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Identification of sweet cherry (*Prunus avium* L.) cultivars using AFLP and SSR markers

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Abstract

The genetic characterization of 19 Lithuania-bred and 5 common sweet cherry (*Prunus avium* L.) cultivars using microsatellite (SSR) and amplified fragment length polymorphism (AFLP) markers is presented. The genetic diversity of sweet cherry cultivars was evaluated using 13 previously published SSR primer pairs and 9 AFLP primer combinations. Based on SSR, AFLP and combined data, three dendrograms were created. Comparison of both marker systems showed that the probability of correct clustering of cultivars in the dendrogram is higher for AFLP markers than for SSR markers. The highest probability of correct clustering of cultivars in the dendrogram was obtained when combined data on fragments obtained using both marker systems was used and this data gives a more comprehensive description of the genome studied.

The SSR primer pairs PCEGA34 and EMPAS06 enable identification of the investigated sweet cherry cultivars group. All studied cultivars were identified using polymorphic fragments amplified with AFLP EcoRI-AC/MseI-CG primer combination with the highest resolving power value. It was established that the 23 AFLP fragments, generated with the EcoRI-AC/MseI-CG primer combination and exhibiting the highest polymorphism information content (PIC) values (from 0.28 to 0.32), were sufficient for sweet cherry cultivar identification. Fragments profile and their number required for the identification of an individual cultivar were different. Molecular profiles with minimal number of SSR and AFLP markers necessary for identification of studied sweet cherry cultivars are presented.

Key words: cultivars identification, fingerprinting, SSR, AFLP.

Introduction

The sweet cherry (*Prunus avium* L.) is a perennial plant, propagated vegetatively; high level of heterozygosity is specific to it. Heterozygosity of genome is increased by gametophytic self incompatibility, which is controlled by multi allele S-locus. Breeding of sweet cherry in Lithuania was started at the Experimental Station of Vytėnai, Lithuanian Institute of Horticulture (currently Lithuanian Research Centre for Agriculture and Forestry) in 1965. The main breeding goals were increased winter hardiness and resistance to fungal diseases, early flowering and higher yield (Lukoševičius, 1998). *In vitro* methods were used to enhance effectiveness of breeding and for seedling selection (Stanys, 1998). Research on sweet cherry haplotypes was performed (Stanys et al., 2008) to assign the Lithuanian sweet cherry cultivars to incompatibility groups, which is useful for growers and breeders.

Modern breeding and selection need objective methods for fast and reliable identification of breeding material and cultivars, for creation of better methods for evaluation of gene pool. These methods would provide information on cultivars quality, which could be useful for performing purposeful crosses thus increasing effectiveness of plant genetic studies and breeding. Full pedigree information on cultivars is usually not available, and morphological traits are not always appropriate because unrelated cultivars and closely related cultivars can display the same morphological characteristics.

The use of molecular techniques that detect variations at deoxyribonucleic acid (DNA) level is more objective. Effective genetic marker systems were developed during the past decades. Many marker systems have been created for various plant species since the first microsatellite marker identification in *Prunus* genus

(Cipriani et al., 1999). The first stone fruit markers were created for apricot (Cipriani et al., 1999; Sosinski et al., 2000) and later they were used in studies of other species from *Prunus* genus (Dirlewanger et al., 2002; Hormaza, 2002). Molecular markers were used for polymorphism studies (Ercisli et al., 2011), identification of population structure (Saltonstall, 2003), for study of seed dispersal (Godoy, Jordano, 2001), creation of genetic linkage maps (Decroocq et al., 2004) and cultivar identification (Aranzana et al., 2002; Schueler et al., 2003).

European Collaborative Programme for Genetic Resources (ECPGR) of *Prunus* group recommended a standard kit of microsatellite markers and a list of standard genotypes in order to create methods for easier comparison of genetic *P. avium* resources. Eight standard genotypes and 16 SSR markers were proposed (Clarke, Tobutt, 2009). Fingerprints for Lithuanian sweet cherry cultivars were lacking.

Our objective was to genetically characterize Lithuanian sweet cherry cultivars and to establish SSR and AFLP markers for identification of sweet cherry cultivars.

Materials and methods

Plant material. The study included 19 landraces and cultivars developed in Lithuania during the last century and 5 common cultivars of foreign origin (Table 1). The plant material was collected at the reference collection of the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry in the year 2010.

