UDC 575.630
https://doi.org/10.2298/GENSR2102783Q
Original scientific article

DETECTING DNA POLYMORPHISM AND GENETIC DIVERSITY IN A WIDE PISTACHIO GERMPLASM BY RAPD MARKERS

Xiaohui QIAN[1]* and Shahram MEHRI[2]

[1]Department of Tourism and Culture, Anhui Finance and Trade Vocational College, Hefei, China
[2]Department of Agronomy and Plant Breeding, ParsAbad Moghan Branch, Islamic Azad University, ParsAbad Moghan, Iran

Qian X. and S. Mehri (2021). *Detecting DNA polymorphism and genetic diversity in a wide pistachio germplasm by RAPD markers*- Genetika, Vol 53, No.2, 783-798.

Assessing the genetic diversity in the population is the prerequisite to start and develop plant breeding projects. *Pistacia vera* is considered as a commercial species of *Pistacia* genus. In Iran, Pistachio export is in the second place in terms of non-oil exports and in the first place among horticultural crops. Therefore, we collected and analyzed 11 pistachio genotype (*Pistacia vera*), from two provinces of Iran regions. Our aims were 1) to assess genetic diversity among some of Irainian pistachio cultivars 2) is there a correlation between species genetic and geographical distance? 3) Genetic structure of populations and taxa. We showed significant differences in quantitative morphological characters in plant species. Akbari cultivars depicted unbiased expected heterozygosity (UHe) in the range of 0.028. Shannon information was high (0.49) in Seifadini cultivars. Akbari cultivars howed the lowest value, 0.029. The observed number of alleles (*Na*) ranged from 0.261 to 2.700 in Shahpasand cultivars and Kalehghoochi cultivars. The effective number of alleles (*Ne*) was in the range of 1.021-1.800 for Akbari cultivars and Moosaabadi cultivars .Gene flow (Nm) was relatively low (0.38) in pistachio cultivars. The Mantel test showed correlation (r = 0.33, p=0.0001) between genetic and geographical distances. We reported high genetic diversity, which clearly shows the among some of Irainian pistachio cultivars can adapt to changing environments since high genetic diversity is linked to species adaptability. Present results highlighted the utility of RAPD markers and morphometry methods to investigate genetic diversity in pistachio cultivars.

Keyword: Gene flow, Random Amplified Polymorphic DNA (RAPD), pistachio cultivars, isolation, morphometry

Corresponding author: Xiaohui Qian, Department of Tourism and Culture, Anhui Finance and Trade Vocational College, Hefei, China, E-mail: xuanxuanww2@163.com; sh.mehri2000@gmail.com

INTRODUCTION

Genetic diversity is a vital feature that helps plant species survive in an ever-changing environment, and it sheds light on understanding the phylogenetic affinity among the species (ERBANO *et al*. 2015). Quite a significant number of genetic resources and materials programs of plant species have been carried out to preserve the plant species worldwide. Scientific data indicate that genetic diversity plays a pivotal role in conservation programs (GOMEZ *et al*. 2005; CONGFEN *et al*., 2021; HAN *et al*., 2021; ZHAO, *et al*., 2021). Iran is considered as one of the main centers for genetic diversity of pistachio including a high diversity of female varieties and male genotypes (TAYEFEH ALIAKBARKHANY *et al*., 2013). Understanding plant genetic diversity is necessary for optimal use of the products, leading to special attention to biodiversity by breeders and researchers (ESMAILPOUR, 2001; ZHANG *et al*., 2020; ZHANG *et al*., 2016; JIANG *et al*., 2021). Studying the genetic diversity of pistachio helps to improve the management and cultivation. Identification and accurate assessment of Iranian pistachios is done to design appropriate improvement programs and ensures the provision of improved cultivars is essential (KAFKAS, 2006; XU, *et al.*, 2021; WANG, *et al.*, 2020).

Pistachio (*Pistacia* sp. L.) belongs to a genus of the Anacardiaceae family. It is one of the most prominent horticultural plants from an economic and commercial point of view, so that vast pieces of land in Iran are allocated to the growth of this plant (ANONYMOUS, 2001). All pistachio species are dioecious and wind-pollinated. The genus *Pistacia* consists of eleven species which only has edible nuts and is commercially important (ZOHARY, 1952). *Pistacia vera* is native to north Afghanistan, northeast Iran, and central Asian republics (BROWIEZ, 1988; KAFKAS, 2006). 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). In addition, there are two other wild species in Iran; *Pistacia atlantica* subsp. *Mutica* and *Pistacia khinjuk*. These species are used mainly as rootstock for *Pistacia vera* and rarely for oil extraction in some countries (KAFKAS and PERL-TREVES, 2002b).

Molecular studies addressing the genus *Pistacia* are few. Most studies regarding the analysis of the genetic diversity of Iranian pistachios have been based on morphological characteristics (KAFKAS *et al*., 2002a; TAJABADIPUR, 1997).

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 them, RAPD (WILLIAMS *et al*., 1990) has been the most commonly used method in pistachio cultivars characterization (HORMAZA *et al.*, 1994; 1998; KAFKAS *et al.*, 2002; KATSIOTIS *et al.*, 2003; GOLAN-GPLDHIRSH *et al.*, 2004; MIRZAEI *et al*., 2005). AFLP and SSR techniques have been also used in pistachio to study genetic relationship among *Pistacia* species and cultivars (GOLAN-GOLDHIRSH *et al.*, 2004; KATSIOTIS *et al.*, 2003; IBRAHIM BASHA *et al.*, 2007; AHMAD *et al.*, 2003; AHMAD *et al.*, 2005; AHMADI AFZADI *et al.*, 2007). Since, there is not much information about genetic identities of male cultivars and genotypes in Iran, therefore the identification of genetic diversity can solve a lot of problems regarding to pistachio pollination (AHMAD *et al.*,

2005). KATS IOTIS *et al*.(2003) studied the effect of pollen type on three pistachio cultivars ('Kalehghoochi', 'Ohadi' and 'Ahmadaghaee'), it was found that pollen type can affect nitrogen, phosphor, potassium, iron, and boron contents of the kernel of pistachio, also According to their study total fruit weight and blankness were affected by pollen type. It has been shown that by using protein marker, male genotypes of pistachio has more polymorphisms compared to the female cultivar (MIRZAEI *et al.*, 2005).

