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Determination of genetic diversity among wild grown apricots from Sakit valley in Turkey using SRAP markers

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Summary

Sequence-related amplified polymorphism (SRAP) marker was employed for the first time to analyze genetic diversity of 57 seed propagated early-maturated wild grown apricot genotypes sampled from different parts of Sakit valley in Mediterranean Region of Turkey. From total 19 primer combinations investigated, 16 could amplify clearly and consistently. They produced a total of 87 fragments, of which 56 (64.3%) were polymorphic bands. All bands obtained from Me3-Em2, Me2-Em10 and Me2-Em6 primers were polymorphic. The cluster analysis revealed that the 57 genotypes were grouped into three major clusters. The similarity ratio among genotypes was between 0.73 and 0.94. There were no identical genotypes. The study revealed that the SRAP marker system is useful for identification and genetic diversity analysis of wild grown apricots.

Introduction

Turkey is one of the richest countries in terms of biological diversity, due to its specific geographic position between Asia and Europe and characteristic ecological, climatic and geomorphologic conditions (Ercisli, 2004).

Apricot trees can be grown both temperate and subtropical zones in the world. China, the Irano-Caucasian region (Turkey and Iran), Central Asia, Europe and North America are the main apricot producer regions in the world (Halasz et al., 2010). Turkey has been dominating world apricot production for a long time and the country is one of the main diversity centres of this unique fruit (Ercisli, 2009). The Central Asia is the oldest and the primary genetic source of apricot groups and Central Asian accessions are self-incompatible; the Irano-Caucasian apricots which are mostly the cultivated ones are mostly self-incompatible, with large fruits and low chilling requirements (Mehlenbacher et al., 1991; Romero et al., 2003; Halasz et al., 2005).

One of the most widely spread wild edible fruits in Turkey are apricots. They are called 'Zerdali' in Turkish. Continuous seed propagation of wild apricots hundreds of years in different agro climatic conditions in Turkey is responsible for high phenotypic variability. Owing to the long-lasting process of natural selection, wild grown apricots have adapted to ecological conditions of habitats and developed natural resistance mechanisms to biotic and abiotic environmental factors (Ercisli, 2009).

Wild grown apricot (*Prunus armeniaca* L.) is an economically important fruit crop in particular for local people living in rural areas in Turkey. It is a multi-purpose fruit tree and besides its fresh edible fruits, it is used in diverse ways because the fruits have distinct taste and aroma. Edible fruits of wild apricots have been used from the past till now as dry fruit, processed into jam, marmalade, fruit juice etc. in Turkey. Traditional uses and drying of apricot fruits have been found to be of great significance in the socio-economy of people in these areas. The bitter seeds of wild grown apricots are valuable material for pharmacology to treat cancer. In Turkey, all apricot cultivars are grafted on seedlings obtained from wild apricot seeds.

More recently, wild grown apricot fruits have been gaining more importance particularly in fruit juice industry in Turkey and there is growing interest in wild grown apricot juice because of its better sugar/acidity balance compared to juice from cultivated apricots.

It is also believed that most of Turkish apricot cultivars were directly selected among wild grown apricots populations within several regions of Turkey (Altindag et al., 2006). The wild grown apricots display great phenotypic diversity in Turkey and they have potential to be used practically in apricot breeding programmes. Natural populations of wild grown apricots in Turkey are mainly found on field borders, some forest edges, and wide areas of lowland and mountains. Besides this, they also can be found in extensive fruit orchards (Ercisli, 2009). In Turkey, there are several valleys such as Sakit valley, Aras valley and Coruh valley which have important wild grown apricot genetic resources (Durgac, 2001; Ercisli, 2009).

