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Characterising the genetic diversity of Lithuanian sweet cherry (*Prunus avium* L.) cultivars using SSR markers

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ABSTRACT

The polymorphisms and characteristics of microsatellite (SSR) loci from 31 sweet cherry (*Prunus avium*) cultivars were analysed. The cultivars were genotyped with 14 DNA primer pairs using a multiplex approach. A total of 20 unique alleles were identified in the Lithuanian cultivars that were absent in the cultivars of foreign origin. The analysis revealed that the expected and observed heterozygosities were higher in the cultivars of Lithuanian origin as compared with those that were introduced. Approximately 2–11 alleles (5.29 on average) with a mean heterozygosity value of 0.68 were established for microsatellite loci from the Lithuanian cultivars.

Four distinct genetic groups of cultivars were defined using cluster analysis. One cluster consisted of Lithuanian landraces, Russian cultivars and their hybrids. The earliest (first generation) Lithuanian cultivars, derived from Lithuanian landraces and Russian cultivars also fitted this cluster. Another cluster consisted of the second generation of cultivars of Lithuanian, Ukrainian and Belarusian origin. The most recent cultivars of Lithuanian origin and the cultivars from Canada and Western Europe were grouped into a different cluster. Fourth cluster consisted of three cultivars: Belobokaya rannaya; its progeny, Meda; and Irema BS of unknown origin.

Characterising the genetic backgrounds of the cultivars revealed the unique features of the sweet cherry grown in the region.

1. Introduction

The sweet cherry (*Prunus avium*) is a popular fruit tree in Lithuania and across Europe. The species originated near the Caspian and Black Sea and spread to the European area of the continent (Webster, 1996). The northern border of the secondary spreading of the species is located in the Baltic region. Populations of wild sweet cherry are found in Western Lithuania. Lithuania is located in a temperate region, and the climatic conditions play an important role in the development of specific plant genotypes grown in that region (Romanovskaja et al., 2009). Previously, the uniqueness of genotypes of the sweet cherry grown in Western Lithuania was revealed through genotyping self-incompatibility alleles (Stanys et al., 2008).

The breeding of the sweet cherry in Lithuania started in 1965 to create cultivars that are resistant to cold and fungal diseases, that ripen early, and that are highly productive (Lukosevicius, 1998). The Lithuanian sweet cherry population, when adapted to local growth conditions, was the source for developing the earliest cultivars. Seeking to improve fruit quality, foreign cultivars were later introduced into crosses with the local sweet cherry.

Efficient breeding and research on sweet cherry genetic resources require new experimental tools and the application of biotechnology. Our previous research demonstrated that the use of *in vitro* techniques in sweet cherry breeding has increased seedling output (Stanys, 1998). The conventional identification of sweet cherry cultivars is based on pomological traits and the description of morphological and horticultural characteristics (IPGRI, 1985; UPOV, 1976) therefore, it is susceptible to subjective and environmental influences. The use of molecular techniques that detect variations at DNA levels enabled the development of cultivar-specific DNA fingerprints. The development of fingerprints using microsatellite markers is less complicated than using the amplified fragment length polymorphism (AFLP) method (Wünsch and Hormaza, 2002). Microsatellite-based molecular markers are used for genotyping plant cultivars or individual plants, assessing the gene flow in populations and mapping the genome. The SSR markers were used for fingerprinting sweet cherry accessions (Struss et al., 2003; Wünsch and Hormaza, 2002), developing genetic linkage maps (Clarke et al., 2009), assessing genetic diversity and

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Table 1
Characteristics of sweet cherry cultivars.

No.	Cultivar	Parents	Origin
1	Agila	Leningradskaya ciornaya × Priusadebnaya	Lithuania
2	Anta	Zemaiciu rozine × Napoleon + Dniprovka	Lithuania
3	Auste	Hedelfinger × Rozine	Lithuania
4	Germa	No. 1106 × Sam	Lithuania
5	Goda	Unknown	Lithuania
6	Irema BS	Unknown	Lithuania
7	Jurga	Dniprovka × Hedelfinger	Lithuania
8	Jurgita	Hedelfinger × Dniprovka	Lithuania
9	Luke	$N_2$1106 × Sam	Lithuania
10	Meda	Belobokaya rannyaya × Hedelfinger	Lithuania
11	Mindauge	Severnaya × Jurgita	Lithuania
12	Rozine	Landrase	Lithuania
13	Seda	Hedelfinger × Rozine	Lithuania
14	Vasare	Leningradskaya ciornaya × Priusadebnaya	Lithuania
15	Vytenu geltonoji	Zemaiciu geltonoji × Zolotaya lositskaya	Lithuania
16	Vytenu juodoji	Zemaiciu raudonoji × Dniprovka	Lithuania
17	Vytenu rozine	Hedelfinger × Rozine	Lithuania
18	Zemaiciu geltonoji	Landrase	Lithuania
19	Zemaiciu juodoji	Landrase	Lithuania
20	Zemaiciu rozine	Landrase	Lithuania
21	Belobokaya rannyaya	Unknown	Russia/Ukraine
22	Dniprovka	Dennisens gelbe Knorpelkirsche × Zabule	Ukraine
23	F12/1	*Prunus avium* selected clone	Unknown
24	Hedelfinger	Unknown	Germany
25	Lapins	Van × Stella	Canada
26	Sam	Natural crossing of Windsor	Canada
27	Severnaya	Unknown	Russia
28	Sunburst	Van × Stella	Canada
29	Van	Seedling of Empress Eugenie	Canada
30	Vega	Bing × Victor	Canada
31	Zolotaya lositskaya	Natural crossing of Dennisens gelbe Knorpelkirsche	Belarus

