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

Simple sequence repeat-based assessment of genetic relationships among *Prunus* rootstocks

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Genet. Mol. Res. 9 (4): 2156-2165 (2010)
Received June 24, 2010
Accepted September 2, 2010
Published November 3, 2010
DOI 10.4238/vol9-4gmr957

ABSTRACT. Ten SSR loci, previously developed for *Prunus*, were analyzed to examine genetic relationships among 23 rootstock candidates for sweet and sour cherries, of the species *P. avium*, *P. cerasus*, *P. mahaleb*, and *P. angustifolia*. Five genotypes of *P. laurocerasus*, not used as rootstock, were included in the molecular analysis. The number of alleles per locus ranged from 8 to 12, with a mean of 9, while the number of microsatellite genotypes varied from 8 to 17, indicating that the SSRs were highly informative. The degree of heterozygosity (0.61) was high. Clustering analysis resulted in two main clusters. The first cluster was divided into two subclusters; the first subcluster consisted of *P. avium* and *P. cerasus*, and the second subcluster consisted of *P. laurocerasus*. The second cluster was divided into two subclusters. The first subcluster consisted of *P. mahaleb* genotypes and the second consisted of *P. angustifolia* genotypes. The reference rootstocks also clustered with their associated botanical species. Unweighted pair-group method with arithmetic mean analysis demonstrated that *P. laurocerasus*

genotypes had less genetic variation and that *P. avium* genotypes were more closely related to *P. cerasus*. The SSR-based phylogeny was generally consistent with *Prunus* taxonomy information, suggesting the applicability of SSR analysis for genotyping and phylogenetic studies in the genus *Prunus*.

Key words: *Prunus*; SSR; Genotyping; Diversity; Rootstock

INTRODUCTION

Prunus is a large genus of trees and shrubs, which includes plums, cherries, peaches, apricots, and almonds. The Prunoideae are traditionally classified as a subfamily within the family Rosaceae (Rehder, 1940). Botanical classification of species within this genus is sometimes controversial, partly because of the ease of interspecific hybridization (Dosba et al., 1994). There are many different types of rootstocks being used for *Prunus* species on a worldwide basis (Rom, 1982). Each one has a particular set of advantages and limitations for adaptation to different geographic regions.

The genomic studies concerning the fruit species have increased enormously in parallel with a renewed interest in fruit germplasm resources and analysis of their genetic diversity including *Prunus* genus (Aranzana et al., 2003; Romero et al., 2003; Ilgin et al., 2009; Yilmaz et al., 2009; Wünsch, 2009). For crop improvement studies, researchers generally desire abundant genetic diversity among materials.

For breeding and commercialization of promising rootstock candidates, a precise determination and discrimination of these materials is desired. In the case of rootstocks, it is very difficult to observe their morphological traits after grafting. In addition, morphological characters are strongly affected by the environment and also developmental stage of plants (Casas et al., 1999). Therefore, markers independent of the environment are necessary for reliable identification and discrimination of genotypes. The superiority of molecular markers over morphological characterization in fruit species is well established and widely accepted (Ercisli et al., 2007; Zamani et al., 2007; Duminil and Di Michele, 2009).

Microsatellites have been extensively used in *Prunus* genetics investigations in the last decade (Wünsch and Hormaza, 2004; Lacis et al., 2009; Cheng and Huang, 2009), and the number of microsatellite loci available particularly for *Prunus* genus has greatly increased. Microsatellite markers, being abundant, multiallelic, and highly polymorphic, provide an efficient and accurate means of detecting genetic polymorphism among fruit species. Most importantly, their codominant nature makes them the markers of choice for population genetic analysis to assess genetic organization in germplasm collections. In *Prunus*, microsatellites have been used for germplasm characterization (Lacis et al., 2009), determination of genetic diversity (Wünsch, 2009), germplasm management (Cheng and Huang, 2009), parentage analysis (Yamamato et al., 2003), cultivar identification (Xuan et al., 2009), and mapping genetic linkage (Lalli et al., 2008). In *Prunus*, microsatellites developed in one species have been used in different species, demonstrating their transferability and ability to detect polymorphism (Wünsch, 2009).

Turkey is an important center for *Prunus* germplasm and diversity. Wild subgenus *Cerasus* forms exhibit all grades of color, shape, taste and, to a certain degree, size. They are found mainly in mixed forests (up to 2000 m), particularly in the Black Sea and Northeast regions of Turkey. These wild germplasms in Turkey have potential in genetic improvement of cherry, primarily as rootstocks (Ercisli, 2004).