Genetic diversity studies are usually tapped due to molecular markers. Molecular markers are an excellent method to disentangle phylogenetic association between species and population. Among molecular methods or markers, RAPD (Random Amplified Polymorphic DNA) are sensitive to detect variability among individuals of species. RAPD method is cost-effective and can work with limited sample quantities. In addition to this, RAPD can amplify and target genomic regions with potential and several markers (ESFANDANI-BOZCHALOYI *et al.*, 2017).

Taxonomical systematics studies were conducted in the past to identify of Irainian pistachio cultivars. According to the best of our knowledge, there is no existing RAPD data on genetic diversity investigations in Iran. We studied 11 samples. Our aims were 1) to assess genetic diversity among of Irainian pistachio cultivars 2) is there a correlation between cultivars and geographical distance? 3) Genetic structure of populations and taxa? These results could benefit Irainian pistachio germplasm collection, conservation and future breeding.

MATERIALS AND METHODS

Plant materials

Eleven specimens belonging to *Pistacia vera* were collected from different localities that were placed between two provinces Rafsanjani Pistachio Research and Semnan, Damghan. Details of geographical populations are given in Table 1, Fig1. Different references were used for the correct identification of species *Pistacia vera* (ZOHARY, 1952; BARONE *et al*., 1993; DOLLO, 1993). Vouchers were deposited at the herbarium of Islamic Azad University, Science and Research Branch, Tehran, Iran (IAUH).

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

Code	Genotypes	Locality	Voucher no.
1	Jandaghi	Kerman, Rafsanjan	IAUH-00001
2	Moosaabadi F	Kerman, Rafsanjan	IAUH-00002
3	Mohseni F	Kerman, Rafsanjan	IAUH-00003
4	Seifadini F	Kerman, Rafsanjan	IAUH-00004
5	Ghafoori F	Kerman, Rafsanjan	IAUH-00005
6	Kalehghoochi F	Kerman, Rafsanjan	IAUH-00006
7	Harati F	Kerman, Rafsanjan	IAUH-00007
8	Phandoghireez F	Kerman, Rafsanjan	IAUH-00008
9	Shahpasand F	Semnan, Damghan	IAUH-00009
10	Akbari F	Semnan, Damghan	IAUH-000010
11	Poostkhormayee F	Semnan, Damghan	IAUH-000011

Morphometry

In total 21 morphological (21 quantitative) characters were studied. Four to twelve samples from each population were randomly studied for morphological analyses (Appendix 1). 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) were used (PODANI, 2000). PAST version 2.17 (HAMMER *et al.*, 2012) was used for multivariate statistical analyses of morphological data.

Random Amplified Polymorphic DNA

We extracted DNA from fresh leaves. Leaves were dried. DNA extraction was carried out according to the previous protocol (ESFANDANI-BOZCHALOYI *et al.*, 2019). DNA quality was checked on an agarose gel to confirm the purity. We amplified the DNA with the aid of RAPD primers (Operon technology, Alameda, Canada). These primers belonged to OPA, OPB, OPC, OPD sets. We selected those primers (10) which could show clear bands and polymorphism (Table 2). Overall, the polymerase chain reaction contained 25µl volume. This 25 volume had ten mM Tris-HCl buffer, 500 mM KCl; 1.5 mM $MgCl_2$; 0.2 mM of each dNTP; 0.2 µM of a single primer; 20 ng genomic DNA and 3 U of *Taq* DNA polymerase (Bioron, Germany). We observed the following cycles and conditions for the amplification. Five minutes initial denaturation step was carried out at 94°C after this forty cycles of 1 minute at 94°C were observed. Then 1-minute cycle was at 52-57°C followed by two minutes at 72°C. In the end, the final extension step was performed for seven to ten minutes at 72°C. We confirmed the amplification steps while observing amplified products on a gel. Each band size was confirmed according to 100 base pair molecular ladder/standard (Fermentas, Germany).

Data analyses

We used an Unweighted pair group method with arithmetic mean (UPGMA) and Ward methods. Ordination methods such as multidimensional scaling and principal coordinate analysis were also performed (PODANI, 2000). The morphological difference among species and population was assessed through analysis of variance (ANOVA). PCA analysis (PODANI, 2000) was done to find the variation in plant population morphological traits. Multivariate and all the necessary calculations were done in the PAST software, 2.17 (HAMMER *et al*., 2001). To assess genetic diversity, we encoded RAPD bands as present and absent. Numbers 1 and 0 were used to show the presence and absence of bands. It is essential to know the polymorphism information content and marker index (MI) of primers because these parameters serve to observe polymorphic loci in genotypes (ISMAIL *et al*., 2019). Marker index was calculated according to the previous protocol (HEIKRUJAM *et al.*, 2015). Other parameters such as the number of polymorphic bands (NPB) and effective multiplex ratio (EMR) were assessed. Gene diversity associated characteristics of plant samples were calculated. These characteristics include Nei's gene diversity (H), Shannon information index (I), number of effective alleles (Ne), and percentage of polymorphism (P% =number of polymorphic loci/number of total loci) (SHEN *et al*.,2017). Unbiased expected heterozygosity (UHe), and heterozygosity were assessed in

GenAlEx 6.4 software (PEAKALL and SMOUSE, 2006). Neighbor-joining (NJ) and networking were studied to fathom genetic distance plant populations (HUSON and BRYANT, 2006; FREELAND *et al*., 2011). The Mantel test was carried out to find the correlation between genetic and geographical distances (PODANI, 2000). As we were interested in knowing the genetic structure and diversity, we also investigated the genetic difference between populations through AMOVA (Analysis of molecular variance) in GenAlEx 6.4 (PEAKALL and SMOUSE, 2006).

