

## SRAP based genetic analysis of some apricot cultivars

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### Abstract

*Apricot (Prunus armeniaca L.) is an important fruit species in Turkey with high level of economic value. Sequence related amplified polymorphism (SRAP) markers were used for the first time to determine genetic diversity and relationships among Turkish and some foreign apricot cultivars. A total 87 bands were obtained from SRAP analysis and 63 of them (73%) were polymorphic. Similarity values among the apricot cultivars were found to be between 0.77 and 0.97. Considerable genetic diversity was determined within and between Turkish and foreign apricot cultivars. Four high chilling requiring cultivars ('Levent', 'Aprikoz', 'Cologlu' and 'Ismailaga') originated from Eastern Turkey clustered apart from the others. There was no clear grouping between European, South African, North American and other Turkish cultivars. Therefore it can be suggested that these cultivars, despite their different geographic origins, have similar genetic background.*

**Keywords:** *Prunus armeniaca*, molecular characterization, fingerprinting, cultivar discrimination, UPGMA

### Introduction

Apricot is supposed to be originated in Central Asia and China, and has been long cultivated in Eastern Asia. Later, through central Asia and Asia Minor, it was introduced into Europe (400 BC). It is cultivated in warm temperate to subtropical regions of all continents. There are four different species and one naturally occurring interspecific hybrid under the generic term of apricot. These are: *P. armeniaca* L., 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 dasycarpa* Ehrh., the black or purple apricot. All apricot species are diploid and include eight pairs of chromosomes (2n=16). The most cultivated apricots belong to the species *P. armeniaca* (HORMAZA & al. 2002 [1], ERCISLI, 2004 [2], UZUN & al. 2007 [3]). Six eco- geographical groups have been proposed in *P. armeniaca*, 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 vigorous trees. Despite its lower variability, most of the progress in apricot breeding has been carried out through hybridization and selection within the European group (HORMAZA & al. 2002 [1]).

Turkey is dominating the apricot production in the world. Apricot has been widely cultivated throughout Anatolia since ancient times for its edible fruit, but mostly in Eastern Anatolia. The most important apricot producing centers in Turkey are Malatya, Erzincan, Aras valley (Iğdir- Kagizman), Mersin (Mut), Elazığ, Sivas, Kahramanmaraş, Kayseri, Niğde, Hatay and Nevşehir provinces (ERCISLI, 2009 [40]). Malatya province, located in Eastern region, is accepted as the capital of apricot not only in Turkey, but also in the world. The region produces half of fresh and 90% of dried apricot of the whole country. Cultivars planted

in this area mature mostly between early June and the beginning of August (ERCISLI, 2004 [2], ASMA & OZTURK, 2005 [4]). Recently, early maturing fresh apricot production has increased in Mersin province located near the Mediterranean Sea. Cultivars produced in this region show low chilling requirements and are harvested from beginning of May, earlier than in Spain, France, Italy and Greece.

A lot of studies have been carried out to determine the genetic diversity in the apricot cultivars regarding some quality parameters studied, fundamentally due to the different genetic origins of the cultured apricot cultivars (RUIZ & EGEEA, 2008 [5]). 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 influences, mainly due to the long generation time and the large size of the fruit trees. Apricots can be adapted to specific microclimates and display significantly different morphologies when moved from one microclimate to another. For these reasons, it becomes imperative to base cultivar identification on more stable traits, such as molecular markers. New methods based on studies at the DNA level must be incorporated into fruit breeding programs, in order to accelerate and optimize genotype fingerprinting and to study the genetic relationships among cultivars (HORMAZA & al. 2002 [1], KRICHEN & al. 2006 [6]).

