

RAPD analysis of genetic diversity in some pomegranate (*Punica granatum* L.) cultivars in Iran

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Abstract

RAPD markers variations were studied in ten pomegranate cultivars. Twenty RAPD primers used produced 323 bands. Nineteen bands were common in all the cultivars while other bands were polymorph. Some specific bands were identified in the cultivars studied which may be used in cultivars discrimination. Primers OPB-07 produced the highest number of polymorphic bands (5.9%[\[U1\]](#)), while primers OPI-16 produced the least number of polymorphic bands (5.2%[\[U2\]](#)). Different similarity coefficients determined among the cultivars studied, showed the highest value of genetic similarity was observed between Abdandan and Suski cultivars while Shasavar and Shahidaneh cultivars differed from the other cultivars and stand far from them in NJ and Bayesian trees. Some specific bands/loci were observed in pomegranate cultivars studied which may be used for cultivar identification.

Key words: Genetic diversity, Iran, pomegranate; RAPD.

Introduction

The pomegranate (*Punica granatum* L.) is among historic native horticultural plants of Iran which have been cultivated in different regions of the country (Figure 1). This plant is native from Iran to the Himalayas in northern India and also cultivated over in all over Mediterranean region (Facciola, 1990). About 550000 hectare of lands has been devoted to the cultivation of pomegranate in Iran producing about 570000 tones of pomegranate fruit (Sheidai and Noormohammadi, 2005; Sheidai et al., 2005; Sarkhosh et al., 2006; Zamani et al., 2007).

. In total 764 cultivars of *P. granatum* have been collected during a germplasm collection in Iran and grown in Saveh and Yazd cities, all of which possess their specific fruit characteristic such as size, color, time of ripening, disease resistance, taste, etc. Till now, there have been limited cytogenetic and genetic studies in pomegranate cultivars of Iran (Raman et al., 1971; Gill et al.,

1981; Xue et al., 1992; Talebbi-Baddaf et al., 2003; Sheidai and Noormohammadi, 2005; Sheidai et al., 2005; Sarkhosh et al., 2006; Yuan et al., 2007; Zamani et al., 2007).

Studying the genetic diversity as well as cultivar identification by using various molecular markers including RAPD (Random Amplified Polymorphic DNA) has been performed in several plant species (Harvey and Botha, 1996; Bautista et al., 2003; Zhao and Pan, 2004). These molecular markers provide an opportunity for direct comparison and identification of different genetic material independent of any influences (Harvey and Botha, 1996; Bautista et al., 2003; Zhao and Pan, 2004).

Since cultivation of the same cultivars for long period of time may lead to the genetic erosion, it is necessary to study the available diversity and introduce new variability as well for planning breeding programs. Therefore, the present study considers RAPD molecular analysis of 10 pomegranate cultivars of Iran for the first time.

Materials and Methods

Ten pomegranate cultivars were used for molecular studies namely, 1 = Bejestoni, 2 = Golnar, 3 = Shirin-poostghermez, 4 = Malas Yazdi, 5 = Abdandan, 6 = Shahidaneh, 7 = Suski, 8 = Toghmalas, 9 = Dokhtar-hamomi and 10 = Shamsavar. For RAPD analysis, fresh leaves were selected randomly from 3-5 plants of each cultivar and DNA extraction was done by use of modified CTAB method (Murry and Tompson, 1980). The PCR reaction mixture consisted of 1 ng template DNA, 1 x PCR buffer (10 mM Tris-HCL pH 8.8, 250 mM KCL), 200 μ M dNTPs, 0.80 μ M 10-base random primers and 1 unit of Taq polymerase, in a total volume of 25 μ l. DNA amplification was performed on a palm cycler GP-001 (Corbet, Australia). Template DNA was initially denatured at 92°C for 3 min, followed by 35 cycles of PCR amplification under the following parameters: denaturation for 1 min at 92°C, primer annealing for 1 min at 36°C and primer extension for 2 min at 72°C. A final incubation for 10 min at 72°C was performed to ensure that the primer extension reaction proceeded to completion. Twenty primers (Operon Technologies Inc, Alameda, USA) were used (Table 1). The PCR amplified products were separated by electrophoresis on a 2% agarose gels using 0.5 X TBE buffer (44.5 Mm Tris/Borate, 0.5 Mm EDTA, pH 8.0) or 12% polyacrylamide gels. The gels were stained with ethidium bromide and visualized under UV light or silver stained for added sensitivity. RAPD markers were named by primer origin, followed with the primer number and the size of amplified products in base pairs. Twenty random primers of Operon technology (Alameda, Canada) were used.

