Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

SSR-based molecular analysis of economically important Turkish apricot cultivars

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Genet. Mol. Res. 9 (1): 324-332 (2010)
Received October 30, 2009
Accepted November 27, 2009
Published February 23, 2010

ABSTRACT. Turkey is not only the main apricot (*Prunus armeniaca*) producer and exporter in the world, but it also has a wide variety of apricot germplasms, owing to its close proximity to the centers of apricot origin. However, there is little or no genetic information on many apricot cultivars that are extensively cultivated in Turkey. We examined the genetic relatedness of 25 Turkish and four exotic apricot cultivars using SSR (simple sequence repeat) markers that were either previously developed for apricot, or for peach (*P. persica*), a close relative of apricot. Allele diversity (with an average allele number of 6.37) at the SSR loci and the heterozygosity rates (with an average Ho value of 0.648) of these cultivars were found to be higher than in previous studies that used the same loci for apricot. This fact might be attributed to the analysis of different numbers of accessions in the different studies. No correlations were found between the genetic relatedness and the geographical distributions of these cultivars. The data reported here will assist in the prevention of confusions in the apricot propagation and breeding in Turkey. The findings can also be directly compared with other studies that used the same SSR markers on apricot.

Key words: Apricot; Simple sequence repeats; Turkey

INTRODUCTION

Apricot (*Prunus armenica* L.), a fruit species of the family Rosaceae, genus *Prunus* L., is widely distributed in the Mediterranean region and the Middle East, as well as Armenia, India, Pakistan, China, and Japan. Apricots have also been taken to the New World by settlers and are now grown mainly in California (Hormaza et al., 2007).

Apricots are consumed as fresh fruit, canned, or frozen, but a large portion of the worldwide apricot production is preserved primarily by drying. Turkey is the top apricot-producing country in the world (http://en.wikipedia.org/wiki/Apricot). It accounts for about 12% (579,000 tons) of world's annual apricot production (Anonymous, 2003). Apart from the Black Sea region and some parts of Eastern Turkey, apricots are widely cultivated in many parts of the country. In the East Anatolia region, apricots are mainly grown to produce dried fruits. Fresh fruit production from apricots mainly occurs in the Marmara (Thrace) region, while apricots are grown for both fresh and dried fruit production in Central Anatolia. Precocious fruits of apricot from the Mediterranean and Aegean regions of Turkey are also consumed freshly in early spring (Asma et al., 2007). The Malatya province located in East Anatolia with diverse apricot germplasm is the center of apricot production, and this region alone provides nearly 50% of all apricot production in Turkey (Anonymous, 2003).

Although Turkey is not the center of origin for apricots, its unique location on the historic Silk Road between Armenia (the center of apricot origin) and the Europe has probably contributed to the formation of a rich genetic diversity of apricots in Turkey (Özbek, 1978). The climatic suitability of this region for apricot production, combined with social and economic factors, has further diversified apricot production. Some of the world's most famous apricot genotypes for dried (e.g., cvs. HacıHaliloğlu, Kabaaşı, Çataloğlu) and fresh (e.g., cvs. Hasanbey, Alyanak, Şekerpare) fruit production are widely cultivated in the region (Hormaza et al., 2007).

Although apricots are important agricultural export commodities for the Turkish economy, the genetic relatedness of current apricot cultivars grown in the country is largely unknown. This information would greatly assist in the identification, breeding and germplasm preservation of Turkish apricots.

Molecular markers, which show independence from the developmental stage and environmental factors, provide highly discriminatory information and, therefore, are frequently used for genetic studies. Randomly amplified polymorphic DNAs (Badenes et al., 1998; Mariniello et al., 2002), amplified fragment length polymorphisms (Hagen et al., 2002; Hurtado et al., 2002; Geuna et al., 2003), and simple sequence repeats (SSRs) (Hormaza, 2002; Zhebentyayeva et al., 2003; Sánchez-Pérez et al., 2005; Maghuly et al., 2005; Pedryc et al., 2009) have been previously used for apricots. However, to the best of our knowledge, no reports have so far been published on genetic characterization of Turkish apricot genotypes.

