GENETIC DIVERSITY OF SOME SWEET CHERRY CULTIVARS BASED ON POMOLOGICAL CHARACTERISTICS AND RAPD MARKERS

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Përmbledhje

Prunus avium L. është një specie drufrutore me rëndësi ekomonike në Shqipëri, frutat e së cilës janë të preferuar për konsum të freskët dhe në industrinë ushqimore. Ky punim synon vlerësimin e diversitetit gjenetik ndërmjet trembëdhjetë kultivarëve të qershisë ku përfshihen kultivarë autoktonë dhe me origjinë të huaj, pjesë e koleksionit ex situ, bazuar në karakteristikat pomologjike dhe të analizës molekulare me anë të markerëve RAPD. Analiza e grupimit duke aplikuar UPGMA dhe koeficientin Dice bazuar në të dhënat e gjeneruara nga analiza molekulare u realizua për të hetuar lidhjet gjenetike ndërmjet gjenotipeve të qershisë. Analiza e tipareve pomologjike identifikoi si më variable, peshën mesatere të frutit, formën dhe ngjyrën e tij, si dhe kohën e pjekjes. 91.86% e variabilitetit total të vlerësuar bazuar në tiparet pomologjike shpjegohet nga tre komponentët e parë PC1, PC2 dhe PC3. Analiza molekulare gjeneroi në total 58 fragmente polimorfike me një mesatare prej 5.8 fragmente për lokus. Gjenotipet u grupuan në dy grupime me një mesatare të ngjashmërisë 50.5%. Nuk pati grupime të gjenotipeve sipas tipareve morfologjike të analizuara. Të dhënat mbi diversitetin gjenetik dhe lidhjet gjenetike të gjenotipeve të qershisë në këtë studim pasurojnë fondin e të dhënave të domosdoshme për një menaxhim efektiv të gjermoplazmës dhe përmirësimit të kësaj specie në terma afatgjatë.

Fjalëkyce: Prunus avium, RAPD, Shqipëri, diversitet gjenetik.

Abstract

Sweet cherry (*Prunus avium* L) is an economically important fruit tree species in Albania. The present study's main objective was to assess the genetic diversity of thirteen sweet cherry cultivars, comprising Albanian autochthonous and some of foreign origin preserved at the ex-situ collection, using pomological traits and RAPD markers. The molecular data were used to cluster the sweet cherry genotypes by applying the UPGMA method. The most variable pomological traits were fruit weight, fruit shape, color, and ripening time. The PCA analysis based on pomological traits showed that the three first principal components, PC1, PC2 and PC3 explained 91.86% of the variability. Genetic analysis based on ten RAPD markers generated 58 polymorphic fragments with

a mean of 5.8 fragments per locus. Cluster analysis divided the investigated genotypes into two groups with a mean similarity of 50.5%. There was no observed grouping of sweet cherry genotypes based on pomological traits as the size, mean weight and color of the fruit. The data provided on the genetic diversity and relatedness of sweet cherry genotypes in the collection would be helpful to breeders and to provide the best collection management practices. The result could make them attractive for future breeding programs and long-term conservation strategies.

Key words: *Prunus avium*, RAPD, Albania, genetic diversity.

Introduction

Sweet cherry (*Prunus avium* L.), is an important fruit tree crop of economic interest, cultivated in Albania for its edible fruits used in the fresh markets and food industry and as rootstocks. The germplasm of sweet cherry maintained in the ex-situ collection of Agricultural Technologies Transfer Centre (ATTC), Vlore, comprises native and foreign introduced cultivars. The characterization of sweet cherry genetic resources is essential for enhancing collection management and improving the breeding process (Patzak *et al*., 2019).

The first step in characterization of genetic resources in sweet cherry cultivars was based on the fruit characteristics, like flavour, aroma, pulp texture, which are appreciated by local and national consumers (Di Vaio *et al*., 2015), fruit bioactive compounds (Di Matteo *et al*., 2016) and many other qualitative and quantitative characteristics following IPGRI or UPOV descriptors. The most valuable pomological traits in determining the fruit quality of sweet cherry cultivars include fruit weight, size, shape and color. However, the concept of fruit quality depends on the consummator preferences (El Baji *et al*., 2021).

This information's relevance increases in native cultivars often preferred and widely cultivated from cherry growers. Hence, phenotypic traits were used to identify sweet cherry cultivars in the ATTC collection (Ferraj *et al*., 2010; Lazaj and Ferraj, 2020). However, studies on phenotypic diversity of sweet cherry in Albania are limited and no studies have been carried out to evaluate genetic diversity of these genotypes to select and conserve the most promising ones. The second step of characterization is DNA fingerprinting analysis by employing molecular markers providing more reliable data compared to the approaches that are affected by environmental conditions as those based on phenotypic, phenological and biochemical properties.

