International Journal of Nuts and Related Sciences 3(1):49-56, 2012
ISSN 2008-9937

Genetic Relationships among *Pistacia* Species Studied By Morphological Characteristics and RAPD Marker

Karimi H.R.[1], Zamani Z.[2], Ebadi A.[2] and Fatahi R.[2]*

[1]Department of Horticultural Science, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, Kerman, Iran

[2]Department of Horticultural Science, Faculty of Agriculture, University of Tehran, Karaj.

Abstract: The aim of this research was to study 33 Pistachio accessions and determine their genetic relationships. Thirty-one morphological characters (17 quantitative and 14 qualitative) together with Randomly Amplified Polymorphic DNA (RAPD) marker data were used for this purpose. Factor analysis was used to determine the effective characteristics and the number of main factors which determined seven main factors. Grouping of pistachio accessions by these factors was performed by Ward's method. Among 77 random decamer primers tested, 12 showed good amplification and polymorphism, and a total of 130 markers were produced that 118 were polymorphism. Grouping by morphological characteristics was compared with the results from RAPD analysis which did not produce a significant correlation.

Keywords: Accession, *Pistacia*, RAPD

INTRODUCTION

The genus *Pistacia* in Anacardiaceae family contains 13 or more species, among them, *Pistacia vera* L. has commercially important edible nuts. The other species grow in wild and their seedlings are used mainly as rootstocks for pistachio [8]. There are two main centers of diversity for *Pistacia*: one comprises the Mediterranean region of Europe, Northern Africa and the Middle East countries. The second is the Eastern part of Zagros Mountains and Caucasus region from Crimea to the Caspian Sea [19].

In the first monograph study of *Pistacia* species, Engler (1881) listed eight species and a few subspecies, however he did not suggest any sectional subdivisions for the species and some species were not completely described by him [19]. So far the most comprehensive taxonomic study of *Pistacia* genus was reported by Zohary (1952), who divided the genus into four sections and 11 species according to leaf characters and nut morphology, however, he provided no find justification to retain *mutica* and *cabulica* as species or subspecies. The classification of *Pistacia* species at molecular level was firstly performed based on chloroplast DNA profiles by Parfitt and Badenes (1997) who divided the genus into two sections: Terebinthus and Lentiscus, as deciduous and evergreen species respectively.

Kafkas and Prel-Treves (2002) characterized *Pistacia* species in Turkey by morphological and molecular data. They revised the miss-identification of samples as *P. eurycarpa* that was described previously by Yaltirik (1967) as *P. khinjuk*. Kafkas and Prel-Treves (2002a) also classified nine *Pistacia* species by RAPD analysis and showed that *P. palaestina* was in fact a subspecies of *P. terebinthus*. Werner et al. (2001) identified *P. saportae* morphologically and at molecular level as a hybrid of *P. lentiscus* and *P. terebinthus*. Recently, Kafkas (2006) characterized *Pistacia* species by AFLP marker. He reported that *P. atlantica* and *P. eurycarpa* have a close genetic

***Corresponding author**: H.R. Karimi, Department of Horticultural Science, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, Kerman, Iran Email: h_karimi1019@yahoo.com

relationship, also *P. atlantica* with *P. mutica* and *P. terebinthus* with *P. palaestina* were the closest paires species. Recently Karimi et al (2008) studied phylogenetic relationship *Pistacia* species by amplified fragment length polymorphism (AFLP). They postulated that *P. eurycarpa* is synonym of the *P. atlantica* subsp. *kurdica* and considered a distinct from *P. atlantica*. Four important *Pistacia* species including *P. vera*, *P. khinjuk*, *P. eurycarpa* (*P. atlantica* sub sp. *kurdica*) and *P. mutica* are wildly growing in Iran [11]. Forests of wild *P. vera* spread to an area of about 75000 ha, in central Asia, near the boarders of Turkmenistan, Afghanistan and northeast of Iran. In Iran *P. vera* grows predominantly in the Sarakhs region covering roughly 17500 ha [2].

