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SHORT COMMUNICATION

Genetic diversity of European pear cultivars (*Pyrus communis* L.) and wild pear (*Pyrus pyraster* (L.) Burgsd.) inferred from microsatellite markers analysis

Łukasz Wolko · Wojciech Antkowiak · Elżbieta Lenartowicz · Jan Bocianowski

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Abstract The aim of this study was to identify the group of highly polymorphic microsatellite markers for the identification of six pear cultivars (*P. communis*) and two individuals of wild pear (*P. pyraster*). From among 40 tested SSR markers, 19 were selected to profile genetic diversity in pear genotypes due to high polymorphisms. These markers showed high heterozygosity levels (0.5–1) and, on average, 6.4 alleles per marker were found. The set of microsatellite markers employed in this study demonstrated usefulness of microsatellite markers for the identification of pear genotypes. The examined wild forms were represented in this study by only two individuals of *P. pyraster*. It can be assumed that these forms were distinctly different from the cultivated pear cultivars.

Keywords Microsatellite · *Pyrus communis* · *Pyrus pyraster* · SSR markers

Ł. Wolko (✉) · E. Lenartowicz
Department of Biochemistry and Biotechnology, Poznań University of Life Sciences, Wołyńska 35, 60-637 Poznań, Poland
e-mail: wolko@o2.pl

W. Antkowiak
Department of Botany, Poznań University of Life Sciences, Wojska Polskiego 71c, 60-625 Poznań, Poland

J. Bocianowski
Department of Mathematical and Statistical Methods, Poznań University of Life Sciences, Wojska Polskiego 28, 60-637 Poznań, Poland

Introduction

SSRs (simple sequence repeats, also designated as microsatellites) have become genetic markers of choice in many plant species due to their abundance, high degree of polymorphism and suitability for automation (Weber and May 1989). SSR markers have several advantages over other molecular markers which ensure a more reliable method for DNA fingerprinting. Among others, they exhibit codominant type of inheritance, a large number of alleles per locus and are abundant in genomes. Due to the fact that the use of SSRs is based on the polymerase chain reaction (PCR) method, the technique is simple and only a small amount of DNA is required.

The limited number of SSR primer pairs from European pear have been reported to date as most work in this field has focused on Japanese pear (Bao et al. 2007; Fernandez-Fernandez et al. 2006; Yamamoto et al. 2001; Yamamoto et al. 2002a, b, c). Although approximately 75% of the SSRs developed in *P. pyrifolia* are polymorphic in European pear, there is a need for more codominant markers for *Pyrus* (Yamamoto et al. 2002a, b, c).

The pear (*Pyrus* spp.) is one of the most important fruit trees, having been cultivated in Europe and Asia for at least 2–3 thousand years and is presently commercially grown in all temperate regions encompassing more than 50 countries of the world. Only one pear species occurs in Poland naturally, namely *Pyrus pyraster* (L.) Burgsd. (= *Pyrus communis* ssp. *pyraster*

L.)–a pear which grows in wild conditions and is widely known as common or field pear. *P. pyraster* species comes from areas of western Black Sea regions, but distribution of this species extend from the British Isles to Latvia (Browicz 1982, Meusel et al. 1965, Paganova 1996, 2001, 2003a, b, c, 2009, Stephan et al. 2003, Terpo and Franco 1968).

The term *Pyrus communis* L. (= *P. domestica* Med.) is generally used to refer to pear trees grown in orchards. Apart from them, however, a hybrid *Pyrus* × *amphigenea* Domin ex Dostálek also occurs which develops as a result of crossing of wild pears with cultivated varieties. In addition, a limited number of representatives of foreign origin can be found, although these are planted primarily in parks and gardens (Dostalek 1989).

It is quite difficult to give an accurate number of pear species in the world because they cross easily with one another and the obtained crosses are allocated various taxonomic positions. Terpo (1985), for example, mentions 52 pear species, while Browicz (1993) gives a list of 38 species and, additionally, 33 examples of interspecific *Pyrus* hybrids as well as 4 examples of intergeneric crosses of *Pyrus* genus with *Sorbus, Cydonia* and *Malus*. Bell (1986) mentions seven other instances of interspecific *Pyrus* hybrids. Kutzelnigg and Silbereisen (1995) maintain that the genus comprises from 20 to 74 species, depending on the type of the adopted approach, all indigenous to Europe, Asia, and the mountainous regions of North Africa.

