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Characterization of microsatellite loci in apple (*Malus* × *domestica* Borkh.) cultivars

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Abstract

Apple fruits constitute an important part of horticultural production in Lithuania and worldwide. Tools for assessment of genetic polymorphism and genotyping are required for breeding and research on apple genetic resources. The aim of the present study was to characterize microsatellite loci of indigenous and traditional Lithuanian apple tree (*Malus* × *domestica* Borkh.) cultivars and select primer pairs suitable for genotyping of the cultivars. Thirty-seven traditional and indigenous cultivars developed during the last century, and, as a reference, a set of eleven standard cultivars available at the collection of apple genetic resources at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry were assessed. Apple genotyping was performed using eleven PCR primers specific to polymorphic microsatellite loci of *Malus* × *domestica* as recommended by the European Cooperative Programme of Plant Genetic Resources (ECPGR) *Malus* workgroup. All microsatellite loci exhibited a high level of polymorphism – 8 to 14 alleles with a mean value of 10.45 for traditional and indigenous cultivars and 5 to 12 alleles 7.55 in average for reference cultivars. The observed heterozygosity varied from 0.70 to 0.97 with an average of 0.84 for traditional and indigenous cultivars and from 0.64 to 1.00 with a mean value of 0.90 for reference cultivars.

CH01g12 was the most polymorphic micorsatellite locus in the group of 37 traditional and indigenous cultivars, while CH02b10 locus exhibited the highest polymorphism in the set of reference cultivars. The polymorphism of the microsatellite markers allowed us to specifically identify 35 of the traditional and indigenous cultivars.

Key words: apple, SSR, genetic polymorphism, genotyping, traditional cultivar, indigenous cultivar, reference cultivar.

Introduction

Apple fruits are important part of horticultural production in Lithuania and a number of traditional cultivars have been cultivated for centuries. For efficient breeding of new apple cultivars and research on apple tree genetic resources tools for genome mapping, assessment of genetic polymorphism and plant genotyping are required. The application of plant biotechnology methods ensures development of efficient approaches for orchard plant genetics research or breeding programs and more reliable procedures for plant quality control.

Polymorphic microsatellite loci are the markers of choice in genetics and breeding studies due to their multi-allelic nature, codominant inheritance, high abundance, reproducibility, transferability over genotypes and extensive genome coverage (Liebhard et al., 2002). A number of microsatellite loci of apple (*Malus* × *domestica* Borkh., *Malus floribunda* 821) were identified and markers developed (Guilford et al., 1997; Gianfranceschi et al., 1998; Liebhard et al., 2002; Silfverberg-Dilworth et al., 2006). During the last two decades microsatellite markers have been extensively used and over forty studies have been published where the markers have been used in genetic polymorphism analysis of traditional cultivars and populations of wild species (including the most recent publication by Garkava-Gustavsson et al., 2008; Gharghani et al., 2009; Richards et al., 2009; Gasi et al., 2010; Van Treuren et al., 2010; Lacis et al., 2011), development of genetic linkage map of apple (Liebhard et al., 2002; Silfverberg-Dilworth et al., 2006) and cultivar genotyping and parentage identification (Melchiade et al., 2007; Moriya et al., 2011).

The collection, breeding and research of apple trees at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry have been carried out since establishment of the Institute in 1938. At present, the Institute maintains a collection of more than 800 of *Malus* sp. cultivars, species and clones. The collection includes 37 indigenous and traditional cultivars that constitute important part of horticultural production and are a popular choice for home gardens, traditional and organic farming.

