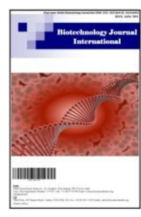

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Genetic Characterization of Domestic Apple Varieties from Tuzla Canton, BiH

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Authors' contributions

This work was carried out in collaboration among all authors. Author ES designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors BS and AH managed the analyses of the study. Authors AA, AD and EI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The basic precondition for apple breeding is the genetic diversity of varieties, which implies a large number of different, positive genes that enable adaptation to different weather conditions, resistance to new diseases and pests. One of the reliable sources of genetic diversity are indigenous varieties of Bosnia and Herzegovina. Their genetic identification is the first step in a process that has as its ultimate goal the collection of genetic material. The main goal of this research is to analyze the genetic variability of five indigenous varieties of apple in Tuzla Canton, Bosnia and Herzegovina in terms of contributing to the management of conservation and expansion of existing genetic resources, spreading this material through registered nurseries.
Study Design: The research included fiveautochthonous apple varieties: "Ovčji nos“ “Dobrić“, "Šarenika“, "Rančica“ and "Petrovača“.
Place and Duration of Study: Samples of young leaves were collected at the site of Donji Moranjci, City of Srebrenik,Tuzla Canton, Bosnia and Herzegovina in the spring of 2019.
Methodology: In order to determine the genetic diversity of five indigenous apple varieties were genotyped ten SSR(Simple Sequence Repeats) markers.

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Results: No cases of synonyms or homonyms were found within the analyzed set.The results of the study indicate a pronounced differentiation, ie all five examined varieties represent unique genotypes.
Conclusion: The examined sets of genotypes possess significant genetic variability, which is important especially when we consider that a relatively small number of samples have been analyzed.

Keywords: Autochthonous apple varieties; genetic diversity; SSR markers.

1. INTRODUCTION

Apple (*Malus sp.)* has a wide range of distribution, it is grown on all continents, in all climates and in all agro-ecological conditions [1]. Many varieties and rootstocks for apples have been obtained by breeding, which are of great economic importance and without which today's apple production cannot be imagined. The most work is done on apple breeding in the world, more than on any other fruit species. The basic precondition for apple breeding is genetic diversity, which implies a large number of different, positive genes that enable apples to adapt to different weather conditions, resistance to new diseases and pests. One of the reliable sources of genetic diversity is the traditional, indigenous varieties of Bosnia and Herzegovina.

For the study of genetic diversity and identification of varieties not only of apples, but also of other plant species, microsatellite markers or simple repeating sequences (SSR - Simple Sequence Repeats) are most often analyzed.Microsatellites are regions of DNA that consist of successive repeats of short nucleotide sequences [2,3,4,5] in all eukaryotic genomes. They serve as indicators of the presence or absence, and often allelic form of a particular gene in the genome of the analyzed varieties. Polymorphism in these markers is due to variations in the number of repeating motives due to a replication error that results in the loss or addition of a repeating motive, which is more common than a point mutation, so these markers are hypervariable. In addition, microsatellites are codominant, frequent, and scattered throughout the genome and are a good tool for genome mapping, population studies, and species and variety identification. The use of SSR markers is based on PCR amplification of repeating nucleotide sequences using a unique set of primers [2,3]. Molecular markers allow the assessment of plant genetic diversity, the detection of duplicates or possible synonyms and / or homonyms, and the ability to manage the collection of plant genetic resources. Several studies based on molecular markers have assessed the diversity of domestic apple varieties in Bosnia and Herzegovina [4,5].

Germplasm represents the total amount of available hereditary material of a particular species and its relatives. Collecting fruit germplasm is imposed as a necessity because there may be a loss of initial genetic material that should be used for further breeding purposes [6].

Nowadays, producers strive to grow highly productive varieties, of which there are very few, to specialize in the choice of growing certain varieties because of their market value, and to make as much profit as possible. As a result, genetic uniformity occurs, ie reduction of genetic variability, which can have catastrophic consequences. The presence of genetic variability of domestic apple varieties is essential for their future improvement, providing breeders with the opportunity to improve existing and create new varieties and hybrids. phenotypic and molecular characterization of varieties. For this purpose, it is necessary to perform identification and inventory of genetic profiles in order to define the material that should be preserved or collected.

