

Molecular analysis of East Anatolian traditional plum and cherry accessions using SSR markers

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ABSTRACT. We conducted SSR analyses of 59 accessions, including 29 traditional plum (*Prunus domestica*), 24 sweet cherry (*Prunus avium*), and 1 sour cherry (*Prunus cerasus*) selected from East Anatolian gene sources and 3 plum and 2 cherry reference accessions for molecular characterization and investigation of genetic relationships. Eight SSR loci [1 developed from the apricot (UDAp-404), 4 from the peach (UDP96-010, UDP96-001, UDP96-019, Pchgms1) and 3 from the cherry (UCD-CH13, UCD-CH17, UCD-CH31) genome] for plum accessions and 9 SSR loci [5 developed from the cherry (PS12A02, UCD-CH13, UCD-CH17, UCD-CH31, UCD-CH21), 3 from the peach (Pchgms1, UDP96-001, UDP96-005) and 1 from the plum (CPSCT010) genome] for cherry accessions were used for genetic identification. A total of 66 and 65 alleles were obtained in the genetic analyses of 31

plum and 28 cherry accessions, respectively. The number of alleles revealed by SSR analysis ranged from 4 to 14 alleles per locus, with a mean value of 8.25 in plum accessions, and from 5 to 10 alleles per locus with a mean value of 7.2 in cherry accessions. Only one case of synonym was identified among the cherry accessions, while no case of synonym was observed among the plum accessions. Genomic SSR markers used in discrimination of plum and cherry accessions showed high cross-species transferability in the *Prunus* genus. Because of their appreciable polymorphism and cross species transferability, the SSR markers that we evaluated in this study will be useful for studies involving fingerprinting of cherry and plum cultivars.

Key words: East Anatolia; Plum; Cherry; Genetic diversity; SSR

INTRODUCTION

Rosaceae, which involves the fruits (apples, strawberries), forest (mazzard) and ornamental (rose) species, is the third most agronomically important plant family in temperate regions. Cherry and the plum are two species belonging to the *Prunus* genus (Rosaceae), which is a large and diverse genus, comprising about 400 species of trees and shrubs, including other economically important species (apricot, almond, plum, and cherry) (Dirlewanger et al., 2002). The wild and cultivated forms of cherries and plums, in Turkey show a relatively large diversity due to their good adaptation to various ecogeographical conditions within the Anatolian region (Onur, 1977; Özakman et al., 1993; Ayanoğlu, 1995).

Plums (*Prunus domestica* L.) are cultivated plants that are mostly classified into three groups, namely European, American, and Asian plums, and are economically important since only some of these species display desirable quality traits (Salesses et al., 1994). Anatolia, which is the Asian part of Turkey, has played a significant role in the sustaining and conserving the cultivated plum types to date (Asma, 2000). With a production figure of 143,705 tonnes, Turkey is the eighth largest producing country in the world after China, USA, Serbia, Romania, Chile, France and Iran (FAOSTAT, 2008). In the coastal areas and in Central, South and East Anatolia, plums are grown for use as table, early grown, deep-frozen and canned forms (Gülcan et al., 2000).

Cherry (*Prunus avium* L.) is an economically viable fruit originating from around the Caspian and Black Sea, as well as Northeast Anatolia, which then spread throughout the world. The distinctiveness among the cherry accessions in breeding and cultivation is fairly important for scientific as well as economic reasons (Struss et al., 2003; Ganopoulos et al., 2011). Cherry production is carried out in the Central Anatolia and Marmara regions, which are suitable regions due to the long harvest time and abundant sunshine (Asma, 2000). Like other species, cherry also has a great genetic diversity potential in Anatolia. Turkey is the leading country in world exportation with a production figure of 531,270 tonnes (FAOSTAT, 2008).

The analysis of genetic diversity of accessions has traditionally been based on morphological traits, although DNA markers do allow for a more precise identification of plant genotypes. Different molecular techniques such as amplified fragment length polymorphism and simple sequence repeats (SSR) have been developed for measuring genetic variability, and

several studies have been performed on the identification of cherry and plum genotypes using molecular markers (Granger et al., 1993; Gerlach and Stösser, 1998; Wunsch and Hormaza, 2002; Struss et al., 2003; Ayanoglu et al., 2007; Mariette et al., 2010).

The objectives of this study were the molecular characterization and establishment of genetic relationships between traditional Turkish cultivated plum and cherry accessions collected from diverse geographical regions in East Anatolia, together with the novelty of the use of SSR markers in the identification of field samples. The data from this study provide a valuable reference for the genetic base of breeding programs and contribute to an international genetic database.

MATERIAL AND METHODS

Plant material and DNA isolation

A total of 59 accessions, namely 29 traditional and 2 reference plum (*Prunus domestica* L.) and 25 traditional [24 sweet cherry (*Prunus avium* L.) and 1 sour cherry (*Prunus cerasus* L.)] and 3 reference cherry (*Prunus avium* L.) accessions, were analyzed in this study. These cultivated plum and cherry accessions were selected from East Anatolia and maintained at the “Horticultural Research Station/Erzincan”. The names of the plum and cherry accessions and the collection sites are presented in Tables 1 and 2, respectively.

Table 1. Accession code, collection sites, allele sizes (bp) of selected traditional plum and accessions at 8 SSR loci.

