Assessment of genetic diversity and search for Plum pox virus resistance alleles of apricot (*Prunus armeniaca* L.) genotypes spread in Azerbaijan using SSR markers

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Abstract: Apricot is one of the most important stone-fruit plants widely cultivated in Azerbaijan. SSR markers were used to evaluate the genetic diversity of 61 apricot genotypes representing the entire gene pool in Azerbaijan. A total of 138 alleles were produced based on 17 SSR markers with an average of 8.1 alleles per primer. The polymorphism information content (PIC) and expected (He) heterozygosity for individual loci were in the range of 0.42–0.8 (mean 0.68) and 0.52–0.82 (mean 0.72), respectively, indicating the abundant genetic variation in Azerbaijani apricot cultivars. The polymorphisms generated by SSRs were enough to differentiate 95% of genotypes, while 4 apricot cultivars exhibited complete similarity between them. NJ tree grouped the 61 genotypes into 7 clusters with 1000 bootstrap values, where the grouping of genotypes was not closely related to the geographic origin or collection site. STRUCTURE analysis identified 4 subpopulations in the collection and, in general, was in agreement with cluster analysis. (SEO)/Harlayne-type resistance alleles of three SSR (PGS1.21-240 bp, PGS1.23-161 bp, PGS1.24-119 bp) and one SSLP loci (ZP002-127 bp) were detected in 3, 15, 15, and 9 accessions, respectively. STRUCTURE analysis could separate genotypes with resistance alleles grouping them in the first two subpopulations. Of the 61 apricot genotypes, 28 had at least one resistance allele. The results demonstrate that Azerbaijani apricots are an important source for breeding PPV-resistant apricots.

Key words: Apricot, genetic diversity, *PPVres*, cluster, structure

1. Introduction

Apricot, *P. armeniaca* L., is one of the top consumed stone-fruit crops grown worldwide (Hormaza et al., 2007). It belongs to the genus *Prunus* in the family Rosaceae (rose) with 240 Mb genome size and 2n = 16 chromosome number (Khan et al., 2008). The fruit is native to Northern China, Central Asia, and also Near-East region as the secondary gene center of origin (Vavilov, 1951; Hormaza et al., 2007). Apricots are a flavorful source of nutrients, such as fiber, minerals, vitamins, and flavonoids, and can be used in fresh, frozen, canned, or dried form throughout the year not only as a dietary but also as a medicinal product (Fratianni et al., 2018). According to the data of 2020, Türkiye is the leading apricot-producing country, followed by Uzbekistan and Iran[1].

Azerbaijani apricots belong to the Near-East origin center and Irano-Caucasian ecogeographical group (Khan et al., 2008; Yilmaz et al., 2012). Apricot is one of the most important fruit plants widely cultivated in Azerbaijan. The cultivated area of apricot orchards in Azerbaijan for 2020 was 3.83 thousand hectares. Apricot production increased by 20.8% compared to 2015 and reached 29.0 thousand tons, and the yield was 85.0 centners/ha²[2]. One of the issues of ensuring food security in the republic is the creation of highly productive and quality fruit varieties, including apricots. The history of apricot cultivation in Azerbaijan goes back to three thousand years. The main areas suitable for apricot cultivation are Nakhchivan, followed by Goranboy, and Terter regions in the midmountain and

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1 World apricot production by country. Website https://www.atlasbig.com/en-us/countries-apricot-production

2 Azerbaycanda erik istehsalı ile bağlı arayış. Website https://azagroinvest.az/wp-content/uploads/sites/131/2021/12/azerbaycanda-erik-istehsali-il58.pdf

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foothill areas. Different varieties of apricots cultivated in the country are distinguished by their taste, fruit size, and high productivity. As a result of long-term folk selection in Azerbaijan, varieties such as Novraste, Badami, Abutalibi, Ag Tabarza, Shalakh, and Girmiziyanaq have been created, which are also well adapted to the dry and continental climate of the regions (Qasımov, 2015).

Plum pox virus (PPV) (or Sharka) is one of the most devastating diseases of *Prunus* species that have a serious economic impact on apricot production (Zhebentyayeva et al., 2008). Although the disease was not detected in Azerbaijan, it is widespread in Europe and reported in Turkey and Russia, the neighboring countries of Azerbaijan (Teber et al., 2019; Gürcan et al., 2019; Gorina et al., 2020). Host resistance is the most promising approach to managing the disease. Most apricot cultivars including the American and European apricots are susceptible to the disease (Kegler and Hartmann, 1998; Karayiannis et al., 1999; Martinez-Gomez et al., 2000); however, several resistant accessions ("Stark Early Orange" (SEO), "Goldrich", "Harlayne", "Stella", and "Harcot") found in North American apricot germplasm were used as resistant resources for breeding (Martinez-Gomez et al., 2000). Likewise, apricot germplasm from Turkey, which is considered a secondary gene center along with Iran and Caucasia (Mehlenbacher et al., 1990), was screened for PPV resistance and a limited number of apricot cultivars and/or types was identified to be PPV-resistant (Gürcan et al., 2019). Contrary to Middle Eastern and Western apricots, a high frequency of Sharka resistance in wild apricots in Central Asia was reported (Decroocq et al., 2016). Meanwhile, segregation and chromosomal location of resistance gene(s) for North American resistance trait (*PPVres*) were investigated and several resistance-associated molecular markers were developed for screening of SEO/North American type resistance. Initially, three simple sequence repeat (SSR) markers cosegregating with *PPVres* (PGS 1.21, 1.23, and 1.24) were developed (Soriano et al., 2012) and used to screen apricot accessions (Decroocq et al., 2014, Rubio et al., 2014; Decroocq et al., 2016; Gürcan et al., 2020). Later, a single sequence length polymorphism (SSLP) marker named ZP002 was developed for the 5 bp deletion in the region that contains the main candidate resistance gene (Decroocq et al., 2014, 2016).

