IDENTIFICATION AND GENETIC DIVERSITY ASSESSMENT OF CHERRY CULTIVARS AND ROOTSTOCKS USING THE ISSR-PCR TECHNIQUE

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ABSTRACT

The ISSR technique was used to determine the genetic similarity between 18 cultivars of sour cherry (*Prunus cerasus* L.), 24 cultivars of sweet cherry (*Prunus avium* L.) and 9 types of rootstocks for plants of these species. In reactions where 35 primers were used, 230 polymorphic DNA fragments diversifying the rootstocks were acquired, as well as 144 polymorphic fragments for the cultivars of sour cherry and 98 of sweet cherry. The highest degree of DNA polymorphism was observed in the case of the rootstocks (71.2%). For sour cherry, it was 50.7% and for sweet cherry 39.5%. It was possible to distinguish between types of rootstocks using two primers (827, 841), cultivars of sour cherry using also two primers (825, 841), whereas in order to distinguish the sweet cherry cultivars, three primers had to be used: 830, 841 and 843. Among the cultivars of sweet cherry, the highest genetic similarity was observed between 'Van' and 'Techlovan', 'Regina' and 'Karina', 'Summit' and 'Sam'. In the case of sour cherry, the most similar genetically proved to be 'Debreceni Bötormo' and 'Ujfehertoi Fürtos' as well as 'Nefris' and 'Safir'. Among the rootstocks, the least disparity demonstrated genotypes of two groups from the GiSeLa and PHL series. Obtained ISSR markers allow the identification of tested genotypes as well as their more accurate characterization. Results of the research may find application in gene banks of *Prunus* genotypes, and in orchard and nursery practice.

Key words: genomic differentiation, microsatellite markers, *Prunus cerasus* L., *Prunus avium* L., *Prunus* rootstocks

INTRODUCTION

Sour cherries and sweet cherries are fruit plants commonly grown in the temperate climatic zone. Meanwhile, the phenotypic distinction (on the basis of morphological traits) of cultivars and rootstocks used for plants of those species is not always possible. Due to those difficulties, the techniques of molecular biology are used in identification works. Molecular biology techniques help explain all the problems connected with nomenclature of cultivars (synonyms) and confusion over cultivar identity (Goulão et al., 2001; Wünsch and Hormaza, 2002a; Cai et al., 2007). These techniques are used to fully characterize genotypes in germplasm collections of sweet cherry cultivars (Lacis et al., 2009) and other species of *Prunus* (Zanetto et al., 2002).

In order to identify the *Prunus* genotypes, various techniques were used: SSR, AFLP and RAPD (Struss et al., 2003; Cai et al., 2007). However, while identifying the sweet cherries cultivars, low polymorphism of DNA was observed (Gerlach and Stosser, 1998; Struss et al., 2003). The ISSR technique (Ziętkiewicz et al., 1994) is used for the genotype identification and phylogenetic tests. This method does not require any knowledge of the genome sequence since primers are used with repeatable motif anchored at the 5' or 3' end by a short (usually 2-4 nucleotides) arbitrary sequence. The ISSR technique allows for acquiring more polymorphic DNA fragments compared to AFLP (Goulão et al., 2001). ISSR markers were useful in identifying cultivars of many species, e.g. grapevine, walnut and strawberry (Moreno et al., 1998; Potter et al., 2002; Arnau et al., 2003), as well as *Prunus* genotypes such as plum (Goulão et al., 2001; Lisek et al., 2007) and almond (Martins et al., 2003).

The aim of the research was to define ISSR markers differentiating cultivars of sour cherries, sweet cherries and rootstocks of those species, also the analysis of DNA polymorphism and the characterization of genetic similarity of tested genotypes.

