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## Characterization of Wild Apricot (*Prunus armeniaca* L.) Genotypes Selected from Cappadocia Region (Nevşehir-Turkey) by SSR Markers

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### ABSTRACT

Cappadocia region of Anatolia hosts the third largest wild apricot population in Turkey. The objective of the study was to characterize 44 wild apricot genotypes selected from Cappadocia Region (Nevşehir-Turkey) as prominent with their late flowering, resistance to spring late frosts, large fruit sizes and/or late fruit ripening characteristics and 5 reference apricot cultivars ('Hacıhaliloğlu', 'Kabaası', 'Hasanbey', 'Aprikoz' and 'Levent') with SSR (simple sequence repeats) markers. A total of 16 SSR primers were used and 13 of them were successfully amplified. 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. There was a quite high genetic diversity among wild apricot genotypes that genetic similarity values varied between 12 and 96%. Homonymous and synonymous genotypes were not encountered.

Keywords: Wild apricot; *Prunus armeniaca*; Genetic diversity; Genetic relationship; Molecular characterization; SSRs

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### 1. Introduction

Vavilov (1951) indicated the origin centers of culture apricots (*Prunus armeniaca* L.) as China, Central Asia and defined Near-East centers extending from Northeastern Iran to Caucasus and Central Anatolia as the secondary origin center of cultured forms. Kostina (1969) divided *P. armeniaca* species into 4 large eco-geographical groups and 13 regional sub-groups and placed Turkey into Iran-Caucasus eco-geographic group (Layne et al 1996; Zhebentyayeva et al 2012). Anatolia (Turkey) is located within the

secondary origin center of apricots, thus has a great genetic diversity (Ercisli 2004). Nevşehir province is located right at the center of Cappadocia region of Anatolia, and the province hosts the 3<sup>rd</sup> largest wild apricot population with about 145000 trees (TUIK 2017). This population is characterized with late flowering, resistance to spring late frosts, large fruits and late ripening. Thus the population exhibits a large variation in fruit physical and quality attributes. Such a diverse population was evaluated for the first time by Dumanoğlu et al (2018) within

the scope of a scientific research project, and superior genotypes were identified. These genotypes were then put under protection in a collection orchard. The genotypes constitute significant materials for apricot breeding studies and genetic relationships among these genotypes should be identified with further molecular techniques. Microsatellites or simple sequence repeats (SSRs) are short repeat sequences (1-6 base length) and have co-dominant characteristics, greater polymorphism ratios, are abundant in genome and have quite high repeatability. Therefore, they have a significant place among DNA markers (Litt & Luty 1989; Gupta et al 1996). These markers are commonly used in identification of species, preservation of genetic materials, population genetics, quantitative trait loci mapping, marker assisted selection and similar studies. SSR markers are also used in genetic characterization of *Prunus* species, including apricots. However, SSR markers were not developed at the same rates for each one of the significant species (apricot, peach, plum, and almond), thus potential use of SSR markers of a species in other *Prunus* species (cross-transferability) have become a significant issue (Hormaza 2002; Romero et al 2003; Zhebentyayeva et al 2003; Hagen et al 2004; Messina et al 2004; Mnejja et al 2005; Sanchez-Perez et al 2005; Ruthner et al 2006; Bouhadida et al 2009; Wünsch 2009; Akpınar et al 2010; Bourguiba et al 2010; Liu et al 2013; Wang et al 2014; Eroglu & Cakir 2015; Gürcean et al 2015; Murathan et al 2017).

In this study, genetic relationships between wild apricot genotypes selected from the wild apricot gene sources of Nevşehir province with regard to late flowering, resistance to spring late frosts, large fruits and or late fruit ripening characteristics were identified with SSR markers developed from *P. armeniaca* and *P. persica*.

## 2. Material and Methods

### 2.1. Plant material and DNA isolation

In this study, 44 wild apricot genotypes selected from Nevşehir (Cappadocia Region-Turkey) locality and the reference apricot cultivars of

‘Aprikoz’, ‘Kabaası’, ‘Hasanbey’, ‘Hacıhaliloğlu’ and ‘Levent’ were used as the plant material. DNA isolations were performed from fresh shoot tips and young leaf samples collected from the genotypes (Lefort et al 1998). DNA purity and concentrations were determined in ND-1000 spectrophotometer and isolated DNA was visually controlled in 1% agarose gel.

