Molecular evaluation of genetic diversity and genetic analysis of Azerbaijan sweet cherries (*Prunus avium* L.) using capillary electrophoresis with fluorescence-labeled SSR markers

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Received: September 25, 2023; Received in revised form: October 26, 2023; Accepted: December 11, 2023

The sweet cherry (*Prunus avium* L.) is one of the world's most commercially important perennial crops and its improvement has been the focus of human effort for thousands of years. A collection of 74 accessions of Prunus avium L. from a research station, across regions in Azerbaijan representing the sweet cherry germplasm in the country, were examined to estimate genetic diversity and to identify genetic relationships among accessions using a set of 12 microsatellite (SSR) markers. Among the primers used, PaCITA 18, pchgmS 2, AK 193, PaCITA 14A, PaCITA 14B, and AK 200 are polymorphic and effective enough to differentiate between cherry genotypes. After calculating the genetic similarity index, a genetic link dendrogram was constructed using phylogenetic and structure analysis. We found the highest number of alleles at the AK 193 locus (12). The PIC value ranged from 0.0322 to 0.7753. Genetic diversity ranged from 0.0312 to 0.8016. We recorded the highest index of genetic diversity for the AK 123 locus. Thus, the results show that Azerbaijan germplasm has good potential for cherry genotypes for further breeding studies. The information obtained from SSR fingerprinting will be useful in optimizing the conservation of sweet cherry genetic resources present in the Azerbaijan region. However, this approach can be applied to optimize the conservation of local genetic resources of other stone fruit tree species.

Keywords: *Prunus avium* L., *SSR markers, genetic diversity, fluorescent capillary electrophoresis, DNA fingerprinting, structure analysis*

INTRODUCTION

Sweet Cherry (*Prunus avium* L.) belongs to the Rosaceae family and is a cross-pollinated, usually diploid (2n=2x=16) tree plant. The natural distribution area is the northern part of Western Eurasia and Africa. Sweet cherry probably originated from the area between the Caspian Sea and The Black Sea and was spread to Europe by birds (Webster, 1996; Blando & Oomah, 2019). The world production of sweet cherry is 2.200.000 tons and it is the fourth most productive after stone fruits such as plum, peach, and apricot. About 60% of the sweet cherry production is concentrated in seven countries (https://www.fao.org/statistics/en/). There are around 1,500 varieties of cherries in the world (https://www.agro.gov.az/az/bitkicilik/coxillikekmeler/gilas). 600 varieties of cherries were collected in France. These cultivars were also collected from the INRA cherry collection of the Prunus Genetic Resources Center near Bordeaux, France. 30% of them belong to the French national cherry collection (Barreneche, 2014). Other varieties are brought from many parts of the world and collected in France (Teribia et al., 2016). In the mid-twentieth century, the introduction of basic molecular technology, the establishment of phylogenetic relationships, the development of genetic markers, and the construction of linkage maps expanded our understanding of key aspects of *Prunu*s genetics (Baek et al., 2018). Sweet cherry collections from 19 European countries were genotyped by

https://doi.org/10.59849/2710-4915.2023.2.83
Available online 31 December 2023

Barreneche and colleagues (2021) using 14 SSRs to assess genetic diversity parameters, estimate the levels of population structure, and identify excessive germplasm. In Japan, more than 200 traditional cherry cultivars are known (Kobayashi 1993), and they show diverse floral characteristics, including traits seldom found in the wild (Kato et al., 2014]. Ohta et al. (2005) characterized the genetic diversity of the flowering cherry *(Prunus* subgenus *aviufro*m) with 85 peaches using SSR markers. Genetic variability among flowering cherries was found to be higher than among peach and cherry cultivars (Ohta et al., 2005). Turkey also possesses a high amount of cherry production and genetic diversity in the cherry population. The genetic diversity of 78 local cherry varieties in Turkey using 4 AFLP and 6 SSR primers was examined (Gulen et al., 2010). According to the dendrogram created out of the UPGMA analysis, the varieties were divided into 18 different groups, and the highest degree of similarity was 70%. Based on the results, the authors concluded that local Turkish varieties have great genetic diversity (Gulen et al., 2010). Marti and colleagues (2012) performed a genetic analysis of a total of 114 cherry genotypes representing commercial and old cultivars from different parts of the world with 40 SNP markers and 7 SSR markers. The results obtained with both markers were compared. As a result, although the average number of alleles per locus, observed heterozygosity, expected heterozygosity, and number of polymorphic points were higher for SSR markers than for SNPs, cherry groups in the dendrogram constructed with both sets of markers showed similarity. Austrian sweet cherry germplasm accessions were genotyped using a harmonized set of 11 simple sequence repeat (SSR) markers optimized in two multiplexed PCR reactions. Thirty-eight distinct allelic profiles were identified (Schuller et al., 2021). Barreneche et al. (2021) genotyped subsets of sweet cherry collections from 19 European countries in Europe A total of 314 accessions, including landraces, early selections, and modern cultivars, were monitored, and 220 unique SSR genotypes were identified. All 14 loci were confirmed to be polymorphic, and a total of 137 alleles were detected with a mean of 9.8 alleles per locus.

