Genetic Polymorphism of Wild Pear Accessions Collected in Lithuania

RYTIS RUGIENIUS[1], ASTA BLAŽYTĖ[1,2], VANDA LUKOŠEVIČIŪTĖ[1], JŪRATĖ BRONĖ ŠIKŠNIANIENĖ[1], BIRUTĖ FRERCKS [1], DALIA GELVONAUSKIENĖ[1], BRONISLOVAS GELVONAUSKIS[2], AUDRIUS SASNAUSKAS[1], DANAS BANIULIS[1] AND VIDMANTAS STANYS[1,3]

[1] Institute of Horticulture Lithuanian Research Centre for Agriculture and Forestry , Kauno str. 30, LT-54333 Babtai, Kaunas distr., Lithuania

[2] Plant Gene Bank, Stoties 2, LT-58343 Akademija, Kėdainiai distr., Lithuania

[3] Aleksandras Stulginskis University, Akademija, Kaunas district, Lithuania

Rugienius, R., Blažytė, A., Lukoševičiūtė, V., Šikšnianienė, J.-B., Frercks, B., Gelvonauskienė, D., Gelvonauskis, B., Sasnauskas, A., Baniulis, D. and Stanys, V. 2013. Genetic Polymorphism of Wild Pear Accessions Collected in Lithuania. *Baltic Forestry* 19(1): 13–21.

Abstract

Wild pear (*Pyrus pyraster* (L.) Burgsd.) is pear species growing naturally in Lithuania and is related to cultivated pear (*Pyrus communis* L.). In some cases, plants identified as *P. pyraster* represent various stages of hybridization between original *P. pyraster* and *P. communis*. Therefore a boundary between cultivated pear, naturalized plants and wild pear is ambiguous. There is limited information on genetic variation and structure of *P. pyraster* population. The aim of our study was to characterize morphological traits and microsatellite loci of pear originally collected from different localities of Lithuania as naturally growing specimens, presently grown in germplasm collection at the Institute of Horticulture Lithuanian, Research Centre for Agriculture and Forestry (IH LRCAF), and establish capacity of the markers to assess genetic structure of the pear population and suitability for species specific identification. Assessment of thirteen morphological parameters revealed high variation among *Pyrus* accessions. Morphological differences among the accessions were quantitative rather than qualitative. Characterization of 9 polymorphic microsatellite loci of 84 pear accessions identified 152 polymorphic alleles. The allele number per locus ranged from 12 to 20. The most polymorphic microsatellite loci were EMPc106, EMPc117, NB109a and CH02c11. High genetic polymorphism was demonstrated by genetic relationship and heterozygosity analysis among accessions. Lower H_o than H_e values indicated possible occurrence of self fertilisation in naturally growing pears. It was shown that morphological variability of *Pyrus* accessions weakly reflects genetic variation among them. The results of the molecular marker analysis of the accessions of free growing pear collection provided information about genetic background of local pear population that would be useful for restoration and maintaining of genetic diversity of forests.

Key words: genetic resources, genetic polymorphism, wild pear, cultivated pear**,** hybrid pear, microsatellite markers

Introduction

Wild pear (*Pyrus pyraster* (L.) Burgsd.) is insect pollinated and rare wild fruit tree species. The species is native to the Central, Western and Southern Europe but with a very scattered occurrence, and it often grows at the margins of hardwood forests in stands mixed with beech (Stephan et al. 2003). It is the only pear species native to Lithuania, which occurs mostly in the South-Eastern regions. It is less common in the Western regions where only single individuals or small groups can be found (Petrokas 2002). The average density of pear is only 30 trees per 1000 ha in the forests of South-Western Lithuania. The absence of cross-pollination owing to territorial isolation and self-incompatibility are the main causes of local extinctions of wild pear trees (Petrokas 2007). *P. pyraster* is considered an important relative of cultivated pear (*Pyrus communis* L.) and is of interest for breeding activities because of the adaptability characteristics, such as growth in heavy clay soils and drought tolerance (Palombi et al. 2007).

In the past, only morphological characters were used for *Pyrus* identification. The genetic variation and structure of *P. pyraster* is not yet known in detail and requires extensive study (Stephan et al. 2003). *Pyrus* accessions maintained at the germplasm collection at the IH, LRCAF originated from different regions of Lithuania (Petryla 1973, Petrokas 2002) and were classified by collector P. Petryla (Petryla 1973) according to morphological trait of fruit weight into 3 groups: 1) wild pear (*P. pyraster* ssp. *pyraster*) clones, 2) culti-

vated pear (*P. communis*) clones, and 3) hybrid clones. However, further evaluation of additional morphological traits (bud and fruit parameters, presence of thorns on shoots) and leaf peroxidase isoforms by R. Petrokas (Petrokas et al. 2007) revealed only several matches to the classification by P. Petryla (Petryla 1973).

An employment of molecular biology tools would provide deeper understanding of genetic background of the wild populations and hybrids of the crop. These new tools include molecular marker techniques, which have been proven useful in identification of the cultivars and hybrids of various crops, including fruit trees (Wunsch and Hormaza 2002). Molecular techniques have been used to elucidate the genetic relationship among *Pyrus* species and cultivars in Portugal, and among genotypes of the Asian and European pear (Monte-Corvo et al. 2000, Teng et al. 2002, Kimura et al. 2003). Genetic analyses based on DNA markers may provide information regarding the immediate ancestor of cultivated pear, and regarding the influence of hybridization. Several different types of DNA markers have been successfully applied for *Pyrus* cultivar identification and the analysis of genetic relationships. Genetic relationships between cultivated pear and related wild species have been investigated using microsatellite, also known as simple sequence repeats (SSR) (Wolko et al. 2010), randomly amplified polymorphic DNA (RAPD) (Monte-Corvo et al. 2000), amplified fragment length polymorphism (AFLP) markers (Monte-Corvo et al. 2000, Dolatowski et al. 2004).

