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GENETIC VARIABILITY STUDY OF ROMANIAN SWEET CHERRY GENOTYPES PRESENT IN THE USAMV OF BUCHAREST ORHARD COLLECTION

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Abstract

Sweet cherry, Prunus avium L. belongs to the Rosaceae family, and nowadays is cultivated worldwide in temperate climates. Currently, plant breeders are using molecular markers, such as RAPD to reduce the time required to create new hybrids and valuable varieties. In the present study, seven RAPD markers were used to study the genetic variability and genetic relationships between 15 sweet cherry genotypes present in the orchard collection of USAMV Bucharest, 14 Romanian ('George', 'Ludovan', 'Bucium', 'Maria', Iașirom', Cociuvaș', 'Alexus', 'Andreiaș', 'Rubin', 'Severin', 'Paulică', 'Boambe de Cotnari', 'Cetățuia', and 'Putna') and the Swedish cultivar 'Rivan'. All RAPD markers proved to be polymorphic, allowing for amplification of 144 loci, with P63 amplifying the highest number of loci (27). The UPGMA dendrogram build on RAPD data grouped the genotypes studied into 2 clusters, one cluster containing mostly descendent of 'Van' and 'Boambe de Cotnari', and the second cluster grouping varieties with German genitors. The present study demonstrates the genetic variability among the Romanian genotypes present in the USAMV of Bucharest orchard collection.

Key words*: genetic diversity, Prunus avium L., genotypes, Random Amplification of Polymorphic DNA.*

INTRODUCTION

The cultivation and use of cherries, *Prunus avium* L., dates back thousands of years, the earliest recorded use of cherries tracing back to the ancient Greeks and Romans (Dirlewanger et al., 2007). Evidence suggests that cherries were known to the Greeks as early as 300 BC, and they were a popular fruit among the Romans, who are credited with spreading cherries throughout Europe and parts of Britain. During the Middle Ages, cherry cultivation continued to be popular in Europe (Campoy et al., 2016; Livarda, 2008). European settlers brought cherry trees to North America in the 1600's, where they became established and started to be widely cultivated and later spread throughout the world (Iezzoni et al., 2017). Romania is among the top countries producing cherries worldwide, most of the commercial sweet cherry orchards being located in the south and north-east of the country (Bujdosó & Hrotkó, 2017)

Over time, selective breeding and agricultural practices have led to the development of the many varieties of cherries we have today. As cherry cultivation was so popular, many European *ex situ* collections include also wild relatives, since these can be a pool of extremely valuable genes, especially in the context of climate change (Antić et al., 2020; Quero-García et al., 2017). The ongoing climate changes force breeders to accelerate the creation of new cultivars that can withstand the new harsh conditions, and this is possible only by incorporating molecular techniques alongside traditional breeding methods (Vicente, 2022).
One of the above-mentioned molecular techniques is Random Amplification of Polymorphic DNA (RAPD) (Udrișteș & Bădulescu, 2019), developed in 1990 (Williams et al., 1990), that uses a single random decamer primer that will attach to both forward and reverse DNA strands, and will amplify the sequences between two annealing sites (Babu et al., 2021).

RAPD technique has multiple applications, from the study of genetic diversity among populations, varieties, species, genera, to checking the genetic stability of plants grown in vitro, study of genetic relationships among cultivars, and cultivar identification within collections (Antić et al., 2020; Ben Tamarzizt et al., 2015; Berindean et al., 2016; Bramhanapalli et al., 2017; Iancu & Chivu, 2021; Zarei et al., 2017).

The goal of the present study is to reveal the genetic variability and genetic relationships among fifteen sweet cherry accessions present in the USAMV of Bucharest orchard collection.

MATERIALS AND METHODS

Plant material

In the present study were used 15 sweet cherry accessions from the orchard collection of USAMV Bucharest, 14 Romanian ('George', 'Ludovan', 'Bucium', 'Maria', Iașirom', Cociuvaș', 'Alexus', 'Andreiaș', 'Rubin', 'Severin', 'Paulică', 'Boambe de Cotnari', 'Cetățuia', and 'Putna') and the Swedish variety 'Rivan' (Figure 1).