Twenty of the traditional cultivars are maintained at the collection of apple tree genetic resources of the Institute. Five of the cultivars are listed in the Na-

tional List of Plant Varieties 2011 (2011). Old cultivars thought to be of local origin include 'Lietuvos pepinas', 'Popierinis', 'Žemaičių grietininis', 'Raudonasis rudeninis dryžuotasis', 'Persikinis', 'Avenarijus', 'Babtų baltasis', 'Panemunės baltasis', 'Panevėžiuko rojinukas', 'Beržininkų ananasas', 'Biržuvėnų žieminis', 'Vytis', 'Jono pepinas', 'Raudoniai' (Tuinyla et al., 1990). Several other cultivars of foreign origin have been cultivated in the region for decades, including 'Pilkasis molinis' (syn. 'Sierinka'), 'Baltasis alyvinis' (syn. 'Belyi naliv'), 'Pupinis' (syn. 'Bohnapfel'), 'Paprastasis antaninis' (clone of 'Antonovka'). The origin of the traditional cultivars is mostly unknown and their genetic background has not been assessed previously.

A number of new breeding lines and cultivars have been developed under apple tree breeding programmes in Lithuania during the last century. The Institute collection maintains 17 such breeding lines and cultivars, 4 of which are included in the National List of Plant Varieties 2011. 'Auksis', released in 1974, is one of the most popular cultivars in the region, and it has been grown in approximately quarter of apple production area. Other prominent cultivars are 'Noris', 'Štaris'. The latest release is 'Aldas' featuring apple scab resistance trait (Sasnauskas et al., 2007) and the promising breeding lines include 'Skaistis' and 'Rudenis' (Sasnauskas et al., 2008).

The aim of present study was to characterize microsatellite loci of Lithuanian traditional and indigenous apple cultivars and to select a set of markers suitable for genotyping of the cultivars. Thirty seven Lithuanian cultivars and eleven reference cultivars were used in the study and microsatellite loci were characterized using a set of eleven PCR primers specific to polymorphic microsatellite loci of apple tree.

Materials and methods

Plant material. Leaf samples were collected from 37 traditional and indigenous cultivars and 11 reference cultivars maintained at the collection of apple genetic resources at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry during the 2008–2010 period and stored at −70°C.

DNA isolation and analysis of microsatellite markers. A hundred milligrams of plant leaves was powdered in liquid nitrogen and genomic DNA was isolated using "DNeasy Plant Mini" kit ("Qiagen" Ltd.) following manufacturer's instructions. DNA samples were stored in TE buffer (100 mM Tris-HCl, 10 mM EDTA and pH 8) at −20°C.

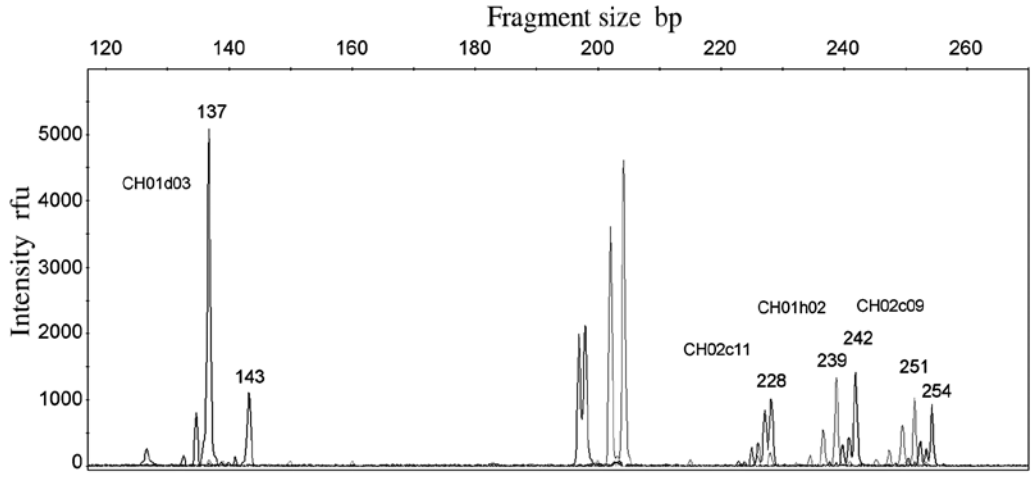
For microsatellite marker analysis, eleven PCR primers shown to be specific to polymorphic microsatellite loci of *Malus* × *domestica* and recommended by ECPGR *Malus/Pyrus* working group were selected: CH01h02, CH02c06, CH02c09, CH02c11, CH02d08, CH04c06, CH04e05, CH01g12, CH02b10 and COL (Liebhard et al., 2002). The multiplex PCR reaction mix was adopted from (Horne et al., 2004): 10 µL volume included 60 ng genomic DNA, 1% polyvnylpyrrolidone (PVP-30), 10 mM dithiothreitol, 0.16 mM dNTP, 1 × PCR reaction buffer, 0.2 U Titan *Taq* DNA polymerase ("BioAtlas" Ltd), 0.35 mM each primer. The conditions for PCR amplification were as described by (Clarke et al., 2003).