The objective of this study was to identify a set of microsatellite loci that are polymorphic in different rootstock candidates of *Prunus* species. It is expected that the information presented here will be useful for selection and more efficient utilization of this germplasm in *Prunus* rootstock breeding programs in the future.

MATERIAL AND METHODS

Plant material

Leaf samples of 20 rootstock candidate genotypes belonging to four *Prunus* species (*P. avium*, *P. mahaleb*, *P. cerasus*, and *P. angustifolia*), which include selections among wild populations as rootstock candidates in Turkey, were used as starting material to carry out a microsatellite marker analysis. Five *P. laurocerasus* genotypes were also included in the analysis just to examine genetic relationships with the other *Prunus* species (Table 1). The genotypes used in this study were obtained from the germplasm collection maintained at the Black Sea Agricultural Research Center in Samsun, Turkey. Three standard *Prunus* rootstocks, SL64, F12/1 and Montmorency, were also included in analysis.

Table 1. List of the subgenera, sections, species, and genotypes included.

Subgenus	Section	Species	English name	Genotypes
cerasus	*eucerasus*	*P. avium* L.	Sweet cherry	*08K53*, *28K20*, *52K42*, *53K08*, *55K92*
cerasus	*eucerasus*	*P. cerasus* L.	Sour cherry	*28V01*, *52V04*, *52V01*, *55V22*, *61V01*
cerasus	*mahaleb*	*P. mahaleb* L.	Mahaleb	*05M07*, *52M05*, *60M44*, *60M16*, *60M37*
cerasus	*laurocerasus*	*P. laurocerasus* L.	Cherry-laurel	*K1*, *K2*, *K3*, *K4*, *K5*
prunus	*prunocerasus*	*P. angustifolia* Marshall	Mountain-cherry	*28T01*, *28T02*, *29T02*, *29T03*, *29T04*

DNA extraction

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

SSR analysis

From an initial screening, 10 simple sequence repeats (SSRs) were selected to check for polymorphism by capillary electrophoresis in 28 genotypes of five different *Prunus* species (Table 1). Polymerase chain reaction (PCR) was conducted using a volume of 10 µL containing 15 ng genomic DNA, 5 pmol of each primer, 0.5 mM dNTP, 0.5 U GoTaq DNA polymerase (Promega), 1.5 mM $MgCl_2$ and 2 µL 5X buffer. The forward primers were labeled 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 at 52-56°C and 2 min at 72°C with a final extension at 72°C for 10 min. The PCR products were first separated on a 3% (w/v) agarose gel

run at 80 V for 2 h. The gel was then stained with ethidium bromide at a concentration of 10 mg/mL. A DNA ladder (100 bp) (Promega) was used for the approximate size determination of the bands. The amplification products were visualized under UV light, and their sizes were estimated relative to the DNA ladder. For further determination of polymorphisms, the PCR products were run on a CEQTM 8800 XL Capillary Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA). The analyses were repeated at least twice to ensure reproducibility of the results. Allele sizes were determined for each SSR locus using the Beckman CEQTM fragment analysis software. In each run, SL64, F12/1 and Montmorency were included as reference rootstocks.

Genetic analysis

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

RESULTS

An SSR analysis of a total of 28 *Prunus* genotypes belonging to five different species showed that SSR markers developed for peach, sweet cherry, cherry, plum, and apricot (Table 2) revealed considerable polymorphism. A total of 108 alleles ranging from 8 to 12 per locus with a mean value of 9 alleles per locus were detected based on clarity and specificity (Table 3). Polymorphic bands were obtained with all loci. PS12A02 locus was the most polymorphic

Table 2. Simple sequence repeat (SSR) primers used in the study.

