USING SIMPLY GENERATED RAPD MARKERS TO DISTINGUISH BETWEEN SWEET CHERRY (*Prunus avium* L.) CULTIVARS

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(Received July 15, 2005/Accepted October 30, 2005)

A B S T R A C T

RAPD analysis was performed on nineteen sweet cherry cultivars. In all, 55 primers were evaluated from the OPB, OPG, OPT and OPU kits manufactured by Operon Technologies, Inc. DNA amplification were observed in reactions with 47 of the primers, meanwhile polymorphic band patterns characteristic for the cultivars analyzed were produced with 26 of them. Thirty-two polymorphic fragments were amplified, which represents 14.6 percent of the total band number. The length of these fragments ranged from about 500 to 1370 bp. Most of these fragments were amplified with primers from the OPB kit. It was possible to distinguish all nineteen cultivars from each other by using six primers which together generated seventeen polymorphic fragments. RAPD is therefore a reliable procedure for distinguishing among sweet cherry cultivars commonly cultivated in Poland.

Key words: sweet cherry, polymorphism, molecular markers, RAPD

INTRODUCTION

The release of valuable new cultivars over the past few years has piqued interest in sweet cherry cultivation among Polish farmers. In this situation proper identification of cherry cultivars is needed to ensure that the product is sufficiently genetically pure to meet the expectations of growers and distributors. Precise cultivar identification is also important in protecting the legal rights of breeders.

Until recently, cultivar identification has relied primarily on comparing phenotypic traits. However, it is often difficult or even impossible to

distinguish between many cultivars because they are so morphologically similar. To complicate matters, morphological changes frequently occur within a single cultivar depending on environmental factors. For this reason, molecular marker techniques have been developed to ensure accurate identification. Even cultivars which are phenotypically extremely similar can be easily distinguished based on differences in their genomes. Molecular identification techniques can be used at any stage of plant development. Furthermore, they are not affected by environmental factors.

PCR techniques have been successfully used to analyze DNA polymorphism in sweet cherry (*Prunus avium* L.) cultivars and rootstocks (Boritzki et al., 2000; Gerlach and Stosser, 1998; Struss et al., 2002; Zhou et al., 2002; Struss et al., 2003; Wunsch et al., 2004; Zhou et al., 2005). Among them, RAPD is a simple and relatively inexpensive PCR based technique which has been used to identify cultivars in other *Prunus* species, such as the peach, the plum and the almond (Warburton and Bliss, 1996; Zhen-Xiang et al., 1996; Ortiz et al., 1997; Bartolozzi et al., 1998; Bellini et al., 1998; Dirlewanger et al., 1998; Shimada et al., 1999).

The aim of this study was to elaborate a simple and reliable procedure for distinguishing among the sweet cherry cultivars commonly cultivated in Poland.

MATERIAL AND METHODS

Plants and genetic material isolation

RAPD analysis was performed on sweet cherries trees growing in the field collection of the Research Institute of Pomology and Floriculture in Skierniewice. The nineteen cultivars used in this study are listed in Table 1.

Genetic material (DNA) was isolated from shoot tips with young leaves using a CTAB-based method (Doyle and Doyle, 1990). Samples weighing 2 grams were mortared in liquid nitrogen and incubated for 30 minutes at 65°C in extraction buffer (2% CTAB; 100 mM Tris HCl pH 8.0; 1.4 M NaCl; 20 mM EDTA; 2% PVP; 0.2% mercaptoethanol). Polysaccharides were removed with 5 N NaCl. Nucleic acids were purified with chloroform/isoamyl alcohol (24:1) and phenol/chloroform/isoamyl alcohol (25:24:1), and then precipitated with isopropanol and dissolved in TE buffer. DNA concentration was measured spectrophotometrically at 260 nm.

PCR conditions

PCR was performed in a volume of 13 µl. The reaction mixture contained 10 x PCR buffer; 2.5 mM $MgCl_2$; 0.1 mM of each nucleotide; 0.325 U *Taq* polymerase; 0.35 µM primer; and 13 ng template DNA. Amplification was performed in an MJ Research Thermocycler (40 cycles: 95°C/30 s, 40°C/45 s, 72°C/90 s). In all, 55 primers were evaluated from the OPB, OPG, OPT and OPU kits manufactured by Operon Technologies, Inc.

