REPUBLIC OF AZERBAIJAN

On the rights of the manuscript

ABSTRACT

of the dissertation presented for obtaining the degree of Doctor of Philosophy

STUDY OF GENETIC DIVERSITY AND SHARKA DISEASE RESISTANCE OF APRICOT PLANT SPREAD IN AZERBAIJAN

Specialty: 2409.01 – Genetics

Field of science: Biology

Applicant: **Amina Marfat Rakida**

BAKU-2024

Dissertaion work was performed at the Departments of "Fruit Plants", "Molecular Genetics and Genomics", "Immunogenetics" of the Institute of Genetic Resources of the Ministry of Science and Education of the Republic of Azerbaijan, and Biotechnology laboratory of the Genome and Stem Cell Center, Erciyes University, in Kayseri, Turkey.

Scientific supervisors: Corresponding member of ANAS, professor
Zeynal Iba Akparov
PhD in biology, Associate professor
Kahraman Idare Gurcan

Official opponents: Doctor of biological sciences, professor
Ramiz Tagi Aliyev

PhD&Doctor of Sciences, Corresponding member of ANAS
Ilham Eyyub Shakhmuradov

Doctor of Philosophy in Biology
Natavan Zohrab Bakhshaliyeva

FD 1.37 Dissertation council of the Supreme Attestation Commission under the President of the Republic of Azerbaijan operating at the Institute of Genetic Resources, MSE RA.

Chairman of the dissertation council:
Doctor of biological sciences, associate professor
Mehraj Ali Abbasov

Scientific secretary of the dissertation council:
Doctor of Philosophy in Biology
Saida Gasim Hasanova

Chairman of scientific seminar:
Doctor of biological sciences, associate professor
Hamlet Beykishi Sadigov

INTRODUCTION

Relevance and degree of development of the topic. Currently, in addition to food rich in carbohydrates, fats and proteins, in ensuring balanced nutrition of the population, more attention is being paid to research on the effective consumption of fruits, which contain vitamins, minerals and essential fatty acids that have a significant effect on the normal functioning of the body. In this regard, the consumption of fruits at the second level of the food chain is of particular importance[1]. Apricot is one of the important fruit plants belonging to the *Rosaceae* family and widely distributed throughout the world (*Prunus sp.*)[2]. Apricot holds an important place in human nutrition due to its delicious fruit. Apricot containing sugars (mainly glucose, fructose and sucrose), organic acids (mainly malic, citric, etc.), phenols, flavonoids, anthocyanins, carotenoids, fibrous foods, bioactive and nutritional compounds such as minerals and vitamins are considered beneficial functional foods in terms of health and disease risk reduction[3]. All studied apricot species are stable diploids[4] with eight pairs of chromosomes (2 n = 16) and have very small genomes of 240 Mb[5]. All of them can be intercrossed in both directions, which makes their classification confusing. This plant has been cultivated in Azerbaijan for more than three thousand years, and

---

[1] Yılmaz, I. The Biological and Pharmacological Importance of Apricot //SOJ Pharmacy & Pharmaceutical Sciences, – 2018. 5(1), – p.1-4.

[2] Moustafa, Kh., Cross, J. Production, pomological and nutraceutical properties of apricot //Journal of Food Science and Technology, – 2019. 56(1), – p.12-23.

[3] Di Vaio, C. Bioactive compounds and fruit quality traits of Vesuvian apricot cultivars (*Prunus armeniaca* L.) and use of skin cover colour as a harvesting index / C. Di Vaio, C. Cirillo, A. Pannico //Australian Journal of Crop Science, – 2019. 13(12), – p.2022-2029.

[4] Zhebentyayeva, T. Chapter 12 Apricot /T. Zhebentyayeva, C. Ledbetter, L. Burgos, [et al] //Handbook of Plant Breeding, Fruit Breeding, – vol. 8. – 2012. – 415-458 p.

[5] Mori, G. D. Resistance to Sharka in Apricot: Comparison of Phase-Reconstructed Resistant and Susceptible Haplotypes of 'Lito' Chromosome 1 and Analysis of Candidate Genes /G. D. Mori, R. Falchi, R. Testolin [et al] //Frontiers in Plant Science, – 2019. 10, – p.1-15.

its cultivation is expanding year by year in a number of regions. Nakhchivan economic zone accounts for 30% of apricot production in Azerbaijan[6]. Among stone fruit crops, apricot occupies an important place in Azerbaijan due to its economic importance and historically this plant has been cultivated since ancient times and has always been in the spotlight due to its diversity and quality. For many years, the folk breeding cultivars of apricot have been created in Azerbaijan, as well as scientific breeding cultivars have been created by conducting research works on its breeding. Examples of these are apricot cultivars such as Shalakh, Badam erik, Girmiziyanag, Abu Talibi, Ag Tabarza (Balyarim), Hagverdi and etc. Despite the genetic diversity of very wide folk, scientific and introduced cultivars and forms of apricot, certain research activities were not carried out towards their targeted collection and creation of a complete collection. In order to create a gene pool of the apricot plant, to assess its genetic diversity, to study its resistance to diseases and pests, to identify *S* genotypes as a result of the characterization of self-(in)compatibility phenotypes and to create new cultivars, it is important to collect its folk and scientific breeding cultivars and to select initial parent forms for the purpose of hybridization.