The objective of the study. The cherry plant cultivated in Azerbaijan has gained fame in Europe and Asia for its high quality (Bekefi et al., 2014). The main reason for this is that nine of the eleven climate types known around the world are in Azerbaijan, which created a good opportunity to grow tropical fruit plants (avocado, mango, etc.) and other plants, especially stone fruit plants (cherry, apricot, plum, peach, etc.). Unfortunately, the genetic characterization of cherry cultivars has not been carried out in Azerbaijan. We investigate the genetic diversity of Azerbaijani cherry varieties, which represent the country's genetic stock, by using SSR markers to reveal the different and similar characteristics from other cherry-producing countries and determine the genetic relationship between them. The originality of this study lies in the fact that this is the first of its kind in Azerbaijan. To date, no research has been conducted to examine genetic variation at microsatellite loci in the local context and introduce cherry cultivars and forms in Azerbaijan. The results obtained with microsatellite markers can be useful in the field of conservation of genetic diversity of sweet cherries, as they will contribute to the reliable classification of samples and the creation of core collections consisting of genetically different cherry varieties. Equally importantly, the characterization of the cherry plant with molecular markers can be used as primary material in future research. Thus, the results of the molecular analysis allow for the categorization of genetically close genotypes and - avoid unnecessary combinations in the selection process in the future. In addition to traditional methods, molecular markers can help guide the search for new genotypes (new allelic diversity) to expand collections. The study's purposes are manifold, and presented below:

a) To determine the areals of varieties and wild forms of local and introduced cherry spread in different regions of Azerbaijan

b) Analyze its current state, identification based on molecular markers, determination of degrees of genetic kinship

c) Preparation of recommendations for effective use in food and breeding programs based on the obtained results

MATERIALS AND METHODS

Plant material. The 74 cherry genotypes used in the study were collected from research stations of 5 regions of Azerbaijan (Guba, Khachmaz,

Sheki, Agdash, and Tartar). Figure 1 shows the regions. Information on the studied genotypes is given in Table 1. Young leaves collected from these genotypes were immediately placed in zip-locked plastic bags containing silica gel for drying.

Fig. 1. Map showing collection sites of cherry (*Prunus avium* L.) sample used for SSR development and population structure analysis (1-Khachmaz; 2-Guba; 3-Aghdash; 4-Shaki; 5-Tartar).

DNA extraction stages. The extraction of nuclear DNA from sweet cherry genotypes was carried out in the biotechnology laboratory of the Genome and Stem Cell Center of Erciyes University in Kayseri, Türkeye. For DNA extraction, the leaf sample was taken from each genotype, crushed in liquid nitrogen, and pulverized. Genomic DNA was extracted from leaves using a modified cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle 1987; Hormaza 1999). 100 mg of the obtained plant powder was placed into a 2 ml tube. 1000 µl of 2xCTAB solution (2% CTAB, 0.1 M Tris HCl (pH = 8.0), 1.4 M NaCl, 20 mM EDTA) preheated to 65ºC and 1% β-mercaptoethanol (pH = 8.0) were added into the tube and mixed well until a homogeneous mass was formed in the Vortex. The obtained suspension was placed in a water bath (65ºC) for 20 minutes-, and inverted every 5 minutes.