The advantage of RAPD markers in assessing genetic diversity is that they are less affected by environmental factors and are almost unlimited in number. They also offer the possibility to observe the genome directly, and thus eliminate the shortcoming inherent to a phenotype observation (Sandalli *et al*., 2005).

The study aimed to assess genetic diversity and genetic relationships among sweet cherry cultivars in the ATTC collection and to identify cultivar-specific RAPD markers, useful in the rapid identification of these varieties in the future.

Material and methods

The plant material was collected ex-situ at Agricultural Technologies Transfer Centre (ATTC), Vlore in Albania. Some of main fruit characteristics as the fruit shape, mean size and weight, fruit skin color, stone shape and the time of ripening were investigated per each cultivar at the stage of fruit technological maturity. The cultivars subject of this study and their investigated pomologic traits are given in the table 1.

Fresh leaves from thirteen cherry cultivars were used for DNA isolation following the cetyltrimethylammonium bromide (CTAB) method described by Kump and Javornik, (1996). Isolated DNA of the sample set was subjected to further analyses by 10 RAPD markers; OPA04, OPA07, OPA08, OPA13, OPA15, OPA16, OPA17, OPB01, OPAG04, OPJ04, OPJ07 and OPJ12 (Operon Technologies). The DNA amplification was carried out in the following PCR reaction, PCR master mix of a volume of 10 µl, (1xPCR buffer, 2mM MgCl2, 0.2mM dNTPs, 0.2µl primer and 0.3U of *Taq* polymerase) was added to 20ng DNA template. The following thermal profile was applied, initial denaturation at 94°C for 1.5 min, followed by 36 cycles of 30s at 94°C denaturation, 45s at 36°C annealing, extension for 1 min at 72°C and the final extension step on 72°C for 5 min. The amplified PCR products were analyzed by 1.5% agarose gel stained in ethidium bromide and visualized under UV light.

The amplified bands were scored as present (1) or absent (0), and a binary matrix was constructed. For each RAPD marker, the total number of bands (TNB), the number of polymorphic bands (NPB), and the percentage of polymorphic bands (PPB). The pairwise genetic similarity was calculated using DICE similarity coefficient (Dice, 1945) and a UPGMA based dendrogram of relationships among cherry cultivars was generated using NTSYS v.2.2 software (Rohlf, 2000). The principal components analysis (PCA) based on pomological characteristics was performed in Past 3.06 software (Hammer *et al*., 2001).

Results and discussion

Pomological characterization and PCA analysis

The sweet cherry cultivars maintained in the ex-situ collection of ATTC, showed a remarkable degree of variability among cultivars in fruit characteristics (Table 1), which are considered very important for their selection in commercial cultivation and use in the breeding programs. The average fruit weight ranged from 8.2g (Sweet Early) to 4.28g (Sciena), the fruit size ranged from medium to large, 53.8% of cultivars had large fruit, which is considered essential for fresh

consumption. The stone shape showed variability across the cultivars. There were identified four types of stone shapes, circular, circular broad, elliptic, elliptic broad, where the shape elliptic broad the most frequent (38.4%). The fruit color varied from yellow to blackish.

Table 1. Sweet cherry cultivars and their fruit characteristics

No	Cultivar	Time of ripening	Fruit shape	Stone shape	Fruit Size	Fruit* weight	Color**
1	Crazy Star	Medium	reniform	circular	large	7.26	5
2	Celeste	Medium	reniform	broad elliptic	large	7.62	5
3	Moro di Cassano	Medium	elliptic	broad elliptic	medium	6.42	4
4	Sweet Early	Early	elliptic	broad elliptic	large	8.2	4
5	Sciena	Medium	elliptic	broad elliptic	medium	4.28	4
6	New star	Medium	oblate	broad elliptic	large	7.89	2
7	Burlat	Medium	cordate	circular	large	8.1	5
8	Unknown	Late	oblate	circular	medium	6.2	1
9	Roze	Late	circular	broad elliptic	medium	5.87	3
10	Italiane	Late	reniform	broad elliptic	medium	6.65	3
11	Napoleon BSh	Medium	reniform	circular	large	7.45	6
12	Napoleon BGj	Late	cordate	circular	large	7.34	6
13	Bukje	Late	oblate	broad elliptic	medium	6.03	2

*Mean fruit weight in gram**Fruit skin fruit color: 1 = yellow; 2 = yellow with blush; 3 = light red; 4 = dark red; 5 = brown-red; 6 = blackish

The Eigenvalues obtained by PCA analysis based on pomological traits indicate that the three first principal components, PC1, PC2 and PC3, explained 91.86% of the total variability. The first principal component (PC1) accounted for

51.96%, it had a positive loading for fruit weight and color, the second principal component (PC2) accounted for 23.22% of total variability, with positive loadings for the fruit shape, time of ripening and fruit weight and color. The third principal component (PC3) accounted for 16.68% of the total variation and was positively correlated with fruit size, time of ripening and fruit color and shape. These descriptors showed to be important in the pomological evaluation of sweet cherry cultivars.