Genetic diversity assessment in apricots using different molecular markers was reported in several countries (Yilmaz et al., 2012; Krichena et al., 2014; Raji et al., 2014; Bakır et al., 2019; Ozrenk et al., 2020). However, to date, only a few Azeri apricots have been genetically characterized (Decroocq et al., 2016). The aim of the research was thus 1) to investigate the genetic diversity of 61 accessions of apricot genotypes representing the entire gene pool in Azerbaijan using SSR markers and 2) to

search for the SEO/harlayne-type Sharka resistance gene in the germplasm.

2. Material and methods

2.1. Plant materials and DNA extraction

Young leaf samples were collected from 61 apricot genotypes (*P. armeniaca* L.) cultivated in four regions (Agdash, Goranboy, Nakhchivan, Terter) of Azerbaijan, which are considered the main apricot-producing regions of the country (Table 1). Leaf samples were brought to Genome and Stem Cell Center Plant Biotechnology Department in Erciyes University Kayseri in Turkey, and further molecular investigations were conducted in this center. DNA was extracted from the samples using the CTAB (cetyltrimethylammonium bromide) protocol (Dolye and Dolye, 1990). Nanodrop (Thermo Scientific, 2000) was used to determine the concentration and quality of extracted DNA.

2.2. Marker analysis

Seventeen SSR primer pairs that were previously used for the characterization of Turkish apricots (Gürcan et al., 2015) were also used in this study. Ten of the SSR are reported as located close to the *PPVres* on LG1 (Soriano et al., 2008; Vera-Ruiz et al., 2011; Soriano et al., 2012). The remaining seven markers (ssrPaCITA16, ssrPaCITA19, ssrPaCITA4, UDAp-404, ssrPaCITA21, pchgms2, and UDP98-412) were selected from different linkage groups (Table 2). Fluorescently labeled M13(-21) primer with 6-FAM, NED, PET, or VIC was used for SSR fragment analysis and PCR was performed following the method described by Schuelke (2000). The PCR reaction mix consisted of 2 µL of 10X PCR buffer, 0.6 µL of 50 mM MgCl$_2$, 2 µL of 10 mM dNTP, 0.15 µL of 10 µM of a sequence-specific forward primer with M13(-21) tail at its 5´ end, 0.35 µL of 10 µM of a sequence-specific reverse primer, and 0.20 µL of 10 µM the universal fluorescent-labeled M13(-21) primer, 0.2 µL 5U/µL Taq DNA polymerase, and 3 µL of 25 ng/µL sample DNA. The total reaction medium was brought up to 20 µL with distilled water. The PCR program consisted of 3 min of an initial denaturation at 94 °C followed by 35 cycles of 30 s denaturation at 94 °C, 40 s annealing at 60 °C, 1 min extension at 72 °C; and 7 min final extension at 94 °C. PCR products were separated with the capillary electrophoresis on an ABI 3500 (Applied Biosystems, Foster City, CA, USA) located in Erciyes University, Kayseri, Turkey. DNA fragment sizes were determined using GeneMapper 4.1 software (Applied Biyosystems, Foster City, Calif., USA). Additionally, the ZP002 marker linked to *PPVres* (Decroocq et al., 2014) was used for resistance screening. PCR for the ZP002 primer pair was conducted without florescent labeling and was visualized on a 3% agarose gel in TBE 1X buffer employing 90 V,

Table 1. Names, regions, and locations of apricot accessions used in the study.

No	Accessions	Region/ location	No	Accessions	Region/ location
1	Zeynebi	Agdash	32	Genotype 4	Terter
2	May Natig	Agdash	33	Ag erik	Terter
3	Ag erik Gulnar	Agdash	34	Genotype 5	Terter
4	Yeni forma 1	Nakhchivan/ Ashabi-Kehf	35	Shemsi	Nakhchivan
5	Jir Zeferani	Nakhchivan Ashabi-Kehf	36	Genotype 6	Goranboy
6	Jir erik	Nakhchivan Ashabi-Kehf	37	Agjanabad 2	Nakhchivan
7	Gaysi	Nakhchivan Ashabi-Kehf	38	Goyche Nabad	Nakhchivan
8	May chicheyi	Nakhchivan	39	Hagverdi 2	Nakhchivan
9	Balyarim	Nakhchivan	40	Genotype 3	Nakhchivan Ordubad
10	Hampa	Nakhchivan	41	Ordubad Sherefi	Nakhchivan Ordubad
11	Yeni forma 2	Nakhchivan	42	Heydari	Nakhchivan Ordubad
12	Jir Nakhchivan	Nakhchivan	43	Ordubad jiri	Nakhchivan Ordubad
13	Yay Sherefi	Nakhchivan	44	Forma 2	Nakhchivan Ordubad
14	Shalakh 1	Nakhchivan	45	Genotype 2	Nakhchivan Ordubad
15	Teberze 1	Nakhchivan	46	Ordubad Nabati	Nakhchivan Ordubad
16	Tokhum Shemsi	Nakhchivan	47	Yeni forma 3	Nakhchivan Ordubad
17	Gejyetishen	Nakhchivan	48	Shalakh 2	Nakhchivan Babek
18	Badami 1	Nakhchivan	49	Alcha erik	Nakhchivan Babek
19	Helena	Nakhchivan	50	Abu Talibi	Nakhchivan Babek
20	Mehmani	Nakhchivan	51	Teberze 2	Nakhchivan Babek
21	Hagverdi 1	Nakhchivan Sherur	52	Ag erik Elchin	Agdash
22	Ag Nabati	Nakhchivan Sherur	53	May Goranboy	Agdash
23	Kurdeshi	Nakhchivan Sherur	54	Mayovka 1	Agdash
24	Talibi	Nakhchivan Sherur	55	Badami 2	Agdash
25	Genotype 1	Nakhchivan Sherur	56	Shalakh 3	Agdash
26	Ag badami	Nakhchivan Sherur	57	Girmiziyanag	Terter
27	Agjanabad 1	Nakhchivan Sherur	58	Genotype 7	Terter
28	Limon erik 1	Nakhchivan Sherur	59	Mayovka 2	Terter
29	Forma 1	Nakhchivan Sherur	60	Limon erik 2	Nakhchivan Babek
30	Ag erik (late ippening)	Goranboy	61	Esgerabat	Nakhchivan Sherur
31	Ag erik (early ippening)	Goranboy			