In this study, 29 economically important apricot genotypes that included 25 genotypes native to Turkey as well as four exotic cultivars were genetically characterized using eight SSR loci. The allele sizes generated by these markers for each cultivar and the genetic relationships among cultivars were determined. The correlation between genetic relatedness of Turkish apricot cultivars and their geographical distributions is also discussed.

MATERIAL AND METHODS

Plant material

The apricot cultivars used in this study were obtained from Horticultural Research Institute, Egirdir, Isparta, Turkey. A list of these cultivars as well as several of their pomological and phenological characteristics are presented in Table 1. Of the exotic apricot genotypes used, Feriana and Beliana were derived from a cross between Hamidi, a Tunisian apricot cultivar, and Canino, a Spanish apricot cultivar, also included in our experiments (Batmaz, 2005). A fourth exotic apricot genotype studied here is Fracasso, an Italian apricot cultivar with unknown descent.

Table 1. Apricot cultivars with their several phenological and morphological characteristics.

No.	Cultivar name	Phenology				Pomology								Origin (city)
		Bud bursting	First blooming	Full blooming	Ripening	Fruit shape	Fruit taste	Pit shape	Kernel flavor	Pit separation	Skin color	Fruit firmness	Usage	
1	Alyanak	17-30M	30M-3A	3-10A	15-19Jy	Ovate	Sourish	Ovate	Bitter	Free	Orange	Soft	F	İzmir
2	Çekirge-52	21-31M	30M-3A	3-9A	13Jy	Ovate	Sweet	Round	Sweet	Semi-Joint	Orange	Soft	F	Bursa
3	Çöloğlu	27-29 M	2-4A	8-10A	12Jy	Round	Sweet	Round	Sweet	Free	Yellow	Soft	F-D	Malatya
4	Çataloğlu	29M-2A	29M-2A	6-8A	15Jy	Ovate	Sweet	Ovate	Sweet	Free	Yellow	Good		Malatya
5	Ethembey	22-30M	27M-3A	31M-10A	13Jy	Oblong	Sweet	Ovate	Bitter	Free	Yellow	Soft	F	Edirne
6	Hacı Haliloğlu	29M-2A	8-10A	12-14A	13-15Jy	Ovate	Sweet	Ovate	Sweet	Free	Yellow	Good	D	Malatya
7	Hacıkız	31M-1A	4-6A	10-14A	14Jy	Ovate	Sweet	Ovate	Sweet	Free	Yellow	Good	F-D	Malatya
8	Hasanbey	27-31M	30M-3A	4-9A	13Jy	Oblong	Sweet	Oblong	Sweet	Free	Yellow	Good	F-D	Malatya
9	İsmailağa	24-30M	28M-4A	3-9A	16Jy	Oblong	Sweet	Oblong	Sweet	Free	Yellow	Good	F-D	Malatya
10	Kabaaşı	18-26M	23-30M	27M-4A	13Jy	Ovate	Sweet	Ovate	Sweet	Free	Yellow	Good	D	Malatya
11	Macar	21-30M	27M-4A	31M-9A	14Jy	-	-	-	-	-	-	-	F	Unknown
12	M. Eriği	30M-2A	3-5A	8-10A	-	Ovate	-	Ovate	Sweet	Free	Yellow	-	F-D	Erzincan
13	Mektep	21-31M	26M-2A	30M-6A	20Jy	-	-	-	-	-	-	-	F	İzmir
14	Sakıt-2	22-27M	27-30M	30M-4A	19Jy	Oblong	-	Ovate	Sweet	Free	Yellow	-	F	Hatay
15	Sakıt-6	21-31M	28M-2A	31M-8A	18Jy	-	-	-	-	-	-	-	F	Hatay
16	Sakıt-7	23-28M	29-31M	1-8 A	16Jy	-	-	-	-	-	-	-	F	Hatay
17	Soğancı	28-30M	2A	5-7A	-	Round	Sweet	Round	Sweet	Semi-Joint	Yellow	Good	D	Malatya
18	Şekerpare	22-28M	30-31M	3-4A	10-13Jy	Ovate	Sweet	Round	Sweet	Free	Yellow	Middle	F	Iğdır
19	Tokaloğlu	22-24M	27-28M	1-4A	-	Ovate	Sweet	Elliptic	Sweet	Semi-Joint	Yellow	Soft	F	Erzincan
20	Şahinbey	-	-	-	-	-	-	-	-	-	-	-	-	Mersin
21	Çağrıbey	-	-	-	-	-	-	-	-	-	-	-	-	Mersin
22	Çağataybey	-	-	-	-	-	-	-	-	-	-	-	-	Mersin
23	Dr. Kaşka	-	-	-	-	-	-	-	-	-	-	-	-	Mersin
24	Alata Yıldızı	-	-	-	-	-	-	-	-	-	-	-	-	Mersin
25	Aprikoz	20M-1A	27M-3A	31M-3A	-	Elliptic	Sweet	Oblong	Sweet	Free	Yellow	Middle	F	Iğdır
26	Beliana*	22-31M	25-29M	31M-4A	23J	Round	Sweet	Round	Bitter	Free	Yellow	Good	F	Unknown
27	Canino	20-26M	26-29M	30M-5A	5Jy	Ovate	-	Ovate	Sweet	Semi-Joint	Orange	Soft	F	Spain
28	Feriana*	21-27M	29M-1A	2-9A	30J-2Jy	Round	Sourish	Oblong	Bitter	Free	Yellow	Good	F	Unknown
29	Fracasso	18-29M	22M-2A	28M-4A	16-17Jy	Round	Sweet	Ovate	Bitter	Joint	Yellow	Soft	F	Italia