Fragment analysis was performed using "3130 Genetic Analyser" ("Applied Biosystems" Ltd.) using 36 cm capillary array and POP-7 polymer. Data was analysed using *GeneMapper* software v.4.0 ("Applied Biosystems" Ltd.).

Data analysis. The parameters used to evaluate the information obtained from analysis of 11 microsatellite markers were: alleles per locus (A), the effective number of alleles per locus (A_e), frequency of alleles (p_i), power of discrimination (PD), expected heterozygosity (H_e) and observed heterozygosity (H_o). The analysis was performed using *PowerMarker* software v.3.25 (Liu, Muse, 2005).

Results and discussion

Eleven polymorphic loci were assessed for 48 Lithuanian and reference apple cultivars. All primer pairs amplified fragments which were clearly separated using the capillary electrophoresis sequencer and no variance was observed in the banding pattern between two to three independent SSR reactions for each DNA sample (Fig.).



Notes. Representative results for cultivar 'Beržininkų ananasas' are shown. PCR and DNA fragment analysis was performed as described in Materials and methods.

Figure. Results of multiplex fragment analysis

For the whole set of cultivars, 121 polymorphic alleles were identified (Table 1).

The allele number per locus varied from 5 to 14 for the 11 microsatellite loci (Table 2). Among the 37 Lithuanian cultivars, 115 polymorphic alleles were identified. The number of polymorphic alleles for each locus varied from 8 to 14 (10.45 in average). For the 11 reference cultivars, 83 polymorphic alleles were identified.

Table 1. SSR profiles of 37 Lithuanian and 11 reference cultivars. SSR alleles in bold apparently occur in duplicate

