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SSR based characterization of peach (*Prunus persica* L.) and apricot (*Prunus armeniaca* L.) varieties cultivated in Hungary

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SUMMARY

The SSR (Simple Sequence Repeat) markers allow the discrimination of the cultivars and determination its specific DNA fingerprints.

The aim of this research was to evaluate fifteen apricot (Prunus armeniaca L.) and fifty-one peach (Prunus persica L.) genotypes cultivated in Hungary to obtain their DNA fingerprints in 6 SSR (Simple Sequence Repeats) loci by allele numbers and sizes.

DNAs were extracted from leaves. PCR was carried out with CY-5 fluorescent labeled Prunus microsatellite markers and the products were separated on polyacrylamide gel with ALF (Automated Laser Flourometer)-Express II.

According to our results, in the case of peach genotypes, all 6 SSRs were able to amplify alleles. UDP 96 005 was the most informative marker and UCDCH 17 was the least due to its monomorphic pattern. Regarding the apricot samples BPPCT 041 did not amplify any allele. In the case of P. armeniaca UDP 96 005 had the highest heterozygosity index as well and the highest number of alleles. The least informative marker was the UCDCH 17. Since the 6 SSR were not enough to discriminate the apricot and peach genotypes, it is suggested to use more SSR primers.

Keywords: *Microsatellite, Prunus, Prunus armeniaca L., Prunus persica L., SSR, Simple Sequence Repeats*

INTRODUCTION

Rosaceous genomes offer one of the best systems for the comparative study of genome evolution. The diploid species representatives of this family (wild strawberry, rose, raspberry, sweet cherry, apricot and peach) have very small genomes of 200–300 Mb; however, they show a broad diversity in growth habit (Surányi et al. 2011, Verde et al. 2013).

Peach (*Prunus persica* (L) Batsch) is considered one of the genetically most well characterized species in the *Rosaceae*, and has been used as a model for genetic and genomic studies within its genus. It has distinct advantages that make it suitable as a model genome species for *Prunus* as well as for other species in the *Rosaceae* (Sosonski et al. 2000, Dhanapal et al. 2012, Nunez-Lillo et al. 2015).

Peach is a diploid plant (n=8) and has a comparatively small genome currently estimated to be 220–230 Mbp based upon the peach v1.0 assembly (Dhanapal and Crisosto 2013).

In contrast to other members of *Prunus*, peach is a self-compatible species with a high level of inbreeding (Warburton and Bliss 1996). According to Testolin et al. (2000) several cultivars with great breeding value show a low degree of heterozygosity (Cipriani et al. 1999, Dirlewanger et al. 2002, Aranza et al. 2003). Diversity of this crop has been drastically reduced by the use of improved varieties with a common genetic base from parents belonging to the same gene pool (Aranzana et al. 2003, Bouhadida et al. 2010).

Apricots with 590 Mbp belong to the family *Rosaceae* as well in the genus *Prunus* L., section *Armeniaca* (Lam.) Koch, which includes eight different species: *P. ansu* Maxima.; *P. armeniaca* L.; *P. brigantiaca* Vill.; *P. mandshurica* (Maxima.); *P. x dasycarpa* Ehrh.; *P. holosericea* (Batal); *P. mume* (Sieb.) and *P. siberica* L. All are interfertile diploid species with eight pairs of chromosome (Maghuly et al. 2005, Surányi et al. 2011).

The origin of apricots is in Middle-Asia and China (Surányi et al. 1981, Wang et al. 2011). They grow commercially worldwide in all temperate and subtropical regions (Maghuly et al. 2005, Surányi et al. 1981, Gürcan et al. 2015). Most of the cultivars are self-incompatible (Szabó and Nyéki 1991); fruits are small to medium and ripen over a long period (Maghuly et al. 2005). However, most of the European cultivars are self-compatible which has been proved that this ability is caused by mutation when pollen or S-allele of the pistil loses its function (Halász et al. 2007).

SSR(microsatellites) are tandemly repeated DNA sequences with a core unit of 1–6 base pairs (bp) which are abundant in prokaryotic and eukaryotic genomes and are ubiquitously distributed in both the protein-coding and non-coding regions (Guichoux et al. 2011, Kalia et al. 2011, Dettori et al. 2015, Cai et al. 2017).

The use of molecular markers for mapping QTLs has become a powerful tool in plant breeding for genetic analysis, early selection, and fingerprinting (Cipriani et al. 1999, Pedryc et al. 2009, Blaker et al. 2013).