PCR products were separated on 1.2% agarose gel, stained with ethidium bromide, and visualized under UV light. Only distinct, reproducible and informative bands were selected for further analysis.

RESULTS AND DISCUSSION

DNA amplification was observed in reaction with 47 out of the 55 primers used. Polymorphic band patterns characteristic for the cultivars analyzed were produced with 26 of these primers. In all, thirty-two polymorphic markers were amplified, which represents 14.6 percent of the total band number. The size of these markers ranged from about 500 to 1370 bp. Most of these markers were amplified with primers from the OPB series. OPB-11 generated four bands, and OPB-06, OPB-12 and OPG-04 each generated three (Tab. 1).

It was possible to distinguish all nineteen cultivars from each other by using six primers which together generated seventeen polymorphic markers (Tab. 2). The use of primer OPB-11 allowed to distinguish cultivars ‘Bing’, ‘Hedelfińska’ and ‘Schneidera Późna’, primer OPB-12 cultivars ’Kareszova’ and ‘Vega’, primer OPB-06 cultivar ‘Pola’. Cultivars ‘Bladoróżowa’, ‘Burlat’, ‘Merton Premier’, ‘Rivan’, and ‘Regina’ were distinguished in reaction with primers OPB-11 and OPB-12, meanwhile ‘Buttera Czerwona’ and ‘Sam’ with primers OPB-06 and OPG-04. Cultivar ‘Buttera Czerwona’ showed specific DNA pattern in RAPD with primers OPB-06 and OPB-12. Distinguishing other cultivars was possible in reaction with the following set of primers: OPB-10,11 and 12 for ‘Kordia’ and ‘Vanda’, OPB 10, 12 and OPG-04 for ‘Summit’ and ‘Star’, OPB-11, OPG-04 and OPT-13 for ‘Van’ (Tab. 2).

Our results agree well with earlier studies using RAPD with *Prunus* species. For example, in one study, 31 plum cultivars could be distinguished from each other by using only three primers (Ortiz et al., 1997). In another study, 18 peach cultivars could be distinguished from each other by using only six primers (Zhen-Xiang et al., 1996). However, some other researchers had difficulty distinguishing closely related sweet cherry genotypes by using RAPD, SSR or AFLP because of the low degree of polymorphism. For example, in one study, fourteen out of eighteen sweet cherry cultivars could be distinguished from each other using 23 selected RAPD primers which together generated 56 polymorphic markers. Of the varieties that could not be distinguished from each other, one was a late ripening cultivar, and two probably were genetically identical to each other (Gerlach and Stosser 1998). In another study, some closely related sweet cherry cultivars and mutants could not be distinguished by several sets of AFLP primers (Zhou et al., 2005). The size of the sweet cherry genome is only 0.7 pg. This may be another reason why the degree of DNA polymorphism in the sweet cherry is so low (Arumuganathan and Earle, 1991; Gerlach and Stosser, 1998).

T a b l e 1 . DNA polymorphism of sweet cherry cultivars in RAPD reactions (1-presence, 0-lack of polymorphic products)

