

Molecular characterization of the Latvian apple (*Malus*) genetic resource collection based on SSR markers and scab resistance gene *Vf* analysis

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Abstract

Apple cultivars are an integral part of the Latvian State Program for Preservation and Sustainable Use of Genetic Resources for Food and Agriculture. Latvian apple ($n = 109$) cultivars of local origin, nominated as National Plant Genetic Resources, were analyzed with eight simple sequence repeat (SSR) markers recommended by the European Cooperative Programme for Plant Genetic Resources (ECPGR) *Malus/Pyrus* working group and a marker for scab resistance gene (*Vf*). All SSR loci exhibited a high level of polymorphism – 12 to 39 alleles, 18.75 in average, with high observed heterozygosity (H_o) ranging from 0.64 to 0.89 and a mean of 0.78. The gene diversity (polymorphism information content value) varied from 0.79 to 0.90, with an average of 0.86. All cultivars could be distinguished with the tested set of SSR loci, with a high frequency of rare (38%) or unique (26%) alleles. For the *Vf* gene, all three possible genotypes (*VfVf*, *Vfvf* and *vfvf*) were detected for 1, 6 and 102 varieties, respectively. The analyzed germplasm showed high genetic diversity, particularly landraces, while the modern cultivars clustered mainly into two groups. Cluster analysis did not reveal a clear pattern with well-defined groups, but confirmed some relationships based on known or putative pedigrees, as well as suggesting the possible parentage of some cultivars.

Keywords: genetic diversity; germplasm; *Malus* spp.; microsatellite

Introduction

Fruit crop genetic resources are very important both for research and breeding purposes. Apple (*Malus* sp.) accessions are an integral part of the Latvian State Program for Preservation and Sustainable Use of Genetic Resources for Food and Agriculture. The Latvia State Institute of Fruit-Growing (LSIFG) pome fruit collection includes ~700 apple accessions, of which 109 accessions belong to the 1st priority group of national genetic resources. This genetic resources group includes cultivars produced from Latvian breeding programmes, as well as clones

and elite hybrids originated and selected in Latvia, with valuable quantitative or qualitative traits; cultivars of local origin (landraces), including foreign cultivars with a long history of cultivation in Latvia.

Evaluation and characterization of apple genetic resources is an important task for further maintenance and utilization activities. Since characterization by phenotype has serious limitations due to environmental influence, limited data exchange and comparison with other researchers, the LSIFG has introduced molecular marker technologies for genetic resource characterization and breeding. Apple genotyping was performed using SSR markers recommended by the European Cooperative Programme for Plant Genetic Resources (ECPGR) *Malus/Pyrus* working group as well as gene-specific molecular markers (e.g. *Vf* resistance gene marker).

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Materials and methods

Total DNA was isolated from young leaves using a Genomic DNA Purification Kit (Fermentas, Lithuania).

PCR were performed in a 20 μ l reaction mixture with 25 ng DNA, 2 μ M each primer, 200 μ M of each nucleotide, 1X PCR buffer and 0.05 U/ μ l REDTaq[®] DNA Polymerase (Sigma, USA) per reaction, in the ep gradient thermal cycler (Eppendorf, Germany). The PCR conditions were the same as already described in the methodology for SSR markers (Guilford *et al.*, 1997; Gianfranceschi *et al.*, 1998) and *Vf* gene detection (Vejl *et al.*, 2003).

PCR products were first checked on 1% agarose gels in 1X Tris–acetate–ethylenediaminetetraacetic acid buffer and visualized by staining with ethidium bromide to test for the presence of PCR products. For SSR genotyping, the same PCR products were subsequently analyzed on an ABI PRISM[®] 3100 Genetic Analyzer (Applied Biosystems, USA) and genotyped using GeneMapper[®] Software v4.0 (Applied Biosystems).

Genetic parameters were calculated using the computer program GENALEX 6.1 (Peakall and Smouse, 2006). Potential population structure was analyzed and phylogenetic tree was constructed based on Nei's genetic distance (Nei, 1973) and Neighbour-Joining clustering method using MEGA version 4 (Tamura *et al.*, 2007).

Results and discussion

The 109 apple varieties nominated as National Plant Genetic Resources were analyzed using a set of eight selected SSR markers (recommended by the ECPGR *Malus/Pyrus* working group) and a marker for scab resistance gene (*Vf*). All SSR loci exhibited a very high level of polymorphism – 12 to 39 alleles, 18.75 in average, with high observed heterozygosity (H_o) ranging from 0.64 to 0.89 and a mean of 0.78. The gene diversity (polymorphism information content (PIC) value) for tested loci varied from 0.79 to 0.90, with an average of 0.86. Results of

genotyping showed high presence of rare or unique alleles, 38 and 26% respectively (Table 1). All cultivars in the collection could be distinguished with the tested set of SSR loci. Internal relationships of apple cultivars were evaluated using SSR data-based dendrogram created applying Nei and Li genetic distances and Neighbour-Joining tree construction method. Cluster analysis did not reveal a clear pattern of clustering with well-defined variety groups, but confirmed some known relationships based on known or putative pedigree. The analyzed Latvian apple germplasm showed high genetic diversity, particularly landraces, while the cultivars developed within the modern breeding program clustered mainly into two groups. The analyzed plant material also included two widely grown cultivars 'McIntosh' and 'Prima', which allowed for checking the accuracy of allele scoring and comparing the results of this study with those presented in previously published studies.

