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MOLECULAR CHARACTERIZATION AND GENETIC RELATIONSHIPS OF WILD APRICOTS (*PRUNUS ARMENIACA* L.) FROM TURKEY USING SSR MARKERS

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Abstract

In the present study we used a total of 29 wild apricot genotypes sampled from 3 regions (Erzincan, Çoruh valley, and Aras valley) which were evaluated by means of simple sequence repeat (SSR) markers. In total, 16 simple sequence repeat (SSR) loci were used and 98 alleles were revealed with a mean number of 6.13 alleles per locus and mean polymorphic information content (PIC) of 0.66 showing a high genetic diversity of Turkish wild apricots. A larger expected heterozygote than the observed was found in the analyzed genotypes. Clustering of individual accessions based on UPGMA dendrogram separated accessions into three major clusters according to their geographic origin and indicating high variability among genotypes. The information obtained in this study implies that the studied genotypes were distant from each other and can be used for apricot breeding strategies and for the management and the conservation of Turkish apricot germplasm.

Key words: molecular characterization, seed propagation, wild apricots

Introduction. Turkey is an important fruit producer in the world and the country has a spot in the global top ten fruit producers. Turkey also plays a part as a fruit export country. With an export of 3.3 million tons of fresh fruit, Turkey is also in the top ten of export countries [1–3].

The seed propagated apricots are called 'wild apricots' in Turkey. This terminology is still 'black box' in apricot producing countries. Extensive variability

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exists among wild apricots and cultivars within and between districts in quality and time of harvest [4, 5]. Although most cultivars mature between the end of May and the beginning of August, and wild apricot populations mature from mid-July until the 20–25th August, some wild apricot forms mature in late September. These forms may be important to extend the harvest period. Late blooming and growth dwarf types have also been reported among the native apricot seedling populations in the Erzincan plain [4].

Plants are characterized by morphological (phenotypic), biochemical and molecular markers. Morphological markers are readily available, require simple equipment to screen and are visible to naked eye. However, they have lots of disadvantages. They exhibit less polymorphism and need experts. They are highly influenced by the environment factors. Often the environmental conditions can influence the expression of a particular characteristic [6].

Microsatellites (or simple sequence repeats (SSRs) are PCR-based molecular markers valued for their abundant and uniform genome coverage, high levels of polymorphism information content as a consequence of their marked mutation rates, and other valuable qualities such as codominant inheritance of DNA amplicons/alleles and request of little amount of DNA for the amplifications [7]. A unique pair of primers defines each SSR marker locus; as a consequence, the molecular information exchange among laboratories is easy and allows individuals to be uniquely genotyped in a reproducible way [8].

To the best of our knowledge, there are no reported studies on SSR analysis of seed propagated wild apricots grown in Eastern Anatolia region of Turkey. Therefore, the objective of this study was to characterize and evaluate the genetic diversity of 29 seed propagated wild apricot genotypes using SSR markers and to develop strategies for preserving the endangered genetic resources of this species.

Material and method. ***Plant material.*** In total, 29 seed propagated wild apricot from Aras valley, Coruh valley, and Erzincan plain of Eastern Anatolia region of Turkey were used and analyzed using SSR markers. The genotypes from C1 to C7 represent samples from Coruh valley, K1 to K9 represent samples from Aras valley and the genotypes E1 to E13 represent samples from Erzincan plain (Table 1).

DNA extraction. Genomic DNA was extracted from young leaf tissues using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI) according to the instructions provided by the manufacturer. Subsequently, an RNAse treatment was performed to the eluted DNA samples. Purity and concentration of the DNA were checked both on 1% (w/v) agarose gel and by NanoDrop® ND-1000 Spectrophotometer.

SSR analysis. A total of 16 SSR loci, were used in the analysis (Table 2).