No.	Cultivar	SSR locus / alleles				
		CH01d03	CH01h02	CH02c09	CH02c11	CH02c06
		Lithuanian cultivars				
1.	'Beržininkų ananasas'	137:143	239:251	242:254	**228**	**255**
2.	'Giedris'	143:150:158	239:247	242:248	219:232	253:255
3.	'Aušris'	**150**	239:243	252:260	222:234	245:253
4.	'Pilkasis molinis'	**137**	239:251	**248**	217:239	217:255
5.	'Babtų baltasis'	145:150	**239**	248:260	228:239	217:265
6.	'Genelis'	141:150	239:247	248:260	232:243	253:255
7.	'Avenarijus'	137:143:150	**239**	248:258	219:228	201:255
8.	'Persikinis'	139:150	239:259	242:246	219:228	245:255
9.	'Tabokinė'	137:158	239:251	248:258	211:222	245:255
10.	'Margutis'	137:143	239:247	242:254	232:239	237:253
11.	'Vytėnų pepinas'	139:143:150	247:251	242:248	**228**	**255**
12.	'Kaunis'	137:150	239:259	242:254	237:239	206:255
13.	'Baltasis alyvinis'	137:150	**239**	248:252	222:228	217:253
14.	'Paprastasis antaninis'	139:150	239:251	248:252	219:228	217:245
15.	'Montvilinis'	137:143	239:251	242:260	237:239	253:255
16.	'Vytenis'	137:143	**251**	248:260	219:234	237:255
17.	'Vytis'	**139**	251:253	**235**	219:234	217:230
18.	'Vytėnų pilkasis'	137:143	239:247	254:258	219:239	206:253
19.	'Saldis'	137:150	239:253	248:260	222:239	**217**
20.	'Auksis'	137:143	247:251	242:260	219:239	217:255
21.	'Skaistis'	137:143	249:251	235:248	222:239	230:241
22.	'Rudenis'	137:143	249:251	246:248	222:234	230:241
23.	'Raudonasis rudeninis dryžuotasis'	**150**	251:259	248:258	**219**	245:255
24.	'Noris'	**150**	249:251	235:260	234:239	**255**
25.	'Popierinis'	137:150	**239**	248:252	222:228	217:253
26.	'Panevėžio rojinukas'	137:143	239:247	249:260	**219**	201:255
27.	'Pupinis'	137:143	247:249	242:252	219:228	230:255
28.	'Štaris'	134:143:150	239:247	**242**	228:237	245:253
29.	'Žemaičių grietininis'	137:141	**251**	248:258	222:239	232:255
30.	'Lietuvos pepinas'	137:143:150	247:259	242:260	232:237	237:255
31.	'Panemunės baltasis'	139:141:143	**239**	248:252	211:222	217:253
32.	'Birutės pepinas'	137:143:150	239:247	248:260	222:237	230:237
33.	'Jono pepinas'	139:143	247:251	254:260	222:243	**245**
34.	'Puikis'	137:143:150	239:251	252:258	237:239	255:265
35.	'Biržuvėnų žieminis'	137:143	**239**	235:258	211:228	253:255
36.	'Raudoniai'	137:143	**247**	242:246	222:232	**255**
37.	'Aldas'	137:141	239:255	246:260	222:234	241:255
		Reference cultivars				
38.	'Empire'	**143**	239:251	235:248	211:234	217:230
39.	'McIntoch'	141:143	**251**	235:260	232:234	230:255
40.	'Geneva Early'	137:139	249:253	235:246	234:243	206:253
41.	'Freedom'	137:158	239:251	248:258	219:239	217:241
42.	'Gala'	143:158	239:251	235:246	222:239	237:241
43.	'Fiesta'	134:137	239:247	235:252	222:234	237:241
44.	'Braeburn'	**137**	**239**	248:260	211:239	241:251
45.	'Prima'	137:139	**251**	248:258	228:232	247:255
46.	'Worcester Pearmain'	137:143	**251**	235:248	228:232	230:232
47.	'Discovery'	137:143	249:251	248:260	228:232	230:247
48.	'Summerred'	137:158	239:251	**260**	219:224	230:237

Table 1 (continued)**.** SSR profiles of 37 Lithuanian and 11 reference cultivars. SSR alleles in bold apparently occur in duplicate

