

## Preliminary results of SSR based characterization of sour (*Prunus cerasus* L.) and sweet cherry (*Prunus avium* L.) genotypes cultivated in Hungary

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**Abstract:** Cherry cultivation in the Carpathian basin area began more than 100.000 years ago. Adapting to the basin specific ecological conditions resulted in high degree of genetic variability among the cherry cultivars. The SSR (Simple Sequence Repeat) markers allow the discrimination of the cultivars and determination their specific DNA fingerprints. Due to the high degree of polymorphism of microsatellite markers, generally only six SSR loci are enough to differentiate the varieties. Microsatellite markers are used not only for cultivar identification but also for the verification of synonyms and homonyms. Owing to their locus specificity and Mendelian codominant inheritance, parentage can be clearly identified, primary and secondary relationships between the cultivars can be discovered.

The aim of this research was to characterize 29 sour cherry (*Prunus cerasus* L.), and 38 sweet cherry (*Prunus avium* L.) genotypes cultivated in Hungary to establish their DNA fingerprints in 6 SSR loci by allele numbers and sizes.

**Keywords:** Microsatellite, *Prunus*, *Prunus avium* L., *Prunus cerasus* L., parent-progeny analysis

### Introduction

Both sour and sweet cherries are important economic dicot stone fruit plants, belonging to the Rosaceae family within the Prunoideae subfamily.

Sour cherry (*Prunus cerasus* L.) is an allopolyploid, spontaneous hybrid species originating from West Asia and South-Eastern Europe. It is supposedly formed from the cross between the diploid sweet cherries (*Prunus avium* L.,  $2n=2x=16$ ) and the tetraploid Mongolian cherries (*Prunus fruticosa* Pall,  $2n=4x=32$ ) (Beaver et al. 1995). Presently, sour cherry still grows wild in various parts of Europe, from Scandinavia and the north of Turkey to the south and shows great genetic diversity.

Sweet cherries (*Prunus avium* L.) are diploid plants ( $2n=2x=16$ ). Primary centers of origin are Pre-Asia (Caspian Sea, Black Sea region) as well as Western China mountains. Europe is considered to be the secondary center of origin (Pór and Fabula 1982; Tropicos 2015).

Hungary is an important sour cherry producing country because of its traditions, varieties, technology, and market opportunities (Nyéki et al. 2003). Towards the end of the 19th century, Pándy and Cigány varieties dominated in sour

cherry production. The quality of Pándy is considered as a standard; it is a unique Hungarian variety (Nyéki et al. 2003).

Sweet cherry production has been doubled in the world since 2001, the average yield in Hungary is 5-6 t/ha. In Hungary ripening period of sweet cherry begins in the third decade of May and ends in the middle of July. According to experts Germersdorfi óriás, Bigarreau Burlat, Van, Margit, Katalin, Linda were the most used cultivars in Hungary in 2012. However, other hybrid species have also become popular such as Carmen, Vera, Rita and other Canadian, American and Italian cultivars (Radóczné 2012).

Microsatellites or simple sequence repeats (SSRs) are co-dominant, abundant, multi-allelic, as well as uniformly distributed over the genome, and can be detected by simple reproducible assays (Powell et al. 1996). Its length ranges from 1- 6 nucleotides (Van Oppen et al. 2000) and can be classified as mono-, di-, tri-, tetra-, penta- and hexanucleotide repeats. With the minimum repeat length of 12 base-pairs they are tandemly repeated usually 5-20 times in the genome (Goodfellow 1992; Vaughan and Lloyd 2003; Ellegren 2004; Prajapati et al. 2017). As a result of their quickness, simplicity, rich polymorphism and stability SSR markers

are highly popular in genetic diversity analysis (Turkoglu et al. 2013; Gürcan et al. 2015; Batnini et al. 2016), construction of fingerprints (Cantini et al. 2001; Rojas et al. 2008; Klabunde et al. 2014; Turet-Sayar et al. 2012; Ivanovych et al. 2017), genetic purity test (Spann et al. 2010), molecular map construction and gene mapping (Ogundiwin et al. 2009; Olukolu et al. 2009; Fan et al. 2010; Pacheco et al. 2014; Rowland et al. 2014; Wang et al. 2014; Eduardo et al. 2015), utilization of heterosis, especially in the identification of species that are genetically related. Microsatellite markers have also been used in several studies to define conserved regions among related species

(Decroocq et al. 2003; Martínez-Gómez et al. 2003; Maghuly and Laimer 2011; Alisoltani et al. 2016) for both plants and animals genome mapping (Weising et al. 1998).

Gustavsson (2014) and Lacis (2014) were of the opinion that SSR markers should be compulsorily used to provide molecular profiles for the cultivars thus detecting duplicates and mislabelling in germplasm collections. In order to ensure that data are compatible with international data bases, there is a marker set which the ECPGR (European Cooperative Programme for Plant Genetic Resources) Prunus WG recommended.

