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Molecular Characterization of *Prunus* Cultivars from Romania by Microsatellite Markers

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Abstract: In Romania, *Prunus* species have great economic and social importance. With the introduction of new cultivars arises the need to preserve and characterize the local *Prunus* germplasm. Thus, a set of 24 polymorphic SSRs were selected for the overall characterization, including 10 peach, 11 apricot and 5 nectarine cultivars. The average number of alleles per locus (Na = 1.958), in addition to overall observed (Ho = 0.299) and expected heterozygosity (He = 0.286) were lower or comparable to those reported in similar studies, probably explained by the smaller number of analyzed cultivars restricted to a smaller geographic area. Among 26 genotypes a total of 101 alleles were identified, of which 46 alleles were in peach, 55 in apricot and 40 in nectarine, respectively. Six alleles from six loci (CPPCT-030, Pchgms-003, Pchgms-004, Pchgms-010, UDP97-401, UDP98-405) were common to all taxonomic groups. The most informative loci were BPPCT-025, Pchgms-021 and UDP96-001 in peach; BPPCT-025, BPPCT-001 and UDP96-001 in nectarine; and BPPCT-002, BPPCT-025, Pchgms-004, Pchgms-020 and Pchgms-021 in apricot. Clustering and genetic similarity analysis indicated that the degree of interspecific divergence in peach and nectarine cultivars was less than that in peach and apricot. These results will be useful to prevent confusion between cultivars, to improve breeding strategies and to benefit the management of *Prunus* cultivars bred in Romania.

Keywords: apricot; germplasm; microsatellites; nectarine; peach; SSR markers

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1. Introduction

Peaches (*Prunus persica* (L.) Batsch var. *persica*), apricots (*Prunus armeniaca* L.), and nectarines (*Prunus persica* (L.) Batsch var. *nucipersica* (Suckow) C. K. Schneid) are among the most important stone fruit crops in temperate climate zones. The centers of domestication of apricot cultivars are Central Asia, China and the Near East [1]. There are hypotheses assuming that several cultivars originated directly from the primary centers and others arose from the hybridization of genotypes from the secondary centers [2]. According to Kostina [3], apricot cultivars are classified into four major eco-geographical groups: Central Asian, Irano-Caucasian (with the richest variability), European and Dzhungar-Zailing (the Tien-Shan area on the border of Kazakhstan and China). The cultivars belonging to the European group show low diversity [4] and similar characteristics that make difficult the

discrimination between cultivars [5] by traditional methods based on morphological descriptors. Thus, molecular markers could provide valuable tools for cultivar discrimination and selection of new genotypes. A different situation can be observed in the case of the Siberian apricot from China, where a relatively high level of genetic diversity was observed among populations, but at the same time, heterozygosity was reduced, most probably as a result of low-level inbreeding [6]. A high genetic diversity among cultivars was also found in Turkey, considered a rich source of diverse apricot germplasm [7], and also in Iran [8].

There is a similar situation in the cases of peaches and nectarines, with a low number of genotypes having been used for the selection of new varieties that have been cultivated around the world [9,10]. The diversity of these crops has been reduced by the use of varieties having parents from the same gene pool [11]. The United States peach cultivars are considered especially limited in their diversity [9], because most commercial cultivars originated from a few parental cultivars used in United States breeding programs in the early twentieth century. The result of these breeding practices was the erosion of the genetic variability of peaches [12–14]. Low level heterozygosity of peaches was also observed in Spain, where traditional varieties have been replaced by new ones from North America [15].

Considering the high genetic similarities among cultivars, priority should be given to find the most effective method of cultivar identification and discrimination. Hence, to avoid misidentification, efficient DNA fingerprinting tools are needed. SSRs (Simple Sequence Repeats) are one of the most powerful marker systems available for such applications because of characteristics such as high levels of polymorphism, codominant inheritance and high discrimination power [16]. Many SSR markers have been developed for different *Prunus* species [17], including peach and nectarine [18–24], and apricot [25,26]. Besides allowing DNA fingerprinting and phylogenetic relationship evaluation in *Prunus* germplasm [27–29], SSR markers have also proved to be useful for the identification of different peach and nectarine hybrids [30], the differentiation of closely related genotypes [31] and the identification of important agronomic traits through QTL mapping [32–34].

Over recent years, most of the Romanian publicly held *Prunus* collections were reorganized and the number of *Prunus* accessions increased, mainly due to the introduction of new selections and hybrids [35–37]. Despite their great importance, most stone fruit cultivars currently present in different Romanian collections have not been characterized by molecular methods. For this reason, we considered it appropriate and essential to perform a comprehensive screening of several apricot, peach and nectarine cultivars using SSR primers typically used for germplasm characterization in other countries, to further support breeding programs and good management practices in local collections.

