Genetic Diversity of Some Sweet Cherry Cultivars Based on Molecular Markers

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ABSTRACT

Sweet cherry (*Prunus avium* L.), originated around the Caspian and Black Sea, is an important fruit tree species of economic interest, and hence, breeding and conservation are requested. Genetic analysis at the molecular level can be used effectively to study molecular polymorphism existing between intraspecific and interspecific tree species and phylogenetic relationships between them and their hybrids. The purpose of this study was to characterize and determine genetic relationships among the sweet cherry native genotypes belonging to Fruit Research & Development Station Bistrita, Romania, using RAPD markers. To eliminate the existence of possible synonyms from national romanian collection, we collect four Van cultivars, from four different national collection. For molecular analysis of the 16 varieties of sweet cherry were considered 13 RAPD primers selected from the literature. They were later used to determine the genetic variability at the molecular level using PAST program, and the dendrogram was generated based on Jaccard's genetic distance. The dendrogram constructed by PAST software. The quantity and quality of the DNA obtained was suitable to achieve PCR amplification step. Only seven out of the 13 RAPD primers have generate polymorphic bands. The rest of seven were monomorphics. The most polymorphic primer was OPB10 which generated 11 bands from which 100% were polymorphic. Seven RAPD primers generated a high level of polymorphism which allowed to divide these cherry varieties into two groups according to their genetic geographical origin and the pedigree.

Keywords: *genotypes, molecular markers, Prunus avium, RAPD.*

INTRODUCTION

Sweet cherry (*Prunus avium* L., *Rosaceae*) is a self-incompatible diploid species, from *Rosaceae* family, with a number of chromosomes $2n = 16$. Originated around the Caspian and Black Sea, it is cultivate in temperate regions of the world for its edible fruits (Wunsch and Hormaza, 2002).

In Romania, old assortment of cherry was continuously improved with the introduction in the culture of foreign and domestic valuable varieties, but also by the selection of clones of existing range.

The main criterion to assess the genetic diversity of fruit varieties is based on phenotypic and morphological analysis of traits. In most of cases it is often difficult or even impossible to distinguish between many cultivars because they

are so morphologically similar (Anna Lisek *et al.*, 2006). Despite its limitations, elaborate and time-consuming, this method has been used for long time to identify genotypes and to design breeding schemes (Di Vaio *et al.*, 2015).

In the world it is possible to find more than 1.500 cultivars of sweet cherry, so it is very possible to find some cultivars highly similar, or to find the same cultivars with different name, or different cultivars with the same name. As a result, the confusion of the cultivars is an important problem (Özbek, 1978; Kaçar, 2001). In China, for example, exists a problem of homonyms and synonyms in the sweet cherry cultivars (Cai *et al.*, 2007).

At Fruit Research & Development Station Bistrița as in all Fruit Development Research Stations from Romania, a large number of Romanian

and foreign genotypes of sweet cherry (varieties, landrace cultivars, selection and hybrids), have only morphological and biochemical evaluation, less a molecular evaluation. All this characteristics frequently depending by the environmental factors. Molecular analysis are not affected by this factors and can detect differences between cultivars on the genomic level. The counterpart of phenotyping is genotyping (Di Vaio *et al.*, 2015).

Marker-assisted selection would be especially advantageous for sweet and sour cherry, respectively, breeding (Stockinger, 1996). As we know, in the breeding program for any plants, the most important technique is genetic variability. PCR analysis based on RAPD markers has been used for identification and determining relationship in many species, including *Prunus*. RAPD primers was used to distinguish mutant cherries and their parents (Yang and Schmitt, 1994). To identify 18 sweet cherry cultivars, Gerlach and Stösser (1998) have used twenty-three RAPD primers. Anna Lisek *et al.* (2006) have used 55 RAPD primers to elaborate a simple and reliable procedure for distinguishing among the sweet cherry cultivars commonly cultivated in Poland. Cai *et al.* (2007) find the specific RAPD markers to study the genetic variation between wild cherry species from a collection held in the Northwest A&F University (NWAUFU). The same markers were used for genotype identification of native Campania sweet cherry cultivars by Vaio., *et al.* (2015).