Various molecular marker analyses have been performed to determine genetic diversity and relationship in apricot. De VICENTE & al., 1998 [7], determined the genetic variability in apricot by using RFLP analysis. RAPD markers have been used widely for genetic analysis in apricot (UZUN & al. 2007 [3], ZHEBENTYAYEVA & SIVOLAP 2000 [8], HORMAZA 2001 [9], MARINIELLO & al. 2002 [10], ERCISLI & al. 2009 [11]). Simple sequence repeats (SSR), (HORMAZA & al. 2002 [1], ZHEBENTYAYEVA & al. 2003 [12], HAYASHI & al. 2008 [13], PEDRYC & al. 2009 [14]) and amplified fragment length polymorphism (AFLP) markers (HAGEN & al. 2002 [15], HURTADO & al. 2002 [16], SALAVA & al. 2002 [17], GEUNA & al. 2003 [18]) have been used for molecular characterization of apricot cultivars. Most of the results obtained by previous studies showed that apricot have a high level of variation.

Sequence related amplified polymorphism (SRAP) is a PCR based marker system as described by LI & OIROS, 2001[19]. The SRAPs is a simple and efficient marker system that can be adapted for a variety of purposes in different crops. It is simple, has reasonable throughput rate, discloses numerous co-dominant markers, targets open reading frames (ORFs), and allows easy isolation of bands for sequencing (LI & QUIROS 2001 [19]). To date, SRAP markers have been used to determine genetic diversity in peach [*Prunus persica* (L) Batsch.] and nectarine (AHMAD & al. 2004 [20]), buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.] (GULSEN & al. 2005 [21]), tomato (*Lycopersicon esculentum* Mill.) (RUIZ & GARCIA-MARTINEZ 2005 [22]), persimmon (*Diospyros kaki* L.f.) (GUO & LUO 2006 [23]), okra (GULSEN & al. 2007 [24]), pea (*Pisum sativum* L. ) (ESPOSITO & al. 2007 [25]), sugarcane (*Saccharum sp.*) (SUMAN & al. 2008 [26]) and citrus (UZUN & al. 2009 [27]). To date, there is no report on determining the genetic diversity and characterization of apricot cultivars by SRAP markers. Recently, we constructed a *Citrus* linkage map based on SRAP markers and found that these markers are evenly distributed throughout *Citrus* genome (GULSEN & al. 2010 [28]). This may apply to the other fruits species and is likely to cause good genome sampling among the apricots.

The objective of this study was to fingerprint and to determine the relationships among some apricot cultivars based on the SRAP markers.

## Materials and Methods

### Plant Materials

A total of 28 apricot cultivars were used in this study. The twenty four of them were grown in Alata Horticultural Research Institute located in Mersin, Turkey and four of them were grown in Fruit Research Institute, Malatya, Turkey. The twenty eight cultivars included 4 new Turkish cultivars, 7 local cultivars, 3 new promising hybrid genotypes, 1 change seedling used for rootstock and 13 cultivars from other countries (Table 1). These cultivars were for table use only.

**Table 1.** List of apricot accessions used in this study

Cultivars	Origin
Aurora	Italy
Bebeco	Greece
Beliana	France
Canino	Spain
Castlebrite	USA
Fracasso	Italy
Harcot	Canada
Ninfa	Italy
P.de Colomer	France
P.de Tyrinthe	Greece
Palstein	South Africa
Priana	France
Roxana	Afghanistan
Alatayildizi*	Turkey
Alyanak	Turkey
Aprikoz	Turkey
Cagataybey*	Turkey
Cagribey*	Turkey
Cologlu	Turkey
Ismailaga	Turkey
Levent	Turkey
Sahinbey*	Turkey
Sakit 2	Turkey
Sakit 6	Turkey
2-89 <sup>+</sup>	Turkey
33-89 <sup>+</sup>	Turkey
7-89 <sup>+</sup>	Turkey
Zerdali	Turkey