2. Materials and Methods

2.1. Plant Material

In this study, 10 peach (*Prunus persica* (L.) Batsch var. *persica*), 11 apricot (*Prunus armeniaca* L.), and 5 nectarine (*Prunus persica* (L.) Batsch var. *nucipersica* (Suckow) C. K. Schneid) cultivars [38,39] were analysed by SSR markers. The cultivars were maintained in the germplasm collection of the Agricultural Research and Development Station, Oradea. Cultivars were described according to specific descriptors (Table 1, Genres Project 61—for peach cultivars, IPGRI—for apricot cultivars).

Table 1. Characterization of apricot, peach and nectarine cultivars according to descriptors used in the genus *Prunus*: 1. Genotype; 2. Country; 3. Vigor of the tree (1. very small; 3. small; 5. medium; 7. great; 9. very great); 4. Blooming (1. extremely early; 2. very early; 3. early; 5. intermediate; 7. late; 8. very slow; 9. extremely late); 5. Harvest time (1. extremely early; 2. very early; 3. early; 5. average season; 7. late; 8. very slow; 9. extremely late); 6. Size of the fruit (1. very small (<20 g); 3. small (21–30 g); 5. medium (31–40 g); 7 great (41–50 g); 8. very big (51–60 g); 9. extremely big (>60 g); 7. Shape of the fruit (Apricot: 1. spherical; 3. ovoid; 5. cordiforme; 6. ovo-conical; 7. oblong; 9. elliptical. Peach: 1. very flat; 2. slightly flattened; 3. ovoid; 4. cordiforme; 5. oblong, 6. elongated); 8. Color of the fruit skin (Apricot: 1. greenish yellowish; 2. open cream; 3. cream; 4. yellow; 5. open orange; 6. orange; 7. dark orange. Peach: 1. green; 2. yellowish green; 3. cream; 4. yellowish cream; 5. yellow; 6. open orange); 9. Extension color coverage (Apricot: 1. absence; 3. reduced; 5. medium expansion; 7. widely extended. Peach: 1. absent; 2. red trail; 3. red stripe; 5. partially red; 6. red extended; 7. red; 8. bright red); 10. Color of the pulp (Apricot: 1. yellowish green; 2. yellowish white; 3. cream; 4. cream; 5. yellow; 6. opened orange; 7. orange; 8. dark orange; 9. Red. Peach: 1. greenish white; 2. white; 3. white yellowish; 4. yellowish; 5. yellow; 6. yellow orange; 7. orange; 8. red); 11. Firmness of the pulp (1. extremely soft; 3. soft; 5. medium; 7. firm; 9. very firm); 12. Taste of the pulp (1. very poor; 3. lower; 5. mediocre; 7. good; 9. excellent); 13. Adhesion of the kernel to the pulp (1. nonadherent; 2. semiadherent; 3. adherent); 14. Origin; 15. Compatibility (SI–Self-incompatible; SC–Self-Compatible).

	Genotype	Country	Vigor of the Tree	Blooming Age	Harvest Maturity	Size of the Fruit	Shape of the Fruit	Color of the Fruit Skin	Extension Color Coverage	Color of the Pulp	Firmness of the Pulp	Taste of the Pulp	Adhesion of the Kernel to the Pulp	Origin	Compatibility
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
								Peach cultivars							
P1	Alexia	RO	5	3	3	8	2	4	3	2	5	9	2	Cross Flacara x Marygold SCDP Baneasa	SC
P2	Collins	USA	5	5	4	6	2	4	6	5	6	6	1	Jerseyland x NJ188 (=Raritan Rose x NJ125). NJ125 = NJ66325 op (=J.H. Halle x Goldfinch)	SC
P3	Amalia	RO	5	3	5	7	3	3	3	3	5	8	1	Roubidoux x Flacara	SC
P4	Jerseyland	USA	7	5	6	7	3	5	8	5	7	7	1	NJ104325 op [= J.H. Halle x NJ41 (=Slappey x Dewey)]	SC
P5	Springold	USA	5	4	2	4	2	4	9	5	7	7	3	Cross [(Fireglow x Hiley) x Fireglow] x Springtime	SC
P6	Cardinal	USA	7	4	4	6	1	5	7	5	7	6	3	Self-fertilization of the Redhaven variety	SC
P7	Antonia	RO	4	3	4	7	3	3	8	3	5	8	2	-	SC
P8	Southland	USA	5	3	6	7	3	4	8	3	5	8	1	Self-fertilization of the Halehaven variety	SC
P9	Redhaven	USA	5	5	6	6	1	5	7	6	5	6	1	Cross Halehaven x Kalehaven	SC
P10	Superbă de toamnă	RO	5	4	7	7	3	3	9	2	5	8	1	Elberta x Mayflower	SC
								Apricot cultivars							
C1	Rareş	RO	3	3	1	6	3	5	5	5	5	7	1	Cross B12/6 x NJA13	SC