No.	Accession code	Collection site (City)	UDAp-404	UDP96-010	UDP96-001	UDP96-019	Pchgms1	UCD-CH13	UCD-CH17	UCD-CH31								
1	24plu001	Erzurum	161	167	87	87	97	101	168	168	166	174	128	136	146	156	164	164
2	24plu002	Iğdır	169	169	75	75	101	101	168	170	164	164	128	136	156	156	164	164
3	24plu003	Gümüşhane	163	163	75	75	97	101	166	170	164	174	128	132	146	156	164	164
4	24plu004	Gümüşhane	167	167	75	75	97	103	170	170	164	164	128	134	148	148	164	164
5	24plu005	Gümüşhane	163	167	63	81	95	101	170	170	164	174	128	132	136	152	166	166
6	24plu006	Gümüşhane	155	165	63	97	95	95	166	170	174	182	128	132	154	154	164	170
7	24plu007	Gümüşhane	161	177	75	87	97	101	166	170	168	174	128	132	150	160	164	170
8	24plu008	Van	159	159	75	87	95	109	166	172	176	182	128	132	150	160	164	170
9	24plu009	Van	171	171	97	97	97	113	166	172	174	182	128	132	148	154	170	170
10	24plu010	Van	161	177	75	99	97	101	166	170	168	174	128	132	150	160	164	170
11	24plu011	Van	175	175	75	89	97	109	166	170	166	174	128	132	148	160	164	170
12	24plu012	Van	167	167	63	85	101	101	168	168	164	164	128	134	148	148	164	164
13	24plu013	Van	159	173	63	87	91	103	172	172	164	174	128	132	146	146	166	170
14	24plu015	Gümüşhane	159	173	75	75	97	101	164	168	174	174	128	128	148	156	164	164
15	24plu016	Erzurum	167	177	63	93	97	101	166	172	164	182	128	132	146	154	164	170
16	24plu017	Artvin	169	169	73	73	97	103	170	186	164	168	128	136	156	164	166	166
17	24plu018	Artvin	167	167	71	71	101	101	170	170	164	164	128	134	156	156	166	166
18	24plu019	Artvin	155	165	85	101	91	103	172	172	164	182	128	132	146	154	164	170
19	24plu020	Erzurum	163	171	73	75	101	101	168	168	172	172	128	136	160	160	166	166
20	24plu021	Erzurum	161	167	75	87	99	113	170	170	164	174	128	132	136	152	166	166
21	24plu022	Erzurum	167	173	75	75	97	111	166	172	166	174	128	132	136	150	164	164
22	24plu023	Erzurum	161	161	71	71	103	103	168	168	164	176	128	136	136	160	166	166
23	24plu024	Erzurum	155	163	91	91	95	101	166	172	164	174	128	132	146	160	164	164
24	24plu025	Gümüşhane	159	159	77	91	97	101	168	168	166	174	128	132	146	160	164	164
25	24plu026	Van	175	175	63	93	101	109	170	170	164	174	128	132	146	156	166	166
26	24plu027	Erzurum	163	163	75	75	97	109	168	172	166	174	128	132	146	154	166	170
27	24plu028	Erzurum	177	177	63	93	101	113	166	170	164	174	128	132	136	150	164	164
28	24plu029	Erzurum	161	173	75	101	97	109	172	172	164	182	128	132	154	154	164	170
29	24plu030	Erzurum	159	177	75	93	97	101	166	170	166	174	128	132	146	150	164	170
30	Stanley	Europe	163	171	91	91	95	101	166	170	174	182	128	132	156	156	164	170
31	Obilnaja	Japan	171	171	75	75	99	99	168	168	170	174	128	136	136	186	150	164

Table 2. Accession code, collection sites, allele sizes (bp) of selected traditional cherry accessions at 9 SSR loci.