Pear genetic resources have not been fully identified due to its low morphological diversity, lack of differentiating characters among species and widespread crossability. Therefore, estimation of genetic diversity among *Pyrus* sp. is often very difficult.

Materials and methods

Six pear cultivars (*P. communis*) were used in this study ('Conference', 'Red Williams', 'Amfora', 'Dicolor', 'Radana', 'Carola'). Every cultivar were represented by two individual trees. All the above cultivars were obtained from the Agro-Horticultural Experimental Farm in Przybroda. Leaves of two wild pear (*P. pyraster*) specimens were collected in the region of Wierzonka (near Poznan). Genomic DNA was extracted from 2 g fresh leaf material following a modified cetyltrimethyl ammonium bromide (CTAB) protocol (Torres et al. 1993). 40 SSR primer pairs were obtained from Fernandez-Fernandez et al. (2006) and Yamamoto et al. (2002a, b). Primer sequences were verified with sequences deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/Genbank/index.html). Microsatellite amplification was conducted using 2 × PCR Master Mix (Fermentas Life Sciences, Canada) [Components: Taq DNA polymerase 0.5 units/μl, $MgCl_2$ 4 mM and dNTP 0.4 mM]. About 50 ng of genomic DNA was mixed with 10 ng of each primer (forward primer labelled with fluorescent chemical FAM) (Table 1), 1 × PCR Master Mix and distilled water to make the final volume of 20 μl. Amplification was performed with 35 cycles at 94°C for 1 min, 42–55°C for 1 min and 72°C for 2 min., for denaturation, annealing and primer extension, respectively. The PCR products were separated and detected using a MegaBACE 1000 (GE Healthcare Life Sciences, USA) sequencer. The size of the amplified bands was determined based on an internal standard DNA (MegaBACE ET550-R Size Standard) with MegaBACE Fragment Profiler Version 1.2 (GE Healthcare).

For the diversity estimation of 19 SSR loci, the polymorphism information content (PIC), observed (HETo) and expected (HETe) heterozygosity and Simpson's Index of Diversity (SID) were calculated as proposed by Gourraud et al. (2005) and Simpson (1949). The coefficient of genetic similarity of the investigated cultivars was calculated using the following formula: $S_{ij} = N_{ij}/N$, where S_{ij} is the genetic similarity between cultivars i and j, N_{ij}–the number of alleles present at i-th and j-th cultivars, N–the number of all alleles, $i, j = 1, 2,\ldots, 8$. Cluster analysis was performed on the basis of the matrix of genetic similarity coefficients. The unweighted pair group method of arithmetic means (UPGMA) was used for clustering by the method of Nei and Kumar (Nei and Kumar 2000) employing for this purpose GenStat v. 7.1 software (Payne et al. 2003). Results of the performed grouping are presented in the form of a dendrogram.

Results

From among 40 tested SSR markers, 19 were selected to profile the genetic diversity among the pear genotypes due to high polymorphisms in the tested pear cultivars and wild pear germplasm (Fernandez-Fernandez et al.

Table 1 SSR genotypes of *P. pyraster* and pear cultivars

Marker	*P. pyraster* 1	*P. pyraster* 2	'Conference'	'Red Williams'	'Amfora'	'Radana'	'Dicolor'	'Carola'
EMPc10	[183][183]	[157][172]	[155][157]	[155][185]	[157][172]	[155][172]	[157][185]	[157][172]
EMPc102	[176][179]	[160][179]	[172][184]	[182][184]	[172][184]	[182][184]	[166][184]	[164][184]
EMPc104	[94][96]	[94][108]	[94][114]	[94][96]	[94][114]	[94][114]	[94][98]	[96][114]
EMPc105	[166][168]	[146][149]	[178]180]	[156][190]	[178]180]	[176][178]	[154][156]	[162][166]
EMPc106	[208][208]	[104][179]	[196][218]	[114][118]	[218][222]	[104][179]	[104][118]	[114][116]
EMPc110	[160][160]	[160][160]	[160][200]	[160][200]	[180][200]	[178][180]	[160][168]	[160][180]
EMPc111	[97][99]	[99][104]	[94][99]	[97][99]	[94][99]	[97][99]	[94][97]	[97][99]
EMPc114	[133][141]	[139][139]	[139][155]	[165][167]	[137][155]	[137][148]	[165][167]	[141][155]
EMPc115	[170][172]	[172][183]	[178][188]	[165][188]	[172][188]	[176][180]	[176][188]	[176][186]
EMPc117	[121][123]	[111][113]	[119][121]	[115][117]	[119][121]	[115][117]	[115][117]	[117][119]
KA14	[180][188]	[188][188]	[188][190]	[180][188]	[188][190]	[180][188]	[180][188]	[188][188]
KU10	[253][253]	[253][253]	[217][253]	[251][253]	[225][253]	[253][253]	[245][253]	[253][253]
BGT23b	[197][210]	[210][214]	[199][208]	[199][208]	[196][199]	[210][214]	[199][208]	[176][197]
NB105a	[140][154]	[140][156]	[142][170]	[156][174]	[142][178]	[142][174]	[156][174]	[156][174]
NB109a	[158][175]	[160][193]	[147][179]	[147][187]	[147][153]	[153][157]	[181][187]	[147][187]
NB113a	[152][154]	[158][160]	[146][150]	[150][154]	[138][150]	[152][154]	[152][154]	[150][154]
NH025a	[75][89]	[79][89]	[95][98]	[70][98]	[70][98]	[70][114]	[80][98]	[94][98]
NH027a	[138][146]	[146][160]	[132][156]	[126][158]	[132][156]	[132][156]	[134][158]	[156][156]
NB141b	[102][104]	[82][140]	[104][132]	[82][94]	[102][104]	[104][132]	[94][104]	[102][104]