Microsatelite markers are highly polymorphic and codominant, these markers have advantages over other molecular markers because of their robustness and reproducibility. Microsatellite marker analysis provides information that is useful for genotyping of individual plants or cultivars and exploring genetic relationship between accessions. Over 100 of microsatelite markers were developed and characterized from genomic DNA of various *Pyrus* species (Yamamoto et al. 2002, Nishitani et al, 2009, Fernández-Fernández et al. 2006). The microsatellite markers were used for identification and characterization of genetic polymorphism of pear cultivars in Europe, North America, Asia and Australia (Basil and Postman 2010, Gasic et al. 2009, Ahmed et al. 2010, Yakovin et al. 2011, Smith et al. 2009).

Free growing *Pyrus* in Lithuania have not been characterized by molecular markers. The aim of our study was to characterize pear accessions maintained at the IH, LRCAF collection based on morphological parameters and using microsatellite markers, and to establish their capacity to assess genetic polymorphism and suitability for species specific identification. Investigation of genetic polymorphism of the pear germplasm collection would provide information about genetic structure of local pear population.

Materials and methods

Plant material. Eighty-four accessions from collection *Pyrus sp.* maintained in germplasm collection at the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry, Babtai, Lithuania. The collection consists of wild (free growing) accessions collected by P. Petryla in different parts of Lithuania during several expeditions in the 1960's and 1970's (Petryla 1973). Accessions No 224 and No 225 were received from Morna, Estonia, No 222 and 223 – from Loshica, Belarus.

Morphological characterization. Morphological parameters were investigated in July of 2011 for the set of 84 accessions and included parameters of length and width of leaf, ratio of leaf length and width, characteristics of leaf blade (shape of apex, shape of base and incisions of margin), leaf color, leaf hairiness and presence of thorns on shoots were described according to descriptors used in UPOV (2000), as well as, leaf shape and glossiness of leaf according to Tuinyla et al. (1990). In addition, data of fruit weight, seed number and fruit setting (five degree score) collected by P. Petryla (1973; and unpublished report) for 50 accessions were included in the analysis.

Microsatellite marker analysis. Fresh leaves of the 84 wild pear clones were sampled in 2010-2011. Leaf samples were frozen and stored at -70°C until used. Genomic DNA was isolated from 100 mg of frozen leaves using "DNeasy Plant Mini kit" ("Qiagen" Ltd.) and manufacturer instructions. Genomic DNA maintained in TE solution (100 mM Tris-HCl, 10 mM EDTA, pH-8.0) at -20°C. Characteristics of nine microsatellite loci were assessed using PCR primers: NB109a, NH025a, (Yamamoto et al. 2002), EMPc11; EMPc105, EMPc106, EMPc115, EMPc117 (Fernández-Fernández et al. 2006), CH01d03, CH02c11 (Liebhard et al. 2002). The multiplex PCR reaction mix was adopted from Horne et al. (2004): 10 µl reaction volume included 60 ng genomic DNA, 1% polyvinylpyrrolidone, 10 mM dithiothreitol, 0.16 mM dNTP, 1× PCR reaction buffer, 0.2 U Titan Taq DNA polymerase (BioAtlas Ltd), 0.35 mM for each primer. Conditions for PCR amplification followed Clarke and Tobutt (2003). Fragment analysis was performed using 3130 Genetic Analyzer (Applied Biosystems Ltd), and data were analyzed appying GeneMapper software v.4.0 (Applied Biosystems Ltd.).

Data analysis. Correlation analysis among the morphological parameters was performed using Statistica software v.10.0. Expected (H_e) and observed (H_o) heterozygosity, polymorphism information content

(PIC) of the microsatellite markers were calculated for each primer pair using PowerMarker software v.3.25 (Liu and Muse 2005). Inbreeding coefficient was estimated by $F = (H_e - H_o)/H_e$. Power of differentiation (PD) and effective number of alleles (Ae) was calculated according to Garkava-Gustavsson et al. (2008). Genetic distances were calculated according to Nei et al. (1983). Subsequent cluster analysis was based on the UPGMA method using PowerMarker software, the dendrogram was constructed and visualized using TreeView software v.1.6.6 (Page 1996).

Results

Morphological characteristics of pear accessions. Assessment of thirteen morphological parameters revealed high variation among *Pyrus* accessions. Intervals of variation for the leaf length, width and the ratio of leaf length and width were 2.7-5.9 mm (on average 3.8 mm), 2.9-8.6 mm (5.1 mm) and 0.9-1.8 (1.4), respectively. Fruit weight number varied from 5 to 90 g (on average 31.7 g) and seed number was from 2 to 9 (on average 6). Approximately half (48%) of assessed accessions were without fruits, and fruit setting score value varied from 1 to 4 for remaining accessions as 38%, 8%, 2% and 6%, respectively.

For qualitative leaf characteristics, the leaf color varied from very light green (23%), to light green (33%), greyish-green (2%), green (19%), dark green (19%) and very dark green (4%), and leaf surface of approximately 98% of accessions were glossy.