No.	Cultivar	SSR locus / alleles					
		CH04c06	CH04e05	COL	CH02d08	CH01g12	CH02b10
				Lithuanian cultivars			
1.	'Beržininkų ananasas'	178:186	175:225	**232**	**219**	108:137	122:143
2.	'Giedris'	172:196	**175**	232:243	213:215	130:152	120:132
3.	'Aušris'	**178**	175:211	222:236	227:232	104:183	130:158
4.	'Pilkasis molinis'	182:196	175:225	**234**	250:258	146:185	130:143
5.	'Babtų baltasis'	189:190	175:225	205:234	**213**	108:135	126:160
6.	'Genelis'	172:196	**175**	232:243	213:215	130:185	120:132
7.	'Avenarijus'	178:182	175:225	222:232	213:227	135:137	122:143
8.	'Persikinis'	**178**	202:225	232:234	225:258	108:137	120:130
9.	'Tabokinė'	**186**	175:211	222:234	**213**	137:146	122:130
10.	'Margutis'	178:196	**175**	222:232	219:250	152:185	132:134
11.	'Vytėnų pepinas'	180:189	198:225	222:234	213:254	135:154	**143**
12.	'Kaunis'	178:196	**175**	**232**	215:258	108:185	112:120
13.	'Baltasis alyvinis'	**178**	204:211	222:245	217:227	104:108	132:158
14.	'Paprastasis antaninis'	**178**	225:229	**232**	**250**	135:137	130:158
15.	'Montvilinis'	170:178	175:211	222:232	213:219	137:150	120:132
16.	'Vytenis'	**189**	175:202	**234**	213:258	135:183	132:143
17.	'Vytis'	182:189	**175**	232:245	**225**	137:170	132:158
18.	'Vytėnų pilkasis'	178:182	**175**	**232**	219:258	108:185	112:132
19.	'Saldis'	178:184	175:204	222:234	213:227	108:137	158:160
20.	'Auksis'	178:182	202:225	234:243	213:258	108:146	120:158
21.	'Skaistis'	182:189	175:200	232:236	213:258	108:154	120:128
22.	'Rudenis'	178:190	175:200	232:236	213:258	108:154	120:128
23.	'Raudonasis rudeninis dryžuotasis'	170:189	175:211	234:245	213:219	137:146	120:130
24.	'Noris'	178:182	**202**	232:243	232:258	137:146	120:145
25.	'Popierinis'	**178**	204:211	222:245	217:227	104:108	132:158
26.	'Panevėžio rojinukas'	178:193	190:198	**232**	**219**	130:155	122:132
27.	'Pupinis'	**178**	**225**	232:243	217:227	**137**	132:143
28.	'Štaris'	**182**	175:202	**243**	213:258	137:150	122:132
29.	'Žemaičių grietininis'	182:186	175:202	**232**	206:213	106:108	136:143
30.	'Lietuvos pepinas'	194:196	**175**	222:232	215:250	150:185	120:134
31.	'Panemunės baltasis'	182:186	175:225	220:222	234:258	104:106	132:160
32.	'Birutės pepinas'	194:196	**175**	222:245	213:215	108:152	130:134
33.	'Jono pepinas'	182:186	175:211	224:234	213:258	106:183	**122**
34.	'Puikis'	186:194	175:202	**222**	213:232	135:150	120:128
35.	'Biržuvėnų žieminis'	178:182	**175**	222:232	227:258	106:108	143:158
36.	'Raudoniai'	182:186	175:198	222:234	219:250	108:185	**143**
37.	'Aldas'	182:196	175:211	224:236	254:258	108:185	120:143
				Reference cultivars			
38.	'Empire'	178:182	175:211	234:243	219:232	108:130	128:158
39.	'McIntoch'	178:182	202:211	234:243	213:232	130:150	128:145
40.	'Geneva Early'	**178**	175:225	205:234	213:258	108:137	112:132
41.	'Freedom'	178:182	175:202	232:234	213:258	137:146	120:158
42.	'Gala'	182:189	**175**	232:234	227:258	146:150	124:130
43.	'Fiesta'	184:190	200:229	232:234	227:258	108:154	118:124
44.	'Braeburn'	182:189	202:204	222:234	219:258	106:108	118:130
45.	'Prima'	182:189	175:202	222:234	225:258	130:135	126:143
46.	'Worcester Pearmain'	182:189	175:202	**232**	213:254	130:150	128:136
47.	'Discovery'	170:182	198:202	232:243	232:254	110:150	**128**
48.	'Summerred'	178:182	175:225	**234**	213:232	137:150	124:128