Sharka disease is one of the most dangerous diseases of stone fruits. The causative agent of this disease, *Plum pox virus* (PPV), infects the leaves and fruits of apricot trees. The causative agent of the disease, *Plum pox virusu* (PPV), is easily transmitted by many aphid species (breeding nursery trade) through grafting and is widespread among *Prunus* species [7]. Although Sharka disease is not observed in Azerbaijan, it is often found in folk and scientific breeding cultivars of apricot in neighboring countries. The presence of this disease in neighbouring countries indicates that there is always a high probability of its transmission to the territory of Azerbaijan. From this perspective, the study of disease resistance of cultivars and forms of apricots cultivated in Azerbaijan, as well as

[6] Züleyxa. Ərik bitkisinin bioloji xüsusiyyətləri//Paralel.az. –2021.

[7] [Elektron resource] URL: https://www.cabi.org/isc/datasheet/42203

the selection and use of sustainable forms in breeding are highly relevant.

Still in, no research has been conducted in Azerbaijan to study the resistance of apricot collections to Sharka disease. This research is the first scientific work on the molecular study of resistance of apricot genotypes common in Azerbaijan to this disease.

From this point of view, it is very important to identify cultivars and forms in which resistance genes to Sharka disease are observed in the apricot collection created in Azerbaijan, and to create a collection of apricot traits; studies conducted in this direction can be used in the future to create cultivars resistant to Sharka disease.

Object and subject of the research. Local and introduced cultivars and forms of the apricot plant were taken as the research object. The subject of the research is the molecular genetic study of the genetic diversity of the apricot collection and its resistance to Sharka disease.

Goals and objectives of the research. The main goal of the research is to create a collection of apricot cultivars and forms existing in Azerbaijan, assess the genetic diversity of the collection using molecular markers, as well as study the resistance of apricot genotypes to Sharka disease.

To this end, the following tasks were set:

- determination of coordinates (GPS) of areas where local and introduced cultivars and forms of apricots are distributed or cultivated and creation of a collection;
- assessment of pomological, phenological and biomorphological traits of apricot cultivars and forms;
- study of genetic diversity of apricot varieties and forms with specific markers;
- creation of core collections of apricot cultivars and forms based on molecular data;
- study of resistance to Sharka disease in apricot collection with gene-specific markers;
- determination of *S* genotypes as a result of characterization of apricot genotypes according to the self-(in)compatibility phenotype.

Research methods. Both classical (assessment of morphological characteristics, pomological analysis, etc.) and modern methods (DNA extraction, PCR reaction, screening using SSR and gene-specific markers, etc.) were applied in the research work.

The main provisions of the defense:

- according to individual morphological and quantitative traits, there is statistically significant high genetic diversity among 61 apricot genotypes cultivated in different environmental conditions;
- According to the results of cluster analysis based on 10 biomorphological, phenological and pomological parameters, the closest genotypes are Agja Nabad 2 (Nakhchivan) and Goyje Nabad (Nakhchivan), and the most distant genotypes are Ag erik Elchin of Agdash origin and Ordubad erik of Tartar origin;
- According to 17 SSR and 1 SSLP markers, among 61 local apricot samples, genotypes from Nakhchivan and Agdash have the highest genetic diversity (PIC=0.65), and samples from Tartar have the lowest genetic diversity (PIC=0.45);
- according to the results of cluster analysis of molecular data, there are 4 subpopulations of 61 local apricot samples grouped into 4 clusters according to the results of STRUCTURE analysis;
- 28 out of 61 apricot samples have sample-specific multiple resistance alleles for 3 SSR and 1 SSLP markers linked to the *PPVres* locus, which provides resistance to the sharka disease;
- according to the self-incompatibility phenotype, at least 9 different *S* genotypes (S_2, S_3, S_6, S_7, S_8, S_{11}, S_{12}, S_{13} and S_c) are present in 61 apricot samples: S_{13} genotype found in 27 samples is the highest, 2- S_{12}, S_3, S_8 and S_C genotypes found in 3 samples have the lowest frequency.

Scientific novelty of the research. For the first time in Azerbaijan, genetic resources of the apricot plant were collected, apricot collection was created and their genetic diversity was assessed, and the resistance of the apricot collection to Sharka disease was evaluated.