Based on characteristics of the leaf blade, 8%, 73%, 18% and 1% of accessions had acute, right-angle, obtuse and rounded shape of apex, respectively; 36%, 38%, 24 and 2% of accessions had right-angle, obtuse, rounded and cordate shape of base, respectively. The incisions of margin were absent for 26% of accessions, and varied from crenate (22%) to bluntly serrate (18%) and sharply serrate (34%). The leaf shape was rounded (20%), oblong (30%), egg-shaped (6%), reverse egg-shaped (18%) and elliptic (26%).

Leaf hairiness was characteristic to 15% of accessions. Thorns on shoots were observed at medium (42%) and high degree (5%).

Correlation analysis among the morphological parameters demonstrated significant correlation at low to moderate degree (r=0.30-0.67, p<0.035) among the six parameters related to leaf shape: leaf length, width, ratio of leaf length and width, shape of leaf blade apex and base, leaf shape. The parameter of leaf glossiness was not included in the analysis because of very low variation (approximately 98% of accessions were glossy).

Among the remaining parameters, weak correlations were found between parameters of leaf hairiness and shape of incisions of leaf blade margin (r=0.34, p=0.014), leaf hairiness and shape of blade base (r=0.30, p=0.037), leaf hairiness and fruit setting (r=0.34, p<0.001), leaf length and presence of thorns on shoots (r=0.39, p=0.005).

Based on the morphological parameters that showed weak correlation, putative morphology of wild pear (presence of leaf hairiness and thorns on shoots,

Table 1. Alleles, characteristic for pears in the studied microsatellite loci. Alleles observed with frequency higher than 10% are shown in bold

Loci	Alleles in bp, (frequency in %)	Number of alleles (A)
EMPc11	115(1); 137(8); 139(5); 141(14); **143(25)**; **145(12)**; 146(1) **147(13)**; **151(14)**; 153(1); 155(3); 159(2)	12
EMPc105	145(3); **155(14)**; 161 (1); 163(1); 165(5); 169(2) 171(2); **175(14)**; **177(13)**; 179(5) 181(2); 183(5); 185(8); 187(2); 189 (5); 193(5); 195(11).	17
EMPc106	94(2); 101(2) 103(8) **105(12)**; 107(5); 109(2) 111(6) 113(7); 115(9); 117(2) **119(11)**; 121(1); 123(6) 127(8); 131(5); 133(3); 139(3); 141(2); 145(5); 146(1).	20
EMPc115	177(7); 175(2); **179(15)**; **181(23)**; 183(4); **185(27)**; 187(2) 189(3); 191(5) 199(4) 201(4); 203(3); 209(2)	13
EMPc117	92(1); 96(4); **98(10)**; **100(11)**; 102 (4); 106(7); 108(5); **110(11)**; 112(4); 114(4); 116(6) 118(3); 120(7); 122(5) 124(5); 126(2) 128(1); 132(1) 134(1); 136(3)	20
NB109a	129(4); 137(2); 139(5); **145(11)**; 151(5); 153(2); 155(2); 157(8); 159(4) 161(3); **163(10)**; 165(6); 167(4); 169(2); **171(14)**; 173(7) 175(5); 177(1); 179(1); 181(4).	20
NH025a	71(1); 73(7); 75(5); **79(18)**; **81(15)**; 83(7); **85(11)**; 87(1); 91(1); 93(3); 95(4); **97(22)**; 99(5)	13
CH01d03	125(1); 129(4); 131(1); 133(4); **135(16)**; 139(2); 141(4); 143(7); **145(10)**; 147 (4); 149(1) **151(14)**; **157(16)**; 159(3); 161(6); 163(2); 165(4); 169(1); 171(3).	19
CH02c11	199(1); 217(10); 221(4); 223(1); **225(13)**; 227(6); 229(10); 231(4); 233(5); 235(2); 237(2); 239(8); **241(17)**; 243(4); 245(1); 247(5); 249(2); 253(4);	18

absence of incisions of margin and obtuse shape of leaf base, length of leaf ≤ 5 cm, poor fruit setting) was characteristic of clones 3, 110, 168 and 189 (except absence of thorns on shoots).

Genetic polymorphism of microsatellite marker loci. Nine polymorphic microsatellite loci were surveyed for the 84 pear accessions, and 152 polymorphic alleles were identified. Only 28 alleles (18.4%) occurred in frequency more than 10% (Table 1). The allele number per locus varied from 12 to 20. The effective number of alleles (Ae) varied from 6.18 to 14.37 (Table 2).

Table 2. Genetic parameters of pear accessions in the studied microsatellite loci

SSR locus	Characteristics						
	Allele size range	Number of alleles (A)	Effective number of alleles (A_e)	Number of genotypes	Expected heterozygosity (H_e)	Observed heterozygosity (H_o)	Inbreeding coefficient *F*
EMPc11	115-159	12	6.95	33	0.86	0.42	0.51
EMPc105	145-195	17	10.77	37	0.91	0.41	0.55
EMPc106	94-146	20	14.05	54	0.93	0.89	0.04
EMPc115	175-209	13	6.18	28	0.84	0.49	0.42
EMPc117	92-136	20	14.37	51	0.93	0.70	0.25
NB109a	129-181	20	13.72	59	0.93	0.60	0.35
NH025a	71-99	13	7.42	37	0.87	0.77	0.11
CH01d03	125-171	19	10.33	44	0.90	0.66	0.27
CH02c11	199-253	18	11.14	53	0,91	0.83	0.09
Mean value		17.00	10.55	44	0.90	0.64	0.29

Microsatellite markers that yield PCR product of only one size were presumed to represent homozygous loci. Seven to forty-nine homozygous genotypes per locus were identified for the studied set of genotypes. The highest number of homozygous genotypes was identified at the loci of EMPc11, EMPc105 and EMPc115, while the lowest number was found at EMPc106 and CH02c11. Among the 84 accessions, number of homozygous loci varied between 0 and 7. Accessions 144, 183 and 224 had the highest number of homozygous loci (7, 6 and 6, respectively). Ten accessions (III2601, 23, 24, 168, 232, 180 213, 166, B69, 136B) had one homozygous locus. The most polymorphic microsatellite loci were EMPc117 (PIC value 0.93), EMPc106 and NB109a (PIC value 0.92).