MATERIAL AND METHODS

Plant material

The analyses were conducted for 18 cultivars of sour cherry (*Prunus cerasus* L.) (Tab. 1), 24 of sweet cherry (*Prunus avium* L.) (Tab. 2) and 9 types of rootstocks/interstock for plants of these species, growing in the germplasm collection in the Research Institute of Pomology and Floriculture in Skierniewice, Poland. Rootstocks tested in the examination are either hybrids or selected types of different species of *Prunus* (Tab. 3). Cultivars selected for the tests were characterized by phenotype traits such as the tree-growth strength, time of fruit ripening, and the size and hue of the fruits.

DNA extraction

The DNA was isolated according to the CTAB method (Doyle and Doyle, 1990). Samples containing 2 g of young leaves were ground in liquid

Table 1. Phenotypic traits and origins of sour cherry cultivars tested in the study

Cultivar	Tree growth	Maturity	Fruit size	Flesh colour	Origin
Agat	medium	early	medium	dark red	Schattenmorelle x Nefris, Poland
Ametyst	strong	late	large	red	Schattenmorelle x Nefris, Poland
Debreceni Bötormo	strong	intermediate	large	red	Seedling or clone of Pandy, Hungary
Ujfehertoi Fürtos	strong	intermediate	large	bright red	Seedling of Pandy, Hungary
Karneol	strong	intermediate	medium	red	Kerezer x Schattenmorelle, Germany
Kelleris 16	strong	intermediate	medium	red	Ostheimer x Fruheste der Mark x free pollination, Denmark
Korund	medium	early	medium	red	Kerezer x Schattenmorelle, Germany
Lucyna	strong	intermediate	medium	red	Schattenmorelle x Schirpotreb, Poland
Schattenmorelle	medium	very late	large	red	Clone of Schattenmorelle, England
Morina	medium	intermediate	medium	red	Kerezer x Reinhardts Ostheimer, Germany
Nana	medium	intermediate	medium	red	Crisana x Morella Neagra, Romania
Nefris	medium	intermediate	medium	dark red	Seedling of Schattenmorelle, Poland
North Star	weak	intermediate	medium	red	Schattenmorelle x Serbian Pie No. 1, USA
Sabina	strong	early	medium	red	Schattenmorelle x Schirpotreb, Poland
Safir	medium	intermediate	medium	dark red	Schattenmorelle x Fanal, Germany
Topas	strong	late	medium	dark red	Fanal x Kelleris 16, Germany
Wanda	medium	intermediate	small	dark red	Nefris x Wołyńska, Poland
Wiśnia Czarna	strong	late	large	dark red	Lack od data

Table 2. Phenotypic traits and origins of sweet cherry cultivars tested in the study

Cultivar	Tree growth	Maturity	Fruit size	Fruit colour	Origin
Burlat	very strong	early	large	red	Unknown, France
Büttners Rote Knorpelkirsche	very strong	very late	large	yellow	Unknown, Germany
Büttners Späte Rote Knorpelkir-sche	strong	intermediate	large	yellow	Unknown, Germany
Hedelfinger	medium	very late	medium	dark red	Unknown, Germany
Karesova	medium	early	large	dark red	Unknown, Czech Republic
Karina	strong	late	large	dark red	Schneiders Späte x Rube, Germany
Kordia	medium	late	large	dark red	Unknown, Czech Republic
Lapins	medium	late	large	red	Van x Stella, Canada
Merchant	medium	intermediate	medium	black	Seedling of Merton Glory, England
Merton Premier	medium	early	medium	dark Red	Emperor Francis x Bedford Prolific, England
Oktavia	medium	late	medium	black	Schneiders Späte x R 57/35, Germany
Pola	medium	late	large	black	Seedling of Schnei-ders Späte, Poland
Rainier	strong	late	large	yellow	Bing x Van, USA
Regina	medium	very late	large	dark Red	Schneiders Späte x Rube, Germany
Rivan	medium	very early	medium	black	Early Riversa x Annonay, Sweden
Sam	strong	intermediate	large	black	Seedling of V-160140, Canada
Schneiders Späte	very strong	late	very large	red	Unknown, Germany
Summit	strong	late	very large	red	Van x Sam, Canada
Sylvia	weak	late	large	dark red	Lambert Compact x Van, Canada
Techlovan	medium	intermediate	large	dark red	Van x Kordia, Czech Republic
Ulster	medium	intermediate	large	red	Schmidt x Lambert, USA
Van	medium	late	large	red	Seedling of Express Eugenie, Canada
Vanda	medium	intermediate	large	dark red	Van x Kordia, Czech Republic
Vega	strong	intermediate	large	yellow	Bing x Victor, Canada

