



Preliminary identification of pear accessions of ‘Lubeničarka’ group using RAPD markers

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Summary

The preliminary identification of six pear (*Pyrus communis* L.) accessions of the ‘Lubeničarka’ group in the collection of the Republic of Srpska Gene Bank was done in order to compare their genetic similarity. Pear accessions were analysed using Randomly Amplified Polymorphic DNA (RAPD) markers. Pear accessions of the ‘Lubeničarka’ group: ‘Lubeničarka’, ‘Lubeničarka Milići’, ‘Lubeničarka Zvornik’, ‘Lubeničarka Vlasenica’, ‘Krupna Lubeničarka’ and ‘Crna Lubeničarka’, were analyzed using eleven primers which amplified base pairs of the length between 400 and 2,000 bp. The obtained results showed that all accessions can be separated into two groups. The first group are accessions with the coefficient of similarity (1.0), namely ‘Lubeničarka’, ‘Crna Lubeničarka’, ‘Lubeničarka Milići’, ‘Lubeničarka Vlasenica’ and ‘Lubeničarka Zvornik’. Only one accession belongs to the second group, namely ‘Krupna Lubeničarka’ accession with genetic similarity coefficient of 0.6.

Keywords

field collection, genetic characterization, genetic similarity, germplasm, duplicates

Introduction

Genetic characterization is a reliable method for determining the degree of genetic similarity of accessions in gene banks. The identification of pear germplasm in gene banks has been successfully performed by applying SSR (Simple Sequence Repeats), AFLP (Amplified Fragment Length Polymorphism) and RAPD (Randomly Amplified Polymorphic DNA) markers. Previous studies for identification of germplasm in gene banks with RAPD markers showed that this method can be successfully used for many species (Kapteyn and Simon, 2002; Bruel et al., 2006; Bayazit et al., 2011; Pavlović et al., 2012; Sadeghi and Cheghamirza, 2012; Marinković et al., 2013; Sharma et al., 2014), but also for pear identification (Botta et al., 1998; Monte-Corvo et al., 2000; Teng et al., 2002; Lin et al., 2011).

Pomological characterisation

Pears of the ‘Lubeničarka’ group have been grown in Bosnia and Herzegovina since ancient times. They are recognizable by their characteristic colour of the flesh which is similar to ripe watermelons (Beširević, 2009). The first pomological characterization of pears of the ‘Lubeničarka’ group (watermelon pear) has been per-

Significance of this study

What is already known on this subject?

- There is a number of pear genotypes with red flesh of fruit under common name ‘Lubeničarka’ in Bosnia and Herzegovina. The pomological characterization done on three genotypes showed clear divergence of one genotype (‘Krupna Lubeničarka’) in relation to other two which also showed some but not clear morphological differences.

What are the new findings?

- Six pear genotypes from ‘Lubeničarka’ group analysed by RAPD markers showed that regardless on morphological variability, this population is classified into two main groups, ‘Krupna Lubeničarka’ with clear pomological characteristics and five genotypes in group ‘Lubeničarka’ with the same coefficient of genetic similarity.

What is the expected impact on horticulture?

- Recognition of genotype ‘Krupna Lubeničarka’ as varieties with stable pomologic and genetic traits. Elimination of duplicate accessions from field Gene Bank.

formed on three genotypes from the Gene Bank of the Republic of Srpska, identified in numerous vegetative progeny as a part of the native assortment of Bosnia and Herzegovina (Mičić et al., 2012). These are, namely, ‘Krupna Lubeničarka’ (common watermelon pear) cultivar which was recommended for the expansion of production at the beginning of the 20th century and two more genotypes, ‘Crna Lubeničarka’ (black watermelon pear) and ‘Bijela Lubeničarka’ (white watermelon pear) that were listed under the common name ‘Lubeničarka’ (Mičić et al., 2012). This study showed different pomological characteristics of ‘Krupna Lubeničarka’ accession as compared to the other ones.