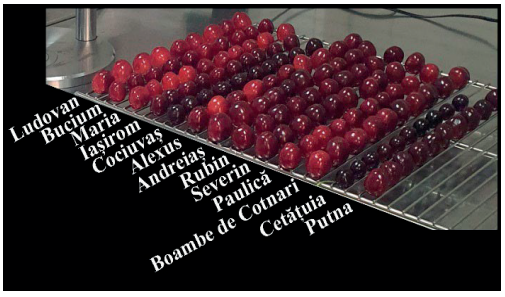

Figure 1. Fruits of 13 sweet cherry varieties

Genomic DNA extraction

Genomic DNA was extracted from young leaves using the Innu PREP Plant DNA I KIT IPC 16 Kit (Analytik Jena) according to the manufacturer instructions, based on the optimization reactions described before (Ionescu et al., 2022). Briefly, tissue was ground to powder with liquid nitrogen, then mixed with SLS lysis solution, and proteinase K, incubated for 1 hour at 60°C, centrifuged to remove plant tissue debris, supernatant treated with RNase A, and transferred to plate for automatic DNA extraction in the InnuPURE C16 System, using the Ext_Lysis_200_C16_04 program.

DNA quality and quantity were checked with Nanodrop 1000 (Biorad).

Measurement of DNA quantity and quality

DNA concentration and quality, based on the A260/A280 and A260/A230 absorbance ratios, were measured with the NanoDrop 1000 spectrophotometer (Biorad).

RAPD reaction

Polymerase chain reaction (PCR) was done with the Platinum™ II Hot-Start PCR Master Mix (2X) (Invitrogen) according to the manufacturer's instructions. PCR reactions were set up in a 10 µl total volume, a final concentration of 2 ng/µl RAPD primer, and 1 ng/µl genomic DNA. For all primers used the annealing step was done at 32ºC. The seven RAPD primers used were P59, P60, P61, P63, P64, P65, and P66, their sequence being presented in Table 1.

Table 1. RAPD primers nucleotide sequence

Primer	DNA sequence
P59	5'-GTTGGTGGCT-3'
P60	5'-GGGAACGTGT-3'
P61	5'-CCGTGACTCA-3'
P63	5'-TGCCGAGCTG-3'
P64	5'-AGGTGACCGT-3'
P65	5'-GTTGGTGGCT-3'
P66	5'-GGGAACGTGT-3'

DNA fragments amplified by the RAPD reactions were separated on 1.5% agarose gel and visualized with the Pharox FX system (BioRad).

Data analysis

The lengths of the amplicons were measured with the Quantity One software (Version 4.6.9., BioRad), based on the 1 Kb Plus DNA Ladder (Invitrogen). Data were converted into a binary matrix and scored as present (1) or absent (0) in a *.csv file, and later analysed with the BIO-R software (Biodiversity Analysis with R for Windows), version 3.0.

RESULTS AND DISCUSSIONS

All decamer primers were polymorphic, and the DNA fragments amplified by PCR had lengths between 170 and 2950 bp, with total number of 144 loci (Table 2). The highest number of loci were observed for the primer P63 (27), and the lowest number of loci was observed for the primer P64 (12).

Table 2. The number of loci and amplicons' lengths corresponding to each decamer primer used

Primer	Number of f loci	Amplicon sizes (bp)
P59	20	1500, 1450, 1200, 1050, 1000, 870, 810, 770, 730, 700, 670, 630, 610, 580, 560, 530, 480, 440, 410, 390
P60	24	2950, 2800, 2400, 2160, 1920, 1800, 1750, 1600, 1500, 1300, 1200, 970, 880, 820, 750, 650, 610, 560, 470, 430, 400, 320, 280, 250
P61	25	2450, 2170, 2000, 1700, 1620, 1530, 1400, 1350, 1250, 1060, 940, 900, 800, 740, 670, 620, 550, 500, 450, 400, 350, 300, 250, 200, 170
P63	27	2670, 2280, 2100, 1990, 1820, 1750, 1650, 1500, 1400, 1300, 1200, 1170, 1050, 900, 870, 810, 760, 740, 700, 650, 610, 550, 530, 480, 420, 400, 360
P64	12	2300, 1500, 1150, 1050, 870, 680, 600, 570, 470, 440, 300, 200
P65	17	2550, 2100, 1800, 1420, 1200, 1100, 950, 850, 700, 600, 550, 500, 430, 400, 350, 320, 300
P66	19	270, 2050, 1850, 1600, 1500, 1260, 1160, 1150, 1060, 900, 850, 730, 630, 590, 530, 440, 400, 220, 190

The calculated Roger's genetic distances are represented in Table 3. The shortest genetic distance, 0.48, is observed between the 'Rivan' and 'Rubin' varieties, whereas the most distantly related are the varieties 'Alexus' and 'Ludovan', followed by 'Alexus' and 'Cetățuia', as it can be also observed from the dendrogram presented in Figure 2.