Because of their appreciable polymorphism and wide cross-species transportability, most of these markers can be integrated into the linkage maps which are currently being constructed in peach, as well as in other stone fruit crops, such as almond, apricot, cherry and plum (Cipriani et al. 1999, Pedryc et al. 2004, Békefi et al. 2015, Makovics-Zsohár et al. 2017).

Microsatellite markers or simple sequence repeat (SSR) markers are codominant, highly polymorphic, easily detectable with PCR procedure, frequent across the genome, and informative across populations, cultivars, and species (Cipriani et al. 1999, Testolin et al. 2000, Dirlewanger et al. 2002, Aranzana et al. 2003, Bouhadida et al. 2007, Lietal 2008, Cheng and Huang 2009, Wünsch 2009, Blaker et al. 2013).

Sosonski et al. (2000) demonstrated that the microsatellites developed in *Rosaceae* species are useful for cross-species amplification and may have utility in both intra- and inter-family comparative mapping analyses.

SSR markers in *Prunus* species were developed previously in peach and used for genetic diversity assessment (Li et al. 2008), cultivar identification (Changwen et al. 2011, Li et al. 2013), trait mapping (Lambert and Pascal 2011, Liu et al. 2009), and phylogenetic studies (Cheng and Huang 2009, Cai et al. 2017). The results depend on the fragment separation method used since the polyacrylamide or agarose gels have different resolution power (Bouhadida et al. 2010).

The aim of this study is not only to identify cultivars, but also to verify synonyms and homonyms, to analyze parent-progeny relationships and to discover primary and secondary relationships between cultivars, as well as to obtain DNA fingerprints of peach and apricot genotypes and to establish SSR database from our results.

MATERIALS AND METHODS

Fifty-one peach (*Prunus persica* L.) from National Food Chain Safety Office and fifteen apricot (*Prunus armeniaca* L.) from Cegléd varieties, hybrids and clones were collected.

The DNA was extracted from leaves using E.Z.N.A OMEGA DNA extraction kit with PVP. The concentration was checked by Nanodrop spectrophotometer.

The SSR based characterization of peach and apricot genotypes the same 6 SSR markers were used (*Table 1*).

PCR in a volume of 10 µL was done in an iCycler equipment (BioRad). The components of the reaction mixture were: 20 ng of template DNA, 0.6 U of WTB-Taq polymerase (WestTeam Biotech, Pécs), 0.1 mM dNTP mix, 0.75 µM of each forward and reverse primer, and 1.25 mM $MgCl_2$ in 1X PCR buffer. For the amplification with the SSR markers we performed touchdown PCR, which consisted of an initiation cycle at 94 °C for two 2 min; 10 cycles of denaturation at 94 °C for 30 seconds, primer annealing at 65 °C for 30 seconds and extension at 72 °C for 1 minute, where the annealing temperature was decreased by 1 °C at each cycle. This was followed by 24 cycles of denaturation at 94 °C for 30 seconds, annealing at 56 °C for 30 seconds and extension at 72 °C for 1 minute. The reaction was completed with a post-polymerization extension cycle at 72 °C for 5 minutes.

Table 1

Six *Prunus* microsatellite which were used in apricot and peach analysis

Locus	Sequence	References
BPPCT 002 F	TCGACAGCTTGATCTTGACC	Dirlewanger
BPPCT 002 R	CAATGCCTACGGAGATAATAGAC	et al. (2002)
BPPCT 030 F	AATTGTACTTGCCAATGCTATGA	Dirlewanger
BPPCT 030 R	CTGCCTTCTGCCACACC	et al. (2002)
BPPCT 041 F	CAATAAGGCATTTGGAGGC	Dirlewanger
BPPCT 041 R	CAGCCGAACCAAGGAGAC	et al. (2002)
UDP 96 001 F	AGTTTGATTTTCTGATGCATCC	Cipriani
UDP 96 001 R	TGCCATAAGGACCGGTATGT	et al. (1999)
UDP 96 005 F	GTAACGCTCGCTACCACAAA	Cipriani
UDP 96 005 R	CCTGCATATCACCACCCAG	et al. (1999)
UCDCH 17 F	TGGACTTCACTCATTTCAGAGA	Struss
UCDCH 17 R	ACTGCAGAGAATTTCCACAACCA	et al. (2003)

The amplification products were separated on 6% polyacrylamide gel (ReproGel™, GE Healthcare, AP Hungary LTD) in a vertical system (ALF-Express II., Amersham Biosciences, AP Hungary LTD, Budapest). Fragments were detected by the Cy-5 fluorescent label attached to the forward primer. The precise size of the amplified SSR regions was determined by applying DNA molecular weight standards and ALFwin Fragment Analyser 1.0 software.