The main goal of this research is to analyze the genetic variability of five indigenous apple varieties in the Tuzla Canton, Bosnia and Herzegovina in terms of contributing to the conservation and expansion of existing genetic resources.

2. MATERIALS AND METHODS

The research included five autochthonous apple varieties: „Ovčji nos“, „Dobrić, Šarenika“, „Rančica“ and „Petrovača“ from the area of Tuzla Canton, BiH. Samples of young leaves were collected at the site of Donji Moranjci, City of Srebrenik in the spring of 2019.

2.1 Sampling

For each examined variety, 10 healthy, undamaged leaves were taken. The material was dried in a digester with homogeneous air flow for 10 days, in the laboratory of the Department of

Biology, Faculty of Science, University of Tuzla. The samples were then stored in paper bags with silica beads and transported to the Institute of Genetic Engineering and Biotechnology, Sarajevo, INGEBINGEB for further molecular genetic analysis.

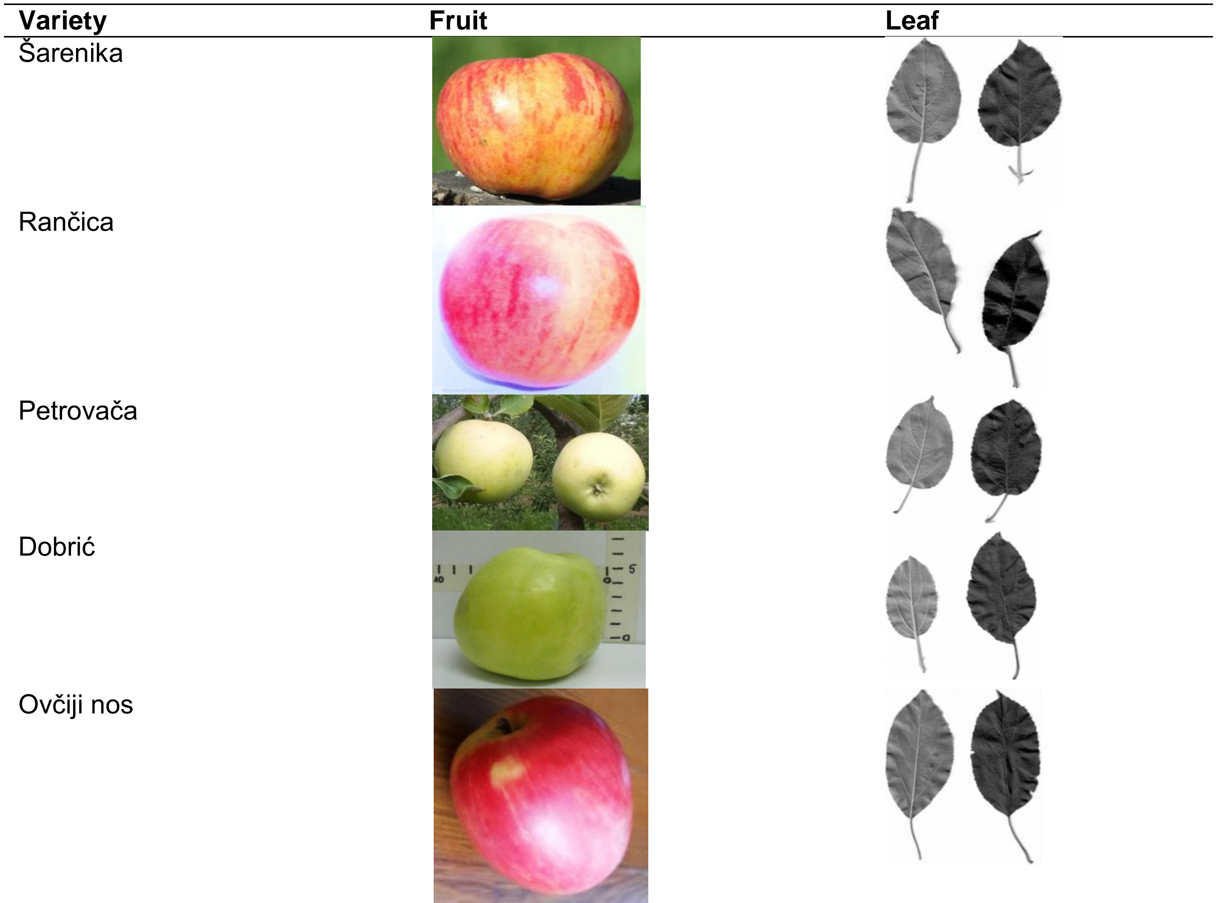
2.2 Isolation of Genomic DNA from Apple Tree Leaves

