Identification of apricot cultivars in Turkey (*Prunus armeniaca* L.) using RAPD markers

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Abstract

This study was carried out to determine genetic relationships among 23 apricot cultivars from Turkey by using randomly amplified polymorphic DNA (RAPD) technology. Among the 40 decamer random primers were used for PCR reactions, 12 primers showed reproducible polymorphic patterns. These primers produced 121 bands, out of which 118 were polymorphic. UPGMA (Unweighted Pair Group Method using Arithmetic average) derived dendrogram, grouped the cultivars into one cluster and one outlier. The main cluster further divided into three subclusters. The highest dissimilarities found between Hacihaliloglu and Kabaasi cultivars (0.108). The variation detected among the closely related genotypes indicates the efficiency of RAPD markers over the morphological and isozyme markers for the identification and construction of genetic linkage maps.

Keywords: *Prunus armeniaca* L.; Cluster analysis; Genetic diversity; RAPD

Introduction

Apricot is cultivated in warm temperate to subtropical regions of all continents in the world. It has been cultivated in China since 2000 BC. During the long periods of cultivation, it moved westwards via Middle and Minor Asia, and arrived in Greece in 300 BC [1]. The gene pool of apricot contains species and varieties with a wide range of adaptation from the cold winters of Siberia to the subtropical climate of North Africa and from the deserts of Central Asia to the humid areas of Japan and eastern China [2]. Although Turkey is not the place of origin of apricot, it has become an important place of world's apricot production. Turkey leads the world apricot production with an average year production of 500.000 tons depending on the influences of late spring frosts, it fluctuates from 18 to 23% of the world's total apricot production [3,4]. Since ancient times, apricot has been cultivated in Inner Anatolia e.g. Malatya, Erzincan, Igdir, for its edible fruit [1]. Malatya is the main apricot center in Turkey followed by Erzincan plain, Igdir and Aegean region [3].

Apricots appear to be unique among the tree fruits in cultivar adaptability; a given cultivar has a very limited area of adaptation. Each apricot growing region in Turkey has different ecological conditions and in each are one or two cultivars adapted. For instance cv. Hacihaliloglu is the cultivar grown in Malatya, cv. Hasanbey in Erzincan and cv. Salak in Igdir [3]. The production relying on one or two major cultivars in each production area is partly responsible for large fluctuations in yield and also makes apricot vulnerable to adverse environmental conditions, diseases, and pests.

The existence of a very large number of rootstocks, cultivars and clones, maintained by vegetative propagation, reinforces the need for a reliable verification system to identity

them properly by the breeders, nurserymen and also the growers. This represents a very important aspect in the fruit industry, as initial planting and establishment orchards incur huge investments of time and money [5]. Accurate identification of varieties and genotypes is also essential for patent protection of these materials. Traditionally, identification of fruit trees including apricot was based on morphological or physiological aspects [6,7]. Biochemical markers like isoenzymes have been used but they have many disadvantages like limited number of polymorphisms detected between close cultivars and variations due to the physiological stage [6]. Hence stable and reproducible marker systems are to be used in apricot for their proper identification. Randomly amplified polymorphic DNA (RAPD) markers used in apricot before [8,9] have proven to be a reliable marker system for genetic fingerprinting and also to determine the genetic relationships among germplasm collections. RAPD markers have the advantages of simplicity and the ability to detect relatively small amounts of genetic variation and also need no prior information on the genome. However, RAPDs do not give information about the genome. The technique has already been successfully applied to estimate genetic relationships in apricot [8], mulberry [10], grape [11] or figs [12].

Since no basic information on the genome of apricot cultivars present in Turkey is available and RAPD technique provides an effective technique of assessment genetic diversity among closely related cultivars, we have selected this marker system to assess the genetic diversity among the 23 main apricot cultivars. Therefore the aim of this study was to characterize and estimate genetic diversity in 23 main apricot cultivars from Turkey. Although this study deals with in Turkey, this data provides a scientific basis for future selection and germplasm management.