Table 2. Genetic diversity estimators of 11 polymorphic SSR loci

SSR locus	Observed allele size range	Number of alleles (A)	Effective number of alleles (A_e)	Power of discrimination (PD)	Number of genotypes	Expected heterozygosity (H_e)	Observed heterozygosity (H_o)
	Lithuanian cultivars / Reference cultivars						
CH01d03	134–158 / 134–158	8 / 6	–	0.81 / 0.69	17 / 8	–	0.86 / 0.75
CH01h02	239–259 / 239–253	8 / 5	3.76 / 2.75	0.89 / 0.76	15 / 6	0.73 / 0.64	0.76 / 0.64
CH02c09	235–260 / 235–260	9 / 6	6.34 / 4.57	0.93 / 0.84	21 / 7	0.84 / 0.78	0.92 / 0.91
CH02c11	211–243 / 211–243	10 / 9	7.19 / 7.56	0.94 / 0.86	22 / 9	0.86 / 0.87	0.89 / 1.00
CH02c06	201–265 / 206–255	11 / 10	5.35 / 7.33	0.94 / 0.89	22 / 10	0.81 / 0.86	0.84 / 1.00
CH04c06	170–196 / 170–190	12 / 6	5.47 / 3.56	0.92 / 0.71	20 / 5	0.82 / 0.72	0.76 / 0.91
CH04e05	175–229 / 175–229	9 / 8	3.33 / 4.32	0.87 / 0.84	14 / 8	0.70 / 0.77	0.70 / 0.91
COL	205–245 / 205–243	9 / 5	4.78 / 3.23	0.91 / 0.83	18 / 7	0.79 / 0.69	0.73 / 0.82
CH02d08	206–258 / 213–258	12 / 7	6.68 / 5.38	0.93 / 0.86	23 / 8	0.85 / 0.81	0.84 / 1.00
CH01g12	104–185 / 106–154	14 / 9	8.61/ 6.54	0.95 / 0.89	25 / 10	0.88 / 0.85	0.97 / 1.00
CH02b10	112–160 / 112–158	13 / 12	8.08 / 7.56	0.95 / 0.91	25 / 11	0.88 / 0.85	0.92 / 0.91
Mean value		10.45 / 7.55	5.96 / 5.28*	0.91 / 0.83	20.18 / 8.09	0.82 / 0.79*	0.84 / 0.90

Notes. *Mean values were calculated for ten SSR loci, excluding CH01d03. CH01d03 primer pair had been identified as multilocus marker (Liebhard et al., 2002), therefore it was not possible to establish frequency of alleles using this multilocus marker and the marker was excluded from allele frequency (p_i) and expected heterozygosity (H_e) analysis.

The number of alleles for each locus ranged from 5 to 12 (7.55 in average). The number of alleles per locus identified in reference cultivars was in agreement with previously published data (Liebhard et al., 2002). Meanwhile, the number of alleles in the set of Lithuanian cultivars was considerably higher and comparable to the results obtained in the studies by Pereira-Lorenzo et al. (2007) and Gharghani et al. (2009). This difference must be related to higher number and diversity of analyzed genotypes.

Among 121 polymorphic alleles, 77 were identified as common to both sets of cultivars. The group of reference cultivars had 6 unique alleles not found among the Lithuanian cultivars. Meanwhile, the latter cultivars had 38 unique alleles that were absent in the set of reference cultivars.

The results of microsatellite marker analysis revealed that all of the cultivars involved in the study had diploid genome. Single microsatellite locus that yield PCR amplification product of only one size was presumed to be homozygous. Meanwhile, CH01d03 primer pair had been identified as multilocus marker (Liebhard et al., 2002); therefore 1–3 alleles were observed for individual genotypes. Except for the genotypes where only single allele was detected, it was not possible to establish frequency of alleles using this multilocus marker and the marker was excluded from allele frequency (p_i) and expected heterozygosity (H_e) analysis. Allele frequencies were distributed unevenly within the remaining 10 loci. The allele frequency was estimated to vary from 1.0% to 46.9% for the whole set of cultivars used in the study. Among the Lithuanian cultivars, the p_i was estimated to range from 1.4% to 50.0%. Most of the unique alleles characteristic of Lithuanian cultivars were rear (p_i < 10%), except for the allele 242 in locus CH02c09 (p_i = 18%), allele 245 in locus CH02c06 (p_i = 11%), alleles 186 and 196 in locus CH04c06 (p_i = 11%) and allele 185 in locus CH01g12 (p_i = 11%). These alleles were spread mostly among traditional cultivars of unknown origin suggesting presence of links to genetic variation that is not common in reference cultivars and might be required to adapt to stringent ecological conditions characteristic of the region.