Table 1. Studied sour cherry genotypes and their parents, origin and self(in)compatibility status (SI, SC)

Genotypes	Parents	Origin	Self-compatibility
3/48 9	Csengődi x Érdi bőtermő	Hungary	
Cigány 59 12	Clone of Cigány	Hungary	SC (S6, S9, Sa, Sb)
Cigány 7 13	Clone of Cigány	Hungary	SC
Cigány C404 9	Clone of Cigány	Hungary	SC
Csengődi	traditional cultivar/landrace	Hungary	SC
Csengődi 11	Clone of Csengődi	Hungary	SC
Debreceni bőtermő	traditional cultivar/landrace	Hungary	SC
Érdi bőtermő	Pándy x Nagy angol	Hungary	SC (S4, S6m, Sa)
Érdi bőtermő 13	Clone of Érdi bőtermő	Hungary	SC
Érdi jubileum	Pándy x Eugénia	Hungary	SC
Érdi nagygyümölcsű	Hankovszky x unknow	Hungary	SI (S1, S12, Sc, Sd)
Favorit	Pándy x Montreulli	Hungary	SC
Kántorjánosi	traditional cultivar/landrace	Hungary	SC
Kántorjánosi 3	Clone of Kántorjánosi	Hungary	SC
Korai pipacs	Pándy x Császár	Hungary	SC
Körösi korai	traditional cultivar/landrace	Hungary	SC
Maliga emléke	Pándy x Eugénia	Hungary	SC
Meteor korai	Pándy x Nagy angol	Hungary	SC
Oblacsinszka	traditional cultivar/landrace	Yugoslav region	SC
Pándy 279	Clone of Pándy	Hungary	SI
Pándy 279 11	Clone of Pándy	Hungary	SI
Pándy 48	Clone of Pándy	Hungary	SI
Pándy 48 10	Clone of Pándy	Hungary	SI
Pándy BB 119	Clone of Pándy	Hungary	SI
Pándy Bb119	Clone of Pándy	Hungary	SI
Paraszt	synonym of cigány?	Hungary	SC
Pipacs 14	unknown origin Hybrid	Hungary	SC
Piramis	M221 x Meteor korai	Hungary	partially SC
Újfehértói fürtös	traditional cultivar/landrace	Hungary	SC (S4, Sd, Se)

Table 2. Studied sweet cherry genotypes, their parents, origin and self(in)compatibility status (SI, SC)

	Parents	Origin	Selfcompatible
Aida	Moldvai fekete (Trusenszkaja 40) x H 236	Hungary	SI ( $S_6S_{12}$ )
Alex	Van x John Innes	Hungary	SC ( $S_3S_3$ )
Anita	Trusenszkaja 2 x H 3	Hungary	SI ( $S_3S_6$ )
Bigarreau Burlat	traditional cultivar/ landrace	France	SI ( $S_3S_9$ )
Botond	German, H 264	Hungary	SI ( $S_3S_4$ )
Canada giant	unknown	Canada	SI ( $S_1S_3$ )
Carmen	Yellow Dragan x H 203	Hungary	SI ( $S_4S_5$ )
Colney	unknow, UK Norfolk, John Innes Centre	United Kingdom	SI ( $S_5S_6$ )
Germersdorfi clone 1	clone of Germersdorfi	Hungary	SI ( $S_3S_{12}$ )
Germersdorfi clone 3	clone of Germersdorfi	Hungary	SI ( $S_3S_{12}$ )
Germersdorfi clone 45	clone of Germersdorfi	Hungary	SI ( $S_3S_{12}$ )
Giorgia	ISF 123 x Caccianese	Germany	SI ( $S_1S_3$ )
Hedelfingeni Óriás	traditional cultivar/ landrace	Germany/HU	SI ( $S_3S_4$ )
Jaboulay	traditional cultivar/landrace	France	SI ( $S_6S_9$ )
Katalin	Germersdorfi óriás x Podjebrad yellow	Hungary	SI ( $S_4S_{12}$ )
Kavics	Germersdorfi óriás x Budakalász	Hungary	SI ( $S_4S_{12}$ )
Krupnoplodnaja	Napoleon Blanc open pollination	Ukraine	SI ( $S_5S_9$ )
Linda	Hedelfingeni óriás x Germersdorfi óriás 3	Hungary	SI ( $S_3S_{12}$ )
Margit	Germersdorfi seedling	Hungary	SI ( $S_4S_{12}$ )
Merchant	traditional cultivar/landrace	United Kingdom	SI ( $S_4S_9$ )
Münchenbergi korai	Flamentier x Márki korai	Germany	SI ( $S_3S_4$ )
Octavia	Schneiders Spate Knorpelkirsche x Rube	Germany	
Pál	Bigarreau Burlat x Stella	Hungary	SC ( $S_4S_9$ )
Péter	Bigarreau Burlat x Stella	Hungary	SC ( $S_3S_4$ )
Regina	Schneiders Spate Knorpelkirsche x Rube	Hungary	SI ( $S_1S_3$ )
Rita	Germersdorfi x Szomolyai fekete	Hungary	SI ( $S_5S_{22}$ )
Samba	2S-84-10 (=Stella 35A) x Stella 16.A.1	Canada	SI ( $S_1S_3$ )
Sándor	Bigarreau Burlat x Stella	Hungary	SC ( $S_4S_9$ )
Solymári gömbölyű	traditional cultivar/landrace	Hungary	SI ( $S_3S_4$ )
Stella	Lambert x St. John Innes 2420	Canada	SC ( $S_3S_4$ )
Sunburst	Van x Stella	Canada	SC ( $S_3S_4$ )
Sylvia	Van x Sam	Canada	SI ( $S_1S_4$ )
Szomolyai fekete	traditional cultivar/ landrace	Hungary	SI ( $S_2S_4$ )
Tünde	Yellow Dragan x Bigarreau Burlat	Hungary	SI ( $S_3S_5$ )
Valeri Cskalov	Rozovaja species open pollination	Romania-Ukraine	SI ( $S_1S_9$ )
Van	Imperatrice Eugenie seedling	Canada	SI ( $S_1S_3$ )
Vega	Bing x Victor	Canada	SI ( $S_2S_3$ )
Vera	Ljana (Truzenszkaja 6) x Van	Hungary	SI ( $S_1S_3$ )