### 2.2. SSR reactions

A total of 16 SSR loci were selected as of 10 *P. armeniaca* (apricot), 4 *P. persica* (peach) and 2 *P. armeniaca* EST-SSR loci (Table 1). Selected SSR loci were tested and the polymorphic ones were used in genetic identifications. PCR amplifications were performed by using M13-tailed primer according to the methods described by Schuelke (2000) in *Prunus* genotypes. A tail (M13 universal sequence (-21), TGTAACGACGGCCAGT) was added to the 5' end of each forward primers. PCR amplifications were performed in 15 µL reaction mixture containing 90 ng genomic DNA, 0.1 µM of each SSR primer, 0.1 µM labelled M13 (-21) universal primer, 0.2 mM of each dNTPs, 1X DreamTaq Green Buffer (includes MgCl<sub>2</sub> at a concentration of 2 mM) (Thermo Scientific) and 0.5 U DreamTaq DNA Polymerase (Thermo Scientific). The amplification program consisted of an initial step of 3 min at 94 °C, followed by 35 cycles of 1 min at 94 °C, 1 min at 50-66 °C, 2 min at 72 °C, followed by 8 cycles of 1 min at 94 °C, 1 min at 53 °C, 2 min at 72 °C, and a final extension at 72 °C for 10 min. The M13 (-21) primer was 5'-fluorescently tagged with HEX, 6-FAM or ROX to facilitate multiplexing. A set of three PCR products (0.5 µL each) was mixed with 0.5 µL GeneScan-600 LIZ size standards (Applied Biosystems, USA) and 9.5 µL Hi-Di™ formamide (Applied Biosystems) and denatured at 95 °C for 5 min, chilled on ice and electrophoresed on the Applied Biosystems Prism 3500 Genetic Analyzer System (Applied Biosystems, USA). GENEMAPPER software v5.0 (Applied Biosystems, USA) was used to determine fragment size.

**Table 1- SSR markers used for characterization of wild apricot genotypes selected from Nevşehir locality**

No	Locus	Primer sequences (5'→3')	Tm (°C)	Species	Reference
1	UDAp-407F*	ttctgctactacaatcgtgttctc	56	<i>Prunus armeniaca</i>	Messina et al (2004)
	UDAp-407R	agagcaccaggctcttctgg			
2	UDAp-410F*	ttgttgacaagaagaaacaaagc	56	<i>Prunus armeniaca</i>	Messina et al (2004)
	UDAp-410R	caacgggttggttcagaag			
3	UDAp-411F*	tcggtggagaagagactgg	56	<i>Prunus armeniaca</i>	Messina et al (2004)
	UDAp-411R	gtccccaccctttacaatg			
4	UDAp-414F*	caagcacaagcgaacaaaat	56	<i>Prunus armeniaca</i>	Messina et al (2004)
	UDAp-414R	ggtggtttcttatccgatgc			
5	UDAp-415F*	aactgatgagaaggggcttg	56	<i>Prunus armeniaca</i>	Messina et al (2004)
	UDAp-415R	actcccgcacattgtgcttc			
6	UDAp-418F*	cagaaatagccccagcacat	56	<i>Prunus armeniaca</i>	Messina et al (2004)
	UDAp-418R	ttcttgcgcaaaaact			
7	UDAp-420F*	ttcctgcttccctcattg	56	<i>Prunus armeniaca</i>	Messina et al (2004)
	UDAp-420R	cccagaactgattctgacca			
8	UDAp-423F*	ccatgtagaaactggctgagg	56	<i>Prunus armeniaca</i>	Messina et al (2004)
	UDAp-423R	cactcgactctctgcctct			
9	AMPA105F*	ctgctctcactcaactcaatgc	55	<i>Prunus armeniaca</i>	Hagen et al (2004)
	AMPA105R	ctcccctaccctctgtatctc			
10	AMPA096F*	ttttgtgccaagtagcatcag	55	<i>Prunus armeniaca</i>	Hagen et al (2004)
	AMPA096R	tcaactaaccaaaaggagtggc			
11	UDP96-010F*	cccattgtgtccacatctc	55	<i>Prunus persica</i>	Cipriani et al (1999)
	UDP96-010R	ttgatgattccatgcgtctc			
12	UDP96-019F*	ttggtcatgagctaagaaaaca	55	<i>Prunus persica</i>	Cipriani et al (1999)
	UDP96-019R	tagtggcacagcaacacc			
13	UDP98-406F*	tcggaaactggtagtatgaacaga	55	<i>Prunus persica</i>	Cipriani et al (1999)
	UDP98-406R	atgggtcgtatgcacagtca			
14	Ma040aF*	agaaattggagtgcacgtaac	55	<i>Prunus persica</i>	Yamamoto et al (2002)
	Ma040aR	acgtgatgagaagtagggag			
	EST-SSR Primers	tcggaaactggtagtatgaacaga	55	<i>Prunus persica</i>	Cipriani et al (1999)
15	AMPA116F*	attgaaggcccttatgtgag	55	<i>Prunus armeniaca-EST</i>	Hagen et al (2004)
	AMPA116R	caaaaaggcgttacagatgatg			
16	AMPA119F*	gtgccacttacctgttttagg	55	<i>Prunus armeniaca-EST</i>	Hagen et al (2004)
	AMPA119R	tcgacgatcagacttgctacag			