characterising sweet cherry collections in several European countries (Ganopoulos et al., 2011; Guarino et al., 2009; Lacis et al., 2009; Wünsch and Hormaza, 2004). The Lithuanian sweet cherry cultivars have not been characterised using SSR markers.

Based on the evolution of genetic resources, the use of high-level characterisation techniques would reveal the breeding history of the Lithuania sweet cherry and improve approaches for the secure conservation of genetic resources.

The aim of this study, therefore, was to assess the genetic diversity and relationship of 31 genotypes of Lithuanian and introduced sweet cherry cultivars using SSR molecular markers. Moreover, the informativeness of the SSR markers for genotype identification was assessed.

2. Materials and methods

2.1. Plant material

The study included 20 local traditional cultivars and Lithuanian cultivars developed during the last century and 11 common cultivars of foreign origin, which are listed in Table 1. The plant material was collected at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry.

2.2. DNA extraction and SSR analysis

The DNA was extracted from leaves using the DNeasy Plant Mini kit (Qiagen) or the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990). Genomic DNA was stored in TE buffer (100 mM Tris–HCl, 10 mM EDTA and pH 8) at −20 °C.

A total of 14 previously published primer pairs were used for SSR analysis: EMPA002, 003, 017, 018 (Clarke and Tobutt, 2003); EMPAS01, 02, 06, 10, 11, 12 (Vaughan and Russell, 2004); PCEGA34 (Downey and Iezzoni, 2000); PS05C03 (Sosinski et al., 2000); UDP98-412 (Testolin et al., 2000) and UCD-CH14 (Struss et al., 2003). The primer pairs were chosen to cover all eight cherry linkage groups (G1-8): G1 – EMPA002, EMPA003; G2 – EMPA017, PCEGA34; G3 – EMPAS02, EMPAS12; G4 – EMPAS06, EMPAS10; G5 – EMPAS11; G6 – EMPAS01, UDP98-412; G7 – UCD-CH14, PS05C03; and G8 – EMPA018 (Clarke et al., 2009). The multiplex PCR reactions were performed in a final volume of 9 μl, containing 60 ng genomic DNA, True Allele PCR Premix (Applied Biosystems) and 0.3 μM of each primer pair. The forward primer was fluorescently tagged with 6-FAM, VIC, NED or PET (Applied Biosystems Instruments, Foster City, CA). The following conditions were used for amplification: 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 25 cycles of 94 °C for 30 s, 55 °C for 45 s, 72 °C for 1 min with a final elongation step of 72 °C for 5 min. The fragment analysis was performed using a Genetic Analyser 3130 (Applied Biosystems, Foster City, CA). The data were analysed using the GeneMapper software v.4.0 (Applied Biosystems Instruments, Foster City, CA).

The presence of null alleles was assessed using Micro-Checker software v.2.2.3 (Van OOsterhout et al., 2004). The frequency of alleles, expected and observed heterozygosity, and polymorphism information content (PIC) were calculated for each SSR primer pair using PowerMarker software v.3.25 (Liu and Muse, 2005). The informativeness of microsatellite loci was established following Botstein et al. (1980), where PIC > 0.5 was considered highly informative, 0.5 > PIC > 0.25 was reasonably informative and PIC < 0.25 was slightly informative. The phylogenetic tree was constructed using the "Nei1983" distance and "UPGMA" tree methods within the PowerMarker programme. To test the reliability of the phylogenetic tree, a bootstrap analysis with 1000 replications was performed using PowerMarker software (Liu and Muse, 2005). The threshold of reliability was set to 50% for the bootstrap values. A consensus tree was constructed using the "consense" programme of the Phylip package v.3.67 (Felsenstein, 1989) and displayed using TreeView software v.1.6.6 (Page, 1996).