Table 1. *Cont.*

	Genotype	Country	Vigor of the Tree	Blooming Age	Harvest Maturity	Size of the Fruit	Shape of the Fruit	Color of the Fruit Skin	Extension Color Coverage	Color of the Pulp	Firmness of the Pulp	Taste of the Pulp	Adhesion of the Kernel to the Pulp	Origin	Compatibility
C2	Mamaia	RO	5	8	7	6	3	6	5	6	5	7	1	Complex hybridization between Marculesti1 (Ananas x Ananas) x Marculesti 5 (Targii de Bucuresti x Ananas)	SC
C3	Comandor	RO	6	7	6	8	3	4	5	5	5	7	1	Cross B 17/52 x Mr 43/1	SC
C4	CR-2-63-1 (Cream Ridge 2–63)	USA												American selection	SI
C5	CR-2-63-2 (Cream Ridge 2–63)	USA	6	4	4	7	2	6	3	7	3	7	1	American selection	SI
C6	CR-24-12-1	USA	7	2	2	8	1	5	5	7	7	9	1	American selection	
C7	CR-24-12-2	USA												American selection	
C8	Saturn	RO	5	6	5	6	2	4	5	7	9	5	2	Hybridization of Marculesti selection 40	SC
C9	Viorica	RO	5	5	3	8	1	6	-	7	7	7	1	Cross B 3/9 (P1) x NJA20	SC
C10	Sirena	RO	5	7	8	7	4	5	4	6	6	7	1	Cross Mr 37/1 x Mr 21/50	SC
C11	Carmela	RO	5	6	5	8	3	5	7	8	6	7	1	Cross Farmigdale x NJA20	SC
								Nectarine cultivars							
N1	Crimsongold	USA	7	3	4	5	3	6	9	5	5	7	2	Nectarine selection x July Elberta	SC
N2	Romamer	RO	7	5	2	5	4	6	3	4	5	5	1	Cross 624029148 x RR 48–153	SC
N3	Delta	RO	5	5	4	5	3	5	7	5	5	8	2	Romania, SCDP-Constanta	SC
N4	Cora	RO	5	5	6	7	3	5	7	5	6	7	2	Romania, SCDP-Constanta	SC
N5	ARK 165	USA			3		1	6	6		6			-	

2.2. SSR Analysis

Five different individuals were analyzed for each cultivar. Leaves were collected and mixed, and genomic DNA was isolated using the CTAB method described by Doyle and Doyle [40]. Allcultivars were initially screened with 34 SSR primers widely used for the molecular characterization of *Prunus* species [18–20,22]. Further analyses were performed with those SSR primers that generated amplicons in all *Prunus* species and which did not amplify unspecific fragments.

The final molecular screening included 24 selected SSR markers (Table 2). Seven microsatellite markers (BPPCT-001, BPPCT-002, BPPCT-014, BPPCT-025, Pchgms-020, Pchgms-021 and UDP96-001) were also screened on an Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). Each forward primer analyzed on 3500 Genetic Analyzer contained a 5′-M13 (TGTAAAACGACGGCCAGT) tail for universal dye labelling [41] (Table 2). PCR amplifications were performed for 24 SSRs using PCR mix containing 12.5 µL 2× DreamTaq Green PCR master mix (Thermo Fisher Scientific, Waltham, MA, USA), 10.25 µL nuclease-free water (Lonza, Switzerland), 25 pmol of each

primer (Eurogentec, Belgium) and 25 ng of genomic DNA in a final volume of 25 µL. DNA amplification was performed according the following program: 1. T = 94 °C, 4 min; 2. T = 94 °C, 30 s; 3. primer annealing at 50–58 °C (variable for different primers), 40 s; 4. elongation T = 72 °C, 40 s; steps 2–4 were repeated 35 times; 5. final elongation T = 72 °C, 5 min. Amplicons were separated on 1.5% agarose (Cleaver Scientific, Warwickshire, UK) gel in 1× TBE buffer (Lonza, Switzerland) and stained with 0.5 µg/mL ethidium bromide (Thermo Fisher Scientific, Waltham, MA, USA). At least 2 independent PCR amplifications were performed for each primer.