\*, Fluorescent labeled primer

### 2.3. Statistical analysis

For each locus, the expected heterozygosity (*He*), observed heterozygosity (*Ho*) and polymorphism information content (PIC) (Nei 1973) were calculated with PowerMarker V3.025 software (Liu & Muse 2005). The neighbor-joining (NJ) and unweighted pair-group method using arithmetic

average (UPGMA) were used to construct and draw a dendrogram from the genetic similarity matrix by using the MEGA6 (Tamura et al 2007) and PowerMarker software programs. Bootstrap analyses with 100 replicates were performed and a consensus tree was obtained to measure the confidence levels for the clusters.

### 3. Results

Allele sizes (bp, base-pair) for 13 SSR loci of wild apricot types selected from Nevşehir locality and reference apricot cultivars are provided in Table 2. Considering the success ratios of 16 SSR loci selected for genetic characterization of wild apricot types, 10-15 of them were thought to be used in genetic analyses. Thirteen SSR loci were successfully amplified. Of the remaining 3 SSR loci, UDP98-406 locus yielded successful PCR reactions, but was not able to be assessed well because of mixed peaks in capillary electrophoresis system. While PCR reactions of AMPA096 locus were unsuccessful, UDP96-019 locus was identified as monomorphic. Therefore, these 3 loci were not used in genetic analyses. The highest number of alleles was observed in UDAp-418 locus with 14 alleles (Table 3). It was followed by both UDP96-010 and AMPA105 loci with 10 alleles and the lowest number of alleles was observed in Ma040 locus with 5 alleles. Total number of alleles was 107 and average number of alleles was 8.23. Average *He* and *Ho* values were calculated 0.722 and 0.669, respectively. *He* values varied between 0.501 and 0.860 and *Ho* values varied between 0.449 and 0.816. Polymorphism information content (PIC) value varied between 0.471 (AMPA119) and 0.845 (UDP96-010) (Table 3).

Genetic similarity dendrogram indicated that genotypes were basically separated into 3 groups (Figure 1). When the dendrogram was evaluated based on the places from where the samples were collected, it was observed that grouping was independent from the sampling locations. Considering the place of reference apricot cultivars in the dendrogram, 'Hasanbey' was placed in the second group, and the other cultivars were placed in 3<sup>rd</sup> group. Reference cultivars were not placed in the first group.

Genetic similarity index values varied between 12 and 96% with the greatest similarity (96%) between wild apricot genotypes of #6 and #61. These genotypes were followed by the #32-#34 and #39-#68 with a similarity ratio of 92%. The lowest

genetic similarity (12%) was observed between the genotypes #13 and #38, #38 and #50, #38 and #64, and #45 and #47. Degree of genetic similarity was independent from the sampling locations. Genotypes #6 and #61, 45 km away from each other (Çavuşin and Gümüşkent, respectively) had the highest genetic similarity (96%) in the study. However, more distantly located (>65 km) genotypes #39 (Gümüşkent) and #68 (Çakıllı), or genotypes #32 and #34 at the same location (Yeşilöz) had the same genetic similarity level of 92%.