Table 3. Origin of *Prunus* rootstocks/interstock analysed in the study

Rootstock/ interstock type	Origin
Frutana	*P. fruticosa* Pall., Poland
Colt	*P. avium* L. x *P. pseudocerasus* Lindl., England
F12/1	Seedling of *P. avium* L., England
GiSeLa 3	*P. cerasus* L. x *P. canescens*, Germany
GiSeLa 5	*P. cerasus* L. x *P. canescens*, Germany
GiSeLa 6	*P. cerasus* L. x *P. canescens*, Germany
PHL A	*P. avium* L. x *P. cerasus* L., Czech Republic
PHL B	*P. avium* L. x *P. cerasus* L., Czech Republic
PHL C	*P. avium* L. x *P. cerasus* L., Czech Republic

nitrogen and incubated for 30 minutes in 65°C extraction buffer (2% CTAB; 100 nM Tris HCl pH 8.0; 1.4 M NaCl; 20mM EDTA; 2% PVP; 0.2% β-mercaptoethanol). Polysaccharides were removed using 5 N NaCl. Nucleic acids were purified with chloroform/isoamyl alcohol (24:1) and the phenol/chloroform/isoamyl alcohol (25:24:1), and then precipitated with isopropanol and dissolved in the TE buffer. DNA concentration was determined spectrophotometrically at 260 nm. Before conducting further analyses, the DNA was diluted to the concentration of 10 ng/µl.

PCR conditions

Polymerase chain reaction (PCR) was carried out in 13 µl of reaction mixture containing 10 x PCR buffer, 2.5 mM $MgCl_2$, 0.1 mM of each nucleotide, 0.325 U of *Taq* polymerase, 0.35 µM of primer and 7 ng of template. Amplifications were performed in DNA Engine Dyad Bio-Rad thermocycler during 40 cycles (95°C/30 s, 55°C/30 s, 72°C/90 s) with the use of 35 microsatellite primers (The University of British Columbia, Canada). PCR products were separated on 1.4% agarose gels, stained with ethidium bromide and visualised in UV light.

Data analysis

For the analysis, only clear, reproducible fragments of the DNA were selected. They were defined as present (1) or absent (0). On the basis of the collected data the number of monomorphic and polymorphic fragments of the DNA was determined, as well as the degree of DNA polymorphism. A similarity matrix was defined using XLSTAT (Addinsoft 2006) based on the Jaccard coefficient. A dendrogram was constructed using the unweighted pair-group mean analysis (UPGMA) method.

RESULTS AND DISCUSSION

Defining the ISSR markers characterizing tested genotypes

Application of the molecular tests allowed for determining the diversity of plant material. In the reactions where 35 primers were used, 230 polymorphic fragments were acquired diversifying the rootstocks. Moreover, 144 fragments diversified the cultivars of sour cherry and 98 of sweet cherry. The size of polymorphic fragments varied from 400 bp to 1950 bp. In reactions with single primers, 3 to 11 polymorphic fragments of rootstocks (6.6 on average per primer), 1 to 9 fragments of sour cherry (4.1 on average) and 0 to 7 fragments of sweet cherry (2.8 on average) were acquired. The largest number of rootstock polymorphic fragments (11) was obtained using primers 822, 823 and 827. The greatest number of polymorphic fragments of sour cherry (9) was obtained with primers 822 and 825. In the case of sweet cherry, the largest number of fragments (6-7) was acquired using primers 830 and 827. Differentiation between rootstock types and sour cherry cultivars was possible with two primers (827 and 841, and 825 and 841 respectively). In the case of sweet cherry, three primers had to be used: 830, 841 and 843.

