

## Evaluation of Genetic Diversity of Iranian Pomegranate Cultivars Using Fruit Morphological Characteristics and AFLP Markers

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### Abstract

The present research evaluated the diversity of a number of Iranian pomegranate cultivars using fruit morphological characteristics and AFLP markers. Thirty-one pomegranate cultivars were collected from Yazd Pomegranate Collection in Iran to study their diversity. Seven AFLP primer combinations were used to amplify a total of 112 polymorphic fragments (47.26%). By use of AFLPs, a low genetic diversity level was detected among cultivars. The relationship between fruit characteristics was analyzed using the principal component analysis (PCA). The cluster analysis based on both fruit characteristics and AFLP data indicated that cultivars were not grouped according to their geographic origins. Moreover, the correlation between the diversity matrix based on fruit characteristics and Dice's genetic similarity coefficient was insignificant ( $r=0.06$ ). The results obtained from this study can improve the conservation and management of pomegranate germplasm resources and could be helpful in optimizing breeding programs.

**Keywords:** AFLPs, fruit characteristics, genetic diversity, Iranian pomegranate, markers

### Introduction

The pomegranate (*Punica granatum* L.), an ornamental plant which has been popular among Mediterranean peoples for centuries (Vazifeshenas *et al.*, 2009) which is native to Iran and the Himalayas, produces delicious and edible fruits, and belongs to the *Punicaceae* family. There exists a local collection of pomegranate cultivars consisting of approximately 760 cultivars in the Yazd province of Iran (Behzadi Shahrabaki, 1997).

Mars and Marrakchi (1999) reported that the fruit morphological characteristics are useful for pomegranate identification; however, these morphological traits are intensely dependent on the environmental conditions. There are some reports using RAPDs markers (Talebi Bodaff *et al.*, 2003; Sarkhosh *et al.*, 2006), SSR markers (Currò *et al.*, 2010; Soriano *et al.*, 2011), RAPDs and morphological markers (Sarkhosh *et al.*, 2009; Zamani *et al.*, 2007), as well as AFLPs markers (Jabir *et al.*, 2008; Yuan *et al.*, 2007) to analyze the genotypic characteristics and genetic relationships of pomegranate cultivars.

Due to the long history of Iranian pomegranate cultivation and the related vegetative propagation, several cases of homonymy and synonymy can be observed among this germplasm. Thus, it is essential to create a reliable classification system for Iranian pomegranates. Moreover, it is very important for using a sensitive and credible molecular technique to detect the DNA variation and identify the pomegranate germplasm, by helping breeders and nurserymen with the selection and propagation of a cultivar.

Having many advantages, such as reproducibility, high levels of polymorphism detection, genome-wide distribution of markers and no requirement for the previous knowledge of the studied genome, have caused AFLPs to be an appropriate technique for genetic diversity among the various molecular markers (Bruna *et al.*, 2007; Polanco and Ruiz, 2002). In addition, Vos *et al.* (1995) reported that AFLP has been known as a more reliable technique than RFLP, RAPD.

AFLP markers have successfully been used to study the genetic diversity at the varietal level in many fruit trees, including apricot (Hurtado *et al.*, 2002), olive (Rotondi *et al.*, 2003) and pear (Bao *et al.*, 2008). Despite, the various studies based on molecular markers in Iran, there is still ambiguities and debates about genetic diversity of pomegranate germplasm in Iran mainly due to different efficiency of different methods. Therefore, the aims of this project were to produce suitable markers for the characterization of pomegranate cultivars and to evaluate the diversity of Iranian pomegranate cultivars using fruit morphological characteristics and AFLPs markers.

### Materials and methods

#### *Plant materials*

Fruit and leaf samples of thirty one pomegranate genotypes were collected from adult trees from the pomegranate collection at Agricultural Research Center of Yazd, Iran (Tab. 1).

Tab. 1. The name, peel color, aril color, taste and origin of 31 pomegranate cultivars