The experiment was repeated for 3 times and reproducible RAPD bands were used for further analysis. Bands obtained were treated as binary characters and coded accordingly (presence =1, absence = 0). Simple matching coefficient and Jaccard coefficients were determined among the cultivars studied and grouping of the genotypes was determined by using UPGMA (Unweighted Paired Groups with Arithmetic Average) and NJ (Neighbor Joining) clustering methods (Podani, 2000; Weising et al., 2005). The fit of dendrograms obtained were checked by bootstrapping. Bayesian clustering was also performed on RAPD data by using Markov chain Monte Carlo (MCMC) method (Hall, 2001; Weising *et al.*, 2005). NTSYS Ver. 2.02 (1998) and PAUP

ver. 4.0b10 (2001) was used for clustering and bootstrapping. Bayesian clustering was performed by MrBayes ver. 3.1 (2005).

Results and Discussion

Twenty RAPD (decamer oligonucleotide) primers used produced 323 bands in total, out of which 304 bands were polymorph and 19 bands (loci) were present in all the cultivars studied (Figure 2). Primers OPB-07 and OPM-11 produced the highest number of bands (17 bands) while, primer OPI-16 produced the lowest number of bands (6 bands).

Some bands were specific for some of the cultivars which may be used in the cultivars discrimination. For example, band OPC02-19 (390 bp) was present only in the cultivar Bajestoni, bands OPC06-11 and OPC08-5 (500 & 1250 bp respectively) occurred only in the cultivar Dokhtar-hamomi, bands OPC08-12, OPC07-14 and OPA13- 3 (500, 700 & 1700 bp respectively) occurred only in Shahidaneh. Bands OPC04-8 and OPC04-9 (600 & 700 bp respectively) also were present in the cultivar Shahidaneh. The presence of specific loci indicates the genetic distinctness of the pomegranate cultivars studied.

There were some loci which were missing only in one cultivar; such loci may also be of use in the pomegranate cultivar differentiation. For example OPA-13 (650 bp), OPC09-8 and 11 loci (625 & 950 bp respectively) were present in all pomegranate cultivars except the cultivar Malas Yazdi, while the band OPC06-4, 5, 7 and 8 (800, 900, 1200 & 1500 bp respectively) were absent only in the cultivar Bejestoni. Band 9(800 bp) of primer OPC-08 and band 7 of OPA-13 (800 bp) were absent only in the cultivar Shasavar. Missing of one particular band is possibly due to genomic recombination and may be of use in cultivar discrimination.

Different similarity coefficients determined among the cultivars studied, showed the highest value of similarity between cultivars Abdandan and Suski (for example $r = 0.73$ in Jaccard similarity, Table 2) while the lowest value of similarity occurred between the cultivars Toghmalas and Shasavar (for example $r = 0.48$ in Jaccard similarity).

Different clustering methods including UPGMA (unweighted paired group with arithmetic average), NJ and Bayesian tree almost produced similar results with good bootstrap and clade credibility values (Figures. 3 & 4). In all trees obtained two cultivars of Abdandan and Suski show high genetic affinity and are placed in a single cluster or clade. Two cultivars of Dokhtar-hamomi and Malas Yazdi join these two with some distance.

Two cultivars of Shirin-poostghermez and Toghmalas show genetic similarity and form a single cluster or clade to which two other cultivars of Bejestoni and Golnar are joined with some distance. Two cultivars of Shahidaneh and Shasavar are placed far from the other cultivars and join them with greater distance indicating their genetic difference with the others. The geographical locality of each cultivar is indicated in NJ dendrogram (Figure 3), as we can see the grouping of the cultivar is not correlated with their geographical origin.