followed by ethidium bromide staining and visualized under UV using gel documentation system BioRad. 'Harlayne' and 'SEO' varieties were used as a positive control for PPV resistance screening.

2.3. Data analysis

The neighbor-joining (NJ) method was used to construct and draw a dendrogram from the genetic similarity matrix based on the SSR data with 1000 bootstrap values. The genetic similarity matrix was created by using PowerMarker software and the NJ tree was created using the DarWin 6.0 (Liu and Muse, 2005; Perrier and Jacquemoud-Collet, 2006) software. The expected heterozygosity (He), observed heterozygosity (Ho), and the polymorphism information content (PIC) were calculated using the PowerMarker. STRUCTURE v2.3.4 (Pritchard et al., 2000) software was used for assigning individuals to the inferred populations. The number of subgroups (K) ranged from 1 to 7, and burn-ins set to 100,000 was performed using the admixture model. The value of k was also estimated by calculating the_K values according to Evanno et al. (2005) using STRUCTURE HARVESTER (Earl and vonHoldt, 2012) and ΔK was used to determine the optimal number of subgroups.

3. Results

3.1. Genetic diversity

DNA of all genotypes was successfully amplified by each of the 17 SSR primer pairs and with ZP002 as well. DNA fragment analysis identified a total of 138 alleles for 61 local apricot genotypes. The number of alleles varied from 4 (Gol061, ssrPaCITA5) to 14 (ssrPaCITA17), with an average of 8.1 per locus (Table 2). The major allele frequency ranged from 0.25 to 0.57, the average was 0.38. The highest frequency (0.92) was noted for 150 bp at locus ssrPaCITA5. One unique allele was found at the UDAp-404 locus in 'Zeynebi' (1). The observed (Ho) and expected (He) heterozygosity for individual loci were in the range of 0.35–1 (mean 0.83) and 0.52–0.82

(mean 0.72), respectively. The observed heterozygosity was higher than the expected heterozygosity, except for PGS1.24, UDAp-404, and ssrPaCITA21 loci. ssrPaCITA4 showed the highest PIC (0.8), while ssrPaCITA5 showed the lowest (0.42). The mean PIC value scored across all SSR alleles for the entire collection was 0.68 (Table 2). Out of 17 markers, 16 revealed PIC values of higher than 0.5. The average PIC value was the highest in accessions from Nakhchivan (0.68), followed by Agdash (0.67) (Table 3).

The values of the genetic dissimilarity index between the local apricot genotypes based on SSR markers varied from 0 to 0.97, the mean was 0.57. Cultivar pairs Teberze 2 (51)–Shalakh 3 (56) and Badami 2 (55)–Limon Erik 2 (60) exhibited complete similarity between them. Genotypes

Table 2. Genetic diversity parameters of 61 apricot cultivars based on 17 SSR markers.

Marker	Linkage group	Major allele freq.	Observed allele number	He	Ho	PIC
Gol061	1	0.40	4	0.69	0.74	0.63
PGS1.03	1	0.32	7	0.77	0.98	0.73
PGS1.20	1	0.34	10	0.80	0.85	0.77
PGS1.21	1	0.37	10	0.77	0.95	0.74
PGS1.23	1	0.39	10	0.76	0.95	0.73
PGS1.24	1	0.49	5	0.68	0.61	0.64
PGS1.252	1	0.50	6	0.59	0.90	0.50
96P10_SP6	1	0.25	9	0.82	0.96	0.79
ssrPaCITA5	1	0.57	4	0.52	0.54	0.42
ssrPaCITA17	1	0.25	14	0.82	0.87	0.79
aprigms18	1	0.37	9	0.78	0.98	0.75
ssrPaCITA16	2	0.49	6	0.69	0.87	0.66
ssrPaCITA19	2	0.31	8	0.75	0.98	0.71
ssrPaCITA4	3	0.26	7	0.82	0.94	0.80
UDAp-404	4	0.34	12	0.76	0.60	0.73
ssrPaCITA21	5	0.45	6	0.61	0.35	0.54
pchgms2	7	0.41	11	0.66	1.00	0.61
Mean		**0.38**	**8.1**	**0.72**	**0.83**	**0.68**
Total			**138**			

Table 3. Genetic diversity parameters within each geographic region.

