Volume 51(1):61-64, 2007
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

RAPD analysis of eleven iranian pomegranate *(Punica granatum* L.) cultivars

Masoud Sheidai[1]*, Z Noormohammadi[2], A Saneghi[1], ZH Shahreiyari[1]

[1]Faculty of Biology Sciences, Shahid Beheshti University, Tehran, Iran, [2]Tarbiat Modarres University, Tehran, Iran

ABSTRACT RAPD markers variations were studied in eleven pomegranate cultivars. Fifteen RAPD primers used out of which 13 primers could produce bands. In total 173 bands were produced out of which 73 bands were common in all the cultivars while 6 bands were specific, which may be used in the cultivars discrimination. Primers OPB12 and OPA13 produced the highest number of polymorphic bands (12 bands out of 16 = 0.75% and 11 bands out of 25 = 0.44), while primers OPR15 and OPA15 produced the least number of polymorphic bands (2 out of 12 = 0.16%). Different similarity coefficients determined among the cultivars studied, showed the highest value of similarity between cultivars Khatooni and Anbari as well as between Khatooni and Atabaki (r = 0.94) while the lowest value of similarity occurred between the cultivars Sefid and Bihaste as well as Sefid and Khatooni (r = 0.62). Different clustering methods showed distinctness ofthe olive cultivars studied. Acta Biol Szeged 51(1):61-64 (2007)

KEY WORDS clusterin9 ^ pomegranate RApD

The pomegranate *(Punica granatum* L.) is native from Iran to the Himalayas in northern India, cultivated over the whole Mediterranean region since ancient times (Facciola 1990) and is one of the most important endemic horticultural plants of Iran. About 550000 hectare of lands has been devoted to the cultivation of pomegranate in Iran producing about 570000 tones of pomegranate fruit.

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. In spite of grate economic importance of pomegranate cultivars in Iran, there have been very limited cytogenetic and genetic studies in them (Gill et al. 1981; Xue et al. 1992; Zhao and Pan 2004; Sheidai and Noormohammadi 2005; Sheidai et al. 2005; Sheidai et al. 2007; Zamani et al. 2007).

Different molecular markers including RAPD (Random Amplihed Polymorphic DNA) have been used in the study of genetic diversity as well as cultivar identihcation in several plant species (Weising et al. 2005). These molecular markers provide an opportunity for direct comparison and identihcation of different genetic material independent of any inhuences (Harvey and Botha 1996; Bautista et al. 2003).

Pomegranate cultivars are grown in different parts of Iran and cultivation of the same cultivars for long period of time may lead to the genetic erosion conhning the subsequent breeding programs. Therefore it is necessary to study the available diversity and introduce new variability as well. For this reason, the present study considers RAPD molecular analysis of 11 pomegranate cultivars of Iran for the first time.

Accepted August 27, 2007
Corresponding author. E-mail: msheidai@sbu.ac.ir

Materials and Methods

Eleven pomegranate cultivars were used for cytogenetic and molecular studies. For RAPD analysis, fresh leaves were selected (0.1 gr) randomly from 3-5 plants of each cultivar and DNA extraction was done by use of NucleoSpin Plant kit (Macherey-Nagel, Germany). The PCR reaction mixture consisted of 1 ng template DNA, 1 x PCR buffer (10 mM Tris-HC1 pH 8.8, 250 mM KC1), 200 pM dNTPs (dineuclotide triphosphosphate), 0.80 pM 10-base random primers (Operon Technology, California, USA) and 1 unit of Taq polymerase (Cinagene Co., Iran), in a total volume of 25 pi. 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 amplihcation 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 hnal incubation for 10 min at 72°C was performed to ensure that the primer extension reaction proceeded to completion. The PCR amplihed products were separated by electrophoresis on a 2% agarose gels (Merck) using 0.5 X TBE buffer (44.5 mM Tris/Borate, 0.5 mM EDTA, pH 8.0) or 12% polyacrylamide geles. The geles 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

Table 1. Specific RAPD primers in pomegranate cultivars.