Dendrograms (Average Linkage) Within Groups were constructed based on the SSR data using SPSS 23 statistical program.

RESULTS

The DNA extraction procedure resulted in sufficient amount of DNA. The precise sizes of the amplified SSR fragments are shown in *Table 2* and *Table 3*. Regarding apricots out of the 6 *Prunus* SSRs BPPCT 041 did not amplified any alleles.

The number of alleles per locus in peaches ranged from 1–7 with a mean value of 3.3 alleles per locus, moreover with the frequency range from 1% to 100%. Markers that have heterozygosity index over 0.5 can be used for separating genotypes; those which are under 0.5 are not so useful. Consequently UDP 96 005 had the highest discrimination power with the heterozygosity index 0.67 (*Table 4*).

The number of alleles per locus in apricots ranged from 1-5 with a mean value of 2.6 alleles per locus, furthermore the frequency range from 2.2% to 100%. UDP 96 005 was the most informative primer pairs in apricot genotypes as well, where the heterozygosity index 0.753 was (*Table 4*).

Table 2

SSR fingerprint of the 51 peach genotypes with 6 *Prunus* primerpairs

Genotypes	BPPCT 002	BPPCT 030	BPPCT 041	UDP 96 001	UDP 96 005	UCDCH 17
Apolka	228:228	175:175	222:222	120:120	172:172	137:137
Olga	228:228	175:175	222:222	120:120	156:172	137:137
Vérbélű	228:228	175:175	222:222	122:122	156:156	137:137
Borota 2000	228:228	175:175	222:222	120:120	168:168	137:137
Kamila	228:228	175:175	222:222	**128:128**	156:156	137:137
Livia	228:228	**160:160**	**212:212**	122:122	172:172	137:137
SB6A-50	228:228	**173:173**	222:222	122:122	156:156	137:137
Vinegold	228:228	175:175	222:222	120:**130**	156:172	137:137
Candor	228:228	175:175	222:222	120:120	156:172	137:137
WB 258*	228:228	175:175	222:222	120:120	156:172	137:137
Royalvee	228:228	175:175	222:222	122:122	156:156	137:137
Veecling	228:228	175:175	222:222	122:**130**	156:172	137:137
Harnaś	228:228	175:175	222:222	120:120	168:168	137:137
Jiztia	228:228	175:175	222:222	120:120	168:168	137:137
Cantadozw	228:228	**171:171**	222:222	120:120	168:168	137:137
Kanadyska	228:228	175:175	222:222	120:120	172:172	137:137
Flamin Fury	228:228	**171:171**	222:222	120:**130**	156:156	137:137
Moulain	228:228	175:175	222:222	120:120	168:168	137:137
Kijowska Wczesna	228:228	175:175	222:222	120:120	168:168	137:137
Harken	228:228	175:175	222:222	120:120	156:172	137:137
Velvetsisters D93 2-10	228:228	175:175	222:222	120:120	156:172	137:137
BL6	228:228	175:175	222:222	120:120	156:156	137:137
Beta	228:228	175:175	222:222	120:120	156:172	137:137
T4*	228:228	175:175	222:222	120:**130**	156:172	137:137
Reliance	228:228	175:175	222:222	122:122	156:156	137:137
T2*	228:228	175:175	222:222	120:**130**	156:172	137:137
Harbringer	228:228	175:175	222:222	120:120	156:172	137:137
Iskara	228:228	175:175	**212:222**	120:120	156:172	137:137
Darbin	228:228	175:175	222:222	120:**124**	172:172	137:137
SB6A-35*	228:228	**171:171**	222:222	122:122	156:156	137:137
T5*	228:228	175:175	222:222	120:**136**	156:172	137:137
PoznaD2	228:228	175:175	222:222	122:122	168:168	137:137
SB6A-40*	228:228	**171:171**	222:222	122:122	156:156	137:137
Redhaven	228:228	175:175	222:222	122:122	156:156	137:137
Erzsébet	228:228	175:175	222:222	120:120	172:172	137:137
Siberian C	228:228	175:175	**212:212**	**130:136**	**138:138**	137:137
9076*	228:228	175:175	222:222	122:122	156:156	137:137
9123*	228:228	175:175	222:222	120:120	172:172	137:137
9286*	**224:224**	**171:171**	222:222	122:**126**	156:172	137:137
9300*	228:228	175:175	**212:222**	120:120	168:168	137:137
9413*	228:228	175:175	**212:222**	120:120	156:156	137:137
9619*	228:228	175:175	**212:222**	120:120	168:168	137:137
Crimson Gold	228:228	175:175	222:222	120:120	172:172	137:137
9674*	228:228	175:175	**212:222**	120:120	156:156	137:137
Vega*	228:228	175:175	222:222	120:120	168:168	137:137
9736*	228:228	175:175	**212:222**	120:120	168:168	137:137
Pit Lane	228:228	175:175	**212:222**	120:120	168:168	137:137
9763*	228:228	175:175	**212:222**	120:120	172:172	137:137
9774*	**224:224**	**171**:175	222:222	120:**128**	172:172	137:137
Harrowbeauty	228:228	175:175	222:222	120:120	168:168	137:137
Super Queen	228:228	175:175	222:222	120:120	172:172	137:137

