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Characterization of new apricot cultivars by RAPD markers

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Abstract

Molecular markers are the most widely used tools in cultivar and species identification. The objective of this study was to characterize some Turkish and European cultivars and new apricot cultivars derived by hybridization between Turkish and European apricot cultivars using RAPD markers. Five new, two local cultivars, and four promising hybrids from Turkey, and 13 cultivars from Europe, North America, South Africa were characterized. Sixty RAPD primers produced 57 polymorphic and 79 monomorphic markers, totaling 136. All the 136 markers were used to construct a dendogram based on UPGMA. All cultivars were distinguished from each other with the similarity value ranging from 0.90 to 0.96. Known hybrids were grouped between or close to either one of parental genotypes. This study may imply narrow genetic diversity among the most widely grown apricot cultivars in the world.

Key words: Apricot, *Prunus armeniaca,* RAPD, molecular markers

Introduction

The apricot is a fruit tree originated in Central Asia and China. It was introduced into Europe through Greece (400 BC), and also later (100 BC) by the Romans. It is accepted that there are four different species and one naturally occurring interspecific hybrid under the generic term of apricot. These are: *P. armeniaca*, the cultivated apricot; *P. sibirica* L., the Siberian apricot; *P. mandshurica* (Maxim.) Koehne, the Manchurian apricot; *P. mume* (Sieb.) Sieb. et Zucc., the Japanese apricot; and *Prunus* x *dasaycarpa* Ehrh., the black or purple apricot. All diploid species include eight pairs of chromosomes (2n=16). The most cultivated apricots belong to the species *P. armeniaca* (Hormaza, 2002).

In *P. armeniaca,* six eco- geographical groups have been proposed, the European group being the most recent and also the least variable of all. This group is characterized by being mostly self-compatible, with a relatively short dormant period, early budbreak and low vigour trees. Genotypes of this group are also grown in North America, Australia and South Africa (De Vicente, 1998). Despite its lower variability, most of the progress in apricot breeding has been carried out through hybridization and selection within the European group (Hormaza, 2002).

Traditional methods to characterize and identify cultivars and rootstocks in fruit tree species are based on phenotypic observations, but this approach is slow and subject to environmental modifications (Staub and Serquen, 1996). Consequently, new methods based on studies at the DNA level must be incorporated into fruit breeding programme to accelerate and optimize genotype fingerprinting and study genetic relationships among cultivars (Hormaza, 2002). For example, De Vicente *et al.* (1998), determined genetic variability in apricot using RFLP analysis. The RAPD procedure is a fast, sensitive method that avoids use of radioactive isotopes and is well suited for studies of many samples. Therefore, RAPD analysis can be used to identify polymorphisms. A number of apple, red raspberry and rose cultivars have been fingerprinted recently using RAPD markers (Lu *et al.*, 1996). Besides, RAPD markers have been used to identify genetic variability in *Prunus* species. Peach rootstock cultivars (Lu *et al.*, 1996), peach (Warburton *et al.*, 1996; Raddova *et al.*, 2003), almond (Bartolozzı *et al.*, 1998; Mirali and Nabulsı, 2003), cherry (Hormaza, 1999; Aka-Kacar and Cetiner, 2001), plum (Heinkel *et al.*, 2000) and apricot (Zhebentyayeva and Sivolap, 2000; Hormaza, 2001; Mariniello *et al.*, 2002) have been identified by RAPD markers. On the other hand, Simple Sequence Repeats (SSR) and Amplified Fragment Length Polymorphism (AFLP) markers have also been used for molecular characterization of *Prunus* species. SSR markers have been used in determination of molecular diversity in apricot (Hormaza, 2001; Hormaza, 2002; Zhebentyayeva *et al*., 2003), *Prunus* rootstock (Serrano *et al.*, 2002), peach (Sosinski *et al.*, 2000), peach and almond (Martinez-Gomez *et al.*, 2003) and genetic diversity in apricot (Hagen *et al.*, 2002; Salava *et al.*, 2002) was revealed by AFLP markers.

Total world apricot production has reached 2.68 million tons. Turkey account 16% of that production (Faostat, 2004). In Turkey, new apricot cultivars for fresh consumption have been developed by hybridization. The objective of this study was to characterize some Turkish and European apricot cultivars and new apricot cultivars derived through hybridization between Turkish and European apricot cultivars using RAPD markers.