For the first time, apricot cultivars grown in Azerbaijan were studied using SSR and SSLP markers, and very high genetic

variation was discovered in the local gene pool. Each accession of the collection was characterized on 17 SSR and 1 SSLP loci, their molecular genetic profile was developed, and synonyms and homonyms were determined.

For the first time, as a result of the study of 61 local and introduced apricot accessions in the collection for 3 SSR and 1 SSLP marker linked to the *Plum Pox Virus* resistance (*PPVres*) locus, accessions with alleles of resistance to Sharka disease were found. In addition, for the first time, the self-(in)compatibility phenotype of the accessions was characterized based on the PCR amplification of the *S-RNase* intron region and the SFB gene in the local apricot gene pool. The *S* genotype of apricot accessions common in Azerbaijan was determined, and it was established that the *Sc* allele, responsible for self-compatibility in the collection, was not present in other genotypes, except for the Mayovka 1 and Forma 2 genotypes, and most of the studied accessions demonstrated self-incompatibility.

Theoretical and practical significance of research. Various genotypes genetically selected in the course of the studies can be used as starting material for obtaining new cultivars. Certification of the apricot collection in accordance with the requirements of the international descriptor and genotyping with molecular markers is of great practical importance from the point of view of creating a core collection. Furthermore, the accessions we have found resistant to Sharka disease are an invaluable genetic resource for creating cultivars resistant to this disease both in Azerbaijan and all over the world. In addition, new resistance sources identified by us will also be useful for dry apricot breeding programs.

Approbation of the work. The main results of the dissertation were discussed in the following scientific seminars and conferences:

- VII International scientific conference on “Innovation Problems of Modern Biology” for young scientists and researchers devoted to the 94th anniversary of the great son and National Leader of Azerbaijani people Heydar Aliyev held at Baku State University of the Ministry of Education of the Republic of Azerbaijan (2017).

- At the conference of young scientists and students “Innovations in Biology and Agriculture aimed at solving global variables” dedicated to the 90th anniversary of academician J.A.Aliyev (2018).

- At the scientific-practical conference on the topic “Actual problems of modern biology” dedicated to the Youth Day and held at the Institute of Microbiology of the Azerbaijan National Academy of Sciences (2019).

- XXVI International Scientific Conference of Students, Postgraduates and Young Scientists “Lomonosov-2019” (2019).

- At the II Karabakh international scientific congress dedicated to applied sciences of the Azerbaijan National Academy of Sciences (2021).

- Reported and discussed at the 4th International conference on advanced engineering technologies held at Bayburt University in Turkey (2022).

Publications. Based on the dissertation materials, 13 scientific works have been published, including 7 articles and 6 theses in local and foreign publications.

Organization in which the dissertation was performed. Dissertaion work was performed at the Departments of “Immunogenetics”, “Fruit Plants”, “Molecular Genetics and Genomics” of the Institute of Genetic Resources of the Ministry of Science and Education of the Republic of Azerbaijan, and Biotechnology laboratory of the Genome and Stem Cell Center, Erciyes University in Kayseri, Turkey.

Total volume of the dissertation with character, in indicating the volume of the structural sections of the dissertation separately. The dissertation is written in Azerbaijani and consists of 158 pages, introduction, 5 chapters, conclusion, results and recommendations, abbreviations, list of literature and appendices. The research work cited 154 literature data, of which 147 were foreign publications. The total volume of the dissertation with characters is 200369 characters (introduction consists of 10192, I chapter-91104, II chapter-11638, III chapter-30356, IV chapter-29923, V chapter-17372, conclusion-7399, results-1909,

recommendation-476). The dissertation contains 25 figures and 28 tables.

MAIN CONTENT OF THE WORK

CHAPTER I. LITERATURE REVIEW

In the introduction, the relevance of the research work is presented based on the analysis of modern literary data, the degree of development of the research topic is noted, the goal and objectives of the work, scientific novelty, theoretical and practical significance of the research are shown and justified. An extensive summary of literature sources is presented with references to the sources on the centers of origin, evolution, taxonomy, distribution, classification, biological characteristics, genome reading, biodiversity, assessment of genetic diversity with molecular markers, diseases, genetics of Sharka disease, genetic bases of resistance to Sharka disease of apricot plant, which is the object of research.

CHAPTER II. MATERIALS AND METHODS OF RESEARCH

61 accessions belonging to apricot (*Prunus armeniaca* L.) species cultivated in different regions of Azerbaijan were used as an object of the research. 44 of the studied accessions were collected from Babek, Sharur, Ordubad regions of the Nakhchivan Autonomous Republic, 6 from Tartar, 3 from Goranboy and 8 from Agdash regions.