Table 1. Information on the used sweet cherry sample

Samples	Sweet cherry-picking region	Origin	GPS
Samba	Guba SRB	Canada	3m-491m
Lapins	Guba SRB	Canada	3m-486m
0900-Ziraat	Guba SRB	Turkey	3m-489m
Jir Gilas	Guba, Zardabi village, y/a	Azerbaijan	4m-610m
Chagrayi Napoleon	Guba, Zardabi village, y/a	Russia	3m-487m
Sari Drogana	Guba SRB	Germany	3m-486m
Tezyetishen Kassini	Guba, Zardabi village, y/a	Germany	3m-331m
Ramon Oliva	Guba, Zardabi village, y/a	France	3m-487m
Regina	Guba, Zardabi village, y/a	Germany	3m-330m
Sweetheart	Guba, Zardabi village, y/a	Canada	3m-329m
Bianka Gozeli	Guba, Zardabi village, y/a	Germany	3m-416m
Sari Denissena	Guba, Zardabi village, y/a	Germany	3m-331m
Jir gilas-2	Guba, Zardabi village, y/a	Azerbaijan	3m-331m
Bigarro Burlat	Guba, Zardabi village, y/a	France	3m-426m
Agh Gilas	Guba, Zardabi village, y/a	Azerbaijan	3m-426m
Early Lory	Guba, Zardabi village, y/a	France	3m-426m
North Vander	Guba, Zardabi village, y/a	Germany	3m-330m
Gara Gilas	Guba, Zardabi village, y/a	Azerbaijan	3m-423m
May Gilasy	Guba, Zardabi village, y/a	Azerbaijan	3m-430m
Krim	Guba, Zardabi village, y/a	Ukraine	3m-493m
Gara Napoleon	Guba, Zardabi village, y/a	Russia	3m-485m
Frans Iosif	Guba, Zardabi village, y/a	Czechia	3m-487m
Gara jir gilas	Sheki Supporting Point	Azerbaijan	3m-423m
Murebbe agh gilas	Sheki Supporting Point	Azerbaijan	3m-329m
Agh jir gilas	Sheki Supporting Point	Azerbaijan	4m-328m
Gara Shabalidi	Sheki Supporting Point	Azerbaijan	3m-334m
Gara Gilas	Sheki Supporting Point	Azerbaijan	3m-333m
Ala Gilas	Sheki Supporting Point	Azerbaijan	3m-339m

Continued Table 1

Samples	Sweet cherry-picking region	Origin	GPS
Jir gilas kesikli	Sheki Supporting Point	Azerbaijan	3m-331m
Okuzureyi agh	Sheki Supporting Point	Azerbaijan	4m-344m
Okuzureyi Gara	Sheki Supporting Point	Azerbaijan	3m-338m
Gizil Gilas	Sheki Supporting Point	Azerbaijan	3m-339m
Kahraba Gilas	Sheki Supporting Point	Azerbaijan	3m-340m
Alij Gilas	Sheki Supporting Point	Azerbaijan	3m-346m
Agh Gilas	Sheki Supporting Point	Azerbaijan	3m-377m
Krim	Sheki, Cheshmali village, y/a	Ukraine	3m-313m
Napoleon- Sheki	Sheki, Cheshmali village, y/a	Russia	3m-313m
Balli Gilas	Sheki, Cheshmali village, y/a	Azerbaijan	3m-314m
Jir gilas aji	Sheki, Cheshmali village, y/a	Azerbaijan	3m-312m
Jir gilas-2	Sheki, Cheshmali village, y/a	Azerbaijan	3m-312m
Mayovka girmizi	Sheki, Cheshmali village, y/a	Ukraine	3m-318m
Dum agh Gilas	Sheki, Cheshmali village, y/a	Azerbaijan	3m-318m
Albali gilas yumru	Sheki, Cheshmali village, y/a	Azerbaijan	3m-314m
Mayovka chil-chil	Sheki, Cheshmali village, y/a	Ukraine	3m-313m
Gara Mayovka	Sheki, Cheshmali village, y/a	Ukraine	3m-322m
Sari Gilas	Sheki, Cheshmali village, y/a	Azerbaijan	3m-322m
Albali gilas agh	Sheki, Cheshmali village, y/a	Azerbaijan	3m-318m
Sari uzun Gilas	Sheki, Cheshmali village, y/a	Azerbaijan	4m-322m
Guzugoren	Sheki, Cheshmali village, y/a	Azerbaijan	4m-322m
Gara Okuzureyi	Tartar, Alasgarli village, y/a	Azerbaijan	3m-197m
Zoghali	Tartar, Alasgarli village, y/a	Azerbaijan	4m-200m
Chal Krim	Tartar, Alasgarli village, y/a	Ukraine	3m-196m
Gejyetishen okuzureyi	Tartar, Alasgarli village, y/a	Azerbaijan	3m-197m
Yabani Gilas	Tartar, Alasgarli village, y/a	Azerbaijan	3m-199m
Napoleon	Tartar, Alasgarli village, y/a	Russia	3m-210m
Shampan Gilas	Tartar, Alasgarli village, y/a	Russia	3m-197m
Agh Krim	Tartar, Alasgarli village, y/a	Ukraine	3m-197m
May gilasi agh	Aghdash, Yukhari Gasil village,y/a	Azerbaijan	3m-43m
Agh Gilas	Aghdash, Yukhari Gasil village, y/a	Azerbaijan	3m-44m
Ala Gilas	Aghdash, Yukhari Gasil village, y/a	Azerbaijan	3m-43m
Gara okuzureyi	Aghdash, Yukhari Gasil village, y/a	Azerbaijan	3m-43m
Tezyetishen Krim	Khachmaz, Gochagli village, y/a	Ukraine	3m-151m
Napoleon	Khachmaz, Gochagli village, y/a	Russia	3m-142m
Krim gejyetishen	Khachmaz, Gochagli village, y/a	Ukraine	3m-141m
Agh Gilas	Khachmaz, Gochagli village, y/a	Azerbaijan	3m-140m
Xrustal	Khachmaz, Gochagli village, y/a	Azerbaijan	3m-141m
Ramon Oliva	Khachmaz, Gochagli village, y/a	France	3m-143m
Tezyetishen Krim	Khachmaz, Gochagli village, y/a	Ukraine	3m-134m
Erken Krasnodar	Khachmaz, Gochagli village, y/a	Russia	3m-137m
Jir Gilas	Khachmaz, Gochagli village, y/a	Azerbaijan	3m-140m
Alyanag	Khachmaz, Gochagli village, y/a	Azerbaijan	3m-142m
En gecyetishen Krim	Khachmaz, Gochagli village, y/a	Ukraine	3m-141m
Gara Krimson	Khachmaz, Gochagli village, y/a	Ukraine	3m-140m
Regina	Khachmaz, Gochagli village, y/a	Germany	3m-140m