No.	Accession code	Collection site (City)	PS12A02		UCD-CH13		UCD-CH17		UCD-CH31		UCD-CH21		Pchgms1		UDP96-001		UDP96-005		CPSCT010	
1	24che001	Erzurum	160	160	136	136	198	206	130	130	109	109	138	138	97	109	119	133	178	180
2	24che002	Van	160	160	128	136	188	198	130	134	109	109	130	138	105	109	119	135	176	176
3	24che003	Erzurum	158	166	128	136	186	186	130	130	109	119	130	138	109	109	115	135	178	178
4	24che004	Erzurum	152	152	128	136	202	202	134	140	107	111	140	184	109	123	135	135	178	178
5	24che005	Van	158	158	128	136	202	202	134	142	107	111	140	184	109	123	135	135	178	178
6	24che006	Erzurum	166	166	128	136	198	198	134	134	111	119	130	138	109	109	119	135	178	182
7	24che007	Van	150	166	122	136	198	198	134	134	111	119	130	138	109	109	119	135	178	182
8	24che008	Van	150	160	126	136	198	198	130	134	109	109	138	138	109	123	119	133	176	176
9	24che009	Erzurum	160	160	128	136	198	198	132	132	109	119	138	138	101	125	123	135	176	176
10	24che010	Erzurum	160	160	128	136	192	204	130	134	111	111	138	152	101	125	119	119	178	178
11	24che011	Van	164	164	128	136	192	198	122	134	109	109	130	138	115	123	115	119	176	176
12	24che012	Van	160	166	128	136	188	204	130	130	109	119	130	130	109	123	109	133	178	178
13	24che013	Erzurum	158	158	128	136	188	200	130	134	111	111	136	156	123	123	119	123	178	178
14	24che014	Erzurum	158	158	128	136	190	202	140	140	109	119	138	138	109	123	119	135	178	182
15	24che015	Gümüşhane	158	158	122	136	188	200	130	134	111	111	136	152	123	123	119	119	178	178
16	24che016	Artvin	158	158	126	136	190	202	142	142	109	119	138	138	109	123	119	135	178	182
17	24che017	Artvin	162	162	128	136	192	198	122	134	109	109	130	138	115	123	115	119	176	176
18	24che018	Artvin	160	160	128	136	198	206	122	130	111	111	130	138	105	123	119	135	176	176
19	24che019	Erzurum	158	166	128	136	188	204	130	130	109	109	138	138	109	109	109	133	178	178
20	K-ST	Unknown	164	176	132	136	192	214	124	124	109	119	138	138	123	123	119	135	176	176
21	K-ZIR	Unknown	158	158	136	136	190	202	142	142	109	119	138	138	109	123	119	135	176	180
22	K-MI	Unknown	162	180	136	136	200	200	124	130	111	117	130	140	105	125	119	135	176	176
23	K-SF	Unknown	164	176	132	136	192	214	124	124	109	119	138	138	123	123	119	135	176	176
24	Vişne-1	Unknown	158	164	128	136	192	200	124	132	107	107	136	136	97	123	115	135	170	176
25	Erz-Macar	Erzincan	158	158	136	136	190	202	142	142	109	119	138	138	109	123	119	135	178	182
26	Lapins	Canada	174	174	126	132	192	214	120	138	117	117	140	184	123	123	119	135	178	178
27	Bing	USA	162	162	128	136	190	190	120	120	109	109	140	184	125	125	119	135	178	178
28	Rainer	USA	160	176	128	136	188	202	120	128	109	109	130	138	123	123	115	135	178	178

DNA was extracted from young leaf tissue following the procedure described by Lefort et al. (1998). About 100 mg young leaves were ground into a fine powder in liquid nitrogen and homogenized. The powder was transferred to a new 1.5-mL polypropylene tube, and 1 mL DNA extraction buffer [50 mM Tris-HCl, pH 8.0, 20 mM EDTA, pH 8.0, 0.7 mM NaCl, 1% (w/v) CTAB (hexadecyltrimethylammonium bromide), 2% (w/v) PVP 40] and 10 μ L 2-mercaptoethanol (1% final concentration) were added. The mixture was vortexed for 5 s and then incubated for 15 min at 65°C in a water-bath. After incubation, an equal volume of chloroform/isoamyl alcohol (24:1) was added and the phases were separated by centrifugation at 16,000 g for 10 min. The aqueous layer was collected and 0.54 volume of cold isopropanol (-20°C) added to precipitate the DNA. The pellet was obtained after centrifugation at 16,000 g for 10 min and resuspended in 100 μ L TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) containing 15 μ g/mL RNase A and incubated for 30 min at 37°C. Proteins were removed by adding 50 μ L 7.5 M ammonium acetate, followed by centrifugation at 16,000 g for 10 min. DNA in the supernatant was precipitated with a 0.54 volume of cold isopropanol; the pellet was dried at room temperature, resuspended in 100 μ L TE and stored at 4°C. The DNA concentration was estimated spectrophotometrically and DNA quality was checked by agarose gel electrophoresis.

SSR analysis

A total of 8 SSR loci, namely 1 developed from apricot (UDAp-404), 4 from peach (UDP96-010, UDP96-001, UDP96-019, Pchgms1) and 3 from cherry (UCD-CH13, UCD-CH17, UCD-CH31), were used in the study for the genetic characterization of the plum accessions (Cipriani et al., 1999; Sosinski et al., 2000; Struss et al., 2003; Messina et

al., 2004). For the genetic characterization of cherry accessions, a total of 9 SSR loci were used: 5 developed from cherry (PS12A02, UCD-CH13, UCD-CH17, UCD-CH31, UCD-CH21), 3 from peach (Pchgms1, UDP96-001, UDP96-005) and 1 from plum (CPSCT010) (Cipriani et al., 1999; Sosinski et al., 2000; Struss et al., 2003; Messina et al., 2004; Mnejja et al., 2005). Polymerase chain reactions (PCR) and SSR analysis were performed as previously described by Şelli et al. (2007). PCR amplifications were performed in a 10- μ L reaction mixture containing 15 ng DNA, 5 pmol of each primer, 0.5 mM dNTPs, 0.5 U GoTaq DNA polymerase (Promega, Madison, WI, USA), and 1.5 mM MgCl₂. 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. The PCR products were diluted with SLS (sample loading solution), followed by the addition of the Genomelab DNA Standard kit-400 and electrophoresed in the CEQ 8800XL capillary DNA analysis system (Beckman Coulter, Fullerton, CA, USA). The allele sizes were determined for each SSR locus using the Beckman CEQ fragment analysis software. The analyses were repeated at least twice to ensure the reproducibility of the results.