To assess the population structure of the pistachio genotypes, a heuristic method based on Bayesian clustering algorithms were utilized. The clustering method based on the Bayesian-model implemented in the software program STRUCTURE (PRITCHARD *et al*., 2000; FALUSH *et al*., 2007) was used on the same data set to better detect population substructures. This clustering method is based on an algorithm that assigns genotypes to homogeneous groups, given a number of clusters (K) and assuming Hardy-Weinberg and linkage equilibrium within clusters, the software estimates allele frequencies in each cluster and population memberships for every individual (PRITCHARD *et al*., 2000). The number of potential subpopulations varied from two to ten, and their contribution to the genotypes of the accessions was calculated based on 50,000 iteration burn-ins and 100,000 iteration sampling periods. The most probable number (K) of subpopulations was identified following 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 (Nm) which were calculated using POPGENE (version 1.31) program (YEH *et al*., 1999). Gene flow was estimated indirectly using the formula: Nm = 0.25(1 - FST)/FST. In order to test for a correlation between pair-wise genetic distances (FST) and geographical distances (in km) between populations, a Mantel test was performed using Tools for Population Genetic Analysis (TFPGA; MILLER, 1997) (computing 999 permutations). This approach considers equal amount of gene flow among all populations.

RESULTS

Morphometry

Significant ANOVA results ($P < 0.01$) showed differences in quantitative morphological characters in plant species. Principal component results explained 80% variation. In the first PCA axis with 55% of total variation, such characters as length of leaves; width of leaves; length of petioles; length of the terminal leaf; width of the terminal leaf; length of inflorescence have shown the highest correlation (> 0.7), fruit length; fruit width; fruit thickness; number of fruit per inflorescence; kernel infestation were characters influencing PCA axis 2 and 3, respectively. Unweighted pair group method with arithmetic mean (UPGMA) showed results (Figure 1).

Based on cluster analysis, the 11 female genotypes were divided into two major groups (Figure 1). Group I consists of six genotypes and was divided into two subgroups. The first subgroup consists of Jandaghi; Moosaabadi; Mohseni; Seifadini 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 subgroup of group I included Ghafoori and Poostkhormayee genotypes. Flowering and maturity time of these genotypes was later than most of the genotypes. Group II consists of five genotypes. This group was also divided into two subgroups. The first subgroup consists of three genotypes (Nos. 6, 8 and 9). Leaf size of these genotypes was the smallest among all genotypes; The genotypes 7, 10 were in the second subgroup, which had

considerably large pistachio nuts. 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.

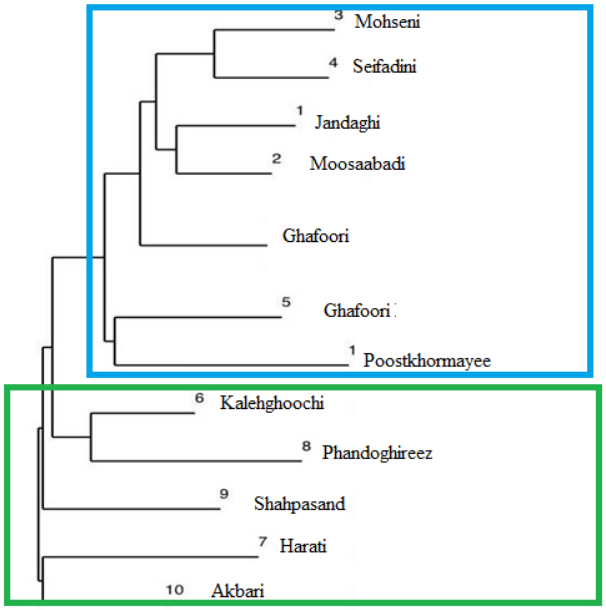

Figure 1. Dendrogram of 11 genotypes and cultivars of female pistachio based on 10 RAPD primers using UPGMA method.

Species Identification and Genetic Diversity

Twenty-five RAPD primers were tested with ten of *Pistacia vera* cultivars as DNA templates; all primers produced amplification products, and only primers showing clear and reproducible band patterns were selected for further analysis. The size of the amplified fragments ranged from 100 to 2500 bp (Fig 2). Ten primers were then chosen for the genotypes identification and phylogenetic analysis. As shown in Table 2, all 10 primers used for RAPD analysis. 80 polymorphic bands were generated and amplified. We recorded the highest polymorphic bands for OPB-02. OPA-05 had the lowest polymorphic bands. The average polymorphic bands ranged to 8 for each primer. The polymorphic information content (PIC) had values in the range of 0.14 (OPB-02) to 0.38 (OPD-08). Primers had 0.32 average polymorphic information content values.

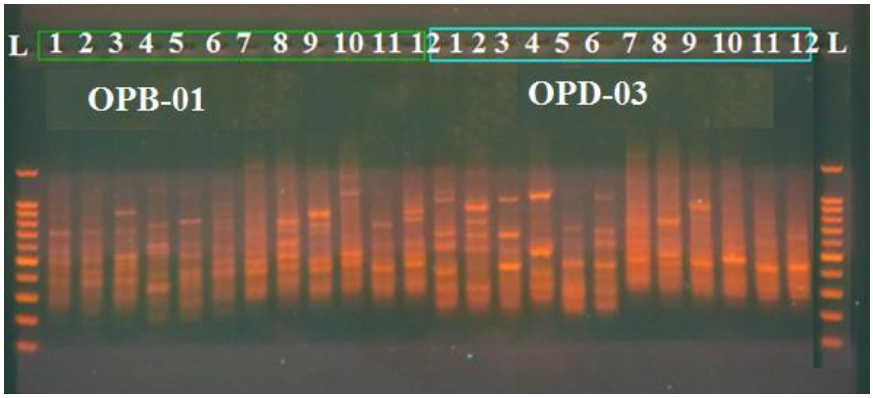

Figure 2. Gel Electrophoresis image of DNA fragments of studied female pistachio populations. L = Ladder 100 bp. Arrows show polymorphic bands.