The present study aimed to reveal the genetic relationships among *Pistacia* species of Iran using the RAPD marker technique, and to compare these with relationships based on morphological characteristics.

MATERIALS AND METHODS

Plant material

A total of 33 accessions belonging to *P. vera*, *P. khinjuk*, all subspecies of *P. atlantica* (*atlantica*, *mutica*, *kurdica* and *cabulica*), three unknown genotypes and three accession proposed to be hybrid from *P. eurycarpa* x *P. mutica* were plant material of this study (Table 1). The accessions were described based on the *Pistacia* descriptor developed by the International Plant Genetic Resources Institute [6] with minor modifications. Thirty one characteristics (17 quantitative and 14 qualitative) were identified for evaluating the chosen samples. Ten rachises were harvested of each tree to measure the rachis length and the number of fruits per rachis. The flower buds were dried and then soaked in water for 12 hours to allow the bud scales to separate to enable counting of the number of scales in the flower buds. Ten fully developed leaves were removed from each tree to evaluate the characteristics of the leaves and leaflets. The shape of the terminal leaflet, terminal leaflet apex, terminal leaflet base, and the nut shape were scored according to the descriptor. One hundred nuts per tree were randomly selected to measure their weight and dimensions. Analysis of variance, means comparison, simple correlations, factor and cluster analysis were carried out using SPSS and SAS software to reveal the relationships between the genotypes. Factor analysis was used to determine the effective characteristics and the number of main factors and grouping of pistachio accessions was performed by Ward's method.

Table 1. Pistachio accession used for molecular and morphological classification and their measured quantitative characteristics

*Obtained from the Iran Pistachio Research Institute

No	Genotype	Species	Location	Leaf length (cm)	Leaf width (cm)	No of leaflets	Terminal leaflet length (cm)	Terminal leaflet width (cm)	Nut length (mm)	Nut width (mm)	Nut thickness (mm)
1	KHI1	*P. khinjuk*	IPRR*	11.35	10.21	3.4	5.15	5	5.9	4.2	2.7
2	KHI2		IPRR*	10.42	8.11	3	4.25	3.41	5.6	5	3.1
3	KHI3		IPRR*	10	9	4	4.17	3.48	6.2	4.3	3.2
4	AAI1	*P. atlantica*	IPRR*	8.65	7.25	6.4	3.84	1.6	4.5	5.7	5.3
5	AAI2		IPRR*	8.72	6.64	7	3.38	1.42	5.5	6.1	5.1
6	AAI3		IPRR*	8.68	6.94	6.7	3.61	1.51	5	6.2	4.6
7	AMI1	*P. atlantica* subsp. *mutica*	IPRR*	11.71	8.1	6	4.35	2.37	6	7.8	5.3
8	AMI2		IPRR*	10.29	10.62	5.6	5.45	2.31	6.2	8.1	5.1
9	AMI3		IPRR*	12.48	8.37	6.2	4.33	2.39	6.4	8.1	5.2
10	BBI1	Hybrid	IPRR*	12	9.87	4.6	4.52	2.9	8	8.7	5.4
11	BBI2		IPRR*	11.4	9	6.6	4.38	2.16	7.5	9.2	5.1
12	BBI3		IPRR*	12	10.2	5	5.56	2	8.5	9.1	5.3
13	BDI1	*P. vera*	IPRR*	14.9	14.8	3	9.5	5.2	20	9	9.1
14	BDI2		IPRR*	14.5	15.6	3	10.3	5	18	10	9.5
15	BDI3		IPRR*	14.6	15.6	4	9.1	4.95	16	11	9.3
16	QZI1		IPRR*	14.4	14.15	4.4	8.25	4.65	19	10	9.6
17	QZI2		IPRR*	12.7	14.9	4.2	8.3	4.85	17	8	9.7
18	QZI3		IPRR*	8.82	12.25	5	7.56	4.45	18	12	9.8
19	SRI1		IPRR*	15.4	11.7	4.4	6.66	3.84	14	11	8.3
20	SRI2		IPRR*	16	16	4.8	7.9	4.96	12	10.2	7.8
21	SRI3		IPRR*	12.7	14.75	4.6	7.9	4.71	13	10.3	8.2
22	AKF1	*P. atlantica* subsp. *kurdica*	Fars	13.97	9.74	6.2	6	1.98	6.2	8	8.7
23	AKF2		Fars	15	11.94	7	5.37	2.53	5.9	6.5	8.1
24	AKF3		Fars	13.7	10.83	6.8	6.1	2.38	6.5	7.3	8.4
25	AKK1		kerman	13.92	12.62	5	6.1	3.33	7.3	7.3	4.1
26	AKK2		kerman	13.33	10	6.4	5.22	3.14	7	7.1	4.3
27	AKK3		kerman	15.87	12.65	6.6	5.63	3.24	7.15	7.2	4.5
28	ACF1	*P. atlantica* subsp. *cabulica*	Fars	17.59	12.95	6.8	6	2.28	7.5	7.3	5
29	ACF2		Fars	14.88	11.71	7.2	5.85	2.26	7.3	7.2	4.9
30	ACF3		Fars	16.23	12.33	7	5.93	2.27	7.7	6.5	5.3
31	UNK1	unknown	Kerman	12.55	9.76	5	5.16	1.95	6.5	6.3	4.5
32	UNK2		Kerman	10.36	9.87	5.25	5.57	2.1	7.5	8.3	5
33	UNK3		Kerman	13.98	9.63	6.6	4.93	2.03	7	7.3	4.7