After shredding the plant tissue, genomic DNA molecule extraction was performed with the Soltis Lab CTAB DNA kit according to the appropriate protocol. In the first isolation step, the samples were incubated in lysis buffer overnight. Then after adding 24:1 chloroform: Isoamyl alcohol the samples were centrifuged. The upper phase supernatant was transferred to a new tube, and after the addition of 7.5M ammonium acetate and isopropanol was placed on ice and centrifuged again. The precipitate was washed with 70% and absolute ethanol, followed by drying of the DNA precipitate at the bottom of the tube. DNA was dissolved in ddH2O and used for amplification.

2.3 SSR Analysis

For amplification of DNA, KAPA3G Plant PCR(Kapa Biosystems) kit was used according to the appropriate protocol. Polymerase chain reaction (PCR) amplifications of microsatellite sequences took place in GeneAmp PCR System 9700 (Applied Biosystems, United States).

To confirm the authenticity of varieties and determine the genetic diversity, ten microsatellite markers used in studies on apple genotyping were used: CHO1H02 (*), CH01H10 (*), CH02CO2a (**), CH0fivee03 (**), CHO1HO1 (*), CH04E02 (**), CH02D08 (* *), CH02CO2B (**), CH0fiveE04 (*) and CH02CO06 (*) The names and sequences of the primers, as well as the conditions for carrying out PCR are described earlier [7,8]. Detection of DNA profiles and fragment sizes was performed using fluorescently labeled primers with the internal standard GeneScan-five00 LIZ, and PCR products were analyzed on.



Picture 1. Autochthonous apple varieties

Picture 2. Collection plant

Automatic sequencer ABI PRISM ®3500 (Genetic Analyzer 3500, Applied Biosystems) with 8 capillaries 50 cm long, which allows separation and analysis of DNA fragments on the principle of capillary electrophoresis and as a detection by CCD camera. All electrophoresis data were collected in the ABI PRISM® Collection software.

2.4 Biostatistical analysis

Biostatic analysis involved calculating the number of detected alleles (DA) and expected (EH) heterozygosity [9] in computer program SPAGeDI 1.3 [10]. The effective allele number (AE) was calculated in the computer program GenAlEx [11]. UPGMA cluster analysis based on the Jaccard similarity coefficient was used to determine similarities between genotypes and was performed in the computer program MEGA 6 (Molecular Evolutionary Genetics Analysis), [12].

3. RESULTS AND DISCUSSION

In all analyzed microsatellite locus, amplification was successful. In general the size of the amplified DNA fragments scored ranged from 88 to 253 bp, Table 1. The average number of detected alleles per locus was 4.9, Table 2. A slightly higher number of alleles per locus 11.3 was recorded in a study conducted by Gasi et al. [13] examining the genetic structure of apples maintained in an ex situ plantation in Bosnia and Herzegovina [10]. Higher values of 13.5 were also recorded by Gasi et al. [13] examining the genetic diversity of apples maintained on farms in Sarajevo and eastern Bosnia. In the study from Kashmir valley [14] a highly informative set of 29 SSR primers was used to distinguish 19 apple cultivars and reported that the average number of alleles per SSR was 7.51.

Effective number of alleles (EA), number of detected alleles per locus (DA), ratio between effective and detected number of alleles per locus (EA / DA) and expected (EH) heterozygosity for 10 SSR locus also shown in Table 2. Among the investigated samples, the mean values of detected alleles (DA) and effective alleles (EA) were 4.59 and 3.90, respectively. The expected heterozygosity (EH) was within 0.36–0.93.

Regarding the average expected heterozygosity obtained in this study (0,78) (Table 2), as value for gene diversity (Nei's genetic diversity) NEI, (1978) [15], it was the same value reported by GASI et al. (2010) (0.78) but somewhat smaller reported by URRESTARAZU et al. (2016) (0.82) [15] and GASI et al. (2013) (0.80).