Subset	Sample size	Observed allele number	He	Ho	PIC
Nakhchivan	44	7.2	0.73	0.84	0.68
Agdash	8	5.5	0.72	0.79	0.67
Terter	6	2.9	0.56	0.84	0.48
Goranboy	3	3.1	0.58	0.80	0.51

'May Natig' (2) and 'Genotype 7 ' (58) were found as genetically most dissimilar, followed by Balyarim (9)–Abu Talibi (50), Helena (19)–Mehmani (20), and Yeni forma 3 (47)–Genotype 7 (58) with a genetic distance equal to 0.91. Genetic relationship among accessions was further represented in an NJ tree, which grouped the 61 genotypes into 7 clusters with 1000 bootstrap values (Figure 1). The clusters are further divided into subclusters with a different number of accessions in each. Cluster 1 was the largest and consisted of 28 genotypes representing almost all regions in this study, while cultivar 'Genotype 2 ' formed a separate cluster 6. Clusters 2 and 3 contained 14 and 11 genotypes, respectively. Cluster 5 consisted of 3 genotypes from three different regions, while the remaining clusters 4 and 7 had two cultivars each.

STRUCTURE analysis identified 4 subpopulations in the collection as the maximum ΔK value was reached at K = 4 (Figure 2) and in general, was in agreement with cluster analysis. Four putative subpopulations with belonging accessions were given as a bar plot in Figure 3. Subpopulations P1 and P2 match up with cluster 2 and the second and third subclusters of cluster 1, while P3 and P4 correspond to the first subcluster of cluster 1 and cluster 3, respectively. Genotypes number 15, 34, 36, 39, 41, 45, 59 which are equivalent to clusters 4, 5, 6, and 7 had the highest admixture and were mainly located at the end of the P4.

3.2. *PPVres* alleles

Three SSRs (PGS1.21, PGS1.23, PGS1.24) used in the current study were reported to produce alleles cosegregated with the *PPVres* locus. In addition, one simple sequence length polymorphism (SSLP) marker - ZP002 identified a 5-bp deletion on the ppb022195m, which is the main candidate gene conferring PPV resistance (Zuriaga et al., 2013). In the current study, the alleles PGS1.21-240 bp, PGS1.23-161 bp, PGS1.24-119 bp, and ZP002-127 bp were detected in 3, 15, 15, and 9 accessions, respectively. Of the 61 genotypes, 28 had at least one resistance allele. Besides 'Harlayne' and 'SEO' control varieties, local cultivars 'Zeynebi (1)' and 'Yeni forma 1 (4)' (Ashabi-Kahf) had all four resistance alleles. In addition, 2 genotypes ('May Natig' (2) and 'Gaysi' (7)) had three and 4 genotypes ('Ag Nabati' (22), 'Genotype 1 Türkiye' (25), 'Forma 1 (Sherur)' (29), and 'Genotype 2 ' (45)) had two resistance alleles, all in heterozygous form (Table 4). Cluster 2 and the second and third subclusters of Cluster1 can be designated as 'Resistant group' as they contained the majority of accessions with PPVresistance allele. STRUCTURE analysis also could separate genotypes with resistance alleles grouping them in the first two subpopulations.

4. Discussion

Transcaucasia, including Azerbaijan, is accepted as one of the native countries of apricot with high diversity and
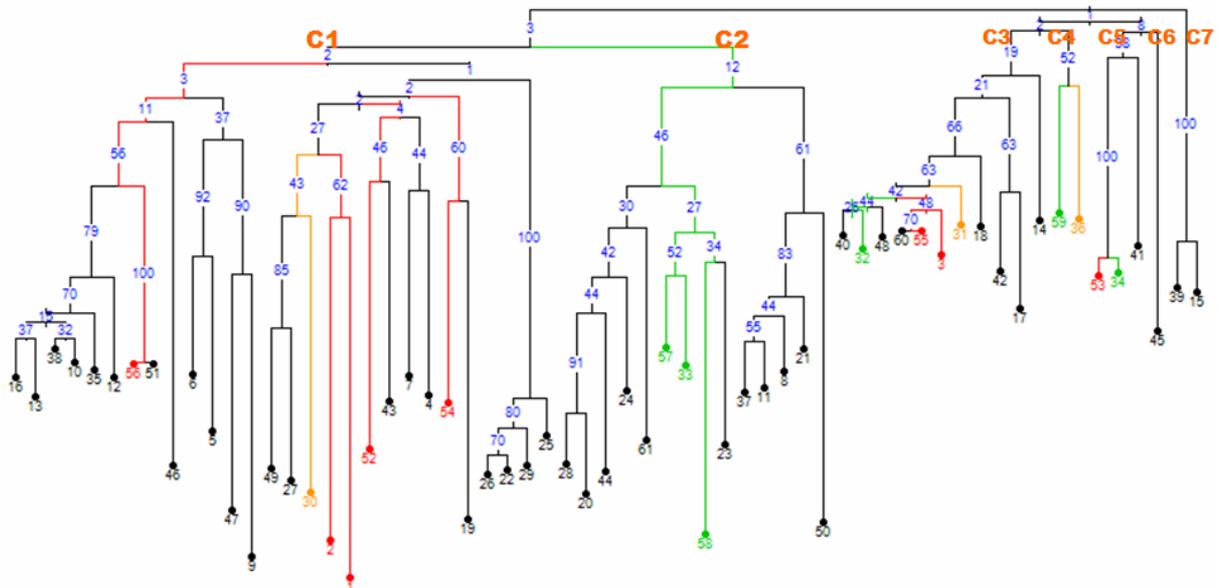