DNA extraction

Genomic DNA was extracted from leaves by Murray & Thompson (1980) method. The purity and quantity of the genomic DNA was determined spectrophotometrically and confirmed by electrophoresis in 0.8% (w/v) agarose gels using known concentrations of bacteriophage lambda DNA.

RAPD markers

Twelve Operon 10-mer primers (Operon Technologies, Alameda, CA, USA) and 100 TIB 10-mer primers (TIBMOLBIOL, Berlin, Germany), were used in this study. Polymerase chain reactions (25 µ) each contained 10 ng template DNA, 1× PCR buffer (CinnaGen, Tehran, Iran), 0.875 mM $MgCl_2$, 200 µM each of dNTPs, 0.2 µM each decamer primer, and 1 unit of *Taq* DNA polymerase (CinnaGen, Tehran, Iran). Amplification reactions were performed in termocycler (iCycler, Bio Rad, Hercules, CA, USA) programmed as follow: 94ºC for 4 min, followed by 35 cycle of 92ºC for 1 min, 37ºC for 1 min, 72ºC for 2 min and a final extension at 72º C for 5 min. Amplified products were separated by electrophoresis in 1.5% (w/v) agarose gels in Tris-borate-EDTA (TBE) buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA- Na_2, pH=8.0), visualized by ethidium bromide staining and photographed under UV light with a Gel Doc system (UVP: Bio Doc, Upland, CA, USA). The Jaccard's similarity

matrix was calculated using numerical taxonomy and multivariate analysis system NTSYSpc Ver 2.11 [15] and the dendrogram produced using the UPGMA.