Table 1. Parentage of sweet cherry cultivars

No.	Cultivar	Parents	Origin
1.	‘Agila’	‘Leningradskaya ciornaya’ × ‘Priusadebnaya’	Lithuania
2.	‘Anta’	‘Žemaičių rožinė’ × ‘Napoleon’ + ‘Dniprovka’	Lithuania
3.	‘Austė’	‘Hedelfinger’ × ‘Rožinė’	Lithuania
4.	‘Germa’	No. 1106 × ‘Sam’	Lithuania
5.	‘Irema BS’	Unknown	Lithuania
6.	‘Jurga’	‘Dniprovka’ × ‘Hedelfinger’	Lithuania
7.	‘Jurgita’	‘Hedelfinger’ × ‘Dniprovka’	Lithuania
8.	‘Lukė’	No. 1106 × ‘Sam’	Lithuania
9.	‘Meda’	‘Belobokaya rannyaya’ × ‘Hedelfinger’	Lithuania
10.	‘Mindaugė’	‘Severnaya’ × ‘Jurgita’	Lithuania
11.	‘Rožinė’	Landrase	Lithuania
12.	‘Seda’	‘Hedelfinger’ × ‘Rožinė’	Lithuania
13.	‘Vasarė’	‘Leningradskaya ciornaya’ × ‘Priusadebnaya’	Lithuania
14.	‘Vytėnų geltonoji’	‘Žemaičių geltonoji’ × ‘Zolotaya lositskaya’	Lithuania
15.	‘Vytėnų juodoji’	‘Žemaičių raudonoji’ × ‘Dniprovka’	Lithuania
16.	‘Vytėnų rožinė’	‘Hedelfinger’ × ‘Rožinė’	Lithuania
17.	‘Žemaičių geltonoji’	Landrace	Lithuania
18.	‘Žemaičių juodoji’	Landrace	Lithuania
19.	‘Žemaičių rožinė’	Landrace	Lithuania
20.	‘Belobokaya rannyaya’	Unknown	Russia/Ukraine
21.	F12	*Prunus avium* selected clone	Unknown
22.	‘Hedelfinger’	Unknown	Germany
23.	‘Sunburst’	‘Van’ × ‘Stella’	Canada
24.	‘Van’	Seedling of ‘Empress Eugenie’	Canada

DNA extraction. The DNA was extracted from leaves using "DNeasy Plant Mini Kit" ("Qiagen", USA) and CTAB method (Doyle, Doyle, 1990). Genomic DNA was stored in TE buffer (100 mM Tris-HCl, 10 mM EDTA and pH-8) at −20°C.

SSR analysis. Thirteen previously published primer pairs were used for SSR analysis: EMPA002, 003, 017, 018 (Clarke, Tobutt, 2003), EMPAS01, 02, 06, 10, 11, 12 (Vaughan, Russell, 2004), PCEGA34 (Downey, Iezzoni, 2000), UDP98-412 (Testolin et al., 2000) and UCD-CH14 (Struss et al., 2003). The multiplex PCR reactions were performed in a final volume of 9 µl, containing 60 ng genomic DNA, "True Allele PCR Premix" ("Applied Biosystems", USA) and 0.3 µM of each primer pair. The forward primer was fluorescently-marked with 6-FAM, VIC, NED or PET ("Applied Biosystems Instruments", USA). The conditions for amplification were as follows: 94°C for 90 s followed by 10 cycles of 94°C for 30 s, 60°C for 45 s (−0.5°C per cycle), 72°C for 1 min and then 25 cycles of 94°C for 30 s, 55°C for 45 s, 72°C for 1 min with an elongation step of 72°C for 5 min. Fragment analysis was performed using a "Genetic Analyser 3130" ("Applied Biosystems").