### 4. Discussion

Hormaza (2002) used 37 SSR loci developed from *Prunus* species to identify the genetic relationships among 48 apricot genotypes collected from different geographical regions. Of these loci, 31 were successfully amplified, 20 had repeatable polymorphic characteristics and a total of 82 alleles were identified in 48 genotypes. The common primer UDP96-100 was also found to be polymorphic in this study. Polymorphic UDP98-406 primer was not able to be assessed because of complex peak profile in our study. In another study, Romero et al (2003) used 16 SSR loci developed from peach genome to identify the relationships among 40 apricot genotypes collected from different eco-geographical regions. Of these loci, 11 presented polymorphism in apricot genotypes and allowed clear identification of each genotype. The common primer UDP96-010 similarly yielded the greatest separation power in this study. Zhebentyayeva et al (2003) tested 30 SSR loci developed through enriched library method from peach genome for 74 apricot genotypes. Of 30 SSR loci tested, 20 were amplified in apricot and 14 were reported to be used in separation of apricot genotypes and apricot germplasm diversity studies. Messina et al (2004) isolated 99 SSR loci from apricots and tested 20 of them in 16 apricot genotypes to determine polymorphism ratios. Of 20 SSR loci tested, 9 (UDAp-401, UDAp-404, UDAp-407, UDAp-410, UDAp-411, UDAp-414, UDAp-415, UDAp-418 and UDAp-420) were recommended to be used in apricot fingerprinting studies. Researchers also implied that 20% of

**Table 2- Allelic data (bp) for wild apricot genotypes of Nevşehir locality and standard apricot cultivars (A, 'Aprikoz'; L, 'Levent'; HB, 'Hasanbey'; HH, 'Hacıhaliloğlu'; KA, 'Kabaşu')**

Genotype #	UDAP411	UDAP415	Ma040	AMPA116	UDAP414	UDAP423						
3	84	110	163	175	225	225	135	165	166	178	184	198
4	88	110	163	175	233	233	135	135	166	186	184	206
6	110	110	169	179	223	241	135	137	166	186	184	184
7	88	110	169	179	223	223	135	137	178	190	184	204
13	88	88	169	169	223	223	135	165	166	166	184	198
14	84	110	175	179	223	233	135	135	178	186	184	204
15	88	110	169	177	223	241	135	165	166	186	184	204
16	84	84	169	169	223	223	135	135	178	186	184	204
17	110	110	163	175	223	223	135	135	178	178	190	206
18	110	110	163	169	223	233	135	135	166	178	184	206
19	88	88	169	175	233	241	135	159	178	186	184	190
20	110	110	169	179	223	241	135	137	166	186	184	184
21	110	110	163	163	233	233	135	153	166	180	180	204
22	110	110	169	169	233	241	135	135	186	186	184	204
23	110	122	163	163	223	225	165	165	166	178	184	206
24	110	122	167	167	223	233	135	153	178	178	198	204
26	84	109	163	163	223	241	135	153	178	186	198	206
27	110	110	163	169	223	223	135	159	178	186	184	206
28	104	104	167	179	223	233	135	137	166	178	184	204
29	110	122	169	175	223	223	135	137	178	190	184	184
31	88	122	163	167	223	223	135	165	166	166	184	198
32	110	110	169	175	223	223	135	153	179	179	184	198
33	110	122	179	179	223	233	135	159	166	178	184	200
34	110	110	169	175	223	223	135	153	178	178	184	198
35	110	110	169	179	223	233	135	135	178	186	184	184
38	110	110	175	181	233	233	145	145	178	178	184	184
39	84	84	169	175	233	233	135	135	178	178	184	184
41	104	110	175	175	223	223	135	135	166	178	184	204
42	88	88	167	175	223	223	135	135	178	186	184	200
43	110	110	167	175	223	233	135	159	166	186	184	204
45	110	110	163	179	223	233	135	135	166	178	184	200
46	84	110	163	179	233	241	135	165	166	178	184	204
47	88	88	175	175	223	223	135	166	166	166	198	204
48	104	104	169	175	223	223	135	137	186	186	186	199
49	88	110	163	167	223	223	135	153	178	178	184	204
50	84	109	163	163	223	233	135	135	166	186	184	206
53	84	104	169	175	223	233	135	137	179	186	186	206
54	88	110	169	175	225	241	135	165	166	186	190	204
59	110	122	163	169	223	223	159	165	166	186	184	204
60	88	110	175	175	223	233	135	135	178	186	184	204
61	110	110	169	179	223	241	135	137	166	186	184	184
64	88	110	169	169	223	223	129	135	184	184	184	200
68	84	84	169	175	233	233	135	135	178	178	184	184
76	88	88	163	175	233	233	135	165	166	186	184	206
A	110	110	163	169	223	225	129	159	184	184	184	198
HB	84	84	175	181	223	233	135	143	166	180	180	198
HH	84	84	167	175	223	223	135	153	180	180	180	204
KA	84	110	163	167	223	223	135	153	180	186	184	204
L	84	110	167	167	223	241	135	153	180	186	204	204