Observed heterozygosity H_o varied from 0.41 to 0.89 with the average value of 0.64. Expected heterozygosity H_e value varied from 0.84 to 0.93 with the average of 0.90. H_o value was lower than H_e value at all investigated loci. Difference between H_e and H_o values was highest for EMPc105 locus and lowest for EMPc106. Inbreeding coefficient *F* varied from 0.04 to 0.55 accordingly (0.29 on average).

Cluster analysis of genetic relationship of pear accessions. Microsatellite marker based cluster analysis of genetic relationship among the accessions revealed high genetic polymorphism among the cultivars in agreement with results of the heterozygosity analysis and high degree of variation of values of morphological parameters among the accessions. Only seven small clusters including two to three closely related accessions were defined in the dendrogram (Fig. 1).

Discussion

The results of previous studies on classification of *Pyrus* accessions collected by P. Petryla (Petryla 1973) raised questions about suitability of morphological traits to distinguish between the two pear species, as well as, genetic structure of the population of wild (free growing) *Pyrus* in Lithuania. Only several accessions identified by P. Petryla (Petryla 1973) as wild or cultivated pear species were confirmed in later study using different morphological traits (bud and fruit parameters, presence of thorns on shoots) and leaf peroxidase isoforms (Petrokas et al. 2007). Only the clone 110 had all traits characteristic to wild pear in the study. In addition, several clones were identified as cultivated pear (clones 157, 168 and 206).

The inability to group the accessions based on morphological markers could suggest that the morphological traits assessed in the studies had no species associated values and reflected only quantitative variation of traits independent of the genetic relationship of the pear accessions. On the other hand, presumption could be made that species specific morphological parameters were not applicable due to highly heterogeneous structure of the population of free growing pear. To solve this ambiguity, we employed a brought spectrum of morphological traits and highly polymorphic molecular markers to establish genetic diversity of the pear population and to assess species specific values of the morphological traits.

In agreement with the previous studies, our evaluation demonstrated a high degree of variation of values of the morphological traits among the *Pyrus* accessions. To assess presence of species specific values of the morphological traits, we made assump-

