

RAPD analysis of genetic relatedness among selected quince (*Cydonia oblonga* Mill.) accessions from different parts of Turkey

S. BAYAZIT¹, B. IMRAK², A. KÜDEN², M. KEMAL GÜNGÖR³

¹Department of Horticulture, Faculty of Agriculture, University of Mustafa Kemal, Antakya, Hatay, Turkey

²Department of Horticulture, Faculty of Agriculture, University of Çukurova, Adana, Turkey

³Minister of Agriculture and Village Affairs (TAGEM), Ankara, Turkey

Abstract

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Quince (*Cydonia oblonga* Mill.) is a minor fruit crop, which is primarily used for marmalade, jam, sauce and as rootstocks for pears. Different cultivated and local quince genotypes are grown in almost all parts of Turkey for fruit usage. In this study, randomly amplified polymorphic DNA (RAPD) technology was used to study the genetic relationships among 13 quince accessions selected from different parts of Turkey. Thirty decamer primers were used and 14 of them did not produce any polymorphism. The remaining 16 primers ranged in their amplification fragments between one (P-402, P-437, OPA 10, OPA 16, OPA 18 and OPA-19) and five (OPA-06 and OPA-07). The size of fragments varied from 100 to 1500 bp. Similarity values among the studied genotypes ranged between 0.483 and 0.925. The resulting dendrogram clustered into two groups (0.69 similarity value) based on evaluation of genetic similarities and differences. The results suggest that RAPD analysis could be used to distinguish and determine genetic variation among quince accessions. Also, the obtained clustering based on RAPD markers agreed to some extent with the geographical origin of the studied set of quince accessions.

Keywords: quince; genetic resource; molecular markers; identification; DNA polymorphism

Quince (*Cydonia oblonga* Mill.) belongs to the *Maloideae* subfamily of the *Rosaceae* family, which includes commercially important fruits such as apples and pears. This subfamily comprises approximately 1,000 species in 30 genera and is characterized by a distinctive fruit, pome, and a base chromosome number of 17 (RODGER, CAMPBELL 2002). Quince was thought to have originated in Persia, Turkistan and the Caucasus. Its tree is used as rootstock for pear cultivars, while its fruit is used for making preserves, jam, marmalade, sauce and

juice (YAMAMOTO et al. 2004). In Turkey, quince has been cultivated for a long time and is one of the most important pome fruits, with the importance and production value increasing over recent years.

Turkey is one of the most important producers of quince with an annual production of nearly 96,282 metric tons (FAO 2009). The most desirable cultivars grown in Turkey are Limon, Ekmek and Eşme. Few breeders or researchers work with quince, and thus detailed descriptions of cultivars are rare in literature. Characteristics of fruit and

