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Assessment of genetic diversity of Czech sweet cherry cultivars using microsatellite markers

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ABSTRACT

We analyzed 24 sweet and wild cherry genotypes collected in Czech Republic to determine genetic variation, using previously described 16 SSR primers to adapt a fast, reliable method for preliminary screening and comparison of sweet cherry germplasm collections. All SSRs were polymorphic and they were able all together to distinguish unambiguously the genotypes. These SSR primers generated 70 alleles; the number of alleles per primer ranged from 2 to 7, with a mean of 4.4 putative alleles per primer combination. The primer UDP-98-412 gave the highest number of polymorphic bands (totally 7), while Empa2 and Empa3 gave the lowest number (2). The allele frequency varied from 2.1% to 87.5%. We observed 10% of unique alleles at different loci. The observed heterozygosity value ranged from 0.25 to 0.96 with an average of 0.72 while expected heterozygosity value varied from 0.22 to 0.75 with an average of 0.59. The PIC value ranged from 0.21 to 0.71 with a mean value of 0.523. Cluster analysis separated the investigated cultivars in two groups. High level of genetic diversity obtained in the collection and proved to be sufficiently genetically diverse and therefore these genotypes would be useful to breeders for the development of new cherry cultivars.

1. Introduction

The sweet cherry (*Prunus avium* L.) is a perennial plant having high level of heterozygosity originated in north eastern part of Turkey, near the Black Sea region (Zohary and Hopf, 2000). At present sweet cherry is cultivated across the Europe and in western Asian areas (Webster, 1996). The excellent favorable climatic conditions potentially increased domestication of wild cherry across the Europe (Wunsch and Hormaza, 2002). The annual production of sweet cherry is continuously increasing due to favorable climatic conditions and continuous demand for export marketing. A number of different sweet cherry varieties are grown at different zones of Czech Republic and also maintained at Research and Breeding Institute of Pomology (RBIP) Holovousy.

The accurate description of genetic diversity in natural and artificial populations and identification of concrete sweet cherry genotypes rely on molecular techniques since morphological traits are easily influenced by environmental and

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agronomical factors (Struss et al., 2001) and also offer several advantages over conventional phenotypic markers and has impact also in breeding programs and orchard establishment (Xuan et al., 2009).

The identity and distinction of cultivars entirely depends upon the easily accessible and reliable genetic markers used for efficient breeding methods such as genetic identification, removal of duplications, fruit quality, tree growth characteristics and other traits (Galli et al., 2005). The genotype grouping and identification has been successfully applied jointly using phenotypic data and molecular markers (Ganopoulos et al., 2011). Isozymes were the first genetic markers applied for sweet cherry characterization and unique genetic profiles were studied by Granger et al. (1993) and Beaver et al. (1995). Later, cultivars were also differentiated based on RAPD markers by Gerlach and Stosser (1997). Apart from isozyme and RAPD, chloroplast and nuclear markers were also developed to study genetic diversity and phylogenetic analysis on cherries (Turkec et al., 2006). Among various molecular markers, SSR are highly polymorphic and abundant in eukaryotic genomes. Therefore, microsatellite markers are the best choice which are codominant and have advantages over other molecular markers due to their robustness and reproducibility. SSR analysis provides useful information for genotyping individual plants or cultivars and exploring genetic relatedness between accessions. Microsatellite markers have been used extensively for finger-printing purposes due to their high polymorphism and reproducibility (Wunsch and Hormaza, 2002; Fajardo et al., 2013).

The most commonly used SSR primers in *Prunus* were derived from peach (Dirlewanger et al., 2002), sweet cherry (Sosinski et al., 2000; Dirlewanger et al., 2002) and sour cherry (Lacis et al., 2009). Several SSRs have been developed to determine the genetic relatedness in all *Prunus* species (Lacis et al., 2009; Antonius et al., 2012). The SSR are transferable among *Prunus* therefore same SSR primers are used for the detection of intra-species variation in related species (Dirlewanger et al., 2002; Wunsch and Hormaza, 2002). In sweet cherries, microsatellites have been used for germplasm characterization (Lacis et al., 2009), determination of genetic diversity (Dirlewanger et al., 2002; Wunsch and Hormaza, 2004), germplasm management (Wunsch and Hormaza, 2002), parentage analysis (Schueler et al., 2003), cultivar identification (Xuan et al., 2009) and mapping genetic linkage (Olmstead et al., 2008).

The objective of this study is comparison of genetic diversity value in modern cultivars, old cultivars and wild germplasm originated from Czech collection at RBIP with the aim to interpret this in connection to present time status of sweet cherry germplasm.

2. Materials and methods

2.1. Plant material and DNA extraction

Twenty two sweet cherry genotypes from breeding collection of the Research and Breeding Institute of Pomology (RBIP) Holovousy (Czech Republic) and two wild cherry genotypes collected from Prague west region were studied. All of the accessions originated from Czech Republic except 'Hedelfingenská' which is of German origin but in the past extensively grown in Czech. Genomic DNA was extracted from fresh young leaves following the protocol described by Hormaza (2002). DNA samples were diluted to concentration 1 ng/μl and stored at −20 °C (Table 1).

Table 1
Studied cultivars of Czech Republic with their pedigree.

Sl.No.	Genotype	Pedigree	Origin
1	Granát	Random seedling	Czech Republic
2	Tim	Krupnoplodnaja x Van	Czech Republic
3	Amid	Kordia x Vic	Czech Republic
4	Sandra	Kordia x Seedling 13	Czech Republic
5	Vilma	Kordia x Vic	Czech Republic
6	Fabiola	Van x Kordia	Czech Republic
7	Hedelfingenská (Hedelfinger)	Random seedling	Germany
8	Falesna Vanda	Van x Kordia	Czech Republic
9	Marta	Kordia x Kaštánka (Early Rivers)	Czech Republic
10	Chlumecká pozdní	Random seedling	Czech Republic
11	Adélka	Knauffs Schwarze x Granát	Czech Republic
12	Halka	Van x Stella	Czech Republic
13	Justyna	Kordia x Starking Hardy Giant	Czech Republic
14	Lívia	Open pollination of Těchlovická I (Ziklova)	Czech Republic
15	Elza	Kordia x Starking Hardy Giant	Czech Republic
16	Felicita	Krupnoplodnaja x Stella	Czech Republic
17	Irena	Kordia x Merton Reward	Czech Republic
18	Christiana	Van x Kordia	Czech Republic
19	Debora	Kordia x Merton Reward	Czech Republic
20	Sylvana	Kordia x Van	Czech Republic
21	Horka	Open pollination of Van	Czech Republic
22	Tamara	Krupnoplodnaja x Van	Czech Republic
23	159 (wild)	Random seedling	Czech Republic
24	161 (Wild)	Random seedling	Czech Republic