Genetic analysis

Number of alleles (N_A), allele frequency, expected (H_E) and observed heterozygosity (H_O), estimated frequency of null alleles (r), 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 "IDENTITY" software was also used to detect identical genotypes. Proportion of shared alleles was calculated by using ps [option 1-(ps)] as described by Bowcock et al. (1994) as genetic dissimilarity in the Microsat (version 1.5) program (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 software NTSYS-pc (Numerical Taxonomy and Multiware Analysis System) (version 2.0) (Rohlf, 1988).

RESULTS

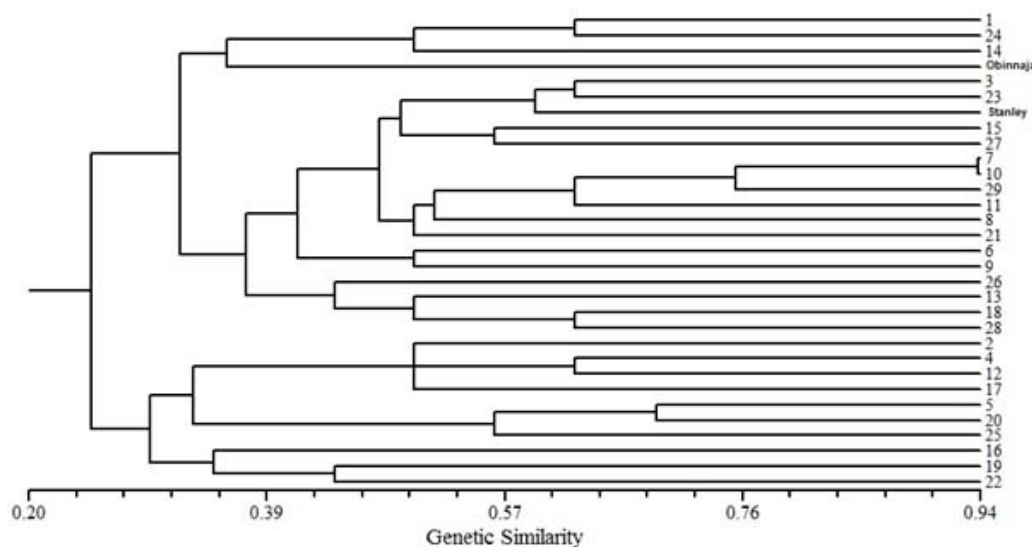
A total of 59 accessions, namely 29 traditional and 2 reference (Stanley, Obilnaja plum (*Prunus domestica* L.) and 25 traditional and 3 reference (Lapins, Bings and Rainer) sweet cherry (*Prunus avium* L.) and sour cherry (*Prunus cerasus* L.) accessions originating from different geographical regions (Erzurum, Van, Gümüşhane, Artvin and Iğdır provinces) of East Anatolia were screened.

Sixty-six alleles were identified in the genetic analyses of 31 plum accessions and the allele sizes are displayed in Table 1. As far as the probability of identity (PI) is concerned, while UDAp-404 (11 alleles, PI: 0.041) locus was the most informative locus, UCD-CH13 locus (4 alleles, PI: 0.364) was identified as the least informative locus (Table 3). H_O ranged from 0.419 (UCD-CH31) to 0.967 (UCD-CH13) and H_E ranged from 0.607 (UCD-CH13) to 0.891 (UDAp-404). Mean H_O and H_E were 0.693 and 0.749, respectively. The PI values ranged from 0.041 (UDAp-404) to 0.364 (UCD-CH13) (Table 3).

Table 3. SSR loci, number of alleles (N_A), expected heterozygosity (H_E), observed heterozygosity (H_O), probability (PI), and the frequency of null alleles (r) of 31 plum and 28 cherry accessions.

SSR Loci	Plum accessions					Cherry accessions					
	N_A	H_E	H_O	PI	r	SSR Loci	N_A	H_E	H_O	PI	r
UDAp-404	11	0.891	0.516	0.041	0.198	PS12A02	10	0.819	0.357	0.095	0.254
UDP96-010	14	0.829	0.548	0.067	0.689	UCD-CH13	5	0.594	0.857	0.350	-0.164
UDP96-001	9	0.795	0.774	0.116	0.011	UCD-CH17	10	0.865	0.678	0.059	0.100
UDP96-019	6	0.755	0.548	0.186	0.117	UCD-CH31	10	0.832	0.5	0.086	0.181
Pchgms1	8	0.754	0.806	0.168	-0.029	UCD-CH21	5	0.700	0.5	0.226	0.117
UCD-CH13	4	0.607	0.967	0.364	-0.224	Pchgms1	7	0.673	0.571	0.205	0.06
UCD-CH17	10	0.866	0.709	0.062	0.083	UDP96-001	7	0.698	0.607	0.230	0.053
UCD-CH31	4	0.632	0.419	0.332	0.130	UDP96-005	6	0.689	0.857	0.254	-0.099
Total	66	6.129	5.287			CPST010	5	0.612	0.285	0.362	0.202
Mean	8.25	0.76	0.66			Total	65	6.469	5.212		
						Mean	7.2	0.718	0.579		

The dendrogram generated from UPGMA cluster analysis of 31 plum accessions based on the Jaccard coefficient of genetic similarity revealed two main groups (Figure 1). Both groups were further divided into two major subgroups containing all of the plum accessions analyzed. No complete ecogeographical clustering was observed within these subgroups. The highest genetic similarity (95%) was observed between accessions 7 (24plu007) and 10 (24plu010). Subsequently, a similarity of 76% was observed between accession 29 (24plu030) and accessions 7 and 10 (24plu007 and 24plu010). Apart from the aforementioned similarities, the plum accessions showed less than 70% similarity with each other. Stanley and Obilnaja which were used as the reference cultivars, were slightly distant from the traditional plum accessions in the dendrogram constructed with SSR data.