RESULTS

Morphological characteristics

Mean values of the studied morphological characters showed large variations between accessions for all traits. Mean values and ranges of variability for the different characters of accessions are presented in Table 2. Significant differences ($p \leq 0.05$) were detected among the species for all characters by analysis of variance. Characteristics showing a greater quantitative range had coefficients of variation (CV) meaning increased possibilities for selection for those characteristics. Split nuts percentage, 100 nut dry weight, terminal leaflet petiole length, nut length, terminal leaflet width, number of fruit per rachis and nut thickness were characteristics with the highest variation. Results from simple correlation analysis showed the existence of significant positive and negative correlations among characteristics (data not shown). Factor analysis was used to determine the number of main factors for reducing the number of characteristics effective to discriminate genotypes (Table 3). Based on factor analysis the characteristics of leaves and nuts accounted for 40% of the variance as the first main factor, and with the other six factors, explained 94% of the total variance. For each factor, a factor loading above 0.65 was considered as significant. Pistachio types were grouped according to these seven factors. Cluster analysis divided accessions into three sub-cluster each consisting of genotypes belonging to species *P. vera*, *P. khinjuk* and *P. atlantica*. Based on the results, *P. khinjuk* located between the other two species, but more resembled to *atlantica* than *vera* species. The *P. atlantica*, with *P. atlantica* subsp. *mutica* located in the same group while *P. atlantica* subsp. *kurdica* separated from them, also hybrid accession located between *P. atlantica* subsp. *kurdica* and *P. atlantica* subsp. *mutica*(Figure1).

Table 2. Pistachio characteristics, range of variability, mean and coefficient of variations for qualitative and quantitative traits

No	Trait	Abbreviation	Unit	Mean	Min	Max	CV(%)*
1	Growth habit of tree	GRHT	1-3	-	1	3	-
2	Trunk color	TRKC	1-3	-	1	3	-
3	Leaf length	LFL	cm	12.91	8.65	17.59	5.12
4	Leaf width	LFW	cm	11.15	6.64	16	6.96
5	Terminal leaflet length	TLFL	cm	5.92	3.38	10.3	7.78
6	Terminal leaflet width	TLFW	cm	3.11	1.51	5.2	11.89
7	Terminal leaflet length / width	TLFL/W	ratio	2.06	1	2.97	5.82
8	Terminal leaflet size	TLSZ	1-3	-	1	3	-
9	Terminal leaflet shape	TLSH	1-5	-	1	5	-
10	Terminal leaflet apex shape	TLAS	1-5	-	1	5	-
11	Terminal leaflet base shape	TLBS	1-4	-	1	4	-
12	Leaf color	LFC	1-3	-	1	3	-
13	Number of leaflets	NLF	-	5.38	3.00	7.20	
14	Leaf texture	LFT	1-2	-	1	2	-
15	Leaf indumentum	LFI	-	-	-	-	-
16	Terminal leaflet petiole length	TLFPL	mm	0.76	0	1.90	21.00
17	Leaf rachis wing	LFRW	1-3	-	1	3	-
18	Petiole shape	PTS	1-3	-	1	3	-
19	Current year shoot color	CYSC	1-3	-	1	3	-
20	Arrangement of scales in flower bud	ASFB	1-3	-	1	3	-
21	Number of scales in flower bud	NSFB	-	13.98	10.00	20.00	8.20
22	Flower bud length	FBL	mm	7.52	6.00	9.20	4.65
23	Flower bud width	FBW	mm	4.73	4.00	5.60	4.04
24	Rachis length	RAL	cm	8.49	5.66	10.25	4.9
25	Number of fruits per rachis	NFPR	-	38.33	14.40	90.00	11.32
26	Nut length	NUL	mm	9.26	4.50	20.00	15.73
27	Nut width	NUW	mm	7.88	4.30	10.30	7.25
28	Nut thickness	NUT	mm	6.25	2.70	9.80	10.77
29	Nut shape	NUS	1-5	-	1	5	-
30	Split nuts percentage	SNUP	%	17	0	90.00	62.94
31	100 Nut dry weight	100NUDW	g	27.55	3.30	82.00	45.54

*CV. Coefficient of variation = (Standard error /Mean) ×100

Table 3. Eigen values and cumulative variance for seven major factors identified from factor analysis.