No.	Cultivars	Peel color	Aril color	Taste	Origin
1	'Shirine Dane Sefide Ferdos'	Red	Red	Sweet	Khorasan
2	'Torshe shahvare Kashmar'	Red	Red	Sour	Khorasan
3	'Shishe Kab'	Red	Red	Sweet-sour	Khorasan
4	'Mazarie Bajestan'	Pink	Yellow	Sweet-sour	Khorasan
5	'Dom Anbaroti'	White	Yellow	Sour	Khorasan
6	'Shirine Dane Ghermeze Ferdos'	Pink	Red	Sweet	Khorasan
7	'Khazar Bajestani'	Red	Red	Sweet-sour	Khorasan
8	'Leili Post Nazok'	Red	Red	Sweet-sour	Khorasan
9	'Leili Post Koloft'	Pink	Yellow	Sweet-sour	Khorasan
10	'Torshe Shahvare Ferdos'	Red	Red	Sour	Khorasan
11	'Bazmanie Post Nazok'	Pink	Pink	Sweet-sour	Sistan o Balochestan
12	'Savehei Post Sefid'	white	Pink	Sweet-sour	Sistan o Balochestan
13	'Savehei Post Ghermez'	Pink	Yellow	Sweet-sour	Sistan o Balochestan
14	'Malase Porbare Saravan'	Pink	Pink	Sweet-sour	Sistan o Balochestan
15	'Malase Mamolie Sarjo'	Red	Red	Sweet-sour	Sistan o Balochestan
16	'Shekanare Post Koloft'	White	Pink	Sweet	Mazandaran
17	'Vahshie Janghalie Ghaemshahr'	Red	Yellow	Sweet-sour	Mazandaran
18	'Mahalie Parande Gorgan'	White	Red	Sour	Mazandaran
19	'Post Sefide Dezfol'	Red	Red	Sour	Khuzestan
20	'Malase Dane Siyahe Ramhormoz'	White	Red	Sweet-sour	Khuzestan
21	'Malase Post Sorkh'	Red	Red	Sweet-sour	Khuzestan
22	'Shirine Post Ghermez'	Red	Red	Sweet	Azarbaejan
23	'Shirine Post Sefid'	Pink	Pink	Sweet	Azarbaejan
24	'Malase Post Nazok'	White	Yellow	Sweet-sour	Azarbaejan
25	'Zagh Yazdi'	Red	Red	Sour	Yazd
26	'Malase Yazdi'	Red	Red	Sweet-sour	Yazd
27	'Post Siyah'	Black	Yellow	Sweet	Yazd
28	'Gorje Shahvar'	Pink	Pink	Sweet	Yazd
29	'Agha Mohammadali'	Red	Red	Sweet	Markazi
30	'Alake Shirine Saveh'	Red	Red	Sweet	Markazi
31	'Malase Saveh'	Red	Red	Sweet-sour	Markazi

#### Morphological and chemical fruit characteristics

Quantitative and qualitative fruit characteristics were evaluated based on morphological and chemical analysis (Mars and Marakchi, 1999; Sarkhosh *et al.*, 2009; Tehranifar *et al.*, 2010), using 20 mature fruit samples per genotype (Tab. 2).

#### DNA extraction and AFLP analysis

Fresh and young fully expanded leaves from each cultivar were collected and ground in liquid nitrogen. Genomic DNA was extracted using DNeasy plant mini kits (Qiagen, Inc., CA, USA). The quantity and quality of isolated genomic DNA was determined using agarose gel [1% (w/v)] electrophoresis and a nano drop spectrophotometer (ND 1000, USA).

AFLP analysis was conducted using the minor modified standard procedure by Vos *et al.* (1995). Approximately 250 ng of genomic DNA was digested by restriction enzymes *EcoRI* and *MseI* and then double standard adaptors were ligated to the obtained fragments to generate templates for amplification. The digest-Ligated DNA

fragments were pre-amplified using *EcoRI*+1 (5'-GACTGCGTACCAATTCA-3') and *MseI*+1 (5'-GATGAGTCCTGAGTAAC-3') primers under the following conditions: 20 cycles of 94°C for 30 s, 56°C for 60 s and 72°C for 60 s and then were used as templates (without dilution). Initially, a total of 35 primer combinations, from which seven primer combinations with the strongest and greatest number of bands were selected for AFLP reaction, were tested. Selective amplification was performed using a pair of *EcoRI*+3 and *MseI*+3 primers. The amplifications consisted of the following steps: one cycle of 94°C for 30 sec, 65°C for 30 sec, and 72°C for 60 sec, followed by 13 cycles at decreasing annealing temperature in decrements of 0.7°C per cycle, then 23 cycles of 94°C for 30 sec, 56°C for 30 sec, and final extension 72°C for 60 sec. The amplification products were resolved by 6% denaturing polyacrylamid gels at 1200 volt for 120 min in 1X TBE (Tris-Borate Ethylenediaminetetraacetic acid). The AFLP markers were visualized by silver nitrate staining according to Sanguinetti *et al.* (1994).

*Data analysis*

After normalizing quantitative morphological data, the mean values of each parameter were estimated for statistical analysis. The average values were utilized to calculate the principal component analysis (PCA) and cluster analysis based on the Euclidean distance between the different genotypes. The principal component analysis was used to compare the influence of each characteristic on the clustering of cultivars. Simply factors loading values equal or greater than 0.5 were considered significant. The dendrogram was conducted using Ward's methods via SPSS for the windows computer software (version, 16).