Likewise, the ISSR technique proved to be successful in distinguishing 45 cultivars of almond, using 5 primers (Martins et al., 2003). Diversification of 48 walnut cultivars required 8 primers (Potter et al., 2002). The obtained results confirm the usefulness of this technique for distinguishing *Prunus* species: cherry, sweet cherry and rootstocks for plants of those species.

Analysis of the DNA polymorphism

The highest degree of DNA polymorphism was observed in the case of rootstocks (71.2%). For sour cherry, it was 50.7% and for sweet cherry 39.5%. Considerable DNA polymorphism of rootstocks is a result of their belonging to hybrids or selected types of different *Prunus* species. A low degree of polymorphism concerning sweet cherry cultivars was also observed using the RAPD technique (Gerlach and Stosser, 1998; Lisek et al., 2006). The low degree of polymorphism in the case of *P. avium* L. may be a result of the small genome of this species (0.7 pg in comparison with *P. cerasus* L. genome – 1.24 pg; Arumuganathan and Earle, 1991). However, the polymorphism of sweet cherry DNA was higher when obtained by the ISSR technique than in the previous test when the RAPD technique was used (Lisek et al., 2006). Repetitive sequences reveal a high degree of polymorphism (Wünsch and Hormaza, 2002a), and that is the reason why their amplification may enable the acquirement of the DNA polymorphism to a still greater extent. Similarly, the degree of polymorphism of the *Vitis* and *Morus* species was higher when obtained by the ISSR technique rather than RAPD (Moreno et al., 1998; Vijayan, 2004). Favourable results for the ISSR technique prove its usefulness for identification

of cultivars which are characterized by a low level of polymorphism, as in the case of sweet cherries.

Assessment of genetic diversity

The sour cherry cultivars 'Debreceni Bötormo' and 'Ujfehertoi Fürtos', both selected as 'Pandy' seedlings or clones (Fig. 1) demonstrated a high degree of genetic similarity. Those cultivars are very similar in terms of phenotypic traits such as the tree--growth strength and time of ripening (Tab. 1). Cultivars originating from the cultivar 'Schattenmorelle' form a separate cluster. Among them, most similar are 'Safir' and 'Nefris' which are marked by average tree--growth strength and intermediate time of ripening. In this group of cultivars, genetic and phenotypic similarity are shown by the Polish cultivars 'Sabina' and 'Lucyna', obtained by crossing 'Schattenmorelle' with 'Schirpotreb'. High genetic and morphological similarity to 'Schattenmorelle', especially in appearance of the fruits was found in the cultivar 'Ametyst'. To this group also belongs the cultivar 'Wanda' which does not originate directly from 'Schattenmorelle' but from a cultivar coming from it. The cultivar 'Topas', also one of this group, genetically resembles 'Safir' and just like it, originates from 'Fanal'. Among cultivars 'Morina', 'Karneol' and 'Korund', originating from the cultivar 'Kerezer', 'Karneol' and 'Korund' are most genetically similar. Fruits of those cultivars look alike but differ in time of ripening. The least similar to the tested sour cherry cultivars was 'Wiśnia Czarna' which can also be noticed in its morphological traits. 'Wiśnia Czarna' was found in expedition in Poland and there is no data concerning its origins (G. Hodun, personal information).