AFLP analysis. AFLP samples were prepared using AFLP pre selective primer mix for regular plant genomes ("Applied Biosystems Instruments"). The PCR conditions for pre selective and selective amplification were performed according to Vos et al. (1995) with some modifications: 1 cycle (an initial denaturing step) of 120 s at 72°C, 20 cycles of 20 s at 94°C, 30 s at 56°C, 120 s at 72°C, 1 cycle (final extension) 30 min at 60°C. Selective amplification was prepared with 9 primer combinations involving fluorescently-marked EcoRI (E) and MseI (M) primers and AFLP amplification core mix ("Applied Biosystems"): 1 cycle (an initial denaturing step) of 120 s at 94°C, 10 cycles of 30 s at 94°C, 30 s at 66°C (−1°C), i.e. the initial temperature was subsequently reduced each cycle by 1°C, 20 cycles: of 30 s at 94°C, 45 s at 56°C, 120 s at 72°C, 1 cycle (final extension) 30 min at 60°C. The nine primer combinations used were E-AC/ M-CG, E-AA/ M-CC, E-AA/ M-CG, E-AA/ M-CAT, E-AG/ M-CAT, E-AA/ M-CTC, E-AA/ M-CTT, E-AT/ M-CC and E-AT/ M-CG. Fragment analysis was performed using a "Genetic Analyser 3130" ("Applied Biosystems"). AFLP analyses were repeated at least twice on all samples in order to check reproducibility of the data.

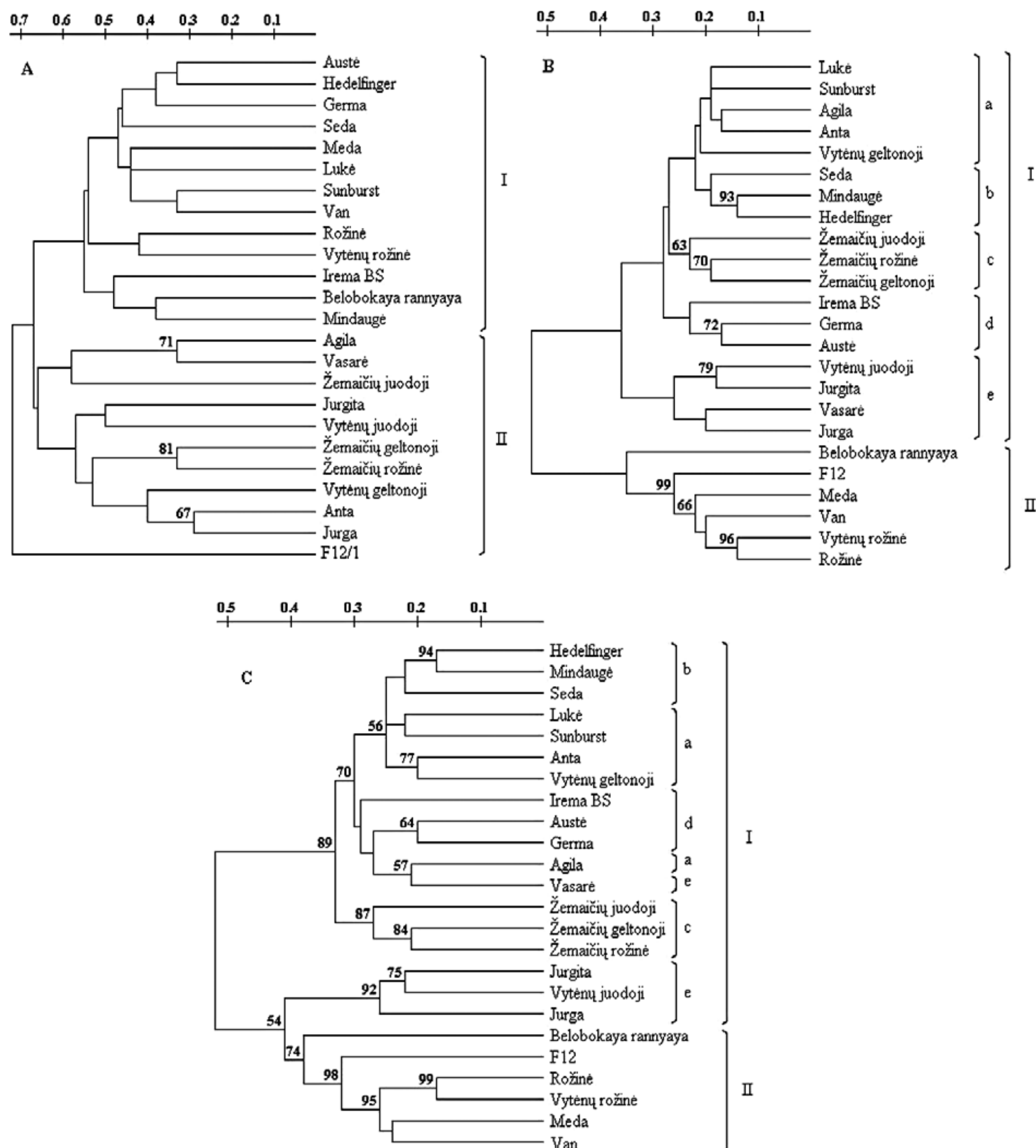
Data analysis. Data from both methods (SSR and AFLP) was analysed using *GeneMapper v.4.0* software ("Applied Biosystems Instruments") and converted to binary matrix to compare both methods and to create the common (SSR with AFLP markers) dendrogram. Three dendrograms according to SSR, AFLP and combined data were performed on *Treecon v.1.3b* software (Van de Peer, De Wachter, 1994), using Nei and Li (1979) distance method and UPGMA tree method. To test the reliability of the dendrograms, a bootstrap analysis with 1000 replications was performed within *Treecon* software. A threshold of reliability was set to 50% for bootstrap values. Additionally, to test the validity of the dendrograms, cophenetic matrices were created using the Nei and Li (1979) distance matrices that were used to construct the dendrograms. Cophetic matrices were created and cophenetic correlation coefficients were calculated using *MultiDendrograms v.2.1* software (Fernandez, Gomez, 2008). Discrimination power and the optimal SSR marker combination were calculated according to Tessier et al. (1999). Resolving power was calculated according to Shen et al. (2010) to determine the most informative AFLP primer combination. Polymorphism information content (PIC) for each polymorphic fragment was calculated according to Shen et al. (2010) to select the smallest set of informative fragments to identify all sweet cherry cultivars used in this study.