The projection of cultivars in the scatterplot (Figure 1) based on the two main principal components (PC1 and PC2), showed differentiation of cultivars ‘Burlat, ‘Napolon BGj and ‘Sweet Early and ‘Napolon Bsh’, ‘Crazy Star’ and ‘Celeste’ based on the PC1 and the cultivars ‘New Star’, ‘Burlat’, ‘Rose’ and ‘Napolon BGj’ based on PC2.

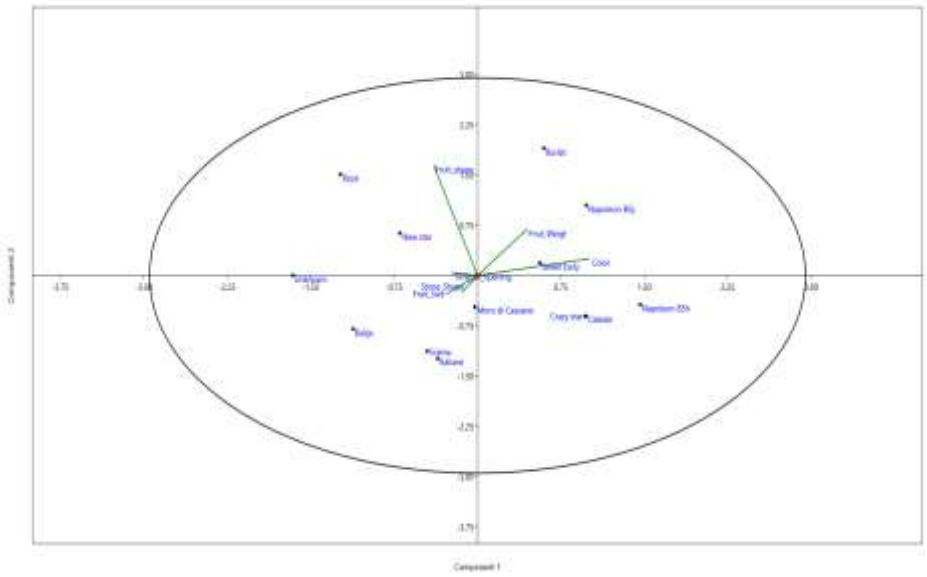

Figure 1. Principal component analysis (PCA) based on pomological traits of cherry cultivars

Genetic diversity and relationships of sweet cherry cultivars

Molecular genotyping of sweet cherry cultivars with ten RAPD markers generated 61 fragments ranging in size from 300 to 2000bp, 95% (58) of these fragments were polymorphic. The mean number of polymorphic fragments per primer was 5.8, ranging from 4 to 10 in OPAG04 and OPA13, respectively (Table 2). The RAPD primer set used in our analysis efficiently differentiated

thirteen sweet cherry cultivars. However, five primers OPAG04, OPA13, OPA15, OPA17 and OPJ12, amplified in ten specific fragments belonging to five specific cherry cultivars ('Napoleon Bishtshkurter', 'Italiane', 'Crazy Star', 'Sciena' and one 'Unknown' local cherry cultivars). The highest number of specific fragments (6) was amplified in the cultivar 'Sciena'.

Four from 13 sweet cherry cultivars amplified one specific reproducible fragment each. The highest number of specific fragments was found in primer OPJ12 (3 fragments) and the lowest in OPAG04 (1 fragment). These specific fragments are important and can be used in the future as potential markers for their specific cultivar identification or selection.

The specific fragments, size, and related specific cherry cultivars are given in Table 3. The efficiency of RAPD markers in identifying and molecular characterization of sweet cherry cultivars was also reported in earlier studies (Demirsoy *at al*., 2008; Di Vaio *et al*., 2015; Berindean *et al*., 2016).