In general, the present study shows the usefulness of RAPD analysis in distinguishing the pomegranate cultivars, particularly identification of the specific bands may be considered important in the pomegranate cultivar identification. If RAPD diversity is combined with fruit and other important agronomic characteristics, performing the similar studies on the other pomegranate genotypes may lead to planning of a better breeding program in the country.

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Table 1. RAPD primers used and their nucleotide sequences.

	Primers	Nucleotide sequence
1	OPA-04	5'AATCGGGCTG 3'
2	OPA-13	5'CAGCACCCAC 3'
3	OPA-18	5'AGGTGACCGT 3'
4	OPB-07	5'GGTGACGCAG 3'
5	OPC-01	5'TTCGAGCCAG 3'
6	OPC-02	5'GTGAGGCGTC 3'
7	OPC-03	5'GGGGGTCTTT 3'
8	OPC-04	5'CCGCATCTAC 3'
9	OPC-05	5'GATGACCGCC 3'
10	OPC-06	5'GAACGGACTC 3'
11	OPC-07	5'GTCCCGACGA 3'
12	OPC-08	5'TGGACCGGTG 3'
13	OPC-09	5'CTCACCGTCC 3'
14	OPC-10	5'TGTCTGGGTG 3'
15	OPI-05	5'TGTTCCACGG 3'
16	OPI-07	5'CAGCGACAAG 3'
17	OPI-16	5'TCTCCGCCCT 3'
18	OPI-18	5'AATGCGGGAG 3'
19	OPM-11	5'GTCCACTGTG 3'
20	OPR-02	5'CACAGCTGCC 3'

Table 2. Jaccard similarity among pomegranate cultivars.

	Jaccard measure									
	1	2	3	4	5	6	7	8	9	10
1	1.0000000									
2	0.6000000	1.0000000								
3	0.6648352	0.6901408	1.0000000							
4	0.5572519	0.6900000	0.5833333	1.0000000						
5	0.6781609	0.5774648	0.6588235	0.5327869	1.0000000					
6	0.6192661	0.5530726	0.6617647	0.5000000	0.5450237	1.0000000				
7	0.4629630	0.5540541	0.4818182	0.4912281	0.4770642	0.5040650	1.0000000			
8	0.6994536	0.6369863	0.6413043	0.5680000	0.6201117	0.6296296	0.5370370	1.0000000		
9	0.6011236	0.5493827	0.6104651	0.4791667	0.6073620	0.6000000	0.4835165	0.6608187	1.0000000	
10	0.6073298	0.5222930	0.6298343	0.4962963	0.5107527	0.7386935	0.4864865	0.6276596	0.5865922	1.0000000

Cultivars abbreviations:

1 = Bejestoni, 2 = Golnar, 3 = Shirin-poostghermez, 4 = Malas Yazdi, 5 = Abdandan, 6 = Shahidaneh, 7 = Suski, 8 = Toghmalas, 9 = Dokhtar-hamomi and 10 = Shahsavar.