Note 1: unique and rare alleles are in bold. Note 2: *under registration process.

Table 3

SSR fingerprint of the 15 apricot genotypes with 6 *Prunus* primerpairs

Genotypes	BPPCT 002	BPPCT 030	BPPCT 041	UDP 96 001	UDP 96 005	UCDCH 17
Mandulakajszi C. 712	187:187	138:146	-	110:110	110:156	128:128
Magyar kajszi C. 1646	187:187	146:146	-	110:110	94:110:124:156	128:128
Bukurija	**189**:**198**	138:146	-	110:110	124:**130**	128:128
Ceglédi óriás	187:187	138:146	-	110:110	110:124:156	128:128
Gönci	187:187	138:146	-	110:110	94:110:124:156	128:128
Ceglédi bíborkajszi C. 244	187:187	138:146	-	110:110	110:156	128:128
Magyar kajszi C. 302	187:187	146:146	-	110:110	94:110:124:156	128:128
Veecot	187:187	146:146	-	110:110	94:110:124:156	128:128
Ceglédi szilárd H-II. 20/6	187:187	146:146	-	110:110	94:110:124:156	128:128
H-II. 25/62	**189:189**	138:146	-	110:110	110:156	128:128
H-II. 25/37	**189:189**	146:146	-	110:110	110:156	**126**:128
H-II. 16/1	187:187	146:146	-	110:110	94:110:124:156	128:128
H-II. 46/45	187:187	138:146	-	110:110	94:110:124:156	128:128
Nyújtó Ferenc emlékére H-II. 25/65	187:187	146:146	-	110:110	110:156	128:128
Rózsabarack C. 320	187:187	138:146	-	110:110	94:124	128:128

Note: unique and rare alleles are in bold.

Table 4

Number of alleles and heterozygosity index per locus in peaches and apricots

Name of the locus	Number of alleles in peaches	Heterozygosity Index (HI) of the primers in case of peaches	Number of alleles in apricots	Heterozygosity Index (HI) of the primers in case of apricots
BPPCT 002	2	0.075	3	0.330
BPPCT 030	4	0.260	2	0.391
BPPCT 041	2	0.208	-	-
UDP 96 001	7	0.517	1	0
UDP 96 005	4	0.670	5	0.753
UCDCH 17	1	0	2	0.064

Dendrograms constructed based on the SSR data of 51 peach and 15 apricot cultivars are shown by *Figure 1–2*.

DISCUSSION

We obtained allele sizes in the same range as it was found in the literature (*Table 5*).

Fifty-one peach genotypes have been examined with the 6 Prunus specific primer pairs. According to the dendrogram (*Figure 1*) constructed from our data shows 3 major groups. Even though our results show small variability in some cases we could found unique alleles for instance in Siberian C at the loci UDP 96 005 or in Livia at the loci BPPCT 030. Furthermore, rare alleles were detected for instance by BPPCT 030, BPPCT 041 (*Table 2*). Concerning our peach genotypes UCDCH 17 was the least informative marker due to its monomorphic pattern. Compare to other authors as *Table 6* shows we have similar results regarding our peach genotypes.