SSRs or microsatellites (Schueler et al. 2003; Höltken and Gregorius 2006) have been developed and successfully applied both in sour and sweet cherries (Downey and Iezzoni

2000; Cantini et al. 2001; Pedersen 2006; SSR markers of *Prunus* origin are polymorphic and transferable within *Prunoideae* (Cipriani et al. 1999; Downey & Iezzoni 2000; Dirlewanger

et al. 2002; Wunsch & Hormaza 2004; Lacis et al. 2009) thus using these SSR markers it was possible to distinguish the cultivars and accessions studied. Our aim was to determine the SSR fingerprints of sour and sweet cherry genotypes to expand databases set by the Institute of Genetics, Microbiology and Biotechnology.

## Materials and methods

### Plant material

The study included 29 sour cherry (*Prunus cerasus* L.) and 38 sweet cherry (*Prunus avium* L.) genotypes (landraces, clones, hybrids, open pollinated variety) developed in Hungary and common cultivars of foreign origin. The plant material was collected at the Fruit Research Institute in Érd, Hungary, and National Food Chain Safety Office NÉBIH, Hungary (Table 1, 2).

### DNA isolation

Young leaves were collected from a single tree for each genotype, stored at -70°C. Total genomic DNA was extracted using the modified CTAB protocol (Van der Beek et al. 1992). The DNA was dissolved in distilled water to a final volume of 200 µl and its concentration was checked with a Nanodrop spectrophotometer.

### Amplification conditions

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<sub>2</sub> in 1X PCR buffer. For the amplification with the SSR primers we performed touchdown PCR, which consisted of an initiation cycle at 94°C for 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.

The amplified 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 Cy-5 fluorescent labelling of the forward primer. The precise sizes of the amplified SSR regions were determined by applying DNA molecular weight standards and ALFwin Fragment Analyser 1.0 software. Dendograms (Average Linkage) Between Groups were constructed based on the SSR data using SPSS 22 statistical program.

### Markers

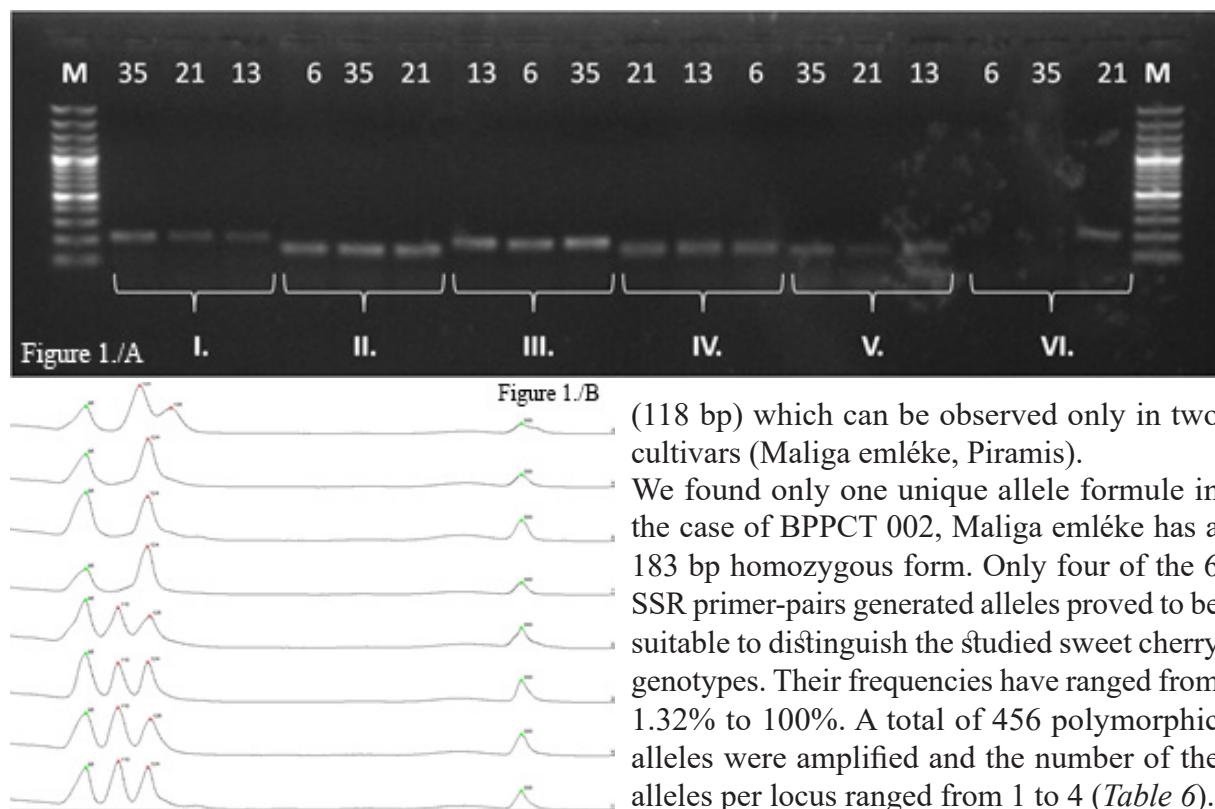
The same 6 SSR primer pairs were used in both sour and sweet cherry (Table 3).