M: March, A: April, J: June, Jy: July, F: Fresh, D: Dried. *These cultivars are derived from a Hamidi x Canino cross; Hamidi is a Tunisian cultivar.

DNA extraction

DNA was extracted from young leaf tissue following the procedure described by Lefort et al. (1998). Concentration and purity of the DNA were determined with a NanoDrop® ND-1000 spectrophotometer.

SSR analysis

Eight SSR markers, namely UDAp-401 and UDAp-404 from apricot (Messina et al., 2004), UDP96-010, UDP96-019, UDP98-406 (Cipriani et al., 1999), Pchgms1, Pchgms2 and Pchgms3 from peach (Sosinski et al., 2000) were used in this study. Polymerase chain reactions (PCR) and SSR analysis were performed as previously described by Şelli et al. (2007). Briefly, PCR amplifications were performed in a reaction volume of 10-µL reaction mixture containing 15 ng DNA, 5 pmoL of each primer, 0.5 mM dNTP, 0.5 unit GoTaq DNA Polymerase (Promega, Madison, WI, USA), including 1.5 mM $MgCl_2$. The forward primers of each pair were labeled with WellRED fluorescent dyes D2 (black), D3 (green) and D4 (blue) (Proligo, Paris, France). The PCR conditions consisted of an initial cycle of 3 min at 94°C, followed by 35 cycles of 1 min at 94°C, 1 min at 55-60°C and 2 min at 72°C, with a final extension at 72°C for 10 min. PCR products were diluted with sample loading solution, followed by the addition of Genomelab DNA Standard Kit-400 and electrophoresed in the CEQ 8800XL capillary DNA analysis system (Beckman Coulter, Fullerton, CA, USA). Allele sizes were determined for each SSR locus using the Beckman CEQ fragment analysis software. In each run, Canino was included as a reference cultivar. The analyses were repeated at least twice to ensure reproducibility of the results.