In order to determine the genetic diversity between the analyzed genotypes, a dendrogram was made based on the allele frequency of the examined microsatellite locus.

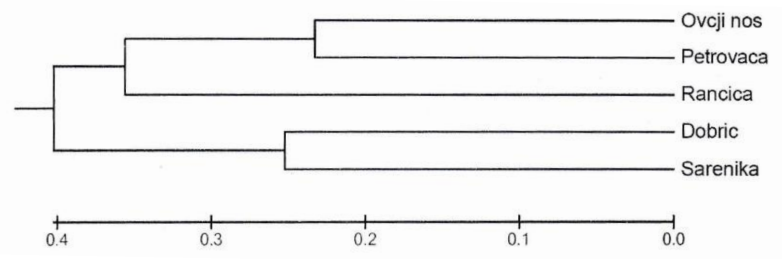
The obtained dendogram indicates a pronounced differentiation, ie each of the five examined varieties represents a unique genotype.

Table 1. Genetic profiles of tested samples by analyzed microsatellite locus

SAMPLE	CH01H02	CH01H02	CH01H10	CH01H10	CH02C02a	CH02C02a	CH0fiveE03	CH0fivee03	CH01H01	CH01H01	CH04e02	CH04e02	CH02D08	CH02C08	ECH0C02B	ECH0fiveE0B	CH0fiveE04	CH0fiveE04	CH02C06	CH02C06
Ovčiji nos	233	247	102	113	144	144	172	172	111	139	1five8	1five8	216	223	109	109	160	160	234	2five3
Dobrić	233	24five	88	9five	180	180	172	172	129	129	1five8	164	208	221	109	113	1five6	1five6	241	2five1
Šarenika	24five	24five	9five	107	160	180	161	16five	117	117	1five8	1five8	204	212	109	113	1five6	1five6	226	249
Rančica	231	231	98	98	167	167	186	192	113	113	1five4	1five8	204	216	109	109	1five8	1five8	226	249
Petrovača	233	233	98	102	144	178	161	172	111	111	1five0	1five0	218	218	109	109	168	168	214	249

Table 2. Range of detected alleles in base pairs, number of detected alleles per locus (DA), effective number of alleles (EA), ratio between effective and detected number of alleles per locus (EA / DA), expected (EH) heterozygosity for 10 SSR locus on five tested apple samples

Locus	Range	DA	EA	EA/DA	EH
CH01H02	231/247	4	3,3	0,83	0,78
CH01H10	88/113	6	five,0	0,83	0,89
CH02C02a	144/180	five	4,2	0,84	0,84
CH0fivee03	161/192	five	3,1	0,62	0,76
CH01H01	111/139	five	4,five	0,90	0,87
CH04e02	1five0/164	4	2,4	0,60	0,64
CH02D08	204/223	7	6,3	0,90	0,93
CH02C02B	109/113	2	1,five	0,7five	0,36
CH0fiveE04	1five6/168	4	3,6	0,90	0,80
CH02C06	214/2five3	7	five,6	0,80	0,91
Average value		**4,9**	**3,9**	**0,80**	**0,78**



Picture 3. UPGMA cluster analysis based on Jaccard's similarity coefficient, performed based on polymorphism of 10 SSR locus within five apple samples

4. CONCLUSION

The results of the research enabled us to identify five analyzed autochthonous apple varieties: „Ovčji nos“, „Dobrić“, „Šarenika“, „Rančica“ and „Petrovača“ from the area of Tuzla Canton, Bosnia and Herzegovina as unique genotypes. No cases of synonyms or homonyms were found within the analyzed set. The examined sets of genotypes possess significant genetic variability, which is important especially when we consider that a relatively small number of samples have been analyzed. Indicating heterogeneous genetics, the results could be useful for future breeding programs of commercial varieties. It would be interesting to conduct further research on these indigenous varieties in terms of the application of markers related to genes of interest for breeding programs. Intensive production strives to use highly productive varieties, which give extremely high quality fruits and the goal of apple breeding is to make the highest quality varieties. However, having in mind the necessity of preserving the germplasm of autochthonous varieties, the results also impose the importance of their preservation and collection in order to avoid the loss of initial genetic material in breeding.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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