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IDENTIFICATION OF GENETIC DIVERSITY AMONG SOME PEARS CULTIVARS WITH ISSR MARKERS

Giancarla VELICEVICI[1], Emilian MADOŞĂ[1], Sorin CIULCĂ[1], Adriana CIULCĂ[1], Dorin CAMEN[1], Carmen BEINŞAN[1], Mihaela MĂLĂESCU[1], Ilinca IMBREA[1], Iuliana CREŢESCU[2]

[1]University of Life Science "King Mihai I" from Timisoara, 119 Aradului Street, Timisoara, Romania
[2]University of Medicine and Pharmacy "Victor Babes", 2 Eftimie Murgu Sq., Timisoara, Romania

Corresponding author email: giancarlavely2000@yahoo.com

Abstract

This study identified genetic diversity in pear cultivars using ISSR techniques. In breeding programs, molecular markers play an important role. Molecular markers are the most efficient tools for the study of taxonomy, genetic variability, phylogenetic analysis, gene tags, gene localization and development of new cultivars. ISSR techniques were used in the identification of the genetic diversity of 10 pear cultivars. Five out of the used primers in this study amplified clear and reproductible bands. The ISSR primers produced 51 bands, and 45 of them were polymorphic, with an average of 10.2 amplicons/primer. The size of the fragments varied from 200 to 1250 bp. The polymorphic bands, registered per primer ranged from 6 (844) to 11 (primer UBC 830). The percentage polymorphism was between 83.33 for UBC808 and 100% for primer 844. Degree of DNA polymorphism was estimated at 89.54% (ISSR). PIC registered values between 0.34 to 844 and 0.75 for UBC814. The primer 844 presented values of discrimination index (PI) 2.06. The obtained results will be useful to serve plant breeding programs.

Key words: *genetic analysis, ISSR markers, pear.*

INTRODUCTION

One of the most diverse and large plant families is the Rosaceae, comprising economically important fruit trees. This one the family consists of over 100 genera and 3,000 species and is considered the third plant family in terms of importance economic importance in temperate regions. (Zarei et al., 2017). The pear with up to 20 species in the world is a species belonging to the *Pyrus* genus of the Rosaceae family of the Rosales team. One of the most widely cultivated fruit crops in the world is the common pear (*Pyrus communis*). In the world, it is the most cultivated fruit species after the apple, (Ünal 2011). Today exist More than 5000 cultivars, although only a small percentage of them are cultivated commercially (Bell et al., 1996). The geographical distribution of pears can be divided into two main groups, including Asian and European pears (Koushesh-Saba et al., 2017; Kumar et al., 2017). European pears are located in Europe, North Africa, Iran, Central Asia, Asia Minor, and Afghanistan, with the most important species *P. communis*. Oriental pears such as the *P. serotina* synonym *P. pryifolia* distributed in Japan have originated from Eastern Asia (Arzani, 2017; Koushesh-Saba et al., 2017; Teng and Tanabe, 2004). Currently, pears are commercially cultivated in more than 50 temperate regions. Over the past decades, efforts have been made to evaluate the genetic diversity of Asian and European pears and other *Pyrus* species using biochemical, morphological, and molecular markers (Koushesh-Saba et al., 2017; Nikzad Gharehaghaji et al., 2014a).Molecular markers have been used for studying the genetic diversity, relationships, and origins of the cultivars, as well as for cultivar discrimination and fingerprinting of several fruit crops (e.g., Cervera et al., 1998; Dirlewanger et al., 1998; Fang and Roose, 1997; Gianfranceschi et al., 1998; Hokanson et al., 1998; Koller et al., 1993). Molecular markers have many advantages compared with phenotypic markers because they are stable, detectable in all tissues irrespective of growth, development and differentiation, and remain unaffected by

fluctuations in environmental conditions, cultural impacts and pleiotropic effects (Gosal et al., 2010). For cultivar identification and taxonomic relationship studies in pears have already been used (Botta et al., 1998; Oliveira et al., 1999; Monte-Corvo et al., 2000) polymerase chain reaction (PCR)-based molecular markers such as randomly amplified polymorphic DNA (RAPD) (Williams et al., 1990) and amplified fragment length polymorphisms (AFLPs) (Vos et al., 1995). For genome studies inter-simple sequence repeat (ISSR) amplification is another microsatellite-based technique useful (Zietkiewicz et al., 1994). The study investigated at the molecular level some of the pear genotypes from different counties to obtain more information about their genetic relationships.