**Table 2 (Continue)- Allelic data (bp) for wild apricot genotypes of Nevşehir locality and standard apricot cultivars (A, ‘Aprikoz’; L, ‘Levent’; HB, ‘Hasanbey’; HH, ‘Hacıhaliloğlu’; KA, ‘Kabaası’)**

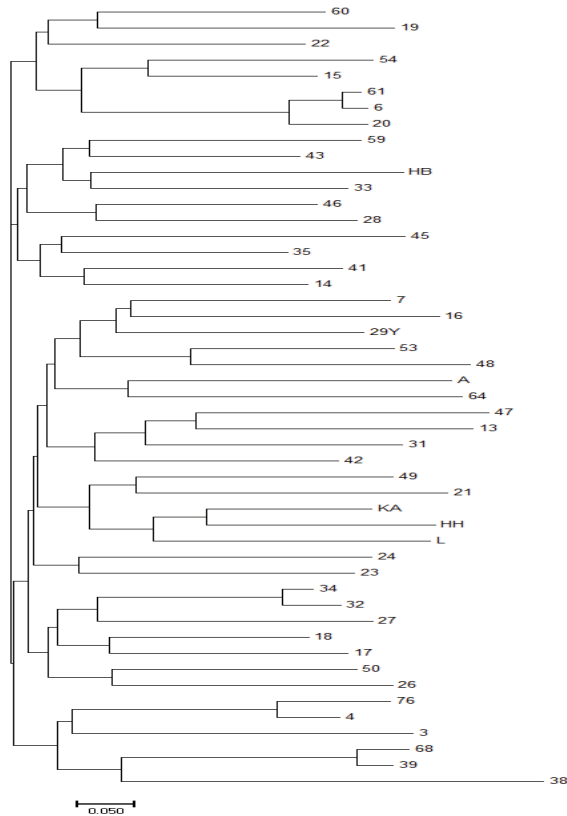
Genotype #	UDAP96-010	UDAP418	AMPA105	AMP119	UDAP420	UDP407	UDP410							
3	93	117	176	180	203	203	114	122	195	195	204	206	142	162
4	93	99	180	180	195	195	120	122	195	195	186	206	140	168
6	113	117	176	182	203	203	116	120	179	195	204	204	140	142
7	99	105	154	170	205	205	116	120	195	195	186	204	166	166
13	117	117	170	170	205	231	120	124	179	179	186	204	142	142
14	105	117	176	180	227	227	120	130	179	195	182	204	136	166
15	93	99	176	180	203	205	116	120	169	195	186	204	142	142
16	113	113	170	180	231	231	116	122	195	195	186	208	162	166
17	99	103	180	180	227	231	120	120	169	195	186	204	136	168
18	93	103	150	180	195	231	120	120	169	171	186	206	142	168
19	97	117	180	180	195	205	116	122	179	195	182	186	140	140
20	114	117	176	184	203	203	120	120	179	195	204	204	140	142
21	99	113	150	152	203	227	120	120	169	195	186	186	136	136
22	103	105	176	180	195	217	120	120	179	195	186	204	140	162
23	113	117	150	180	195	227	116	120	169	195	186	204	140	168
24	105	117	170	180	195	227	116	120	179	195	204	209	140	162
26	93	117	152	176	217	231	120	120	169	179	186	206	140	168
27	99	103	176	176	205	217	120	120	189	195	206	206	140	166
28	93	113	174	180	195	195	120	120	171	195	182	186	136	142
29	91	117	176	180	205	227	120	122	195	195	186	208	162	162
31	103	117	180	180	205	227	120	120	171	195	204	204	142	168
32	103	117	176	180	195	195	120	120	169	195	182	206	140	162
33	99	105	182	182	195	205	120	120	169	195	182	206	136	142
34	103	117	176	180	195	195	120	120	169	195	182	206	140	162
35	97	105	176	180	205	227	116	120	187	195	186	186	162	168
38	97	97	170	180	205	205	129	129	195	195	209	209	144	144
39	105	105	154	164	195	195	120	120	195	195	186	209	140	162
41	93	93	176	180	227	231	120	130	183	195	186	186	142	166
42	105	117	176	180	195	227	120	120	171	179	186	204	142	162
43	97	99	176	176	195	205	116	120	179	195	182	186	142	166
45	97	97	150	150	226	226	120	130	171	195	182	182	140	168
46	93	97	154	180	195	195	120	120	169	195	186	186	140	166
47	103	117	164	176	205	231	116	122	169	169	186	208	142	166
48	91	117	150	180	227	231	120	122	169	171	186	206	166	166
49	91	105	152	176	203	227	120	120	169	185	186	186	166	166
50	97	117	176	181	195	231	120	122	169	195	184	186	140	162
53*	105	117	150	170	205	217	120	120	169	195	186	206	136	162
			180			227								
54	97	99	154	176	195	205	120	120	169	195	204	204	142	168
59	97	105	176	176	195	205	120	120	169	169	182	186	136	168
60	105	113	154	184	195	205	116	120	183	195	186	204	140	140
61	113	117	176	182	203	231	116	120	179	195	204	204	140	142
64	113	113	170	170	191	191	120	120	169	171	186	204	136	140
68	105	105	154	154	195	195	120	122	195	195	186	209	140	162
76	93	99	164	182	195	195	120	122	195	195	186	206	140	168
A	105	113	169	176	227	227	120	122	169	195	186	205	162	162
HB	97	105	164	182	195	205	116	120	169	195	186	206	142	168
HH	99	105	164	180	205	205	120	120	185	195	204	206	140	168
KA	105	105	162	164	205	227	120	120	171	195	204	206	140	142
L	99	103	148	180	227	227	120	120	169	185	186	204	142	168