Materials and methods

Plant materials: A total of 24 genotypes were used in this study. Plant materials were obtained from Alata Horticultural Research Institute located in Mersin, Turkey. The twenty-four genotypes included 5 new cultivars, 2 local cultivars, 13 European cultivars, and 4 new promising hybrid genotypes (Table 1). Hybrid genotypes have desirable traits such as early maturing and coloring. These all cultivars and genotypes are

Table 1. List of the 24 apricot cultivars used in the study and their geographic or genetic origin

Cultivars	Origin	Parents
Errani	Italy	Chance seedling
Bebeco	Greece	Chance seedling
Bulida	Spain	Chance seedling
Canino	Spain	Chance seedling
Feriana	France	Hamidi x Canino
Fracasso	Italy	Chance seedling
Priana	France	Hamidi x Canino
Goldrich	USA	Chance seedling
Harcot	Canada	Chance seedling
Palstein	South Africa	Chance seedling
Precoce de Tyrinthe	Greece	Chance seedling
Precoce de Colomer	France	Chance seedling
Sakıt-2	Turkey	Chance seedling
Sakıt-6	Turkey	Chance seedling
Alatayildizi	Hybrid	Sakıt-6 x P. de Colomer
Cagataybey	Hybrid	Sakıt-2 x P. de Colomer
Cagribey	Hybrid	Sakıt-6 x P. de Colomer
Dr. Kaska	Hybrid	P. de Colomer x 07-K-11
Sahinbey	Hybrid	Sakıt-6 x J. Foulon
2-89	Hybrid	P. de Colomer x 07-K-11
28-89	Hybrid	Sakıt-1 x Fracasso
33-89	Hybrid	Sakıt-1 x Cafano
15-90	Hybrid	J. Foulon x Sakıt-1
7-89	Hybrid	Sakıt-6 x J. Foulon

Table 2. List of the 14 RAPD primers amplifying fragments, number of markers scored, number of polymorphic markers, and estimated size of the polymorphic markers

RAPD primers	Number of fragments scored	Number of polymorphic fragments	Polymorphic fragment sizes (bp)
OPAA 01	10	4	700, 750, 900, 1000
OPAA 02	10	7	370, 800, 850, 1050, 1350, 1400, 1500
OPAA 09	6	2	730, 740
OPAA 14	5	3	600, 650, 1200
OPAA 17	10	8	900, 1200, 1300, 1350, 1400, 1450, 1600, 2000
OPAA 18	4	1	740
OPAA 19	5	3	500, 900, 1350
OPAH 06	5	5	800, 900, 1250, 1400, 1500
OPAH 09	3	2	500, 1700
OPAH 14	3	2	700, 750
OPAH 16	11	4	350, 550, 1000, 1500
OPAH 17	10	6	300, 600, 650, 800, 1450, 1500
OPAH 18	8	5	1400, 1600, 2000, 2100, 2200
OPAH 20	10	7	300, 500, 550, 600, 700, 800, 1500

commercially important.

DNA extraction: Genomic DNA was isolated from leaf samples using DNA extraction procedure described by Dellaporta *et al.* (1983) with some modifications. Three gram of young leaves were crushed in liquid nitrogen and were ground in centrifuge tube with 30 µL mercaptoethanol solution. Tubes were set for few minutes at room temperature. Then, 15 mL extraction buffer [for 800 mL extraction buffer, 200 mL stock (4x), 400 mL of pure water 40 g PVP, 80 mL SDS (2%)] was added and incubated at 65°C for 30 min. Extraction solution was kept in ice for 2 min, 6 mL 5M KAc (potassium acetate) was added and kept on ice for 5 min. The samples was centrifuged (Suprafuge 22, Heraeus-Sepatech) at 13,475 rpm for 15 min at 4°C and aqueous phase was transferred into clean tube containing 15 mL of cold isopropanol. It was kept at room temperature for 15-30 min and centrifuged at 5000 rpm for 20 min. Liquid phase was removed from tube. Five mL of washing buffer (76% ETOH, 10 mM NH_4Ac) was added on pellet. After 20 min, it was centrifuged at 5,000 rpm for 10 min at 4°C, liquid phase was removed, 1.5 mL TE (50 mM Tris-HCl, 10 mM EDTA, pH 7.4) was added. Shaking, it was incubated at 37°C for 1 h. One-hundred and fifty µL of 3 M NaAc was added and waited on ice for 20 min. After centrifuged at 12,000 rpm for 15 min, aqueous phase was transferred to new tube and mixed with 15 mL of cold isopropanol. After incubation for 30-60 min, sample was centrifuged at 6,000 rpm for 10 min. Upper phase was removed and pellet was mixed with 5 mL of washing buffer (76% ETOH, 10 mM NH_4Ac). Centrifuged at 10,000 rpm for 10 min and upper phase was removed from tube. Pellet was air-dried at 37°C for 30 min, then mixed with 200 µL TE until completely dissolved. RNAs were removed from pellet by adding RNAse at a final concentration of 10 µg/mL, and incubation at 37°C for 30 min. Then 200 µL of TE and 3 M of NH_4Ac at final concentration of 2.5 M (pH 7.7), 500 µL of cold ethanol was added. DNA pellet was recovered by centrifuging at 14000 rpm for 2 min. After air-drying, DNA pellet was dissolved in 200 uL of TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.4). DNA concentrations were measured using spectrophotometer.