Morphological, pomological and phenological traits of apricot accessions were studied[8] based on international (IPGRI and CEC, 1984) descriptors and analyzed based on multidimensional statistical programs such as correlation, principle component and cluster.

[8] Caliskan, O., Bayazit, S., Sumbul, A. Fruit Quality and Phytochemical Attributes of Some Apricot (*Prunus armeniaca* L.) Cultivars as Affected by Genotypes and Seasons //Notulae Botanicae Horti Agrobotanici Cluj-Napoca, – 2012a. 40(2), – p.284-294.

To study the genetic diversity of apricot accessions at the nuclear genome level using SSR primers, DNA extraction was performed based on the CTAB (cetyltrimethylammonium bromide) protocol proposed by Doyle J.J and Doyle J.L (1990), purity of DNA was checked by Nanodrop device, diluted to appropriate concentration and PCR was performed using selected primers, PCR products were separated with the capillary electrophoresis on an ABI 3500 (Applied Biosystems, Foster City, CA, USA), DNA fragments were electrophoresed on a 3% agarose gel[9] (Figure 1). Molecular-genetic analyzes were performed at the Departments of "Molecular Genetics and Genomics", the "Fruit Plants" of the Institute of Genetic Resources of the Ministry of Science and Education of the Republic of Azerbaijan, and the Genome and Stem Cell Center, Erciyes University in Kayseri, Turkey.

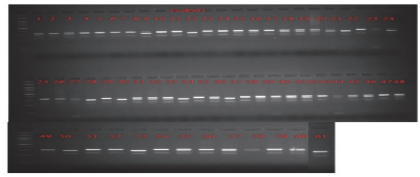

***Figure 1**. 3% agarose gel electrophorogram of PCR products obtained with Gol061 primer pair*

Statistical analyses. Computer programs such as SPSS, XLSTAT, PowerMarker V3.25, DarWin 6 and Structure 2.3.4 were used in the dissertation work.

CHAPTER III. COLLECTION AND ASSESSMENT OF APRICOT ACCESSIONS

3.1. Collection of apricot genotypes

Expeditions were organized to various regions of Azerbaijan (Tartar, Agdash, Goranboy) and the Nakhchivan Autonomous

[9] Doyle, J.J., Doyle, J.L. Isolation of plant DNA from fresh tissue //Focus, – 1990. 12(1), – p.13–15.

Republic. Cultivation location and coordinates of 44 apricot cultivars and forms from the NAR, 6 apricot cultivars and forms from Tartar, 8 apricot cultivars and forms from Agdash, 3 apricot cultivars and forms from Goranboy regions were determined. Cutting material was taken from all cultivars and forms and brought to the Institute of Genetic Resources, and the apricot gene pool, consisting of all cultivars, was created for the first time by ingrafting to rootstocks.

3.2. Pomological, phenological and biomorphological assessment of apricot cultivars and forms

The research was conducted on apricot accessions collected from the Nakhchivan Autonomous Republic, Tartar, Agdash and Goranboy regions of Azerbaijan. Ecological factors in these regions create favorable conditions for the normal development of apricot. 44 cultivars and forms of apricot were collected from the Nakhchivan Autonomous Republic, 6 from Tartar region, 3 from Goranboy region, and 8 from Agdash region. They were delivered to the Institute of Genetic Resources and their pomological, morphological and phenological characteristics were evaluated according to 10 parameters. Among the studied collection accessions, a big difference in yield, sugar content and fruit weight was revealed. Thus, most of the cultivars and forms of apricots distributed in the Nakhchivan Autonomous Republic had small fruits, only “Hampa” and “Limon erik 2” cultivars had a fruit weight of more than 50 grams. In general, the fruits were characterized by yellow skin ground color and flesh color and total soluble solids (TSS). Five accessions had orange, two white, and three greenish-yellowish fruit skin ground color (SGC).

The weight of the fruits of the cultivars and forms cultivated in Tartar, Goranboy and Agdash varied from 11.1 to 100.2 g. SGC was orange for 4 cultivars: Ag erik Elchin, Badami 2, Ordubad erik, Mayovka 2. The skin ground color of Ag erik Gejyetishen was white, and its flesh color was cream. Finally, the skin ground color of the Girmiziyanag has a greenish-yellowish color.

3.3. Determination of correlative relationships between biomorphological, phenological and pomological characteristics of apricot accessions and assessment of biodiversity

Correlation analysis was carried out to identify linear dependence between the biomorphological, pomological and phenological traits of the accessions. Among the Nakhchivan accessions, a high correlation has been established among a number of morphological, pomological and phenological traits (Table 3.3). As expected, the highest correlations with the Pearson correlation index were recorded between bud break season and blossom season, fruit weight and length, blossom season and harvest season. Thus, larger sized fruits with maximum FL and FW had higher mass. Pearson correlation indices of fruit weight with fruit length and width were determined as r=0.894 and r=0.868, respectively. As can be seen from the results of PK analysis, the first three components accounted for 76.69% of the total variation. In apricot accessions cultivated in Tartar, Goranboy and Agdash regions, the highest Pearson correlation index was recorded between bud break season and blossom season, bud break season and harvest season, bud break season and leaf fall season, blossom season and harvest season, blossom season and leaf fall season, harvest season and leaf fall season ($r = 1.00$; $p < 0.05$). As can be seen from the results of PK analysis, the first three components accounted for 87.02% of the total variation.