\* new cultivars, <sup>+</sup> promising hybrids

### DNA extraction and SRAP analysis

Genomic DNA was extracted from young leaves of 28 accessions by the CTAB method as described by UZUN & al. 2009 [27]. DNA concentration was measured with a microplate spectrophotometer (BioTek Instruments, Inc. Vinooski, USA), and 10 ng/mL DNA templates were made using TE (10 mM Tris– HCl, 0.1 mM EDTA, pH 8.0). A total of 16 SRAP primer combinations were used in this study (Table 2). PCR reaction components and PCR cycling parameters for SRAP analysis was performed as described by UZUN & al. 2009 [27]. Each of 15 mL reaction consisted of 1.33 mM of primers, 200 µM of each dNTP, 1.5 µL of 10X PCR Buffer (Biorun, Nantes, France), 2 mM of MgCl<sub>2</sub>, 0.8 µg/µL Bovine

serum albumine (Biological Industries, Beit Haemek, Israel) 5.8  $\mu$ L ddH<sub>2</sub>O, 1 unit of Taq polymerase (Biorun, Nantes, France) and 20 ng of template. DNA Thermal Cycler (Sensoquest Progen Scientific Ltd. Mexborough, South Yorkshire, UK) was used and cycling parameters included 2 min of denaturing at 94 °C, five cycles of three steps: 1 min of denaturing at 94 °C, 1 min of annealing at 35 °C and 1 min of elongation at 72 °C. In the following 35 cycles the annealing temperature was increased to 50 °C, and for extension, one cycle 5 min at 72 °C. PCR products were separated on 2% agarose gel in 1X TBE buffer (89 mM Tris, 89 mM Boric acid, 2 mM EDTA) at 115 volt for 2.5-3 h. The fragment patterns were photographed under UV light for further analysis. A 100 bp standard DNA ladder as the molecular standard in order to confirm the appropriate markers were used for SRAP analysis.

### 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 program ver. 2.11) software package (ROHLF, 2000 [29]). A similarity matrix was constructed by using SRAP data based on DICE, 1945 [30] coefficient. Then, the similarity matrix was used to construct a dendrogram using the UPGMA (unweighted-pair group method arithmetic average) to determine genetic relationships among the studied accessions. The genetic similarity matrix and ultrametric distance matrix produced from UPGMA-based dendrogram with COPH module nested in the same software was compared using Mantel's matrix correspondence test (MANTEL, 1967 [31]). The result of this test is a cophenetic correlation coefficient,  $r$ , that indicates how well dendrogram represents similarity data. Polymorphism information content (PIC) values were calculated according to SMITH & al. 1997 [32], using the following formula for all primer combinations:

$$PIC = 1 - \sum f_i^2,$$

Where  $f_i$  is the frequency of the  $i$ th allele. PIC provides an estimate of the discriminatory power of a locus by taking into account, not only the number of alleles that are expressed, but also the relative frequencies of those alleles (SMITH & al. 1997 [32]). PIC values range from 0 (monomorphic) to 1 (very highly discriminative, with many alleles in equal frequencies).

## Results

Eighty-seven fragments were obtained from 16 SRAP primer combinations, and were used for analyses in this study. The number of bands per primer ranged from 3 to 9 with mean value of 5.4. Maximum number of fragments found in em7/me12 and em10/me9 and the highest polymorphic fragments were obtained from em10/me9 primer combination (Table 2). All bands (3) obtained from em6/me6 and em15/me6 primer combinations were polymorphic. Ratio of polymorphic SRAP markers was 73%. The number of fragments per PCR reaction for apricot cultivars was reported as 3.5 (RUTHNER & al. 2006 [33]) and 4.1 (SANCHEZ-PEREZ & al. 2005 [34]) for SSR markers, as 6.5 (UZUN & al. 2007 [3]) and 9.8 (ERCISLI & al. 2009 [11]) for RAPD markers. The PIC values for the 16 primer combinations ranged from 0.14 (em1/me4) to 0.80 (em15/me6), with a mean of 0.48 (Table 2). We also determined unique fragments present or absent for cultivars. A 280-bp fragment amplified by primer em11/me12 was observed only in '2-89' and 'Castlebrite'. The fragments of 650 bp produced by em6/me4 observed only in 'Roxana' and 450 bp absent only in 'Cologlu'. 'Ninfa' differed from the others with absence of 320 bp fragment and 'Sahinbey' with absence of 280 bp fragment of em10/me2. Primer em4/me4 produced a 950 bp fragment for only 'Sakit 2' and 'Harcot'. Also 'Harcot' distinguished from the other with the presence of 320 bp band for em2/me3 and '33-89' distinguished with the presence of a 400 bp fragment of em15/me6. For primer em10/me8, '33-89' differed from the others by the presence of a 900 bp, 'P. de