For the SSR markers screened on Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA) a 12.5 µL PCR reaction mixture was used, containing about 25 ng of template DNA, 200 nM of M13 5′-labeled with a fluorescent dye (FAM, VIC, NED, PET), 65 nM of M13-tailed locus-specific primer, 200 nM of untailed locus-specific primer, 1 U of *Taq* polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 1× PCR buffer with 2 mM $MgCl_2$, and 200 µM of each dNTP (Thermo Fisher Scientific, Waltham, MA, USA). Finally, 1 µL of PCR product from each of the four M13 dyes was pooled with 0.24 µL of LIZ500 standard ladder (Thermo Fisher Scientific, Waltham, MA, USA) and 12 µL of formamide to create three SSRs multiplexes in total. The pooled mixture was analyzed on an Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA), and fragment sizes were scored using GeneMarker V2.7.0 (SoftGenetics LLC, State College, PA, USA).

The Excel Macro AutoBin v0.9 [42] developed by Franc Salin was used to analyze the raw data set of amplified microsatellite markers and to determine the 'bin' size for each allele.

GenAlEx v6.5 (Peakall, Smouse, 2012) [43] was used to estimate at each locus, over all loci and for each *Prunus* accession, the observed (Na) and effective (Ne) number of alleles, the Shannon's information index (I), and the observed (Ho) and expected (He) heterozygosity, weighted on the number of samples (UHE).

A two-dimensional plot of principal coordinates analysis (PCoA) of the studied *Prunus* cultivars was generated by the GenAlEx v6.5 [43].

The unweighted pair group with arithmetic mean (UPGMA) dendrogram was produced with Poppr v2.8.2 [44]. The polymorphic information content (PIC) was calculated using the on-line Gene-Calc Tool [45].

A genetic similarity matrix was generated using Jaccard's similarity coefficient [46] in the NTSYSpc SIMQUAL module [47].

Table 2. Number of observed alleles per locus among *Prunus* cultivars.

Locus	Primer Sequence (5′→ 3′)	Repeat Motif	References	Size-Range (bp)	Peach	Apricot	Nectarine	No. of Unique Alleles	PIC
BPPCT-001	* AAT TCC CAA AGG ATG TGT ATG AG CAG GTG AAT GAG CCA AAG C	(GA)27	[22]	133–176	4	2	3	6	0.8076
BPPCT-002	* TCG ACA GCT TGA TCT TGA CC CAA TGC CTA CGG AGA TAA AAG AC	(AG)25		196–221	3	9	2	12	0.9102
BPPCT-004	CTG AGT GAT CCA TTT GCA GG AGG GCA TCT AGA CCT CAT TGT T	(CT)22		175–230	1	3	1	4	0.7005
BPPCT-010	AAA GCA CAG CCC ATA ATG C GTA CTG TTA CTG CTG GGA ATG C	AG)4 GG(AG)10		125–150	1	2	1	3	0.5907
BPPCT-014	* TTG TCT GCC TCT CAT CTT AAC C CAT CGC AGA GAA CTG AGA GC	(AG)23		204–233	2	1	1	3	0.5916
BPPCT-025	* TCC TGC GTA GAA GAA GGT AGC CGA CAT AAA GTC CAA ATG GC	(GA)29		168–215	5	4	4	9	0.8769
CPPCT-022	CAATTAGCTAGAGAGAATTATTGGACAAG AAGCAAGTAGTTTG	(CT)28CAA (CT)20	[20]	250–800	2	2	1	4	0.6363
CPPCT-029	CCAAATTCCAAATCTCCTAACATGATCAA CTTTGAGATTTGTTGAA	(CT)24		80–200	2	1	1	3	0.5513
CPPCT-030	TGAATATTGTTCCTCAATTCCTCTAGG CAAGAGATGAGA	(CT)30		175–220	2	2	2	3	0.5897
Pchgms-001	GGG TAA ATA TGC CCA TTG TGC AAT C GGA TCA TTG AAC TAC GTC AAT CCT C	(AC)12(AT)6	[18]	160–200	1	2	1	3	0.5898
Pchgms-003	GGA TCA TTG AAC TAC GTC AAT CCT C CAA CCT GTG ATT GCT CCT ATT AAA C	(CT)14		200–220	2	2	1	3	0.592
Pchgms-004	ATC TTC ACA ACC CTA ATG TC GTT GAG GCA AAA GAC TTC AAT	(CT)21		150–200	2	3	2	4	0.7002
Pchgms-010	GGTCACGCATCCTTTCATTT GACACCTCCATTTGTATCAAAGC	T19A10	[48]	180–200	1	2	1	2	0.3743
Pchgms-011	AAGCAATAAAACCAGCAGCAA TCAATCAATTGGCATGTTCG TTGAGGCCCACTTATTAGCC CCCCCATTATTCAAACTTCTG	(TA)11		250–300	1	1	1	3	0.5907
Pchgms-012	CGACACTTAGCTAGAAGTTGCCTTA TCAAGCTCAAGGTACCAGCA	(CT)9(TC)20(CA)9		200–450	2	3	2	7	0.8245