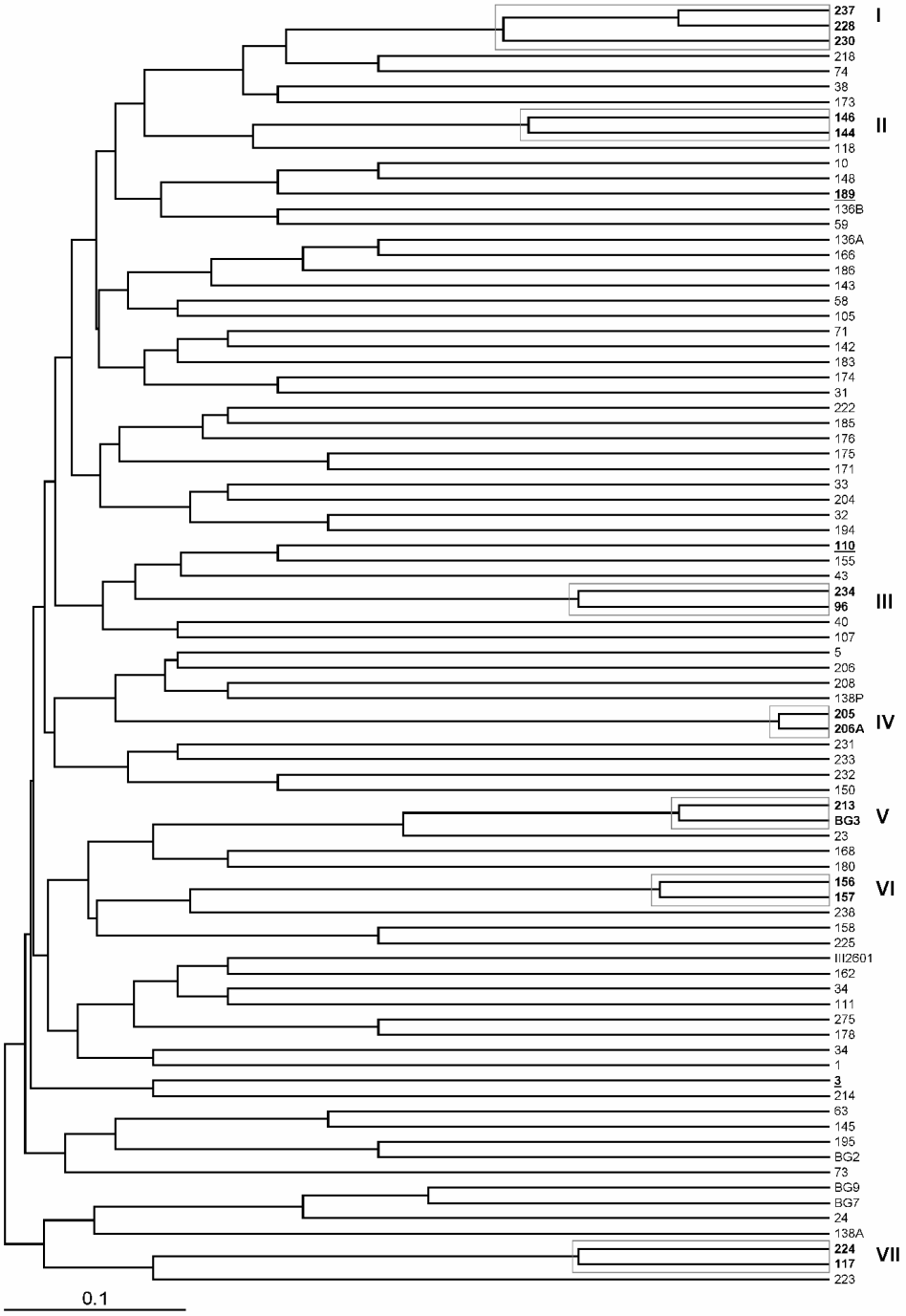

Figure 1. Genetic relationship of pear accessions. Clusters of closely related accessions are indicated by Roman numerals. Accessions identified as putative wild pear genotypes based on morphological traits are underlined

tion that there should be species specific association among distribution of values of different morphological parameters. Therefore this relationship should be manifested by correlation of the values among the pear accessions of wild and cultivated pear. Meanwhile, in case of highly heterogeneous group of accessions with only quantitative variation of values of the morphological traits independent of the genetic relationship, low degree of correlation could be expected.

The correlation analysis among the morphological traits demonstrated that moderate correlation was present only among the directly related parameters associated to shape of leaf (leaf length, width, ratio of leaf length and width, shape of leaf blade apex and base, leaf shape). For unrelated parameters, only six weak correlations were found and accessions with putative species specific traits could be distinguished. As expected, putative morphology of wild pear was charac-

teristic of clone 110 in agreement with both of the previous studies (Petryla 1973, Petrokas et al. 2007). In addition, wild pear morphology was observed for clone 168 that was identified as cultivated pear by the previous studies. However, significance of the correlation between the parameters with low level of variation of values, such as in case of leaf hairiness (absence of trichomes was characteristic of 85% of accessions), is uncertain. Further ambiguity was brought by comparison of the molecular marker data. The results of cluster analysis (Figure 1) demonstrated large genetic distance among these putative wild pear clones. Since molecular marker data that represents genetic relationship did not support the results of morphological analysis, the similarities of the values of morphological traits could be interpreted as coincidence.

High level of polymorphism among the pear accessions was revealed by cluster analysis of the microsatellite marker data. Only seven small clusters of two to three closely related genotypes were identified that included 15 accessions (Figure 1). The remaining pear accessions were highly heterogeneous. One could expect that similar values of morphological traits among accessions of the same cluster could be associated with genetic relationship among the accessions. Although similarity of the accessions within clusters was obvious, match of values of morphological traits was inconsistent (Table 3).

It has been demonstrated that plants of genus *Pyrus* have undergone extensive historical dispersal, leading to generation of numerous ambiguous interspecific hybrids with limited phenotypic variation (Yamamoto et al. 2002). In agreement, our molecular marker data demonstrated that the population of free growing pear in Lithuania is highly heterogeneous. Although morphological and phenological characterization provides basic features of the ecotypes, it is not sufficient to assess genetic diversity of pear genotypes because of the low phenotypic variation among species and varieties (Ahmed et al. 2010).

Molecular markers provide resolution sufficient to distinguish specific genotypes and to assess genetic diversity between germplasm pools (Smith et al. 2009, Stanys et al. 2012, Sikorskaite et al. 2012). Martinelli et al. (2008) report the value of discrimination power (D) 0.8288 for microsatellite markers as compared to morphological characters 0.5019. In this study, the analysis of genetic polymorphism of microsatellite loci proved the efficiency of microsatellite markers for the assessment of genetic diversity. The assessment of genetic diversity of *Pyrus* accessions using 9 microsatellite primer pairs identified 152 polymorphic alleles. The majority (121) of alleles had frequency lower than 10%. For all markers except EMPc11, amplified fragments were found to be of similar size range for most of the unique and common alleles. For EMPc11 marker allele No. 115 characteristic of wild pears, DNA fragment size was considerably smaller than the size of other alleles of this locus. The loci EMPc106 and EMPc117 were found most informative for the popu-

Table 3. Morphological parameters of closely related and putative wild accessions of *Pyrus* collection

Cluster	Geno type	Character*											
		Leaf length, mm	Leaf widh, mm	Proportion of leaf length and width	Shape of leaf base	Shape of apex	Leaf shape	Incisions of leaf margin	Leaf colour	Leaf hairiness	Thorns	Fruit setting	Fruit weight, g**	Seed number**
I	**230**	5.5	3.4	1.6	2	2	2	2	2	1	1	0	70	6
I	**237**	5.8	3.3	1.8	2	2	2	1	1	1	1	0	45	6
I	**228**	4.4	3.1	1.4	2	1	2	3	4	1	1	0	70	6
II	**144**	4.0	3.8	1.1	4	3	1	3	2	1	2	0	16	8
II	**146**	4.6	4.1	1.1	3	3	1	4	1	1	1	4	30	7
III	**234**	5.3	3.7	1.4	2	2	5	2	4	1	1	0	90	4
III	**96**	6.0	4.1	1.5	3	1	2	3	4	1	2	1	22	7
IV	**205**	3.9	3.3	1.2	3	2	3	4	1	1	2	0	26	7
IV	**206A**	4.0	3.2	1.3	2	2	4	4	2	1	2	1	25	7
V	**213**	4.1	3.2	1.3	3	2	2	1	4	1	2	1	-	-
V	**BG3**	6.5	4.7	1.4	2	2	5	1	4	1	1	1	-	-
VI	**157**	6.2	3.8	1.6	2	2	2	4	5	1	1	0	40	7
VI	**156**	7.1	5.2	1.4	2	2	2	4	3	1	1	1	42	7
VII	**117**	5.0	4.6	1.1	4	2	2	4	2	1	2	0	12	4
VII	**224**	5.9	4.3	1.4	3	2	5	2	1	1	1	1	7	-