The diversity in apricots has been studied with pomological, morphological and phenological characteristics for a long time (Guerriero and Watkins, 1984). In recent years, DNA-based markers are widely used to clarify the genetic relationship among the apricot accessions (Romero et al., 2003; Yilmaz et al., 2012). For germplasm characterization and breeding and commercialization of promising apricot cultivars, precise characterization and discrimination of the genotypes are prerequisite. Different types of marker such as morphological, molecular, biochemical systems have been used for identification in horticultural plants (Ercisli et al., 2007; Kafkas et al., 2008; Pedryc et al., 2009). However, due to the effects of environmental factors, assessment of morphological and pomological traits may be ambiguous. Therefore, markers independent from the environment are necessary for reliable identification and discrimination of genotypes and cultivars. DNA markers are independent from environmental interactions and they show high level of polymorphism. They are considered invaluable tools for determining genetic relationships/diversity. Various types of DNA markers are now available. Among them, RAPD developed by Williams et al. (1990) has been commonly used in apricot to assess genetic variability and relationships among cultivars (Marinello et al., 2002; Ercisli et al., 2009). More recently, ISSR (Chenjing et al., 2005; Yilmaz et al., 2012), AFLP (Krichen et al., 2006; Yuan et al. 2007), SSR (Maghuly et al., 2005; Pedryc et al., 2009) and SRAP (Uzun et al., 2010) techniques have frequently been used in apricot to characterize different genotypes belonging to diverse eco-geographical groups.

Sequence related amplified polymorphism (SRAP) is a PCR based marker system as described by Li and Oiros, 2001. 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 and Quiros, 2001). This marker system has been used to determine genetic diversity in several horticulture plants such as

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peach [*Prunus persica* (L.) Batsch.] and nectarines (Ahmad et al., 2004), persimmon (*Diospyros kaki* L.f.) (Guo and Luo, 2006), apricots (Uzun et al., 2010) and citrus (Uzun et al., 2009).
To date, there is no report on determining the genetic diversity and characterization of wild grown apricots by SRAP markers in Turkey and in the world. This study aimed to determine the genetic diversity among seed propagated apricots sampled from Sakit valley in Mediterranean region of Turkey.

Material and methods

Study area and plant material

The study area, Sakit valley, one of the famous wild apricot growing areas, is located in the east Mediterranean region of Turkey. The altitude of this valley is ranging from 120 to 1150 meters above sea level (m a.s.l.). A total of 57 Sakit apricot genotypes were collected from different part of the Sakit valley. All 57 genotypes are preselected according to their high yield capacity. They were free from pests and diseases.

DNA extraction and SRAP analysis

Genomic DNA was extracted from young leaves of 57 wild apricot genotypes by the CTAB method as described by Uzun et al. (2009). DNA concentration was measured with a microplate spectrophotometer (BioTek Instruments, Inc. Vinooski, USA), and DNA was diluted to 10 ng/mL using TE (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). A total of 19 SRAP primer combinations were used in this study (Tab. 1). PCR reaction components and PCR cycling parameters for SRAP analysis was performed as described by Uzun et al. (2009). Each of the 15 μL reactions 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 $MgCl_2$, 0.8 μg/μL Bovine serum albumine, 5.8 μL ddH_2O, 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 ºC, five cycles of three steps: 1 min of denaturing at 94 ºC, 1 min of annealing at 35 ºC and 1 min of elongation at 72 ºC. In the following 35 cycles, the annealing temperature was increased to 50 ºC, and the extension per cycle was set to 5 min at 72 ºC. PCR products were separated on a 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 was used as the molecular standard to confirm the appropriate markers 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). A similarity matrix was constructed by using SRAP data based on (Dice, 1945) 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 genotypes.