* SRB (Scientific Research base); y/a (yard area)

After cooling at room temperature for 5 min, 700 µl of chloroform: isoamyl alcohol (24: 1) (CHIA) was added to the suspension and kept on ice for 30 minutes after inversion 20-25 times. In this case, all the components containing protein and phenol are dissolved, except for DNA and RNA. The mixture was centrifuged at room temperature at 14000 rpm for 5 min and the supernatant was transferred to another 2 ml tube. The stage was repeated by adding CHIA again. To precipitate DNA, 800-850 µl of cold isopropanol was added to the supernatant, and the tube was

sealed with paraffin, carefully mixed, and kept at -20^{0}C for 1 day. Samples were kept at -20^{0}C and centrifuged at 14000 rpm for 1 minute. After transferring the supernatant to a new tube, it was centrifuged twice by adding a cold washing solution (76% ethanol and 10 mM ammonium acetate). It was kept at room temperature for 30 minutes for drying and then 100 µl of TE (ph: 8) was added. This DNA solution was used as a reserve. DNA was quantified using a spectrophotometer, then 1 µl of RNAse was added and stored at 37°C for 30 min.

SSR primers and polymerase chain reaction (PCR) amplification. Initially, 157 SSR primers were available in the laboratory and used for apricot studies. Initial screening of the 157 SSR primers on eight cherries was performed with PCR reactions in 20 µl volumes containing 2 µl 10 x PCR Buffer (100 mM Tris-HCl pH 8.0 at 25ºC, 500 mM KCl, 0.8% (v/v) Nonidet), 2 µl dNTP, 2 µl $MgCl_2$, 0.25 µl forward primer (F), 0.25 µl reverse primer (R), 2 µl genomic DNA, 0.2 µl Taq polymerase and 11.3 µl ddH_20. The PCR amplification procedure was conducted at 94ºC for 3 min, followed by 35 cycles of 94ºC for 45 s, 55ºC for 1 min, and then 72ºC for 1 min, and a final extension step at 72ºC for 5 min. PCR products were separated by 3% agarose gel 1 x TBE buffer at 180 V for 2 h. The bands in the gel were stained with the ethidium bromide solution and examined under ultraviolet light. The SSRs producing polymorphic, robust, bright bands for the 8 cherry genotypes were selected according to the agarose gel view.

Among the 157 SSRs, 12 primer pairs producing clear, simple, and repeatable bands were selected to analyze the 74 cultivars (Table 2).

Allele sizing, cluster, and structure analysis. Fluorescently labeled SSR genotyping and PCR were performed for the 12 SSRs according to the method described by Schuelke (2000) for allele sizing and characterization of the loci that appeared polymorphic in the initial screening on agarose gels. PCR reactions were performed including M13 primer fluorescently labeled with 6-FAM, NED, PET, or VIC amplifying the same 74 cherries.