Results and discussion

Genetic relationship of sweet cherry cultivars. Genetic relationship dendrograms were created in order to use as many genetically different Lithuanian sweet cherry cultivars as possible in breeding process and to predict heterosis effect in hybrids. The first dendrogram (SSR) was created using 107 fragments generated with 13 microsatellite primer pairs (Fig. A). Sweet cherry cultivars clustered into two groups. *Prunus avium* clone F12 was separated into distinct branch. The first group consisted of cultivars, derived in Lithuania, Canada, Western and Eastern Europe; however, significant differences were not identified. The second group consisted of archaic landraces from the western part of Lithuania (Samogitia) and the first generation of Lithuanian cultivars (hybrids between Lithuanian landraces and Russian cultivars). The significant associations in this group were between cultivars 'Agila' and 'Vasarė', 'Žemaičių geltonoji' and 'Žemaičių rožinė' and between cultivars 'Anta' and 'Jurga'.

The second dendrogram (AFLP) was constructed using 344 polymorphic fragments, obtained with 9 AFLP primer pairs (Fig. B). Two main groups were established. The first group consisted of five subgroups. Significant associations were not identified in the first (a) subgroup. Cultivars 'Mindaugė' and 'Hedelfinger' were significantly clustered into the second (b) subgroup, since cultivar 'Mindaugė' is the offspring of cultivar 'Hedelfinger'. Cultivars 'Žemaičių juodoji', 'Žemaičių rožinė' and 'Žemaičių geltonoji', which are landraces from the western part of Lithuania (Samogitia), were significantly separated into the third (c) subgroup. 'Germa' and 'Austė', cultivars derived from crosses of the same parental plants, were clustered into the fourth (d) subgroup. Cultivars 'Vytėnų juodoji' and 'Jurgita' were separated into the fifth (e) subgroup; both of these cultivars have common parental cultivar 'Dniprovka' (Fig. B). Second, the main AFLP dendrogram group consisted of five sweet cherry cultivars and *Prunus avium* selected clone F12. Cultivars 'Rožinė' and 'Vytėnų rožinė' were significantly clustered, because 'Vytėnų rožinė' is the offspring of 'Rožinė'.

Dendrograms based on both AFLP and SSR markers were compared and eight significant associations (bootstrap values >50%) were obtained in the AFLP dendrogram, while only three in the SSR dendrogram.