Cultivars	OPB12-1 2	OPA15-12	OPA18-5	OPA13-8	OPA13-12	OPA13-20
1- Pustsiah Ardestan	0	0	0	0	0	0
2- Pustsefid Shomal	0	0	0	0	1	1
3- Anbari daneghermeze	0	0	1	1	0	0
4- Atabaki Jahrom	0	0	0	0	0	0
5- Meykhosh pustghermez	0	0	0	0	0	0
6- Bihaste Ardestan	1	1	0	0	0	0
7- Shirinnar Pave	0	0	0	0	0	0
8- Goltorsh Taft	0	0	0	0	0	0
9- Khatooni daneghermez	0	0	0	0	0	0
10- Dadashi Ashkezar	0	0	0	0	0	0
11- Pustsiah Ashkezar	0	0	0	0	0	0

of amplified products in base pairs. Fifteen random primers of Operon technology (Alameda, Canada) were used.

RAPD bands 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 different clustering methods and ordination based on principal coordinate analysis (PCO; Ingrouille 1986; Chatfield and Collins 1995). Cophenetic correlation was determined for different clustering methods. NTSYS Ver. 2.02 (1998) was used for clustering and PCO analyses.

Results and Discussion

The RAPD experiment was repeated 3 times and the well reproduced bands were scored for further analysis. The results of RAPD analysis of 11 pomegranate cultivars are presented in Tables 1-3 and Figs 1-3. Fifteen RAPD primers used out of

Table 2. The RAPD bands missing only in one pomegranate cultivars.

Cultivars	OPB12-11	OPB12-13	OPB07-3	OPA15-8	OPA18-2	OPA04-1	OPA04-3	OPA04-4	OPA04-5
1-Pustsiah Ardestan	1	1	1	1	1	1	1	1	1
2- Pustsefid Shomal	0	0	1	0	0	0	0	0	0
3- Anbari daneghermeze	1	1	1	1	1	1	1	1	1
4- Atabaki Jahrom	1	1	1	1	1	1	1	1	1
5- Meykhosh pustghermez	1	1	1	1	1	1	1	1	1
6- Bihaste Ardestan	1	1	1	1	1	1	1	1	1
7- Shirinnar Pave	1	1	1	1	1	1	1	1	1
8- Goltorsh Taft	1	1	1	1	1	1	1	1	1
9- Khatooni daneghermez	1	1	1	1	1	1	1	1	1
10- Dadashi Ashkezar	1	1	0	1	1	1	1	1	1
11- Pustsiah Ashkezar	1	1	1	1	1	1	1	1	1

Cultivars	OPA04-11	OPB05-6	OPB05-9	OPB05-1	OPB12-12	OPA15-12	OPA18-5	OPA13-8	OPA13-12	OPA13-20
1	1	1	1	1	0	0	0	0	0	0
2	0	1	1	1	0	0	0	0	1	1
3	1	1	1	1	0	0	1	1	0	0
4	1	1	1	1	0	0	0	0	0	0
5	1	0	0	0	0	0	0	0	0	0
6	1	1	1	1	1	1	0	0	0	0
7	1	1	1	1	0	0	0	0	0	0
8	1	1	1	1	0	0	0	0	0	0
9	1	1	1	1	0	0	0	0	0	0
10	1	1	1	1	0	0	0	0	0	0
11	1	1	1	1	0	0	0	0	0	0

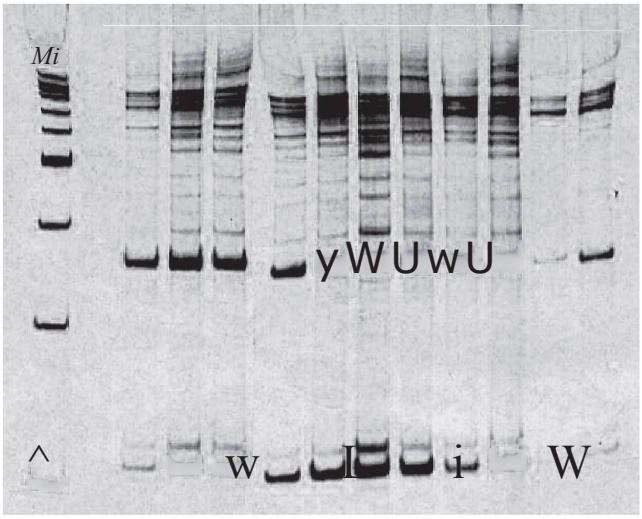

Figure 1. RAPD profile of pomegranate cultivars by primer OPR12. (Columns from right to left: pomegranate cultivars as in Table 1, No DNA and Molecular marker.