Factor	1	2	3	4	5	6	7
Cumulative variance(%)	39.99	60.41	73.93	80.53	86.35	90.38	94
Eigen value	12.39	6.33	4.19	2.04	1.80	1.24	1.12

Characteristics	Units	Factor loading						
Rachis length	cm	-0.470	0.169	0.686**	0.376	0.217	-0.172	-0.216
Number of fruits per rachis	-	-0.547	0.227	-0.247	0.07	0.240	-0.677	0.217
Leaf length	cm	0.493	-0.04	0.857**	0.03	0.04	0.07	-0.09
Leaf width	cm	0.824**	0.279	0.458	0.113	0.09	0.08	-0.05
Terminal leaflet length	cm	0.878**	0.359	0.268	0.09	-0.01	0.08	0.09
Terminal leaflet width	cm	0.614	0.664**	0.07	0.327	.225	-0.07	-0.04
Terminal leaflet petiole length	mm	0.188	0.711**	-0.318	0.496	0.05	-0.181	-0.223
Number of leaflets	-	-0.334	-0.766**	0.223	-0.322	-0.08	0.06	0.248
Terminal leaflet shape	-	-0.392	0.07	-0.516	0.216	0.587	-0.368	-0.209
Terminal leaflet apex shape	-	-0.05	0.457	-0.244	0.672	-0.385	-0.07	-0.08
Terminal leaflet base shape	-	0.272	0.776**	0.03	0.103	0.235	0.231	0.04
Terminal leaflet size	-	0.02	0.853**	-0.195	0.07	-0.09	-0.08	-0.06
Leaf indumentums	-	-0.549	0.480	0.261	0.173	-0.151	-0.297	-0.01
Petiole shape	-	0.01	-0.274	-0.163	-0.216	-0.92**	-0.01	0.136
Leaf color	-	0.385	-0.07	0.221	0.640	0.438	0.300	0.188
Current year shoot color	-	-0.314	-0.585	0.154	-0.581	-0.05	-0.209	0.377
Terminal leaflet length / width	-	-0.173	-0.535	0.187	-0.658	-0.395	0.112	0.04
Nut length	mm	0.908**	0.383	-0.03	0.113	-0.05	0.09	-0.01
Nut width	mm	0.853**	-0.124	0.03	0.346	0.03	0.257	-0.08
Nut thickness	mm	0.949**	0.130	-0.134	0.07	-0.05	0.161	-0.03
100 nut dry weight	g	0.929**	0.309	-0.08	0.117	-0.07	0.06	0.03
Nut shape	-	-0.239	-0.08	0.369	-0.247	0.709**	0.355	-0.219
Growth habit	-	0.329	0.273	0.178	0.06	0.230	.819**	0.05
Trunk color	-	0.06	-0.252	0.09	-0.04	-0.312	-0.06	0.876**
Arrangement of scales in flower bud	-	-0.264	-0.673**	0.176	-0.03	0.272	0.104	0.410
Number of scales in flower bud	-	-0.189	-0.295	0.789**	-0.360	0.05	0.212	0.217
Split nuts percent	%	0.803**	0.453	-0.135	0.05	-0.305	0.07	0.166
Flower bud length	mm	0.09	-0.345	0.828**	-0.03	0.200	0.136	0.239
Flower bud width	mm	0.422	0.705**	0.178	0.303	0.195	0.176	0.331
Leaf texture	-	-0.190	-.305	-0.05	-.913**	-0.05	0.09	-0.01
Leaf rachis wing	-	-0.233	-.736**	0.04	-.264	-.100	-0.423	0.174

**Significant factor loadings (considered values above 0.65)

Table 4. List of the most informative primers and the degree of polymorphism obtained among 33 Pistachio genotypes studied