Note. A – SSR dendrogram based on data of 13 SSR primer pairs, B – AFLP dendrogram with all polymorphic fragments of 9 primer combinations, C – AFLP-SSR dendrogram, based on combined data of SSR and AFLP markers.

Figure. Cluster analysis of 24 sweet cherry accessions based on different molecular marker methods

The different grouping of cultivars in SSR and AFLP dendrograms can be caused due to the fact that these markers in the genome are differently distributed. SSR markers are mostly present in non-coding DNA (Struss et al., 2003) and rare in protein coding sequences (Dokholyan et al., 2000). However, AFLP markers are widely distributed throughout the genome and are often concentrated in centromeric regions (Meudt, Clarke, 2007). Significant association of cultivars 'Žemaičių geltonoji' and 'Žemaičių rožinė' into one group in both dendrograms show that these cultivars are very similar genetically and their genomes are similar both in coding and non-coding parts of nucleolus DNA. Cultivars 'Rožinė' and 'Vytėnų rožinė' were grouped in both dendrograms, but the association was significant in AFLP dendrogram only. This shows that the number of SSR markers used in this study was insufficient for significant association of these cultivars. Another reason may be the lack of common markers in non-coding DNA sequences in these cultivars, so their genetic relationship may not be identified using SSR markers.

Common dendrogram using AFLP and SSR data was constructed (Fig. C). Four cultivars ‘Žemaičių geltonoji’ with ‘Žemaičių rožinė’ and ‘Rožinė’ with ‘Vytėnų rožinė’ were grouped similarly as in SSR dendrogram. Clustering of cultivars in common dendrogram is similar to that in AFLP dendrogram. Cultivars ‘Agila’ and ‘Vasarė’ were clustered into separate subgroup in the common dendrogram (Fig. C), while cultivar ‘Agila’ belonged to subgroup (a), and cultivar ‘Vasarė’ belonged to the subgroup (e) of the first AFLP dendrogram group (Fig. B). High significance was obtained for all branches of common dendrogram. This shows that different DNA markers should be used for establishment of cultivars’ genetic relationship, because a combination of different marker systems characterizes genome more comprehensively.

The cophenetic correlation coefficient indicates the degree of agreement between distance values implied by the dendrogram and the original distance matrix. The cophenetic coefficient was higher for common dendrogram (r = 0.871) and AFLP dendrogram (r = 0.869), than for SSR dendrogram (r = 0.659). This indicates again that the use of larger number of different DNA markers increases the validity of the dendrogram.

Cultivar identification. To determine the minimal combination of SSR primer pairs for cultivar identification, the SSR primer pairs were ranked according to the highest discrimination power (D) value. SSR primer pairs PCEGA34 and EMPAS06 were most informative for sweet cherry cultivars tested (D value 0.92 and 0.87, respectively). The combination of these both primer pairs enabled identification of all sweet cherry cultivars. Eleven alleles were identified in sweet cherry using microsatellite primer pair PCEGA34 (Table 2). Five alleles were common (>10%) and six were rare (<10%). Fifteen unique combinations of these alleles were found and their usage enabled identification of 15 out of 24 sweet cherry cultivars. Seven alleles were amplified in sweet cherry cultivars using microsatellite primer pair EMPAS06. Six of these alleles were common and one was rare. Thirteen unique allele combinations were found and 13 sweet cherry cultivars could be identified using this SSR marker. The sweet cherry molecular profiles generated using PCEGA34 and EMPAS06 primer pairs are presented in Table 3. Unique combinations of alleles are cultivar-specific and may be used in identification of these cultivars. After amplification of sweet cherry DNA with both primers, it is possible to identify 21 out of 24 studied cultivars according to distribution and combinations of unique alleles. The remaining 3 unidentified cultivars may be discriminated according to other, non-unique combinations of DNA fragments. That shows great informative and differentiation power of these primer pairs. The study of Greek sweet cherry cultivars (Ganopoulos et al., 2011) also demonstrated high information content of PCEGA34 locus.

