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GENETIC DIVERSITY AMONG SOME PEARS GENOTYPES FROM WEST PART OF ROMANIA ON THE BASE OF RAPD MARKERS

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Abstract

An important tool in fruit tree breeding programs is genetic variation. In present work were studied the genetic diversity of some pears genotypes from West part of Romania. For this way we used 10 randomly amplified polymorphic DNA (RAPD) primers. Five out of the 10 primers used in this study amplified clear and reproducible bands. The RAPD primers produced 62 bands, and 46 of them were polymorphic, with an average of 12.4 amplicons/primer. The polymorphic bands, registered per primer ranged from 6 (OPA18) to 10 (primer OPA12). Total polymorphism generated by a certain primer (PIC), registered values between 0.39 P11 0.45 OPA18. The discrimination index (PI), presented values among 2.72 for the primer OPA18 and 5.38 for P-25 primer, which had the highest capacity to generate polymorphic bands to genotypes being studied. Results showed the suitability of RAPD analysis in genetic diversity studied of pear landraces.

Key words*: genetic diversity, RAPD, pears.*

INTRODUCTION

Rosaceae is one of the large and diverse family of plants that includes a multitude of important plant species, such as some fruit trees that are economically important. This family is the third most important plant family in temperate zones with over 100 genera and 3,000 species (Dirlewanger et al., 2002; Shulaev et al., 2008; Lo et al., 2012). *Pyrus communis* is the most commonly cultivated pear species in Europe, America, and Africa (Bell, 1990). In previous decades, the identification and assessment of pear species was based on botanical and chemotaxonomic characters (Challice and Westwood, 1973). In recent decades, the introduction of modern and more productive varieties of pears grown in special orchards has led to a drastic decline in diversity and genetic erosion of ancient varieties. Local varieties of pears "took refuge" on the hills and at the foot of the mountains, where it stubbornly continues to grow in people's orchards, in small clumps and compact, forming a real "resistance movement" in the face of "invasion" very productive improved hybrids and varieties. In horticulture Randomly amplified (RAPD) polymorphic DNA techniques have been used extensively for germplasm identification and progeny testing (Gogorcena et al., 1994; Polito et al., 1994; Tancred et al., 1994; Bartolozzi et al., 1998). Sustainable use and conservation of plant genetic resources is a necessity for future food security. Advances in biotechnology have created new opportunities for genetic resources, conservation and use (Rao, 2004). The environment has an influence on botanical characteristics of plants, making this technique (molecular biology) more valuable because it is based directly on genetic structure (Morell et al., 1995). Attempts to characterize pear genetic variability by DNA molecular markers have been rare. In fruit tree RAPD have been used to analyse different aspects of plant genomes, including taxonomic classification and genetic diversity (Chaparro et al., 1994; Lisek et al., 2010; Oliveira et al., 1999) construction of genetic maps (Liebhard et al., 2003),

identification of progenies of cross pollination (Zamani et al., 2010), marker-assisted selection (Zhang et al., 2014), population structure (Walisch et al., 2015). These multilocus markers are not just fast, simple, and sensitive, but also universal, making useful for comparing related genera and species at the DNA level. For future food security conservation and sustainable use of plant genetic resources is a necessity. New opportunities for genetic resources, conservation and use, have been generated with the help of advances in biotechnology (Rao, 2004). DNA marker based on (PCR) - are versatile tools in different aspects of genomic studies. It is possible to analyse, closely related genera in order to evaluate their phylogenic relationships by using DNA molecular markers. The aim of the study was to investigate at the molecular level some of the pear landraces from different county to obtain more information about the genetic relationships of these trees.

MATERIALS AND METHODS

In this study were used a total of 10 pear landraces belonging to three county from West part of Romania (Table 1).