Table 3. Six *Prunus* microsatellite primer pairs used in the marker analyses

Locuscode	Primer sequences (5'-3')	References
BPPCT 041 F	CAATAAGGCATTGGAGGC	Dirlewanger et al. (2002)
BPPCT 041 R	CAGCCGAACCAAGGAGAC	
BPPCT 030 F	AATTGTACTTGCCTAATGCTATGA	Dirlewanger et al. (2002)
BPPCT 030 R	CTGCCTTCTGCCACACC	
BPPCT 002 F	TCGACAGCTTGATCTTGACC	Dirlewanger et al. (2002)
BPPCT 002 R	CAATGCCTACGGAGATAATAGAC	
UDP 96 005 F	GTAACGCTCGTACCCACAAA	Cipriani et al. (1999)
UDP 96 005 R	CCTGCATATCACCAACCCAG	
UDP 96 001 F	AGTTTGATTCTGATGCATCC	Cipriani et al. (1999)
UDP 96 001 R	TGCCATAAGGACCGGTATGT	
UCDCH 17 F	TGGACTTCACTCATTCAGAGA	Struss et al. (2003)
UCDCH 17 R	ACTGCAGAGAATTCCACAAACCA	

## Results and Discussion

The modified CTAB DNA extraction procedure (Van der Beek et al. 1992) resulted in sufficient amount of DNA (40-80 ng/ $\mu$ l). The PCR products tested on agarose gel and the size of the amplified SSR fragments determined with ALFwin Fragment Analyser 1.0 software can be seen in *Figure 1*. The precise sizes of the amplified SSR fragments are shown in *Table 4, 5, 6* and *7*.



*Figure 1. A:* Agarose gel electrophoresis results with six SSR markers on sweet cherry genotypes **M**: GeneRuler 100bp+; **I.**: BPPCT 041, **II.**: BPPCT 030, **III.**: BPPCT 002, **IV.**: UDP 96 005, **V.**: UDP 96 001, **VI.**: UCD-CH 17; **6**: Carmen, **13**: Kavics, **21**: Regina, **35**: Conley

**B:** ALFwin Fragment Analyser 1.0 software results; Samples order (row number, primer, name of the samples) **4**: UDP 96 005 Jaboulay, **5**: UDP 96 001 Hedelfingeni óriás, **6**: UDP 96 001 Katalin, **7**: UDP 96 001 Krupnoplodnaja, **8**: UDP 96 001 Münchenbergikorai, **9**: UDP 96 001 Margit, **10**: UDP 96 001 Sunburst, **11**: UDP 96 001 Van

All of the 6 SSR markers displayed polymorphic pattern. Because of the allopolyploid origin of sour cherry we got 1-3 different alleles, however 4 different alleles were not found, alleles represent

2 heterozygous and 1 homozygous alleles, meaning that one of the loci was heterozygous while the other homozygous. In absence of reference data we were not able to decide which allele belongs exactly to the homozygous locus. We could calculate the allelic frequency from the numbers of alleles (*Table 4*). The number of the alleles were 2-5 and the frequency of the alleles were between 2.3%-85.3%. In the case of UDP 96 005 primer there was a rare allele

(118 bp) which can be observed only in two cultivars (Maliga emléke, Piramis).

We found only one unique allele formule in the case of BPPCT 002, Maliga emléke has a 183 bp homozygous form. Only four of the 6 SSR primer-pairs generated alleles proved to be suitable to distinguish the studied sweet cherry genotypes. Their frequencies have ranged from 1.32% to 100%. A total of 456 polymorphic alleles were amplified and the number of the alleles per locus ranged from 1 to 4 (*Table 6*).

UDP96 001 and UCDCH 17 primers resulted in 2 different alleles, both in homozygous or heterozygous form. Four alleles were identified in cherry genotypes using UDP96 005 locus-specific primers (*Table 6*). Two alleles were common and two were rare. Cultivars Botond, Regina and Octavia had unique combinations of these alleles and they could be identified by using this marker. In the case of BPPCT 002 primer we got an allele (179 bp) in high frequency, the almost monomorphic pattern was broken only in 2 cultivars, Antia and Hedelfingeni óriás contained a rare allele (183 bp) (*Table 7*).

We observed the same range of alleles like

Table 4. Microsatellite loci, alleles and their frequency in sour cherry genotypes cultivated in Hungary

UDP 96 001		UDP 96 005		BPPCT002		BPPCT030		BPPCT041		UCDCH 17	
Fragment size bp	Frequency %										
101	14.3	106	22.4	167	41.8	140	48.3	201	85.3	178	40.8
115	59.2	110	22.4	179	23.9	158	33.3	229	14.7	182	14.1
125	26.5	118	2.3	183	34.3	162	18.4			188	14.1
		120	34.1							198	31
		136	18.8								

Table 5. SSR fingerprint of the 29 sour cherry genotypes with 6 *Prunus* primer pairs