Genetic analysis

Number of alleles, allele frequency, expected (He) and observed heterozygosity (Ho), estimated frequency of null alleles, and probability of identity (PI) were calculated for each locus using the "IDENTITY" 1.0 program (Wagner and Sefc, 1999) according to Paetkau et al. (1995). The proportion of shared alleles was calculated using ps (option 1 - (ps)) as described by Bowcock et al. (1994) as genetic dissimilarity by the Microsat program (version 1.5) (Minch et al., 1995). These data were then converted to a similarity matrix, and a dendrogram was constructed with UPGMA (unweighted pair-group method with arithmetic mean) (Sneath and Sokal, 1973), using the NTSYS-pc software (Numerical Taxonomy and Multiware Analysis System) (version 2.0) (Rohlf, 1988).

RESULTS

Allele sizes (bp) generated by 8 SSR markers on 29 apricot cultivars are given in Table 2. A total of 51 alleles were obtained by these 8 SSR markers. The number of alleles ranged from 4 (UDP98-406) to 10 (UDAp-404), with an average allele number of 6.37. The lowest and the highest He values were 0.392 and 0.839 for UDP96-019 and UDP96-010, respectively, with an average He value of 0.657. The lowest Ho for UDAp-401 was 0.379 while the highest one was 0.896 for UDP96-010, with an average Ho value of 0.648 (Table 3).

Table 2. Allele sizes (bp) of apricot cultivars at 8 simple sequence repeat loci.

No.	UDAp-401		UDAp-404		UDP96-010		UDP96-019		UDP98-406		Pchgms1		Pchgms2		Pchgms3	
1	205	205	150	158	84	94	165	165	88	88	160	166	145	145	193	195
2	173	205	150	150	86	86	165	209	88	102	160	166	145	145	187	193
3	213	213	152	158	80	86	165	209	84	88	166	170	151	173	191	195
4	211	211	152	158	80	86	165	209	88	102	166	174	173	173	187	193
5	173	205	150	150	84	86	165	209	88	102	160	166	145	145	187	193
6	205	205	150	150	84	86	165	209	88	102	160	166	145	145	187	193
7	173	215	158	158	80	80	165	209	98	102	170	174	159	173	193	195
8	201	215	146	170	78	86	165	165	84	88	160	166	145	173	187	195
9	215	215	180	182	86	94	165	165	102	102	166	174	173	173	187	195
10	213	213	180	182	86	86	165	165	84	102	166	170	145	173	193	195
11	173	205	148	150	84	86	165	209	88	102	160	166	145	145	187	193
12	205	205	158	158	84	86	165	209	84	88	160	166	159	159	193	195
13	205	205	158	170	94	98	165	165	88	88	160	160	145	159	187	195
14	205	205	150	158	80	94	165	165	88	98	160	166	145	145	195	195
15	205	205	158	158	98	100	165	165	88	98	166	166	145	145	193	195
16	205	205	158	182	94	100	165	165	84	88	166	174	145	159	187	195
17	213	213	158	182	86	98	165	185	88	98	160	170	159	173	195	195
18	173	215	158	158	78	80	165	165	88	98	166	166	145	159	193	195
19	173	205	158	158	84	94	165	165	84	88	160	174	159	171	195	195
20	173	205	146	158	98	100	165	165	88	98	160	166	145	163	195	195
21	205	205	146	158	78	80	165	165	88	98	166	168	145	163	195	195
22	201	205	158	158	80	100	165	181	98	98	166	168	145	159	193	195
23	171	201	146	182	80	84	181	209	88	98	160	166	159	163	193	195
24	205	205	146	146	78	80	165	165	88	88	160	166	145	159	193	195
25	205	205	158	158	94	98	165	209	88	88	160	170	145	159	193	195
26	205	205	168	170	80	100	165	165	102	102	160	160	159	173	195	197
27	205	213	146	156	80	96	165	189	88	88	166	166	145	147	195	195
28	205	205	168	170	78	86	165	165	102	102	160	166	159	173	195	195
29	205	205	158	158	94	100	165	165	88	88	166	174	145	173	193	195

Table 3. Simple sequence repeat (SSR) loci, number of alleles (n), expected heterozygosity (He), observed heterozygosity (Ho), probability of identity (PI), and the frequency of null alleles (r) of 29 cultivars analyzed at 8 SSR markers.