Materials and Methods

Plant Material and Sample Collection

Fruit properties were determined randomly selected 30 fruits at harvest time. The leaf samples from total 23 apricot (*Prunus armeniaca* L.) cultivars were collected in Malatya Fruit Research Institute. Each cultivar had cultivar identification label on tree. Seventh leaves from the top of 90 day old primary branches of 23 cultivars were collected separately and stored immediately at –80 ^{0}C for DNA extraction.

DNA extraction

Genomic DNA was extracted from powdered (grounded with liquid nitrogen) plant materials using a modified method described by Lin et al., [13]. Approximately 10-15 mg tissue samples from each plant species were snap frozen in liquid nitrogen in 2 ml Eppendorf tubes. 1000 μl DNA extraction buffer [100mM Tris-HCl (pH 8.0); 50mM EDTA (pH 8.0); 500mM NaCl; 2% SDS (w/v); 2% 2-mercaptoethanol (v/v); 1% PVP (w/v)] was added and mixed well. The mixture was incubated at 65°C in a water bath for 40 min with intermittent shaking at 5 minute intervals. The mixture was centrifuged at 12 000 g for 15 min at 4°C, the supernatant was transferred into a new 1.5 ml tube and mixed with equal volume of phenol:chloroform:isoamylalchol (25:24:1), and centrifuged. The supernatant was collected and mixed with 1/10 volume 10% CTAB-0.7M NaCl in a new tube. After centrifugation, the supernatant was collected and equal volume of chloroform:isoamylalchol (24:1) was added and mixed gently. The DNA was precipitated by the addition of 0.6 volume of freezer-cold isopropanol, left at -20°C for 10min. The DNA was pelleted by centrifugation (12 000xg,10min) and the isopropanol was poured off; the DNA was allowed to air-dry before being dissolved in 100 μl of TE buffer.

PCR amplification with RAPD primers

Samples were screened for RAPD variation using standard 10-base primers supplied by Operon. A 30μl of reaction cocktail was prepared as follows: 10x Buffer 3.0 μl, dNTPs 10mM, magnesium chloride 25mM, primer 5μM, *Taq* polymerase (5unit) 0.4 μl, water 19.2 μl, sample DNA 100ng/μl. Total 34 RAPD primers were tested in this study and polymorphism obtained primers were shown in Table 2.

The thermolcycler (Eppendorf Company) was programmed as follows: 2 min at 95°C; 2cycles of 30 sec. at 95°C, 1 minute at 37°C, 2 minute at 72°C; 2 cycles of 30 sec. at 95°C, 1 minute at 35°C, 2 minute at 72°C; 41 cycles of 30 sec at 94°C, 1 minute at 35°C, 2 minute at 72°C; followed by a final 5 minute extension at 72°C then brought down to 4°C.

All primers tested using all cultivars and markers were checked two times for reproducibility.

Electrophoresis

The PCR products (27 μl) were mixed with 6x gel loading buffer (3 μl) and loaded onto an agarose (1.5% w/v) gel electrophoresis in 0.5XTBE (Tris-Borate- EDTA) buffer at 70 V for 150 min. The gel was stained in ethidium bromide solution (2 μl Etbr/100ml 1xTBE buffer) for 40 min and visualized under UV in Bio Doc Image Analysis System with Uvisoft analysis package (Cambrige, UK).

Data Analysis

The positions of scorable RAPD bands were transformed into a binary character matrix ('1' for the presence and '0' for the absence of a band at a particular position), which was entered in the RAPD distance computer program [14]. These data were used for calculation of pairwise genetic distances among cultivars using the Jaccard coefficient [11]. (The computer program calculated the degree of genetic similarity between each pair of the 23 cultivars using the simple equation: JC =1-a/(a+b+c), where „a" is the number of bands shared by plant „x" and plant „y", „b" is the number of bands in plant „x", and „c" is a number of bands in plant „y". The Jaccard coefficient ignores absence matches. The distance matrix was used for cluster analysis using the unweighted pair-group method with arithmetic averages (UPGMA).