Figure 1. Genetic relationships among 61 apricot cultivars generated using 17 SSR markers. Black, Nakhchivan; red, Agdash; green, Terter; yellow, Goranboy. Accession numbers are given in Table 1. The Roman numerals indicate the cluster numbers.
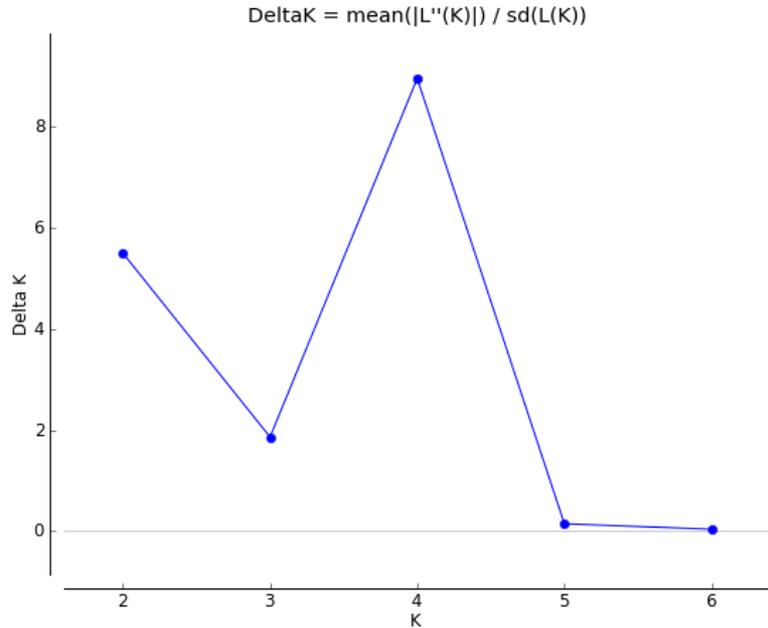
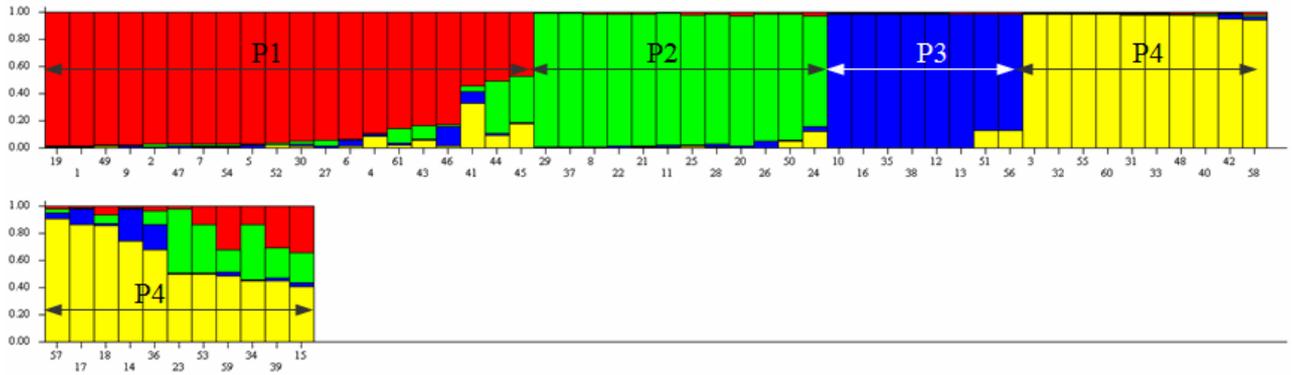*Figure 2.* ΔK vs. K plotted as proposed by Evanno et al. (2005).

Figure 3. Population structure of the apricot accessions. The bar lengths represent Q value. P1 - P4: different populations.

larger fruits (Khan et al., 2008). Thus, knowledge about the genetic structure of Azerbaijani apricots is very useful for both their effective use and future improvement and breeding strategies. SSR markers were frequently used for studying genetic diversity and taxonomic relationships among different species and apricot cultivars (Hormaza, 2002; Zhebentyayeva et al., 2003; Krichen et al., 2006; Pedryc et al., 2009; Akpınar et al., 2010; Bakır et al., 2019; Ozrenk et al., 2020; Sheikh et al., 2021).

In the current study, genetic diversity of local apricot cultivars was studied using 17 SSR markers. These primer pairs were previously used for the characterization of Turkish apricots (Gürcan et al., 2015) and showed high

polymorphism. At the present study, the total number of alleles for 17 microsatellite loci came to 138, with the overall mean of 8.1 per locus, which is twice higher than that obtained by Hormaza (2002) and Romero et al. (2003) with 19 and 16 microsatellites, respectively. Only one cultivar in our study had a unique allele. Thus, an allele 182 bp of UDAp-404 locus was found only in cultivar 'Zeynebi' (1). However, out of 138 alleles, 30 were rare with frequencies less than 1%. Primers UDAp-404, pchgms2, and ssrPaCITA17 were the loci with the highest rare alleles. These alleles can be used as molecular identity data for separate apricot cultivars. On the other hand, the average PIC and the expected heterozygosity were 0.68 and 0.72,

Table 4. Allelic profile of resistant (SEO, Harlayne) and resistant accessions at 3 SSR and 1 SSLP loci placed close to the *PPVres* locus on LG1.