Materials and methods

Plant material

The comparison of the six pear accessions of the ‘Lubeničarka’ group, namely ‘Lubeničarka’, ‘Lubeničarka Milići’, ‘Lubeničarka Zvornik’, ‘Lubeničarka Vlasenica’, ‘Krupna Lubeničarka’ and ‘Crna Lubeničarka’ was done by using RAPD markers. The sampling was performed in the field collection of the Gene Bank of the Republic of Srpska, which is located in the Genetic Resources Institute of the University of Banja Luka.

DNA extraction

The DNA was extracted from young leaves, following a modified CTAB protocol described by Williams et al. (1991). Concentration of DNA was determined spectrophotometrically at the wavelength of 260 nm. For future analyses, DNA dilutions of 20 nm μl^{-1} were prepared.

PCR condition

The PCR amplification was carried out in a total volume of 25 μl . Each reaction contained 20 ng of DNA, 10 \times PCR buffer (Fermentas), 0,2 mM of each of four nucleotides (Fermentas), 3,5 mM MgCl_2 , 0,5 μM primer (Metabion) and 0,25 U Taq DNA polymerase (Fermentas).

The amplification was performed in a thermocycler, model SureCycler 8800 (Agilent Technologies, USA). Eleven decamer primers were used in order to establish presence or absence of polymorphism between the accessions, by performing molecular analysis. The first step of the PCR reaction was carried out at 94°C for 5 min. The DNA amplification was done in 40 cycles of denaturation (30 s at 94°C), annealing (30 s at 37°C) and extension (1 min at 72°C). After completion of all cycles, the final elongation step was performed at 72°C for 8 min. The amplification products were separated by electrophoresis on 1% agarose gels in 1 \times TBE buffer at 90 V for 120 min. The gels were stained with ethidium bromide and visualised by UV transillumination.

Statistics

The electrophoregram analysis was performed and amplified bands were given numerical value "1", while non-amplified loci were given numerical value "0". In this way, the input data were provided for the analysis of similarity/dissimilarity of the observed accessions. The coefficient of genetic similarity was calculated according to Jaccard (Jaccard, 1908) and phylogenetic tree was generated by cluster analysis using SPSS software.

Results

RAPD analysis

In examining genetic similarity between the six accessions of the 'Lubeničarka' group, 41 bands were amplified using 11 oligonucleotide primers (Table 1). The percentage of polymorphism between the analysed accessions ranged from 25%–50%. The highest degree of polymorphism (50%) was observed for primers OPA-01, OPA-15 and OPM-01. The lowest level of polymorphism (25%) was found

Table 1. List of the selected primers and the degree of polymorphism obtained among 6 pear accessions from 'Lubeničarka' group.

Primer	Nucleotide sequence	Number of amplified bands	Percentage of polymorphic bands
OPA-01	CAGGCCCTTC	4	50
OPA-05	AGGGGTCTTG	3	33
OPA-08	GTGACGTAGG	3	33
OPA-11	CAATCGCCGT	5	40
OPA-15	TTCCGAACCC	4	50
OPD-06	ACCTGAACGG	3	33
OPD-11	AGCGCCATTG	4	25
OPD-14	CTTCCCCAAG	3	33
OPM-01	GTTGGTGGCT	4	50
OPM-02	ACAACGCCTC	3	33
OPM-05	GGTGGTCAAG	5	40

with primer OPD-11. Lengths of fragments in base pairs were determined by comparing the amplified DNA fragments with fragments of known length (marker GeneRuler TM100 bp Plus DNA Ladder). The lengths of DNA fragments obtained by PCR with 11 primers in the 6 pear accessions ranged from 400 to 2.000 base pairs (Table 1).

By analysing the electrophoregrams, numerical values were used to calculate the coefficient of genetic similarity by Jaccard (Table 2).

Based on the similarity coefficient, the six pear accessions can be divided into two groups. The first group are accessions which showed no difference between them, i.e., they have the same coefficient of genetic similarity. It refers to the following accessions: 'Lubeničarka', 'Lubeničarka Milići', 'Lubeničarka Zvornik', 'Lubeničarka Vlasenica' and 'Crna Lubeničarka', with genetic similarity coefficient of 1.0. The genetic similarity coefficient of 'Krupna Lubeničarka' is 0.60 and it is classified into the second group because it is different from the above-mentioned accessions (Figures 1 and 2).

