IDENTIFICATION OF PEAR CULTIVARS WITH RAPD AND ISSR MARKERS

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A B S T R A C T

RAPD and ISSR techniques were used in identification of 26 pear cultivars. As a result of reactions carried out with RAPD 25 primers, 103 polymorphic DNA fragments were obtained. The largest number of polymorphic DNA fragments (7-8) was produced in reactions with the following primers: OPT 15, OPG 16 and OPG 19. Identification of cultivars and rootstocks was achievable with the use of OPT 15, OPG 19 and OPU 07 primers. In the reactions performed with 22 ISSR primers, 135 markers of pear cultivars were obtained. The size of fragments varied from 280 to 1790 bp. The greatest number of polymorphic products (9) was obtained in the reactions with primers: 830, 840 and 844. Primers 840 and 844 enabled identification of all tested genotypes. Degree of DNA polymorphism was estimated at 56.3% (RAPD) and 71.5% (ISSR). Results of the research confirm the usefulness of both techniques in identifying pear cultivars.

Key words: *Pyrus communis* L., molecular markers, DNA polymorphism

INTRODUCTION

Pear is widely cultivated in temperate climates and its fruit is higly valued by consumers on account of its flavour. The production of fruit requires the ability to distinguish one cultivar from the other in nurseries and orchards. Assessment performed on the basis of morphological traits may prove misleading due to substantial similarity in appearance of trees and fruits. New cultivars are constantly being introduced, which may cause further difficulties in their identification. The possibility of erroneous determination of the cultivar's name made it necessary to use

molecular techniques allowing precise identification of genotypes. Isoenzymatic markers were used to identify the *Pyrus communis* pear (Manganaris and Tsipouridis, 2002). Techniques based on amplification of DNA in the PCR reactions were also used to identify the *Pyrus communis* pear. Such techniques include: RAPD (Sharifani and Jackson, 2000; Kim et al., 2005; Monte-Corvo et al., 2000, 2002), SSR (Wünsch and Hormaza, 2007; Yamamoto et al., 2002; Brini et al., 2008), AFLP (Monte-Corvo et al., 2002) and ISSR (Monte-Corvo et al., 2001, 2002).

The advantage of RAPD and ISSR techniques is their versatility and lack of necessity of having to know the sequence of a genome. Moreover, the ISSR technique, based on the analysis of repetitive fragments of genome, finds application in identification of genotypes with high genetic similarity or presenting low degree of DNA polymorphism (Zietkiewicz et al., 1994).

The aim of the work was to identify pear cultivars using RAPD and ISSR techniques.

MATERIAL AND METHODS

Plant material

Tests were conducted for 26 *Pyrus communis* L. cultivars grown in Poland (Tab. 1). Cultivars which may be introduced into production in the future because of their appeal to both producers and consumers were also included. For analysis, 1 g of young leaves from apical parts of shoots was taken from the trees planted in the collection at the Institute of Pomology and Floriculture in Skierniewice, Poland.

DNA extraction

DNA was isolated by method of Doyle and Doyle (1990). Concentration of DNA was determined spectrophotometrically at the wavelength of 260 nm. For further analyses, DNA dilutions of 10 ng/µl were prepared.

PCR conditions

Reaction was conducted in 13 µl of mixture containing 1 x PCR buffer, 2.5 mM $MgCl_2$, 0.1 mM of each nucleotide, 0.325 U of Taq polymerase (Fermentas), 0.35µM of primer and 10 ng of template. Amplification of DNA was performed in termocycler Engine Dyad Bio-Rad with 40 RAPD cycles (95°C/30s, 40°C/30s, 72°C/90s) and 42 ISSR cycles (95°C/30s, 55°C/30s, 72°C/90s). We used 25 RAPD primers (Operon Technologies Inc.) from the OPB, OPG, OPT and OPU series as well as 22 microsatellite primers (The University of British Columbia, Canada). DNA fragments were separated in 1.4% agarose, stained with ethidium bromide, and visualised under ultraviolet light.

Data analysis

For the analysis, only clear, repetitive DNA fragments were selected. Size and presence or absence in the profiles of tested cultivars were then determined. Number of mono- and polymorphic DNA fragments was defined on the basis of electrophorograms, just like the degree of the DNA polymorphism.