Genetic similarity of sweet cherry cultivars was observed in the case of genotypes originating from the cultivar 'Van', with 'Techlovan' most resembling it (Fig. 2). Those cultivars are marked by average growth strength. 'Techlovan' has large fruits of a darker hue (Tab. 2). 'Techlovan' also shows genetic and phenotypic similarity to the cultivar 'Kordia', present in its origins. Those cultivars are characterized by average tree-growth strength and similar, dark-red fruit colour. 'Vanda' shows similar genetic disparity to cultivars it originates from – 'Van' and 'Kordia'. Morphologically, 'Vanda' resembles 'Kordia' more, which is particularly visible in the fruit colour. The cultivar 'Summit', which originated from 'Sam' and 'Van', is more genetically similar to 'Sam'. Phenotypic traits are varied here, 'Summit' and 'Sam' have similar tree-growth strength, whereas colour of fruits is closer between 'Summit' and 'Van'. Similarity between 'Summit' and 'Sam' was noted with the use of the SSR technique (Wünsch and Hormaza, 2002b). Among genotypes originating from the cultivar 'Schneiders Späte', 'Regina' and 'Karina' most resemble each other genetically which can also be seen in morphological traits – 'Karina' bears fruit late, 'Regina' very late, and the fruits are similar,

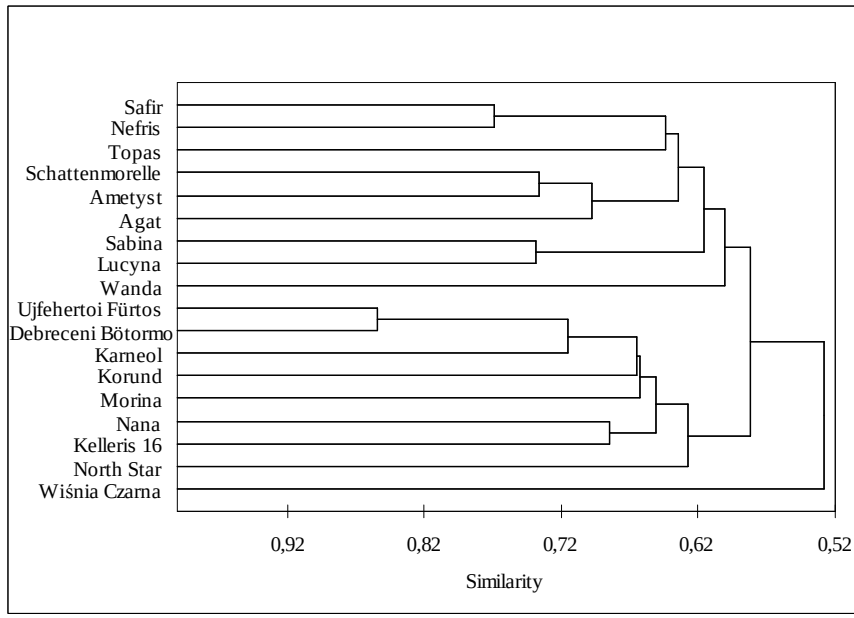

Figure 1. Dendrogram representing genetic similarity of sour cherry cultivars based on ISSR arkers