Table 2. *Cont.*

Locus	Primer Sequence (5′→ 3′)	Repeat Motif	References	Size-Range (bp)	Peach	Apricot	Nectarine	No. of Unique Alleles	PIC
Pchgms-020	* AATTGCATCACAGCAAGAGC GGGGGTTTGGTTAAGATCG CCCTTACCCCCTTACCACTT	(TA)15(TC)11		265–280	2	4	2	6	0.8101
Pchgms-021	* ACCACCATTTTGGCTCTCTG ACCACCACAACCAAACCATT	(TA)14		289–306	3	1	3	4	0.703
Pchgms-022	ATAATCCGGCAGGGGTCTTA TTGGGGTTTGTCAGTATTTTACA	(GA)14(AT)9		100–500	1	1	1	2	0.2392
Pchgms-023	CTGCCGAAAGCATTTTGAAT GAGCTCATGGCAACACAGAA	(TTC)5		300–500	1	2	1	3	0.5769
UDP96-001	* AGTTTGATTTTCTGATGCATCC TGCCATAAGGACCGGTATGT	(CA)17	[19]	123–146	3	1	3	6	0.8096
UDP97-401	TAAGAGGATCATTTTTGCCTTG CCCTGGAGGACTGAGGGT	(GA)19		100–150	1	2	1	2	0.3648
UDP97-402	TCCCATAACCAAAAAAAACACC TGGAGAAGGGTGGGTACTTG	(AG)17		125–170	1	1	2	3	0.5874
UDP97-403	CTGGCTTACAACTCGCAAGC CGTCGACCAACTGAGACTCA	(AG)22		100–110	1	1	1	2	0.3744
UDP98-405	ACGTGATGAACTGACACCCA GAGTCTTTGCTCTGCCATCC	(AG)9		100–150	2	3	2	4	0.6975
Total					46	55	40	101	
Average					1.9	2.3	1.7	4.2	

* The forward primer that contained a 5′-M13 (TGTAAAACGACGGCCAGT) tail.

3. Results

The selected markers amplified a total of 101 alleles in the analysed *Prunus* cultivars, with a mean allelic richness of 55 alleles in apricot, 46 alleles in peach and 40 alleles in nectarine cultivars (Table 2). The percentage of polymorphic loci ranged from 66.67% in apricot cultivars to 45.85% in nectarines. The mean number of alleles per locus (Na) was higher in apricot cultivars (2.292), followed by peach (1.917) and nectarine cultivars (1.667). The most polymorphic loci were BPPCT-002 (12 alleles), BPPCT-025 (9 alleles), Pchgms-012 (7 alleles), BPPCT-001 (6 alleles), Pchgms-020 (6 alleles), and UDP96-001 (6 alleles) (Table 2). The electrophoretic patterns of several polymorphic markers are shown in the supplementary material (Figures S1–S8). Six common alleles were identified among all taxonomic groups as revealed by six loci (CPPCT-030, Pchgms-003, Pchgms-004, Pchgms-010, UDP97-401, UDP98-405) (Table S1). The estimated genetic diversity indices are shown in Table S2. The average value of unbiased heterozygosity (uHe) was highest in apricot cultivars (uHe = 0.369), followed by peach (uHe = 0.295) and nectarine (uHe = 0.253) (Table S2). Overall, 26 multilocus genotypes (MLGs) were observed in our data set and 67 private alleles identified. The highest number of private alleles was registered in apricot cultivars (43 private alleles/22 loci), while the lowest number of private alleles (6 private alleles/4 loci) was found in nectarine cultivars (Table S3). The analyzed SSR markers were highly polymorphic with PIC values ranging from 0.24 to 0.91 with an average of 0.63 (Table 2).

The UPGMA clustering analysis revealed two main groups in the analyzed *Prunus* germplasm (Figure 1). Cluster I was further divided into two specific clusters: Ia comprising peach cultivars and Ib with nectarine cultivars. Cluster II was also subdivided in two sub-groups comprising apricot cultivars with combined Romanian and North American origin (Figure 1). This clustering is justified by Jaccard's similarity coefficient, with the highestvalues present between peach and nectarines, ranging from 0.26 to 0.39. The lowest genetic similarity was registered between apricot and peach cultivars, varying between 0.015 to 0.086 (Table S4).

