mirzaei et al. (2005) could be attributed to differences in the tested genotypes and the selected primers. Katsiotis et al. (2003) obtained 82.41% polymorphism and of a total of 22.11, there were 18.2 polymorphic bands. In a study reported by Golan-Goldhirsh et al. (2004) in assessing polymorphisms among 28 Mediterranean *Pistacia* accessions, twenty seven selected primers produced 259 total bands (average 9.59) and 86.1 of them were polymorphic.

KHADIVI (2018) revealed high level of polymorphism among the studied genotypes. The seven SSR primer pairs generated a total of 18 alleles that 13 of them were polymorphic among the genotypes. The range of the polymorphic alleles was 1 (for Ptms9, Ptms40, Ptms41, and Ptms42) to 5 (for Ptms7 locus) with an average of 2.57. The amplified allele sizes ranged from 120 to 250bp. Pair-wise genetic similarity coefficients varied from 0.20 to 0.75.

The present study indicated that a higher genetic diversity was found in the older genotypes. This fact confirms our speculation that pistachio cultivations have increasingly led to the reduction of their genetic variation due to deployment of improved cultivars and to the availability of private or public grafted seedling nurseries for pistachio, as well as the changing livelihood conditions. Recently, the method of pistachio cultivation is changing leading towards an increased reduction of crop diversity deployed on farm. In the past, pistachio diversity was maintained high in the field through a number of cultivation practices, s. a. use of male varieties derived from seed, use of wild *Pistacia* species to boost pollination and hence the fruit setting,

use of natural populations of wild *Pistacia* (*P. atlantica*) as a rootstock due to their well-known resistance to stony and calcareous soils. In order to utilize different genotypes of pistachio efficiently, it is important to know the genetic variation and genetic relationships that exist between them. The SSR primer pairs used in the present study revealed moderate levels of polymorphism in Iranian pistachios.

AHMAD *et al*. (2003, 2005) reported moderate diversity among the Syrian, Turkish, and American pistachios. Protection against the loss of genetic diversity is required urgently (HAMRICK and GODT, 1996). In traditional areas of pistachio cultivation, contact between the cultivated clones and wild species is quite common and has existed for hundreds or even thousands of years (ZOHARY, 1996). As a result, interspecific hybridization of *P. vera* with other *Pistacia* species led to the development of various hybrids with different backgrounds (MAGGS, 1973; BARAZANI *et al*., 2003).

In conclusion, although Iran is one of the two major centers of *Pistacia* diversity and the main pistachio producer in the world, the Iranian pistachio industry has a very narrow genetic base. The present results demonstrated that the study of genetic diversity among some *Pistacia* genotypes and cultivars using ISSR markers provided information that is relevant for the conservation of pistachio germplasm. The current results showed that Iranian genotypes have a moderate genetic variation and therefore are very important for genetic conservation and the planning of future breeding programs. The present results may be used for the conservation, core collection and future breeding of the pistachio.

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GENETIČKI DIVERZITET I STRUKTURA POPULACIJE IRANSKIH KULTIVARA PISTAĆA (*Pistacia vera* L.)

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Izvod

Iran ima bogatu germplazmu pistaća, pa je diverzitet i broj sorti iranskog pistaća jedinstven u svetu. Genetska raznolikost je presudna za održivo korišćenje genetičkih resursa i njihovo očuvanje. Kao jedna od najstarijih useva orašastih plodova u istoriji čovečanstva, plodovi pistaća imaju visoku hranljivu vrednost i komercijalno su važni. U ovoj studiji, genetska varijacija genotipova pistaća istražena je nuklearnim ISSR markerima. Genetska veza između 11 sorti procenjena je korišćenjem 12 ISSR prajmera. Ukupno 53 trake, od kojih je 44 (83%) polimorfno, amplifikovano je sa 12 prajmera, sa prosečno 4,4 trake po prajmeru. Ukupan broj amplifikovanih fragmenata bio je između 2 i 6, a broj polimorfnih fragmenata bio je u rasponu od dva do šest. Amplifikovni aleli imali su veličinu od 300 do 1600 bp. Parovi koeficijenta genetske sličnosti varirali su od 0,70 do 0,95. UPGMA dendrogram razdvojio je genotipove u dva glavna klastera. Mantelov test pokazao je korelaciju između genetske i geografske udaljenosti. AMOVA je otkrila značajnu genetsku razliku među sortama i pokazala je da je 35% ukupnih genetskih varijacija nastalo zbog raznolikosti unutar sorti. Sadašnji rezultati mogu se koristiti za konzervaciju, stvranje *core* kolekcija i buduće oplemenjivanje pistaća.

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