Table 2. Primers used in simple sequence repeat (SSR) analysis of cherry cultivars with fluorescent capillary electrophoresis

Locus	Forward and reverse primer sequences (5′-3′)	T_m, ºC
SSR PaCITA 18	F:CACGACGTTGTAAAACGACGCCGGTAGCTTTCGATTTCAAAC R:CCTAGGCTTCTATTCCCCTCACGAC	55
SSR pchcms2	F:CACGACGTTGTAAAACGACAGGGTCGTCTCTTTGAC R:CTTCGTTTCAAG91GCCTG	53
SSR AK 69	F:TGAAACTGAGGACGATGACG R:CGTCTTCCGGATTTGCTTTA	50
SSR AK 123	F:TGACATGCGCACTCTTCTCT R:CAGTTGGTAGGCCCTGGTAA	50
SSR PaCITA 10	F:CACGACGTTGTAAAACGACGGTGAGGTCTGTGCTGAATATGCCA R:CGATTAAAGAAATAAGAAAAAGAGC	55
SSR PaCITA 12	F:CACGACGTTGTAAAACGAGACACCCCAACCCACCCATCATGT R:GGTSTTGGAAATGTGGAAAGAAATG	56
SSR PaCITA 14A	F:CACGACGTTGTAAAACGACCCTTCAATGCTGGCATGGTTTCTTC R:GGAGAGAGGGTAGCTAGGGGGAGG	55
SSR PaCITA 14B	F:CACGACGTTGTAAAACGACCCTTCAATGGTGGCATGGTTTCTTC R:GGAGAGAGGGTAGCTAGGGGGAGG	55
SSR AK 193	F:GCAAATCAGCTAGTGAAAGA R:TACCACTTTACGATGTGTCGTT	53
SSR AK 200	F:CCAGTAGATTGGGTGCTACT R:CTACGTCCAAGAACAAGATT	53
SSR pchgms2	F:CACGACGTTGTAAAACGACGTCAATGAGTTCAGTGTCTACACTC R:AATCATAACATCATTCAGCCACTGC	53
SSR AK 178	F:GCACCAACTGTTCCATTTGA R:TGTCTTGATGTGAACCATGC	55

An electronic version of a test table was made, and the machine table was generated automatically. A mixture of 980 μL HIDI and 20 μL LIZ 600 was placed in a 96-well reaction plate with a continuous pipette, with each well having a volume of 10 μL. The well plate was sealed with sealing plate film, and placed in a flat plate centrifuge. In the PCR instrument, the denaturation process was conducted at 94^0 C for 5 min, without heating the hot cover, and at the end of the procedure, the 96-well plate was placed immediately on iced water. Once cooled, the well plate was placed in a flat plate centrifuge and exposed to an RCF. A 1 μL aliquot was loaded into an ABI 3500 capillary electrophoresis instrument (Applied Biosystems, Foster City, CA, USA) (Chao et al., 2007).

A genetic similarity matrix based on the proportion of shared alleles was generated and the expected heterozygosity (He), observed heterozygosity (Ho), and polymorphism information content (PIC) were calculated using the PowerMarker V3.25 software (Liu and Muse, 2005). The neighbor-joining (NJ) method used a matrix using the MEGA6 (Tamura et al., 2007) and PowerMarker V3.25 software programs. Both were used to identify the relationship between species.

The genetic structure in different of the 74 cherry cultivars was analyzed using 12 SSR primer pairs by model-based Software Structure v.2.3.4. by Pritchard et al. (2000). The most likely number of genetic clusters (k) in the Azerbaijan cherry population was also estimated by calculating the Δk values in line with Evanno et al. (2005) using STRUCTURE HARVESTER (Earl and vonHoldt 2012).

RESULTS AND DISCUSSION

As research material, 31 local, 34 introduced and 9 wild forms of 74 samples of sweet cherry discovered as a result of scientific expeditions in the Guba, Khachmaz, Sheki, Agdash, and Tartar regions of Azerbaijan, belonging to separate farms, backyards, scientific research and experimental bases were used, and their areals were determined using GPS technology (Table 1). Twelve SSR loci which could be scored with confidence for eight cherries were further used for the fingerprinting of the 74 cherries. The PCR products of 12 SSR loci were obtained, and their fragments were analyzed. The alleles were scored, and the SSR characteristic values, namely the number of alleles (n), He, Ho, and PIC, for the loci were calculated. Twelve microsatellite primers (SSR) were used to characterize sweet cherry (*Prunus avium* L.) samples. The genetic parameters for each locus - allele number (n), allele frequency (%), expected heterozygosity (He), observed heterozygosity (Ho), and detection probability (PI) described in the paper, were determined by the IDENTITY 1.0 (Wagner and Sefc 1999) program and calculated using the Paetkau vd. Method (1995). After calculating the genetic similarity index, a genetic link dendrogram was constructed using the UPGMA method (Sneath and Sokal 1973). Based on the genetic parameters obtained, 6 alleles were expected at the PaCITA 18 and AK 200 loci, while 5 alleles were detected at the pchcmS 2 and pchgmS 2 loci. The largest number of alleles was found in the AK 193 locus (12). A total of 54 alleles were found in 12 SSR loci. The PIC value ranged from 0.0322 to 0.7753. Genetic diversity ranged from 0.0312 to 0.8016. The allele frequency ranged from 0.2929 to 0.9841. The highest index was recorded for the AK 123 locus. Thus, all genetic parameters are listed in Table 3.