Cultivar	Length of polymorphic fragment (bp)																
	primer OPB 06			primer OPB 10		primer OPB 11				primer OPB 12			primer OPG 04			primer OPT 13	
	1340	1300	1240	950	910	820	790	680	<564	1020	910	740	1250	1100	860	710	690
Bing	1	0	0	0	0	1	0	0	0	1	1	0	0	1	1	1	0
Bladoróżowa	1	0	0	1	1	1	0	1	1	0	1	1	0	0	0	0	0
Burlat	1	0	0	1	0	1	0	1	0	0	1	1	1	1	1	0	0
Büttnera Czerwona	0	0	1	1	0	1	0	1	1	0	1	0	0	1	1	0	0
Büttnera Czerwona Późna	0	0	0	1	1	1	0	1	1	0	1	0	0	1	0	1	0
Hedelfińska	1	0	0	1	0	1	1	1	1	1	1	0	0	1	1	0	0
Kareszova	0	0	1	1	0	1	0	1	1	0	0	1	0	1	1	0	0
Kordia	1	0	0	1	0	1	0	1	1	1	1	0	0	1	1	0	0
Merton Premier	1	0	1	1	0	1	0	1	0	0	0	0	0	1	1	0	0
Pola	1	1	0	1	0	1	0	1	1	0	1	0	1	1	1	1	0
Regina	1	0	0	1	0	1	0	1	1	0	0	0	0	1	1	0	0
Rivan	1	0	0	1	1	1	0	1	0	1	1	0	0	1	1	0	1
Sam	0	0	0	1	0	1	0	1	1	0	1	0	0	1	1	0	0
Schneidera Późna	1	0	1	1	0	0	0	1	1	0	1	0	0	1	1	0	0
Star	1	0	0	0	0	1	0	1	1	0	1	0	1	1	1	1	0
Summit	1	0	0	0	0	1	0	1	1	0	1	0	0	1	1	0	0
Van	1	0	0	1	0	1	0	1	1	0	1	0	0	1	1	1	0
Vanda	1	0	0	0	0	1	0	1	1	1	1	0	0	1	0	0	0
Vega	1	0	0	1	0	1	0	1	1	1	1	1	1	1	1	1	0

T a b l e 2. Primers selected for differentiation of analysed 19 sweet cherry cultivars X – the presence of specific, polymorphic DNA fragment generated in RAPD-reaction with described primer

Cultivar	Primer					
	OPB 06	OPB 10	OPB 11	OPB 12	OPG 04	OPT 13
Bing			X			
Bladorożowa			X	X		
Burlat			X	X		
Büttnera Czerwona	X			X		
Büttnera Czerwona Późna	X				X	
Hedelfińska			X			
Kareszova				X		
Kordia		X	X	X		
Merton Premier			X	X		
Pola	X					
Regina			X	X		
Rivan			X	X		
Sam	X				X	
Schneidera Późna			X			
Star		X		X	X	
Summit		X		X	X	
Van			X		X	X
Vanda		X	X	X		
Vega				X		

In our study, the degree of polymorphism calculated on the basis of RAPD analysis was 14.6%. This agrees well with earlier studies, in which the degree of polymorphism was found to be 19% using AFLP, and between 17 and 21% using SSR (Zhou et al., 2002; Boritzki et al., 2000; Struss et al., 2003). However, even this low degree of polymorphism was enough to distinguish between all of the nineteen cultivars we examined.

RAPD is therefore a reliable procedure for distinguishing among the sweet cherry cultivars commonly cultivated in Poland. The data collected will be useful in developing DNA fingerprinting techniques for routine use in the orchard.

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ODRÓŻNIANIE ODMIAN CZEREŚNI (*Prunus avium* L.) Z UŻYCIEM MARKERÓW RAPD

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STRESZCZENIE

Analizowano polimorfizm DNA 19 odmian czereśni *Prunus avium* L. przy zastosowaniu markerów RAPD. Przetestowano 55 starterów z firmy Operon Technologies z grup OPB, OPG, OPT i OPU. Amplifikację DNA przeprowadzono w temocyklerze MJ Research (40 cykli: 95°C/30 s, 40°C/45 s, 72°C/90 s). DNA amplifikowało w reakcji 47 spośród 55 starterów. Polimorfizm DNA obserwowano w RAPD z 26 starterami. Łącznie uzyskano 32 polimorficzne fragmenty DNA (14,6 % uzyskanych fragmentów) o długości od 1370 pz do około 500 pz. Większość polimorficznych produktów uzyskano z użyciem starterów OPB-06, OPB-12 i OPG-04. Odróżnienie wszystkich badanych odmian było możliwe przy zastosowaniu 6 starterów, generujących 17 fragmentów polimorficznych: OPB-06, OPB-10, OPB-11, OPB-12, OPG-04 i OPT-13. Uzyskane wyniki potwierdzają przydatność markerów RAPD do odróżniania odmian czereśni i znajdą zastosowanie przy opracowaniu wzorów prążkowych (fingerprinting) dla poszczególnych odmian.

Słowa kluczowe: czereśnia, polimorfizm DNA, markery molekularne, RAPD