Colomer' by the presence of a 450 bp, 'Sakit 6' and '2-89' differed having a 400 bp fragment while only a 310 bp fragment was absent for 'Aurora'.

**Table 2.** List of SRAP primers used in this study, their numbers of total and polymorphic fragments and percentage of polymorphism, and polymorphism information contents (PIC)

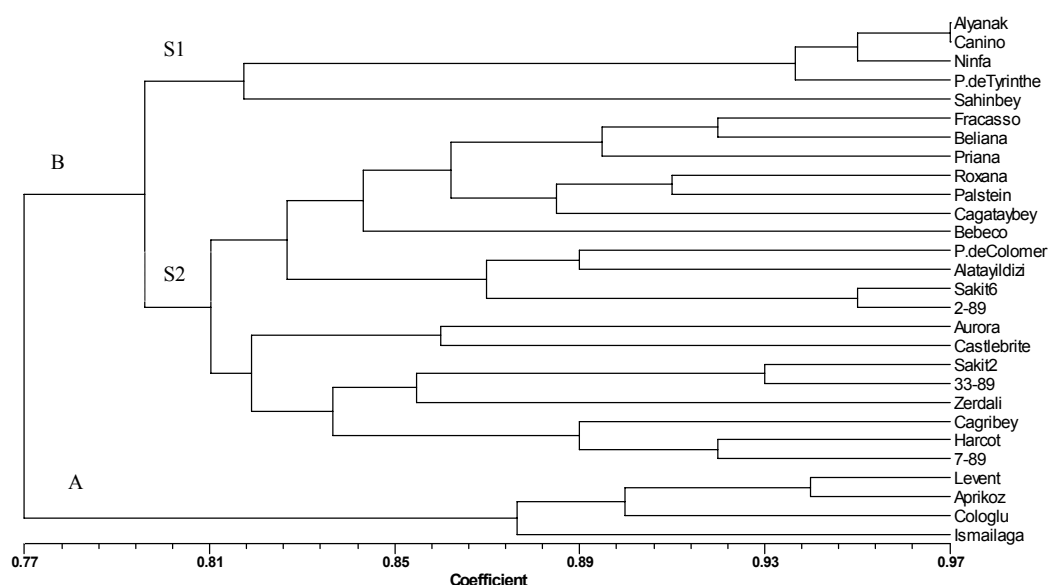
Primer combinations	Total fragments	Polymorphic fragments	Polymorphism (%)	PIC
em1 me2	7	5	71	0.52
em1 me4	3	2	67	0.14
em2 me3	6	5	83	0.66
em4 me4	5	2	40	0.22
em4 me9	7	5	71	0.51
em6 me4	6	4	67	0.39
em6 me6	3	3	100	0.55
em7 me12	9	6	67	0.43
em7 me8	3	2	67	0.49
em7 me9	6	4	67	0.54
em10 me2	8	5	63	0.47
em10 me9	9	8	89	0.66
em11 me12	3	2	67	0.45
em14 me4	3	2	67	0.36
em15 me 13	6	5	83	0.52
em15 me6	3	3	100	0.80
Mean	5.4	3.9	73	0.48
Total	87	63	-	-