As a result of cluster analysis using Ward's method, apricot genotypes were grouped into 3 main groups based on 10 biomorphological, phenological, pomological indicators at Euclidean distance. A dendrogram reflecting the result of cluster analysis is shown in Figure 2.

42 genotypes (Agja Nabad 2, Goyje Nabad, Mehmani, Esgerabat, Shalakh 3, Ordubad Nabati, Abu Talibi, Iran sortu, Badami 1, Agjanabad 1, Yeni forma 2, Hagverdi 1, Forma 2, Maychicheyi, Limon erik 1, Talibi, Balyarim, Hagverdi 2, Gejyetishen, Helena, Shemsi, Ag Nabati, Yay Sherefi, Forma 1, Kurdeshi, Ag badami, Teberze 1, Ordubad Sherefi, Irandan gelme,

Yeni Forma 3, Teberze 2, Badam erik 2, Limon erik 2, May Natig, Ag erik Gejyetishen, Hampa, Shalakh 1, Turkiye sortu, Shalakh 2, Heydari, Mayovka 1, Girmiziyanag) located in the first group made up 68.9% of all genotypes. This group is divided into 2 subclusters. There were 31 genotypes in the first subcluster, and 11 genotypes in the second subcluster. The genotypes included in this group had TSS compared to other groups.

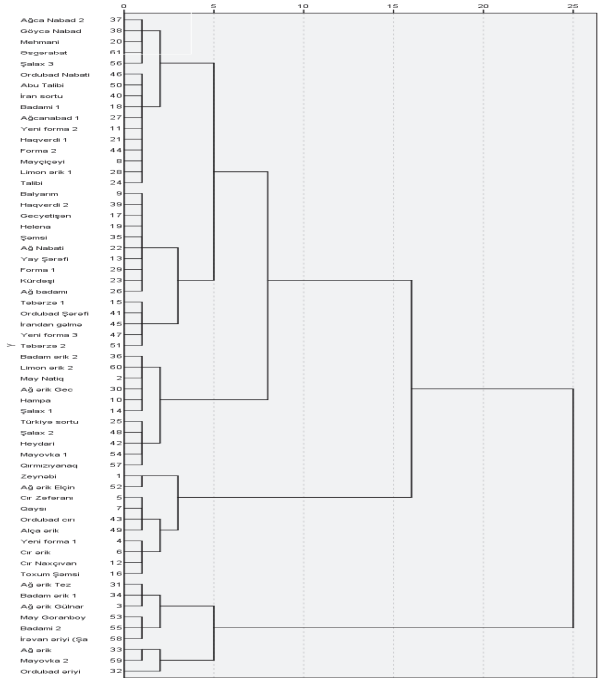

Figure 2. *Grouping of apricot genotypes according to morphological and pomological quantitative indicators.*

In the second group, 10 genotypes are localized. The genotypes included in this cluster accounted for 16.4% of all genotypes. Apricot cultivars grouped in this cluster, such as Zeynebi, Ag erik Elchin, Jir Zeferani, Gaysi, Ordubad jiri, Alcha erik, Yeni forma 1, Jir erik, Jir Nakhchivan, Tokhum Shemsi, were distinguished with high values of fruit length from other accessions.

The third group contains apricot genotypes such as Badam erik 1, Ag erik Tezyetishen, Ag erik Gulnar, May Goranboy, Badami 2, Iravan erik (Shalakh), Ag erik, Mayovka 2, Ordubad erik. The values of fruit weight, fruit length and fruit width parameters were high in these genotypes. Cluster analysis combined the genotypes listed above into a third group and made it possible to separate them from other genotypes.

CHAPTER IV. ASSESSMENT OF GENETIC DIVERSITY OF APRICOT ACCESSIONS OF DIFFERENT ORIGINS BY MEANS OF 17 SSR AND 1 SSLP MARKERS

4.1. Assessment of the genetic diversity in apricot collection based on 17 SSR and 1 SSLP loci

Table 1.