Marker index (MI) values were 3.22 (OPD-02) to 6.77 (OPB-01), with an average of 5.5 per primer. Effective multiplex ratio (EMR) values are useful to distinguish genotypes. In our study, we reported 8.43 (OPD-03) to 13.34 (OPB-01) EMR values. EMR values averaged 11.5 per primer (Table 2). All the necessary genetic features calculated of populations of *Pistacia vera* are shown (Table 3). Akbari cultivars depicted unbiased expected heterozygosity (UHe) in the range of 0.028. Shannon information was high (0.49) in Seifadini cultivars. Akbari cultivars howed the lowest value, 0.029. The observed number of alleles (*N*a) ranged from 0.261 to 2.700 in Shahpasand cultivars and Kalehghoochi cultivars. The effective number of alleles (*Ne*) was in the range of 1.021-1.800 for Akbari cultivars and Moosaabadi cultivars. Gene flow (Nm) was relatively low (0.38) in cultivars of *P. vera.*

Analysis of Molecular Variance (AMOVA) test highlighted genetic differences among the studied genotypes (P = 0.001). AMOVA showed that 80% of genetic variation was among the genotypes. Relative less variation (20%) was reported within the genotypes. Genetic similarity and dissimilarity assessed through Genetic statistics (GST) showed significant differences i.e., (0.888, P = 0.001) and D_est values (0.198, p = 0.001). Moreover, pair-wise AMOVA revealed significant genetic difference almost among all the studied genotypes. These results indicate that of pistachio genotypes are genetically differentiated and we can use such genetic difference in future breeding programs of this valuable plant species. The results of this study showed that there is a relatively low level of genetic diversity in the studied samples which are expected in view of the dioecius and outbreeding nature of the cultivated pistachio cultivars and high level of heterozygosity due to the cross-pollinating nature of the plant established during the evolution and domestication processes which have been conserved by the propagation of clones through vegetative reproduction.

Table 2. RAPD primers and other parameters. Note: 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.

Primer name	Primer sequence (5'-3')	TNB	NPB	PPB	PIC	PI	EMR	MI
OPA-05	5′-AGGGGTCTTG-3′	8	6	92.31%	0.34	3.21	10.23	4.55
OPA-06	5′-GGTCCCTGAC-3′	10	10	100.00%	0.17	2.32	11.55	4.44
OPB-01	5′-GTTTCGCTCC-3′	9	8	96.89%	0.23	3.56	13.34	6.77
OPB-02	5′-TGATCCCTGG-3′	13	12	95.81%	0.14	4.21	10.60	5.22
OPC-04	5'-CCGCATCTAC-3'	7	7	100.00%	0.27	3.37	9.55	3.25
OPD-02	5′-GGACCCAACC-3′	10	10	100.00%	0.36	4.86	11.88	3.22
OPD-03	5′-GTCGCCGTCA-3′	9	7	84.99%	0.33	3.51	8.43	3.85
OPD-05	5′ -TGAGCGGACA-3′	12	10	93.84%	0.26	3.66	11.33	4.11
OPD-08	5′-GTGTGCCCCA-3′	10	9	94.91%	0.38	1.21	11.50	5.65
OPD-11	5′-AGCGCCATTG-3′	11	8	95.74%	0.27	2.66	9.57	5.12
Mean		9.5	8	80.44%	0.32	3.5	11.5	5.5
Total		95	80					

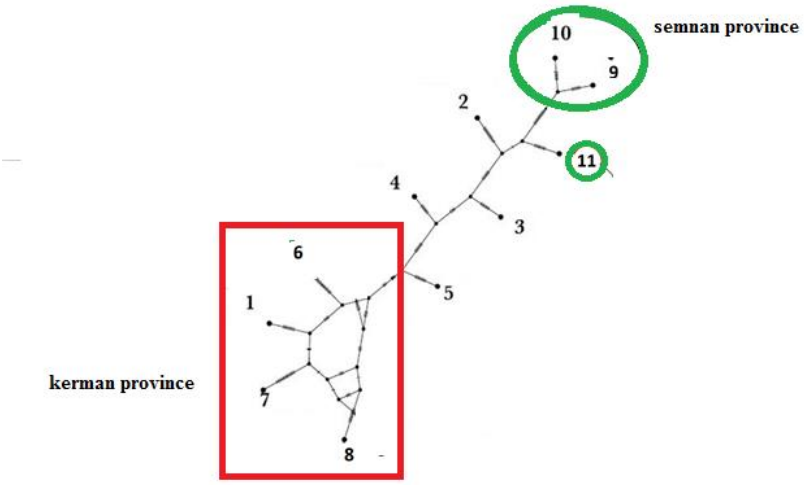

Figure 3. Integer NJ net tree produced while using RAPD data.

Table 3. Genetic diversity 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 in populations).

Pop	N	Na	Ne	I	He	uHe	%P
pop1	10.000	1.500	1.311	0.279	0.267	0.187	40.00%
pop2	9.000	2.333	1.800	0.270	0.233	0.217	53.33%
pop3	12.000	1.500	1.441	0.330	0.233	0.233	40.00%
pop4	13.000	1.333	1.232	0.496	0.300	0.343	73.33%
pop5	10.000	1.167	1.078	0.083	0.150	0.053	66.67%
pop6	15.000	2.700	1.462	0.337	0.290	0.240	47.00%
pop7	15.000	1.433	1.196	0.150	0.183	0.090	55.00%
Pop8	8.000	0.499	1.067	0.38	0.271	0.30	39.33%
Pop9	9.000	0.261	1.024	0.192	0.23	0.23	36.67%
Pop10	6.000	0.555	1.021	0.029	0.025	0.028	30.00%
Pop11	10.000	0.431	1.088	0.23	0.22	0.23	33.53%

TCS network supported NJ relationship, and revealed the presence of 2 main groups (Fig. 3). The first group was formed by Jandaghi; Moosaabadi; Mohseni; Seifadini; Ghafoori; Kalehghoochi, Phandoghireez and harati of Kerman province genotype.The second group was composed of Shahpasand; Akbari and Poostkhormayee (Semnan Province).