\*. Genotype #53 has 3 alleles and shows triploidy for the loci of UDAP418 and AMPA105



**Table 3- Genetic parameters for wild apricot genotypes of Nevşehir Locality (number of alleles (n), expected (He) and observed (Ho) heterozygosity, polymorphism information content, PIC)**

Locus	n	He	Ho	PIC
UDAp-411	6	0.675	0.449	0.634
UDAp-415	7	0.787	0.714	0.755
Ma040	5	0.601	0.469	0.546
AMPA116	9	0.607	0.673	0.585
UDAp-414	7	0.751	0.673	0.710
UDAp-423	9	0.715	0.816	0.684
UDP96-010	10	0.860	0.796	0.845
UDAp-418	14	0.821	0.755	0.801
AMPA105	10	0.812	0.571	0.788
AMPA119	7	0.501	0.510	0.471
UDAp-420	8	0.682	0.755	0.641
UDAp-407	8	0.747	0.735	0.710
UDAp-410	7	0.825	0.776	0.801
Total	107	9.385	8.694	8.971
Average	8.23	0.722	0.669	0.690



**Figure 1- UPGMA (Unweighted pair group method with arithmetic average) based dendrogram showing genetic similarity between 44 wild apricot genotypes of Nevşehir locality and 5 reference apricot cultivars (A, Aprikoz; L, Levent; HB, Hasanbey; HH, Hacıhaliloğlu; KA, Kabaası) based on 13 SSR loci**

SSR loci were successfully amplified in different *Prunus* species including peaches, nectarine, almond, European plum, Japanese plum, cherry and sour cherry. Of the relevant primers, UDAp-401 and UDAp-404 were also used in the present study and successfully amplified and had quite high polymorphism ratios. Akpınar et al (2010) used 10 SSR loci of which 2 were developed from apricots and 8 from peaches for genetic identification of 25 local apricot genotypes and 4 foreign reference cultivars. In the present study, the primer UDP96-010 developed from the peaches had also similar polymorphism. Ullah et al (2017) employed 20 SSR loci for genetic characterization of 12 apricot genotypes significant for the economy of Pakistan and reported polymorphic bands for 18 of them.

Zhebentyayeva et al (2003) characterized 74 apricot genotypes with 30 SSR markers and reported the number of alleles between 2 (Pchgms 17) and 16 (UPD 96-001) and total number of alleles as 107 with an average number of alleles as 7.64. Messina et al (2004) tested 20 of 99 SSR loci isolated from apricots in 16 apricot genotypes to determine polymorphism ratios and reported the number of alleles between 2 and 9, expected heterozygosity between 0.26 and 0.82. Liu et al (2013) developed 19 microsatellite loci for marker assisted selection (MAS) of *P. sibirica* L. with regard to late flowering and characterized them in 40 genotypes. Researchers reported the number of alleles between 3 and 11, expected and observed heterozygosity ratios between 0.063 and 0.917, and between 0.295 and 0.876, respectively. In another study carried out with *P. sibirica* L. species, 31 SSR loci were used to assess genetic diversity and population structure. The number of alleles were reported between 5 and 33 with an average value of 19.323, and average expected and observed heterozygosity ratios were reported 0.639 and 0.774, respectively (Wang et al 2014). Gürcan et al (2015) assessed 278 apricot genotypes with 20 SSR loci and reported the number of alleles between 5 and 25 with an average number of alleles as 12.78, expected and observed heterozygosity values of 0.75 and 0.63, respectively. Study, 49 *P. armeniaca*

genotypes including wild apricot genotypes and reference cultivars were screened through 13 SSR markers and total number of alleles was identified as 107, average number of alleles per locus as 8.23, polymorphism information content as 0.69.