2006: EMPc10, EMPc102, EMPc104, EMPc105, EMPc106, EMPc110, EMPc111, EMPc114, EMPc115, EMPc117; Yamamoto et al. 2002a: KA14, KU10, BGT23b; Yamamoto et al. 2002b: NB105a, NB109a, NB112a, NH025a, NH027a, NB141b) Genotyping results enable to identify 32 unique alleles for *P. pyraster* genotypes and 31 unique alleles in six pear cultivars genomes. This result reflects diversity between two pear genotypes (Table 1). Polymorphism varied considerably; specific alleles per locus ranged between 3 and 11 (Table 2). As expected, the range of sizes amplified by each primer pair across different cultivars was considerable (20 bp in EMPc111 up to 120 bp size range in EMPc106). The observed heterozygosity (0.5–1), expected heterozygosity (0.45–0.95), polymorphism information content (PIC) (0.42–0.89) and Simpson's Index of Diversity (SID) (0.45–0.95) values were calculated (Table 2). These calculations indicated that these primers provide useful tools for fingerprinting, sample identification and studying the diversity of *Pyrus* germplasm and, also, for linkage mapping and marker-assisted selection.

The cluster analysis clearly identified the genetic relationship between pear genotypes and demonstrated the potential and ability of microsatellite markers for genome analysis (Fig. 1 and Table 3) The genetic tree divides into two main clusters. The first cluster groups all pear cultivars and the second one contains forms of wild pear. The highest genetic relationships were observed between cultivars 'Conference' and 'Amfora' as well as between 'Red Williams' and 'Dicolor'. The above results confirm the origin of these cultivars, because 'Amphora' was created by crossing 'Conference' with a Czech cultivar 'Holenicka'; 'Dicolor' derived from cross-breeds of 'Red Williams' and the same 'Holenicka' cultivar (Rejman 1994). It is possible to partially reconstruct the 'Holenicka' genotype by analyzing genotypes of these four cultivars (both alleles for 9 loci and one for 7 loci).

Discussion

The microsatellite markers were able to distinguish between studied pear genotypes. The high degree of polymorphism of microsatellite markers allowed a rapid and efficient identification of pear cultivars. The high polymorphism information content (PIC) value of microsatellite markers (with the average of over 0.62) makes them an ideal tool for differentiating

Table 2 Locus information relating to 19 loci SSR, where *k* is the number of individuals in which the locus was amplified, *n* is the number of alleles, *g* is the number of unique genotypes, *p* is the frequency of the most prevalent genotype, *HETo* is observed heterozygosity, *HETe* is expected heterozygosity and *SID* is Simpson's Index of Diversity