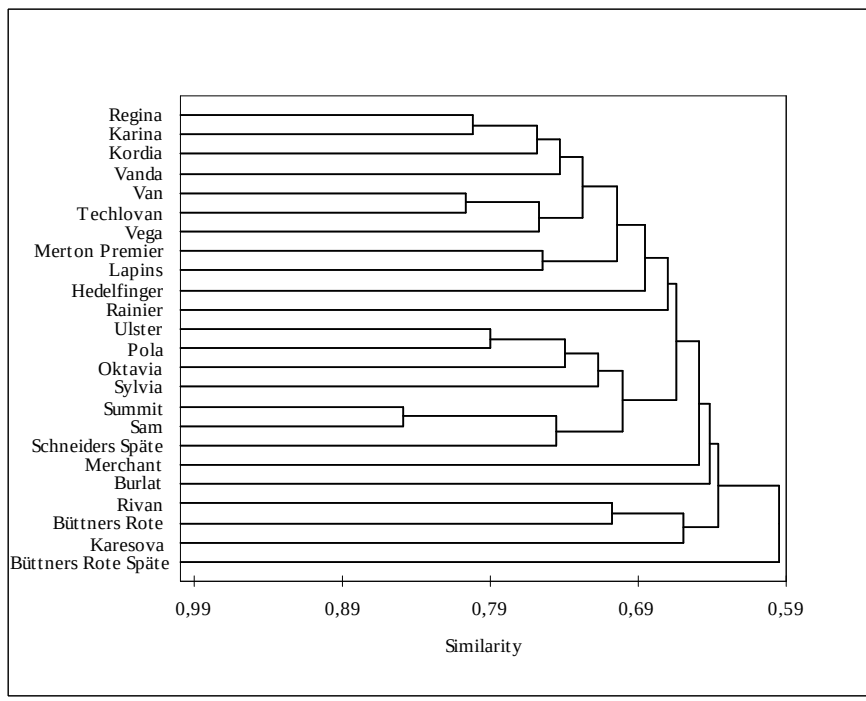

Figure 2. Dendrogram representing genetic similarity of sweet cherry cultivars based on ISSR markers

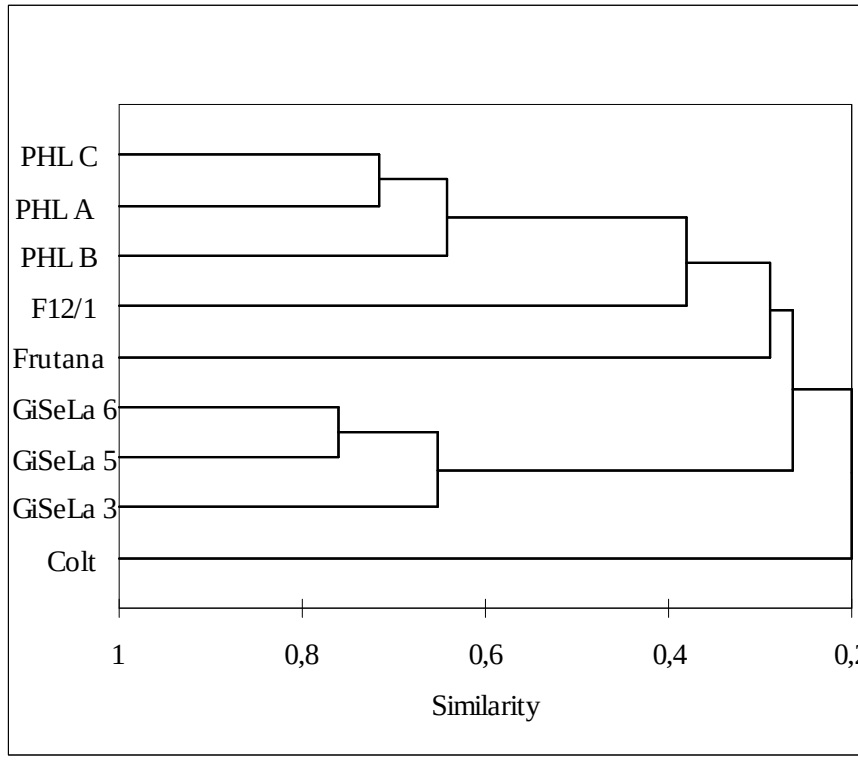

Figure 3. Dendrogram representing genetic similarity of rootstock/interstock types based on ISSR markers

large and dark-red. 'Kordia' whose origins are unknown bears a genetic and phenotypic resemblance to the cultivar 'Regina'. The similarity of those two cultivars was also observed using SSR markers (Dirlewanger et al., 2002). Great genetic similarity was noted between cultivars 'Pola' and 'Oktavia,' which are also characterized by resemblance of morphological traits such as growth strength, time of ripening as well as hue of fruits.