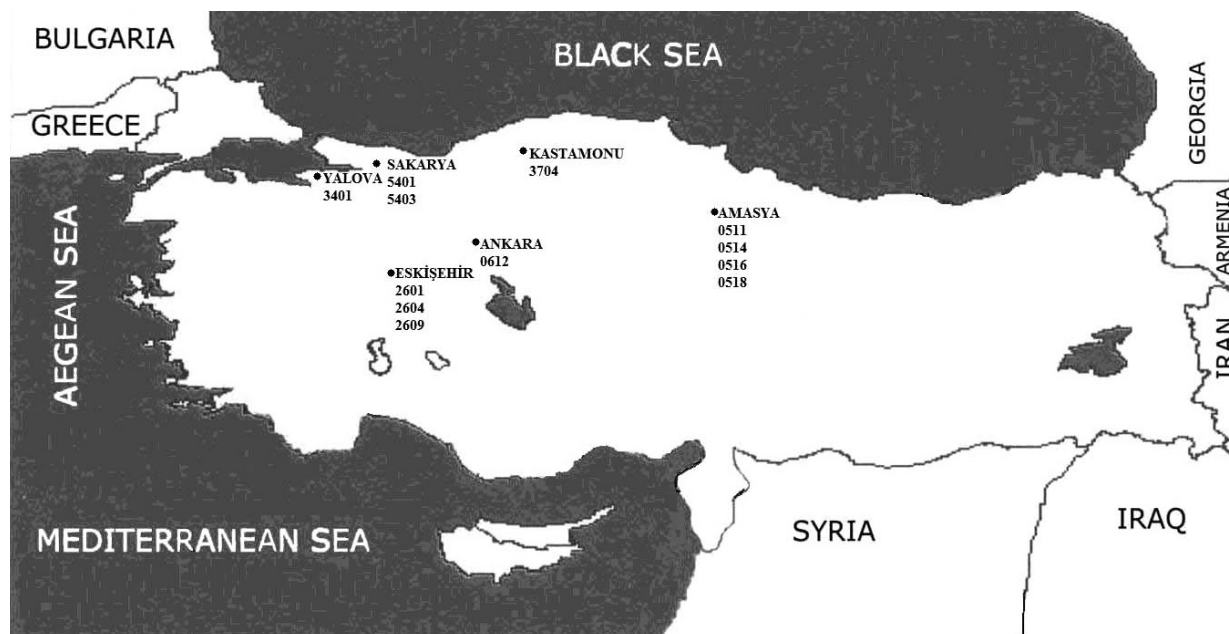


Fig. 1. Selected regions of 13 Turkish quince accessions sampled from different parts of Turkey

tree are so similar that it is very difficult to make a reliable classification. Differentiation of cultivars is possible based on the shape of fruit: apple-shaped (*Cydonia maliformis* Mill. Schneid.) and pear-shaped (*Cydonia pyriformis* Dierb.-F. Zinn) but the morphology of fruit could be influenced by the fruit set, the number of seeds and environmental factors (ÖZBEK 1978). All over the world, approximately 40–50 cultivars are propagated but only 10–15 of them are well-known internationally (SZABÓ 1998).

Turkey is very rich in local quince varieties; however, the frequent use of synonyms is a major problem in Turkish quince fruit production. There are many quince cultivars with similar morphological characters, which are cultivated in different locations under different names in Anatolia: an important deficiency for the region. One solution to the problem of the lack of differentiation of cultivars is creating genetic definitions for all the cultivars with molecular markers such as RAPD, amplified fragment length polymorphism (AFLP) or simple sequence repeats (SSR).

DNA-based molecular markers have become increasingly popular in the characterization and identification of genetic resources because they are not influenced by environmental factors and are more polymorphic. However, there are only three reports (YAMAMOTO et al. 2004; DUMANOĞLU et al. 2009; HALÁSZ et al. 2009) on the genetic study of quince cultivars using SSR markers and to our

knowledge, there are no reports on the genetic study of quince cultivars using RAPD markers. This suggests that these markers could be used in the genus *Cydonia*, where no significant work has been published in this field. Compared with other fruit trees, the identification and isolation of genes from *Cydonia* is very limited and in the last few years the genetic resource of this genus has been eroded through the loss of local cultivars, although some effort has been made in establishing collections, particularly in Turkey.

The RAPD technique is fast, cheap and easy, since it does not require knowledge of the sequences of the markers and can produce abundant polymorphic fragments. RAPD analysis is one of the techniques that was successfully used to reveal genetic variations of apple (KOLLER et al. 1993; MULCAHY et al. 1993; ZHU et al. 1997; ROYO, ITOIZ 2004; ADEBAYO et al. 2009; ERTÜRK, AKÇAY 2010) and pear (CHEVREAU, SKIRVIN 1992; YE et al. 1996; CHEVREAU et al. 1997; OLIVEIRA et al. 1999; SCHILIRO et al. 2001; LISEK, LOZPARA 2010).

The objective of this study was to identify the 13 selected quince accessions from different parts of Turkey. Since no molecular information is available regarding quince genotypes grown in Turkey, this study aimed at discriminating between and determining the genetic relationships between the most important quince genotypes selected from different parts of Turkey and grown at the Pozantı Agricultural Research Centre of Çukurova Univer-

Table 1. The accession number, cultivar name and selection regions of 13 Turkish quince accessions sampled from different parts of Turkey

Accession No.	Cultivar name	Selection region
3401	Altınayva	Atatürk Central Horticultural Research Institute, Garden Collection, Yalova
0511	Acem	Göllü Bağları Location, Central District, Amasya
0514	Limon	Gökhöyük Tigem* Farm, Amasya
0516	Tekeç	Gökhöyük Tigem Farm, Amasya
0518	İstanbul	Gökhöyük Tigem Farm, Amasya
0612	Kalecik	Emenağaç Location, Kalecik District, Ankara
1924	Osmancık	Unknown
2601	Çengelköy	Alpagut Village, Sarıcakaya District, Eskişehir
2604	Ekmek	Karaoğlan Village, Sarıcakaya District, Eskişehir
2609	Ekmek	Alpagut Village, Sarıcakaya District, Eskişehir
3704	Bardacık	Aşağı Çayır Village Taşköprü District, Kastamonu
5403	Eşme	Umurbey Village, Geyve District, Sakarya
5401	Eşme	Yukarı Dere Village, Central District, Sakarya

*Tigem, Ministry of Agriculture of the Republic of Turkey, General Directorate of Agricultural Enterprises

sity, Adana, Turkey using the RAPD technology. The information obtained from this work may be useful for better germplasm management, identification of accessions and also in avoiding duplications or mislabeling of the genotypes studied. The determination of genetic diversity and relationships between accessions, as well as the application of the data obtained for accessions identification, are also discussed.