which 13 primers produced 173 bands in total. All 13 primers produced polymorphic bands. Seventy-three bands (loci) were present in all the cultivars studied and may be considered as the common bands in pomegranate cultivars. Six RAPD loci were specific in some of the cultivars which may be used in the cultivars discrimination. For example, OPA05-12 and OPB12-12 loci were present only in the cultivar Bihaste, while OPA13-8 and OPA18-5 loci were present only in the cultivar Anbari. The OPA-13-12 and OPA-13-20 loci were present only in the cultivar Sefid. The presence of specific loci indicates the genetic distinctness of the pomegranate cultivars studied.

Table 3. Jaccard similarity among pomegranate cultivars. (Cultivars number as in Table 1).

Cultivars	1	2	3	4	5	6	7	8	9	10	11
1	1.00										
2	0.75	1.00									
3	0.75	0.64	1.00								
4	0.76	0.66	0.94	1.00							
5	0.73	0.65	0.85	0.85	1.00						
6	0.72	0.62	0.87	0.89	0.84	1.00					
7	0.77	0.64	0.87	0.90	0.82	0.85	1.00				
8	0.72	0.67	0.88	0.89	0.82	0.84	0.91	1.00			
9	0.72	0.62	0.91	0.91	0.82	0.87	0.89	0.90	1.00		
10	0.70	0.66	0.82	0.83	0.78	0.80	0.80	0.86	0.84	1.00	
11	0.74	0.63	0.85	0.84	0.75	0.81	0.83	0.85	0.87	0.80	1.00

The primers OPB12 and OPA13 produced the highest number of polymorphic bands (12 bands out of 16 = 0.75% and 11 bands out of 25 = 0.44), while the primers OPR15 and OPA15 produced the least number of polymorphic bands (2 out of 12 = 0.16%).

There were 16 loci which were missing only in one cultivar; such loci may also be of use in the pomegranate cultivar differentiation. For example OPB07-3 loci was present in all pomegranate cultivars except the cultivar Bihaste, while the bands OPB05-1, 6 and 9 were absent only in the cultivar Meykhosh. The other 12 loci were absent only in the cultivar Sefid.

Different similarity coefficients determined among the cultivars studied, showed the highest value of similarity between cultivars Khatooni and Anbari as well as between Anbari and Atabaki (for example r = 0.94 in Jaccard similarity) while the lowest value of similarity occurred between the cultivars Pustsefid and Bihaste as well as Pustsefid and Khatooni (for example r = 0.62 in Jaccard similarity).

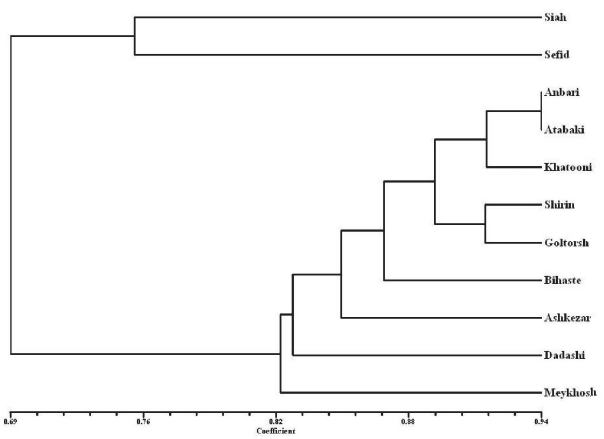

Figure 2. UPGMA clustering of pomegranate cultivars based on RAPD markers.