Most genetic disparity can be seen between the rest of the sweet cherry cultivars and 'Büttners Rote Späte', grown for some 200 years and distinctly different morphologically from other cultivars (Tab. 2). The 'Burlat' cultivar is also genetically dissimilar. Such results were also obtained using the AFLP technique (Zhou et al., 2005). 'Burlat' is a cultivar grown for some 100 years and is marked by very early ripening of fruits.

A considerably greater genetic diversity was observed among rootstock types than in the group of sweet cherry and sour cherry cultivars. The biggest differences were noted between rootstocks from the 'GiSeLa' series and the 'F12/1' rootstock as well as between 'Frutana' and both 'Colt' and 'F12/1' rootstocks (Fig. 3). This is most likely because

those genotypes belong to different species of *Prunus* (Tab. 3). The highest similarity among rootstocks was seen in genotypes within the 'GiSeLa' and 'PHL' series.

The results of the conducted research indicate the usefulness of the ISSR technique for identification of sour cherry and sweet cherry cultivars and rootstocks for the plants of these species. The possibility of distinguishing all tested sweet cherry cultivars, a species of low polymorphism levels, shows that the ISSR technique is more effective than others. The definition of genetic similarity allows for a more complete, fuller characterization of *Prunus* genotypes gathered in various germplasm collections. DNA markers may find use in ensuring the security of licenses and in fruit farming and nursery practice.

REFERENCES

Arnau G., Lallemand J., Bourgoin M. 2003. Fast and reliable strawberry cultivar identification using inter simple sequence repeat (ISSR) amplification. EUPHYTICA 129: 69-79.

Arumuganathan K., Earle E.D. 1991. Nuclear DNA content of some important plant species. PLANT MOL. BIOL. REP. 9: 208-218.

Cai Y.L., Cao D.W., Zhao G.F. 2007. Studies on genetic variation in cherry germplasm using RAPD analysis. SCI. HORT. 111: 248-254.

Dirlewanger E., Cosson P., Tavaud M., Aranzana M.J., Poizat C., Zanetto A., Arus P., Laigret F. 2002. Development of microsatellite markers in peach [*Prunus persica* (L.) Batsch] and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L). THEOR. APPL. GENET. 105: 127-138.

Doyle J.J., Doyle J.L. 1990. Isolation of plant DNA from fresh tissue. FOCUS 12: 13-15.

Gerlach H.K., Stosser R. 1998. Sweet cherry cultivar identification using RAPD-derived DNA fingerprints. ACTA HORT. 468: 63-69.

Goulão L., Monte-Corvo L., Oliveira C.M. 2001a. Phenetic characterization of plum cultivars by high multiplex ratio markers: Amplified Fragment Length Polymorphisms and Inter-simple Sequence Repeats. J. AMER. SOC. HORT. SCI. 126(1): 72-77.

Lacis G., Rashal I., Ruisa S., Trajkovski V., Iezzoni A.F. 2009. Assessment of genetic diversity of Latvian and Swedish sweet cherry (*Prunus avium* L.) genetic resources collections by using SSR (microsatellite) markers. SCI. HORT. 121: 451-457.

Lisek A., Korbin M., Rozpara E. 2006. Using simply generated RAPD markers to distinguish between sweet cherry (*Prunus avium* L.) cultivars. J. FRUIT ORNAM. PLANT RES. 14: 45-52.

Lisek A., Korbin M., Rozpara E., Żurawicz E. 2007. Plum cultivar DNA polymorphism generated with RAPD and ISSR markers. ACTA HORT. 734: 281-285.

Martins M., Tenreiro R., Oliveira M.M. 2003. Genetic relatedness of Portuguese almond cultivars assessed by RAPD and ISSR markers. PLANT CELL REP. 22: 71-78.

Moreno S., Martin J.P., Ortiz J.M. 1998. Inter-simple sequence repeats PCR for characterization of closely related grapevine germplasm. EUPHYTICA 101: 117-125.