MATERIAL AND METHODS

Plant material

Thirteen quince accessions selected from different parts of Turkey (Fig. 1) and grown at the Pozantı Agricultural Research Institute of University of Çukurova in Adana Province were studied for characterization. Leaf samples were taken from the accessions listed in Table 1, in 2007.

DNA extraction

DNA from fresh, newly expanded, healthy leaves was extracted following the protocol of DELLAPORTA et al. (1983). DNA concentration and purity were measured using a spectrophotometer Milton Roy Spectronik 1201 (Milton Roy Co., NY, USA).

An initial screening with 30 primers (10-mers) from the kits OPA, UBC and P from Operon Technologies Inc. (Huntsville, USA) was carried out on two accessions. Sixteen informative primers were selected due to their ability to produce polymorphic, unambiguous and stable RAPD markers.

RAPD analysis and gel electrophoresis

RAPD amplification was performed in a 25- μ l reaction volume containing 200 ng genomic DNA, 10 \times buffer (25mM MgCl₂, 2.5mM dNTPs, 200 ng primer (Operon Technologies Inc., Huntsville, USA), and 0.3 U Taq DNA polymerase (Promega, Madison, USA). The thermocycler was programmed as follows; initial cycle of 5 min at 94°C followed by 35 cycles of 30 s at 94°C, an annealing temperature of 30°C to 38°C for 1 min, elongation step of 1 min 45 s at 72°C, and a final extension step of 8 min at 72°C.

PCR products were separated by gel electrophoresis on 1.5% agarose gels with 1 \times TBE (Trisma base, boric acid, EDTA) buffer, at 100 V/cm for 4 h. The gel was stained with 0.25 μ g/ml ethidium bromide and photographed black and white on Polaroid type 665 film. RAPDs was often criticized for low reproducibility; in order to avoid this phenomenon we used highly constant conditions and all reactions were repeated at least twice (Kocsis et al. 2005).

Table 2. List of primers selected from Kit A, C, E (Operon Technologies Inc., Huntsville, USA) used to study the genetic relatedness of 13 Turkish quince accessions sampled from different parts of Turkey

Name	Sequence (5' to 3')	No. of bands	No. of polymorphic bands	Polymorphism (%)	Size (bp)(min–max)
UBC 238	CTCTCCAGCA	4	2	50.0	600–1,500
UBC 340	AGGGAGTTCC	5	2	40.0	200–1,000
P402	CCGCTTGACG	3	1	33.3	200–1,500
P437	CGGATCGACA	4	1	25.0	200–1,000
OPA 03	AGTGAGCCAC	6	3	50.0	300–1,500
OPA 05	AGGGGTCTTG	4	3	75.0	600–1,500
OPA 06	GGTCCCTGAC	5	5	100.0	400–1,500
OPA 07	GAAACGGGTG	6	5	83.3	200–1,400
OPA 09	GGGTAACGCC	4	2	50.0	200–1,500
OPA 10	GTGATCGCAG	4	1	25.0	200–900
OPA 11	CAATCGCCGT	6	4	66.6	200–1,000
OPA 16	AGCCAGCGAA	3	1	33.3	100–1,500
OPA 18	AGGTGACCGT	4	1	25.0	200–1,000
OPA 19	CAAACGTCGG	3	1	33.3	200–900
P 123	GGGATTTCGAC	4	3	75.0	300–1,000
P 166	GTGACGGACT	5	3	60.0	200–1,500
Total		70	38	54.2	

Data analysis

To ensure the absence of artifacts, bands were carefully selected from replicated amplifications. Amplified bands were designated by their primer code and their size in base pairs. Data were recorded as discrete variables: 1 for the presence and 0 for the absence of a similar band. Only intense and reproducible bands appearing on the gel were scored. Jaccard's coefficient was used to calculate the genetic distance (Dps) between cultivars. MVSP software v. 3.1 (Kovach Computing Services, Wales, UK) was used to calculate the similarity index. These indices were converted into a dendrogram by using the UPGMA cluster.