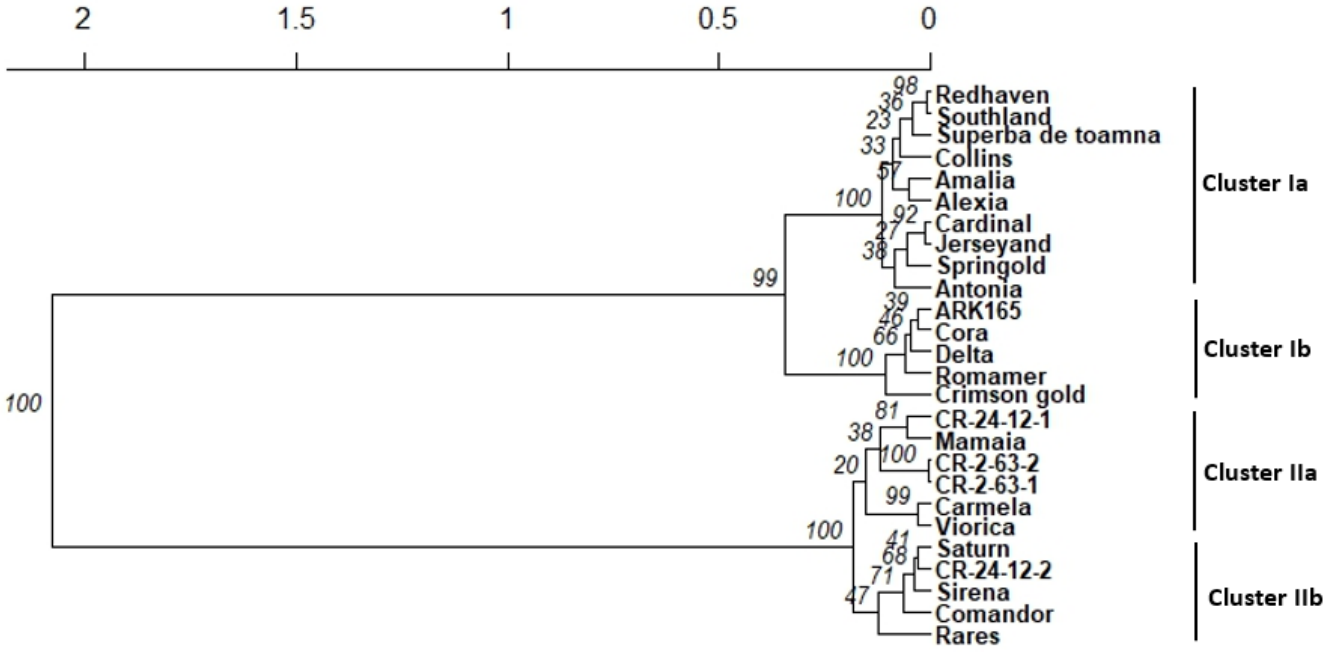

Figure 1. Unweighted pair group method with arithmetic mean (UPGMA) tree based on Nei'genetic distances calculated from 24SSRs. Generated with Poppr v2.8.2 [44].

This genetic clustering was also confirmed by principal coordinate analysis (PCoA) (Figure 2). The first and second principal coordinates accounted for 51.75% and 15.35% of

the total variation, respectively, explaining the total genetic variation across 24 SSR loci in different *Prunus* cultivars.