Structure and phylogenetic analysis. Population structure analysis by STRUCTURE software (Pritchard et al. 2000) revealed four sub-groups in Azerbaijan cherries for all the group number (k) tested from two to seven. For an illustration of four sub-groups in cherry in Azerbaijan, the graphs of estimated sub-populations are depicted in Figure 2 for k=4. The sup-population number was also estimated by calculating Δ k values as described by Evanno et al. (2005). As a result, the maximum Δ k value was reached at k=4.

Consequently, the most likely number of subpopulations in cherry samples collected in Azerbaijan is four. The four sub-groups in Cherry were named P1, P2, P3, and P4. Populations are shown in Figure 3B. A UPGMA cluster based on the shared allele distance was generated. The dendrogram is shown in Figure 3A. The cherry samples showed considerable spread in the dendrogram, with a low tendency to cluster by geographical origin. Thirty-three accessions were assigned to the P1 subpopulation, and most of the genotypes (16) assigned to P1 were clustered in the Shaki region of the UPGMA tree.

Table 3. Sequences of the 12 microsatellites (SSR) primers used for the characterization of 74 cherries (*Prunus avium* L.) accessions including the number of alleles per locus, allele frequency, expected heterozygosity, observed heterozygosity, genetic diversity, and polymorphism information content (PIC)

Primers and source plant	Source	Allele frequency	Number of alleles	Gene diversity	Heterozygous	PIC
PaCITA 18	Lopes et al. (2002)	0.4923	6	0.6347	0.8769	0.5721
pchcms2	Sosinski et al., (2000)	0.4727	5	0.5893	0.1636	0.5038
AK69	Kose et al. (2017)	0.9833	2	0.0328	0	0.0322
AK123	Kose et al. (2017)	0.9841	2	0.0312	0	0.0308
PaCITA10	Lopes et al. (2002)	0.7576	4	0.4008	0	0.3716
PaCITA12	Lopes et al. (2002)	0.9583	4	0.0807	0.0556	0.0791
PaCITA14A	Lopes et al. (2002)	0.4931	3	0.5136	0.9861	0.3952
PaCITA14B	Lopes et al. (2002)	0.5071	2	0.4999	0.9857	0.3749
AK193	Kose et al. (2017)	0.2929	12	0.8016	0.7857	0.7753
AK200	Kose et al. (2017)	0.4914	6	0.5489	0.9483	0.4473
pchgms2	Sosinski et al., (2000)	0.5069	5	0.5262	0.9167	0.4147
AK178	Kose et al. (2017)	0.9514	3	0.0935	0.0139	0.0909
Total		7.8911	54	5.2214	5.7325	4.0879
Mean		0.6576	4.5	0.3961	0.4777	0.3407