Table 1. Biological material

Landraces	County	Landraces	County
1. Par rosu	Caras-Severin	6. Lubenicarka	Mehedinti
2. Lubinite	Caras-Severin	7. Par de Balvanesti	Mehedinti
3. Malaiete	Caras-Severin	8. Par de Malovat	Mehedinti
4. Albe de Sf. Petru	Timis	9. Mici galbene	Timis
5. Limunka	Mehedinti	10. Marganesc	Caras-Severin

Leaf samples of pears were stored at -80ºC until use. Genomic DNA was extracted from pear leaves according to CTAB method (by Doyle, 1987). The PCR reaction was performed with the following protocol: two minutes for 94°C, for 40 cycles. Each cycle consisted of 1 min at 95°C, 10 sec at 50°C, 15 sec at 45°C, 20 sec at 40°C, 1 min at 35°C, 30 sec at 45°C and 1 min 45 sec at 72°C and a final extension step for 5 min at 72°C. Finally, the mixture was kept at 4°C until electrophoresis. Following amplification, the PCR products (10 μL) were loaded in 2% agarose gels, stained with ethidium bromide in Tris-acetate-EDTA (TAE) buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0), and separated by electrophoresis, and photographed on an ultraviolet trans illuminator. Each gel was analysed, by scoring the presence (1) or absence (0) of bands for the genetic relationship analysis. A dendrogram was constructed based on the similarity matrix. In view of the potential characterization of different molecular marker systems to evaluate inter population variability in the studied genotypes, different parameters were calculated:

- the total polymorphism generated by a certain primer (PIC = Polymorphic Information Content) which indicates its discriminatory power.

$$PIC = 1 - \sum_{i=1}^{n} P_{ij}^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2$$

Pi - frequency of allele i; Pj - frequency of allele j; Pij - frequency of allele i for locus j; n - the total number of loci.

- the discrimination index (PI), which certifies the effectiveness of a particular primer in detecting polymorphism.

$$PI = \sum PIC$$

Genetic similarity among genotypes studied calculated through coefficient Jaccard, which was recommended to be used for dominant markers ISSR, RAPD, taking in view that the absence of a bands was associated to a homozygous loci. JC= a/(a+b+c), where a, b, c, represented the commons and un-commons of those genotypes (Dangi et al., 2004). On base of genetic similarity matrix among landraces, it was made the dendrogram using the method of clusters average.

RESULTS AND DISCUSSIONS

For the analysis of the genetic polymorphism of the pears landraces included in the study, 10 RAPD primers were tested. Of these primers, 5 primers produced clear, reproducible, and informative patterns were scored. A total of 63

fragments (loci) were generated. The fragments length ranged from 100 to 1750 base pairs (bp). This is in accordance with the results reported by (Teng et al. 2002). Each primer generated a number of bands which varied from 11 in OPA18 to 14 in OPA12 respectively, with an average of 12.6 bands/primer (Table 2). These findings are also in line with those reported by Oliveira et al. (1999) and Monte-Corvo et al. (2000) in studies related to pear identification using RAPD markers. Among the 63 amplified loci, 46 showed polymorphism with an average of 9.2 polymorphic bands/primer that was higher than those reported by Cho et al. (2012), while lower than what was reported by Monte-Corvo et al. (2000). The total polymorphism generated by a particular primer (PIC) recorded values between 0.392 for the P11 primer and 0.453 for the OPA18. The efficiency of a particular primer in detecting polymorphism is given by the discriminatory index (PI), which recorded values from 2.72 for the OPA18 primer and 5.38 for the P25 primer, which has the highest capacity to generate polymorphic bands.

Table 2. Polymorphism rate through RAPD primers

Primer	Primer sequence 5'- 3'	Length of the fragments	Number of amplified fragments	Number of polymorphic fragments	% de polymor phism	PIC $\overline{x} \pm s_{\overline{x}}$	PI
P25	GCACTGAGTA	175-1200	14	12	85.71	0.448±0.046	5.38
P11	GCTGCTCGAG	150-1050	13	10	76.92	0.392±0.057	3.92
OPA 13	CAGCACCCAC	150-1500	11	8	72.72	0.412±0.061	3.3
OPA 12	GGGTAACGCC	150-1750	14	10	71.42	0.418±0.053	4.18
OPA18	AGGTGACCGT	100-1450	11	6	54.54	0.453±0.073	2.72
	Total		63	46			
	No bands/primer		12.6	9.2			