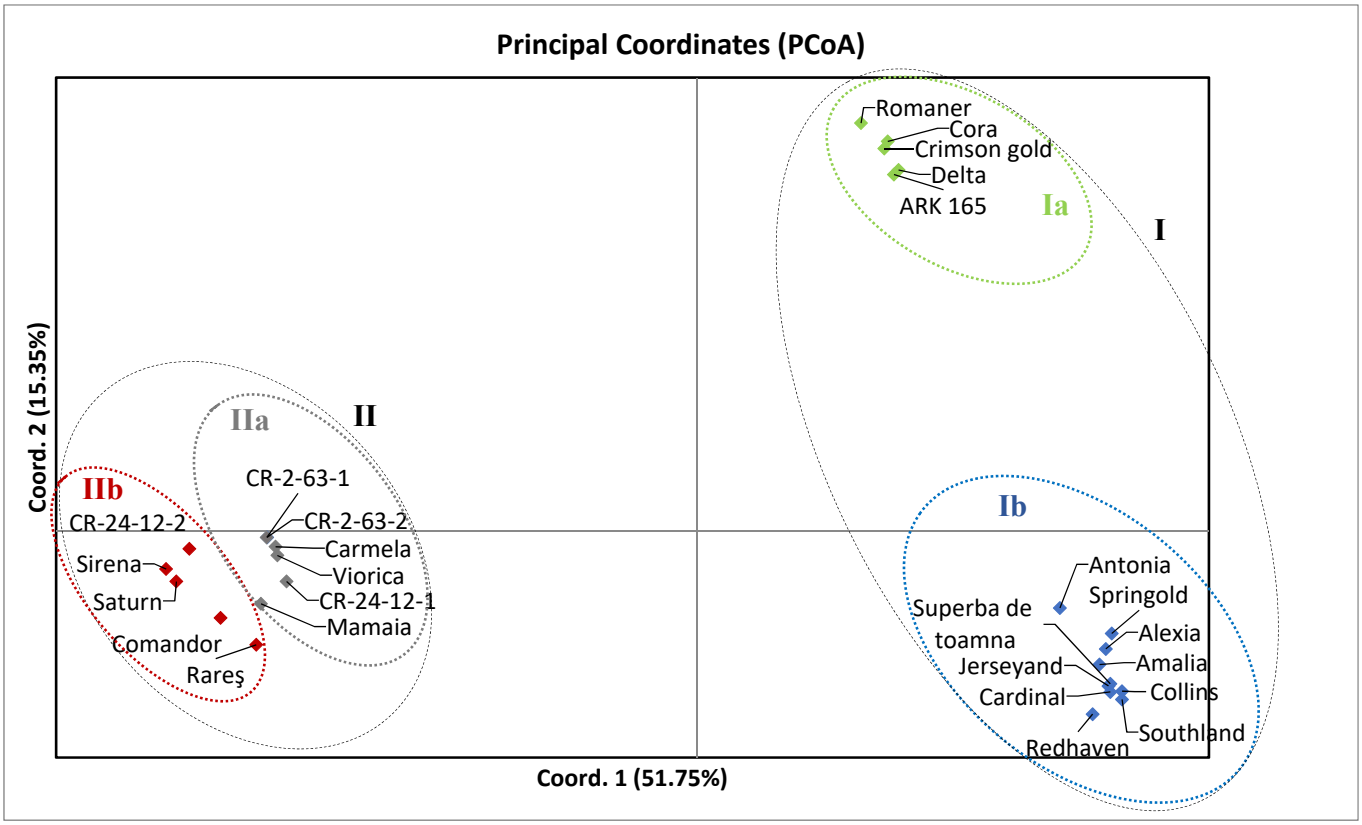

Figure 2. A two-dimensional plot of the Principal Coordinate Analysis (PCoA) of SSR data showing the clustering of 26 *Prunus* cultivars: 10 peach (cluster I, subcluster Ia), 5 nectarine cultivars (cluster I, subcluster Ib) and 11 apricot cultivars (cluster II with two subclusters: IIa and IIb). Generated with GENALEX 6.5 program [43].

4. Discussion

The primary aim of this study was to test and select a set of SSR markers suitable for analyzing genetic diversity among 26 *Prunus* genotypes pertaining to three different species (peach, apricot and nectarine) from a local Romanian collection, towards their conservation and utilization in future breeding programs. In this regard, 24 SSR primer pairs generating amplicons in all analyzed cultivars were tested, some of the band patterns being polymorphic.

These markers allowed the discrimination of 26 multilocus genotypes (MLGs) among analyzed *Prunus* cultivars. Altogether a total of 101 alleles were identified, of which 67 were private alleles and 34 were shared among the three *Prunus* species, with only 6 alleles common to all taxonomic groups.

The allele size in our study ranged from 133 to 176 bp for marker BPPCT-001, 196 to 221 bp for BPPCT-002, and 168 to 215 bp for BPPCT-025 (Tables S1 and S3). Other studies reported values between 128–168 bp [22], 166–229 bp [49] and 226–238 bp for BPPCT-002, and 178–202 bp for BPPCT-025 [22].

Our collection exhibited variable levels of overall genetic diversity compared to previous studies. The mean values for overall expected heterozygosity (He) in our collection ranged from 0.22 in nectarine to 0.28 in peach and 0.35 in apricot (Table S2). These data are comparable with those reported by Dettori et al., 2015 [17] (0.31 for peach and 0.27 for apricot), where 90 *Prunus* accessions belonging to five species were analyzed with 26 SSRs. Overall, the expected heterozygosity in *Prunus* accessions cultivated in Romania is weaker

compared to that registered in Chilean (0.46 in nectarine and 0.57 in peach) [50] or Chinese (0.79 in apricot) [51] collections. Moreover, allelic richness is lower (Na = 1.6 to 2.3) than had earlier been reported (Na = 2.4 to 15.14) [4,51–53]. This might be explained by the lower number of genotypes, restricted to a smaller geographic area, analyzed in our study.