No	VARIETIES	UDP 96 001	UDP 96 005	BPPCT002	BPPCT030	BPPCT041	UCDCH 17
1	Csengődi	115	110/120/136	167/183	140/158	201	178/198
2	Érdi bőtermő	101/115/125	106/120/136	167/183	140/162	201	178/198
3	Pándy 279	115	106/110/120	167/179/183	140/158	201	178/182/198
4	Pándy 48	115	106/110/120	167/179/183	140/158	201	178/182/198
5	Pándy BB 119	115	106/110/120	167/179/183	140/158	201	178/182/198
6	Újfehértói fürtös	115	106/110/120	167/179/183	140/158	201	178/182/198
7	Pipacs	115	106/110/120	167/183	140/162	201	178/188
8	Érdi nagygyümölcsű	115	120/136	167/183	140/162	201	178/188
9	Hybrid 3/48 9	115/125	106/120/136	167/183	140/162	201	178/188/198
10	Csengődi 11	115	110/120/136	167/183	140/158	201	178/198
11	Maliga emléke	115/125	106/120/136	183	140/162	201	178/198
12	Korai pipacs	101/115/125	106/120/136	167/183	140/162	201	182/188
13	Érdi jubileum	101/115/125	120/136	167/183	140/158/162	201	182/198
14	Meteor korai	115	106/118/120	167/183	140/158/162	201	182/198
15	Kántorjáncsi 10	115	106/110/120	167/179/183	140/162	201	178/182/198
16	Cigány C404	115/125	110/120/136	167/179	140/158	201/229	178/188
17	Cigány 7 13	115/125	110/120/136	167/179	140/158	201/229	178/188
18	Debreceni bőtermő	101/115	106/110/120	167/179/183	140/158	201	178/182/198
19	Piramis	115/125	106/118/120	167/183	140/158	201	178/188/198
20	Kántorjáncsi 3	101/115/125	106/110/120	167/179/183	140/158	201	178/182/198
21	Paraszt	115/125	110/120/136	167/179	140/158	201/229	178/188
22	Körösi korai	115	110/120/136	167/179	140/158	201	178/198
23	Cigány 59 12	115/125	110/120/136	167/179	140/158	201/229	178/188
24	Pándy Bb119 14	115	106/110/120	167/179/183	140/158	201	178/182/198
25	Favorit	115	106/120/136	167/183	140/162	201	178/198
26	Pándy 279 12	115	106/110/120	167/179/183	140/158	201	178/182/198
27	Pándy 48 13	115	106/110/120	167/179/183	140/158	201	178/182/198
28	Oblecsinszka	101/115/125	110/120/136	167/179	140/158	201/229	178/188/198
29	Érdi bőtermő 13	101/115/125	106/120/136	167/183	140/162	201	178/198

Table 6. Microsatellite loci, alleles and their frequency in sweet cherry genotypes cultivated in Hungary

UDP 96 001		UDP 96 005		BPPCT002		BPPCT030		BPPCT041		UCDCH 17	
Fragment size bp	Frequency %										
110	27.6	110	1.4	179	96.1	140	100	201	100	188	32.9
125	72.4	118	3.9	183	3.9					198	67.1
		120	42.1								
		136	52.6								

Table 7. SSR fingerprint of the 38 sweet cherry genotypes with 6 *Prunus* primer pairs

No	VARIETIES	UDP 96 001	UDP 96 005	BPPCT002	BPPCT030	BPPCT041	UCDCH 17
1	Anita	125/125	120:120	<b>179:183</b>	140:140	201:201	198:198
2	Aida	110/125	120:120	179:179	140:140	201:201	198:198
3	Alex	110/125	136:136	179:179	140:140	201:201	188:188
4	Bigarreau Burlat	110/125	120:136	179:179	140:140	201:201	188:198
5	<b>Botond</b>	125/125	<b>110:120</b>	179:179	140:140	201:201	188:188
6	Carmen	125/125	120:136	179:179	140:140	201:201	188:188
7	Germ.3	110/125	120:136	179:179	140:140	201:201	188:198
8	Germ. 45	110/125	120:136	179:179	140:140	201:201	188:198
9	<b>Hed. Óriás</b>	125/125	120:136	<b>183:183</b>	140:140	201:201	188:188
10	Kavics	110/125	120:136	179:179	140:140	201:201	188:188
11	Katalin	125/125	136:136	179:179	140:140	201:201	188:188
12	Krupnoplodnajaa	125/125	120:136	179:179	140:140	201:201	198:198
13	Linda	125/125	120:136	179:179	140:140	201:201	188:198
14	Münch. Korai	110/125	136:136	179:179	140:140	201:201	198:198
15	Margit	110/125	120:136	179:179	140:140	201:201	188:188
16	Pál	125/125	120:136	179:179	140:140	201:201	188:188
17	Péter	110/125	120:136	179:179	140:140	201:201	188:188
18	<b>Regina</b>	110/125	<b>118:136</b>	179:179	140:140	201:201	188:198
19	Rita	110/125	120:136	179:179	140:140	201:201	188:198
20	Sándor	110/125	136:136	179:179	140:140	201:201	188:198
21	Solymári gömb.	125/125	120:136	179:179	140:140	201:201	188:188
22	Stella	125/125	120:136	179:179	140:140	201:201	188:188
23	Sunburst	110/125	120:136	179:179	140:140	201:201	188:188
24	Szom. Fekete	125/125	120:136	179:179	140:140	201:201	188:188
25	Tünde	110/125	120:136	179:179	140:140	201:201	198:198
26	Val. Eskavol	110/125	120:136	179:179	140:140	201:201	198:198
27	Van	110/125	120:136	179:179	140:140	201:201	188:188
28	Vega	110/125	136:136	179:179	140:140	201:201	188:188
29	Vera	125/125	120:136	179:179	140:140	201:201	188:198
30	Canada giant	125/125	120:136	179:179	140:140	201:201	188:188
31	Germ. 1	110/125	120:136	179:179	140:140	201:201	188:198
32	Conley	125/125	136:136	179:179	140:140	201:201	188:188
33	Sylvia	110/125	120:136	179:179	140:140	201:201	188:188
34	Samba	125:125	120:136	179:179	140:140	201:201	188:188
35	<b>Octavia</b>	125:125	<b>118:118</b>	179:179	140:140	201:201	198:198
36	Merchart	125:125	120:136	179:179	140:140	201:201	198:198
37	Jabolay	110:125	120:136	179:179	140:140	201:201	188:188
38	Giorgia	110:125	120:136	179:179	140:140	201:201	188:188

Note. – numbers in bold are unique allele combinations.