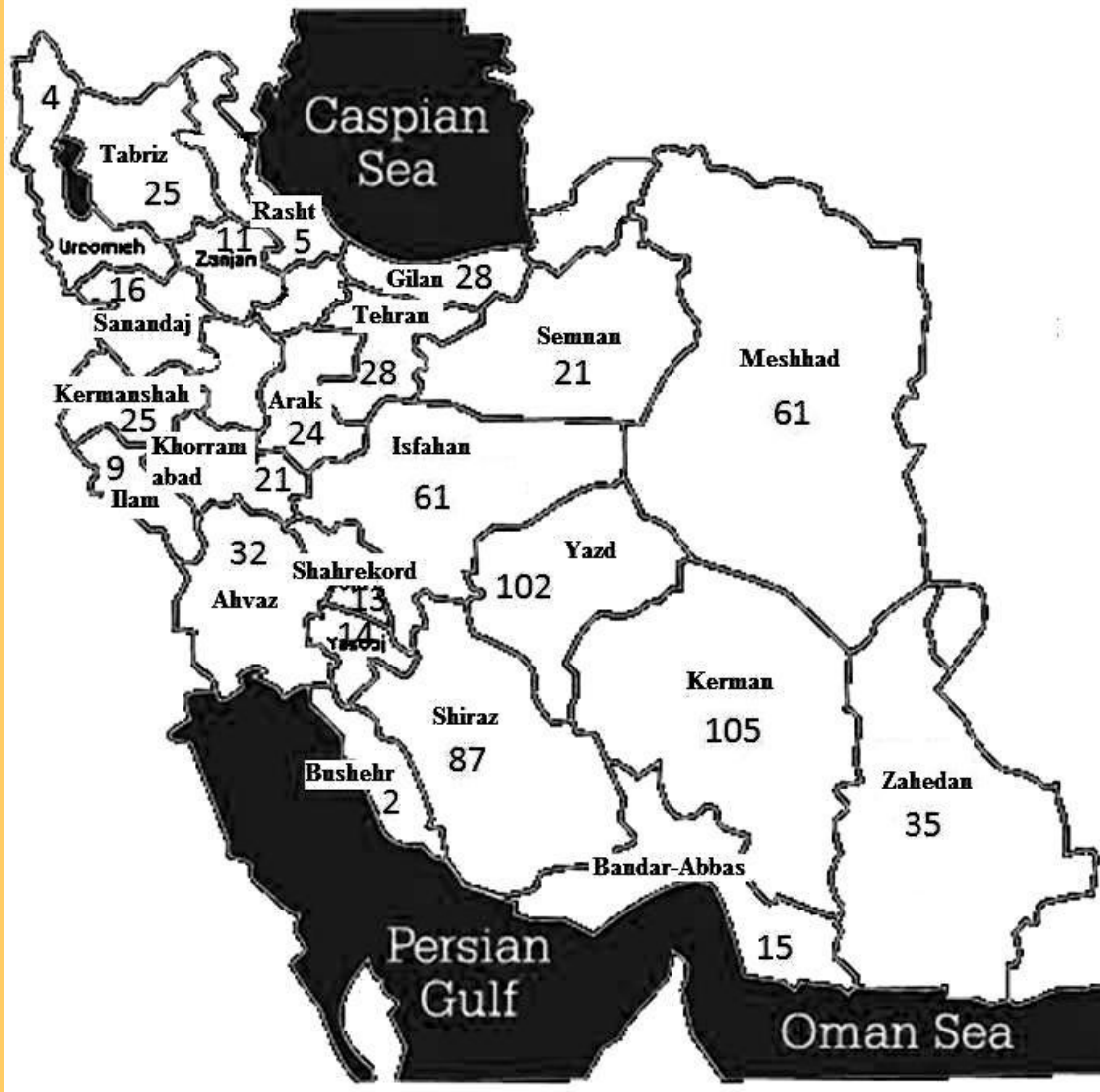
Legends to Figures 1-4.

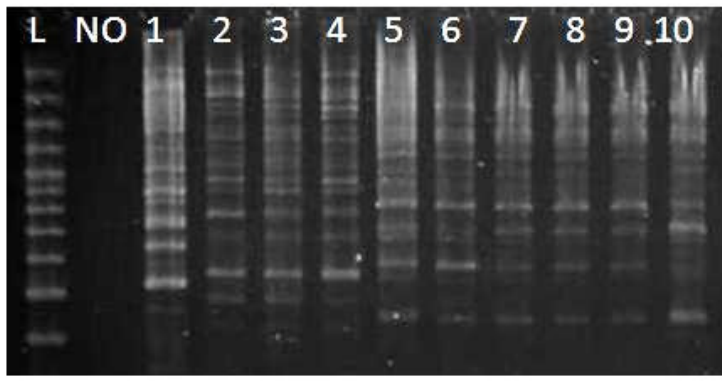
Figure 1. Geographical distribution of pomegranate cultivars in Iran. (Numbers indicate the numbers of cultivars cultivated in the region).