17 microsatellites studied in 61 apricot genotype and synthesized alleles on 1 SSLP loci

Name of primer	Synthesized alleles	Name of primer	Synthesized alleles
Gol061	4	ssrPaCITA17	14
PGS1.03	7	aprigms18	9
PGS1.20	10	ssrPaCITA16	6
PGS1.21	10	ssrPaCITA19	8
PGS1.23	10	ssrPaCITA4	7
PGS1.24	5	UDAp-404	12
PGS1.252	6	ssrPaCITA21	6
96P10_SP6	9	pchgms2	11
ssrPaCITA5	4	ZP002	2
Mean value	7.8		

The apricot collection was evaluated based on 17 SSR and 1 SSLP loci placed in 6 different linked groups, a total of 140 bands were synthesized, the number of alleles synthesized with each primer varied from 2 to 14 and made 7.8 alleles on average. As a result of the analysis, it was determined that the number of alleles determined

for each locus is high in relation to the total number of genotypes. As mentioned, the most informative microsatellite locus was at the $(GA)_{24}$ locus, and 14 alleles were amplified for 61 genotypes on this number (Table 1).

4.2. Determination of genetic distance indices of apricot accessions, their grouping according to the degree of genetic relationship and certification based on SSR profiles

The distribution frequency of 140 alleles in the studied collection is given. Allele frequencies among accessions varied between 0.008 and 0.92. Since the occurrence frequency of alleles is below 1% in 29 allelic variants (20.7%) and above 20% in 36 allelic variants (25.7%) out of 140, rare (I) and high-frequency (III) alleles, respectively, were included in the group of common alleles (II), since it ranged from 1-20% in 75 allelic variants (53.6%). High-frequency and common alleles were found in almost all loci and the number of these alleles was varied between 1 (ssrPaCITA16, ssrPaCITA4, ZP002) and 3 (Gol061, PGS1.03, ssrPaCITA19) in group III, between 1 (Gol061, ssrPaCITA19, ZP002) and 8 (PGS1.23) in group II. In I group, 1, 2, 1, 2, 6, 1, 4, 1, 5 and 6 rare alleles were registered on 10 loci (PGS1.03, PGS1.20, PGS1.21, 96P10_SP6, ssrPaCITA17, aprigms18, ssrPaCITA19, ssrPaCITA4, UDAp-404, pchgms2), respectively, and the number of alleles varied from 1 to 6. Note that a unique allele for the $(GA)_{21}$ locus was observed in the Zeynabi genotype of Agdash origin with the primer UDAp-404. Rare alleles can be used as a criterion for the recognition and certification of genotypes, as well as for protecting the copyright of breeders in breeding work.

Genetic diversity among the genotypes we studied was examined and analyzed in two directions - for each microsatellite locus and geographical origin.

The primary criterion for assessing genetic diversity using markers is the ability to adequately reflect the number of studied loci and their entire genome. The markers used in our study covered

different loci of the apricot genome to one degree or another, being included in 6 different linked groups.

Table 2 shows the number of alleles (Na) which allow to quantitatively assess the genetic diversity among accessions, observed heterozygosity (Ho), expected heterozygosity (He) and as well as obtained values of polymorphism information content (PIC) parameters for different loci. Among 18 loci, the weakest variability was observed with the ZP002 primer, with 2 alleles, and the highest variability was observed with the ssrPaCITA17 primer, with 14 alleles. The variability in the number of alleles per locus (2-14) may be due to the presence of locus-specific mutations of different frequency, which, in turn, affect the allelic or genetic diversity of SSR loci. The average value of alleles for all loci was equal to 7.8 (Table 2). In our study, the locus-specific genetic diversity coefficients ranged from 0.15 to 0.82.

Table 2.

Variability parameters of 17 SSR and 1 SSLP markers in 61 studied apricot cultivars

Marker	**No. of obs.**	**Na**	**He**	**Ho**	**PIC**
1	**2**	**3**	**4**	**5**	**6**
Gol061	61	4	0.69	0.74	0.63
PGS1.03	52	7	0.77	0.98	0.73
PGS1.20	59	10	0.80	0.85	0.77
PGS1.21	61	10	0.77	0.95	0.74
PGS1.23	61	10	0.76	0.95	0.73
PGS1.24	49	5	0.68	0.61	0.64
PGS1.252	60	6	0.59	0.90	0.50
96P10_SP6	53	9	0.82	0.96	0.79
ssrPaCITA5	59	4	0.52	0.54	0.42
ssrPaCITA17	61	14	0.82	0.87	0.79
aprigms18	61	9	0.78	0.98	0.75
ssrPaCITA16	52	6	0.69	0.87	0.66
ssrPaCITA19	60	8	0.75	0.98	0.71
ssrPaCITA4	52	7	0.82	0.94	0.80

1	2	3	4	5	6
UDAp-404	52	12	0.76	0.60	0.73
ssrPaCITA21	55	6	0.61	0.35	0.54
pchgms2	58	11	0.66	1.00	0.61
ZP002	56	2	0.15	0.16	0.14
Mean	56.8	7.8	0.69	0.79	0.65

Number of alleles (Na), expected heterozygosity (He), observed heterozygosity (Ho), polymorphism information content (PIC).