Table 2. Coefficient of the Similarity between six pear accessions from 'Lubeničarka' group according to Jaccard.

Accession*	Coefficient of the Similarity					
	L	LM	LZ	LV	LC	LK
L	1.00					
LM	1.00	1.00				
LZ	1.00	1.00	1.00			
LV	1.00	1.00	1.00	1.00		
LC	1.00	1.00	1.00	1.00	1.00	
LK	0.60	0.60	0.60	0.60	0.60	1.00

* L: 'Lubeničarka'; LM: 'Lubeničarka Milići'; LZ: 'Lubeničarka Zvornik'; LV: 'Lubeničarka Vlasenica'; LC: 'Lubeničarka Crna'; LK: 'Lubeničarka Krupna'.

Discussion

A low degree of polymorphism was recorded in this study. Of the six analysed pear accessions, 5 had the same genotype, while 'Krupna Lubeničarka' accession was clearly separated from the others. Accessions which had same coefficient of genetic similarity were collected from the municipalities located in the eastern part of the Republic of Srpska.

According to Đurić et al. (2009, 2014) there have been frequent migrations in this region, and fruit tree seedlings were taken to new areas where the transferred varieties often received new names. Thus, over years, numerous private garden collections of fruit trees have been established in Bosnia and Herzegovina, where the same cultivar exists under different names and often different cultivars occur with the same name. Studies conducted since 2004 have proved this fact since a large number of different accessions have been found in a small area (Đurić et al., 2009). The genetic characterization of this rich gene pool is necessary before the establishment of collections in order to prevent duplicate accessions in the gene bank. The results of genetic characterization of pear accessions of the 'Lubeničarka' group (Figures 3–5) established that 'Krupna Lubeničarka' is a separate genotype and confirmed the results obtained by morphological and pomological characterization of ac-

cessions of this group (Mičić et al., 2012), i.e., that 'Krupna Lubeničarka' has stable pomological features which clearly and reliably determine this variety.

Previous studies of morphological and pomological characterization of pears from the 'Lubeničarka' group (Mičić et al., 2012) showed that 'Crna Lubeničarka' and 'Bijela Lubeničarka' genotypes are characterised by certain pomological distinctions that clearly make them different, but also by some similarities, the variability of which raises the question of their reliable pomological and genetic characterization. In this study, accessions of the 'Lubeničarka' group which exhibit certain morphological differences do not differ at this level of observation.

By applying the RAPD markers, it is possible to determine the level of genetic similarity in order to eliminate duplicate accessions in the pear collections and these markers can be used for preliminary identification of pear cultivars before their multiplication and introduction to a collection. Coupled with the morphological characterization which preceded this study, it was confirmed that 'Krupna Lubeničarka' accession is different in pomological characteristics and genetic structure from the other accessions of the 'Lubeničarka' group. In future research, it is necessary to apply other types of molecular markers which produce a larger degree of polymorphism.