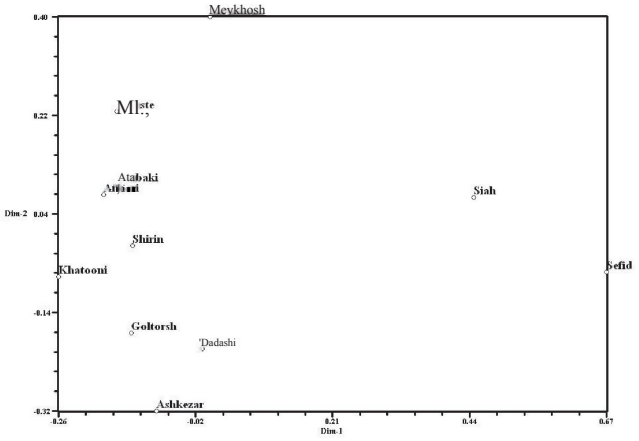

Figure 3. PCO ordination of pomegranate cultivars based on RAPD markers.

Different clustering methods showed distinctness of the olive cultivars studied, for example, UPGMA (unweighted paired group with arithmetic average), single linkage and complete linkage methods using the Jaccard similarity and Nei and Li coefficients produced similar results, supported by PCO ordination of the cultivars (Figs. 2 and 3). The cophenetic correlation determined showed the highest value for UPGMA method (r = 0.92), indicating a good ht of clustering to the original similarity of the cultivars. Therefore the result of UPGMA clustering along with PCO ordination is discussed bellow.

In general two major clusters or groups are formed (Figs. 2 and 3). The hrst major cluster is comprised of the cultivars Pustsiah and Pustsehd which are placed far from the other pomegranate cultivars. The second major cluster or group is comprised of the other cultivarsjoined each other with grate distances. Among these the cultivars Anbari and Atabaki show more similarity and along with Khatooni form the hrst subcluster, while Shirinnar and Goltorsh cultivars form the second sub-cluster,joined to the members of the hrst sub-cluster with some distance. The other cultivarsjoin this cluster step by step, out of which the cultivar Meykhosh differs the most andjoin with a grater distance to the other cultivars.

In general, the present study shows the usefulness of RAPD analysis in distinguishing the pomegranate cultivars, particularly identihcation of the specihc bands may be considered important in the pomegranate cultivar identihcation. 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.

References

Bautista R, C'anovas FM, Claros MG (2003) Genomic evidence for a repetitive nature of the RAPD polymorphisms in *Olea europaea* (olive-tree). Euphytica 130:185-190.

Chatfield C, Collins AJ (1995) Introduction to Multivariate Analysis. Chapman & Hall, London.

Facciola S (1990) Cornucopia: a source book of edible plants. Kampong Publications 166-167.

Gill B, Bir SS, Bediy S (1981) Cytological studies on woody Euphorbiaceae from North and Central India. New Botanist 8:35-44.

Harvey M, Botha FC (1996) Use of PCR-based methodologies for the determination of DNA diversity between *Saccharum* varieties. Euphytica 89:257-265.

Raman VS, Manimekaliai G, Sreerangaswamy SR (1971) Chromosome behavior at meiosis in *Punica granatum* L. Cytologia 36:400-404.

Sheidai M, Noormohammadi Z (2005) Chromosome pairing and unreduced gamete formation in nineteen pomegranate *(Punica granatum* L.) cultivars. Cytologia 70(3):257-265.

Sheidai M, Khandan M, Nasre-Esfahani Sh (2005) Cytogenetical study of some Iranian pomegranate *(Punica granatum* L.) cultivars. Caryologia 58(2):132-139.

Sheidai M, Khandan M, Nasre-Esfahani Sh (2007) B-chromosomes in Iranian pomegranate *(Punica granatum* L.) cultivars. Caryologia (in press).

Ingrouille MJ (1986) The construction of cluster webs in numerical taxonomic investigations. Taxon 35:541-545

Weising K, Nybom H, Wolf K, Kahl G (2005) DNA fingerprinting in plants. Principles, Methods, and Applications. Second Edit., Taylor and Francis, New York.

Xue BS, Weng RF, Z-Zhang M (1992) Chromosome numbers of Shanghai plants I. Investigatio et Studium Naturae 12:48-65.

Zamani Z, Sarkhosh A, Fatahi R, Ebadi A 2007. Genetic relationships among pomegranate genotypes studied by fruit characteristics and RAPD markers. J Hort Sci Biotech 82(1):11-18.

Zhao W, Pan Y (2004) Genetic diversity of genus moms revealed by RAPD Markers. Int J Agri Biol 6(6):950-956.