Results and discussion

Genetic diversity

The SRAP primers, total fragments, polymorphic fragments and polymorphism percentage are given in Tab. 1.
In the present study, a total of 87 bands were presented from the 19 selected SRAP primers among 59 individual wild grown apricot genotypes. Of these, 56 fragments (64.3%) were polymorphic (Tab. 1). The primers Me3-Em2, Me2-Em10 and Me2-Em6 gave the highest polymorphism ratio (100%) while Me1-Em6, Me6-Em2 and Me7-Em3 did not give polymorphic bands (0%). The number of bands per primer ranged from 1 to 9 with mean value of 4.6 (Tab. 1). The results obtained in this study show that there are high levels of polymorphism in wild grown apricots in Sakit valley of Turkey and all of them were distinguished with the SRAP markers. Previously, the number of fragments per PCR reaction for apricot cultivars was reported as 3.5 (Ruthner et al., 2006) and 4.1 (Sanchez-Perez et al., 2005) for SSR markers, as 6.5 (Uzun et al., 2007) and 9.8 (Ercisli et al., 2009) for RAPD markers and as 5.4 for SRAP markers (Uzun et al., 2010). Turkish germplasm was studied by Akpinar et al. (2010), Uzun et al. (2010) and Yilmaz et al. (2012) and genetic diversity and relationships among the accessions were determined using RAPD, ISSR, SRAP and SSR markers. They found high genetic diversity among Turkish apricot cultivars. Yuan et al. (2007) reported a 72% polymorphism in apricot based on AFLP data. This is comparable to the SRAP based analysis performed in this study. In apricot, the high level of genetic differentiation could be explained by the mating system and by low migration rates. Most 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 et al., 2005).

Tab. 1: List of SRAP primers used in this study, their numbers of total and polymorphic fragments and percentage of polymorphism

SRAP Primers	Total fragments	Polymorphic fragments	Polymorphism (%)
Me8-Em6	5	3	60
Me3-Em2	4	4	100
Me7-Em10	4	2	50
Me2-Em10	4	4	100
Me2-Em6	5	5	100
Me1-Em6	2	0	0
Me3-Em12	4	3	75
Me2-Em12	6	5	83
Me5-Em8	4	2	50
Me6-Em5	6	1	17
Me2-Em2	6	2	33
Me7-Em6	8	6	75
Me7-Em3	4	2	50
Me4-Em12	3	2	67
Me5-Em2	3	2	67
Me6-Em2	2	0	0
Me7-Em3	1	0	0
Me9-Em10	7	5	71
Me13-Em15	9	8	89
Mean	4,6	2,9	64.3
Total	87	56	

Genetic relationships among the accessions

The data obtained from SRAP analyses were used to perform genetic similarity analysis among the 57 wild grown apricot genotypes.

The similarity matrix was calculated using 87 SRAP fragments according to Dice's coefficient method (DICE, 1945). Then, the similarity matrix was used to perform UPGMA cluster analysis. All genotypes used in this study were distinguished. The analyzed wild grown apricot genotypes had similarity levels ranging from 0.73 to 0.94 (Fig. 1).

In the Dice's coefficient based UPGMA dendrogram, wild grown apricot genotypes clustered into three main groups (I, II and III) (Fig. 1). Group I, II and III consisted of five, fourty-seven and five genotypes, respectively. Group I, II and III also further divided into 2 subgroups. There were no identical genotypes on the dendrogram (Fig. 1). The most closely related genotypes in this study were '34' and '36'; '27, 31 and 38'; '17 and 21'; '14 and 26'; '13 and 29' and '44 and 45' with 98% similarity ratios. UZUN et al. (2010) reported genetic similarity between 0.77 and 0.97 among 28 Turkish apricot cultivars.

MAGHULY et al. (2005) 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 et al., 2002). In previous studies, it was observed that heterozygosity among apricot cultivars decreased from China to Middle Europe and the Middle European and Chinese apricots were clarified to be distantly related (PEDRYC et al., 2009). It can be assumed that cultivars from east of Turkey are closer to Irano-Caucasian group than to European group and the ones from west of Turkey including Sakit valley are closer to European group (HALASZ et al., 2010).