Tab. 2. List of fruit characteristic observed in the 31 pomegranate cultivars, range of variability, means and coefficient of variation

Trait code	Trait	Min	Mean	Max	CV%*
FW	Fruit weight (gr)	194.38	243.90	314.52	11.60
FL	Fruit length (mm)	69.50	75.86	81.57	4.55
FD	Fruit diameter (mm)	64.99	78.97	86.88	5.18
FL/FD	Fruit length/ Fruit diameter (Ratio)	0.88	0.98	1.61	12.43
FV	Fruit volume (ml)	204.25	262.49	341.36	11.63
FDe	Fruit Density (gr/cm <sup>3</sup> )	0.68	0.93	0.9	2.76
CL	Calix length (mm)	13.45	19.53	24.0	12.73
CD	Calix diameter (mm)	12.52	17.71	24.18	16.10
CL/CD	Calix length/ Calix diameter (Ratio)	0.81	1.13	1.72	17.11
PT	Peel thickness (mm)	3.13	4.12	6.51	19.01
PW	Peel weight (gr)	63.61	114.81	185.14	21.60
PW/F	Peel weight /Fruit (%)	32.72	47.21	75.18	18.69
AW	Aril weight (gr)	64.48	125.25	170.43	19.81
AL	Aril length (mm)	11.29	11.97	13.17	4.26
AD	Aril diameter (mm)	6.49	7.45	8.24	5.84
AL/AD	Aril length/ Aril diameter (Ratio)	1.40	1.61	1.79	5.57
A/F	Aril/Fruit (%)	26.29	51.26	65.81	16.28
SW	Seed weight (gr)	21.23	33.74	59.59	25.89
SW/F	Seed weight/ Fruit (%)	9.45	13.82	20.56	20.75
JV	Juice volume(ml)	37.28	82.17	112.50	20.84
JW	Juice weight(gr)	39.31	86.41	117.75	20.95
JD	Juice density(gr/cm <sup>3</sup> )	1.03	1.05	1.06	0.81
J/F	Juice/Fruit (%)	16.06	35.43	47.15	18.85
PH	pH	2.87	3.65	4.21	9.29
TSS	Total soluble solids (%)	11.0	13.62	15.38	8.21
TA	Titrate acidity (%)	0.33	1	2.44	52.98
MI	Maturity index(-)	5.03	18.12	47.07	56.78
AC	Ascorbic acid(mg/100g)	7.19	13.76	18.42	22.20
ANA	Anthocyanin absorbance (O.D. <sub>510</sub> )	5.55	9.60	30.12	58.10
AN	Antioxidant (%)	15.98	30.06	54.37	33.04
JTP	Juice total phenol (mg/100g)	159.79	3301.84	3195.33	83.14
TS	Total sugar (mg/100g)	13.23	18.68	21.72	9.69

\*CV, coefficient of variation = (standard deviation/mean) × 100

Manually, AFLP fragments were scored according to their presence (1) or absence (0) to form a raw data matrix. The statistical analysis was constructed using the NTSYS software version 2.02 (Rohlf, 1998). The genetic similarities between all cultivars were estimated based on the Dice's coefficient (Nei and Li, 1979). In order to construct a dendrogram, the similarity matrix was calculated by the unweighted pair-group method of the arithmetic average (UPGMA) using the SAHN clustering model. The cophenetic coefficient was computed in order to test the goodness of fit between the cluster in the dendrogram and the similarity coefficient matrix. The Mantel test was applied to calculate the correlation between the two dendrograms produced by morphological and AFLP data (Mantel, 1967).

**Results and discussion***Fruit characteristics*

Thirty-one cultivars were characterized by a large variability in quantitative morphological traits including fruit shape, color and juice (data not shown). The range of the mean values of each studied cultivar exhibited a significant diversity in the fruit characteristics. The mean, maximum, minimum and coefficient of the variation values for each characteristic among all genotypes were illustrated in Tab. 2. Among all quantitative characteristics titratable acidity (TA), Juice total phenol (JTP) and Anthocyanin absorbance (ANA) showed higher CV values indicating a high level of variation. PCA results indicated that the first component related to fruit weight (FW), fruit length (FL), fruit volume (FV), peel thickness (PT), peel weight/fruit (PW/F ratio), aril weight (AW), aril length (AL), aril diameter (AD), aril length/aril diameter (AL/AD ratio), aril/fruit (A/F ratio), seed weight (SW), juice volume (JV), juice density (JD), juice/Fruit (J/F ratio), total sugar (TS) and total soluble solids (TSS) accounted for 31.84% of the total variation and grouped cultivars based on most of the studied physical characteristics. The second component which explained 19.24% of the total variation is dominated by five other physical characteristics explaining 95.01 of the total variance (Tab. 3). According to the aforementioned seven factors, thirty-one cultivars fall into the main five clusters at a distance of 10 (Fig. 1).

*AFLP analysis*

A total 112 polymorphic bands from 237 fragments, ranging in size from 50 to 800 bp, were generated using seven primer combinations (Fig. 2). The percentage of polymorphic DNA bands ranged from 40% (E-ACT, M-CTT) to 58.06% (E-AAC, M-CAA) with an average of 47.26% (Tab. 4).