SSR primer	Sequence (5'-3')	Species origin	Reference
Pchgms1	GGG TAA ATA TGC CCA TTG TGC AAT C GGA TCA TTG AAC TAC GTC AAT CCT C	Peach	Sosinski et al., 2000
UDP96001	AGT TTG ATT TTC TGA TGC ATC C TGC CAT AAG GAC CGG TAT GT	Peach	Cipriani et al., 1999
UDP96005	GTA ACG CTC GCT ACC ACA AA CCT GCA TAT CAC CAC CCA G	Peach	Cipriani at al., 1999
UCDCH17	TGG ACT TCA CTC ATT TCA GAG A ACT GCA GAG AAT TTC CAC AAC CA	Sweet cherry	Struss et al., 2003
UDAP401	AAA CCC TAG CCG CCA TAA CT GCT AAA GGC CTT CCG ATA CC	Apricot	Messina et al., 2004
UCDCH21	TTG TTG ACC ATC GAA TAT GAA G GAA GGT ACA TGG CGT GCC	Sweet cherry	Struss et al., 2003
UDAP404	CAT GAA CAG GGT CAA AAG CA TAT ATC CTT ACG CGG CCT CA	Apricot	Messina et al., 2004
CPSCT010	TTG GGT AAA TAC TTT ATC ATT TCC TCC CTG AAT AAG GGT TGT GC	Plum	Mnejja et al., 2005
UCDCH31	TCC GCT TCT CTG TGA GTG TG CGA TAG TTT CCT TCC CAG ACC	Sweet cherry	Struss et al., 2003
PS12A02	GCCACCAATGGTTCTTCC AGCACCAGATGCACCTGA	Cherry	Downey and Iezzoni, 2000

Table 3. List of microsatellites that produced polymorphic amplification patterns among the genotypes studied.

Locus name	No. of alleles	He	Ho	PI	r
PS12A02	12	0.81	0.63	0.089	0.0943
UCDCH17	8	0.86	0.42	0.068	0.2380
Pchgms1	11	0.72	0.34	0.142	0.2193
UDAP401	8	0.73	0.63	0.177	0.0539
UCDCH31	8	0.77	0.89	0.146	-0.0663
UCDCH21	8	0.69	0.38	0.175	0.1860
UDAP404	8	0.61	0.82	0.373	-0.1315
UDP96001	11	0.81	0.54	0.084	0.1447
UDP96005	11	0.85	0.82	0.072	0.0175
CPSCT010	10	0.64	0.58	0.190	0.0404
Total	108	8.64	7.31		
Average	9	0.72	0.61		

Ho = observed heterozygosity; He = expected heterozygosity; PI = probability; r = null allele frequencies.

among the 10 loci, with the highest effective number of alleles (12 alleles), and was followed by Pchgms1, UDP96001 and UDP96005 (11 alleles) (Table 3).

Observed heterozygosity calculated by direct counts for the putative locus, identified by each primer pair, ranged between 0.34 and 0.89 with a mean value of 0.61. Among the loci, the Ho values were the highest (0.89) for UCDCH31, while the lowest (0.34) were for the Pchgms1 locus (Table 3). The most informative locus, with regard to the PI, was PS12A02 (12 alleles per locus, PI = 0.089), whereas the least informative locus was UDAP404 (8 alleles per locus, PI = 0.373) (Table 3). The 10 selected primer pairs generated distinctive products in the range of 93-272 bp in the five different taxonomic groups (Table 3). The number of microsatellite in the different genotypes ranged from 8 (UDAP401, UCDCH21) to 17 (UCDCH31) with an average of 11.1 and a total of 111 (Table 4).

The genetic similarity measured within and among species ranged 0.95-1.00 within *P. laurocerasus*, 0.25-0.50 within *P. avium*, 0.60-1.00 within *P. cerasus*, 0.65-0.85 within *P. mahaleb*, and 0.60-0.80 within *P. angustifolia* genotypes (Table 5). The average similarity ratios within species in descending order were *P. laurocerasus* (0.97) > *P. cerasus* (0.76) > *P. mahaleb* (0.75) > *P. angustifolia* (0.68) > *P. avium* (0.35), respectively. The similarity ratio between standard SL64 and *P. mahaleb* genotypes, F12/1 and *P. avium* genotypes, and Montmorency and *P. cerasus* genotypes ranged from 0.40 to 0.65, 0.25 to 0.40, and 0.55 to 0.60, respectively (Table 5).

With respect to similarity between species, the average similarity ratios considering the average of five genotypes of each species was 0.12, 0.10, 0.05, and 0.01 between *P. laurocerasus* and *P. avium*, *P. cerasus*, *P. mahaleb*, and *P. angustifolia* genotypes, respectively. These ratios were 0.34, 0.02 and 0.03 between *P. avium* and the other three species (*P. cerasus*, *P. mahaleb* and *P. angustifolia*, respectively). *P. cerasus*-*P. mahaleb* and *P. cerasus*-*P. angustifolia* had 0.04 and 0.06 average similarity ratios, respectively (Table 5).