Based on genetic similarity, pears landraces were classified hierarchically into two clusters, with an average diversity of about 37%. The first group has a complex structure and includes four landraces that own about 68% of the common alleles of the five primers. The 78% genetically similar Limunka and Mărgănesc landraces make up a first subcluster, along with the St. Peter's White landraces, which differs by about 26% from the two previously presented. The populations in the first subcluster have flattened yellow spherical fruits ripening at the end of August. The population of St. Peter collected from Timis county has greenish yellow pear-shaped fruits. The second cluster is composed of the landraces from Mehedinti County, Malovăţ pear and Lubenicarka, between which there is a genetic similarity of 73%, together with the landraces of Mălăieţe and Lubiniţe from Caras-Severin. The populations of this cluster are characterized by green-skinned fruits with red spots and red flesh. The fruits are small, pear-shaped. There is an average diversity of about 35% between the populations in these two clusters. Bălvăneşti pear and Small yellow landraces represent a separate group that owns approximately 75% of the common alleles of the five primes. These populations, even if they come from different areas, have fruits with similar shapes and color, small yellow. The interpopulation similarity for the alleles of the five RAPD markers had values ranging from 53.97% between Păr roşu and Lubiniţe to 79.37% between Limunka and Mărgănesc.

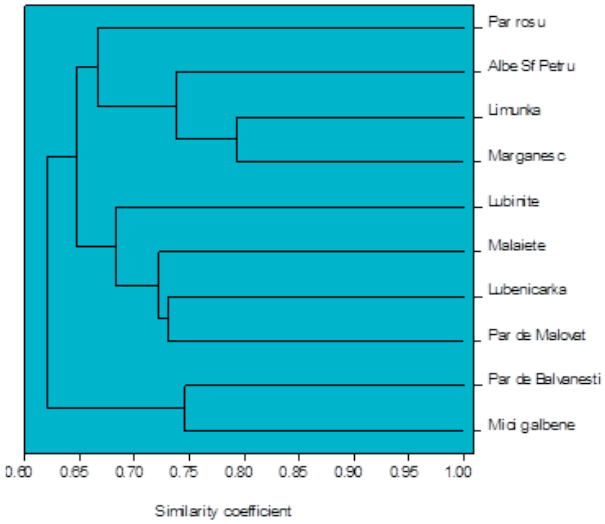

Figure1. UPGMA clustering of pear landraces using RAPD primers

Table 3. Similarity matrix between pear landraces using RAPD primers

Landraces	1	2	3	4	5	6	7	8	9
1. Par rosu	1								
2. Lubinite	0.5397	1							
3. Malaiete	0.6190	0.6984	1						
4. Albe de Sf.Petru	0.6508	0.6032	0.6508	1					
5. Limunka	0.7825	0.6032	0.5873	0.7460	1				
6. Lubenicarka	0.6825	0.6349	0.7143	0.6190	0.6825	1			
7. Par de Balvanesti	0.6190	0.6032	0.5873	0.6508	0.6825	0.5556	1		
8. Par de Malovat	0.6349	0.7143	0.7302	0.6349	0.6984	0.7302	0.5714	1	
9. Mici galbene	0.5873	0.7302	0.7460	0.6508	0.5556	0.5556	0.7460	0.5714	1
10. Marganesc	0.6667	0.6508	0.6984	0.7302	0.7937	0.7302	0.7467	0.7143	0.6032

CONCLUSIONS

RAPD markers can be used in studies concerning the genetic variability in pears. The RAPD primers generated 63 bands, and 45 of them were polymorphic, with an average of 12.4 amplicons/primer. The polymorphic bands, registered per primer ranged from 5 (OPA18) to 10 (primer OPA12). Total polymorphism generated by a certain primer (PIC), registered values between 0.39 to P11 and 0.54 to OPA18. The discrimination index (PI), presented values among 2.72 for the primer OPA18 and 5.38 for P-25 primer, which had the highest capacity to generate polymorphic bands to genotypes being studied. Results showed the suitability of RAPD analysis in genetic diversity study of pear. The interpopulation similarity for the alleles of the five RAPD markers had values ranging from 53.97% between Păr roşu and Lubiniţe to 79.37% between Limunka and Mărgănesc. The obtained results will be useful for plant breeding programs.

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