The range of the similarity matrix obtained by the Dice coefficient varied between 0.793 and 0.997 with an average of 0.944 (Tab. 5). The genetic relationship between 31 cultivars based on the Dice's similarity coefficient is shown

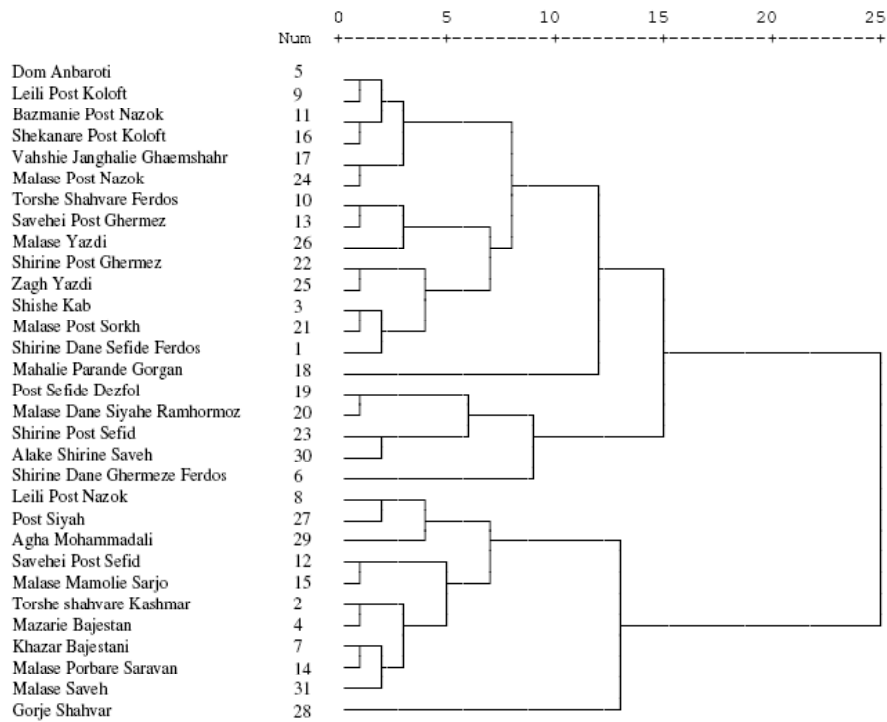


Fig. 1. The principle component analysis dendrogram of 31 pomegranate cultivars obtained from fruit characteristics data

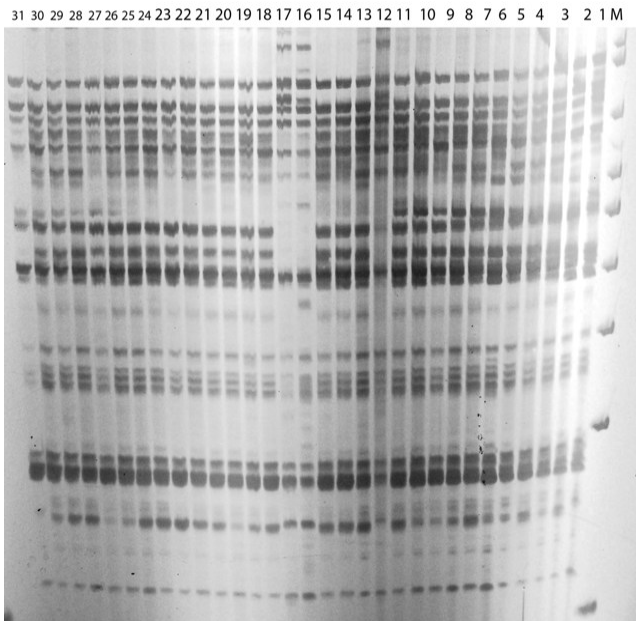


Fig. 2. An example of AFLP profile using the E-ACT / M-CAA selective primer combination. M is 50 bp standard sizes Marker

in a dendrogram (Fig. 3). The cophenetic correlation coefficient calculated between the similarity matrix and cophenetic matrix, which were obtained from dendrogram data, was very high ( $r=0.99$ ). According to Dice's similarity matrix and the UPGMA clustering method, the dendrogram exhibited two main groups (A-B) that were identified at the 0.81 similarity level (Fig. 3). Group A consisted of two subgroups, one containing the cultivar No. 5 that

was identified at the 0.90 similarity level and the other, including two cultivars (No. 4 and No. 7) and showed the morphological relationship. Group B contained all other 28 cultivars, which were identified at the 0.92 similarity level. Twenty-eight morphologically distinct cultivars are completely close to one another in the genetic analysis by AFLPs. Except for the three cultivars (No. 4, No. and No. 7), which were separated by a similarity coefficient of 0.81, most of them had a similarity coefficient up to 0.95. No obvious relationships were detected between the morphology, the origins and the estimated genetic traits. According to the genetic analysis conducted by AFLP, most of the cultivars were composed of simply one group in spite of their distinct origin and morphology. The three remaining cultivars, which did not fall into this group, were morphologically distinct. According to the dendrogram (Fig. 3) and similarity matrix (Tab. 5), a relatively low genetic diversity was observed among the studied cultivars.