MATERIALS AND METHODS

A total of 10 pear cultivars belonging to three counties from West part of Romania were used in this study (Table 1).

Table 1. Biological material

Population	County	Population	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

DNA was isolated from young leaves using method of Doyle and Doyle (1990). The PCR reaction was performed with the following protocol: 42 ISSR cycles (95ºC/30s, 55ºC/30s, 72ºC/90s). Following amplification, the PCR products (10 µL) were loaded in 1.5% 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. Were selected for the analysis, only clear, repetitive DNA fragments. 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.

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

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 locus. 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 genotypes, it was made the dendrogram using the method of clusters average.

RESULTS AND DISCUSSIONS

As a result of reactions carried out with 5 ISSR primers, 45 polymorphic DNA fragments were obtained, their size varying from 200 to 1250 bp. The largest number of polymorphic fragments was produced in reactions with primers UBC 830, UBC 831 and UBC 808. The average of amplicons/primer was 10.2. The polymorphic bands, registered per primer ranged from 6 (844) to 11 (primer UBC 830). The percentage polymorphism was between 83.33 for UBC 808 and 100% for primer 844. Degree of DNA polymorphism was estimated at 89.54% (ISSR). These results were similar to the one presented by Monte-Corvo et al., 2001, where degree of DNA polymorphism was (79.5%). PIC registered values between 0.34 to 844 and 0.75 for UBC 814. The primer 844 presented values of discrimination index (PI)2.06.

Table 2. Polymorphism rate through ISSR primers

	Primer sequence 5'- 3'	No. of fragments amplifyed	Polymor-phic band	% polymor-phism	Pi	PIC $\bar{x} \pm s_{\bar{x}}$
UBC-831	(CT) 8T	11	10	90.9	4.54	0.454±0.044
UBC-808	(AG) 8C	12	10	83.33	4.1	0.41±0.05
UBC-830	(TG) 8G	13	11	84.61	5.08	0.462±0.049
UBC 814	(CT) 8A	9	8	88.88	6	0.75±0.068
844	(CT) 8RC	6	6	100	2.06	0.343±0.044
	Total	51	45	89.54		

Based on genetic similarity, pears populations were hierarchically classified into three clusters between which there is an average diversity of approximately 46%. The first group included four populations that possess approximately 65% of the common alleles of the five primers. The populations Par roşu and Mălăiețe, genetically similar to a degree of 72.55%, make up a first subcluster, while the populations Mici galbene and Mărgănesc differ from each other to a degree of approximately 31%. The second cluster is composed of the Limunka and Păr de Malovaţ populations, between which there is a genetic similarity of 61%. Between the populations of these two clusters, there is an average diversity of approximately 35%. The Lubinite, Albe de SFP, Păr de Bălvăneşti, and Lubenicarka populations represent a separate group that possesses approximately 65% of the common alleles of the five primers.

The interpopulation similarity (Table 3) for the alleles of the five ISSR primers had values ranging from 27.45% between Par de Malovat and Lubinițe to 78.43% between Par de Bălvăneşti and Albe de SfP.