Table 1. DNA polymorphic fragments of pear cultivars obtained with RAPD and ISSR technique

Cultivar	Size of polymorphic DNA fragments				
	RAPD			ISSR	
	Primer OPT 15	Primer OPG19	Primer OPU 07	Primer 840	Primer 844
Beurre Bosc	1050, 490, 470	990, 720	890, 640	1160, 1030, 900, 670, 280	870, 530
Beurre Hardy	890, 470	720	890, 640	1160, 1030, 900, 280	1150, 870, 530
Bojniczanka	490	1150, 990, 800, 720	890, 760, 640	1160, 370	1150, 870, 540, 430
Carola	490, 470	1150, 990	890, 760, 640	1160, 1070, 370, 280	1290, 580, 530
Clapp's Favourite	800, 490	1760, 990, 800, 720	890, 760, 640	1070, 670, 370, 280	1150, 390
Comice	490	720	890, 640	1160, 900, 280	1150, 870, 580, 430
Concorde	890, 660, 490, 470	1150, 770	890, 760, 720	1310, 280	580, 540, 430
Condo	1050, 890, 660, 490, 470	770	890, 720, 640	1310, 1160, 280	830, 580, 540, 430
Conférence	1050, 890, 490	770	890, 760	1310, 1160, 280	830, 580, 430
Cure	1050, 770, 490, 470	1760, 1150, 990, 800	-	1310, 1160, 1000, 900, 670, 280	1290, 870, 540, 390
David	490, 470	1760	890, 760, 720	1160, 1030, 670, 370, 280	1150, 870, 430, 390
Dolores	1050, 890, 490	720	-	1310, 1160, 1070, 280	1150, 830, 580
General Leclerc	1050, 890, 490, 470	990, 720	890, 720	1310, 1160, 1070, 900, 280	540, 430
Hortensja	490	1760, 990, 770	890, 760, 640	1030, 670, 370, 280	1150, 430, 390
Hnidzik	1050, 490	1760, 800, 720	890, 640	1070, 1000, 370, 280	1150, 870, 830, 390
Isolda	490	1760	890, 640	900, 670	1150, 870, 430, 390
Lipcówka Kolorowa	490	1760, 700	890, 640	1160, 1070, 900, 280	1150, 870, 430, 390
Lukasówka	1050, 490	1760, 1150, 720	890, 760, 720, 640	1310, 1160, 900, 280	1150, 870, 390
Nojabrskaja	890, 470	990, 720	890, 760	1160, 370, 280	870
Packham's Triumf	470	1760, 1150, 990	890, 760	1160, 1070, 280	1150, 870, 580, 390
Patten	840, 770, 490	800, 720	890, 640	1070, 1000, 820, 280	1150, 580
Radana	890, 800, 490	1760, 990, 800, 700	890, 760, 640	1160, 1070, 1030, 900, 670, 280	1150, 390
Salisbury	1050, 890, 800, 470	-	760	1030, 370, 280	1150, 870
Trewinka	490	720	640	1160, 1030, 1000, 900, 280	1150, 870, 580
Verdi	660, 490, 470	1150	890	1160, 1030, 1000, 280	1150, 870, 540
William's	490	1760, 990, 800, 720	890, 760, 640	1160, 670, 370	1150, 870, 580, 430, 390

RESULTS AND DISCUSSION

As a result of reactions carried out with 25 RAPD primers, 103 polymorphic DNA fragments were obtained, their size varying from 390 to 1990 bp. The largest number of polymorphic fragments was produced in reactions with primers OPT 15, OPG 16 and OPG 19, while identification of cultivars was possible using primers OPT 15, OPG 19 and OPU 07 (Tab. 1). Cultivars 'Beurre Bosc', 'Concorde', 'Condo', 'Cure', 'Clapp's Favourite', 'General Leclerc', 'Patten', 'Radana', 'Verdi' and 'Salisbury' were identified with the use of primer OPT 15. Differentiating cultivars 'Bojniczanka', 'Carola', 'Hortensja', 'Hnidzik', 'Lukasówka', 'Packham's Triumf' and 'Lipcówka Kolorowa' was achieved in reactions with primer OPG 19. Primers OPG 19 and OPU 07 were necessary to identify cultivars 'Trewinka', primers OPT 15, OPG 19 and OPU 07 identified cultivar 'Comice', while OPT 15 and OPG 19 primers enabled identification of the cultivars 'Beurre Hardy', 'Dolores', 'William's', 'David', 'Conference', 'Nojabrskaja' and 'Isolda'.