RESULT AND DISCUSSION

Thirteen selected accession were genotyped by RAPD markers. Sixteen informative primers were selected due to their ability to produce polymorphic RAPD markers.

A total of 70 bands were evaluated from the 16 primers used; 38 polymorphic bands were found and the level of DNA polymorphism established between quince accessions was 54.29%. From the primers OPA-06 and OPA-07, the maximum number of polymorphic bands (five) were produced. The lowest number of polymorphic bands (one) was obtained using OPA-10, OPA-16, OPA-18, OPA-19, P-402 and P-437 primers. When the ratios of polymorphic bands are examined on the basis of primers, the highest ratio (100%) was determined with OPA-06 and the lowest ratio (25%) was obtained with P-437, OPA-10 and OPA-18 (Table 2). According to THIS et al. (1997) some primers seem to be more efficient than others in producing stable and reproducible DNA fingerprints.

Primer selection is essential for discrimination analysis. Obviously, the more bands scored and plants studied, the higher the statistical significance of the calculation. About 100 bands should be enough to obtain statistically significant results (KOCIS et al. 2005), however, KOLLER et al. (1993) described 11 apples cultivars by using only two

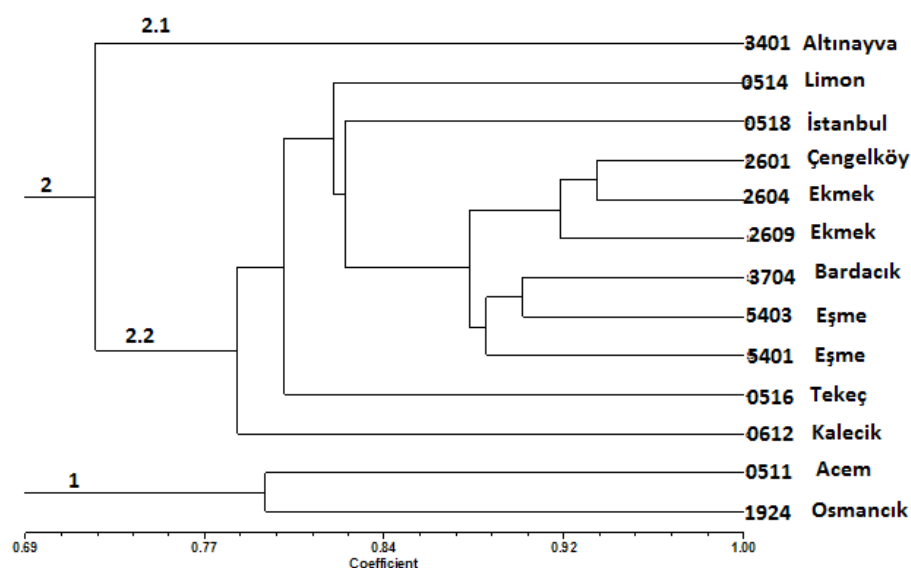


Fig. 2. UPGMA dendrogram of 13 quince accessions sampled from different parts of Turkey based on 16 random RAPD primers

RAPD primers. TANCRED et al. (1994) also used only two RAPD primers in order to distinguish a new apple genotype from three commercial apple cultivars. AUTIO et al. (1998) identified 15 apple rootstocks using only two RAPD primers and ORAGUZIE et al. (2001) described 155 new and old apple genotypes by using only nine RAPD primers.

When the dendrogram determining the rate of relativity between genotypes on the basis of coefficient of similarity was examined, it was observed that the similarity ratio among the quince accessions was high. The dendrogram generated by RAPD analysis showed two distinct groups. While the first group included only two genotypes (0511 and 1924), 11 quince accessions were in the second group (Fig. 2). The second group was divided into two subclusters, the first contained variety 3401 (selected from Altınayva population, Yalova Province of Marmara Region, Turkey). The second subcluster contained the related varieties: 2604 and 2609 which were 0.906 similar (both were clonal selections from cv. Ekmek quince populations, Eskişehir Province of Central Anatolia, Turkey), and 5403 and 5401 which were 0.885 similar (both were clonal selections from cv. Eşme quince populations, Sakarya Province of Marmara, Turkey). These findings suggest that the clustering based on RAPD markers agreed to some extent with the geographical origin of the studied set of quince accessions (Fig 2).