Table 2. RAPD primers, the approximate range of amplified fragments and their polymorphism data

Primer name	**Molecular weight of bands (bp)**	**TNB**	**PNB**	**PPB**
OPA07	500-1500	7	7	100
OPA08	400-900	7	6	85.7
OPA13	300-1500	10	10	100
OPA15	400-1000	6	5	83.3
OPA16	400-2000	5	5	100
OPA17	400-1000	5	5	100
OPB01	400-1000	5	5	100
OPAG04	300-2000	5	4	80
OPJ04	500-2000	5	5	100
OPJ12	300-2000	6	6	100
Mean		**6.1**	**5.8**	**94.9**

Table 3. Selected primers for differentiation of 5 cherry cultivars in our sample set

Cultivar	OPAG04	OPA13		OPA15			OPA17		OPJ12		
	0.2	0.4	0.7	0.4	0.9	1.0	0.4	1.2	0.6	0.3	
Napoleon BSH					+						
Italiane		+									
Crazy Star	+										
Sciena			+	+			+	+	+	+	
Unknown						+					

* The presence of a specific DNA fragment in a given size (in kbp)/primer for each genotype

Based on the obtained RAPD profiles, the genetic relatedness analysis among sweet cherry cultivars was performed by applying Dice's similarity coefficients and UPGMA method of clustering, presented in figure 2.

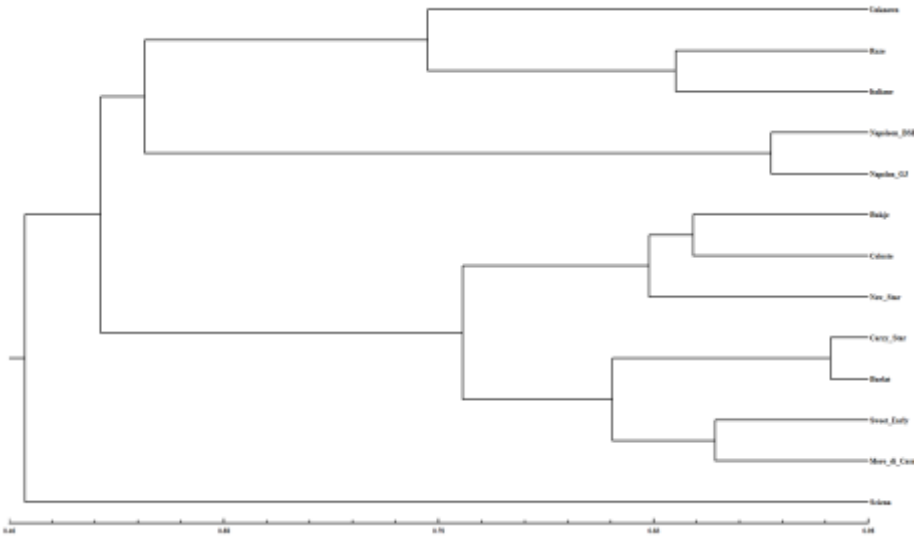

Figure 2. Genetic relationships among sweet cherry cultivars based on RAPD data

The study revealed a significant level of genetic diversity of sweet cherry collection maintained in ATTC. Among cultivars, Dice's similarity coefficients values ranged from 32 % to 93%, with a mean of 59 %. The highest level of similarity was observed between 'Crazy Star' and 'Burlat', while the lowest similarity was exhibited between 'Napoleon Bishtgjate' and 'Celeste'. The identity of one genotype that in the collection was coded as 'Unknown' could not be revealed in this study, however, it showed higher similarity (74%) with 'Bukje'. Cultivars were classified into two main clusters in the relatedness analysis based on their RAPD profiles.

The most distant, not clustered in any group appeared the 'Sciena' genotype. Other genotypes were clustered into two main groups and into several small subgroups. The first group comprised five cultivars that show a mean similarity of 64%, among them; 'Unknown', 'Roze', 'Italiane', 'Napoleon Bishtshkurter', Napoleon Bishtshgjate', whereas the second group comprised seven cultivars, 'Bukje', 'Celeste', 'New Star', Crazy Star', 'Burlat', 'Sweet Early', 'Moro di Cassano', which share a mean similarity of 77%.

Conclusions

In conclusion, the sweet cherry genotypes showed high variability in pomological traits, where the most variable traits were fruit mean weight, shape, color and the time of ripening. The three first principal components, PC1, PC2 and PC3 explained 91.86% of the variability. Relatedness analysis clustered cultivars into two main groups based on their RAPD profile, with a mean similarity of 50.5%.

There was no observed grouping of sweet cherry genotypes based on pomological traits as the size, mean weight and the color of the fruit. The results of the genetic characterization of sweet cherry cultivars held at the ATTC collection contribute to a better understanding of the genetic diversity and the identification of cultivar-specific RAPD fragments, which may be used as a tool in the future rapid genotype identification and selection. Thus, providing relevant data for planning efficient collection conservation and use programs.

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