The aim of this study was to establish the genetic relationships among the sweet cherry native genotypes belonging to Fruit Research & Development Station Bistrita, using RAPD markers and to eliminate the possible synonyms between "Van" sweet cherry cultivars from different collection from our country.

MATERIALS AND METHODS

In present study we analyzed 16 varieties of sweet cherry, of these, a group of 13 representative, native and foreign cultivars (Rosii de Bistrita, Uriase de Bistrita, Timpurii de Bistrita, Negre de Bistrita, Ana, Gloria, Iva, Ivona, Jubileu 30, Rubin, Kordia, Stella and Van, were from collection of the Fruit Research & Development Station Bistrita, Romania and another three Van cultivars were collected from other three different Stations of Fruit Research from Romania. The cultivars chosen

and their geographic and genetic origin are show in Tab. 1.

For PCR analysis, DNA was isolated from young leaves using the protocol described by Lodhi *et al.*, 1994, and modified by Pop *et al.*, 2003. DNA were quantified spectrophotometrically at 260 nm using a NanoDrop ND 1000 and diluted for a finally concentration of 50 ng/μl in nuclease-free water (Promega).

To distinguish all cherry varieties we used 13 primers RAPD (Operon Technologies Inc., USA). For optimization of PCR conditions and for initial screening were used only four varieties of cherry and only seven primers was selected based on polymorphic bands. The RAPD primers used are presented in Tab. 2.

PCR reactions was performed in a volume of 15 μl, with the following component: 50 ng template DNA, 2xPCR master-mix (ready to use, Bioline), 0.2 μM/primer and free water. Amplification was carried out in a 96 Well Gradient Palm-Cycler CG1-96 (Corbett Research), with the following program: initial denaturation step of 94°C for 3 min. follow by 45 cycles of 1 min at 93°C, 1 min. at 34°C and 1 min. at 72°C. The program was completed with the finally extension at 72°C for 10 min. After amplification the PCR product was stored at 4°C prior to electrophoresis. Amplified DNA fragment were separated in 1% agarose gel with TAE buffer 1%, stained with etidium bromide. Gels were run for 1,5 h at 80V and visualized under UV light (UVP BioImaging Systems). Each product was visualized on gel with 100 bp DNA Step Ladder molecular marker (Promega) to calculate the bands sizes.

The electrophoresis patterns were analyzed using TotalLab 120. For data matrix, bands were scored as present (1) or absent (0). Genetic distance between varieties was calculated using Jaccard (1908) coefficient of similarity. Cluster analysis was obtained using the PAST software and a dendrogram was generated based on genetic distance.

RESULTS AND DISCUSSION

The quantity of DNA yield obtained from sweet cherry cultivars was between 196.84 - 789.75 ng/μl with purity between 2.15-2.21 nm.

Out of 13 primers used for amplification, only seven (54%) generated a polymorphic band, which was used for genotyping of cherry cultivars, and these were OPE14, OPA03, OPA04, OPA11,