### Genetic relationships among the apricot cultivars

The data obtained from SRAP analyses were used to perform genetic similarity analysis among the 28 apricot cultivars. The similarity matrix was calculated using 87 SRAP fragments according to Dice's coefficient (DICE, 1945 [30]) method. Then, the similarity matrix was used to perform UPGMA cluster analysis. Cophenetic correlation between ultrametric similarities of tree and the similarity matrix was  $r = 0.72$ ,  $P < 0.01$ . All of accessions used in this study were distinguished. The apricot cultivars studied had similarity levels ranging from 0.77 to 0.97 (Figure 1). In the UPGMA dendrogram based on the Dice's coefficient, apricot cultivars clustered into two main groups (A and B), at similarity value of 0.77. Group A consisted of four cultivars originated as change seedlings in the Eastern Turkey. Three of them, 'Levent', 'Cologlu' and 'Ismailaga', were from Malatya province. 'Levent' is a late maturing cultivar, harvested in September, while 'Cologlu' and 'Ismailaga' are harvested in July (YILMAZ, 2008 [35]). ERCISLI & al. 2009 [11] also found 'Cologlu' and 'Ismailaga' cultivars in the same branch based on the RAPD data. On the other hand, AKPINAR & al. 2010 [39] notified these two cultivars in close subgroups based on SSR data. 'Aprikoz' originated in Igdir province and is harvested at the end of June. These four cultivars are cultivated in temperate zone of the Eastern Turkey, and show higher chilling requirements than the other studied cultivars.

Group B included 24 cultivars, 11 of them from Turkey and 13 of them from other countries. Most of these cultivars are suitable for Mediterranean-like climates with early maturing. This group consisted of two subgroups (S1 and S2). 'Alyanak', 'Canino', 'Ninfa', 'P. de Tyrinthe' and 'Sahinbey' (new cultivar obtained from 'Sakit-6' × 'J. Foulon') were nested in S1. The most closely related cultivars in this study were 'Alyanak' originated from

Aegean region in Turkey and ‘Canino’ from Spain with a similarity level of 0.97. ‘P.de Tyrinthe’ and ‘Ninfa’ were two most cultivated early maturing cultivars in Turkey. Subgroup 2 was separated into two clusters with a similarity level of 0.81. Small branch consisted of eight accessions. In this branch, ‘Aurora’ (Italy), ‘Castlebrite’ (USA) and ‘Harcot’ (Canada) were from Europe and North America. ‘Aurora’ was clustered with cultivars from USA in previous study (GEUNA & al. 2003 [18]). The promising hybrids, ‘33-89’ (‘Sakit 1’ × ‘Cafano’) and ‘7-89’ (‘Sakit 6’ × ‘Foulon’), were obtained from hybridization of local and foreign cultivars. ‘Cagribey’ (‘Sakit 6’ × ‘P.de Colomer’), an early maturing new cultivar, and ‘7-89’ are progenies of ‘Sakit 6’ and were nested closely. In the large branch of subgroup 2, ‘Beliana’ and ‘Priana’ from the same parents (‘Hamidi’ × ‘Canino’) were found to be closely related. For two new early maturing cultivars, ‘Cagataybey’ (‘Sakit 2’ × ‘P.de Colomer’) and ‘Alatayildizi’ (‘Sakit 6’ × ‘P.de Colomer’) were nested between their parents. The other promising hybrid, ‘2-89’ (‘P.de Colomer’ × ‘07-K-11’), was closely nested to ‘P. de Colomer’. In addition, this branch included some other foreign cultivars, ‘Fracasso’ (Italy), ‘Roxana’ (Afghanistan), ‘Palstein’ (South Africa) and ‘Bebeco’ (Greece).



**Figure 1.** Dendrogram of the 28 apricot accessions using UPGMA method obtained from SRAP markers

## Discussion

The SRAP marker system is becoming the marker of choice for characterization and genetic diversity studies in a wide range of plants. The study described in this paper shows that SRAP analysis is a powerful tool also for the characterization of apricot cultivars. In our study the SRAP markers were used for the first time in apricot and distinguished cultivars efficiently with high level of polymorphism. On the other hand, some cultivars were distinguished by only one primer combination.