A significant part of the cultivars grown in Latvia include in their pedigree the cultivars derived from 'McIntosh' ('Cortland', 'Lobo', 'Melba' etc.), local cultivars 'Sīpoliņš', 'Baltais Dzidrais' ('Yellow Transparent'), 'Antonovka', 'Rēveles Bumbierābele' ('Revaler Birnapfel') or 'Cukuriņš' ('Korobovka'). The obtained dendrogram did not always show clear relationships according to known pedigrees or morphological features, but helped to confirm some known ones or to cast doubt upon reported pedigrees, as well as suggesting possible previously unknown parentages (Fig. 1). For example, the Daugmale clone of 'Baltais Dzidrais' comes out as a possible seedling, not as a clone. The same may be true for the two supposed clones of the old cultivar 'Ničnera Zemeņu' ('Nitschners Erdbeerapfel'). Also, 'Robežnieku Sīpoliņš' is too far distanced from 'Sīpoliņš' to be a clone. On the other side, our results confirmed the genetic difference between Dobeles and Pūres clones of 'Ādamābele', which was already previously doubted. The dendrogram confirmed also the supposed origin of cv. 'Sārīsa Agrā' from 'Baltais Dzidrais' and cv. 'Vigo' from 'Sīpoliņš'.

Table 1. Genotyping results for the Latvian apple accessions using SSR markers

Locus	No. of homozygous plants	No. of alleles	H_o	PIC value	No. of genotypes	Discrimination power	Allele length range (bp)
CH02d08	19	17	0.826	0.849	37	0.958	168–258
CH04c07	32	13	0.706	0.871	46	0.963	94–140
CH01e12	28	39	0.725	0.837	71	0.975	89–223
CH01h01	36	12	0.670	0.789	28	0.935	102–130
CH02c09	13	14	0.881	0.876	36	0.961	219–259
CH02c06	12	16	0.890	0.890	59	0.980	202–267
CH01f02	14	17	0.872	0.903	53	0.982	62–224
NZ05g8	39	22	0.642	0.877	49	0.964	68–246
Average	24.1	18.8	0.777	0.862	47.4	0.965	



Fig. 1. Genetic relatedness dendrogram of Latvian apple genetic resources accessions. Group of origin: ○, ‘McIntosh’; ●, ‘Sipoliņš’; ●/○, ‘McIntosh’ and ‘Sipoliņš’, □, ‘Antonovka’; ■, ‘Baltais Dzidrais’ (‘Yellow Transparent’); △, ‘Rēveles Bumbierābele’ (‘Revaler Birnapfel’); ▲, ‘Cukuriņš’ (‘Korobovka’); ◇, ‘Delicious’.

The origin of landraces and amateur cultivars usually is either unknown or unclear. Our results, together with known pedigree data, helped to suggest the possible parentage of several local cultivars, e.g. for ‘Celmiņu Dzeltenais’ (‘Ananasrenette’ × ‘Dzeltenais Dzidrais?’), ‘Vilka Rožābele’ (‘Suislepp’ × ‘Ničnera Zemeņu?’), ‘Ugunda’ and ‘Līčiņu Ziemas’ as both derived from ‘Chernoguz’, ‘Lizuma Ziemas’ as derived from the ancient landrace ‘Zaķapurni’, established a close link between cultivars ‘Velte’ (‘James Grieve’ × pollen mix) and ‘Aivariņš’ (unknown), etc.

While the claimed pedigree of ‘Korta’ is ‘Cukuriņš’ × ‘Rēveles Bumbierābele’, our study shows that the second parent could be ‘Baltais Dzidrais’ instead. The close linking between ‘Ella’ and ‘Ventspiliete’ creates doubt about the precision of data about their parentage (‘Anoka’ × ‘Golden Delicious’ and ‘James Grieve’ × ‘Serinka’, respectively). The phylogenetic analysis also supported the previous doubts about the parentage of the modern cultivar ‘Ligita’ (‘Alkmene’ × ‘Bogatyr?’); its pollen parent may be a scab-immune cultivar with ‘McIntosh’ in the pedigree.

The grouping of *Malus sylvestris* (sample collected at Slitere Nature Reserve) with several ‘McIntosh’-type cultivars was surprising, as well as the similarity of red-leaved (f. *niedzwetskyana*) local foundling ‘Carnikava’ with cv. ‘Rāja’ (‘Cortland’ × ‘Serinka’), and the close relationship between the highly unsimilar landraces ‘Majoru Saldais’ and ‘Vasaras Citronu’. More precise results may be obtained when other known or possible parent cultivars are included in the study; for example, ‘Antonovka’, ‘Revaler Birnapfel’, ‘Suislepp’ and ‘Treboux’.

For the *Vf* gene, all three possible genotypes (*VfVf*, *Vfvf* and *vfif*) were detected for 1, 6 and 102 cultivars, respectively. Cultivar ‘Prima’ was used as a positive control for apple scab resistance gene (*Vf*) detection. Cultivars with *VfVf* and *Vfvf* genotypes have been selected as valuable sources for further apple scab resistance breeding. All cultivars where the dominant *Vf* allele was found have been produced as a result of the modern breeding programme.

Application of eight well-tested microsatellite markers as well as *Vf* gene-specific markers on Latvian *Malus* genetic resources showed that:

- (1) The tested microsatellite markers showed suitability for evaluation of genetic diversity and relatedness of Latvian apple genetic resources, and ensured discrimination of all accessions including varieties sharing the same pedigree.
- (2) Application of eight tested microsatellite markers showed suitability for confirmation or prediction of possible parentage of apple varieties.
- (3) Old local Latvian apple varieties do not contain the *Vf* resistance gene.

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