Polymerase Chain Reaction (PCR). PCR was conducted in a volume of 10 µL and contained 15 ng genomic DNA, 5 pmol of each primer, 0.5 mM dNTP, 0.5 unit GoTaq DNA polymerase (Promega), 1.5 mM $MgCl_2$ and 2 µL 5X

Table 1

Tree growth habit, harvest date, fruit shape and fruit skin and flesh colour of wild apricots

Genotypes	Tree Growth Habit	Ripening Time	Fruit Shape	Ground Colour	Over Colour	Flesh Colour
C1	Upright to Spreading	Early	Oblong	Light Yellow	Light Orange	Deep Orange
C2	Spreading	Medium	Oblong	Yellow	Deep Orange	Orange
C3	Spreading	Early	Round	Light Yellow	Orange	Deep Yellow
C4	Spreading	Early	Ovate	Light Yellow	Orange	Orange
C5	Spreading	Medium	Ovate	Yellow	Orange	Deep Yellow
C6	Spreading	Medium	Round	Yellow	Deep Orange	Orange
C7	Spreading	Medium	Ovate	Creamy	Deep Orange	Deep Orange
K1	Spreading	Early	Oblong	Yellow	Orange	Orange
K2	Spreading	Early	Oblong	Light Yellow	Orange	Orange
K3	Upright to Spreading	Early	Round	Light Yellow	Deep Yellow	Light Orange
K4	Spreading	Early	Ovate	Yellow	Light Orange	Light Orange
K5	Spreading	Early	Ovate	Yellow	Dark Yellow	Light Orange
K6	Spreading	Early	Ovate	Light Yellow	Light Orange	Orange
K7	Spreading	Early	Ovate	Yellow	Orange	Orange
K8	Upright to Spreading	Early	Oblong	Light Yellow	Deep Orange	Deep Yellow
K9	Upright to Spreading	Early	Round	Creamy	Orange	Light Orange
E1	Spreading	Medium	Oblong	Yellow	Deep Orange	Orange
E2	Spreading	Medium	Round	Light Yellow	Orange	Orange
E3	Spreading	Early	Ovate	Yellow	Orange	Light Orange
E4	Spreading	Medium	Oblong	Light Yellow	Orange	Orange
E5	Spreading	Medium	Ovate	Yellow	Deep Orange	Deep Orange
E6	Spreading	Late	Oblong	Light Yellow	Deep Yellow	Orange
E7	Spreading	Early	Ovate	Yellow	Light Orange	Deep Yellow
E8	Spreading	Medium	Round	Light Yellow	Deep Orange	Light Orange
E9	Spreading	Late	Round	Yellow	Yellow	Orange
E10	Spreading	Medium	Ovate	Light Yellow	Orange	Deep Orange
E11	Spreading	Late	Ovate	Yellow	Deep Orange	Orange
E12	Spreading	Late	Round	Yellow	Orange	Light Orange
E13	Upright to Spreading	Very late	Elliptic	Light green	Orange	Orange

buffer. The forward primers were "labelled" with WellRED fluorescent dyes D2 (black), D3 (green) and D4 (blue) (Proligo, Paris, France). Reactions without DNA were included as negative controls. PCR amplification was performed using the Biometra® PCR System. The amplification conditions consisted of an initial denaturation step of 3 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min

Table 2

Simple sequence repeats (SSRs), number of detected alleles, observed heterozygosity (Ho), expected heterozygosity (He) and polymorphism information content (PIC) of 16 SSR markers on 29 wild apricot genotypes

SSR Primers	Number of alleles	Observed heterozygosity (Ho)	Expected heterozygosity (He)	PIC
pchgms1	5	0.55	0.71	0.58
pchgms2	8	0.62	0.80	0.70
pchgms3	6	0.50	0.69	0.53
pchgms4	6	0.71	0.70	0.55
PS12A02	9	0.52	0.75	0.73
UDP96-001	5	0.55	0.63	0.62
UDP96-003	6	0.53	0.62	0.54
UDP96-005	7	0.66	0.68	0.77
UDP96-008	5	0.59	0.59	0.55
UDP96-010	7	0.48	0.60	0.84
UDP96-019	5	0.60	0.71	0.59
UDP97-402	6	0.66	0.68	0.61
UDP98-021	6	0.49	0.56	0.60
UDP98-405	5	0.64	0.74	0.75
UDP98-406	7	0.58	0.70	0.68
UDP98-409	9	0.47	0.65	0.82
Average	6.13	0.58	0.68	0.66

at 52–56 °C and 2 min at 72 °C with a final extension at 72 °C for 10 min. For determination of polymorphisms, the PCR products were run on CEQ$_{\text{TM}}$ 8800 XL Capillary Genetic Analysis System (Beckman Coulter, Fullerton, CA). The analyses were repeated at least twice to ensure reproducibility of the results.