One to thirteen homozygous loci were identified for the whole set of cultivars used in the study, meanwhile the number of homozygous loci varied from 1 to 11 and from 0 to 4 in the sets of Lithuanian and reference cultivars, respectively. No homozygous loci were identified in 8 cultivars, including 5 of Lithuanian cultivars. These were mostly cultivars (‘Auksis’, ‘Aldas’, ‘Skaistis’, ‘Rudenis’) newly developed from crosses of foreign or/and traditional cultivars. Four was the highest number of homozygous loci characteristic of ‘Beržininkų ananasas’ and ‘Vytis’.

Observed heterozygosity H_o value among the Lithuanian cultivars varied from 0.70 to 0.97 (0.84 on average). Expected heterozygosity H_e value varied from 0.70 to 0.88 with a mean value of 0.82. Among the reference cultivars, the range of H_o value was from 0.64 to 1.0 (0.9 on average) and the range of H_e value was from 0.64 to 0.87 (0.79 on average). Among Lithuanian cultivars, H_o value was equal or higher than H_e value for seven loci out of ten used in the analysis. $H_o < H_e$ ratio was found for CH04c06 and COL loci. $H_o \geq H_e$ ratio was characteristic of all of the loci of reference cultivars. High and similar H_o and H_e values confirm the fact that self-incompatibility in apple tree reduces the possibility of inbreeding. However, H_o values were found to be higher for the set of reference cultivars (0.90) as compared to Lithuanian cultivars

(0.84), suggesting slightly lower genetic diversity among the Lithuanian cultivars. In comparison to other studies, the mean H_o for Lithuanian cultivars was comparable to the value reported by Pereira-Lorenzo et al. (2007) (H_o = 0.83) and higher than those reported by Larsen et al. (2006) (H_o = 0.77), Gharghani et al. (2009) (H_o = 0.63) and Coart et al. (2003) (H_o = 0.68, 0.73 and 0.75) for wild, ornamental and domestic apple populations. The mean H_o values for referece cultivars were higher as compared to those reported in the studies mentioned above. The mean H_e values for both, Lithuanian and reference cultivars, were comparable to those reported by Pereira-Lorenzo et al. (2007) (H_e = 0.80), Larsen et al. (2006) (H_e = 0.78) and Coart et al. (2003) for ornamental (H_e = 0.78) and domestic apple populations (H_e = 0.84). However, the values were slightly higher than those reported by Coart et al. (2003) for wild apple populations (H_e = 0.72) and the H_e was lower than values reported by Gharghani et al. (2009) (H_e = 0.86).

Analysis of the genetic relationship among the Lithuanian and reference cultivars using cluster analysis revealed five groups of distantly related cultivars (Sikorskaite et al., submitted for publication). Assessment of this data in combination with characteristics of resistance to apple scab of indigenous, traditional and progenitor cultivars revealed putative links to genetic background involved in response to apple scab infection.

A unique fingerprint of alleles of microsatellite loci has been identified for most of the Lithuanian cultivars included in the study. Only 'Baltasis alyvinis' and 'Popierinis' could not be discriminated using the eleven microsatellite primer pairs. The identity of the two cultivars has been the subject for debate among pomologists, and 'Popierinis' being the genetic variant of 'Baltasis alyvinis' is the established opinion.

Power of discrimination (PD) values varied from 0.81 to 0.95 in the set of Lithuanian cultivars, and the highest PD value was identified for CH01g12 and CH02b10 loci. A minimum set of PCR primers required to distinguish Lithuanian cultivars with the exception of 'Baltasis alyvinis' and 'Popierinis' was identified and includes three options: CH01g12, CH02b10 and CH02c11; CH01g12, CH02b10 and CH02c06; CH01g12, CH02b10 and CH02c09 markers.