No	Accessions	PGS1.21 240 bp	PGS1.23 161 bp	PGS1.24 119 bp	ZP002 127 bp
1	Zeynebi	194/240	159/161	119/123	127/132
2	May Natig	208/210	159/161	119/119	127/132
4	Yeni forma 1	194/240	161/163	119/121	127/132
7	Gaysi	194/240	155/163	119/121	127/132
8	Maychicheyi	212/214	161/163	-	132/132
20	Mehmani	212/214	159/161	-	132/132
21	Hagverdi 1	212/214	155/161	123/123	132/132
22	Ag Nabati	198/216	147/153	119/121	127/132
23	Kurdeshi	194/214	147/161	121/121	132/132
24	Talibi	212/214	155/161	121/121	132/132
25	Genotype 1	198/216	147/153	119/121	127/132
26	Ag badami	198/216	147/153	121/149	127/132
27	Agjanabad 1	194/194	155/159	119/121	132/132
28	Limon erik 1	212/214	159/161	-	132/132
29	Forma 1	198/216	147/153	119/121	127/132
30	Ag erik (late rippening)	194/216	159/161	-	132/132
31	Ag erik (early rippening)	194/214	153/155	119/121	132/132
36	Genotype 6	194/214	153/155	119/121	132/132
37	Agjanabad 2	212/214	155/161	-	132/132
40	Genotype 3	194/214	155/161	121/121	132/132
44	Forma 2	212/214	159/161	-	132/132
45	Genotype 2	212/214	147/161	119/121	132/132
49	Alcha erik	194/194	155/159	119/121	132/132
52	Ag erik Elchin	194/194	155/161	121/121	132/132
54	Mayovka 1	194/220	153/155	119/123	132/132
57	Gyrmiziyanag	194/214	153/155	119/121	132/132
59	Mayovka	194/214	153/155	119/121	132/132
61	Esgerabat	194/214	155/163	123/123	127/132
	SEO	194/240	155/161	119/121	127/132
	Harlayne	194/240	155/161	119/121	127/132

respectively, which both indicate rich genetic diversity in local apricot germplasm and confirm the effectiveness of used SSR primers. The PIC values were significantly correlated and increased with the number of alleles at a given locus (r = 0.7; p < 0.05). Our results concur well with the earlier reports of Zhebentyayeva et al. (2003) and are higher than the values reported by Martínez-Mora et al.

(2009), Bourguiba et al. (2012), and Akpınar et al. (2010). Zhang et al. (2013) analyzed 94 apricot cultivars from China using 21 SSR markers and showed a high level of genetic diversity, where the mean expected heterozygosity was 0.792. In our experiment, null alleles were found in 10 out of 17 loci, with a frequency range from 1.6% to 19%; however, in the vast majority of the loci the Ho values

were higher than He values, indicating high heterozygosity level and favorable allele richness in the studied local apricot collection. Zhang et al. (2013) assert that the high frequency of null alleles can also be attributed to the domestication center as they are a result of mutations in the flanking region used for primer binding.

The apricot germplasm in the current study was collected from four different regions of Azerbaijan. However, the majority of cultivars (72%) represent the Nakhchivan Autonomous Republic (AR), which is distinguished by rich flora and has ancient horticultural traditions. A large number of local, as well as introduced, apricot varieties are widespread in the region. Thus, high diversity found in the studied collection was expected and can be mainly related to the wide and rich ecogeographical diversity of Nakhchivan AR and hybridizations among local materials conducted by local gardeners for centuries. Agdash region represented by only eight cultivars was the second most diverse, while genetic diversity among 6 Terter (He = 0.56) and 3 Goranbay (He = 0.58) cultivars were relatively the same but still high (Table 3). Vavilov (1951) mentioned Iran, Turkey, and the Caucasus as one of the possible origin centers of apricot, while Kostina (1946) suggested that apricots were transferred from Central Asia to Iran and the Transcaucasian area, including Azerbaijan and have undergone further improvement here. Both statements, to some extent, explain and justify the high diversity found in the current study. However, a high level of genetic variation could also be due to the right choice of highly polymorphic markers.

All markers, except ssrPaCITA5, showed PIC values above 0.5, which can be considered highly informative according to Botstein et al. (1980). However, in the current study, a subset of 10 loci representing high heterozygosity and PIC values (above 0.7) from 4 different linkage groups which is preferred for better genome coverage can be recommended with confidence for future fingerprinting studies.