Table 4. Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among populations.

pop ID	1	2	3	4	5	6	7	8	9	10	11
1	****	**0.8855**	0.9073	0.8908	0.8343	0.8846	0.8541	0.7927	0.8233	0.8264	0.7822
2	0.0774	****	0.9008	0.9093	0.8842	0.8821	0.8466	0.8277	0.8565	0.8051	0.7886
3	0.0973	0.1044	****	**0.8700**	0.8897	0.8644	0.8830	0.8269	0.8453	0.7871	0.7369
4	0.1156	0.0950	0.0834	****	0.8611	0.8844	0.8829	0.8527	0.8745	0.7947	0.7825
5	0.1811	0.1231	0.1169	0.1495	****	0.8883	0.8808	0.8603	0.8594	0.8678	0.8608
6	0.1226	0.1254	0.1457	0.1229	0.1185	****	0.8888	0.8407	0.9259	0.9124	0.8907
7	0.1577	0.1665	0.1244	0.1246	0.1269	0.1179	****	0.8455	0.8775	0.7921	0.8232
8	0.2323	0.1891	0.1901	0.1594	0.1505	0.1735	0.1678	****	0.8360	**0.7562**	**0.7694**
9	0.1944	0.1549	0.1681	0.1341	0.1515	0.0879	0.1307	0.1792	****	0.8225	0.8351
10	0.1907	0.2168	0.2394	0.2298	0.1418	0.0916	0.2330	0.2795	0.1953	****	0.9054
11	0.2456	0.2375	0.2524	0.2453	0.1499	0.1157	0.1945	0.2621	0.1802	0.0775	****

Gene flow (Nm) was relatively low (0.38) in pistachio genotypes. Genetic identity and phylogenetic distance in the pistachio genotypes members are mentioned (Table 4). Kalehghoochi and Shahpasand genotypes were genetically closely related (0.925) to each other. Poostkhormayee genotypes and Mohseni genotypes were dissimilar due to low (0.736) genetic

similarity. The mantel test showed correlation (r = 0.33, p=0.0001) between genetic and geographical distances.

Populations genetic structure

The number of genetic groups was determined by two methods of 1—K-Means clustering which is based on the maximum likelihood approach, and 2—Evanno test which is based on STRUCTURE analysis and is a Bayesian approach based method. K-Means clustering based on pseudo-F and BIC (Bayesian Information Criterion) recognized 2 and 4 genetic groups, respectively. This is in agreement with AMOVA result, showing significant genetic difference among date populations of *Pistacia vera*.

Evan test based on delta k (Fig. 4) identified the optimum number of genetic groups 2. We performed STRUCTURE analysis based on k = 2, to identify the genetic groups (Fig. 5). In the plot of k = 3, the cultivars of Jandaghi; Moosaabadi; Mohseni; Seifadini; Ghafoori; Kalehghoochi, Phandoghireez and harati of Kerman province (green colored) are placed in the first genetic group, while the populations of Shahpasand; Akbari and Poostkhormayee (Semnan Province) (yellow colored) formed the second genetic group. These different genetic groups may be used in future breeding and hybridization programs of Iranian date *Pistacia vera* genotypes.

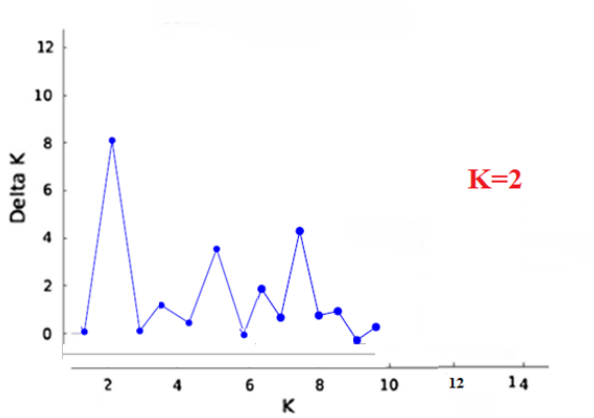

Fig. 4. Delta k plot of Evanno's test based on STRUCTURE analysis.

The mean Nm = 0.33 was obtained for all RAPD loci, which indicates low amount of gene flow among the populations and supports genetic stratification as indicated by K-Means and STRUCTURE analyses. This result is in agree with grouping we obtained with PCA plot, as these populations were placed close to each other. 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 of *Pistacia vera* genotypes.

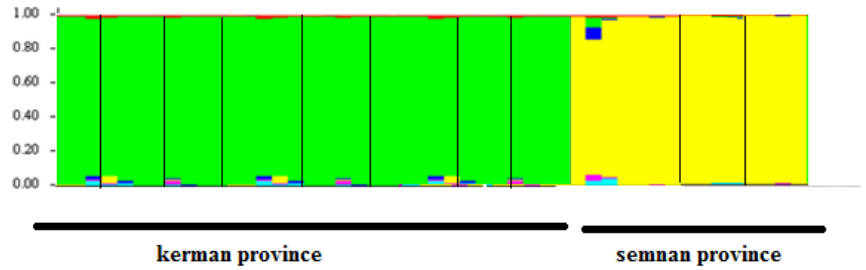

Fig. 5. STRUCTURE plot of *Pistacia vera* populations based on k = 2, Numbers are according to Table 1.

DISCUSSION

The *Pistacia vera* is a relatively complex taxonomic group, and several morphological characters make it difficult to identify and classify *Pistacia vera* species (MIRZAEI *et al*., 2005). Given the complexity, it is necessary to explore other methods that could complement the traditional taxonomical approach (ERBANO *et al*., 2015). Advent and developments in molecular techniques have enabled plant taxonomists to utilize molecular protocols to study plant groups (ERBANO *et al*., 2015). We examined genetic diversity in *Pistacia vera* genotypes by morphological and molecular methods. We mainly used RAPD markers to investigate genetic diversity and genetic affinity in *Pistacia vera* genotypes. Our clustering and ordination techniques showed similar patterns. Morphometry results clearly showed the utilization or significance of morphological characters in *Pistacia vera* genotypes. UPGMA method results also confirmed the application of morphological characters to separate *Pistacia vera* genotypes. The present study also highlighted that morphological characters such as the ratio of the pistachio kernel to the testa; length of the pistachio kernel; width of the pistachio kernel thickness of the pistachio kernel could delimit the *Pistacia vera* genotypes. We argue that such a dissimilarity was due to differences in quantitative and qualitative traits.

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 (MIRZAEI *et al*., 2005).

In our study, morphology and genetic diversity in 11 cultivar of *Pistacia vera* genotypes are given in detail for the first time. The aim of the present study was to find diagnostic features to separate *Pistacia vera* genotypes in Iran. Morphological characters are considered as an useful tool for the identification of the species, as indicated previously AHMAD *et al*. (2005).