SSR markers are used for various purposes (genetic mapping and etc.), especially for genetic characterization of *Prunus* species, including apricots. However, SSR markers were not able to be developed at the same rates for each one of the significant species (apricot, peach, plum, almond), thus potential use of SSR markers of a species in other *Prunus* species (cross-transferability) have become a significant issue. In this study, two different sources were preferred in selection of SSR loci for genetic assessment of wild apricot genotypes. Of these loci, while only one of 10 SSR loci (AMPA096) was unsuccessful, peak quality was poor in UDP98-406 locus developed from *P. persica*, and the UDP96-019 locus developed from the same source was monomorphic. EST-SSR primers developed from *P. armeniaca* were successfully amplified and identified as polymorphic.

## 5. Conclusions

In this study, 16 SSR primers developed from apricot and peaches were used. Thirteen SSR primers were successfully amplified, and they had quite high polymorphism ratios. These 13 SSR loci were found sufficient and successful for characterization and identification of selected wild apricot genotypes. There was a quite high genetic diversity among the genotypes. Genetic similarity varied between 12 and 96%, and homonymous and synonymous genotypes were not encountered.

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## References

- Akpınar A E, Koçal H, Ergül A, Kazan K, Selli M E, Bakır M, Aslantas Ş, Kaymak S & Sarıbas R (2010). SSR-based molecular analysis of economically important Turkish apricot cultivars. *Genetics and Molecular Research* 9: 324-332
- Bouhadida M, Casas A M, Gonzalo M J, Arus P, Morena M A & Gogorcena Y (2009). Molecular characterization and genetic diversity of *Prunus* rootstocks. *Scientia Horticulturae* 120: 237-245
- Bourguiba H, Khadari B, Krichen L, Trifi-Farah N, Santoni S & Audergon J M (2010). Grafting versus seed propagated apricot populations: two main gene pools in Tunisia evidenced by SSR markers and model-based Bayesian clustering. *Genetica* 138: 1023-1032
- Cipriani G, Lot G, Huang W G, Marrazzo M T, Peterlunger E & Testolin R (1999). AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L) Batsch] isolation, characterization and cross-species amplification in *Prunus*. *Theoretical and Applied Genetics* 99: 65-72
- Dumanoglu H, Bakır M, Ernim C & Macit T (2018). Evaluation of wild apricot (*Prunus armeniaca* L.) genetic materials around Nevşehir province for late blooming, resistance to late spring frosts, large fruit size and late fruit maturity. The Final Report of Scientific Research and Technological Research Project (114O279). TUBITAK, Ankara
- Ercisli S (2004). A short review of the fruit germplasm resources of Turkey. *Genetic Resources and Crop Evolution* 51: 419-435
- Eroglu D & Cakir B (2015). Molecular characterization of sweet cherry genotypes and rootstocks by using *Prunus* SSR sequences. *Austin Journal of Biotechnology & Bioengineering* 2(3): 1044
- Gupta P K, Balyan H S, Sharma P C & Ramesh B (1996). Microsatellites in plants: a new class of molecular markers. *Current Science* 70: 45-54
- Gürcan K, Öcal N, Yılmaz K U, Ullah S, Erdogan A & Zengin Y (2015). Evaluation of Turkish apricot germplasm using SSR markers: Genetic diversity assessment and search for Plum pox virus resistance alleles. *Scientia Horticulturae* 193: 155-164
- Hagen L S, Chaib J, Fady B, Decroocq V, Lambert J P & Audergon J M (2004). Genomic and cDNA microsatellites from apricot (*Prunus armeniaca* L.). *Molecular Ecology Notes* 4: 742-745
- Hormaza J I (2002). Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequence repeats. *Theoretical and Applied Genetics* 104: 321-328
- Kostina K F (1969). The use of varietal resources of apricots for breeding. *Trudy Nikitiskogo Botanicheskogo Sada* 40: 45-63
- Layne R E C, Bailey C H & Hough L F (1996). Apricots. In: J Janick & J N Moore (Eds.) *Fruit Breeding: Tree and Tropical Fruits*, Jhon Wiley & Sons, Inc. New York, pp.79-111
- Lefort F, Lally M, Thompson D & Douglas G C (1998). Morphological traits, microsatellite fingerprinting and genetic relatedness of a stand of elite oaks (*Q. robur* L.) at Tuallynally, Ireland. *Silvae Genetica* 47: 5-6
- Litt M & Luty J A (1989). A hypervariable microsatellite revealed by *in vitro* amplification of a dinucleotide repeat within the cardiac muscle actin gene. *The American Society of Human Genetics* 44: 397-401
- Liu H U, Liu J, Wang Z, Ma L Y, Wang S Q, Lin X G, Wu R L & Pang X M (2013). Development and characterization of microsatellite markers in *Prunus sibirica* (Rosaceae). *Applications in Plant Sciences* 1(3): apps.1200074
- Liu K & Muse S V (2005). PowerMarker: an integrated analysis environment for genetic marker analysis. *Bioinformatics* 21: 2128-2129
- Messina R, Lain O, Marrazzo M T, Cipriani G & Testolin R (2004). New set of microsatellite loci isolated in apricot. *Molecular Ecology Notes* 4: 432-434
- Mnejja M, Garcia-Mas J, Howad W & Arus P (2005). Development and transportability across *Prunus* species of 42 polymorphic almond microsatellites. *Molecular Ecology Notes* 5: 531-535
- Murathan Z T, Kafkas S, Asma B M & Topçu H (2017). S allele identification and genetic diversity analysis of apricot cultivars. *The Journal of Horticultural Science and Biotechnology* 92: 251-260
- Nei M (1973). Analysis of gene diversity in subdivided populations. *The Proceedings of the National Academy of Science* 70: 3321-3323
- Romero C, Pedryc A, Munoz V, Llacer G & Badenes M L (2003). Genetic diversity of different apricot geographical groups determined by SSR markers. *Genome* 46: 244-252