**Figure 1.** UPGMA Cluster analysis of the SSR data from plum accessions based on Jaccard's coefficient of genetic similarity.

A total of 65 alleles were obtained in the genetic analyses of 28 cherry accessions, and the specific allele sizes revealed by these primers are presented in Table 2. With regard to the probability of identity (PI), while UCD-CH17 (10 alleles, PI: 0.059) locus was the most informative locus, CPSCT010 locus (5 alleles, PI: 0.362) was identified as being the least informative. H_o ranged from 0.285 (CPSCT010) to 0.857 (UCD-CH13, UDP96-005), and H_e ranged from 0.594 (UCD-CH13) to 0.865 (UCD-CH17). Mean H_o and H_e were 0.571 and 0.729, respectively. The PI values ranged from 0.059 to 0.36 (Table 3).

The dendrogram generated from the UPGMA cluster analysis based on the Jaccard coefficient of genetic similarity classified all 28 cherry (27 sweet cherry and 1 sour cherry) accessions included in this study into two main groups, which are depicted in Figure 2. In terms of genetic similarities between the accessions, the highest genetic similarity (94%) was observed between the cherry accessions 16 (24che016) and 25 (Erzincan Macar), excepting the synonymous accessions. At the same time, these accessions could probably be considered as clonal mutants, showing one or 2 allele differences. There was 89% similarity identified between accessions 11 (24che011) and 17 (24che017), as well as between accessions 6 (24che006) and 7 (24che007). There was 83% similarity identified between accessions 21 (K-ZİR), 16 (24che016) and 25 (Erz-Macar) and between accessions 13-15 and 4-5. Accessions 20 (K-ST) and 23 (K-SF) were identified as being synonymous. Apart from the above mentioned similarities, the cherry accessions showed less than 80% similarity with each other. The similarity between the sour cherry accession (Vişne 1) and sweet cherry accessions ranged from 11 to 39%. The sour cherry accession 24 (Vişne1) and accession 22 (K-M1) formed a separate group and displayed a distinct branching pattern.

A dendrogram was generated on the basis of the selected common SSR loci (Pchgms1, UCD-CH13, UCD-CH17, UCD-CH31, UDP96-001) of the plum and cherry accessions. As indicated by the dendrogram, plum and cherry accessions were clustered in separate groups (Figure 3).

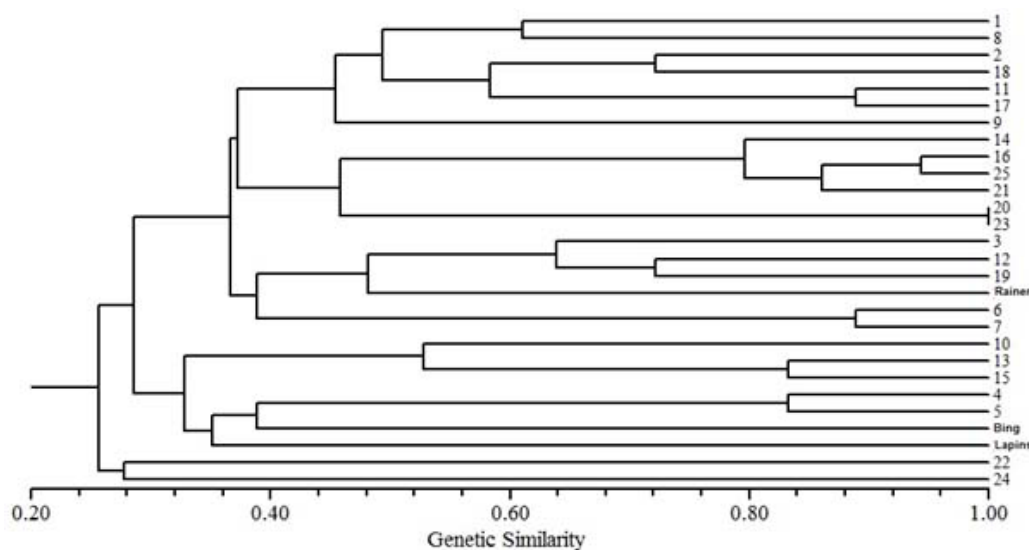


Figure 2. UPGMA Cluster analysis of the SSR data from cherry accessions based on Jaccard's coefficient of genetic similarity.