PGS1.20, PGS1.21, PGS1.23, pchgms2, UDAp-404, ssrPaCITA17 loci showed high allelic diversity and the listed markers were determined to be more informative (GD> 0.6) for the studied cultivar accessions. The highest index was recorded at $(TC)_{11}N_{32}(CT)_{11}A(TC)_{6,}$ $(GA)_{24}$, $(GA)_{17}$ loci, respectively, for 96P10_SP6, ssrPaCITA17, ssrPaCITA4 (GD=0.82) primers, and the lowest index for ZP002 (GD=0.15).

Expected heterozygosity (He) among loci varied from 0.15 at ZP002 to 0.82 at 96P10_SP6, ssrPaCITA17 and ssrPaCITA4 with an average of 0.69 units. For comparison, some authors have reported that the average expected heterozygosity for apricot cultivars is equal to 0.645, 0.741, 0.626 and 0.752 ([10],[11],[12],[13]). During our experiment, the observed heterozygosity (Ho) varied from 0.16 in ZP002 to 1.00 in pghms2 and averaged 0.79 units. According to Zhebentyayeva and her colleagues, the heterozygosity observed in 74 apricot cultivars belonging to different ecogeographical groups varied between 0.18

[10] Zhebentyayeva, T. Simple sequence repeat (SSR) analysis for assessment of genetic variability in apricot germplasm /T. Zhebentyayeva, G. Reighard, V. Gorina [et al] //Theoretical and Applied Genetics, – 2003. 106(3), – p.435–444.

[11] Maghuly, F. Microsatellite variability in apricots (*Prunus armeniaca* L.) reflects their geographic origin and breeding history /F. Maghuly, E. B. Fernandez, S. Ruthner [et al] //Tree Genetics & Genomes, – 2005. 1(4), – p.151–165.

[12] Bourguiba, H. Loss of genetic diversity as a signature of apricot domestication and diffusion into the Mediterranean Basin Bourguiba et al. /H. Bourguiba, J. M. Audergon, L. Krichen, [et al] //BMC Plant Biology, – 2012a. 12(49), – p.1-16.

[13] Pedryc A. Genetic diversity of apricot revealed by a set of SSR markers from linkage group G1 /A. Pedryc S. Ruthner R. Hermán [et al] //Scientia Horticulturae, – 2009. 121(1), – p.19–26.

and 0.84 and was equal to 0.52 units on average[10]. The mean value of PIC was 0.65, with a minimum of 0.14 at the ZP002 locus and a maximum of 0.80 at the ssrPaCITA4 locus. This value was close to the PIC value (0.586) determined by Bourguiba et al. in apricot[14].

For example, while the ssrPaCITA17 marker is characterized by a high number of alleles (14 alleles) for 61 genotypes and, accordingly, a high GD coefficient (0.82), only 2 alleles were synthesized with the ZP002 marker, and the GD coefficient was correspondingly very low, and amounted to 0.15 units.

The maximum genetic diversity was recorded in genotypes of Nakhchivan (GD=0.70), Agdash (GD=0.69) and Goranboy origin (GD=0.54), and the minimum diversity in genotypes of Tartar origin (GD=0.53) (Table 3).

Table 3.

Genetic diversity observed for each geographic region

Regions where accessions were collected	**Genetic diversity**	
	Limit of variation	**Mean value**
Nakhchivan	0.16-0.82	0.70
Tartar	0.32-0.72	0.53
Goranboy	0.44-0.72	0.54
Aghdash	0.22-0.82	0.69

Although genotypes of Nakhchivan and Agdash origin are characterized by closer values of GD coefficients, if the relatively high variation limit of GD is considered, then the studied accessions of Nakhchivan origin can be considered more diverse and rich from a genetic point of view.

[14] Bourguiba, H. Loss of genetic diversity as a signature of apricot domestication and diffusion into the Mediterranean Basin Bourguiba et al. /H. Bourguiba, J. M. Audergon, L. Krichen, [et al] //BMC Plant Biology, -2012a. 12(49), -p.1-16.

Region-specific differences in marker polymorphism were also recorded. For example, while the PGS1.21 locus is more polymorphism in genotypes of Nakhchivan origin (GD=0.80), the GD coefficient determined with this marker among Tartar genotypes is only 0.50 units. Another locus – $(CT)_{24}$ was distinguished by its high polymorphism (GD=0.78) among Agdash accessions, this indicator is relatively reduced (respectively, GD=0.64 and GD=0.72) in genotypes of Nakhchivan and Goranboy origin, and the lowest indicator (GD=0.58) was recorded in Tartar genotypes.