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Table 8. The origin, and the observed allele sizes of the 6 SSR primers

Primer	Origin	Reference	Allele size range in sour cherry (bp)	Allele size range in sweet cherry (bp)	Allele size range according to our results (bp)	
					sour cherry	sweet cherry
UDP 96 001	peach	Cipriani et al. (1999)	99-113 (Turkoglu et al. 2010)	97-125 (Öz et al. 2013) 105-125 (Turkoglu et al. 2010)	101-125	110-125
UDP 96 005	peach	Cipriani et al. (1999)	115-135 (Turkoglu et al. 2010)	109-135 (Öz et al. 2013) 115-135 (Turkoglu et al. 2010)	106-136	110-136
BPPCT 002	peach	Dirlewanger et al. (2002)	168-182 (Antonius et al. 2011)	179-185 (Dirlewanger et al. 2002)	167-183	179-183
BPPCT 030	peach	Dirlewanger et al. (2002)		140 (Dirlewanger et al. 2002)	140-162	140
BPPCT 041	peach	Dirlewanger et al. (2002)		201 (Dirlewanger et al. 2002)	201-229	201
UCD-CH17	sweet cherry	Struss et al. (2003)	178-202 (Turkoglu et al. 2010)	186-190 (Struss et al. 2003) 186-214 (Öz et al. 2013) 180-202 (Turkoglu et al. 2010)	178-198	188-198

in the literature (Table 8), we found only 1-2 bp differences, but it is general because of the different gelectrophoresis methods (acrylamid or capillary). Dendograms constructed based on the SSR data of 29 sour cherry and 38 sweet cherry cultivars are shown in Figure 2 and 3.

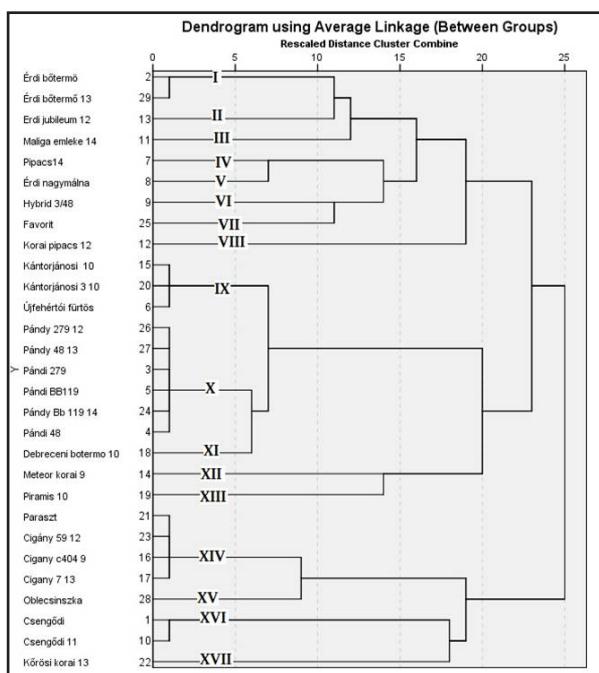


Figure 2. The SSR results of the analyzed 29 sour cherry genotypes

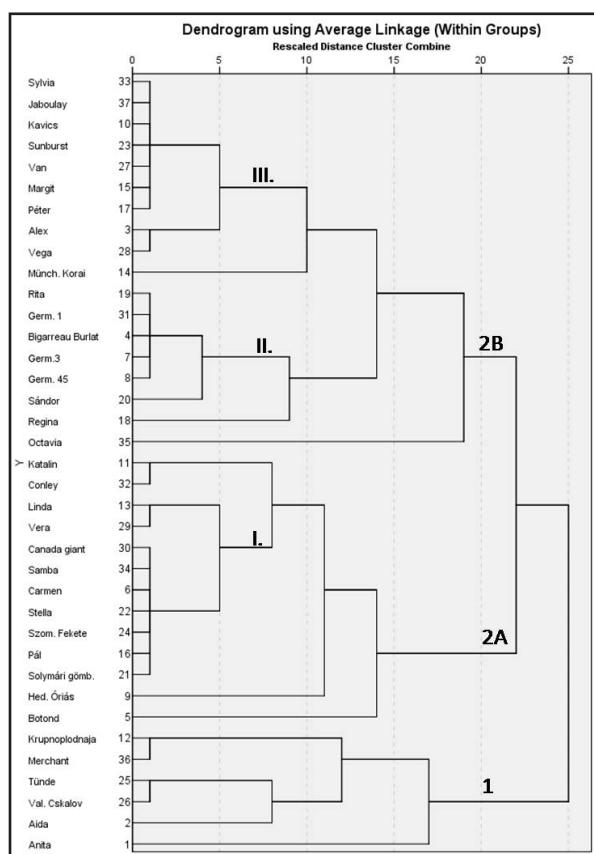


Figure 3. The SSR results of the analysed 38 sweet cherry genotypes

Table 9. Parent-progeny relationship of Van, Stella and Sunburst

Primers	Van	Sunburst	Stella
BPPCT 041	201	201	201
BPPCT 030	140	140	140
BPPCT 002	179	179	179
UDP 96 005	120:136	120:136	120:136
UDP 96 001	110:125	110:125	125
UCDCH 17	188	188	188