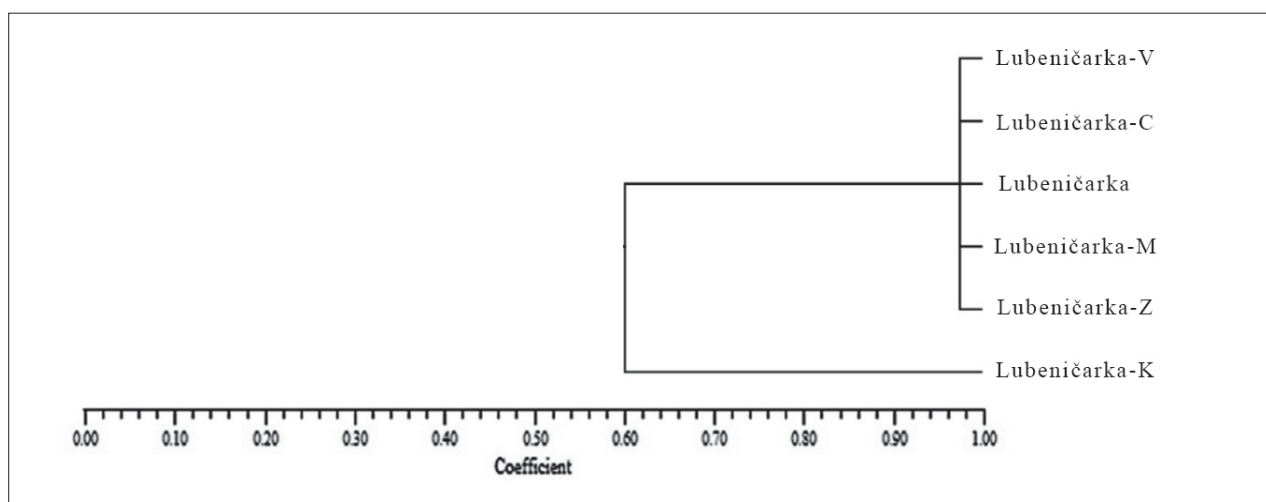


Figure 1. Dendrogram generated using UPGMA analysis, showing genetic relationship of 6 pear accessions.

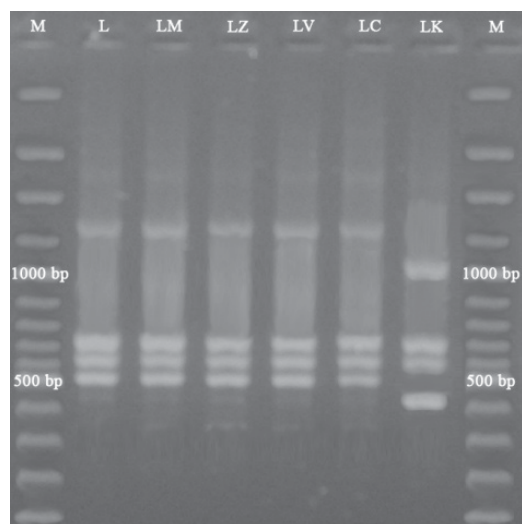


Figure 2. RAPD profile for 6 accessions of pear from 'Lubeničarka' group obtained with primer OPA-01 (M: marker GeneRuler TM100 bp Plus DNA Ladder; L: 'Lubeničarka'; LM: 'Lubeničarka Milići'; LZ: 'Lubeničarka Zvornik'; LV: 'Lubeničarka Vlasenica'; LC: 'Lubeničarka Crna'; LK: 'Lubeničarka Krupna').



Figure 3. Pear accession 'Krupna Lubeničarka' (Photo: N. Mičić, orig.).



Figure 4. Pear accession 'Crna Lubeničarka' (Photo: N. Mičić, orig.).

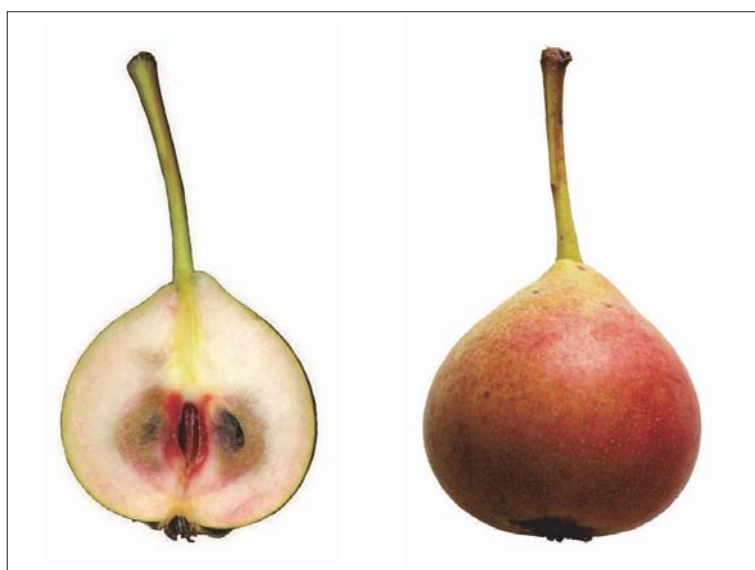


Figure 5. Pear accession 'Lubeničarka' (Photo: N. Mičić, orig.).

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