In the present work, 11 *P. vera* cultivars were characterized with 10 RAPD markers. The results confirm the efficiency of microsatellite markers for fingerprinting purposes. Our results demonstrated that the the average polymorphic bands ranged to 8 for each primer. The polymorphic information content (PIC) had values in the range of 0.14 (OPB-02) to 0.38 (OPD-

08). Primers had 0.32 average polymorphic information content values. Marker index (MI) values were 3.22 (OPD-02) to 6.77 (OPB-01), with an average of 5.5 per primer. Effective multiplex ratio (EMR) values are useful to distinguish genotypes. In our study, we reported 8.43 (OPD-03) to 13.34 (OPB-01) EMR values. EMR values averaged 11.5 per primer. These values were higher than those reported by ARABNEJAD *et al*. (2008), who detected an average of 3.69 alleles per primer pairs and an average PIC of 0.46 detected in 20 commercial cultivars of Iranian pistachio; and also higher than those reported by BAGHIZADEH *et al*. (2010) (an average of 2.75 alleles per primer pairs and an average of 0.44 for detected in 31 Iranian pistachio cultivars) and by AHMAD *et al*. (2005) (an average of 3.30 alleles per locus in 17 pistachio cultivars). KOLAHI-ZONOOZI (2014) assessed genetic diversity of 45 commercially Iranian cultivars using 12 nSSR markers and detected that PIC varied from 0.19–0.56 with an average of 0.33 and the mean of Ho and He were 0.49 and 0.35, respectively. MIRZAEI *et al*. (2005) reported 80.00%polymorphism among 22 Iranian pistachio cultivars and wild pistachio species. In a study reported by GOLAN- GOLDHIRSH *et al*. (2004) in assessing polymorphisms among 28 Mediterranean pistachio accessions, 27 selected primers produced 259 total bands (an average of 9.59).

Some cultivars in different locations have the same name and some morphological identity, while molecular results showed differences between them. For instance, Badami-Zarand cultivar was differentiated from Badami- Kaj and Badami-Zoodras. Also, Ghazvini-Zodras showed differences with Ghazvini. These differentiations can be due to the intrinsic nature of RAPD, since it is very unlikely that the microsatellites amplified correspond to the mutated DNA region when they have been randomly isolated from the whole genome. The results from this study showed that the studied cultivars had high genetic variation due to the species' dioeciously and cross-pollination nature (Ahmad *et al*., 2005).

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 RAPD markers used were useful for determination of genetic diversity among pistachio cultivars in Iran.

Received, June 29th, 2020
Accepted March 22nd, 2021

REFERENCES

ARABNEJAD, H., M., BAHAR, A., TAJ (2008): Genetic diversity of Pistacia khinjuk Stocks by SSRmarkers. Agri Sci Tech., *12*:207–217.

ATTAR, F., S. ESFANDANI-BOZCHALOYI M. MIRTADZADINI, & F. ULLAH (2018). Taxonomic identification in the tribe Cynoglosseae (Boraginaceae) using palynological characteristics, Flora, 249, 97–110.

AHMAD, R., L., FERGUSON, S.M., SOUTHWICK (2003): Identification of pistachio (Pistacia vera L.) nuts with microsatellite markers. J. Am. Soc. Hortic. Sci., *128*:898–903.

AHMAD, R., L., FERGUSEN, S.M., SOUTHWICK (2005): Molecular marker analysis of pistachio rootstocks by simple sequence sepeats and sequence-selated Amplified solymorphisms. J. Hort. Sci. Biotech., *80*: 382-386.

BAGHIZADEH, A., S., NOROOZI, M., JALALI-JAVARAN (2010): Study on genetic diversity of some Iranian Pistachio (Pistacia vera L.) cultivars using random amplified polymorphic DNA (RAPD), inter sequence repeat (ISSR) and simple sequence repeat (SSR) markers: A comparative study. Afr. J. Biotech., *9*:7632–7640.

BROWIEZ, K. (1988): Chorology of trees and shrubs in south-west Asia and adjacent regions. Polish Scientific Publication, Warsaw, Poland.

BARONE, E., L., DIAMARCO, F.P., MARRA, M., SIDARI (1993): Isozymes and multivariate analysis to discriminate male and female Sicilian germplasm of pistachio. IXth Groupe de Recherches et d'Etudes Mediterraneen pour le Pistachier et l'Amandier, Agrigento, pp. 73–79

CONGFEN, Z., L. YINGXIAO, H. LINFENG, S. FENGJUN, Y.WEITANG,,... L.XUEGANG (2021): Effects of Uranium on the Antioxidant Responses of Chinese Oak Silkworm, Antheraea pernyi. Pakistan Journal Of Zoology, 1-8.

DOLLO, L. (1993): An isozyme study of Sicilian Pistacia species, varieties, and offspring from artificial pollination. IXth Groupe de Recherchesetd'EtudesMediterraneen pour le Pistachieretl'Amandier, Agrigento, pp. 80–88.

ERBANO, M., G.S.E., SCHÜHLI, É.P.D., SANTOS (2015): Genetic variability and population structure of Salvia lachnostachys: implications for breeding and conservation programs. Int. J. Mol. Sci., *16*(4):7839-7850.

ESFANDANI-BOZCHALOYI, S., M., SHEIDAI, M. KESHAVARZI, Z. NOORMOHAMMADI (2017): Genetic and morphological diversity in *Geranium dissectum* (Sec. Dissecta, Geraniaceae) populations. Biologia, *72*(10):1121- 1130.

ESFANDANI-BOZCHALOYI, S., M. SHEIDAI, M. KESHAVARZI, Z. NOORMOHAMMADI (2018a): Species Relationship and Population Structure Analysis In *Geranium* Subg. *Robertium* (Picard) Rouy With The Use of ISSR Molecular Markers. Act. Bot. Hung., *60*(1–2):47–65.

ESFANDANI-BOZCHALOYI, S., M. SHEIDAI, M. KESHAVARZI, Z, NOORMOHAMMADI (2018b): Species Identification and Population Structure Analysis In *Geranium* Subg. *Geranium* (Geraniaceae). Hacquetia, *17*(2):235–246.

ESFANDANI –BOZCHALOYI, S., M. SHEIDAI, M. KESHAVARZI, Z. NOORMOHAMMADI (2018c): Morphometric and ISSR-analysis of local populations of *Geranium molle* L. from the southern coast of the Caspian Sea. Cytol. Genet., *52*(4):309–321.