PCR Amplification: Sixty RAPD primers were used for amplification of isolated apricot DNA. 20 RAPD primers were described by Hormaza (2001), 40 primers were Operon KIT AA and KIT AH primers (Operon Technologies Inc., Alameda, CA, USA). Primers and other PCR components (in 0.5 mL eppendorf tube were as follows: 20 ng of genomic DNA, 12.5 µL of 2x PCR master mix (Promega Cat.no: M7502) (Promega Corp., Madison, WI, USA), 1 µL of 25 mM $MgCl_2$, 20 ng primer, 0.2 unit of Taq DNA polymerase and ddH_2O). PCRs were carried out in Perkin Elmer Cetus 480. Cycling parameters were as described by Hormaza (1999).

Amplified DNA fragments were analyzed by gel electrophoresis in 1.5% agarose (Sigma, A5093) at 70 V for 3 h. After electrophoresis gels were stained with 0.1% ethidium bromide for 30 min. Then visualized under UV with an Polaroid type 667 black-white film (Polaroid MP 4+ Instant Camera System). A 1 kb ladder (GeneRulerTM DNA Ladder Mix, Fermentas) was used to estimate the approximate molecular weight of the amplified products.

Data Analysis: Each band was scored as present (1) or absent

(0) and data were analyzed with the Numerical Taxonomy Multivariate Analysis System (NTSYS-pc) version 1.8 software package (Exeter Software, Setauket, N.Y., USA) (Rohlf, 1993). A similarity matrix was constructed based on Dice`s coefficient (Dice, 1945), which considers only one to one matches between two taxa for similarity. The similarity matrix was used to construct a dendogram using the unweighted pair group method arithmetic average (UPGMA) to determine genetic relationships among the germplasm studied. To provide a goodness-of-fit test for the similarity matrix to cluster analysis, first, COPH module was used to transform the tree matrix to a matrix of ultrametric similarities (a matrix of similarities implied by the cluster analysis) and then, MXCOMP module was used to compare this ultrametric similarities to the similarity matrix produced.

Results and discussion

Out of 60 primers tested, 39 gave non-scorable bands, 7 produced non-polymorphic fragments. The remaining 14 primers amplified from 1 to 8 polymorphic fragments ranged 300 to 2200 bp. OPAA 17 primer amplified the highest number of fragments, 8 bands followed by OPAH 20 and OPAA 2 (7 fragments), OPAH 17 (6 fragments), OPAH 5 and OPAH 18 (5 fragments) primers (Table 2). The number of polymorphic markers was 59 out of 136 (43.4%). On an average, each primer gave 6.47 scorable markers and 4.21 polymorphic markers per amplification. Cophenetic correlation between ultrametric similarities of tree and similarity matrix was found to be high ($r = 0.70$, $P<0.01$). No two apricot cultivars were found to be identical. However, the similarity level was high among the cultivars studied, ranging from 0.90 and 0.96 (Fig.1). There were two groups at the similarity level of 0.90. The lower branch included three cultivars (Priana, Feriana, and Canino) and one promising genotype (28-89). Feriana and Canino were found to be the most closely related genotypes among all cultivars with the similarity level of 0.96. Cultivar Feriana and Priana are progeny of Canino (Yilmaz, 2002). This indicates the strength of RAPD markers in detecting relationships and diversity among germplasm. The upper branch contained twenty cultivars including five newly developed apricot cultivars and four other promising hybrids. These genotypes were derived from known parental combinations (Table 1). They were placed between or close to either one of the parental genotypes. This finding may indicate that RAPD markers can successfully determine genetic relationships among genotypes, and identify cultivars.

The similarity value among 24 genotypes from diverse geographic regions ranged from 0.90 to 0.96. This may imply low genetic variability among the 24 apricot cultivars studied. This narrow variability is a drawback from the point of view of breeders, because they need high genetic variability to improve agronomic traits and the cultivars are selected only based on very few agronomic traits such as maturation time, coloring, and yield.