Data analysis and genetic relationships. The genetic analysis program "IDENTITY" 1.0 [9] was used for the calculation of number of alleles, expected and observed heterozygosity. Genetic similarity was determined by the program "MICROSAT" (version 1.5) using proportion of shared alleles, which was calculated by using "ps (option 1 – (ps))". The results were then converted to a similarity matrix, and a dendrogram was constructed with the UPGMA method [10] using the software NTSYS-pc (Numerical Taxonomy and Multiware Analysis System, version 2.0). Polymorphic Information Content (PIC) was calculated according to Kalinowski et al. [11]. The principal coordinate analysis (PCoA) was performed to show the relationships and differentiation of the wild apricot genotypes in a three-dimensional array of eigenvectors using the DCENTER and EIGEN modules of NTSYS-pc 2.10e software.

Results and discussion. A set of 16 SSR markers evenly distributed in 8 chromosomes was used to identify 29 wild apricot genotypes. All of the SSR markers produced clear bands and thus the polymorphism of wild apricot germplasm was effectively analyzed.

In total, the markers revealed 98 alleles ranging from five allele per loci (pchgms1, UDP96-001, UDP96-008, UDP96-019 and UDP98-405) to 9 alleles per loci (PS12A02 and UDP98-409), with an average of 6.13 alleles per loci across all markers (Table 2). Akpinar et al. [12] characterized 25 Turkish and 4 foreign apricot genotypes using eight SSR loci. They reported the number of alleles from 4 to 10, with an average allele number of 6.37 indicating slightly higher values than our results. Gurcan et al. [13] found very high alleles per marker (12.78 alleles per marker) in 278 apricot accessions originated from Turkey, USA, EU and Pakistan. Yilmaz et al. [14] used 96 apricot genotypes in SSR analysis and 16 SSR primers yielded 83 polymorphic bands, with an average of 5.19. Among the SSR loci, UDP98-409 and pchgms-2 generated the highest number of alleles (7), while locus UDP96-019 had the lowest one (2 alleles) which indicates similarities with our study. Khadivi-Khub et al. [15] reported a lower average number of alleles per loci (4.62 alleles) across 13 SSR markers while examining the genetic diversity among 37 Iranian apricot genotypes. The genetic diversity decreases from wild to cultivated apricots as well from East to West. In Europe most of the apricot cultivars are self-compatible showing narrow genetic diversity [5]. Hormaza [16] obtained an average of 4.10 alleles per locus from 20 loci in 48 apricot cultivars in Spain. Romero et al. [17] reported 34 alleles with an average of 3.10 per locus in 40 apricot cultivars grown in Spain. Zhang et al. [18] reported high allele diversity per locus, with an average allele number of 15.14 in China by using 97 wild apricots implying that Chinese apricot germplasm presented a high level of genetic diversity. Bakir et al. [19] studied Cappadocia region of middle Anatolia that hosts the third largest wild apricot population in Turkey. They used 44 wild apricot genotypes and 5 reference apricot cultivars for SSR (simple sequence repeats) analysis. They reported that the total number of alleles was 107, average number of alleles was 8.23; average He and Ho values were 0.722 and 0.669, respectively. Polymorphism information content (PIC) values varied between 0.471 and 0.845. They also found a high genetic diversity among wild apricot genotypes.

The lowest and the highest He values were 0.56 and 0.80 for UDP96-021 and pchgms2, respectively, with an average He value of 0.68. The lowest Ho for UDP98-409 was 0.47 while the highest one was 0.71 for pchgms4, with an average Ho value of 0.58 (Table 2). The high levels of observed and expected heterozygosities in the set of genotypes tested indicate the potential use of this set of SSR markers to investigate the population genetic structure in *P. armeniaca*. Zhang et al. [18] reported average Ho and He values as 0.55 and 0.61 in China by using 97 wild apricot genotypes. They showed that high genetic diversity indices

support that the apricot population in the Tien-Shan Mountains still maintains a high level of genetic diversity. Compared to the wild Siberian apricot ($He = 0.71$), the genetic diversity of apricot in our study is slightly lower, which may be the result of years of deforestation, overgrazing, and reduced areas of apricot resources, leading to reduced population genetic diversity [20]. Gurcan et al. [13] used 278 apricot accessions originated from Turkey, USA, EU and Pakistan and reported average He and Ho values as 0.75 and 0.63, respectively. Akpinar et al. [12] characterized 25 Turkish and 4 foreign apricot genotypes using eight SSR loci. They found average He and Ho of 0.66 and 0.65, respectively, indicating some similarities with our study.