A wide range of genetic distance (0 to 0.97) was found within the studied germplasm, once again underlining the abundant genetic variation in Azerbaijani apricot cultivars. The polymorphisms generated by SSRs were enough to differentiate 95% of genotypes. Only 4 apricot cultivars could not be discriminated against using these SSR loci as they were identical at 18 loci (17 microsatellites and 1 SSLP) with a similarity coefficient of 1.0. NJ tree grouped 61 local apricot varieties into 7 clusters. The major cluster 1 is further subdivided into three subclusters, where the first subcluster is composed of a very homogenous group of 12 cultivars from Nakhchivan and 1 from Agdash and the third cluster is represented exclusively by samples from Nakhchivan. Five Agdash genotypes were also located in this cluster, of which Zeynebi (1) and May Natig (2) were the closest. Fourteen cultivars from Nakhchivan and

Terter formed three nearly homogenous subclusters within cluster 2, whereas cluster 3 was the most heterogeneous in terms of geographical regions and contained genotypes from all four regions. Three genotypes fell into cluster 5; however, an accession namely 'Ordubad sherefi' (41) was found quite distinct from the other cultivars as it clustered apart from the two genotypes with GD values 0.35 and 0.38. Genotype 6 (Goranboy) (36) and Mayovka 2 (Terter) (59) formed cluster 4, and the last cluster 7 included genotypes Teberze 1 (15) and Hagverdi 2 (39), both from Nakhchivan, which exhibited high similarity (GD = 0.09). Furthermore, cluster 6 is formed only by one accession 'Genotype 2 ' (45), which indicates the remoteness of this variety. The genetic distance between this genotype and the rest of the cultivars varied from 0.41 to 0.79. Thus, genotypes from Nakhchivan are dominated in all clusters, which is certainly due to the largest sample size from this region. Despite the genotypes with the same origin implying a low genetic diversity, genetic distance value among cultivars from Nakhchivan was equal to 0.54. As mentioned due to varied geographic and climatic zones, Nakhchivan has abundant apricot germplasm resources (Qasımov, 2015). The apricot gardens in Nakhchivan are located in the valleys of rivers running from the mountains into the Araz river, and sometimes in highlands around 1800–2000 m altitude, which also leads to the emergence of rich diversity and differentiation at the same time. Cultivars from other regions were also placed in different subclusters and diffused into each other, indicating that grouping based on genetic parameters was not closely related to the geographic origin or collection site and significant germplasm exchange and transfer took place among these regions. Thus, three cultivars used from Goranboy and collected from the same/very close locations were quite different and distributed among clusters 1, 3, and 4 with a GD range of 0.31–0.61. The 8 cultivars from Agdash also did not cluster together: five were in cluster 1, two in 3, and one was in cluster 5. Such occurrence was due to the diverse genetic background among Agdash cultivars, which was also supported by the high number of alleles and high PIC value (Table 3). Genetic distances among them varied from 0.06 to 0.83. At the same time, the cultivars from Terter fell into 4 different clusters. However, three of them formed a separate subcluster within cluster 2 together with 'Kurdeshi' (23) from Nakhchivan, indicating some portion of shared alleles. Gürcan et al. (2015) studied the genetic diversity of the Turkish apricot germplasm together with accessions from Europe and Pakistan and pointed out that the high genetic similarity does not reflect the ecogeographical grouping of apricots. Similarly, Malaki et al. (2006) suggested that accessions collected from the different geographic areas did not necessarily have different genetic backgrounds.

The most confusing result was the identity between cultivars from different regions. Thus, 'Teberze 2' (51) from Nakhchivan showed 100% similarity with 'Shalakh 3' (56) from Agdash, whereas another variety form Agdash 'Badami 2' (55) had the same SSR profile as 'Limon Erik 2' (60) from Nakhchivan. This fact makes them potential synonyms based on studied microsatellite loci. Another case that should be noted in the study is the existence of plant materials with the same name introduced from various locations. What is surprising, all these cultivars had different fingerprints and thus can be considered 'homonyms'. Thus, based on the SSR data from 17 loci 10 accessions in the studied collection had one or two more homonyms, which are distinguished by numbers 1, 2, and 3 in Table 1. For instance, three genotypes under the Shalakh name (2 from Nakhchivan and one from Agdash) had completely different molecular profiles (GD range 0.53–0.59), while 'Shalakh 3' (56) from Agdash was the same as 'Teberze 2' (51), most probably excluding the latter being 'Shalakh'. The same was noted for Limon Erik 1 (28) and Limon Erik 2 (60) with a genetic distance equal to 0.71, while the second one was identical with 'Badami 2' (55) as mentioned. As 'Shalakh 3' (56) and 'Teberze 2' (51) could be distinguished from each other with neither morphological (flesh color, skin ground color, and total soluble solids) nor SSR markers, they seem to be the same cultivar. Moreover, only a slight difference in skin ground color was observed between 'Badami 2' (55) and 'Limon Erik 2' (60). Anyway, additional morphological and genotyping analyses are required to determine the correct names for these cultivars. Due to its multiallelic and codominant nature, SSR markers can be quite useful in this effort. The synonyms and homonyms can be arisen due to mistakes both at the source and during propagation (Gökirmak et al., 2009). These varieties are cultivated by local farmers in their gardens for many decades and were probably distributed among other farmers without proper descriptions and correct names, thereby names used for the same cultivar changed in different growing areas. On the other hand, similarity in pomological and other traits may lead to confusion and miscalling different varieties with the same name.

According to ∆K value of STRUCTURE analysis, 61 local apricot accessions were divided into four different subpopulations. The majority of accessions were strongly assigned to their corresponding subpopulations with high Q values, whereas about 15% of cultivars were high admixture, pointing to their complex pedigrees. Bar clusters were correlated with grouping in the NJ tree. Twenty accessions were assigned to the P1 subpopulation, of which 16 represent Cluster 1, while P3 is completely composed of genotypes of Cluster 1 (first subcluster) with Q values 0.85–0.99. The majority of accessions in

subpopulation P2 (67%) were placed in cluster 2 in the NJ tree. The remaining 21 apricot accessions were assigned to P4, of which 11 fell into cluster 3, and 4 into cluster 2. In addition, clusters 4 and 7, each with two accessions also consigned to P4. By now, STRUCTURE is widely used in many studies aiming to understand the population structure of the apricot. Bourguiba et al. (2012) found that apricot accessions of Mediterranean countries form three main populations, namely Irano-Caucasian, North Mediterranean and South Mediterranean Basins. Krichena et al. (2014) revealed that Tunisian apricot germplasm is divided into two distinct gene pools, indicating two distinct origins.