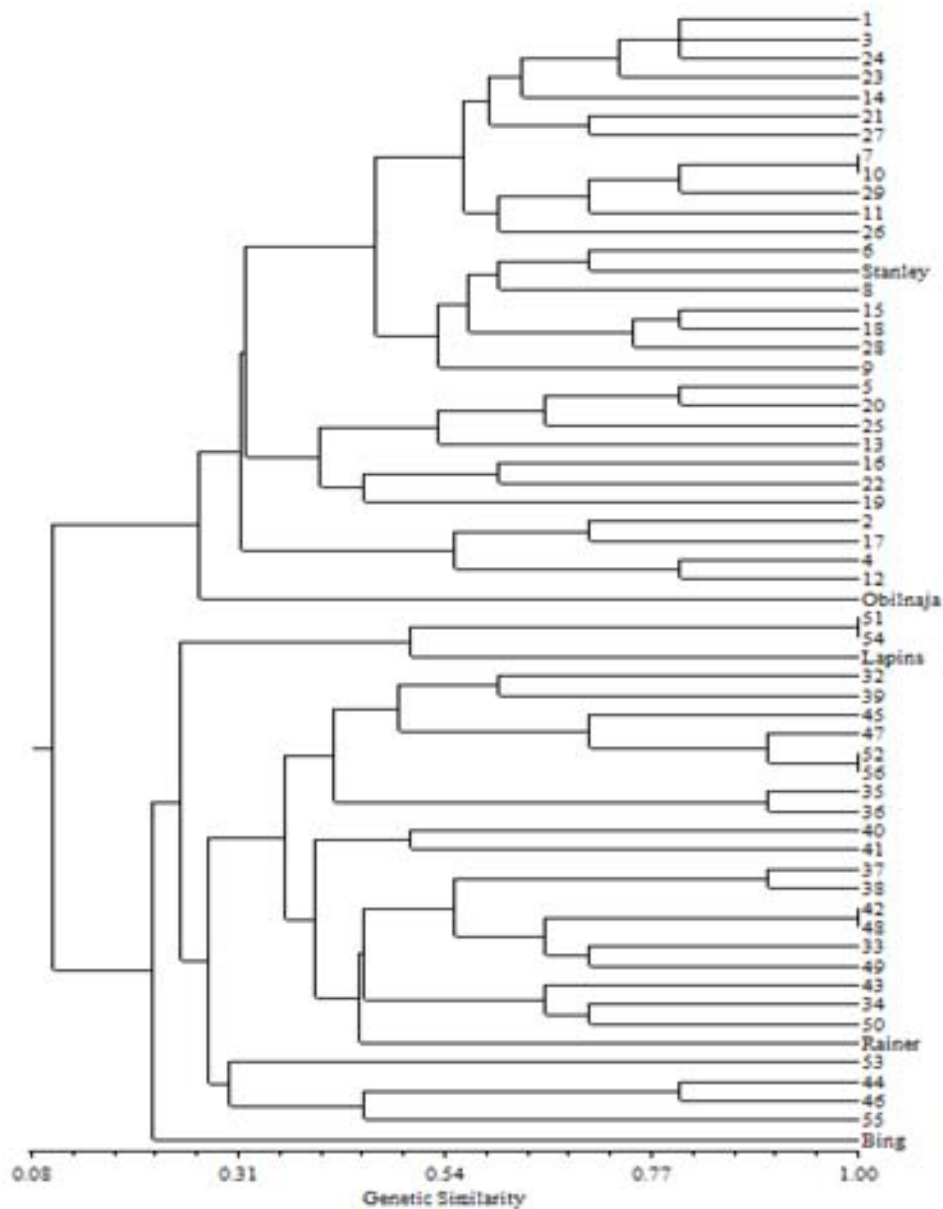


Figure 3. UPGMA cluster analysis of the SSR data between cherry and plum accessions based on Jaccard's coefficient of genetic similarity (1-29 plum and 32-56 cherry).

DISCUSSION

In this study, we report the genetic characterization of traditional cultivated cherry and plum accessions in East Anatolia with the use SSR markers. The results obtained from the

present study showed that microsatellites can be effectively used for fingerprinting purposes in *Prunus* species. While respectively 4 and 19 alleles were detected at the loci UCD-CH13 and UCD-CH17 in North American plums (Rohrer et al., 2004), we found respectively 4 and 10 alleles in the East Anatolian plum accessions and 5 and 10 alleles in the cherry accessions. The numbers of alleles in the plum and cherry accessions were either equal or similar for the UCD-CH13 locus, but they were remarkably lower for UCD-CH17 locus compared to the findings of Rohrer et al. (2004). UCD-CH21 and UCD-CH31 loci were previously used by Rohrer et al. (2004) in the analysis of North American plums, but they were not scored. In the current study, which analyzed 28 cherry accessions, 5 and 10 alleles were scored, respectively. The same loci were also used by Struss et al. (2003) for the SSR-based genetic characterization of 27 cherry accessions; where 4 each alleles were observed. The 4 alleles were detected by the use of the UCD-CH31 locus in this study for the analysis of 31 plum accessions. The number of alleles for the above mentioned 2 loci was remarkably low for the utilization of the cherry and plum gene sources compared to the findings of Rohrer et al. (2004). While 7 alleles were detected at the UDP96-001 locus by Rohrer et al. (2004), the locus produced 9 alleles and 7 alleles, respectively, in the plum and cherry accessions used in our study (Cipriani et al., 1999). The allele number for the PS12A02 locus used in this study for the analysis of the cherry accessions was similar to that found by Downey et al. (2000). In their study on the SSR analysis of cherry (10 alleles), polymorphism was high. However, this locus produced a smaller number of alleles (4 alleles) in the study by Hormaza (2002) on apricot. A genetic similarity of about 95% between 7 and 10 (24plu007 and 24plu010) in the plum accessions and between 16 and 25 (24che016 and Erz-Macar) in the cherry accessions indicated a possible clonal variational relationship between the accessions. These accessions might have originated from either of the two provinces and might have formed ecotypes through transportation and cultivation, or the genotypes may have a common ancestor.