In this study, both dendrograms obtained from the morphological and AFLP markers were not consistent with the local name and geographical origin. Furthermore, the results of this study were in agreement with the others (Jabir *et al.*, 2008; Narzary *et al.*, 2009; Yuan *et al.*, 2007), showing that the clustering of the cultivars is not related to the geographical distance.

The level of the genetic diversity highly correlated with the sample size; therefore, it would be worth mentioning that the used sample size was small in the present study. Also, another reason for the low genetic diversity could be due to the vegetative propagation. Over a period of 2500 years (Behzadi Shahrabaki, 1997), there has been more

Tab. 3. Eigen values, cumulative variance and factor loadings for each variable of the components of PCA analysis for 31 pomegranate cultivars

Factor	1	2	3	4	5	6	7
Eigen value	11.13	6.74	4.86	3.78	2.83	2.23	1.69
%of variance	31.81	19.24	13.89	10.80	8.07	6.37	4.82
Cumulative variance	31.81	51.06	64.94	75.74	83.82	90.19	95.01
Characteristics	Factor		Loading				
Fruit weight	0.72 <sup>*</sup>	0.28	0.54 <sup>*</sup>	0.03	-0.15	0.17	0.07
Fruit length	0.76 <sup>*</sup>	0.46	0.20	-0.09	-0.02	0.31	0.08
Fruit diameter	0.12	-0.33	-0.02	-0.86 <sup>*</sup>	-0.28	0.15	-0.16
Fruit length/ Fruit diameter	0.23	0.53 <sup>*</sup>	0.15	0.76 <sup>*</sup>	0.24	0.00	0.11
Fruit volume	0.70 <sup>*</sup>	0.39	0.51 <sup>*</sup>	-0.13	-0.17	0.16	0.03
Fruit Density	0.17	-0.54 <sup>*</sup>	0.10	0.74 <sup>*</sup>	0.07	0.16	0.20
Calix length	0.35	0.63 <sup>*</sup>	0.42	-0.41	-0.13	0.05	0.31
Calix diameter	-0.41	-0.33	0.47	-0.01	0.59 <sup>*</sup>	-0.37	-0.05
Calix length/ Calix diameter	0.49	0.55 <sup>*</sup>	-0.11	-0.29	-0.46	0.28	0.26
Peel thickness	-0.75 <sup>*</sup>	0.22	0.45	0.20	0.27	0.15	0.24
Peel weight	-0.48	0.61 <sup>*</sup>	0.52 <sup>*</sup>	0.05	-0.04	0.26	0.09
Peel weight / Fruit	-0.87 <sup>*</sup>	0.41	0.22	-0.01	0.04	0.16	0.06
Aril weight	0.99 <sup>*</sup>	-0.12	0.11	-0.00	-0.05	-0.02	0.01
Aril length	0.88 <sup>*</sup>	-0.37	-0.23	0.01	0.03	-0.12	-0.06
Aril diameter	0.78 <sup>*</sup>	0.43	-0.04	0.27	0.08	0.31	-0.06
Aril length/ Aril diameter	0.64 <sup>*</sup>	0.35	-0.39	0.34	0.16	0.30	-0.12
Aril/Fruit	0.91 <sup>*</sup>	-0.34	0.14	-0.07	-0.08	-0.10	-0.02
Seed weight	0.90 <sup>*</sup>	-0.36	0.15	-0.06	-0.07	-0.10	0.01
Seed weight/ Fruit	0.21	-0.48	0.49	0.16	0.22	-0.09	0.56 <sup>*</sup>
Juice volume	0.75 <sup>*</sup>	-0.60 <sup>*</sup>	-0.14	-0.06	0.01	-0.22	-0.05
Juice weight	0.08	0.74 <sup>*</sup>	0.11	-0.19	0.40	-0.06	-0.49
Juice density	-0.56 <sup>*</sup>	0.68 <sup>*</sup>	-0.27	-0.17	0.18	-0.11	-0.23
Juice/Fruit	0.78 <sup>*</sup>	-0.01	0.46	-0.04	0.19	0.05	-0.26
pH	0.45	0.37	-0.54 <sup>*</sup>	0.16	-0.21	-0.03	-0.31
Total soluble solids	0.58 <sup>*</sup>	-0.07	-0.56 <sup>*</sup>	-0.04	0.37	-0.28	0.33
Titration acidity	-0.29	-0.69 <sup>*</sup>	-0.19	-0.01	0.01	0.57 <sup>*</sup>	0.01
Maturity index	0.31	0.75 <sup>*</sup>	-0.07	0.45	0.14	-0.31	0.02
Ascorbic acid	0.01	0.06	-0.67 <sup>*</sup>	0.65 <sup>*</sup>	-0.13	0.17	0.09
Anthocyanin absorbance	0.24	0.28	-0.84 <sup>*</sup>	-0.24	0.20	-0.03	0.23
Antioxidant	-0.08	-0.55 <sup>*</sup>	0.10	-0.01	0.46	0.4	-0.32
Juice total phenol	0.41	-0.43	0.44	0.12	0.31	0.38	-0.12
Total sugar	-0.62 <sup>*</sup>	-0.28	-0.30	0.02	-0.52 <sup>*</sup>	0.14	0.26
Aril color	-0.14	-0.12	0.51 <sup>*</sup>	0.26	-0.71 <sup>*</sup>	-0.28	-0.14
Peel color	-0.01	-0.11	0.35	0.52 <sup>*</sup>	-0.46	-0.50 <sup>*</sup>	-0.29
Taste	0.30	0.29	0.12	-0.49	0.33	-0.47	0.33