### The SSR results of sour cherry

The dendrogram (*Figure 2*) displays two major groups. The first large group includes I-XIII clusters and the second small group contains the XIV-XVII clusters with the Cigány clones, the cultivars Csengődi, Paraszt, Oblecsinszka and Körösi korai. The Oblecsinszka of Serbian

origin is close to Cigány. The larger group consists of two small ones: the first contains the I-VIII clusters while the other the IX-XIII clusters. The Érdi varieties (Érdi bőtermő, Érdi jubileum, Érdi nagymálna), the Pipacs, Korai pipacs, Maliga emléke, Hybrid 3/48 and Favorit are in the first group. These cultivars have the same parent or grandparent which was the Pándy. Clones of Pándy, Debreceni bőtermő, Piramis, Meteor korai the Kántorjánosi and the Újfehértói fürtös are in the second group. These cultivars originate from natural and landscape selection. The Kántorjánosi clones and the Újfehértói fürtös could not be differentiated. These two cultivars were selected by the staff of Research and Extension Centre for Fruit Growing Ltd.

Based on the dendrogram it can be seen that the 6 SSR primers could discriminate the

Table 10. Parent-progeny relationship of Pándy (P1) and its offsprings Érdi jubileum, Érdi bőtermő, Maliga emléke, Korai pipacs, Favorit

Primers	Cultivars/ Clones	P1	Primers	Cultivars /Clones	P1
<b>Primers</b>	<b>Érdi jubileum</b>	<b>Pándy</b>	<b>Primers</b>	<b>Korai pipacs</b>	<b>Pándy</b>
BPPTC002	167:183	167:179:183	BPPTC002	167:183	167:179:183
BPPCT030	140:158:162	140:158	BPPCT030	140:162	140:158
BPPCT041	201	201	BPPCT041	201	201
UDP96-001	101:115:125	115	UDP96-001	101:115:125	115
UDP96-005	120:136	106:110:120	UDP96-005	106:122:136	106:110:120
UDC-CH017	182:198	178:182:198	UDC-CH017	182:188	178:182:198
<b>Primers</b>	<b>Érdi bőtermő</b>	<b>Pándy</b>	<b>Primers</b>	<b>Favorit</b>	<b>Pándy</b>
BPPTC002	167/183	167/179/183	BPPTC002	167/183	167/179/183
BPPCT030	140/162	140/158	BPPCT030	140/162	140/158
BPPCT041	201	201	BPPCT041	201	201
UDP96-001	101115/125	115	UDP96-001	115	115
UDP96-005	106/122/136	106/110/120	UDP96-005	106/122/136	106/110/120
UDC-CH017	178/198	178/182/198	UDC-CH017	178/198	178/182/198
<b>Primers</b>	<b>Maliga emléke</b>	<b>Pándy</b>			
BPPTC002	183	167:179/183			
BPPCT030	140/162	140/158			
BPPCT041	201	201			
UDP96-001	115/125	115			
UDP96-005	106/122/136	106/110/120			
UDC-CH017	178/198	178/182/198			

cultivars except Kántorjánosi and Újfehértói fürtös. The clones gave the same microsatellite fingerprints. Because of the tetraploidy of the sour cherry we have to use more SSR primers to distinguish all cultivars as this has been confirmed by the participants of FA 1004 COST action. The Cigány clones and the Paraszt were indistinguishable at the chosen 6 SSR loci. According to several references these two cultivars might be synonyms. To answer this question either more polymorphic SSRs (about 32) are necessary for a better resolution of the relationships or e.g. SNP markers should be applied since according to Fernandez et al. (2009) SNPs are more efficient tools for cultivar fingerprinting and identification than SSRs.

#### *The SSR results of sweet cherry*

On the dendrogram (*Figure 3*) 3 main groups could be observed. The group (1) consists of Krupnoplodnaja, Merchant, Tünde, Valerij Cskalov, Aida and Anita. Tünde, Aida and Anita are Hungarian cultivars, Sándor Brózik and János Apostol were their breeders. One of the parents of Aida and Anita is a Trusenszkaja clone which was brought about in the former Soviet Union. Valerij Cskalov derives from open pollination of Rozojna and it was made in the former Soviet Union, too. Krupnoplodnaja originates from open pollination of Napoleon Blanc in Ukraine. This group (1) could be classified into the Slavic ancestor group except Merchant (which originates from the United Kingdom) and Tünde (Hungarian cultivar).

The remaining two groups (2A, 2B) contain mostly the Van (III), Germersdorfi (II) and Stella offsprings. with a few exceptions. There are cultivars (Münchenberg korai, Regina, Octavia, Hedelfingeni óriás, Botond) forming separate individual groups outside the main group.

Though Sándor, Pál as well as Péter have the same parents, only Sándor and Péter are in the same group (II) together with one of their parents (Bigarreau Burlat). Pál got in an other group (I) with the other parent, Stella.