Because AFLPs are highly reproducible dominant markers (Agarwal et al., 2008), the large number of fragments gives them a high statistical power (Meudt, Clarke, 2007), so they are well suited for distinguishing between closely related genotypes. The highest resolving power (31.74) was established for EcoRI-AC/MseI-CG primer combination, when nine AFLP primer combinations were studied. Fifty-four polymorphic fragments were generated using this primer combination, and all studied cultivars were distinguished. PIC values for fragments obtained with this primer pair ranged from 0.05 to 0.32, 0.24 in average. In order to establish minimal subset for this primer pair, fragments with the highest PIC value were selected for analysis. At the beginning, six fragments with maximum PIC value (0.32) were used and fragments were added to analysis till all the sweet cherry cultivars were distinguished. It was established that the 23 AFLP fragments with a PIC value ranging from 0.28 to 0.32 are sufficient for identification of sweet cherry cultivars. Fragments profile and their number required for identification of particular cultivars were different depending on the genotype: the largest number of AFLP fragments was required for identification of cultivar ‘Seda’ (18). Six AFLP fragments were enough for identification of cultivars ‘Austė’, ‘Germa’, ‘Jurga’, ‘Vytėnų juodoji’ and ‘Belobokaya rannyaya’ (Table 3).

Table 2. Microsatellite loci alleles and their frequency in sweet cherry cultivars

EMPAS06		PCEGA34	
Fragment size bp	Frequency %	Fragment size bp	Frequency %
203	12.5	136	16.7
205	6.2	140	2.1
207	16.7	144	27.1
218	10.4	146	10.4
220	12.5	152	6.2
222	22.9	154	10.4
229	18.7	156	6.2
		158	4.2
		162	4.2
		164	10.4
		216	2.1

In comparison with other sweet cherry studies, 68 cultivars (89.5% of the studied cultivars) were discriminated using nine SSR markers (Wünsch, Hormaza, 2002), 90 cultivars (71.4%) were discriminated using three SSR markers (Lacis et al., 2009), we established the smallest SSR primer pairs combinations set (2), which was able to distinguish all tested sweet cherry cultivars. In the sweet cherry studies combining both marker systems, 15 cultivars (100%) were discriminated with fifteen SSR and four AFLP markers (Struss et al., 2003), 78 cultivars (100%) were discriminated with six SSR and four AFLP markers (Gulen et al., 2010). In our study, we found out the minimal marker set of two SSR primer pairs and one AFLP primer combination that was able to distinguish all the tested sweet cherry cultivars.

Table 3. Minimal DNA marker (SSR and AFLP) profiles required for identification of sweet cherry cultivars

Cultivar	SSR[1]		AFLP markers, generated using CG-AC primer pair (fragment size, bp)
	EMPAS06 (fragment size, bp)	PCEGA34 (fragment size, bp)	
‘Agila’	203:222	**146:162**	152, 154, 199, 211, 233, 272, 275, 283, 291, 321, 323, 328, 464
‘Anta’	**205:207**	144	117, 154, 199, 211, 233, 243, 260, 272, 275, 283, 291, 310, 321, 323, 328, 464
‘Austė’	**207:220**	**136:158**	211, 272, 291, 313, 321, 328
‘Germa’	**220:222**	**146:158**	152, 211, 233, 291, 321, 328
‘Jurga’	207:229	**144:164**	117, 233, 283, 291, 313, 326
‘Jurgita’	207:229	**144:154**	117, 152, 172, 283, 297, 310, 326, 333
‘Lukė’	207:222	**136:162**	154, 199, 211, 233, 243, 260, 272, 283, 291, 321, 323, 326, 328, 365, 464
‘Meda’	218:222	144	117, 152, 260, 297, 310, 326, 333, 365
‘Mindaugė’	**220:229**	144	81, 117, 172, 199, 211, 243, 260, 272, 275, 283, 291, 321, 323, 326, 328, 464
‘Irema BS’	**203:207**	**136:146**	152, 154, 211, 243, 272, 275, 291, 321, 323, 328, 464
‘Seda’	**229**	**140:216**	117, 152, 154, 172, 199, 211, 243, 260, 272, 275, 283, 291, 310, 321, 323, 328, 365, 464
‘Rožinė’	**218:222**	154:156	81, 117, 152, 233, 260, 297, 310, 326, 333, 365
‘Vasarė’	203:222	**144:152**	152, 211, 233, 283, 291, 310, 321, 326, 328
‘Vytėnų geltonoji’	207:229	144	154, 199, 211, 260, 272, 275, 283, 291, 323, 326, 328, 333, 365, 464
‘Vytėnų juodoji’	**222:229**	**156:164**	117, 152, 297, 310, 326, 333
‘Vytėnų rožinė’	218:229	154:156	81, 152, 297, 310, 326, 333, 365
‘Žemaičių geltonoji’	207:222	**152:164**	81, 152, 154, 172, 211, 233, 272, 275, 291, 310, 321, 323, 328, 464
‘Žemaičių juodoji’	203:222	**136:152**	81, 152, 211, 233, 272, 283, 291, 310, 321, 323, 328, 464
‘Žemaičių rožinė’	**205:222**	**164**	117, 152, 154, 172, 211, 272, 275, 291, 297, 310, 321, 323, 328, 365
F12	**203**	144	81, 117, 152, 172, 310, 326, 333, 365
‘Belobokaya rannyaya’	**222**	**146:146**	152, 297, 310, 333, 365, 464
‘Hedelfinger’	218:229	**154**	117, 154, 172, 199, 211, 243, 272, 275, 283, 291, 297, 321, 323, 326, 328, 365, 464
‘Sunburst’	**218:220**	136	152, 154, 172, 199, 211, 243, 260, 272, 275, 291, 310, 321, 323, 326, 328, 464
‘Van’	**205:220**	136	81, 152, 260, 297, 310, 326, 333, 365