*Values of morphological characters: shape of leaf base: 1 – acute, 2 – right-angled, 3 – obtuse, 4 – truncate, 5 – cordate; shape of apex: 1 – acute, 2 – right -angled, 3 – obtuse, 4 – rounded; leaf shape: 1 – rounded, 2 – oblong, 3 – egg-shaped, 4 – reverse egg-shaped, 5 – elliptic; incisions of leaf margin: 1 – absent, 2 – crenate, 3 – blunty serrate, 4 – sharply serrate; leaf color: 1 – very light green, 2 – light green, 3 – greyish-green, 4 – green, 5 – dark green, 6 – very dark green; thorns on the shoots: 1 – absent, 2 – present; fruit setting 0-5 scale (0 – no fruits, 5 – very many fruits)
**Data according Petryla, 1973 and unpublished report

lation of the wild pear. In agreement, these loci were identified as most informative in our previous study on traditional and standard pear cultivars (Lukoseviciute et al. in press).

Mean observed heterozygosity H_o was considerably lower as compared to expected heterozygosity H_e. It corresponds well with the studies of *Pyrus usuriensis* (Katayama et al. 2007) and different *Pyrus* genotypes (Yakovin et al. 2011). The values of H_o and H_e and difference between the two values vary among the loci and genotypes. In our study, respective values of H_e and H_o values were 0.91 and 0.41 for locus EMPc105, while 0.93 and 0.89 for EMPc106. In the study of European pears by Fernandez-Fernandez et al. (2006), the corresponding values were 0.90 and 0.75 for locus EMPc105, and 0.91 (equal H_e and H_o) for EMPc106.

The difference between observed and expected heterozygosity obtained in our study could be caused by the presence of null alleles in one of the homologous chromosomes in some cultivars (Brookfield 1996) or linkage of these loci with functional genes subjected to natural or artificial selection. However, it is very likely that this phenomenon was associated with the existence of genetically isolated groups within pear populations (Yakovin et al. 2011). In such cases, observed heterozygosity was lower than expected heterozygosity H_e due to the limited number of alleles or limited gene flow.

Most of the pear cultivars are cross-pollinating. Self-pollination is eliminated and inbreeding is prevented through a self-incompatibility mechanism. Low expected heterozygosity and high inbreeding coefficient for a cross pollinating species like *Pyrus* observed in our study experiments could mean that self-incompatibility barrier might be overcome in several cases in pedigree of investigated pears or inbreeding took place more often than expected in outbreeders. Such phenomenon could be explained by the fact that wild pears in Lithuania occurs as isolated single trees or small groups consisting of close relatives (Petrokas et al. 2007). This implication is supported by the highest number of homozygous loci found in accession No 224 which comes from Polli research station Estonia. Density of wild pear tree population in Estonia is even lower than in Lithuania and cross pollination possibilities are lower.

Conclusions

Assessment of genetic polymorphism of the collection of wild (free growing) pear originating from different parts of Lithuania and surrounding regions using morphological and microsatellite markers supported previous observations of highly heterogeneity of pear populations. High degree of variation of morphological parameters was observed among the pear accessions. Based on results of correlation analysis among the morphological traits and comparison to microsatellite analysis data, we conclude that morphological parameters were found to be unreliable indicators of genetic background and could not be used to explicitly identify pear species; meanwhile the molecular markers were useful to describe genetic diversity of the wild population of pear.

Using a set of 9 microsatellite markers, 152 polymorphic alleles (12 to 20 alleles per locus) among the 84 studied accessions were identified. The most polymorphic microsatellite loci were EMPc106, EMPc117, NB109a and CH02c11. Among the polymorphic alleles, 18.4% occurred in frequency more than 10%. High genetic polymorphism was demonstrated by analysis of heterozygosity and genetic relationship among the accessions. Lower *Ho* than *He* values indicates a presence of self fertilisation consanguineous mating in naturally growing pears that may be due to the low population density.

The diversity of genetic background of free growing pear population revealed by the results of the microsatellite marker analysis represents important guidelines for restoration and maintaining of genetic diversity of forests. Estimate of genetic relationship among the accessions highlighted genetic uniqueness of the majority accessions stored in the collection of wild pear that would be of high value for the restoration of population of free growing pear in Lithuania. To represent genetic structure of current population, a high number of different genotypes should be used in restoration of forest diversity.

Acknowledgement

The research was funded by the Research Council of Lithuania grant No. MIP-72/2010.

References

Ahmed, M., Anjum, M. A., Khan, M. Q., Ahmed, M. J. and Pearce, S. 2010. Evaluation of genetic diversity in *Pyrus* germplasm native to Azad Jammu and Kashmir (Northern Pakistan) revealed by microsatellite markers. *African Journal of Biotechnology* 9(49): 8323-8333.

Bassil, N. and Postman, J. D. 2010. Identification of European and Asian pears using EST-SSRs from Pyrus. *Genetic Resources and Crop Evolution* 57: 357-370.

Brookfield, J.F. 1996. A Simple New Method for Estimating Null Allele Frequency from Heterozygote Deficiency. *Molecular Ecology* 5:453-455.