As it can be seen on the dendrogram there are varieties which could not be distinguished

from each other (Katalin-Conley, Valerij Cskalov-Tünde, Péter-Margit-Van-Kavics-Sunburst-Sylvia-Jaboulay, Alex-Vega, Canada giant-Samba-Carmen-Stella-Szomolyai fekete-Pál-Solymári gömbölyű). We need to include more polymorphic SSR loci in order to be able to discriminate them.

#### *Parent-progeny relationship*

Due to their Mendelian codominant inheritance, microsatellites can be used for pedigree identification of cultivars. The parent-progeny relationships may be clearly identified even if the actual or assumed crossing partners are heterozygous in the given microsatellite locus, because the progeny will receive one allele from one parent and the other allele from the other one.

Table 11. Parent-progeny relationship Hedelfingeni óriás, Germersdorfi óriás 3 and Linda

Primers	Hedelfingeni óriás	Linda	Germersdorfi óriás
UDP 96 005	120/136	120/136	120/136
UDP 96 001	125	125	<b>110:125</b>
UCDCH 17	188	188/198	188/198

Table 12. Parent-progeny relationship of Van, Stella and Sunburst

Primers	Van	Sunburst	Stella
<b>UDP 96 005</b>	<b>120/136</b>	<b>120/136</b>	<b>120/136</b>
UDP 96 001	110/125	110/125	125
UCDCH 17	188	188	188

Table 13. Parent-progeny relationship of Bigarreau Burlat and Stella and their offsprings: Péter; Sándor, Pál

Primers	Bigarreau Burlat	Péter	Stella
UDP 96 005	120/136	120/136	120/136
UDP 96 001	110/125	110/125	125
UCDCH 17	188/198	188	188
Primers	Bigarreau Burlat	Sándor	Stella
UDP 96 005	120/136	136	120/136
UDP 96 001	110/125	110/125	125
UCDCH 17	188/198	188/198	188
Primers	Bigarreau Burlat	Pál	Stella
UDP 96 005	120/136	120/136	120/136
UDP 96 001	110/125	125	125
UCDCH 17	188/198	188	188

Table 14. Parent-progeny relationship of Stella and its offspring, Samba

Primers	P1	Offspring
	Stella	Samba
UDP 96 005	120/136	120/136
UDP 96 001	125	125
UCDCH 17	188	188

Table 15. Parent-progeny relationship of Bigarreau Burlat and its offspring, Tünde

Primers	P1	Offspring
	Bigarreau Burlat	Tünde
UDP 96 005	120/136	120/136
UDP 96 001	110/125	110/125
UCDCH 17	188/198	198

Table 16. Relationship between Yellow Dragan's offsprings (Tünde; Carmen)

Primers	Tünde	Carmen
UDP 96 005	120/136	120/136
UDP 96 001	110/125	125
UCDCH 17	198	188

Table 17. Analysis of Germersdorfi offsprings' SSR fingerprints clones and seedlings (Margit), Germersdorfi x Szomolyai fekete

Primers	Germersdorfi clone 3	Germersdorfi clone 1	Germersdorfi clone 45	Margit
UDP 96 005	120:136	120:136	120:136	120:136
UDP 96 001	110:125	110:125	110:125	110:125
UCDCH 17	188:198	188:198	188:198	188
Primers	P1	Offspring	P2	
	Szomolyai fekete	Rita	Germersdorfi clone 1	
UDP 96 005	120:136	120:136	120:136	
UDP 96 001	125	110:125	110:125	
UCDCH 17	188:198	188:198	188:198	

Table 18. Parent-progeny relationship of Van and its offsprings

Primers	P1	Offspring	Offspring	Offspring
	Van	Alex	Vera	Sylvia
UDP 96 005	120/136	136	120/136	120/136
UDP 96 001	110/125	110/125	125	110/125
UCDCH 17	188	188	188:198	188

Table 19. Schneiders Spate Knorpelkirsche and Rube offsprings' realationship

Primers	Offspring	Offspring
	Regina	Octavia
UDP 96 005	118/136	118
UDP 96 001	110/125	110/125
UCDCH 17	188/198	198

The following tables (*Table 9-15, 17, 18*) present the parent-progeny results of the sour and sweet cherry cultivars. In some cases we could examine only one of the putative parents (*Table 16 and 19*).

The SSR data obtained at 6 loci do not contradict the putative parentage of the following cultivars: Linda, Sunburst, Péter, Sándor, Pál, Samba, Tünde, Carmen, Germersdorfi clones (3 clones), Margit, Rita, Alex, Vera, Sylvia, Regina, Octavia (*Table 11-19*). However, exact determination would require to add more (~30) microsatellite loci to the analysis.

## Conclusion

Five (UDP 96 001, UDP 96 005, BPPCT 002, BPPCT030, BPPCT041) of the 6 SSR primers were developed for peach. They could be applied in sour and sweet cherry genotyping together with the sweet cherry-specific UCDCH 17. While the sour cherry varietes could be distinguished (expect Kántorjánosi and Újfehértói fürtös), several sweet cherry varietes

had the same SSR patterns. BPPCT primers described by Dirlewanger et al. (2002) did not give appropriate polymorphisms in sweet cherry despite these primers were more discriminative in sour cherry. There are two BPPCT primers which produced monomorphic pattern in the case of sweet cherry, while these primers amplified 2-4 different alleles in sour cherry. The UDP primers presented satisfactory level of polymorphism

both in sweet and sour cherry. According to our results more UDP primers with known map position are advisable to use for discriminating the non-distinguishable varieties.

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