No	Primer	Sequence 5´→3´	Total no. of bands	No. of polymorphic bands	Polymorphic bands (%)
1	OPAD-02	CTGAACGCTG	10	10	100
2	OPAE-10	CTGAAGCGCA	9	8	88
3	OPB-10	CTGCTGGGAC	15	14	93.30
4	OPG-02	GGCACTGAGG	13	11	84.60
5	OPZ-10	CCGACAAACC	15	13	86.60
6	TIBMBB-12	GTGTGCCCCA	7	7	100
7	TIBMBC-04	CCACGTGCCA	16	16	100
8	TIBMBC-13	TCGGTGAGTC	8	6	75
9	TIBMBE-05	GGAACGCTAC	9	7	77.70
10	TIBMBE-08	GGGAAGCGTC	10	10	100
11	TIBMBE-17	GGGAAAAGCC	12	10	83.30
12	TIBMBE-19	AGGCCAACAG	6	6	100
Total		-	130	118	-
Mean		-	10.83	9.83	90.68

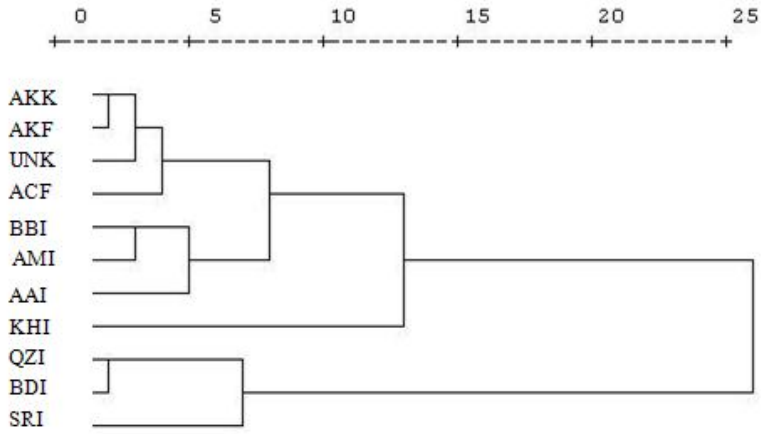

Fig.1. Dendrogram representing morphological relationships among *Pistacia* genotype using ward method

AKK: *P. atlantica* subsp. *kurdica*, Kerman
AKF: *P. atlantica* subsp. *kurdica*, Fars
ACF: *P. atlantica* subsp. *cabulica*, Fars
AAI: *P. atlantica* subsp. *atlantica*, Institute
BDI: *P. vera* cv. Badami Riz, Institute
AMI: *P. atlantica* subsp. *mutica*, Institute
UNK: Unknown, Kerman
BBI: Garden mastic, Institute
KHI: *P. khinjuk*, Institute
QZI: *P. vera* cv. Qazvini, Institute
SRI: *P. vera* var. Sarakhs, Institute

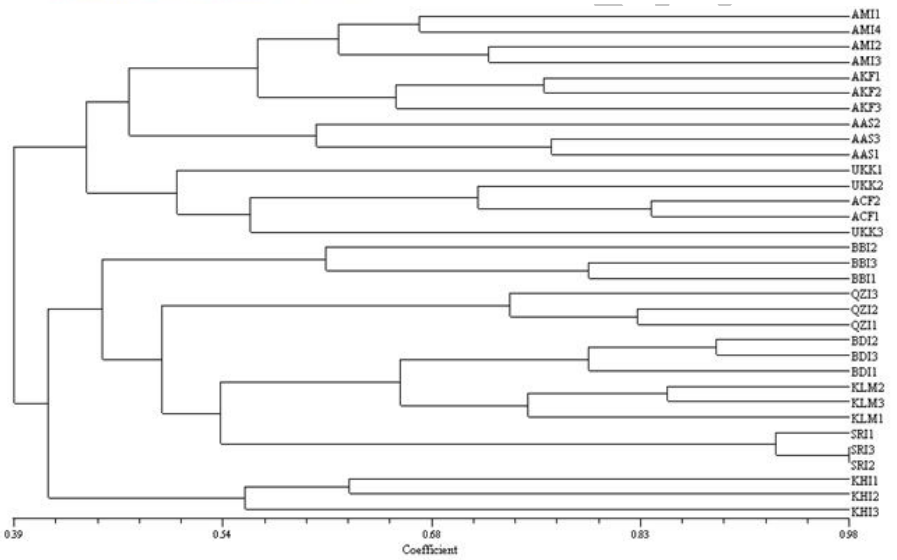

Fig.2. Dendrogram representing morphological (upper) and molecular (lower) relationships among *Pistacia* genotype using UPGMA method