Conclusions

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 analy-

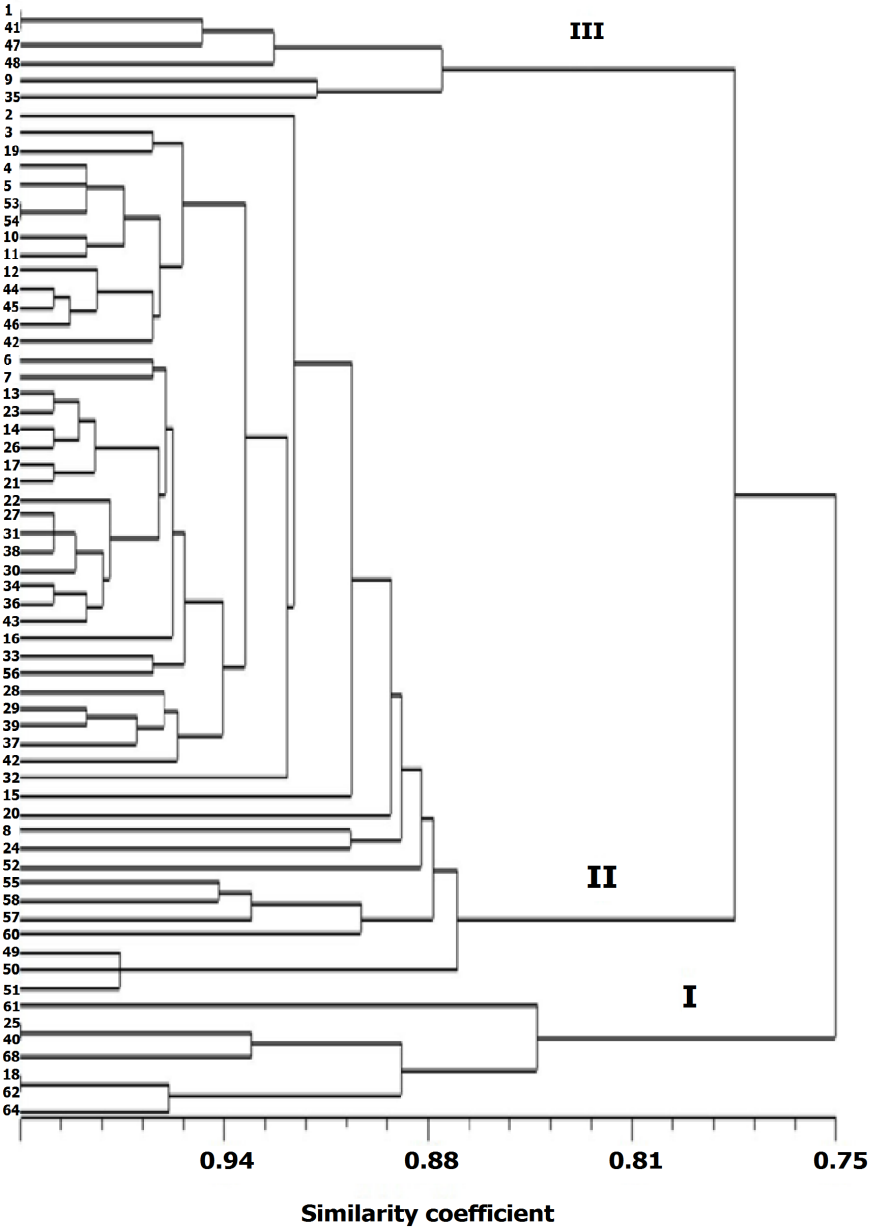

Fig. 1: Dendrogram of the 57 wild grown apricot genotypes using UPGMA method obtained from SRAP marker

sis is a powerful tool for the characterization of wild grown apricot genotypes. In our study, the SRAP markers were used for the first time in wild grown apricot and distinguished genotypes efficiently with high level of polymorphism. Genetic variation among the wild grown 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 wild grown apricot genotypes was probably due to seed propagation. It can be concluded that investigated wild growing fruit species have a great future potential in genetic research, as well as in biodiversity research. It is necessary to carry out further inventorisation and evaluation of investigated wild growing apricots to utilize them in the most appropriate way, as well as conservation of interesting accessions in the gene banks. The most valuable collected specimens should be involved in plant breeding programmes that can create new cultivars.

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