The most informative primers in our study were those targeting BPPCT-025, BPPCT-001, BPPCT-002, Pchgms-021, and UDP96-001 loci in peach cultivars, BPPCT-025, BPPCT-001 and UDP96-001 in nectarines, and BPPCT-002, BPPCT-025, Pchgms-004, Pchgms-020 and BPPCT-004 in apricot cultivars. Some of these primers generated either monomorphic or polymorphic patterns, as shown in previous studies [18,22,54–56].

Based on these results, we may conclude that the genetic diversity parameters reported here are lower or comparable with those previously described in different *Prunus* germplasms, showing a wide range of variation depending on the set of SSR markers used, the number of analyzed genotypes and the origin of analyzed accessions. These variations may be also influenced by the method of detection used [17].

Clustering analysis indicated a lower degree of interspecific divergence between peach and nectarine cultivars than between peach and apricot cultivars, also shown by Jaccard's similarity coefficient. However, there was a separation of peach cultivars (subcluster Ia) from nectarines (subcluster Ib). Similar clustering results were reported by Rojas et al. (2008) [50]. The UPGMA cluster analysis shows the existence of two subclusters in apricot cultivars, with mixed Romanian and North American cultivars (Figures 1 and 2). Subcluster IIb is composed mainly of Romanian validated apricot cultivars (Saturn, Sirena, Comandor and Rares) that were phenotypically selected for fruit quality within the improvement program performed during the period 1983–2006 at the Băneasa Research and Development Station for Fruit Tree Growing [16,57]. Subcluster IIa is composed of Romanian apricot cultivars (Carmela, Viorica) that were selected mainly for resistance to disease by hybridizing with North American varieties obtained from the Rutgers Fruit Research and Extension Center, Rutgers University (CR-2-63-1, CR-2-63-2, CR-24-12-1, CR-24-12-2, NJA13 and NJA20) [37,58]. In recent years, most apricot breeding programs in Europe used PPV-resistant cultivars from North America to introduce this trait into local germplasms [59]. While some associations between analyzed *Prunus* genotypes might be explained by complex crosses between local cultivars and American germplasm, for several genotypes this information is unknown.

5. Conclusions

In this study we used a set of 24 previously published SSRs markers for *Prunus* species and assessed their versatility in DNA fingerprinting 10 peach, 11 apricot and 5 nectarine cultivars from Romania. The average number of alleles per locus, and the overall observed and expected heterozygosity were lower or comparable to those reported in similar studies. Clustering and genetic similarity analysis showed that all genotypes are clustered in their respective taxonomic groups, outlining a lower interspecific divergence between peach and nectarine than between peach and apricot cultivars.

To our knowledge, this is the first study assessing the usefulness of microsatellite markers in the characterization of local *Prunus* collections comprising apricot, peach and nectarine cultivars. The results will support breeding programs to improve the management practices of local collections.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae8040291/s1. Table S1. Multilocus genotype summary inferred from 24 SSRs in 26 accessions belonging to three different *Prunus* species (10 peach, 11 apricot and 5 nectarine cultivars). Table S2. The main parameters over 24 SSR loci analyzed in peach, apricot and nectarine cultivars. Table S3. Summary of private alleles per locus for peach, apricot and nectarine cultivars. Table S4. Jaccard's similarity coefficients among *Prunus* genotypes. Figure S1. *Prunus* electropherograms of polymorphic microsatellite marker BPPCT-001). Figure S2. *Prunus* electropherograms of polymorphic microsatelite marker BPPCT-002. Figure S3. *Prunus* electropherograms of polymorphic microsatelite marker BPPCT-014. Figure S4. *Prunus* electrophero-

grams of polymorphic microsatelite marker BPPCT-025. Figure S5. *Prunus* electropherograms of polymorphic microsatelite marker Pchgms-020. Figure S6. *Prunus* electropherograms of polymorphic microsatelite marker Pchgms-021. Figure S7. *Prunus* electropherograms of polymorphic microsatelite marker UDP96-001. Figure S8. SSR markers in peach (a-BPPCT038; b-PMS067), apricot (c-pchcm3, d-pchcm10) and nectarine cultivars (e-CPPCT022, CPPCT029, CPPCT030; f-pchcm1, pchcm3, pchcm4); separation on 1.5% agarose gel stained with 0.5 μg/mL ethidium bromide. The order of the samples is shown in Table 1.

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