In general, cultivar breeding programmes do not involve resistance to biotic and abiotic factors. Hence, outbreaks of disease and other pests destroy whole tree or crop in many instances. Increasing genetic variability is crucial to breeding

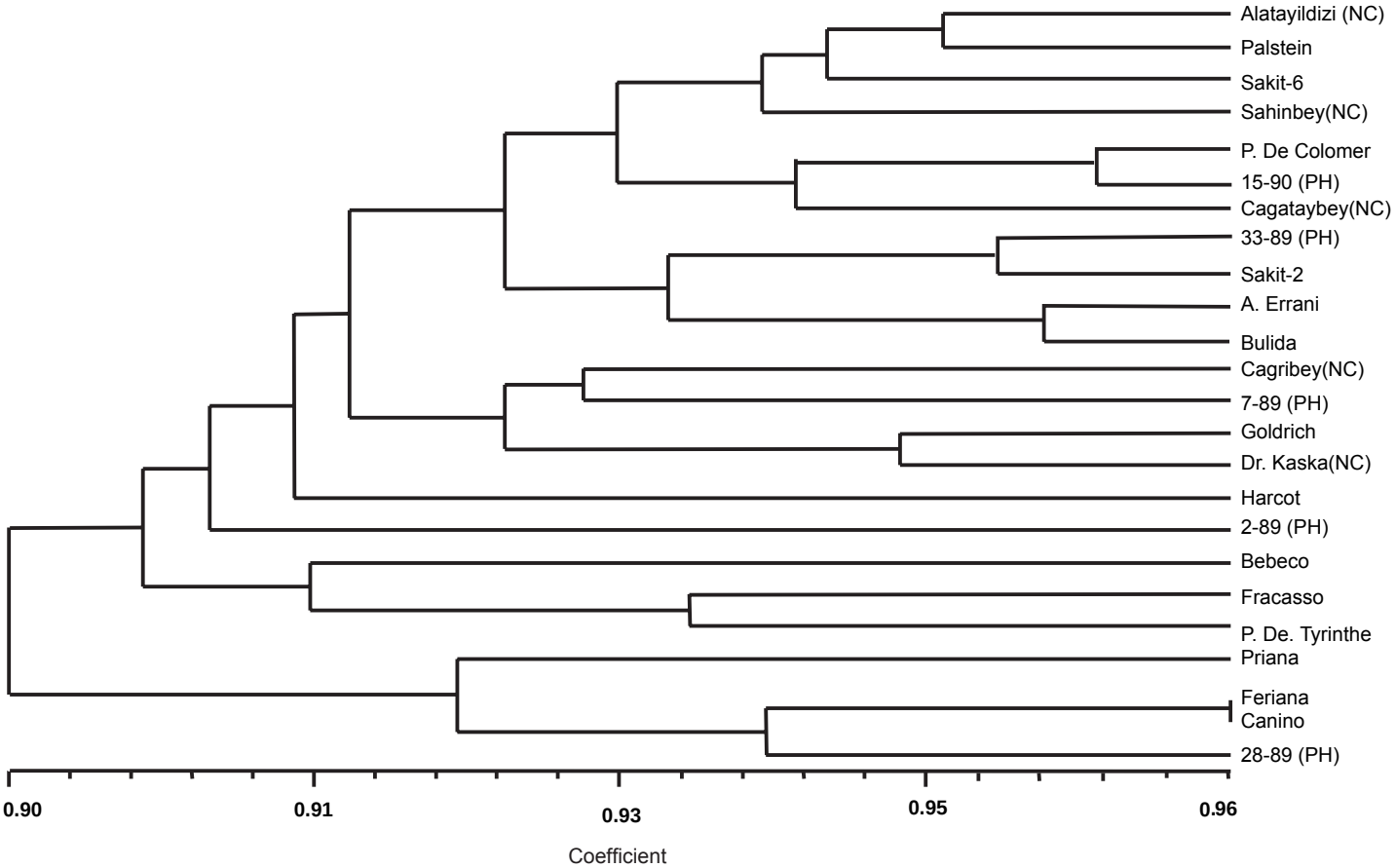

Fig. 1. Dendrogram based on the shared polymorphic amplification product resultant from the use of 21 primers on 24 apricot cultivars and genotypes using UPGMA. Abbreviations are as follows: NC, new cultivar; PH, promising hybrid.

programs. For this aim, wild apricots or the closely related species could be the useful source of variation for plant breeders because within the genus interspecific crosses are usually feasible and efficient in terms of selection pressure. Studies evaluating the disease resistance and fruit quality characteristics of germplasm collections are essential for future breeding programme (Hagen *et al.,* 2002). In conclusion, using genotypes from different ecogeographical groups in breeding programme will allow widening of genetic base in apricot.

The RAPD markers efficiently discriminated all cultivars in this study. Hence, they may be readily used in understanding relationship level, establishing germplams collections, and integrating markers into genetic linkage maps establishing germplasm core collections. As cheap and quick markers, RAPD markers can be used for genetic map of apricots, and used in marker aided selection. RAPDs may have potential in developing resistant cultivars via marker- aided selection, which would further enhance apricot improvement opportunities.

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