Several research groups working on different fruit trees such as plums (SHIMADA et al. 1999), pistachio

(HORMAZA et al. 1998), apple (LANDRY et al. 1994) and grapevine (VIDAL et al. 1999) reported agreements between RAPD data and morphological and agronomical criteria. In our study, origin of the genotypes mentioned in Table 1, has some agreement with the dendrogram. However, the similarity of the data obtained in this study agree, to some extent (at least for the same origin) with the geographical distribution of the tested genotypes. Cv. Ekmek varieties 2604 and 2609, cv. Eşme varieties 5403 and 5401 were originally selected from same population and same geographical region. The first two (cv. Ekmek varieties 2604 and 2609) and second two (cv. Eşme varieties 5403 and 5401) were very similar to each other (0.906 and 0.885) (Table 3).

YAMAMOTO et al. (2004) found that RAPD analysis was not polymorphic for quince cultivars whereas SSRs were more polymorphic for genetic characterization of quince cultivars. However, HALÁZS et al. (2009) reported that SSR markers used for identification of apple genotypes gave low allele number per locus in quince.

The RAPD method can solve one of the major problems of varietal identification in pome fruits: the existence of homonyms and synonyms, particularly with regard to varieties that were cultivated for centuries and are widely distributed (BORREGO et al. 2002). The RAPD technique is fast and easy to use, since it does not require knowledge of the sequences of the markers and can produce abundant polymorphic fragments. RAPD analysis is one of the

Table 3. Similarity coefficient among of 13 quince accessions sampled from different parts of Turkey and analyzed by 16 RAPD primers

	3401	0511	0514	0516	0518	0612	1924	2601	2604	2609	3704	5403	5401
Altınayva	1.00												
Acem	0.511	1.00											
Limon	0.661	0.483	1.00										
Tekeç	0.620	0.682	0.684	1.00									
İstanbul	0.607	0.537	0.810	0.755	1.00								
Kalecik	0.627	0.551	0.719	0.684	0.759	1.00							
Osmancık	0.660	0.690	0.632	0.723	0.636	0.627	1.00						
Çengelköy	0.698	0.537	0.842	0.788	0.855	0.759	0.698	1.00					
Ekmeç	0.698	0.566	0.842	0.755	0.821	0.759	0.731	0.925	1.00				
Ekmeç	0.679	0.577	0.825	0.769	0.836	0.774	0.712	0.906	0.906	1.00			
Bardacık	0.686	0.580	0.772	0.816	0.815	0.784	0.686	0.885	0.849	0.902	1.00		
Eşme	0.667	0.566	0.780	0.788	0.759	0.727	0.667	0.855	0.821	0.870	885	1.00	
Eşme	0.720	0.580	0.772	0.745	0.750	0.750	0.720	0.849	0.815	0.902	843	0.885	1.00

techniques that was used successfully to reveal genetic variations in many studies (KOCŞİS et al. 2005; LIŞEK et al. 2006; KOC et al. 2009). Notwithstanding the limitations, RAPD markers proven to be a highly effective and efficient method for the genetic analysis (LUO et al. 2002; ULANOVSKY et al. 2002). Large numbers of data sets can be generated because different RAPD primers are commercially available (FANIZZA et al. 2000). Our results confirmed that RAPD analysis can be used to differentiate cultivars from obtained quince material like SSR analysis previously reported by YANG and SCHMIDT (1994). Our results confirmed the potential of RAPD technology as a reliable, rapid and inexpensive screening method to discriminate quince genotypes.

CONCLUSION

Quince has been cultivated in Turkey for a long time, possibly for many centuries. It is grown at present in each of the nine agricultural regions. Quinces are least frequently grown in the south eastern and northeastern regions and most widespread in the north and west of our country in the Aegean, Marmara and Central Northern agricultural regions. These regions are very important for quince production. Different quince cultivars and local genotypes are grown in these regions. It can be generalized that genotypes selected from these regions most likely represent the majority of genotypes cultivated or local varieties in Turkey. KÜDEN et al. (2006) reported the pomological characterization of these quince genotypes but included no information about molecular characterization. These results indicate that RAPD is useful for analysing genetic variation and geographic distribution of quince accessions. Consequently our studies were very informative molecular characterization of quinces genotypes of Turkey. The addition of the novel molecular data to previously made pomological analyses completed the characterization of our Turkish quince advance selection collection. The genotypes from this collection will be used in the forthcoming cross breeding studies. The characterization results will be of great importance on the selection of the parents and the combinations.

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Corresponding author:

Dr. SAFDER BAYAZIT, University of Mustafa Kemal, Faculty of Agriculture, Department of Horticulture, 31034 Antakya, Hatay, Turkey
phone: + 90 326 245 5845/1043, fax: + 90 326 245 5832, e-mail: sbayazit30@gmail.com, sbayazit@mku.edu.tr