Table 3. Roger distances calculated with the Bio-R software

NAME	George	Ludovan	Bucium	Maria	Iașirom	Cociuvaș	Alexus	Andreiaș	Rubin	Severin	Paulică	Boambe de Cotnari	Cetățuia	Putna	Rivan
George	0.00	0.57	0.52	0.60	0.51	0.53	0.64	0.59	0.60	0.54	0.53	0.60	0.60	0.61	0.60
Ludovan	0.57	0.00	0.55	0.51	0.61	0.53	0.69	0.63	0.65	0.62	0.57	0.61	0.54	0.61	0.61
Bucium	0.52	0.55	0.00	0.58	0.53	0.51	0.63	0.61	0.60	0.56	0.56	0.60	0.57	0.61	0.57
Maria	0.60	0.51	0.58	0.00	0.62	0.55	0.65	0.65	0.62	0.62	0.55	0.63	0.61	0.62	0.58
Iașirom	0.51	0.61	0.53	0.62	0.00	0.54	0.63	0.54	0.56	0.60	0.59	0.58	0.60	0.58	0.58
Cociuvaș	0.53	0.53	0.51	0.55	0.54	0.00	0.60	0.59	0.61	0.55	0.51	0.61	0.61	0.65	0.59
Alexus	0.64	0.69	0.63	0.65	0.63	0.60	0.00	0.57	0.59	0.60	0.61	0.60	0.67	0.61	0.55
Andreiaș	0.59	0.63	0.61	0.65	0.54	0.59	0.57	0.00	0.58	0.59	0.60	0.58	0.57	0.61	0.57
Rubin	0.60	0.65	0.60	0.62	0.56	0.61	0.59	0.58	0.00	0.55	0.58	0.53	0.60	0.60	0.48
Severin	0.54	0.62	0.56	0.62	0.60	0.55	0.60	0.59	0.55	0.00	0.57	0.58	0.62	0.60	0.54
Paulică	0.53	0.57	0.56	0.55	0.59	0.51	0.61	0.60	0.58	0.57	0.00	0.57	0.63	0.62	0.53
Boambe de Cotnari	0.60	0.61	0.60	0.63	0.58	0.61	0.60	0.58	0.53	0.58	0.57	0.00	0.58	0.60	0.51
Cetățuia	0.60	0.54	0.57	0.61	0.60	0.61	0.67	0.57	0.60	0.62	0.63	0.58	0.00	0.49	0.58
Putna	0.61	0.61	0.61	0.62	0.58	0.65	0.61	0.61	0.60	0.60	0.62	0.60	0.49	0.00	0.55
Rivan	0.60	0.61	0.57	0.58	0.58	0.59	0.55	0.57	0.48	0.54	0.53	0.51	0.58	0.55	0.00

The colour gradient ranges from Blue to Red, short to long distance

The UPGMA dendrogram built with the Bio-R software grouped the genotypes into two main clusters, the first one with 7 varieties, and the second one with 8 varieties (Figure 2).

In the first cluster, 'Bucium' and 'Cociuvaș' are grouped together having as common genitor 'Boambe de Cotnari'. 'Ludovan' and 'Maria' have the common genitor the variety 'Van'. In addition, four out of seven varieties in this cluster have as genitor the variety 'Van'. The varieties present in the second cluster have a larger pool of genitors, however many of these

are of German origin, and again the 'Cetățuia' and 'Putna' varieties, having as common genitor 'Van', are grouped together.