Polymorphic information content ranged from 0.53 (pchgms3) to 0.84 (UDP96-010) with an average PIC of 0.66 across all markers. The discrimination power (average PIC) of the markers observed in the present study was lower than that of Gurcan et al. [13] who found higher PIC values (average 0.71) in 278 apricot accessions originated from very diverse geographical regions including Turkey, USA, EU and Pakistan. Khan et al. [21] used 34 apricot accessions from 14 countries representing the European, Irano-Caucasian, Central Asian and North American cultivars and one sample of *P. x dasycarpa*, *P. brigantiaca* and reported average He and Ho values as 0.82 and 0.64, respectively. The higher PIC observed in the current study supports evidence of greater allelic richness and gene diversity in wild apricots than in cultivated or landrace genotypes [18]. This was expected because of seed propagation in wild apricot populations compared to cultivated apricots, which are primarily clonally propagated. In all individuals, the observed heterozygosity (Ho) was lower than the expected heterozygosity (He), which can result from an intensive selection process.

Genetic analysis of 29 wild apricot genotypes revealed three distinct clusters (Fig. 1) and the examined genotypes were differentiated based on similarity coefficients. The dendrogram revealed a clear separation of most Turkish wild apricot genotypes (Fig. 1). Cluster I contained genotypes sampled from Aras region (K1–K9) and further diverged into two sub-clusters. The closest genotypes within cluster I were K4 and K5 with 0.91 similarity ratio. Cluster II contained only the genotype of samples from Coruh valley (C1–C7) and further diverged into two sub-clusters. The closest genotype pairs were C1 and C3 with 0.84 similarity ratio. Cluster III was the largest and was divided into two sub-clusters, and contained only the genotypes from Erzincan plain. The closest genotype pairs were E3 and E7 with 0.90 similarity ratio (Fig. 1). The dendrogram obtained after processing the experimental data clearly indicates the heterogeneity and geographical isolation of the seed propagated wild apricot genotypes (Fig. 1). The results also clearly revealed that the highest proportion of genetic variation is between regions rather than within regions. In other words genotypes are clustered in the same group reflecting the low degree of variability. We found some similarities with SSR and morphological data. For example the closest genotypes in cluster I K4

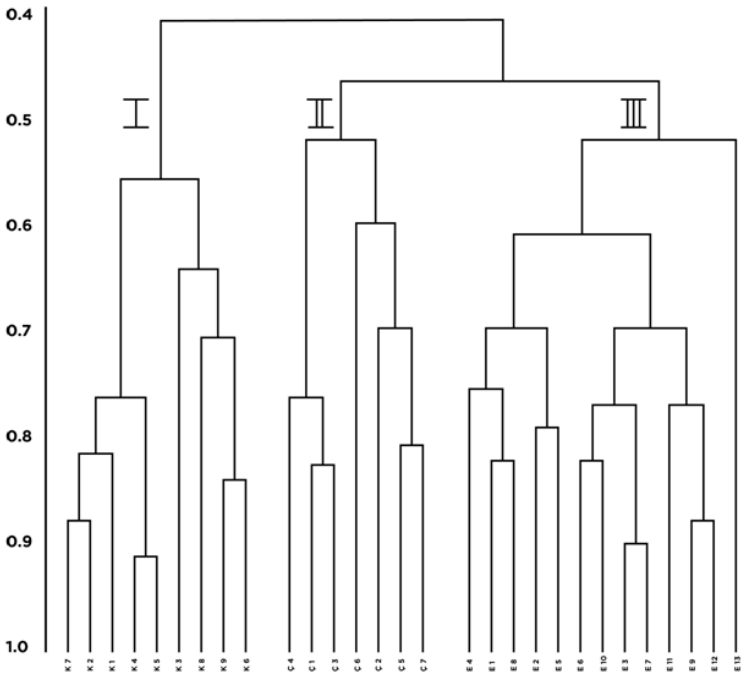


Fig. 1. The UPGMA dendrogram based on simple matching similarity matrix obtained using 16 SSR markers, illustrating the relative similarity among 29 wild apricot genotypes

and K5 and in cluster III E3 and E7 had early ripening, ovate fruit and spreading tree growth habit characteristics. E3 genotype distinctly separated from the other genotypes (Fig. 1). It has also distinct morphological characters such as upright to spreading tree growth habit, very late ripening time and light green fruit ground colour (Table 1).