Potter D., Gao F., Aiello G., Leslie C., McGranahan G. 2002. Inter simple

sequence repeat markers for fingerprinting and determining genetic relationships of walnut (*Juglans regia*) cultivars. J. AMER. SOC. HORT. SCI. 127: 75-81.

Struss D., Ahmad R., Southwick S.M. 2003. Analysis of sweet cherry (*Prunus avium* L.) cultivars using SSR and AFLP markers. J. AMER. SOC. HORT. SCI. 128: 904-909.

Vijayan K. 2004. Genetic relationship of Japanese and Indian mulberry (*Morus* spp.) genotypes revealed by DNA fingerprinting. PLANT SYST. EVOL. 243: 221-232.

Wünsch A., Hormaza J.I. 2002a. Cultivar identification and genetic fingerprinting of temperate fruit tree species using DNA markers. EUPHYTICA 125: 59-67.

Wünsch A., Hormaza J.I. 2002b. Molecular characterisation of sweet cherry (*Prunus avium* L.) genotypes using peach [*Prunus persica* (L.) Batsch] SSR sequences. HEREDITY 89: 56-63.

Zanetto A., Maggioni L., Tobutt K.R., Dosba F. 2002. *Prunus* genetic resources in Europe: Achievement and perspectives of a networking activity. GENET. RESOUR CROP. EVOL. 49: 331-337.

Zhou L., Kappel F., Wiersma P.A., Hampson C., Bakkeren G. 2005. Genetic analysis and DNA fingerprinting of sweet cherry cultivars and selections using amplified fragment length polymorphisms (AFLP). ACTA HORT. 667: 37-44.

Ziętkiewicz E., Rafalski A., Labuda D. 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. GENOMICS 20: 176-183.

IDENTYFIKACJA I OCENA ZRÓŻNICOWANIA GENETYCZNEGO ODMIAN ORAZ PODKŁADEK CZEREŚNI I WIŚNI Z UŻYCIEM TECHNIKI ISSR-PCR

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S T R E S Z C Z E N I E

Technikę ISSR zastosowano do identyfikacji oraz określenia podobieństwa genetycznego 18 odmian wiśni (*Prunus cerasus* L.), 24 odmian czereśni (*Prunus avium* L.) oraz 9 typów podkładek dla roślin tych gatunków. W reakcjach PCR z użyciem 35 starterów uzyskano 230 polimorficznych fragmentów DNA różnicujących podkładki oraz odpowiednio 144 i 98 fragmentów polimorficznych dla odmian wiśni i czereśni. Największy stopień polimorfizmu DNA z użyciem obu technik obserwowano dla podkładek (71,2%), mniejszy u wiśni (50,7%), a najmniejszy u czereśni (39,5%). Odróżnienie typów podkładek oraz odmian wiśni było możliwe stosując dwa startery (827, 841 i odpowiednio 825, 841), natomiast do odróżnienia odmian czereśni ko-

nieczne było użycie trzech starterów: 830, 841 i 843. Wśród odmian czereśni największe genetyczne podobieństwo obserwowano między odmianami ‘Van’ i ‘Techlovan’, ‘Regina’ i ‘Karina’ oraz ‘Summit’ i ‘Sam’. Największym podobieństwem genetycznym odmian wiśni wykazały się odmiany ‘Debreceni Bötormo’ i ‘Groniasta z Ujfehertoi’ oraz ‘Nefris i Safir’. Wśród podkładek najmniejszym dystansem charakteryzowały się genotypy w obrębie dwóch grup: z serii GiSeLa oraz z serii PHL. Uzyskane markery ISSR umożliwiają identyfikację testowanych genotypów oraz pełniejszą ich charakterystykę. Wyniki badań mogą znaleźć zastosowanie w kolekcjach gromadzących genotypy *Prunus* oraz w praktyce sadowniczej i szkółkarskiej.

Słowa kluczowe: różnicowanie genomów, markery mikrosatelitarne, *Prunus cerasus* L., *Prunus avium* L., podkładki *Prunus*