ESFANDANI-BOZCHALOYI, S., M. SHEIDAI, M. KESHAVARZI, Z NOORMOHAMMADI (2018d): Molecular diversity and genetic relationships among *Geranium pusillum* and *G. pyrenaicum* with inter simple sequence repeat (ISSR) regions. Caryologia, *71*(4):1-14.

ESFANDANI-BOZCHALOYI, S., M. SHEIDAI, M. KALALEGH (2019): Comparison of DNA extraction methods from Geranium (Geraniaceae). Acta Bot. Hung., *61*(3-4):251-266.

ESFANDANI-BOZCHALOYI S, M. SHEIDAI, M. KESHAVARZI (2018E) MACRO- AND MICRO-MORPHOLOGICAL STUDY OF FRUITS AND SEEDS IN THE GENUS *GERANIUM* (GERANIACEAE), Phytotaxa, 375(3):185 – 204. DOI.ORG/10.11646/PHYTOTAXA.375.3.8

ESFANDANI-BOZCHALOYI S, W. ZAMAN (2018f).Taxonomic significance of macro and micro-morphology of *Geranium* L. species Using Scanning Electron Microscopy. Microsc Res Tech,8 1, 12(652-666). DOI: 10.1002/jemt.23159 october

EVANNO, G., S., REGNAUT, J., GOUDET (2005): Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol., *14*:2611–2620.

ESMAIL-POUR, A. (2001): Distribution, use and conservation of pistachio in Iran. In: Padulosi S, Hadj-Hassan A (eds) In towards a comprehensive documentation and use of Pistacia genetic diversity in central and West Asia, North Africa and Europe. Report of the IPGRI workshop, 14–17 December 1998, Ibrid, Jordan. IPGRI, Rome, Italy.

FAOSTAT (2004): FAOSTAT database. FAO statistics database on The World Wide Web. http://apps.fao.org (accessed December 2004).

FALUSH, D., M., STEPHENS, J.K., PRITCHARD (2007): Inference of population structure using multilocus genotype data: dominant markers and null alleles. Mol. Ecol. Notes, *7*:574−578.

FREELAND, J.R., H., KIRK, S.D., PETERSON (2011): Molecular ecology (2nd ed). UK: Wiley-Blackwell.

GOMEZ, A., G.G., VENDRAMIN, S.C., GONZÁLEZ-MARTÍNEZ, R., ALIA (2005): Genetic diversity and differentiation of two Mediterranean pines (Pinus halepensis Mill. and Pinus pinaster Ait.) along a latitudinal cline using chloroplast microsatellite markers. Divers Distrib., *11*(3): 257-263.

GHOLAMIN, R. and M. KHAYATNEZHAD (2020a): Assessment of the Correlation between Chlorophyll Content and Drought Resistance in Corn Cultivars (Zea Mays). Helix, *10*: 93-97.

GHOLAMIN, R. and M. KHAYATNEZHAD (2020b): The effect of dry season stretch on Chlorophyll Content and RWC of Wheat Genotypes (Triticum Durum L.). Biosci. Biotech. Res. Comm., *13*: 1833-1829.

GHOLAMIN, R. and M. KHAYATNEZHAD (2020c): Study of Bread Wheat Genotype Physiological and Biochemical Responses to Drought Stress. Helix, *10*: 87-92.

GHOLAMIN, R. and M. KHAYATNEZHAD (2020d): The Study of Path Analysis for Durum Wheat (Triticum durum Desf.) Yield Components. Biosci. Biotech. Res. Comm., *13*: 2139-2144.

GOLAN-GOLDHIRSH, A., O, BARAZANI, Z.S. WANGL (2004): Genetic relationships among Mediterranean Pistacia species evaluated by RAPD and AFLP markers. Plant Syst. Evol., *246*:9–18.

HORMAZA, J.I., L, DOLLO, V.S. POLITO (1994): Identification of RAPD marker linked tosex determination in Pistacia vera using bulked segregant analysis. TAG, *89*:9–13.

HAMMER, O., D.A.T., HARPER, P.D., RYAN (2001): PAST: paleontological statistics software package for education and data analysis. Palaeontol. Electron., *4*:9.

HEIKRUJAM, M., J., KUMAR, V., AGRAWAL (2015): Genetic diversity analysis among male and female Jojoba genotypes employing gene targeted molecular markers, start codon targeted (SCoT) polymorphism and CAAT box-derived polymorphism (CBDP) markers. Meta Gene, *5*:90-97.

HUSON, D.H., D., BRYANT (2006): Application of phylogenetic networks in evolutionary studies. Mol. Biol. Evol., *23*:254–267.

HAN, X., Z. WEI, B, ZHANG, Y. LI, T. DU,,... H.CHEN (2021): Crop evapotranspiration prediction by considering dynamic change of crop coefficient and the precipitation effect in back-propagation neural network model. Journal of hydrology (Amsterdam), 596:126104.

ISMAIL, N.A., M.Y., RAFII, T.M.M., MAHMUD, M.M., HANAFI, G., MIAH (2019): Genetic Diversity of Torch Ginger Germplasm Revealed by ISSR and SSR Markers. Bio. Med. Res., *2019*:5904804.

JIANG, L., B, ZHANG, S. HAN, H. CHEN, & Z. WEI (2021): Upscaling evapotranspiration from the instantaneous to the daily time scale: Assessing six methods including an optimized coefficient based on worldwide eddy covariance flux network. Journal of hydrology (Amsterdam), *596:* 126135.

KOLAHI-ZONOOZI, S.H., M. MARDI, M, ZEINALABEDINI (2014): Development of 12 new SSR markers for genetic diversity and structure analysis in pistachio (Pistacia vera L.) J. Hort. Sci. Biotech., *89*:707–711.

KAFKAS, S., E. KAFKAS, R. PERL-TREVES (2002): Morphological diversity and germplasm survey of three wild Pistacia species in Turkey. Gen. Res. Crop Evol., *49*:261–270.

KAFKAS, S., A. KASKA, A.N., WASSIMI, S. PADULOSI (2006): Molecular characterisation of Afghan pistachio accessions by amplified fragment length polymorphisms (AFLPs). J. Hort. Sci. Biotech., *81*(5):864–868.