Results and Discussion

Results of RAPD analysis are summarized in Table 2, 3 and Fig 1 and 2. A total of 40 decamer oligonucleotide primers were used to investigate twenty-three apricot cultivars all belongs to *Prunus armeniaca* L. But 28 primers did not produce any polymorphic bands or did not amplify clear products. In total, 12 primers which produced good and reproducible polymorphic bands among the 23 apricot cultivars were used for further analysis.
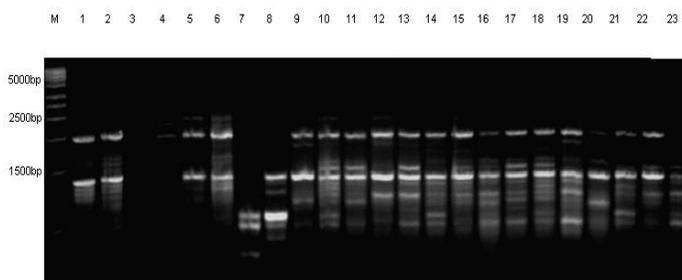

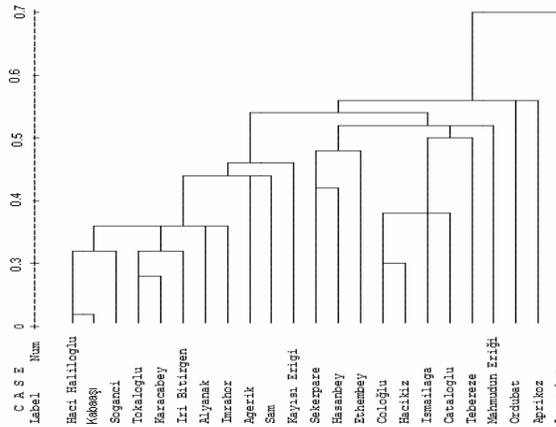**Fig. 1**. Identification of 23 *Prunus armeniaca* cultivars with primer OPH-18

Fig. 2. UPGMA dendrogram of 23 apricot cultivars based on 12 random RAPD primers

Table 1. Morphological features of apricot cultivars used in this study

Nr.	Cultivars	Growing Region	Fruit Weight (g)	Skin Color	Fruit Shape	Kernel Taste	Flesh Color
1	Haci Haliloglu	Malatya	34.60±1.45	Orange	Round	Sweet	Yellow
2	Kabaasi	Malatya	36.14±2.34	Orange	Round	Sweet	Yellow
3	Soganci	Malatya	38.99±1.65	Yellow	Round	Sweet	Yellow
4	Ethembey	Malatya	35.31±3.13	Orange	Cylindiric	Bitter	Orange
5	Cologlu	Malatya	32.33±2.98	Yellow	Round	Sweet	Yellow
6	Ismailaga	Malatya	46.62±4.33	Yellow	Oblong	Sweet	Yellow
7	Cataloglu	Malatya	30.73±2.59	Yellow	Round	Sweet	Yellow
8	Hacikiz	Malatya	33.47±3.11	Yellowish	Oval	Sweet	Cream
9	Tokaloglu-Izmir	Aegean	36.61±4.68	Orange	Oval	Bitter	Orange
10	Karacabey	Aegean	43.98±3.65	Orange	Cylindiric	Bitter	Orange
11	Iri Bitirgen	Aegean	40.41±4.44	Yellow	Oval	Sweet	Yellow
12	Alyanak	Aegean	46.57±5.35	Orange	Oval	Bitter	Orange
13	Imrahor	Aegean	38.40±3.76	Orange	Round	Bitter	Yellow
14	Sam	Aegean	31.00±3.21	Orange	Round	Bitter	Orange
15	Kayisi Erigi	Erzincan	30.12±4.23	Yellow	Round	Bitter	Yellow
16	Sekerpare	Erzincan	25.11±1.78	Cream	Round	Sweet	Yellow
17	Hasanbey	Erzincan	43.62±6.65	Yellow	Cylindiric	Sweet	Yellow
18	Mahmudun Erigi	Erzincan	42.81±5.65	Red	Round	Sweet	Yellow
19	Agerik	Igdir	41.03±4.27	White	Round	Sweet	White
20	Salak (Aprikoz)	Igdir	60.33±5.87	Yellow	Cylindiric	Sweet	Yellow
21	Tebereze	Igdir	39.00±4.33	Orange	Round	Sweet	Yellow
22	Ordubat	Igdir	25.33±1.51	Orange	Cylindiric	Sweet	Yellow
23	Agcenabat	Igdir	53.69±4.21	Cream	Round	Bitter	Yellow