On the other hand, SSR markers developed from the other *Prunus* species were successfully used for genetic characterization of plum and cherry cultivars in this study. However, the average numbers of alleles detected in our study in both cherry and plum by these markers were different than those detected in peach and apricot. The Pchgms1 locus has produced 4 alleles in genetic identifications of both peach (Sosinski et al., 2000) and apricot (Hormaza, 2002), while we found 7 alleles in cherry and 8 alleles in plum. As indicated by the findings of the study, this locus showed a higher number of alleles in both the selected traditional Turkish plum and cherry accessions compared to other *Prunus* species (peach and apricot). On the other hand, H_o of this locus was identified as being higher than those in all the aforementioned studies. The findings reveal that the region has a rich and sustainable genetic diversity. While the UDP96-010 locus produced 2 alleles and an average heterozygosity of 0.21 in peach (Cipriani et al., 1999), it produced 8 alleles and an average heterozygosity of 0.54 in plums used in our study. The allele number and heterozygosity obtained in plum were found to be remarkably higher compared to the findings of other studies on other *Prunus* species, which utilized the same locus (Hormaza, 2002; Romero et al., 2003; Sánchez-Pérez et al., 2005). There were 4 alleles identified in the peach sequenced UDP96-019 locus developed by Cipriani et al. (1999), whereas there were 6 alleles identified at this locus in the plum accessions. While 6 alleles were detected in the peach sequenced (Cipriani et al., 1999) UDP96-005 SSR locus in their study on 84 wild cherry genotypes (Schueler et al., 2003), in the current study, the locus produced 6 alleles. There were 11 alleles ($H_o = 0.516$) detected in the UDAp-404 locus used

in the current study for the genetic analyses of the plum accessions, and the number of alleles was higher compared to the study by Messina et al. (2004) (who developed this locus) on apricot genotypes (6 alleles, $H_o = 0.44$). At the same time, an SSR locus (UCDCH13) developed from cherry produced the lowest number of alleles in plum cultivars, while a SSR locus (CPSCT010) developed from plum produced the lowest number of alleles in cherry cultivars in this study. As a result, cross-species transferability of UCDCH13 and CPSCT010 loci does not seem to be completely compatible between cherry and plum cultivars.

The dendrogram based on the selected common SSR loci of the plum and cherry accessions (Figure 3) indicated that these cultivars were mainly classified into two groups and that the cherry and plum accessions formed distinct subgroups as expected. The alleles obtained from protected SSR sites were highly similar at the UCD-CH13 locus. The low internal similarity rates of the plum and cherry accessions and the low numbers of synonymous and homonymous accessions indicated a highly protected genetic diversity in the region. Additionally, a remarkably low number of synonymy cases (100% similarity) indicated that continuous transportation of material is either non-existent or very seldom in the region. No errors were detected, and the findings were verified after a comparison between the morphological denominations of both the plum and cherry accessions and genetic data.

On the other hand, the results of this study on the identification of the East Anatolian plum and cherry gene sources revealed a high potential for rich genetic diversity in this region. This region is of great importance since it has a cold climate and harbors genotypes that comprise a cold tolerant germplasm and since these plum and cherry gene sources evaluated here do not take part in other national germplasms in East Anatolia. Therefore, our findings reported here are important in adding to the international genetic database and identification of cultivar origins, together with the novelty of the use of the selected SSRs.

REFERENCES

- Asma BM (2000). Kayısı Yetiştiriciliği. Evin ofset, Malatya.
- Ayanoğlu H (1995). Doğu Akdeniz Bölgesinde Sofralık Erik Seleksiyonu. PhD thesis, Çukurova University.
- Ayanoğlu H, Bayazit S, Inan G, Bakir M, et al. (2007). AFLP analysis of genetic diversity in Turkish green plum accessions (*Prunus cerasifera* L.) adapted to the Mediterranean region. *Sci. Hortic.* 114: 263-267.
- Bowcock AM, Ruiz-Linares A, Tomfohrde J, Minch E, et al. (1994). High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* 368: 455-457.
- Cipriani G, Lot G, Huang WG, Marrazzo MT, et al. (1999). AC/GT and AG/CT microsatellite repeats in peach [*Prunus persica* (L.) Batsch]: isolation, characterization and cross-species amplification in *Prunus*. *Theor. Appl. Genet.* 99: 65-72.
- Dirlwanger E, Cosson P, Tavaud M, Aranzana J, et al. (2002). Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.). *Theor. Appl. Genet.* 105: 127-138.
- Downey SL and Iezzoni AF (2000). Polymorphic DNA markers in black cherry (*Prunus serotina*) are identified using sequences from sweet cherry, peach and sour cherry. *J. Am. Soc. Hort. Sci.* 125: 76-80.
- FAOSTAT (2008). FAO Statistics Database on the World Wide Web. Available at [http://faostat.fao.org]. Accessed November 2010.
- Ganopoulos IV, Kazantzis K, Chatzicharisis I, Karayiannis I, et al. (2011). Genetic diversity, structure and fruit trait associations in Greek sweet cherry cultivars using microsatellite based (SSR/ISSR) and morpho-physiological markers. *Euphytica* 181: 237-251.
- Gerlach HK and Stösser R (1998). Sweet cherry cultivar identification using RAPD-derived DNA fingerprints. *Acta Hortic.* 468: 63-69.
- Granger AR, Clarke GR and Jackson JF (1993). Sweet cherry cultivar identification by leaf isozyme polymorphism. *Theor. Appl. Genet.* 86: 458-464.