- Ruthner S, Pedryc A, Krska B, Romero C & Badenes M L (2006). Molecular characterization of apricot (*Prunus armeniaca* L.) cultivars using cross species SSR amplification with peach primers. *International Journal of Horticultural Science* 12: 53-57
- Sanchez-Perez R, Ruiz D, Dicient F, Egea J & Martínez-Gómez P (2005). Application of simple sequence repeat (SSR) markers in apricot breeding: molecular characterization, protection, and genetic relationships. *Scientia Horticulturae* 103: 305-315
- Schuelke M (2000). An economic method for the fluorescent labelling of PCR fragments. *Nature Biotechnology* 18: 233-234
- Tamura K, Dudley J, Nei M & Kumar S (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* 24: 1596-1599
- TUIK (2017). Number of wild apricot trees in Nevşehir. <https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr> (Retrieved in 15.12.2017)
- Ullah S, Aish M, Iqbal H, Hafeez R & Muhammad Z H (2017). Genetic analysis of economically important apricot cultivars in Gilgit Baltistan based on SSR molecular markers. *Romanian Biotechnological Letters* 22: 12456-12463
- Vavilov N I (1951). The Origin, variation, immunity and breeding of cultivated plants (Translated by S. K. Chestitee). *Chronica Botanica* 13: 1-366
- Wang Z, Kang M, Liu H, Gao J, Zhang Z, Li Y, Wu R & Pang X (2014). High-level genetic diversity and complex population structure of Siberian apricot (*Prunus sibirica* L.) in China as revealed by nuclear SSR markers. *PLoS One* 9, e87381. doi:10.1371/journal.pone.0087381
- Wünsch A (2009). Cross-transferable polymorphic SSR loci in *Prunus* species. *Scientia Horticulturae* 120: 348-352
- Yamamoto T, Mochida K, Imai T, Shi Y Z, Ogiwara T & Hayashi T (2002). Microsatellite markers in peach [*Prunus persica* (L.) Batsch] derived from an enriched genomic and cDNA libraries. *Molecular Ecology Notes* 2: 298-301
- Zhebentyayeva T N, Reighard G L, Gorina V M & Abbott A G (2003). Simple sequence repeat (SSR) analysis for assessment of genetic variability in apricot germplasm. *Theoretical and Applied Genetics* 106: 435-444
- Zhebentyayeva T, Ledbetter C A, Burgos L & Llàcer G (2012). Apricot. In: M L Badenes & P H Byrne (Eds.) *Fruit Breeding*, Springer, New York, pp. 415-458