Clarke, J.B. and Tobutt, K.R. 2003. Development and characterization of polymorphic microsatellites from *Prunus avium* 'Napoleon'. *Molecular Ecology Notes* 6: 453-455.

Dolatowski, J.N.J., Podyma, W., Szymanska, M. and Zych, M. 2004. Molecular studies on the variability of Polish semi-wildpears (*Pyrus*) using AFLP. *Journal of Fruit and Ornamental Plant Research*, Special ed. 12: 331-337.

International Union for the Protection of New Varieties of Plants (UPOV). 2000. Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability. Pear (*Pyrus communis* L.). UPOV, Geneva, Switzerland. 40 pp.

Fernández-Fernández, F., Harvey, N.G. and James, C.M. 2006. Isolation and characterization of polymorphic microsatellite markers from European pear (*Pyrus communis* L.). *Molecular Ecology Notes* 6: 1039-1041.

Garkava-Gustavsson, L., Kolodinska Brantestam, A., Sehic, J., and Nybom, H. 2008. Molecular characterisation of indigenous Swedish apple cultivars based on SSR and S-allele analysis. *Hereditas* 145:99-112

Gasic, K., Han, Y., Kertbundit, S., Shulaev, V., Iezzoni, A. F., Stover, E. W., Bell, R. L., Wisniewski, and M. E. and Korban, S. S. 2009. Characteristics and transferability of new apple EST-derived SSRs to other Rosaceae species. *Molecular Breeding* 23:397-411.

Horne, E.C., Kumpatla, S.P., Patterson, K.A., Gupta, M. and Thompson, S.A. 2004. Improved high-throughput sunflower and cotton genomic DNA extraction and PCR fidelity. *Plant Molecular Biology Reporter* 22: 83a-83i.

Katayama, H., Adachi, S., Yamamoto, T. and Uematsu, C. 2007. A wide range of genetic diversity in pear (*Pyrus ussuriensis var. Aromatica*) genetic resources from Iwate, Japan revealed by SSR and chloroplast DNA markers. *Genetic Resources and Crop Evolution* 54:1573-1585.

Kimura, T., Sawamura, Y., Kotobuki, K., Matsuta, N., Hayashi, T., Ban, Y. and Yamamoto, T. 2003. Parentage analysis in pear cultivars characterized by SSR markers. *Journal of the Japanese Society for Horticultural Science* 72(3):182-189.

Liebhard, R., Gianfranceschi, L., Koller, B., Ryder, C.D., Tarchini, R., Van De Weg, E. and Gessler, C. 2002. Development and characterisation of 140 new microsatellites in apple (*Malus* x *domestica* Borkh.). *Molecular Breeding* 10: 217-241.

Liu, K. and Muse, S.V. 2005. PowerMarker: Integrated analysis environment for genetic marker data. *Bioinformatics* 21(9): 2128-2129.

Lukoseviciute, V., Gelvonauskiene, D., Baniulis, D., Frercks, B., Sasnauskas, A., Stanys, V. and Rugienius, R. 2012. Characterization of microsatellite marker loci in Lithuanian pear *(Pyrus communis)* cultivars. *Acta Horticulturae* in press.

Martinelli, F., Busconi, M., Camangi, F., Fogher, C, Stefani, A. and Sebastiani, L. 2008. Ancient Pomoideae (*Malus domestica* Borkh. and *Pyrus communis* L.) cultivars in "Appenino Toscano" (Tuscany, Italy): molecular (SSR) and morphological characterization. *Caryologia* 61(3): 320-331.

Monte-Corvo, L., L. Cabrita, C.M., Oliveira, and Leitão, J. 2000. Assessment of *Pyrus* cultivars by RAPD and AFLP markers. *Genetic Resources and Crop Evolution* (47):257-265.

Nei, M., Tajima, F. and Tateno, Y. 1983. Accuracy of estimated phylogenetic tree from molecular data. II. Gene frequency data. *Journal of Molecular Evolution* 19: 153-170.

Nishitani, C., Terakami, S., Sawamura, Y., Takada, N. and Yamamoto, T. 2009. Development of novel EST-SSR markers derived from Japanese pear (*Pyrus pyrifolia*). *Breeding Science* 59: 391-400.

Page, R. 1996. TreeView: An application to display phylogenetic trees on personal computer. *Computer Applications in the Biosciences* 12(4): 357-358.

Palombi, M.A., Lombardo, B. and Saboni, E. 2007. *In vitro* regeneration of wild pear (*Pyrus pyraster* Burgsd) clones tolerant to Fe-chlorosis and somaclonal variation analysis by RAPD markers. *Plant Cell Reports* 26: 489-496.

Petrokas, R. 2002. Savaime plintančių kriaušių skiriamieji požymiai [Distinctive characters of self-spreading pears]. *Sodininkystė ir daržininkystė* 21(2): 22-31. (In Lithuanian)

Petrokas, R., Gelvonauskis, B. and Šikšnianienė, J. B. 2007. The taxonomic identity of scattered pear trees of South-western Lithuania. *Miškininkystė* 1(61): 50-58.

Petryla, P. 1973. Laukinių kriaušių išplitimas LTSR ir kai kurios jų augimviečių sąlygos [Distribution of wild pears in LSSR and some conditions of its growing places] *Lietuvos žemdirbystės mokslinio tyrimo instituto darbai* 16: 75-84. (In Lithuanian)

Sikorskaite, S., Gelvonauskiene, D., Stanys V. and Baniulis, D. 2012. Characterization of microsatellite marker loci in local apple (*Malus* x *domestica*) cultivars in Lithuania. *Zemdirbyste-Agriculture* 99(2): 131-138.