Table 2. List of the selected primers and the degree description of the polymorphism obtained among 23 apricot cultivars, percentage of polymorphic markers (P) for each primer

Primer code	Sequence 5'→3'	Size (bp) Min-max	Polymorphic Bands	Monomorphic Bands	Percentage of Polymorphic markers
OPA- 1	CAGGCCCTTC	1300- 2500	10	0	100
OPA- 2	TGCCGAGCTG	1600- 2800	9	0	100
OPA- 4	AATCGGGCTG	1000- 3000	15	0	100
OPA- 13	CAGCACCCAC	1200- 2800	6	3	66,7
OPH- 14	ACCAGGTTGG	500- 3000	12	0	100
OPH- 17	CACTCTCCTC	1500- 2000	5	0	100
OPH- 18	GAATCGGCCA	1400- 3000	13	0	100
OPW- 11	CTGATGCGTG	1500- 2700	11	0	100
OPW- 13	CACAGCGACA	1600- 2800	6	0	100
OPW- 17	GTCCTGGGTT	1200- 2200	12	0	100
OPW- 18	TTCAGGGCAC	1200- 3000	9	0	100
OPW- 20	TGTGGCAGCA	1500- 3000	10	0	100
Total		500- 3000	118	3	97.5

The high number polymorphic markers detected in this study could be result of high diversity among the material used. It is previously showed that there is high diversity among apricot cultivars by using AFLP markers [15]. However, Hormaza [16] reported not so high diversity among apricot cultivars by using SSR markers. RAPDs and AFLPs are anonymous dominant markers and SSRs are genetic co-dominant markers. The differences could be result of nature of material because different materials used in both studies as well. On the other hand the fact that apricots are less cultivated than, for instances, peaches may explain higher diversity. The size of the amplified fragments ranged from 500 to 5000 bp. Each primer generated from 5 to 13 RAPD bands. OPA-1, OPA-2, OPA-4, OPA-13, OPH-14, OPH-17, OPH-18, OPW-11, OPW-13, OPW-17, OPW-18, OPW-20 produced 10, 9, 15, 6, 12, 5, 13, 11, 6, 12, 9, and 10 polymorphic bands, respectively. In total 97,5% of the bands were polymorphic.

The genetic distance matrix (Table 3) showed that the highest genetic distance was between cv. Iri Bitirgen and cv. Agcenabat (0.713) and the least was between cv. Hacihaliloglu and cv. Kabaasi (0.108). This indicates that the cv. Hacihaliloglu and cv. Kabaasi are genetically closer than the other cultivars and cv. Kabaasi could be bud mutation of traditional cv. Hacihaliloglu. In a previous study, it was found that cv. Hacihaliloglu and cv. Kabaasi have similar better agronomical characters for example it bears more quality fruit than the other cultivars in Malatya region [3].