possibility that an intensive exchange of propagation material, such as cutting, all around provinces of Iran. Also, it is assumed that a large proportion of valuable cultivated pomegranates have lost through the continuous removal of old plantations, and commercially seedlings propagated from a few cultivars by cuttings. As a low level of polymorphism detected by SSR markers in pomegranate

Tab. 4. AFLP primer combinations, total numbers of fragments generated by each primer set, number of polymorphic fragments detected, and percentages of polymorphic fragments used in this study of 31 pomegranate cultivars

Primer	Total no. of bands	No of polymorphic bands	% of polymorphic bands
E ACT + /M + CAA	37	21	56.76
E ACT + /M + CTT	30	12	40
E ACT + /M + CCT	40	18	45
E ACT + /M + CTA	32	14	43.75
E ACC + /M + CAA	32	13	40.62
E AAC + /M + CAA	31	18	58.06
E AAC + /M + CTT	35	16	45.71
Total	237	112	
Average	33.8	16	47.26

genotypes (Currò *et al.*, 2010), these Authors reported that higher levels of polymorphism could be detected by analyzing larger collections or natural populations in the origin areas.

A very poor correlation was obtained between the morphological distance matrix and AFLP similarities matrix ( $r=0.06$ ) (Fig. 4). This result was in agreement with the results obtained by Talebi Boddaf *et al.* (2003), Zamani *et al.* (2007) and Sarkhosh *et al.* (2009) and confirmed the insignificant correlation between the morphology and the RAPD markers. In order to provide a better matching of the relationship between the morphological traits and the molecular markers, more morphological characteristics such as phenological traits of leaves, flowers and fruits are required to be estimated.

The first possible explanation for the lack of correspondence between morphological traits and molecular markers are that these morphological differences such as the fruit color, fruit shape, height, form of trees and branching habit are probably the result of alleles that were not detected by the present molecular markers. Wen *et al.* (2004) and Zahuang *et al.* (2004) proposed that post-transcriptional effects, translation, environmental changes and non-nuclear inheritance can lead to the lack of the correspondence between morphological traits and molecular marker. In some studies the low correlation between these markers are observed (Heidary *et al.*, 2009; Martinez, 2003; Rotondi *et al.*, 2003) and the others (Cavagnaro *et al.*, 2006) it has been showed that there is a significant correlation between these markers. Another explanation would be the relatively low number of markers used in this study resulted in inadequate genome coverage (De Langhe *et al.*, 2005).

To prepare a better matching of the relationship between morphological and molecular markers, more primers or an extended set of primer combinations must be utilized. More studies with both morphological and other markers such as Co-dominate markers, might solve this issue.

In conclusion, the results demonstrated that AFLP profiles are valuable tools with great potential for classi-