Smith, S. L., Wang, J., Liu, S., Richards, S., Cogan, N.O.I, Forster, J.W. and Smith, F. 2009. Assessment of genetic diversity in cultivars and germplasm pools of European and Asian pear (*Pyrus* spp.) using SSR markers. *SABRAO Journal of Breeding and Genetics* 41: 1

Stanys, V., Baniulis, D., Morkunaite-Haimi, S. , Siksnianiene, J.B., Frercks, B., Gelvonauskiene, D., Stepulaitiene, I., Staniene, G. and Siksnianas, T. 2012. Characterising the genetic diversity of Lithuanian sweet cherry (*Prunus avium* L.) cultivars using SSR markers. *Scientia Horticulturae* 142: 136-142.

Stephan, B.R., Wagner, I. and Kleinschmit, J. 2003. EUFORGEN Technical Guidelines for genetic conservation and use for wild apple and pear (*Malus sylvestris* and *Pyrus pyraster*). International Plant Genetic Resources Institute, Rome, Italy 6 pp.

Teng, Y, Tanabe, K., Tamura, F. and Itai, A. 2002. Genetic relationships of Pyrus species and cultivars native to East Asia revealed by randomly amplified polymorphic DNA markers. *Journal of the American Society for Horticultural Science* 127: 262-270.

Tuinyla, V., Lukoševičius, A. and Bandaravičius, A. 1990. Lietuvos pomologija: Atlasas. T. 1. Obelys ir kriaušės [Polmology of Lithuania. Atlas. Vol. 1. Apple and Pear Trees]. Mokslas, Vilnius, 333 pp.

Wolko, Ł., Antkowiak, W., Sips, M. and Słomski, R. 2010. Self-incompatibility alleles in Polish wild pear (*Pyrus pyraster* (L.) Burgsd.): a preliminary analysis. *Journal of Applied Genetics* 51(1): 33-35.

Wunsch, A. and Hormaza, J.I. 2002. Cultivar identification and genetic fingerprinting of temperate fruit tree species using DNA markers. *Euphytica* 125, 59-67.

Yakovin, N. A., Fesenko, I. A., Isachkin, A. V. and Karlov, G. I. 2011. Polymorphism of Microsatellite Loci in Cultivars and Species of Pear (*Pyrus* L.). *Russian Journal of Genetics* 47(5): 564–570.

Yamamoto, T., Kimura, T., Shoda, M., Imai, T., Saito, T., Sawamura, Y., Kotobuki, K., Hayashi, T. and Matsuta, N. 2002. Genetic linkage maps constructed by using an interspecific cross between Japanese and European pears. *Theoretical and Applied Genetics.* 106:9–18.

Received 20 December 2011
Accepted 07 June 2013

ГЕНЕТИЧЕСКИЙ ПОЛИМОРФИЗМ ПОПУЛЯЦИИ ДИКОЙ ГРУШИ В ЛИТВЕ

Р. Ругиенюс, А. Блажите, В. Лукошявичюте, Ю. Б. Шикшняниене, Б. Фрерцкс, Д. Гелвонаускиене, Б. Гелвонаускис, А. Саснаускас, Д. Банюлис и В. Станис

Резюме

Дикорастущая лесная груша (*Pyrus pyraster* (L.) Burgsd.) – вид распространенный в Литве и родственный культивируемой обыкновенной груше (*Pyrus communis* L.). Некоторые растения, идентифицированые как *P. Pyraster*, являются разной степени гибридами между *P. pyraster* и *P. communis.* Исходя из того, граница между гибридами и видами груши весъма расплывчата. Информация о генетической вариации и структуре популяций *P. pyraster* недостаточна.

Генетические ресурсы груши недостаточно изучены из-за малой морфологической изменчивости, недостатка дифференциирующих признаков между видами и частого взаимного скрещивания. Традиционные методы определения видов, основанные на агрономических и морфологических параметрах, зависят от влияния условий окружающеи среды и фенологической фазы растений. Поэтому исполъзование молекулярных методов дает возможность более глубокого познания генетических основ диких популяций и происхождения гибридов груши.

Целью иследований было характеризовать микросателлитные локусы свободно произростающих образцов груши, собранных в разных местностях Литвы и содерживаемых в коллекционных садах института Садоводства и Овощеводства Литовского Центра Аграрных и Лесных Наук и оценить возможные связи между генетическими и морфологическими маркерами груши.

Микросателлитный анализ выполнен на 84 клонах груши которые согласно преобладающим морфологическим признакам были распределены на три группы: дикую, культивируемую и гибридную грушу. В девяти полиморфных микросателлитных локусах груши идентифицированы 153 полиморфные аллели. Число аллелей отдельного локуса варьировало от 12 до 20. Идентифицированы наиболее полиморфные микросателлитные локусы (EMPc106, EMPc117, NB109a и CH02c11) и уникальные аллели, характерны каждой из упомянутых трех групп. Результаты анализа гетерозиготности и генетических связей показали высокий генетический полиморфизм изученных образцов. Идентифицированное более низкое значение *Ho*, чем *He* указывает на сравнительно частые случаи самоопыления естественно произрастающих груш.

Показано, что морфологическая изменчивость образцов в роде *Pyrus* слабо отражает их генетическую изменяивость. Такие признаки как вес плодов и ворсистость молодых листьев можно использовать как морфологические маркеры генетического родства коллекционных образцов.

Ключевые слова: генетические ресурсы, генетический полиморфизм, дикорастущая груша, культивируемая груша, гибридная груша, микросателлитный анализ