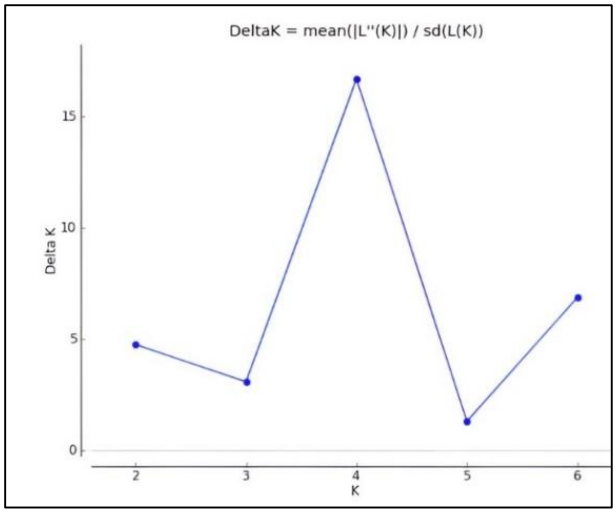
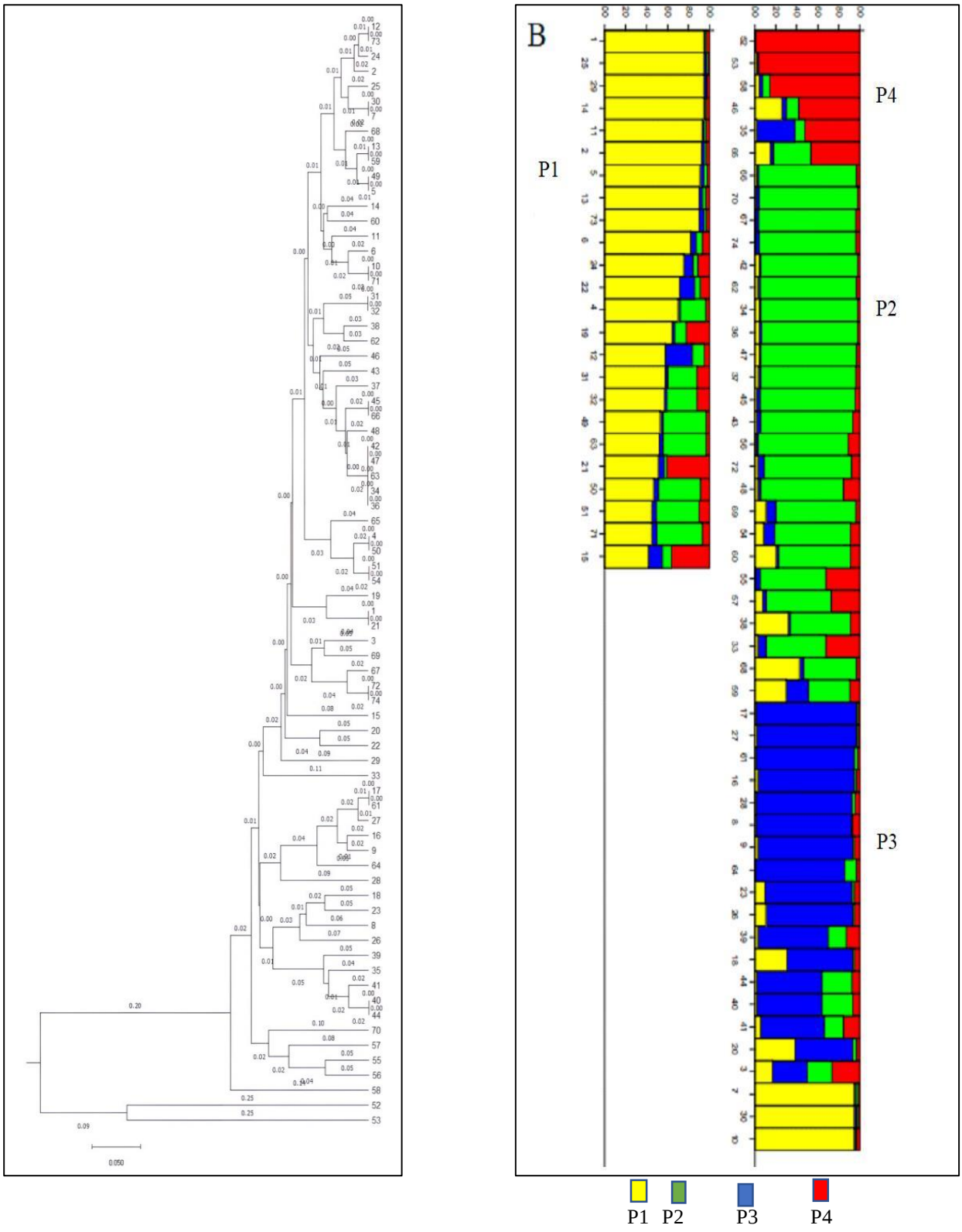


Fig. 2. Estimation of Dk in the 74 cherry accessions (K is the number of populations)

The eighteen samples of the P2 subpopulation were suggested by the structure analysis clustered. Considering P2, again like the P1 subpopulation, mostly the samples were not clustered together according to their geographic origin or their collection sites. In the sixteen genotypes of the P3 population, in brief, both the UPGMA tree and the structure analysis put together some geographically close samples but also placed some other geographically distant areas samples into the same group. The seven samples of the P4 subpopulation suggested by the structure analysis also clustered together on the lower part of the UPGMA tree. Five of the 7 samples of the P4 subpopulation were collected from Tartar. However, the other two samples of the P4 population Aghdash (1 sample), and Khachmaz (1 sample) were collected from distant locations.



(A) **(B)**

Fig. 3. UPGMA clusters (A), and Population STRUCTURE analysis (B) of 74 cherry genotypes based on twelve SSR loci.