KATSIOTIS, A., M. HAGIDIMITRIOU, A., DROSSOU, C., PONTIKIS, M., LOUKAS (2003): Genetic relationships among species and cultivars of Pistacia using RAPDs and AFLPs. Euphytica, *132*:279–286.

MEHLENBACHER, S.A. (2003): Progress and prospects in nut breeding. Acta Hortic., *622*: 57–79.

MIRZAEI, S., M BAHAR., B. SHARIFNABI (2005): A phylogenetic study of Iranian wild pistachio species and some cultivars using RAPD marker. Acta Hortic., *726*:39–43.

MEIRMANS, P.G. (2012): AMOVA-based clustering of population genetic data. J. Heredity, *103*:744–750.

PRITCHARD, J.K., M, STEPHENS, P. DONNELLY (2000): Inference of population structure using multilocus genotype data. Genetics, *155*:945−959.

PEAKALL, R., P.E., SMOUSE (2006): GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes. 6:288–295.

PODANI, J. (2000): Introduction to the exploration of multivariate data [English translation]. Leide (Netherlands): Backhuyes.

SHEN, Z., K. ZHANG, L. MA, J. DUAN, Y. AO (2017): Analysis of the genetic relationships and diversity among 11 populations of Xanthoceras sorbifolia using phenotypic and microsatellite marker data. Elect. J. Biotech., *26*:33-39.

SHAKOOR, A., Z. FANG, Z. GUL, L, WUYANG, L, XINCAN, S. ESFANDANI-BOZCHALOYI (2021): Morphometric analysis and sequence related amplified polymorphism determine genetic diversity in Salvia species, Notulae Botanicae Horti Agrobotanici Cluj-Napoca, *49*: 12153-12153.

TAYEFEHALIAKBARKHANY, S., A.R.,TALAIE FATAHI, M.R. MOGHADAM (2013): Investigation of genetic diversity among Pistacia Vera In the Khorasan by using molecular. Mod. Genet., *8*:169–176.

YEH, F.C., R., YANG, T. BOYLE (1999): POPGENE. Microsoft Windows-based freeware for population genetic analysis. Release 1.31. University of Alberta 1-31.

WANG, L., T. YANG, B. WANG, Q, LIN, S. ZHU, C. LI, F. YU (2020): RALF1-FERONIA complex affects splicing dynamics to modulate stress responses and growth in plants. Science advances, 6(21), z1622.

XU, J., L. JI, Y. RUAN, Z. WAN, Z. LIN, S .XIA,..... X.CAI (2021): UBQLN1 mediates sorafenib resistance through regulating mitochondrial biogenesis and ROS homeostasis by targeting PGC1尾 in hepatocellular carcinoma. Signal transduction and targeted therapy, *6(1)*: 190.

ZHANG, W., Y. HU, J. LIU, H, WANG, J. WEI, P.SUN, H.ZHENG(2020): Progress of ethylene action mechanism and its application on plant type formation in crops. Saudi Journal of Biological Sciences, *27*(6): 1667-1673.

ZHANG, B., D. XU, Y. LIU, F.,LI, J.CAI, L.DU (2016): Multi-scale evapotranspiration of summer maize and the controlling meteorological factors in north China. Agricultural and Forest Meteorology, *216*: 1-12.

ZHAO, H., X. LIU, L. YU, S. LIN, ZHANG, C., H.XU,,... L.WANG (2021): Comprehensive landscape of epigenetic-dysregulated lncRNAs reveals a profound role of enhancers in carcinogenesis in BC subtypes. Molecular Therapy-Nucleic Acids, *23:* 667-681.

ZOHARY, M. (1952): A monographic study of the genus Pistacia. Palest J. Bot. Jerus. Ser., *5*:187–228.

DETEKCIJA DNK POLIMORFIZMA I GENETIČKOG DIVERZITETA KOD GERMPALZME PISTAĆA POMOĆU RAPD MARKERA

Xiaohui QIAN[1]* i Shahram MEHRI[2]

[1]Departman za turizam i kulturu, Anhui stručna škola za finansije i trgovinu, Hefei, Kina
[2]Department of Agronomy and Plant Breeding, ParsAbad Moghan Branch, Islamic Azad University, ParsAbad Moghan, Iran

Izvod

Procena genetičkog diverziteta populacije je preduslov za započinjanje i razvoj projekata oplemenjivanja biljaka. *Pistacia vera* se smatra komercijalnom vrstom roda *Pistacia*. U Iranu je izvoz pistaća na drugom mestu po izvozu nenaftnih proizvoda i na prvom mestu među hortikulturnim vrstama. Stoga smo prikupili i analizirali 11 genotipova pistaća (*Pistacia vera*) iz dve provincije u Iranu. Cilj nam je bio 1) da procenimo genetički diverzitet među nekim iranskim sortama pistaća 2) da li postoji korelacija između genetske i geografske udaljenosti vrsta? 3) Genetska struktura populacija i taksona. Pokazali smo značajne razlike u kvantitativnim morfološkim karakterima biljnih vrsta. Sorte Akbari su prikazivale očekivanu heterozigotnost (UHe) u opsegu od 0,028. Informacije o Šenon indeksu bile su visoke (0,49) kod sorte Seifadini. Sorte Akbari zabeležile su najnižu vrednost, 0,029. Uočeni broj alela (Na) kretao se od 0,261 do 2 700 kod sorti Shahpasand i Kalehghoochi. Efektivni broj alela (Ne) bio je u rasponu od 1.021-1.800 za sorte Akbari i Moosaabadi. Protok gena (Nm) bio je relativno nizak (0.38) u sortama pistaća. Mantelov test pokazao je korelaciju (r = 0,33, p = 0,0001) između genetske i geografske udaljenosti. Izvestili smo o velikom genetičkom diverzitetu, što jasno pokazuje da se neke od sorti iranskog pistaća mogu prilagoditi promenljivom okruženju, jer je velika genetska raznolikost povezana sa prilagodljivošću vrsta. Sadašnji rezultati ukazali su na korisnost RAPD markera i morfometrijskih metoda za istraživanje genetičkog diverziteta pistaća.

Primljeno 29. VI.2020.
Odobreno 22. III. 2021.