The genetic diversity detected among the five species studied divided them into two main groups depicted in Figure 1, which are in agreement with their current taxonomic classification and their morphological characteristics. The first cluster was also divided into two subclusters: the first subcluster consisted of *P. avium* and *P. cerasus*, and the second subcluster consisted of *P. laurocerasus*. The second cluster was also divided into two subclusters: the first subcluster consisted of *P. mahaleb* genotypes, and the second consisted of *P. angustifolia* genotypes. The reference rootstocks also clustered with their associated botanical species (Figure 1).

Table 4. Allele sizes, in bp, of 10 microsatellite loci in 28 *Prunus* genotypes.

Accession No.	Species	Genotype	PS12A02	UCDCH17	Pchgms1	UDAP401	UCDCH31	UCDCH21	UDAP404	UDP96001	UDP96005	CPSCT010
1	*P. laurocerasus*	52 K 12	132:138	160:174	142:142	106:116	84:94	95:109	154:154	103:103	93:101	162:168
2		52 K 17	132:138	160:174	142:142	106:116	84:94	95:109	154:154	103:103	93:101	162:168
3		54 K 03	132:138	160:174	142:142	106:116	84:94	95:109	154:154	103:103	93:101	162:168
4		28 K 04	132:138	160:174	142:142	106:116	84:94	95:109	154:168	103:103	93:101	162:168
5		52 K 18	132:138	160:174	142:142	106:116	84:94	95:109	154:168	103:103	93:101	162:168
6	*P. avium*	08 K 0053	160:160	180:190	136:142	260:266	132:132	109:109	154:168	115:123	119:131	176:176
7		28 K 0020	166:176	188:202	130:152	270:270	132:134	109:109	154:168	109:123	115:115	176:180
8		52 K 0042	158:158	180:200	138:138	262:266	130:142	109:117	154:168	105:123	119:133	176:182
9		53 K 0008	162:174	200:200	138:146	266:266	132:140	109:113	154:168	115:125	115:131	176:180
10		55 K 0092	158:166	188:198	138:138	262:266	124:134	111:111	154:168	105:123	115:135	176:176
11	*P. cerasus*	28 V 0001	146:160	180:188	138:180	262:266	124:132	103:109	154:168	99:113	119:135	170:176
12		52 V 0004	146:160	180:188	138:180	262:266	124:132	103:109	154:168	99:113	119:135	170:176
13		52 V 0001	146:160	180:188	138:180	262:266	124:132	103:109	154:168	99:113	119:135	170:176
14		55 V 0022	146:160	178:202	168:180	262:272	130:142	103:113	154:168	99:113	115:135	170:176
15		61 V 0001	146:160	178:202	168:180	262:272	130:142	103:113	154:168	99:113	115:135	170:176
16	*P. mahaleb*	05 M 0007	146:146	164:164	188:188	138:146	100:100	95:95	172:172	117:117	123:129	212:232
17		52 M 0005	162:162	164:164	188:188	138:146	106:106	95:95	170:170	117:117	113:123	212:232
18		60 M 0044	146:146	164:164	188:188	138:146	100:100	95:95	170:170	117:117	113:123	212:232
19		60 M 0016	146:146	164:164	188:194	138:146	100:126	95:95	170:170	117:117	123:129	212:232
20		60 M 0037	146:146	164:164	188:188	138:146	100:122	95:95	170:170	119:119	113:129	212:232
21	*P. angustifolia*	28 T 0001	134:134	154:154	168:180	146:162	160:162	95:95	174:182	111:111	109:113	178:178
22		28 T 0002	134:134	154:154	168:180	146:162	162:162	111:111	162:186	111:111	109:109	178:178
23		29 T 0002	134:134	154:154	168:180	146:162	162:162	111:111	156:184	111:111	111:111	178:178
24		29 T 0003	134:134	154:154	156:180	146:162	162:164	111:111	174:182	109:109	111:111	178:178
25		29 T 0004	134:134	160:160	156:180	146:162	158:162	111:111	156:184	109:109	109:113	178:178
26		SL64	142:142	164:164	188:188	138:146	124:124	111:111	170:170	119:119	113:113	206:228
27		F12/1	156:176	202:202	138:138	262:272	124:130	109:109	154:168	123:123	127:135	180:180
28		Montmorency	158:164	180:200	138:178	266:266	130:142	103:109	154:168	99:113	115:135	170:176
Allele sizes (bp)			132-176	154-202	130-194	106-272	84-164	95-117	154-186	99-125	93-135	162-232
Number of alleles per genotype			13	12	10	8	17	8	7	13	14	9