In the last decade, the availability of reliable molecular markers is of great importance for plant breeding. In this study, genetic variation in sweet cherry cultivars of Azerbaijan has been assessed using SSR markers. The results are compared with the recently published genetic analysis of sweet cherry: Krmpot et al. (2020) investigated the genetic variability of 14 genotypes of sweet cherries using 26 SSR markers. Eight autochthonous sweet cherry genotypes from four different locations and six virus-free reference cherry varieties were included in the study. The average genetic distance between them was 0.43. The number of alleles per locus ranged from two to eight. The minimum number of two alleles of polymorphic loci showed EMPa003 and EMPa002, while the highest number of eight loci alleles had PceGA34 and UDP97-402 (Tanja Krmpot et al., 2020). Farsad and Ashari characterized 23 important Iranian sweet cherries (Prunus avium) cultivars collected from different provinces of Iran and 1 foreign cultivar, which was used as control, considered for breeding programs by using 21 microsatellite markers. Out of 21 SSR markers, 16 were polymorphic, producing 177 alleles that varied from 4 to 16 alleles (9.35 on average) with a mean heterozygosity value of 0.82 that produced successful amplifications and revealed DNA polymorphisms. Allele size varied from 95 to 290 bp (Farsad and Esna-Ashari, 2016).

Cherry breeding programs and the development of superior cherry cultivars are important needs in Azerbaijan and other cherry-rich countries.

The findings of this analysis must be considered in light of a few limitations. The low number of SSRs used in this study can be a drawback and the reason why samples are not separated according to their geographic origin on the Structure analysis and UPGMA dendrogram. Moreover, this approach may also be due to cross-regional migration or breeding and cultivation in different regions, indicating the complex nature of the history of sweet cherry domestication.

However, it is worth mentioning that for the old genetic studies on wild cherry, the result of the structural analysis using the data obtained with 8 SSR primers of 278 cultivars from 11 populations of wild cherry in Italy was found to be K=11. It has been reported that there is no specific geographical structure in the populations (Rogatis et al., 2012). The K value was determined as 2 in the structural analysis made with the data obtained as a result of the analysis of 131 wild cherry accessions sampled from 5 populations in northern, north-west and central Spain with 9 SSR loci (Fernandez-Cruz et al., 2014). The K value was found to be 5 as a result of the structural analysis performed with 11 SSR loci in 93 samples from 5 populations of wild cherry in Greece (Ganopoulos et al., 2011).

The genetic relatedness reflects the difference in genetic background between cultivars, so it is possible to breed elite cultivars by selecting genetically distant cultivars as hybrid parents. Genetic relatedness and genetic distance enable the development of optimal cultivars and rootstocks into superior cultivars.

In conclusion, the genetic structure and polymorphic SSR loci for cherry varieties cultivated in Azerbaijan have not been reported to date. In our study, the size and structure of genetic diversity in 74 sweet cherry trees sampled from 4 subpopulations of Guba, Khachmaz, Shaki, Tartar, and Aghdash regions were analyzed using SSR markers. As a result of the analyses and assessments, important information was obtained on the studied populations of sweet cherries.

CONCLUSION

This study represents an important step in the improvement protection and conservation of the cherry tree, which is gradually declining in the forests of Azerbaijan due to various factors. The study of the cherry gene pool using molecular markers allows both scientific and practical organization of their effective use. Thus, as a result of our studies, it was confirmed that microsatellite loci are a powerful tool for determining genetic differences between cherry genotypes. Geneticists and breeders can use this information, which reflects the degree of relatedness of the samples, to select varieties to cross for genetic studies. Despite its potential benefits, the study's authors have several recommendations for future studies. Thus, in addition to these populations, it is recommended to identify different populations belonging to the sweet cherry tree in Azerbaijan. In addition, the results obtained are recommended to geneticists

and breeders in the selection of samples to be crossed for genetic studies. Selected high-yielding, large-fruited, good-looking sweet cherry genotypes that meet market demand (Samba, Ziraat 0900, Regina, Ramon Oliva, Gara Okuzureyi, Agh gilas, Chahrayi Napoleon) can be used in breeding programs to create new varieties. Nuclear microsatellites AK 193, PaCITA 10, and PaCITA 18 are recommended for the characterization of genetic diversity with SSR primers. The early-ripening Chal Crimea and late-ripening Okuzureyi genotypes with rare alleles can be used as initial material for the improvement of new varieties.

ACKNOWLEDGEMENT

This research was supported by the General Directorate of the Institute of Genetic Resources, Ministry of Science and Education of Azerbaijan.

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