The results obtained in this study showed that there were high levels of polymorphism in Turkish and foreign apricot cultivars and all of them were distinguished with the SRAP data. High level of polymorphism in Turkish apricot cultivars was previously reported based on their RAPD data by ERCISLI & al. 2009 [11] and based on SSR data (AKPINAR & al. 2010 [39]). Genetic variation among the apricot cultivars was determined by using various marker systems. HURTADO & al. 2002 [16] reported that there was a gradation of polymorphism from RFLPs (low) to RAPDs (high) to AFLPs (very high) in apricot and a

better resolution of relatedness among cultivars was obtained from AFLP. YUAN & al. 2007 [36] found a 72% polymorphism in apricot based on AFLP data. This is comparable to the SRAP based analysis performed in this study.

Considerable diversity was detected among the apricots. In addition, the selected apricots were successfully fingerprinted. High allele number and heterozygosity reflect the ability of SSR marker to provide a unique genetic profile for individual genotypes in apricot (MAGHULY & al. 2005 [37]). In apricot, the high level of genetic differentiation could be explained by the mating system and by low migration rates. Most of fruit trees under cultivation are derived from allogamic wild progenitors in which cross-pollination was maintained by self-incompatibility. Genetically, domestication of fruit trees means changing the reproductive biology by shifting from sexual reproduction (in the wild) to vegetative propagation (MAGHULY & al. 2005 [37]).

Genetic variation among the apricots may have potential for breeding new cultivars by selection and hybridization for high yield, quality and resistance to biotic and abiotic stress conditions. The diversity determined between apricot cultivars was probably due to crosses between wild and cultivated apricots and cultivars from different geographic origin. The apricots studied originated in Turkey, Europe, North America, South Africa and Afghanistan. In the UPGMA analysis, cultivars were not distinctly clustered in respect of their geographic origin. In comparison, cultivars from North America (Castlebrite and Harcot) were clustered as apart relatively from most of European cultivars. It is reported that in the past, breeding programs in North America generally were concentrated by hybridization involving only the European eco-geographical group and then introduction of apricot to North America, following at least two routes, one Asian and the other European germplasms. Consequently, American cultivars have European and Asian ancestors. On the other hand, the majority of European apricot cultivars most likely originated from a few forms brought from the near-Eastern region (KHAN & al. 2008 [38]). MAGHULY & al. 2005 [37] reported that the east European, west European and Irano-Caucasian groups were very similar compared to Central Asian cultivars and other apricot species. The European genetic base (Mediterranean Basin and Continental Europe) was reported as much narrower and European cultivars share a common genetic base and are interrelated, which makes classification based on genetic distances difficult (HAGEN & al. 2002 [15]). In previous study it was observed that heterozygosity among the apricot cultivars decreased from China to Middle Europe and the Middle European and Chinese apricots were clarified to be distantly related (PEDRYC & al. 2009 [14]). It can be suggested that differences among the apricots sampled relatively from close distances were small and therefore grouping pattern do not associate with samples geographic origin, but can be clustered distinctly if sampled from relatively long distances. However, the Turkish cultivars were grouped in respect of their geographic distribution. Cultivars originated in Eastern Turkey and show higher chilling requirement (group A in the dendrogram) were distinct from others that originated in South of Turkey. It can be assumed that cultivars from East of Turkey are closer to Irano-Caucasian group than to European group. On the other hand, 'Alyanak' from the West of Turkey (in subgroup 1) separated from other Turkish cultivars.

## Conclusions

The apricot cultivars used in this study showed a high level of polymorphism. This is promising from the point view of maintaining diversity. We observed no grouping based on the countries of origin, with the exception of a few Turkish cultivars. These results may indicate the potential of the studied molecular technique to discriminate among the apricot genotypes, very useful in selecting the parental genotypes used in hybridization breeding

programs. In addition, the SRAP markers, by successfully discriminating among apricot cultivars, can be used for characterization and diversity analyses of apricot genetic resources.

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