Figure 2. RAPD profile of pomegranate cultivars by primer OPB-07 (A), OPC-02 (B) and OPC-09 (C). Abbreviations: L = Molecular ladder, NO = No DNA, 1 = Bejestoni, 2 = Golnar, 3 = Shirin-poostghermez, 4 = Malas Yazdi, 5 = Abdandan, 6 = Shahidaneh, 7 = Suski, 8 = Toghmalas, 9 = Dokhtar-hamomi and 10 = Shahsavar.

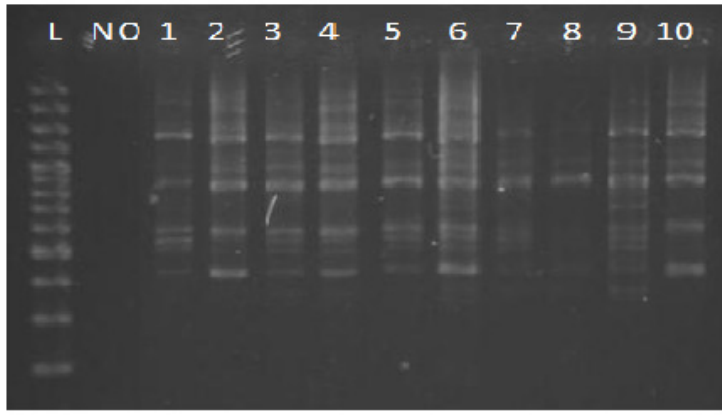
Figure 3. NJ tree of pomegranate cultivars based on RAPD markers. (Values above branches indicate bootstrap values).

Figure 4. Bayesian tree of pomegranate cultivars based on RAPD markers. (Values above branches indicate clade credibility values).