Similarly, the RAPD technique, with the use of 25 primers, was successful in the identification of 25 pear cultivars, where – depending on the primer used – 8 to 17 total bands were obtained, their size varying from 400 to 1500 bp (Monte-Corvo et al., 2000). Nine primers, generating from 5 to 11 total bands, enabled identification of 16 genotypes belonging to different species of *Pyrus*, including 4 cultivars *Pyrus communis* L. (Sharifani and Jackson, 2000).

In this work, using the RAPD technique, degree of DNA polymorphism of pear cultivars came to 56.3%. Monte-Corvo et al. (2000, 2002) observed higher degree of DNA polymorphism (73.8%-84%,), but, at the same time, noticed substantial differences in the degree of polymorphism depending on the primer used (40%-100%). DNA polymorphism within the range of 14% to 42.8% was obtained by Sharifani and Jackson (2000), and it also depended on RAPD primers. Results show that RAPD primers should undergo precise selection if a higher degree of polymorphism is to be produced.

In reactions with 22 ISSR primers, 135 polymorphic fragments of pear cultivars were produced, sized from 280 bp to 1790 bp. The greatest number (9) of polymorphic fragments was produced in reactions with primers 830, 840 and 844; the degree of polymorphism came to 71.5%. Primers 840 and 844 enabled identification of all tested genotypes. Cultivars 'Beurre Hardy', 'Beurre Bosc', 'Bojniczanka', 'William's', 'Carola', 'Concorde', 'Condo', 'Cure', 'Dolores', 'General Leclerc', 'Hortensja', 'Hnidzik', 'Comice', 'Conference', 'Lukasówka', 'Nojabrskaja', 'Patten', 'Trewinka', 'Packham's Triumf', 'Verdi' and 'Salisbury' were identified in reactions with primer 844, while primers 840 and 844 were necessary to

identify cultivars 'Clapp's Favourite', 'Radana', 'David', 'Isolda' and 'Lipcówka Kolorowa'.

The ISSR technique was successful in identifying 24 pear cultivars, using each of the 8 tested primers (Monte-Corvo et al., 2001) and degree of DNA polymorphism (79.5%) achieved by these authors was similar to the one presented in this work (71.5%).

Results of the research show that ISSR and RAPD techniques proved useful for identification of pear cultivars. Both techniques are not very complicated, and their costs are also similar. Developed RAPD and ISSR markers may be applied for identification of pear cultivars in nurseries and orchards, as well as in germplasm collections of this species.

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ZASTOSOWANIE MARKERÓW RAPD I ISSR DO IDENTYFIKACJI ODMIAN GRUSZY

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

Grusza jest gatunkiem powszechnie uprawianym w klimacie umiarkowanym, a owoce jej są cenione przez konsumentów ze względu na walory smakowe. Produkcja owoców gruszy wymaga umiejętności odróżniania odmian w szkółkach i w sadach. Ocena dokonywana na podstawie cech morfologicznych może być bardzo trudna z powodu dużego podobieństwa w wyglądzie drzew i owoców, dlatego do precyzyjnej identyfikacji genotypów stosuje się techniki biologii molekularnej. Celem pracy było odróżnienie 26 odmian gruszy z użyciem technik RAPD i ISSR przez określenie markerów DNA identyfikujących testowane odmiany. W wyniku przeprowadzonych reakcji z 25 starterami RAPD otrzymano 103 polimorficzne fragmenty DNA. Najwięcej fragmentów polimorficznych (7-8) otrzymano w reakcjach ze starterami OPT 15, OPG 16 i OPG 19. Odróżnienie odmian i podkładek było możliwe przy użyciu starterów OPT 15, OPG 19, OPU 07. W reakcjach z 22 starterami ISSR uzyskano 135 markerów odmian gruszy. Wielkość fragmentów wahała się od 280 pz do 1790 pz. Najwięcej polimorficznych produktów (9) uzyskano w reakcjach ze starterami 830, 840 i 844. Użycie starterów 840 i 844 umożliwiło odróżnienie wszystkich testowanych genotypów. Stopień polimorfizmu DNA określono na poziomie 56,3% (RAPD) i 71,5% (ISSR). Wyniki badań potwierdzają przydatność obu technik do rozróżniania odmian gruszy.

Słowa kluczowe: *Pyrus communis* L., markery molekularne, polimorfizm DNA