Conclusions

1. Assessment of 11 polymorphic loci of 48 apple cultivars identified 121 polymorphic alleles. The allele number per locus varied from 5 to 14. The CH01g12 was the most polymorphic microrsatellite locus in the group of 37 Lithuanian cultivars, while CH02b10 locus exhibited the highest polymorphism in the set of reference cultivars.

2. Among 121 polymorphic alleles, 77 were identified as common to both sets of cultivars. Lithuanian cultivars had 38 unique alleles that were absent in the set of reference cultivars. Most of the unique alleles were rare (p_i < 10%), except for the allele 242 in locus CH02c09 (p_i = 18%), allele 245 in locus CH02c06 (p_i = 11%), alleles 186 and 196 in locus CH04c06 (p_i = 11%) and allele 185 in locus CH01g12 (p_i = 11%). These alleles were most common among traditional cultivars of unknown origin.

3. The number of homozygous loci varied from 1 to 11 in the set of Lithuanian cultivars, and from 0 to 4 in the set of reference cultivars. Among the cultivars that contained no homozygous loci, the most common were the newly developed cultivars obtained from the crosses of foreign or/and traditional cultivars ('Auksis', 'Aldas', 'Skaistis', 'Rudenis'). The highest number of homozygous loci was characteristic of 'Beržininkų ananasas' and 'Vytis'.

4. High heterozigosity values were found for all ten loci used in the analysis. H_o values were found to be higher for the set of reference cultivars as compared to Lithuanian cultivars, suggesting slightly lower genetic diversity among the traditional cultivars.

5. The identified minimum set of PCR primers required to distinguish 35 Lithuanian cultivars (except 'Baltasis alyvinis' and 'Popierinis') includes CH01g12, CH02b10 and CH02c11 (CH01g12, CH02b10 and CH02c06 or CH01g12, CH02b10 and CH02c09) markers.

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Naminės obels (*Malus* × *domestica* Borkh.) veislių mikrosatelitų sekų charakteristika

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Santrauka

Obuoliai sudaro svarbią dalį sodininkystės produkcijos Lietuvoje ir pasaulyje. Genotipuojant obels genetinius išteklius ir vykdant genetinio polimorfizmo tyrimus, taikomi molekulinės biologijos metodai. Tyrimo tikslas – charakterizuoti lietuviškų vietinės kilmės bei tradicinių naminės obels veislių mikrosatelitų sekų lokusus ir nustatyti pradmenų poras, tinkamas šioms veislėms genotipuoti. Tirtos 37 tradicinės bei vietinės kilmės veislės, sukurtos per pastarąjį šimtmetį, ir 11 standartinių veislių, saugomų LAMMC Sodininkystės ir daržininkystės instituto genetinių išteklių kolekcijoje. Mikrosatelitų sekoms apibūdinti panaudota vienuolika porų pradmenų, pritaikytų *Malus* × *domestica* polimorfiškų mikrosatelitų sekų analizei ir rekomenduojamų *ECPGR Malus* darbo grupės. Lietuviškų veislių grupėje identifikuota nuo 8 iki 14 alelių (vidurkis – 10,45), įvertinto heterozigotiškumo reikšmė – 0,92. Standartinių veislių grupėje identifikuota nuo 5 iki 12 alelių (vidurkis – 7,55), įvertinto heterozigotiškumo reikšmė – 0,90. Lietuviškų veislių grupėje didžiausias polimorfiškumas būdingas CH01g12 ir CH02b10 lokusams (PD vertė – 0,95). Tirtų mikrosatelitų sekų polimorfiškumas leido specifiškai identifikuoti 35 vietines ir visas tirtas standartines veisles. Nustatytas pradmenų rinkinys, tinkamas diferencijuoti 35 vietines obels veisles: CH01g12, CH02b10 ir CH02c11 (CH01g12, CH02b10 ir CH02c06 arba CH01g12, CH02b10 ir CH02c09).

Reikšminiai žodžiai: obelis, genetinis polimorfizmas, genotipavimas, tradicinė veislė, vietinės kilmės veislė, standartinė veislė.