Table 5. The similarity matrix of species and genotypes belonging to *Prunus* species.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	1																											
2	1.00	1																										
3	1.00	1.00	1																									
4	0.95	0.95	0.95	1																								
5	0.95	0.95	0.95	1.00	1																							
6	0.15	0.15	0.15	0.20	0.20	1																						
7	0.10	0.10	0.10	0.15	0.15	0.30	1																					
8	0.10	0.10	0.10	0.15	0.15	0.40	0.25	1																				
9	0.10	0.10	0.10	0.15	0.15	0.40	0.30	0.35	1																			
10	0.05	0.05	0.05	0.10	0.10	0.30	0.40	0.50	0.30	1																		
11	0.10	0.10	0.10	0.15	0.15	0.45	0.25	0.45	0.35	0.45	1																	
12	0.10	0.10	0.10	0.15	0.15	0.45	0.25	0.45	0.35	0.45	1.00	1																
13	0.10	0.10	0.10	0.15	0.15	0.45	0.25	0.45	0.35	0.45	1.00	1.00	1															
14	0.05	0.05	0.05	0.10	0.10	0.20	0.25	0.30	0.25	0.30	0.60	0.60	0.60	1														
15	0.05	0.05	0.05	0.10	0.10	0.20	0.25	0.30	0.25	0.30	0.60	0.60	0.60	1.00	1													
16	0.05	0.05	0.05	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.05	0.05	0.05	0.05	0.05	1												
17	0.05	0.05	0.05	0.05	0.05	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.65	1											
18	0.05	0.05	0.05	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.05	0.05	0.05	0.05	0.05	0.85	0.80	1										
19	0.05	0.05	0.05	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.05	0.05	0.05	0.05	0.05	0.80	0.70	0.85	1									
20	0.05	0.05	0.05	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.05	0.05	0.05	0.05	0.05	0.70	0.65	0.80	0.75	1								
21	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.05	0.05	0.05	0.10	0.10	0.05	0.10	0.10	0.05	0.10	1							
22	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.05	0.05	0.05	0.10	0.10	0.05	0.05	0.05	0.05	0.05	0.80	1						
23	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.05	0.05	0.05	0.10	0.10	0.05	0.05	0.05	0.05	0.05	0.75	0.80	1					
24	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.10	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.70	0.60	0.70	1				
25	0.05	0.05	0.05	0.05	0.05	0.00	0.05	0.00	0.00	0.10	0.05	0.05	0.05	0.05	0.05	0.05	0.10	0.10	0.05	0.10	0.60	0.55	0.60	0.65	1			
26	0.05	0.05	0.05	0.05	0.05	0.00	0.00	0.00	0.00	0.05	0.05	0.05	0.05	0.00	0.00	0.40	0.55	0.55	0.45	0.65	0.10	0.05	0.05	0.05	0.10	1		
27	0.10	0.10	0.10	0.15	0.15	0.25	0.40	0.40	0.25	0.40	0.35	0.35	0.35	0.35	0.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	1	
28	0.10	0.10	0.10	0.15	0.15	0.30	0.25	0.55	0.45	0.40	0.60	0.60	0.60	0.55	0.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.30	1