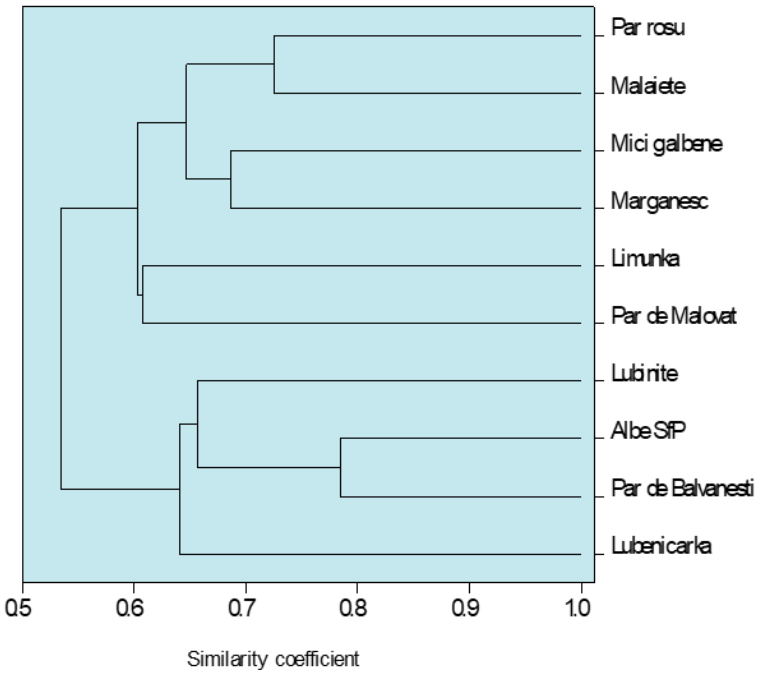

Figure.1. UPGMA clustering of pears population using ISSR primers

Table 3. Similarity matrix between pear cultivars using ISSR primers

Population	1	2	3	4	5	6	7	8	9
1. Par rosu	1								
2. Lubinite	0.4314	1							
3. Malaiete	0.7255	0.4314	1						
4. Albe de Sf. Petru	0.6078	0.6275	0.5686	1					
5. Limunka	0.6078	0.4706	0.6471	0.4902	1				
6. Lubenicarka	0.6275	0.6471	0.6667	0.5882	0.4706	1			
7. Par de Balvanesti	0.6667	0.6863	0.5882	0.7843	0.5098	0.6863	1		
8. Par de Malovat	0.6078	0.2745	0.6863	0.4902	0.6078	0.4706	0.4314	1	
9. Mici galbene	0.5882	0.6471	0.6275	0.5882	0.549	0.5294	0.6078	0.5098	1
10. Marganesc	0.7059	0.5294	0.6667	0.5490	0.5882	0.6078	0.5686	0.6275	0.6863

Table 4. Analysis of variance for pear populations concerning the bands of ISSR primer

Population	Between groups		Within groups		F test
	SS	DF	SS	DF	
1. Par rosu	0.20	1	9.96	49	0.98
2. Lubinite	9.83	1	2.80	49	172.27**
3. Malaiete	0.50	1	10.48	49	2.36
4. Albe de Sf. Petru	0.95	1	10.03	49	4.63*
5. Limunka	0.16	1	12.00	49	0.64
6. Lubenicarka	1.30	1	10.63	49	5.98*
7. Par de Balvanesti	1.92	1	8.67	49	10.86**
8. Par de Malovat	4.32	1	7.83	49	27.05**
9. Mici galbene	1.97	1	9.37	49	10.29**
10. Marganesc	0.05	1	8.57	49	0.31

Regarding the populations being studied (Table 4), a notable contribution to the total variability regarding the spectrum of the different fragments amplified by the ISSR primers was observed in the case of the Lubinite and Par de Malovat populations, which is highlighted by a different allelic structure. The lowest variance values were recorded in the Mărgănesc and Păr roşu populations. The highest variability of the polymorphic bands within the first cluster was recorded in the Mălăieţe population, respectively in the Limunka population for the second cluster. Lubenikarka and Albe de Sf. Petru populations show a high influence on diversity at the level of the third cluster.

Table 5. Correlation coefficients between the similarity matrices given by different ISSR primer

Primer	UBC808	UBC830	UBC814	UBC844
UBC831	0.052 *p=0.733*	0.186 *p=0.22*	-0.091 *p=0.551*	0.418** *p=0.004*
UBC808		0.069 *p=0.648*	0.245 *p=0.105*	0.111 *p=0.466*
UBC830			-0.120 *p=0.432*	0.087 *p=0571*
UBC814				0.386** *p=0.009*

The low and statistically uncertain value of the correlation coefficient between the genetic similarity matrices identified using the different ISSR primers, attests that in general, the respective primers provide complementary information regarding the diversity of the analyzed pears populations.

CONCLUSIONS

The largest number of polymorphic fragments was produced in reactions with primers UBC 830, UBC 831 and UBC 808. The percentage polymorphism was between 83.33 for UBC 808 and 100% for primer 844. Degree of DNA

polymorphism was estimated at 89.54% (ISSR). Based on genetic similarity, pears populations were hierarchically classified into three clusters between which there is an average diversity of approximately 46%. The first group included four populations that possess approximately 65% of the common alleles of the five primers. The second cluster is composed of the Limunka and Păr de Malovaţ populations, between which there is a genetic similarity of 61%. The Lubinite, Albe de SFP, Păr de Bălvăneşti, and Lubenicarka populations represent a separate group that possesses approximately 65% of the common alleles of the five primers. The obtained results with the ISSR analysis will be useful for plant breeding programs.

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