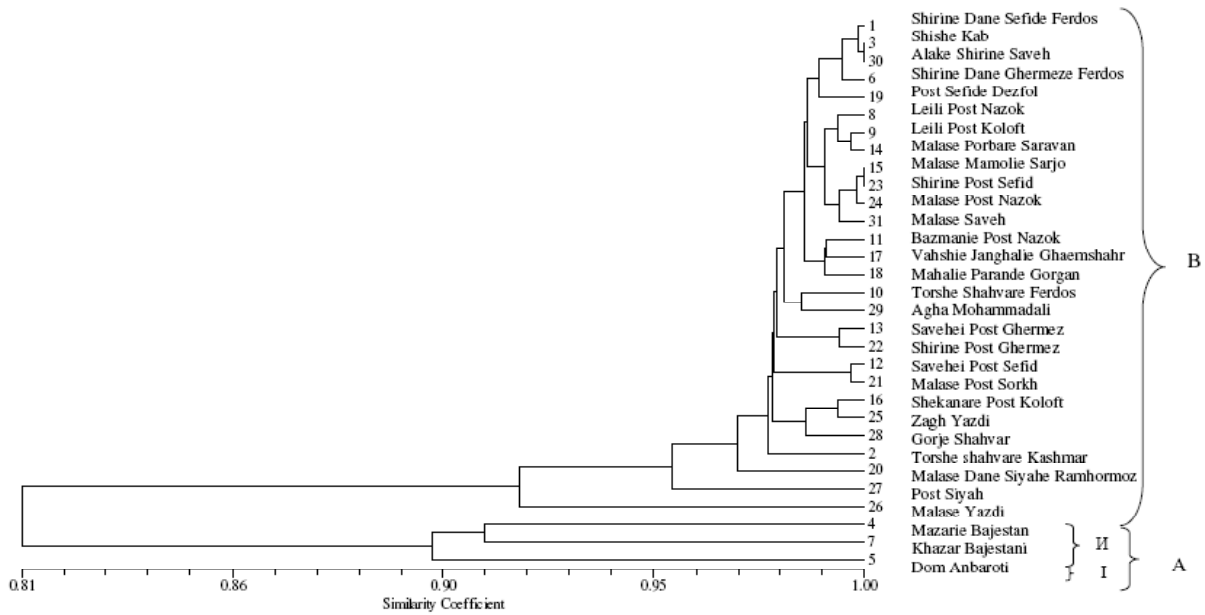


Fig. 3. UPGMA dendrogram constructed using the Dice's similarity (Nei and Li, 1979) coefficient analysis based on molecular profiles revealed by AFLP marker

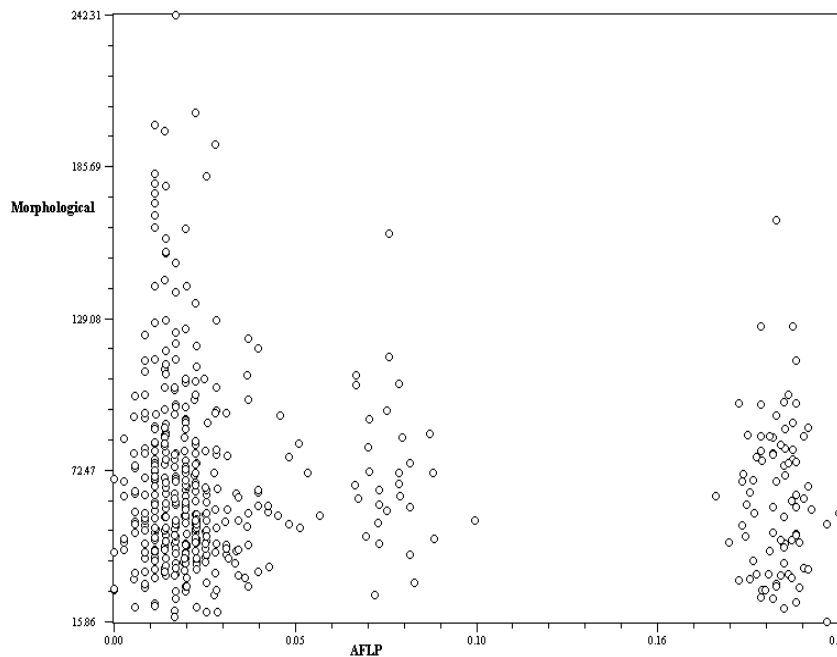


Fig. 4. Scatter plots showing pair wise comparisons between the Dice's similarity coefficients (Nei and Li, 1979) as calculated from AFLP analysis and distance using fruit characteristics

fications of Iranian pomegranate. In addition, complete comprehension of the genetic diversity within cultivars would contribute to a more efficient use of germplasm in plant breeding programs. Furthermore, it is essential to investigate other molecular markers linked closely to the morphological traits of pomegranate in the future.

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**References**

Bao L, Chen K, Zhang D, Li X, Teng Y (2008). An assessment of genetic variability and relationships within Asian pears based on AFLP (amplified fragment length polymorphism) markers. *Sci Hortic* 116:374-380.  
 Behzadi Shahrabaki H (1997). Genetic diversity of pomegranate genotypes in Iran. Agriculture Education Pub.