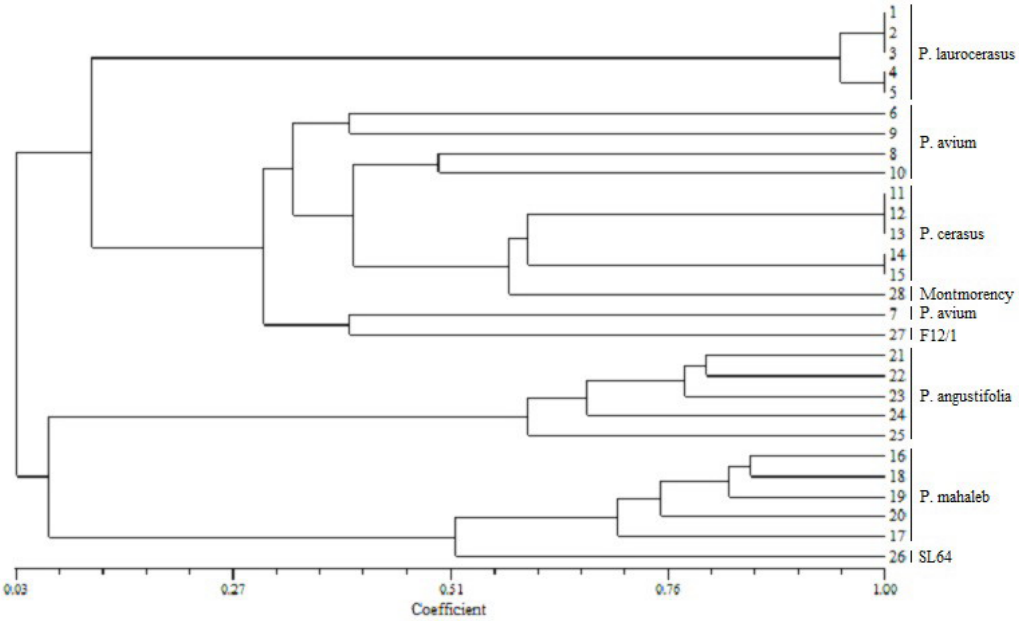

Figure 1. Dendrogram of 28 *Prunus* genotypes based on UPGMA analysis using the similarity matrix generated by the Nei and Li coefficient after amplification with 10 pairs of microsatellite primers.

DISCUSSION

In this study, we report for the first time the use of SSR markers for assessing genetic relatedness among 20 promising cherry rootstocks selected from the Black Sea and Northeast regions of Turkey and five *P. laurocerasus* and three standard rootstocks (SL64, F12/1 and Montmorency). The results obtained in the present study show that microsatellites can be effectively used for fingerprinting purposes in *Prunus*. In fact, all microsatellite primer pairs tested produced good and various levels of amplifications. As expected, the PS12A02 locus developed for *cerasus* was the most polymorphic among the six loci with the highest effective number of alleles (12 alleles) and was followed by Pchgms1, UDP96001 and UDP96005 (11 alleles). The results confirm the high transferability of the SSR used among different *Prunus* species. This transferability across *Prunus* species was already confirmed by different authors (Dirlewanger et al., 2002; Wünsch and Hormaza, 2002; Bouhadida et al., 2009). Previously, the PS12A02 locus was found to be the most informative in some studies (Downey and Iezzoni, 2000; Wünsch and Hormaza, 2004). The second most informative locus, Pchgms1, was also found to be very informative (11 alleles) in sweet cherries (Wünsch et al., 2004). According to Sefc et al. (2000), the PI value should be over 0.05, and all loci used in this study had PI values higher than 0.05, indicating that the selected loci were highly polymorphic for the materials used.

Previously, SSR markers have been widely used for molecular characterizations and similarity relationships among *Prunus* accessions and have revealed high polymorphism level to discriminate these accessions (Sosinski et al., 2000; Dirlewanger et al., 2002; Wünsch et al., 2004; Maghuly et al., 2005).

The higher levels of within-group variation observed within *P. avium* genotypes suggest a complex history of development of *P. avium* along the Black Sea and Northeast Anatolia in Turkey. The introduction and spread of wild and semi-domesticated *Prunus* species, especially from its native Near East range, domestication of indigenous wild *Prunus* species, natural hybridization between indigenous and introduced plants, and human selection may have contributed to this high variation.

The highest genetic similarity was detected between *P. avium* and *P. cerasus* with an average similarity value of 0.34. This was expected as *P. avium* is one of the ancestors of *P. cerasus.*

The observed and expected heterozygosities averaged over the 10 SSR loci were 0.61 and 0.72, respectively, indicating higher mean values than those reported for SSRs in *Prunus* species (Aranzana et al., 2003; Bouhadida et al., 2009). High allele number and high heterozygosity obtained in the present study reflect the ability of SSR markers to provide a unique genetic profile for individual plant genotypes.

Such high levels of heterozygosity are commonly observed among clonally propagated, outbred, perennial species, since they are favored during selection and are known to confer greater adaptability, vigor and productivity in clonal varieties (Aradhya et al., 1998; Sefc et al., 2000).

In summary, the gene pool of *Prunus* surveyed along the Black Sea and Northeast Anatolia in Turkey has significant amounts of genetic variation. In regard to germplasm management, our results show that the germplasm collection is highly variable and that most variation is common to all genetic groups identified. The *Prunus* germplasm from the region could have economically important adaptive traits that can potentially be incorporated into *Prunus* breeding programs. Hence, it is expected that the results of this study will assist current *Prunus* rootstock breeding efforts in Turkey, as well as maintaining the genetic integrity of the genetic resources. These results also demonstrate the high potential of SSR analysis in cherry rootstock identification and studies on diversity in *Prunus* species.

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