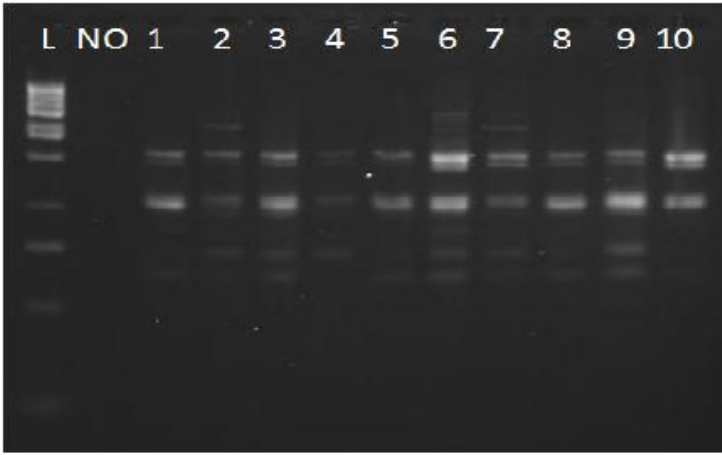




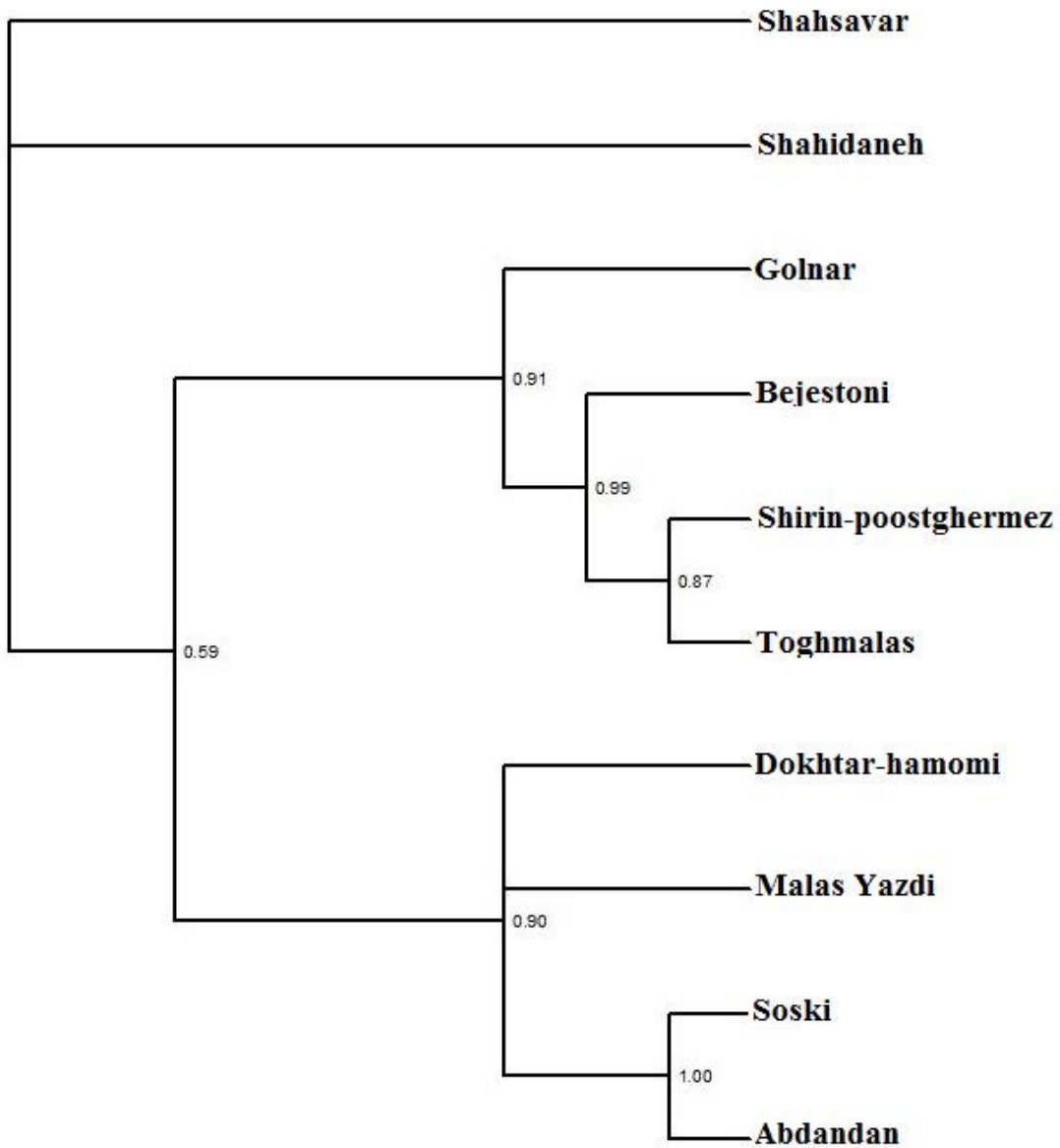
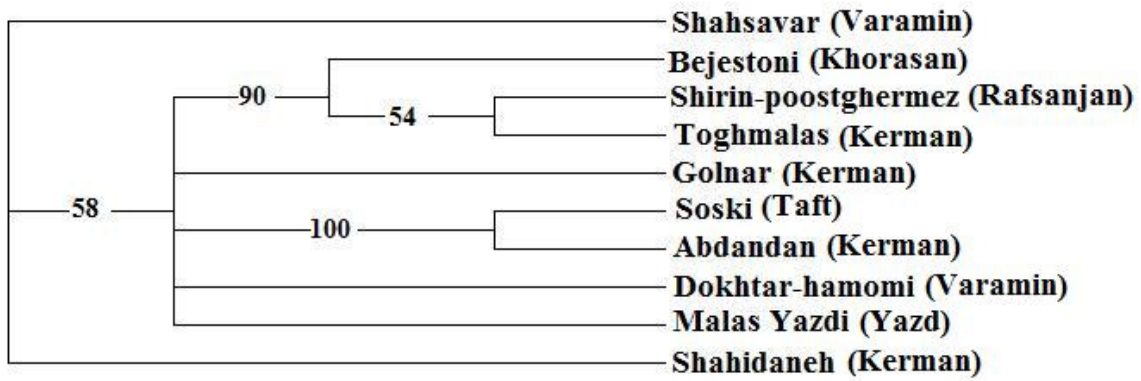
A



B



C



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