Table 4. Varieties' origin

Nr. crt.	Variety	Origin
1.	George	'Cireșe de octombrie' x 'Fromm'*
2.	Ludovan	'Van' x 'Boambe de Cotnari'*
3.	Bucium	'Van' x 'Boambe de Cotnari'*
4.	Maria	'Van' x 'Stella'*
5.	Iașirom	'Van' x 'Boambe de Cotnari'*
6.	Cociuvaș	'Boambe de Cotnari' x 'Bigarreau Moreau'*
7.	Alexus	Polenizare liberă 'Lijana'*
8.	Andreiaș	HC.27/4 x Boambe de 'Cotnari'*
9.	Rubin	'Hedelfinger' x 'Germersdorf'*
10.	Severin	'Thurn und Taxis' x 'Germersdorf'*
11.	Paulică	'Bigarreau Drogan' x 'Fromm'*
12.	Boambe de Cotnari	Local selection**
13.	Cetățuia	'Van' x 'Boambe de Cotnari'*
14.	Putna	'Van' x 'Muncheberger Fruhe'*
15.	Rivan	'Early Rivers' x 'Van'***

*(Ștefan et al., 2018)
**(Blaja et al., 1965)
***(Budan & Grădinariu, 2000)

It is interesting to note that in the first cluster, three varieties, 'Iașirom', 'Bucium', and 'Ludovan' have the same genitors: 'Van' x 'Boambe de Cotnari', however they are separated in the three subclusters.
In addition, the 'Cetățuia' variety located in the second cluster also has as genitors 'Van' x 'Boambe de Cotnari'.
These apparent differences among close relatives could be because RAPD markers are dominant, so they cannot differentiate between homozygous and heterozygous loci, and furthermore, amplicons of similar lengths do not have necessarily the same DNA sequence (Amiteye, 2021), so some varieties may appear closer related than they really are.
Nevertheless, for the purpose of this study, the seven RAPD markers did highlight the genetic variability among the varieties studied and generated genetic fingerprints variety-specific.

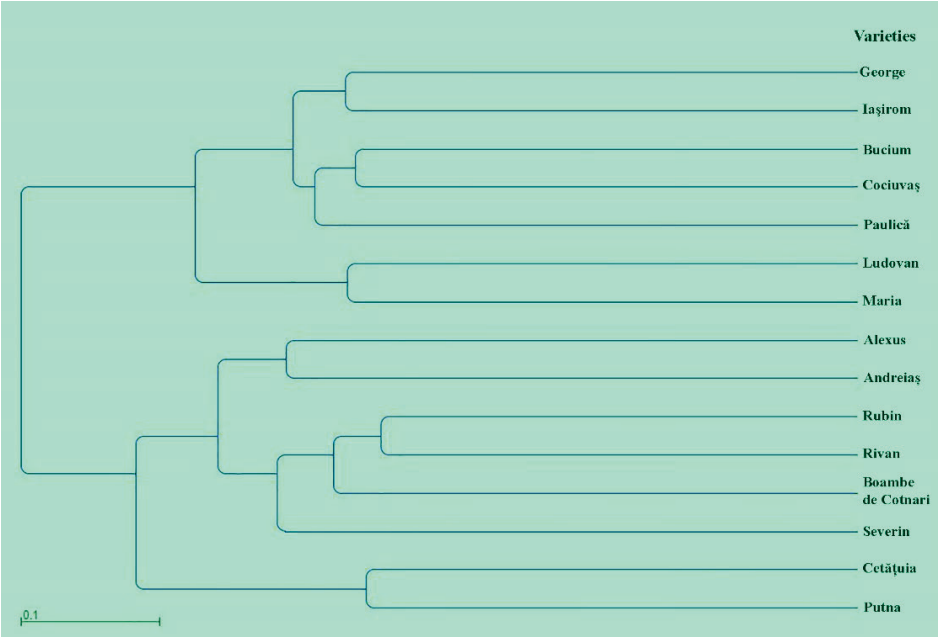

Figure 2. Dendrogram based on the RAPD data generated with the Bio-R software

CONCLUSIONS

RAPD analysis of the fifteen accessions from the USAMV of Bucharest orchard revealed that:

- ✓ All random decamer primers identified polymorphic RAPD loci.
- ✓ P63 marker identified the highest number of loci, making it the most suitable marker to discriminate among the fifteen accessions.
- ✓ Analysis of the data generated from the RAPD reactions using the seven decamers was used to construct a UPGMA

dendrogram, and to demonstrate the genetic variability among the cultivars studied.

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