AKF: *P. atlantica* subsp. *kurdica*, Fars
ACF: *P. atlantica* subsp. *cabulica*, Fars
AAI: *P. atlantica* subsp. *atlantica*, Institute
BDI: *P. vera* cv. Badami Riz, Institute
AMI: *P. atlantica* subsp. *mutica*, Institute
KLM: *P. vera* var. Sarakhs, khorasan
BBI: Garden mastic, Institute
KHI: *P. khinjuk*, Institute
QZI: *P. vera* cv. Qazvini, Institute
SRI: *P. vera* var. Sarakhs, Institute
UNK: Unknown, Kerman

Of the 77 Operon 10-mer primers and 100 TIB 10-mer primers were used in this study, 12 primers produced good polymorphic bands in the studied pistachio genotypes. These 12 primers produced 130 discrete DNA fragments, of which 118 showed polymorphism in genotypes. Most polymorphism was shown by TIBMBC-04 (Table 4). According to the dendrogram (Figure 2), 33 accession of Pistachio divided in to five groups at a similarity coefficient of 0.51, each consisting of accession belonging to species *P. khinjuk*, *P. vera*, *P. atlantica* subsp. *cabulica*, *P. atlantica*, *P. atlantica* subsp. *kurdica* and *mutica*. Based on the results *P. atlantica* subsp. *kurdica* and *mutica* separated from *P. atlantica*.

In some cases, based on RAPD markers, accession in clusters did not agree with those in clusters based on morphological characteristics. The of accession *P. atlantica* sub sp. *kurdica* and *P. atlantica* sub sp. *mutica* that were placed in the same group whereas different in morphological characteristics. Based on the data's RAPD, *P. vera* grouped with of accession *P. khinjuk*, but was grouped alone morphologicaly.

DISCUSSION

A very low correlation was observed between similarity matrices obtained based on morphological characteristics and RAPD markers for the pistachio accession studied ($r_{=+}$0.25). In similar studies taxonomic relationship and genetic variation of wild *Pistacia* germplasms in Turkey using morphological data and RAPD analysis studied, cluster analysis, based on morphological data revealed that the closest species to *P. vera* was *P. eurycarpa* (*P. atlantica* subsp. *kurdica*) but *P. eurycarpa* and *P. atlantica* were the closet pair of species to *P. vera* based on molecular data [7]. Dissimilarity in grouping using RAPD markers and pheno-taxonomic characteristics was also reported in other crops [16]. However, genetic diversity in pistachio which was analyzed by RAPD, AFLP and morphological characteristics, showed a significant correlation between the different marker systems [12]. Lack of fit between morphological characteristics and clustering by RAPD markers can be related to the evaluation of mostly non-coding regions of the genome while coding (expressed) sequences and their interactions create morphological characteristics. Also, those parts of the genome amplified using RAPD primers might not be in gene that code for morphological characteristics [4]. Results from these experiments indicate that the RAPD technique is useless to identify ultra specific in the *Pistacia*. Applying more informative markers, such as SSRs and AFLPs, would also improve our understanding of the genetic relationship of *Pistacia* species. Alternatively, to analyses genotypes according to their morphological characters and relate these to molecular can provide more precise results.

REFERENCES

1. Al- Yafi, J.; (1978) New characters differentiating *Pistacıa atlantica* subspecies. Candollea.; 33: 201- 208.

2. Behboodi, B.; (2003) Ecological distribution study of wild pistachios for selection of rootstock. Options Mediterranean's, Series A, Numero.; 63**:** 61- 67.