Table 3. Phenetic distance matrix among of 23 apricot cultivars based on 12 RAPDs markers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1	-																						
2	.108	-																					
3	.323	.381	-																				
4	.387	.473	.423	-																			
5	.346	.440	.502	.289	-																		
6	.365	.337	.433	.405	.429	-																	
7	.407	.395	.391	.396	.471	.501	-																
8	.503	.390	.437	.400	.366	.461	.378	-															
9	.485	.402	.531	.423	.491	.508	.411	.503	-														
10	.447	.473	.547	.404	.450	.399	.407	.614	.422	-													
11	.533	.437	.607	.503	.504	.512	.540	.399	.447	.361	-												
12	.607	.537	.581	.531	.561	.486	.503	.628	.519	.606	.426	-											
13	.594	.471	.496	.476	.466	.407	.390	.614	.504	.483	.440	.483	-										
14	.643	.581	.631	.493	.502	.467	.607	.507	.430	.544	.516	.507	.416	-									
15	.535	.483	.400	.391	.421	.509	.442	.547	.623	.518	.487	.422	.561	.288	-								
16	.447	.391	.428	.422	.602	.444	.426	.364	.487	.460	.536	.504	.480	.440	.502	-							
17	.637	.567	.569	.420	.621	.397	.501	.418	.541	.500	.604	.486	.539	.396	.391	.370	-						
18	.541	.427	.609	.516	.418	.418	.471	.510	.470	.480	.581	.607	.411	.447	.402	.509	.502	-					
19	.525	.561	.581	.580	.518	.563	.567	.491	.641	.561	.602	.384	.503	.528	.369	.503	.626	.447	.509	-			
20	.601	.510	.540	.570	.606	.589	.621	.486	.510	.447	.547	.506	.480	.542	.602	.694	.368	.581	.462	-			
21	.627	.610	.613	.611	.588	.624	.576	.511	.422	.510	.610	.607	.514	.600	.609	.536	.541	.436	.388	.608	-		
22	.661	.570	.680	.596	.610	.594	.489	.506	.540	.620	.447	.605	.540	.580	.627	.480	.486	.384	.503	.491	.572	-	
23	.687	.593	.692	.581	.701	**.713**	.622	.588	.571	.664	.588	.546	.622	.524	.633	.629	.567	.526	.476	.549	.637	.487	-

The average genetic distance of 0.596 among the cultivars clearly shows that significant genetic diversity exists among the apricot cultivars. Hence, these cultivars are to be preserved as valuable genetic resources for breeding. The high genetic diversity present among these cultivars clearly suggests that they must have originated from genetically divergent parents or have a long history of adaptation to their respective micro-climatic regions.

Earlier studies using RAPD [8], SSR [9] and AFLP [18] techniques showed large genetic variations present among different apricot cultivars in Italy and Spain.

The dendrogram obtained from the RAPD markers grouped the 23 cultivars into one cluster and one outgroup. The main cluster further divided into 3 subclusters visible

representing 4 major apricot production regions in Turkey. Similarity rate varied among groups of region; however dissimilarity rate was very low within the groups indicating relatively homogenous cultivar properties which agree with the fact that these cultivars have poor adaptability to other regions. Our findings also support the fact that apricot cultivars are severely restricted in their ecological adaptation [2].

In general, cultivars from same ecological region showed similar profiles (Figure 2). However, it could be observed that many of the cultivars identified as genetically closer as cv. Hacihaliloglu and cv. Soganci have different morphological characters such as fruit weight and fruit color, cv. Tokaloglu and cv. Karacabey have different fruit shape, and cvs. Sam and Agerik had different flesh color (Table 1).

This could be due to accumulation of somatic mutation during the vegetative propagations. Guleryuz et al. [3] reported that among the fruit characteristics used for grouping of apricot, those related to fruit size, skin and flesh color, kernel taste and juice parameters have more consistency and, thus, discriminating values. They could detect homoenymes in some cases, however, they suggested that use of other biochemical and molecular markers such as RAPD could supply complementary useful information.

Differences in results obtained from grouping with molecular markers and grouping with phenotaxonomical characters were also reported in apricot [15,19,20] and the other fruit species such as mulberry [7] and pomegranate [21].

This disagreement between dendrograms from molecular and morphological data could be attributed to a number of reasons; one is the effects of different climatic conditions on morphological traits, which do not influence RAPD markers [22,23]. It is known that some mutational changes could easily expressed phenotypically such as fruit color and shape but that may not affect the primer binding sites. Garcia et al. [24] reported that tree size, shape and branching habit may not be detectable by application of molecular markers. It should be also noted that post-transcriptional modifications and non-nuclear inheritance of some characteristics can cause the lack of fitting of morphological markers with molecular markers [21,23].

As a conclusion, results of this study indicate that in Turkey the level of polymorphism among apricot cultivars is appreciably high and these cultivars can be used in breeding programs aimed at developing region specific apricot cultivars.

This is the first attempt to use molecular markers to investigate the genetic relationships of apricot cultivars grown under different temperate agro ecological conditions in Turkey and the information generated in this study is of much use in the improvement of apricot through breeding in Turkey, which is needed now to sustain apricot cultivation for the higher fruit production.

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