- Karaj, Iran, 265 p (In Farsi).
- Bruna S, Portis E, Cervelli C, De Benedetti L, Schiva T, Mercuri A (2007). AFLP-based genetic relationships in the Mediterranean myrtle (*Myrtus communis* L.). *Sci Hortic* 113:370-375.
- Cavagnaro PF, Cavagnaro JB, Lemes JL, Masuelli RW, Passera CB (2006). Genetic diversity among varieties of the native forage grass *Trichloris crinita* based on AFLP markers, morphological characters, and quantitative agronomic traits. *Genome* 49(8):906-918.
- Currò S, Caruso M, Distefano G, Gentile A, La Malfa S (2010). New microsatellite loci for pomegranate, *Punica granatum* (*Lythraceae*). *Am J Bot* 97:58-60.
- De Langhe E, Pillay M, Tenkouano A, Swennen R (2005). Integrating morphological and molecular taxonomy in Musa: the African plantains (*Musa* spp. AAB group). *Plant System Evol* 255:225-236.
- Jbir R, Hasanaoui N, Mars M, Marrakchi M, Trifi M (2008). Characterization of Tunisian pomegranate (*Punica granatum* L.) cultivars using amplified fragment length polymorphism analysis. *Sci Hortic* 115:231-237.
- Heidary S, Marashi H, Farsi M, Mirshamsi Kakhki A (2009). Assessment of genetic structure and variation of native *Berberis* populations of Khorasan provinces (Iran) using AFLP markers versus morphological markers. *Iran J Biotechnol* 7 (2):101-107.
- Hurtado MA, Westman A, Beck E, Abbott GA, Llacer G, Badenes ML (2002). Genetic diversity in apricot cultivars based on AFLP markers. *Euphytica* 127:297-301.
- Levin GM (1994). Pomegranate (*Punica granatum* L.) plant genetic resources in Turkmenistan. *Plant Gene Res Newslet* 97:31-36.
- Mantel NA (1967). The detection of disease clustering and a generalized regression approach. *Cancer Res* 27:209-220.
- Mars M, Marrakchi M (1999). Diversity of pomegranate (*Punica granatum* L.) germplasm in Tunisia. *Genetic Res Crop Evol* 46:461-467.
- Martinez L, Cavagnaro P, Masuelli R, Rodriguez J (2003). Evaluation of diversity among Argentine grapevine (*Vitis vinifera* L.) varieties using morphological data and AFLP markers. *Plant Biotechnol* 6(3):244-253.
- Narzary D, Mahar KS, Rana TS, Ranade SA (2009). Analysis of genetic diversity among wild pomegranates in Western Himalayas, using PCR methods. *Sci Hortic* 121:237-242.
- Nei M, Li W (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc Natl Acad Sci USA* 76:5269-5273.
- Polanco C, Ruiz ML (2002). AFLP analysis of somaclonal variation in *Arabidopsis thaliana* regenerated plants. *Plant Sci* 162:817-824.
- Rohlf FJ (1998). NTSYS-PC. Numerical taxonomy and multivariate analysis system, version 2.00. Exeter Software, Setauket, NY.
- Rotondi A, Magli M, Ricciolini T, Baldoni L (2003). Morphological and molecular analysis for the characterization of a group of Italian olive cultivars. *Euphytica* 132:129-137.
- Sanguinetti CJ, Dias Neto E, Simpson AJG (1994). Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques* 17:915-919.
- Sarkhosh A, Zamani Z, Fatahi R, Ranjbar H (2009). Evaluation of genetic diversity among Iranian soft-seed pomegranate accessions by fruit characteristics and RAPD markers. *Sci Hortic* 121:313-319.
- Sarkhosh A, Zamani Z, Fatahi R, Ebadi A (2006b). RAPD markers reveal polymorphism among some Iranian pomegranate (*Punica granatum* L.) genotypes. *Sci Hortic* 111:24-29.
- Soriano JM, Zuriaga E, Rubio P, Llácer G, Infante R, Badenes ML (2011). Development and characterization of microsatellite markers in pomegranate (*Punica granatum* L.). *Mol Breeding* 27:119-128.
- Talebi Baddaf M, Sharifi Neia B, Bahar M (2003). Analysis of genetic diversity in pomegranate cultivars of Iran, using random amplified polymorphic DNA (RAPD) markers (in Farsi). In: *Proc Third National Congress of Biotechnology*, 2:343-345.
- Tehranifar A, Zarei M, Nemati Z, Esfandiyari B, Vazifeshenas MR (2010). Investigation of physico-chemical properties and antioxidant activity of twenty Iranian pomegranate (*Punica granatum* L.) cultivars. *Sci Hortic* 126:180-185.
- Torre A, Lopez C, Yglesias E, Cornelius JP (2008). Genetic (AFLP) diversity of nine *Cedrela odorata* populations in Madre de Dios, southern Peruvian Amazon. *For Ecol Manage* 255:334-339.
- Vazifeshenas MR, Khayyat M, Jamalians S, Samadzadeh AR (2009). Effects of different scion-rootstock combinations on vigor, tree size, yield and fruit quality of three Iranian cultivars of pomegranate. *Fruits* 64(6):343-349.
- Vos P, Hogers R, Bleeker M, Reijmans M, Van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res* 23:4407-4414.
- Wen XP, Pang XM, Deng XX (2004). Characterization of genetic relationships of *Rosa roxburghii* Tratt and its relatives using morphological traits, RAPD and AFLP markers. *J Hortic Sci Biotechnol* 79:189-196.
- Yuan Z, Yin Y, Qu J, Zhu L, Li Y (2007). Population Genetic Diversity in Chinese Pomegranate (*Punica granatum* L.) Cultivars Revealed by Fluorescent-AFLP Markers. *J Genet Genom* 34(12):1061-1071.
- Zahuang FY, Chen JF, Staub JE, Qian T (2004). Assessment of genetic relationships among *Cucumis* spp. by SSR and RAPD marker analysis. *Plant Breed* 123:167-172.
- Zamani Z, Sarkhosh A, Fatahi R, Ebadi A (2007). Genetic relationships among pomegranate genotypes by RAPD markers and morphological characters of fruit. *J Hortic Sci Biotechnol* 82:11-18.