3. Engler, A.; (1881) Burseraceae et Anacardiaceae. In: De Candolle AC (ed.) Monographie Phanerogamarum, Volume 4. Paris, pp 284-293.

4. Garcia, M. T.; Ontivero, M.; Dlazriccl, J. C.; Castagnaaro, A.; (2002) Morphological traits resolution RAPDs and morphological markers. Genome.; 44**:** 787-790.

5. Golan- Goldhirsh, A.; Barazani, O.; Wang, Z. S.; Khadka, D. K.; Saunders, J. A.; Kostiukovsky, V.; Rowland, L. J.; (2004) Genetic relationships among Mediterranean *Pistacıa* species evaluated by RAPD and AFLP markers. Plant Systematic and Evolution 246**:** 9-18.

6. IPGRI., (1998) Descriptors for *Pisatacia spp*. (excluding *P. vera* L.): International Plant Genetic Resources Institute, Rome, Italy.

7. Kafkas, S.; Perl-Treves, R.; (2002) Interspecific relationship in the genus *Pistacia* L (Anacardiaceae) based on RAPD fingerprinting. Journal of Horticultural Science.; 37**:** 168-171.

8. Kafkas, S.; EBRU, K.; Perl-Treves, R.; (2002) Morphological diversity and germplasm survey of three wild *Pistacia* species in Turkey. Genetic Resources and Crop Evolution.; 49**:** 261-270.

9. Kafkas, S.; (2006) Phylogenetic analysis of the genus *Pistacia* by AFLP markers. Plant Systematic and Evolution.; 262: 113 – 124.

10. Karimi, H. R.;, Kafkas, S.; Zamani, Z.; Ebadi, A.; Fatahi, M. R.; (2008) Genetic relationships among species and cultivars of *Pistacia* using AFLP marker. Plant Systematic and Evolution.; 279: 21-28.

11. Karimi, H. R.; Zamani, Z.; Ebadi, A.; Fatahi, M. R.; (2008) Morphological diversity of *Pistacia* species in Iran. Genetic Resources and Crop Evolution.; 56: 561-571.

12. Katsiotis, A.; Hagidimitriou, M.; Drossou, A.; Partikis, C.; Loukas, M.; (2003) Genetic relationships among species and cultivars of *Pistacia* using RAPDs and AFLPs. Euphytica.; 132**:** 279-286.

13. Murray, M.; Thompson, W. F.; (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acid Research.; 8**:** 4321-4315.

14. Parfitt, D.; Badens, M. L.; (1997) Phylogeny of the genus *Pistacia* as determined from analysis of the chloroplast genome. Proceedingof the National Academy of Science, USA, 1997.

15. Rohle, J. F.; (2004) NTSYS - pc: 2.11 Numerical taxonomy and multivariate analysis system, version 2.11. Exeter Publishing, Ltd., Setauket, New York.

16. Uma, S.; Sudha, S.; Saraswathi, M. S.; Nickavasagam, M.; (2004) Analysis of genetic diversity and phylogenetic relationships among indigenous and exotic Silk (AAB) group of bananas using RAPD markers. Journal of Horticultural Science and Biotechnology.; 79: 523-527.

17. Werner, O.; Sanchez-Gomez, P.; Guerra, J.; Martinez, J. F.; (2001) Identification of *Pistacia saportae* Brrant (Anacardiaceae) by RAPD analysis and morphological characters. Scientia Horticulture.; 91**:** 178–186.

18. Yaltirik, F.; (1967) Anacardiaceae. In: Davis P. H. (ed.) Flora of Turkey.; 2: 544 – 548.

19. Zohary, M.; (1952) A monographical study of the genus *Pistacia*. Palestine Journal of Botany Jerusalem Series.; 5: 187 – 228.