Note. [1] – numbers in bold are unique allele combinations.

Our data show that it is possible to identify all investigated Lithuania-bred sweet cherry cultivars using both marker systems. Identification of cultivars using SSR and AFLP markers may be performed at any stage of plant development and is independent on the environment impact.

Conclusions

1. Microsatellite (SSR) primer pairs PCEGA34 and EMPAS06 and amplified fragment length polymorphism (AFLP) markers EcoRI-AC/MseI-CG are informative and have a high genotype differentiation power. Usage of these markers enables identification of Lithuanian-bred sweet cherry cultivars.

2. Compared with one marker system, the use of several deoxyribonucleic acid (DNA) markers enables a more precise characterization of the genome for establishment of cultivar genetic relationship.

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Trešnės (*Prunus avium* L.) veislių apibūdinimas, naudojant mikrosatelitų ir pagausintų fragmentų ilgio polimorfizmo molekulinius žymeklius

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Santrauka

Naudojant 13 anksčiau publikuotų paprastųjų pasikartojančių sekų (PPS) pradmenų porų ir 9 pagausintų fragmentų ilgio polimorfizmo (PFIP) pradmenų kombinacijas, apibūdinta 19 Lietuvoje sukurtų ir 5 paplitusios trešnės (*Prunus avium* L.) veislės. Remiantis PPS bei PFIP žymeklių sistemų ir jungtiniais abiejų sistemų duomenimis, buvo sudarytos trys dendrogramos. Palyginus PPS ir PFIP molekulinių žymeklių sistemas nustatyta, kad veislių taisyklingo sugrupavimo tikimybė yra didesnė PFIP molekulinių žymeklių pagrindu sudarytoje dendrogramoje. Didžiausia taisyklingo sugrupavimo tikimybė nustatyta dendrogramoje, sudarytoje panaudojant abiejų žymeklių sistemų jungtinius duomenis, išsamiau apibūdinančius tiriamą genomą.
PPS pradmenų poros PCEGA34 ir EMPAS06 leido identifikuoti visas tirtas trešnės veisles. PFIP EcoRI-AC/MseI-CG pradmenų kombinacijos skiriamoji geba buvo didžiausia. Naudojant šios pradmenų kombinacijos generuotus polimorfinius fragmentus buvo identifikuotos visos tirtos veislės. Nustatyta, kad veislių identifikacijai pakanka 23 PFIP fragmentų, generuotų su EcoRI-AC/MseI-CG pradmenų kombinacija ir turinčių didžiausią polimorfizmo informacijos kiekio vertę, kuri svyravo nuo 0,28 iki 0,32. Fragmentų kombinacija ir jų skaičius vienai veislei identifikuoti skyrėsi. Pateikti tirtų veislių molekuliniai profiliai naudojant minimalų PPS ir PFIP žymeklių kiekį, kurio reikia trešnės veislėms identifikuoti.

Reikšminiai žodžiai: veislės identifikavimas, DNR profilis, PPS, PFIP.