- Gülcan R, Tekintaş FE, Misirli A, Sağlam H, et al. (2000). Meyvecilikte Üretim Hedefleri. Türkiye Ziraat Mühendisleri V. Teknik Kongresi, Ankara, 587-615.
- Hormaza JI (2002). Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequence repeats. *Theor. Appl. Genet.* 104: 321-328.
- Lefort F, Lally M, Thompson D and Douglas GC (1998). Morphological traits, microsatellite fingerprinting and genetic relatedness of a stand of elite oaks (*Q. robur* L.) at Tullynally, Ireland. *Silvae Genet.* 47: 257-262.
- Mariette S, Tavaud M, Arunyawat U, Capdeville G, et al. (2010). Population structure and genetic bottleneck in sweet cherry estimated with SSRs and the gametophytic self-incompatibility locus. *BMC Genet.* 11: 77.
- Messina R, Lain O, Marrazzo MT, Cipriani G, et al. (2004). New set of microsatellite loci isolated in apricot. *Mol. Ecol. Notes* 4: 432-434.
- Minch E, Ruiz-Linares A, Goldstein DB, Feldman M, et al. (1995). Microsat (Version 1.4d): A Computer Program for Calculating Various Statistics on Microsatellite Allele Data. Stanford University, Palo Alto.
- Mnejja M, Garcia-Mas J, Howad W and Arús P (2005). Development and transportability across *Prunus* species of 42 polymorphic almond microsatellites. *Mol. Ecol. Notes* 5: 531-535.
- Onur S (1977). Yerli ve Yabancı Erik Çeşitlerinin Seçimi. *Yalova Bahçe Kültürleri Aras. Enst. Derg.* 8: 57-64.
- Özakman S, Onaz K, Karabiyik N and Ozkarataş I (1993). Erik Çesit Adaptasyon Projesi. Aegan Agricultural Research Institute of the Ministry of Agriculture and Rural Affairs, Izmir. Annual Report for 1993.
- Paetkau D, Calvert W, Stirling I and Strobeck C (1995). Microsatellite analysis of population structure in Canadian polar bears. *Mol. Ecol.* 4: 347-354.
- Rohlf FJ (1988). NTSYS-PC Numerical Taxonomy and Multivariate Analysis System, Version 2.0, User's Guide. Exeter Software, Setauket.
- Rohrer JR, Ahmad R, Southwick SM and Potter D (2004). Microsatellite analysis of relationship among North American plums (*Prunus* sect. *Prunocerasus*, Rosaceae). *Plant. Syst. Evol.* 244: 69-75.
- Romero C, Pedryc A, Munoz V, Llacer G, et al. (2003). Genetic diversity of different apricot geographical groups determined by SSR markers. *Genome* 46: 244-252.
- Salesses G, Grassely C and Bernhard R (1994). Utilisation des espèces indigènes et exotiques pour l'amélioration des *Prunus* cultivés, variétés et portegreffe. *C. R. Acad. Agric. Fr.* 80: 77-88.
- Sánchez-Pérez R, Ruiz D, Dicenta F, Egea J, et al. (2005). Application of simple sequence repeat (SSR) markers in apricot breeding: molecular characterization, protection, and genetic relationships. *Sci. Hortic.* 103: 305-315.
- Schueler S, Tusch A, Schuster M and Ziegenhagen B (2003). Characterization of microsatellites in wild and sweet cherry (*Prunus avium* L.) - markers for individual identification and reproductive processes. *Genome* 46: 95-102.
- Şelli F, Bakir M, Inan G and Aygun H (2007). Simple sequence repeat-based assessment of genetic diversity in "Dimrit" and "Gemre" grapevine accessions from Turkey. *Vitis* 46: 182-187.
- Sneath PHA and Sokal RR (1973). Numerical Taxonomy. Freeman, San Francisco.
- Sosinski B, Gannavarapu M, Hager LD, Beck LE, et al. (2000). Characterization of microsatellite markers in peach [*Prunus persica* (L.) Batsch]. *Theor. Appl. Genet.* 101: 421-428.
- Struss D, Ahmad R and Southwick SM (2003). Analysis of sweet cherry (*Prunus avium* L.) cultivars using SSR and AFLP markers. *J. Am. Soc. Hort. Sci.* 128: 904-909.
- Wagner HW and Sefc KM (1999). Identity 1.0. Centre for Applied Genetics. University Agricultural Sciences, Vienna.
- Wünsch A and Hormaza JI (2002). Molecular characterisation of sweet cherry (*Prunus avium* L.) genotypes using peach [*Prunus persica* (L.) Batsch] SSR sequences. *Heredity* 89: 56-63.