In conclusion, we need to use more SSR primers to be able to distinguish all of our genotypes.

Out of the 6 *Prunus* specific primer pairs only one (BPPCT 041) did not amplify any alleles in our 15 apricot samples. With the remaining 5 SSR markers we made an SSR database. After analyzing our results we constructed a dendrogram (*Figure 2*) which displays two major groups, one of them (II) only containing Bukurija due to its two unique alleles at the loci BPPCT 002 and UDP 96 005. These make Bukurija well distinguishable in the case of our samples. Regarding the other major group (I) it can be divided into 2 subgroups. The first subgroup (1) includes the clones of Magyar kajszi and Veecot Canadian variety which can not be discriminated with these 6 *Prunus* SSRs. Furthermore, it contains some hybrids and Hungarian traditional cultivars. The subgroup 2 displays clones and hybrids of traditional Hungarian cultivars.

Namezi et al. (2016) analyzed 27 apricot genotypes with SSR markers. According to their results UDP 96 001 was the most informative locus with 0.71 and UDP 96 005 was middle strong with 0.48 heterozygosity index. However, regarding our results UDP 96 001 was the least informative since it gave us monomorphic pattern and UDP 96 005 was the most informative with 0.753 heterozygosity index (*Table 4*).

Sanchez-Perez et al. (2005) had similar results in allele sizes to ours concerning UDP 96 001 and UDP 96 005. They also found UDP 96 005 to be multilocal when some genotypes showed four alleles (*Table 3*).