PPV resistance in apricot is controlled by the major locus in the upper part of LG1 and by minor loci in LG3 and LG5 (Lambert et al., 2007; Lalli et al., 2008; Sicard et al., 2008; Soriano et al., 2008). Several molecular markers were located close to them (Dondini et al., 2011; Vera-Ruiz et al., 2011; Soriano et al., 2012), of which three (PGS1.21, PGS1.23, and PGS1.24) have been noted to be closely linked to the *PPVres* loci (Soriano et al., 2012). We observed one base increment (PGS1.21-240 bp, PGS1.23-161 bp, PGS1.24-119 bp) for the studied alleles in our study compared to Soriano et al. (2012) which may be due to the reading chemistry as also mentioned by Gürcan et al. (2015). The use of molecular markers linked to the gene of interest can accelerate the selection and breeding of desired genotypes with one or more resistance loci. Our research revealed that nearly half of the studied apricot accessions (46%) possessed a resistance allele to PPV. Twenty apricot cultivars had one resistance allele, whereas 2 genotypes, namely 'Zeynebi' (1) and 'Yeni forma 1' (4) had all four resistance alleles (Table 4). Cultivars 'May Natig' (2) from Agdash and 'Gaysi' (7) from Nakhchivan had three alleles; however, allele combination has differed; PGS1.21-240 bp was absent in May Natig (2), while PGS1.23-161 bp was not detected in Gaysi (7). Moreover, 'May Natig' was homozygous in the PGS1.24 locus. Cultivars 'Ag Nabati' (22), 'Genotype 1 ' (25), and 'Forma (Sherur)' (29) possessed two desired alleles (119 bp and 127 bp) for markers PGS1.24 and ZP002 and 'Genotype 2 ' (45) (161 bp and 119 bp) for markers PGS1.23 and PGS1.24. The potential of these three markers for MAS studies was investigated in several studies. Thus, Gürcan et al. (2015) reported that only 1.7% of Turkish apricot accessions have (SEO)/Harlayne-type resistance alleles, while it was very high in the Pakistani accessions (41.7%). Screening of 31 accessions containing resistant and susceptible apricot plants showed the presence of the desired allele for each marker at the three loci in all resistant varieties (Soriano et al., 2012). However, this allele was absent in the susceptible germplasm. Rubio et al. (2014) screened 80 apricot cultivars for PGS1.21, PGS1.23, and PGS1.24 loci

and observed a close relationship between the phenotypes and allele combinations of resistant accessions. However, a few susceptible cultivars also showed these alleles. Despite the tight linkage, several studies have concluded that the use of markers colocalizing with the *PPVres* locus is not fully reliable (Decroocq et al., 2014). However, according to Passaro et al. (2017), it still represents an effective tool for the preliminary screening of research material. Due to the absence of phenotypic data on Azerbaijani accessions, we were not able to test the reliability of the markers.

Despite that the NJ tree was constructed based on 17 SSR loci, of which only three are closely linked to *PPVres* loci, a tendency of colocation of resistant accessions in the dendrogram was observed. Thus, while all the 13 accessions in the first subcluster of cluster 1 did not have any desired allele for each of the four marker loci, the *PPVres* allele was observed in most of the accession of the second and third subclusters and cluster 2. Moreover, within the second subcluster genotypes with 3 and 4 desired resistance alleles were the closest, whereas 3 of 4 accessions forming the third subcluster had two resistance alleles. In other words, 23 out of 28 genotypes exhibited resistance alleles clustered together in the second cluster and two subclusters of the first cluster and showed similar SSR patterns, which indicates that they have common (shared) alleles not only for *PPVres* but also for other SSR loci studied. Similar results were also obtained in STRUCTURE analysis. Thus, 22 out of 28 apricot accessions with *PPVres* alleles were located in P1 and P2 bar clusters. The rest of 6 resistant cultivars fell into P4. Similarly, Gürcan et al. (2015) have concluded that 'Harlayne', 'SEO', and resistant accession found in the study had a common heritage as they were grouped together in the dendrogram. In a like manner, eight putative subpopulations were found based on the STRUCTURE analysis, which nicely separated the resistant cultivars and the Pakistani accessions from the rest.

To summarize, SSR profiles of 61 Azerbaijani apricot genotypes have been described for the first time and high diversity and genetic differentiation were observed within the local genepool. The results indicate the importance of the current collection for apricot improvement and breeding programs as a source of useful alleles and for facilitating the introgression of these desired alleles into new apricot varieties. In addition, SSR data obtained provides reliable and valuable information for effective conservation and management strategies, including duplicate (synonyms and homonyms) identification. The results demonstrate that *PPVres* alleles are abundant in the Azerbaijani apricots and they can serve as a valuable source for breeding and can assist breeders in choosing suitable parents for future selection strategies on Sharka resistance.

Competing interests
There is not any conflict of interest between the authors.

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