Marker	k	*n*	g	*p*	HETo	HETe	PIC	SID	Range of allele size
EMPc10	8	5	6	0.375	0.875	0.825	0.753	0.825	150–190
EMPc102	8	8	6	0.250	1.000	0.850	0.792	0.850	150–190
EMPc104	8	5	5	0.375	1.000	0.750	0.689	0.750	90–120
EMPc105	8	11	7	0.250	1.000	0.950	0.889	0.950	145–190
EMPc106	8	9	7	0.250	0.875	0.933	0.873	0.933	100–220
EMPc110	8	5	6	0.250	0.750	0.717	0.670	0.717	155–210
EMPc111	8	4	4	0.500	1.000	0.717	0.626	0.717	90–110
EMPc114	8	8	7	0.250	0.875	0.917	0.855	0.917	125–170
EMPc115	8	9	8	0.125	0.875	0.900	0.840	0.900	165–200
EMPc117	8	7	5	0.375	1.000	0.875	0.808	0.875	105–130
KA14	8	3	3	0.500	0.750	0.567	0.470	0.567	170–205
KU10	8	5	5	0.500	0.500	0.450	0.420	0.450	210–260
BGT23b	8	8	5	0.375	1.000	0.883	0.818	0.883	170–220
NB105a	8	7	6	0.375	1.000	0.867	0.809	0.867	130–190
NB109a	8	10	7	0.250	1.000	0.917	0.857	0.917	135–210
NB113a	8	7	5	0.375	1.000	0.842	0.776	0.842	130–165
NH025a	8	9	7	0.250	1.000	0.883	0.826	0.883	60–120
NH027a	8	8	6	0.375	0.875	0.875	0.813	0.875	125–170
NB141b	8	6	5	0.375	1.000	0.825	0.757	0.825	75–140

between *Pyrus* genotypes. Similarly, high values of polymorphism were reported by other authors (Struss and Plieske 1998; Yamamoto et al. 2002a, b, c).

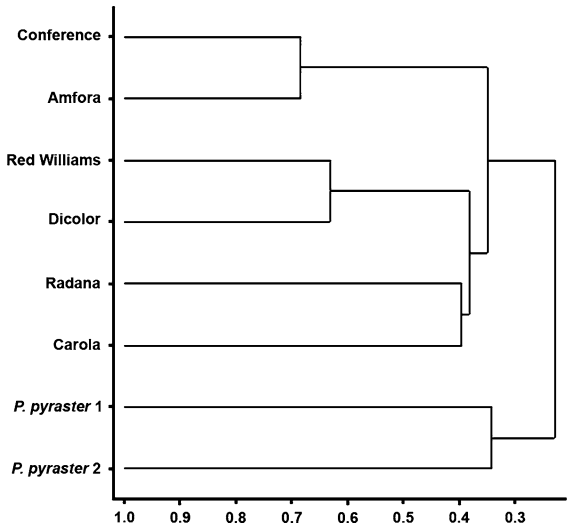

Fig. 1 UPGMA dendrogram describing genetic similarity of the examined cultivars

More heterozygotes than expected means excess outbreeding in the studied population and suggests the same kind of disassortative mating. The example of this form of disassortative selection is self-incompatibility (SI) in plants (mechanism described and observed in *Pyrus* species (Kim et al. 2002, 2006; Zisovich et al. 2004). Self-incompatibility is a genetic system that prevents self-fertilization through the recognition and rejection of pollen expressing the same allelic specificity as that expressed in the pistils (Castric and Vekemans 2004; Ma and Oliveira 2002). A key to understanding this variation is to investigate the evolutionary properties of the genes controlling in self-incompatybility plant mating systems. Self-incompatibility prevents inbred in wild pear populations and generates observed high level of variations.

The examined *Pyrus* wild forms were represented in this study by only two individuals of *P. pyraster*. It can be assumed that these forms were distinctly different from the pear cultivars. Our intention was to provide a molecular tool to identify and characterize *Pyrus* varieties and species estimating the genetic diversity. The set of microsatellite markers employed in this

Table 3 Coefficients of genetic similarity of the studied cultivars

Similarity	*P. pyraster* 1	*P. pyraster* 2	'Conference'	'Red Williams'	'Amfora'	'Radana'	'Dicolor'	'Carola'
P. pyraster 1	1							
P. pyraster 2	0.3421	1						
'Conference'	0.1842	0.1842	1					
'Red Williams'	0.2368	0.1842	0.3684	1				
'Amfora'	0.2105	0.1842	0.6842	0.3158	1			
'Radana'	0.2895	0.2632	0.3421	0.3684	0.4211	1		
'Dicolor'	0.2368	0.1842	0.3158	0.6316	0.2632	0.3684	1	
'Carola'	0.3421	0.2368	0.3421	0.4211	0.4211	0.3947	0.3684	1

study demonstrates the usefulness of microsatellite markers for the identification of pear genotypes.

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