SSR locus	N	He	Ho	PI	r
UDAp-401	7	0.635	0.379	0.215	0.156
UDAp-404	10	0.759	0.568	0.125	0.098
UDP96-010	8	0.839	0.896	0.084	-0.031
UDP96-019	5	0.392	0.448	0.477	-0.039
UDP98-406	4	0.659	0.655	0.266	0.002
Pchgms1	5	0.672	0.827	0.273	-0.092
Pchgms2	7	0.694	0.655	0.236	0.023
Pchgms3	5	0.613	0.758	0.333	-0.089
Total	51	5.256	5.186		
Average	6.37	0.657	0.648		

As far as the PI values are considered, the most informative loci were UDAp-404 (PI: 0.125) with 10 alleles and UDP96-010 (PI: 0.084) with 8 alleles. UDP96-019 (PI: 0.477) with 5 alleles was found to be the least informative locus (Table 3).

As for allele frequencies, the 165-bp allele at the UDP96-019 locus was the most frequently observed allele with a frequency of approximately 76%. The least frequent loci (with a frequency of 1.7%) were as follows: the 171-bp allele at the UDAp-401 locus, the 148- and 156-bp alleles at the UDAp-404 locus, the 96-bp allele at the UDP96-010 locus, the 191- and

197-bp alleles at the UDP96-010 locus, and the 147-, 151- and 171-bp alleles at the Pchgms2 locus (Table 4).

Table 4. Allele frequencies of 8 simple sequence repeat loci.

No.	UDAp-401	*alf*	UDAp-404	*alf*	UDP96-010	*alf*	Pchgms3	*alf*	UDP98-406	*alf*	Pchgms1	*alf*	UDP96-019	*alf*	Pchgms2	*alf*
1	171	*0.017*	146	*0.120*	78	*0.086*	187	*0.155*	84	*0.103*	160	*0.327*	165	*0.758*	145	*0.448*
2	173	*0.120*	148	*0.017*	80	*0.206*	191	*0.017*	88	*0.500*	166	*0.448*	181	*0.034*	147	*0.017*
3	201	*0.051*	150	*0.155*	84	*0.120*	193	*0.275*	98	*0.172*	168	*0.034*	185	*0.017*	151	*0.017*
4	205	*0.568*	152	*0.034*	86	*0.241*	195	*0.534*	102	*0.224*	170	*0.086*	189	*0.017*	159	*0.241*
5	211	*0.034*	156	*0.017*	94	*0.137*	197	*0.017*			174	*0.103*	209	*0.172*	163	*0.051*
6	213	*0.120*	158	*0.431*	96	*0.017*									171	*0.017*
7	215	*0.086*	168	*0.034*	98	*0.086*									173	*0.206*
8			170	*0.068*	100	*0.103*										
9			180	*0.034*												
10			182	*0.066*												

alf: allele frequency

Genetic similarity of apricot genotypes ranged from 18 to 94%. Native apricot cultivars in general showed a low level of similarity to exotic ones. Nevertheless, Fracasso, an Italian cultivar, clustered with the Turkish cultivar Sakıt-6 (15). For exotic cultivars, the highest similarity (75%) was found between Belina and Feriana, constituting a dual group in the dendrogram shown in Figure 1. In native genotypes, the highest similarity was found between Ethembey (5)-Hacıhaliloğlu (6), Ethembey (5)-Macar (11) and Ethembey (5)-Çekirge52 (2), with a genetic similarity of 94% (Figure 1).

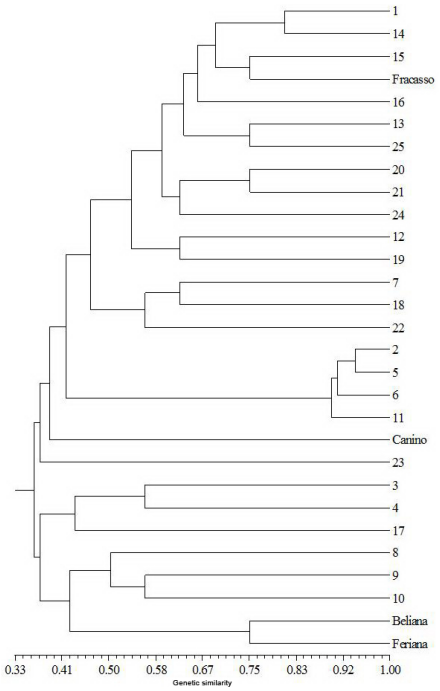

Figure 1. Genetic similarity (%) dendrogram of apricot cultivars used in the present study.