In this study, microsatellite markers could potentially provide consistent information for identification and are valuable tools for studying the genetic relationships between apricot genotypes. More primers and other Turkish or foreign genotypes may be necessary for future researchers and should be compared with the present assay.

Principal coordinate analysis (PCoA) was applied to the raw data from the SSR "1" and "0" matrix by using NTSYS 2.10e software, and the contribution rates of the first 3 principal coordinates were 39.67%, 22.47%, and 13.80%, respectively, accounting for 75.94% of the genetic similarity variance. These wild apricot genotypes samples were partitioned into 2 distinct groups. PCoA Group 1 included 12 samples. PCoA Group 2 included 17 samples (Fig. 2) and in general were consistent with the results of cluster analysis. Group 1 was characterized by early ripening time. The genetic variation was higher in Group 2 than in Group 1. The markers employed here should be useful for the characterization and comparison

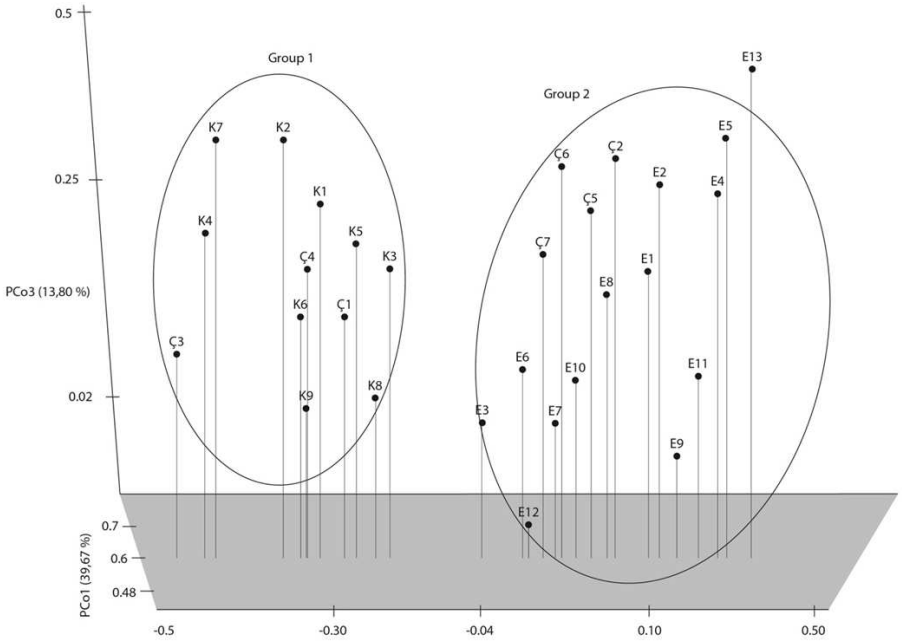


Fig. 2. PCoA plot for the 29 analyzed wild apricot genotypes (the genotypes number codes on the plot refer to Table 1)

of wild apricot germplasm collections and for the detection of propagation errors. The evaluation of the molecular diversity of wild apricot genetic resources is important for the optimal development of programmes aimed at conservation. These markers were able to identify uniquely all the wild apricot genotypes studied.

Conclusion. The results of the current study revealed significant genetic diversity among wild apricot accessions in Coruh valley, Aras valley, and Erzincan plain in Eastern Turkey. It is therefore imperative to undertake germplasm conservation while paying attention to the distinct accessions from each locality. Furthermore, consumer-oriented morphological traits observed in these accessions will greatly benefit breeding efforts to improve existing apricot cultivars. In our set of apricot cultivars, SSR markers detected a considerable polymorphism and were efficiently used for fingerprinting purposes. High allele number and heterozygosity reflect the ability of SSR markers, to provide unique genetic profiles for individual plant genotype. The high diversity of apricots available in Eastern Turkey should be included in a responsible manner into breeding programmes.

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