Tab.1. Native and foreign cultivars of sweet cherry used and their origins

Probe name	Cultivar name	Genetic origin	Origin
C1	Rosii de Bistrita	Hybrid (Hedelfinger x Ramon Oliva)	FRDS Bistrita
C2	Uriase de Bistrita	Hybrid (Hedelfinger x Germersdorf)	FRDS Bistrita
C3	Timpuriu de Bistrita	Hybrid (Bigarreau Donissen x Ramon Oliva)	FRDS Bistrita
C4	Negre de Bistrita	Hybrid (Hedelfinger x Germersdorf)	FRDS Bistrita
C5	Ana	Hybrid (Hedelfinger x Ramon Oliva)	FRDS Bistrita
C6	Gloria	Hybrid (Boambe de Cotnari x Van)	FRDS Bistrita
C7	Iva	Hybrid (Hedelfinger x Ramon Oliva)	FRDS Bistrita
C8	Ivona	Hybrid (Hedelfinger x Van)	FRDS Bistrita
C9	Jubileu 30	Hybrid (Hedelfinger x Ramon Oliva)	FRDS Bistrita
C10	Rubin	Hybrid (Hedelfinger x Ramon Oliva)	FRDS Bistrita
C11	Kordia	Unknown	Czech Republic/FRDS Bistrita
C12	Stella	Lambert x John Innes Seedling 2420	Summerland,B.C./ FRDS Bistrita
V_BN	Van	Hybrid (Seedling of Empress Eugenie)	Summerland,B.C./ FRDS Bistrita
V_IS	Van	Hybrid (Seedling of Empress Eugenie)	Summerland,B.C./ FRDS Iasi
V_P-M	Van	Hybrid (Seedling of Empress Eugenie)	Summerland,B.C./ RIFG Pitesti-Maracineni
V_CJ	Van	Hybrid (Seedling of Empress Eugenie)	Summerland,B.C./ FGRS Cluj-Napoca

FRDS: Fruit Research & Development Station; FGRS: Fruit Growing Research Station; B.C: British Columbia

Tab.2. The RAPD primers used in this study, their sequence, the number of bands from which polymorphic and the molecular weight of the bands

No.	Primer name	Sequence 5'- 3'	Polymorphic/ Monomorphic	Bands (no.)	Polymorphic bands (no/%)	Molecular weight of bands (pb)
1	OPAB 11	GTGCGCAATG	Monomorphic	-	-	-
2	OPB 17	AGGGAACGAG	Monomorphic	-	-	-
3	OPB 11	GTAGACCCGT	Monomorphic	-	-	-
4	OPA 04	AATCGGGCTG	Polymorphic	9	6 (67%)	324-1326
5	OPA 11	CAATCGCCGT	Polymorphic	10	10 (100%)	862-2152
6	OPB 10	CTGCTGGGAC	Polymorphic	11	11 (100%)	1043-2513
7	OPX 03	TGGCGCAGTG	Monomorphic	-	-	-
8	AB 11	CAATCGCCGT	Polymorphic	10	7 (70%)	403-1270
9	OPB 09	TGGGGGACTC	Monomorphic	-	-	-
10	OPX 03	TGGCGCAGTG	Polymorphic	11	7 (64%)	175-1160
11	OPB 08	GTCCACACGG	Monomorphic	-	-	-
12	OPA 03	AGTCAGCCAC	Polymorphic	12	11 (92%)	508-1267
13	OPE 14	TGCGGCTGAG	Polymorphic	10	8 (80%)	337-1320

OPB10, OPX03 and AB11, the rest of primers were monomorphics. The total number of bands obtained with seven selected primers was 73,

the average number of bands per primer was 10.4. Among the 73 fragments, 61 (83.56%) were polymorphics with the average bands per primer

Fig.1. RAPD amplification profile obtained using OPB10 primers (see the probe name in tab.1); M-molecular marker (100 bp DNA Step Ladder)

of 8.7. The remaining 12 (16.4%) corresponded to monomorphic loci. This number of monomorphic bands confirms the common genetic origin of the most analyzed varieties of cherry.

The molecular weight of the bands ranged from 175 pb to 2513 pb. The most polymorphic primer was OPB10 which generated 11 bands from which 100% were polymorphics, while the primer OPA04 generated only 9 bands from which 67% were polymorphics (Tab. 2). The primer OPB 10 were previously reported to distinguish the following cultivars "Kordia" and "Vanda", "Summit" and "Star" (Anna Lisek *et al.*, 2006) and yielded consistent and reproducible polymorphism between accessions (Mayol and Rosselló, 2001). The OPB 10 electrophoresis pattern of this primer is represented in Fig. 1.