Figure 1: **SSR based dendrogram of the analyzed 51 peach genotypes**

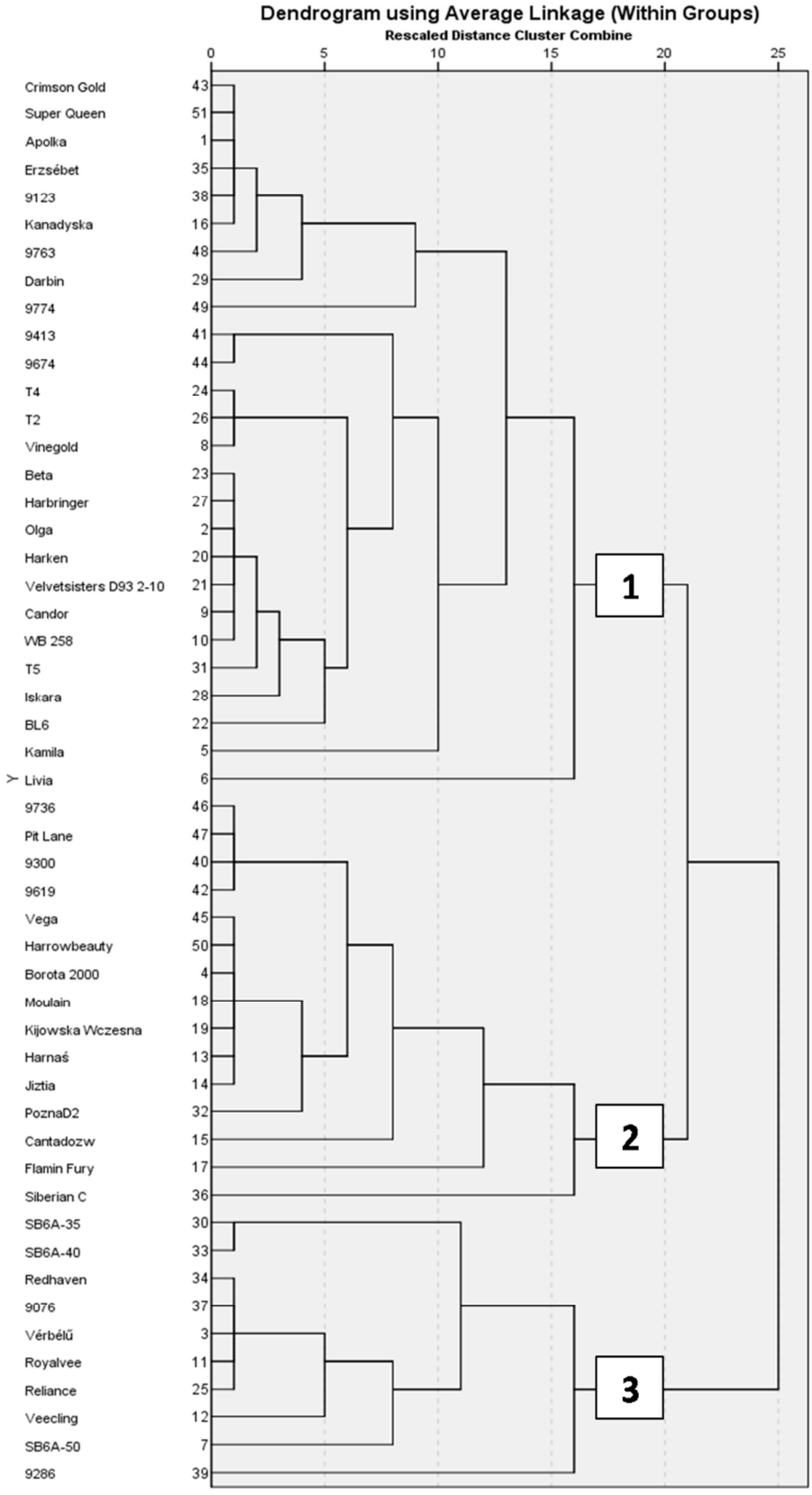

Figure 2: **SSR based dendrogram of the analyzed 15 apricot genotypes**

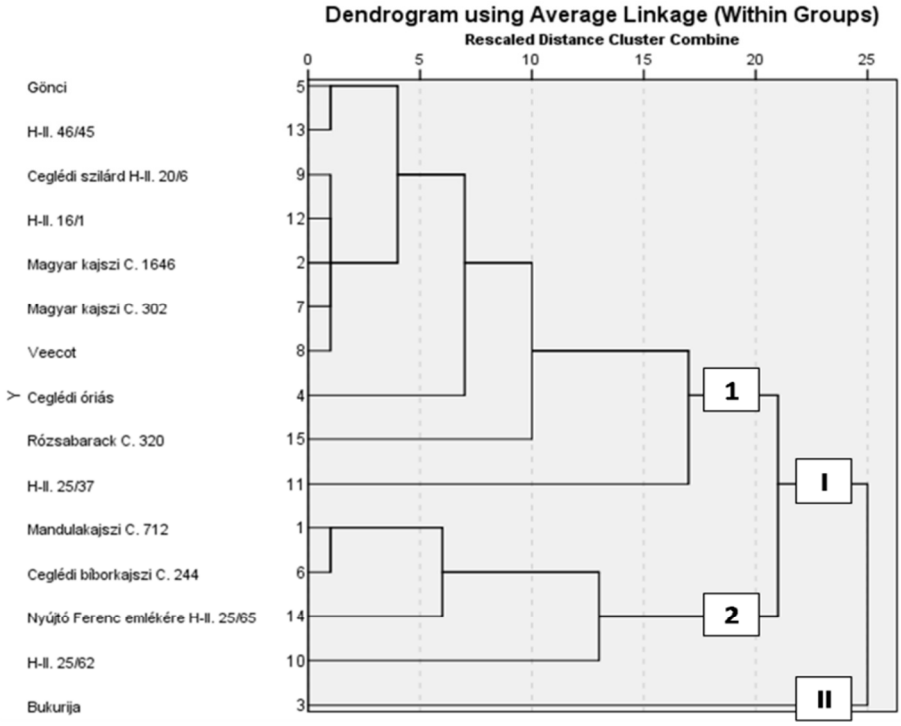

Table 5

Allele size ranges in the references and in the present study

Primer	Origin	Reference	Allele size ranges in peach (bp)	Allele size ranges in the present study, peach (bp)	Reference	Allele size range in apricot (bp)	Allele size ranges in the present study, apricot (bp)
UDP 96 001	peach	Cipriani et al. (1999) Ahmed et al. (2004) Cai et al. (2017)	127–129 120–124 117–125	120–136	Sanchez-Perez et al. (2005)	104	110
UDP 96 005	peach	Cipriani et al. (1999) Cai et al. (2017)	145–147 156–173	138–172	Sanchez-Perez et al. (2005)	82–164	94–156
BPPCT 002	peach	Dirlewanger et al. (2002)	226–238	224–228			187–198
BPPCT 030	peach	Dirlewanger et al. (2002)	158–180	160–175			138–146
BPPCT 041	peach	Dirlewanger et al. (2002)	210–220	212–222			-
UCD-CH17	sweet cherry	Ahmed et al. (2004)	193	137			126–128

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