DISCUSSION

In this study, we were able to amplify DNA fragments from apricot using SSR markers, some of which have been previously developed for peach, another *Prunus* species (Cipriani et al., 1999; Sosinski et al., 2000). However, the average number of alleles detected in our study from apricot by these markers was different from peach. For example, Pchgms1, Pchgms2 and Pchgms3 produced 4, 2 and 3 alleles, respectively, for peach (Sosinski et al., 2000), while only 2, 1 and 2 alleles, respectively, for cherry (*Prunus avium* L.) (Wünsch and Hormaza, 2002). In apricot genetic identification studies, the same loci (Pchgms1, Pchgms2 and Pchgms3) yielded 4, 5 and 3 alleles (Hormaza, 2002) while in the present study, the numbers of alleles revealed were 5, 7 and 5, respectively. These findings show that Pchgms1, Pchgms2 and Pchgms3 produced more alleles in apricot than in the other *Prunus* species. There is also evidence that Ho rates for these loci were higher in the present study than those found in earlier studies (Sosinski et al., 2000; Wünsch and Hormaza, 2002).

The SSR loci used in this study revealed higher heterozygosity rates in Turkish apricots than those in other *Prunus* species, including apricots from other regions of the world, suggesting that the apricot germplasm used in this study was probably more diverse (or heterozygous) than those used in other studies (Sosinski et al., 2000; Wünsch and Hormaza, 2002; Hormaza, 2002; Romero et al., 2003; Sánchez-Pérez et al., 2005). The high heterozygosity levels and allele numbers observed in the current study were particularly useful for efficient genetic identification of Turkish apricot germplasm. The high level of genetic identity (94%) found between Ethembey (5) and Çekirge-52 (2), and between Ethembey (5) and Hacıhaliloğlu (6) also correlated well with several common pomological properties of these cultivars, such as taste, color and seed shape (Table 1).

The relatively high genetic similarity (75%) between Çağrıbey (21; a Sakıt-6 x P. de Colomer cross) and Çağataybey (22; a Sakıt-2 x P. de Colomer cross) could be attributed to the fact that these two cultivars had the same pollinator (Batmaz, 2005). The relatively high similarity (75%) between Beliana (a Hamidi x Canino cross) and Feriana (a Hamidi x Canino cross) could also be due to the fact that these cultivars had the same pollinator (Batmaz, 2005).

Sakıt-2 (14), Sakıt-6 (15) and Sakıt-7 (16) were relatively less similar genetically and formed a homonymous group. It is interesting that Sakıt-6 (15) and Sakıt-7 (16) were also substantially similar to the exotic cultivar Fracasso, although no association between these cultivars has been previously reported.

The He values of UDAp-401, UDAp-404, UDP98-406, and Pchgms2 were higher than the Ho values. Previous reports by Zhebentyayeva et al. (2003) and Messina et al. (2004) also found relatively high He in some of these SSR loci in apricots. In this study, the frequency of null alleles at these four loci was positive, but these low values suggest the absence of null alleles (Table 3). Except for the above-mentioned apricot cultivars, in general, the genetic similarity among the cultivars was low and no synonymous cultivars were found, implying that Turkey is a rich source of diverse apricot germplasm. No correlation was found between the genetic relatedness and the geographical distributions of the cultivars.

Our findings reported here would be useful for better management of Turkish apri-

cot germplasm. Notably, the data reported here could be directly compared to other studies, which have used or will be using the same SSR markers in other apricot genotypes or could be integrated into future studies investigating the genetic diversity of apricots from a broader geographical region.

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