With a set of seven RAPD primers it was possible to distinguish the cultivars and seems large enough to represent phylogenetic relationship among all 12 varieties of sweet cherry and to observe the similarity between the rests of four Van cultivars. The results from this study are in agreement with earlier studies using RAPDs in sweet cherry (Anna Lisek *et al.*, 2006; Leyla Demirsoy *et al.*, 2008; Vaio *et al.*, 2015;), and other plant species, like, *Juglans* cultivars, *Rosa* cultivar or walnut cultivars (Pop Iulia Francesca *et al.*, 2010; 2011; 2013), or *Attalea speciosa* (Santos *et al.*, 2015), even in *Pseudomonas* strains (Jaipreet *et al.*, 2015).

Using Jaccard coefficient was generated a similarity matrix for all polymorphic primers. The dendrogram constructed by PAST software is

represented in fig.2. According to the dendrogram results produced by cluster analysis, varieties are separated in two main groups with a similarity value of 0.96 showing a good fit. RAPD primers clustered the varieties according to their geographical origin and almost to their pedigree.

Within the Romanian origin group, all the varieties created at Fruit Research & Development Station Bistrita were clustered together in a group 1 (Rosii de Bistrita, Uriase de Bistrita, Timpuriu de Bistrita, Negre de Bistrita, Ana, Gloria, Iva, and Ivona). In the second group we found the foreign cultivars, all four Van cultivars, Stella cultivar which is Nord American origin, more exactly from Summerland B.C. (Lapins, 1971) and Kordia cultivar which is European origin (Czech Republic) but with unknown genetic origin (<http://treefruit.wsu.edu/variety/attika-kordia>). The fact that all four Van varieties are grouped together eliminate the problem of the existence of possible the synonyms in the Romanian national collections.

If regarding more exactly in group 1, all the cultivars were grouped together because they have at least one of the parents common. Here can be observed a subgroup includes 'Rosii de Bistrita' and 'Timpuriu de Bistrita' which have the highest similarity value between them (0.87). One of the parents of them is Ramon Oliva cultivar.

A high value of similarity is found between 'Rosii de Bistrita' and 'Uriase de Bistrita' (0.84) and common genitor of them is variety of German origin – Hedelfinger. The same variety Hedelfinger is found as one of genetic genitor for 'Negre de Bistrita', 'Ana', 'Ivona', 'Jubileu 30' and 'Rubin'.

Fig. 2. Dendrogram generated using PAST software, based on the genetic relationships between sweet cherry varieties and the similarity calculated using Jaccard coefficient

`Iva` cultivar, which have the same genetic origin (Hedelfingen x Ramon Oliva) is near `Ana` and `Gloria`. The similarity value between them is 0.80 (`Iva` and `Gloria`) and 0.72 (`Iva` and `Ana`). `Ivona` cultivar, as shown in dendrogram is at the limit between the groups, because of the second parent – Van, the similarity value between them is 0.56. But the highest value of similarity is between `Ivona` and `Iva` (0.70). That confirms the one of the common parent of these cultivars which is Hedelfingen variety.

Subgroup `Rubin` and `Kordia` grouped together in the second group. The similarity value between them is 0.65. A question mark the presence of `Rubin` cultivar in this group because it is a native from Fruit Research & Development Station Bistrita, obtained from crossing between

Hedelfinger x Ramon Oliva. But `Kordia` cultivar with the unknown genetic origin, all we know is his geographical origin, Czech Republic. They have some similar agronomical characteristics, like medium vigor of the tree, high productivity, “Kordia, produced high and very large fruits, with good quality (Kazantzis *et al.*, 2011), late ripening varieties of both (June 25th – July 5th for Kordia and the last decade of June for Rubin). Also, in Australia, `Kordia` cultivar is widely grown for good fruits size, flavor and quality (Bound *et al.*, 2014).

CONCLUSION

The RAPD primers used in analysis could identify the relationship between cultivars from FRDS Bistrita, based on their genetic origin.

But to explain in detail some other aspects is recommended to repeat the analysis using more RAPD primers or extend the molecular analysis with another technique which offers an “image” more exactly on this situation.

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