As already mentioned, a rich diversity of apricot plants in the Nakhchivan Autonomous Republic was once again confirmed due to the discovery of significant genetic diversity (GD=0.70) among apricot genotypes of Nakhchivan origin during our studies. Observed genetic diversity is the result of the combined effects of mutation, migration, breeding, and gene flow. Artificial selection by humans in cultivated plants plays a major role in changing the structure of populations. However, natural selection in populations consisting of traditional varieties found in many natural conditions, under the influence of biotic and abiotic factors of a heterogeneous environment, is one of the main reasons for the formation of population differences. The identified genetic diversity and variety of apricot accessions studied in the Nakhchivan Autonomous Republic may also be associated with the wide and rich ecological and geographical diversity of this region.

Based on the index of genetic distance among genotypes, a dendrogram was compiled reflecting the genetic relationship of apricot accessions studied by Darwin 6 cluster analysis. The compiled dendrogram grouped 61 apricot genotypes into 4 main clusters (Figure 3).

The population structure of the accessions was estimated using the STRUCTURE 2.3.4 computer program. Population structure was used to obtain the most likely number of genetic subpopulations and their K values were calculated [15]. The ΔK value corresponding to each K was

[15] Evanno, G., Regnaut, S., Goudet, J. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study August// Molecular Ecology, – 2005. 14(8), – p.2611-2620.

calculated and the corresponding scatter plot was constructed (Fig. 4). According to the ΔK value of the STRUCTURE analysis, 61 local apricot accessions were divided into 4 different subpopulations. In general, while the STRUCTURE results confirmed the groupings shown in the NJ dendrogram, some differences were observed among them. Looking at the history of breeding of resistant cultivars, STRUCTURE analysis produces better results than NJ cluster analysis.

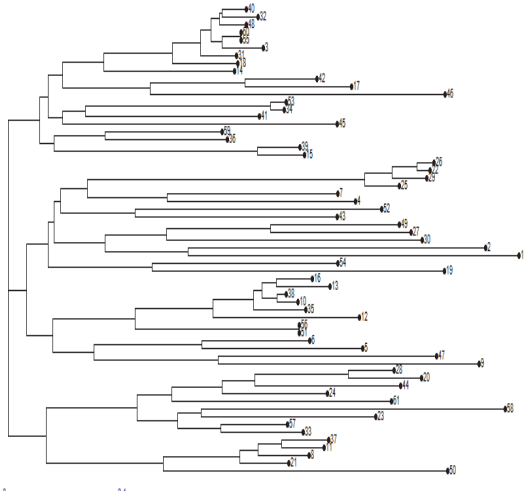

Figure 3. *Dendrogram showing the genetic proximity of 61 apricot accessions of different origins according to 17 SSR and 1 SSLP loci*

So far, STRUCTURE analysis has been widely used in many studies aimed at understanding the population structure of apricots. Bourguiba et al (2012)[16] determined that apricot accessions from Mediterranean countries constitute three main populations (Iran-Caucasus, Northern Mediterranean and Southern Mediterranean). Krichena et al. (2014)[17] revealed that Tunisian apricot germplasm

[16] Bourguiba, H. Loss of genetic diversity as a signature of apricot domestication and diffusion into the Mediterranean Basin Bourguiba et al. /H. Bourguiba, J. M. Audergon, L. Krichen, [et al] //BMC Plant Biology, – 2012a. 12(49), – p.1-16.

[17] Krichena L, Audergonb J. M, Trifi-Faraha N. Assessing the genetic diversity and population structure of Tunisian apricot germplasm //Scientia Horticulturae, – 2014. 172(5), – p.86–100.

had two different origins and separated into two different gene pools.

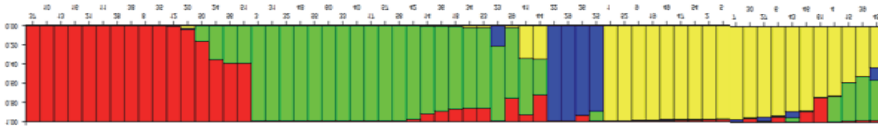

Figure 4. *Population structure of apricot accessions*

Thus, certification of the apricot genotypes studied by us based on SSR profiles will not only improve the management of the apricot gene bank, but also provide the world scientific community with reliable information about the gene pool and diversity of apricot in Azerbaijan. SSR profiles will be used to certify varieties, create new varieties and enrich core collections and assist breeding strategies.

CHAPTER V. ASSESSMENT OF RESISTANCE OF APRICOT ACCESSIONS TO SHARKA DISEASE

5.1. Screening of resistance genes to Sharka disease

Our study examined three SSR (PGS1.21, PGS1.23 and PGS1.24) and one SSLP (ZP002) marker placed in LG1 near the *Plum Pox Virus* (*PPVres*) resistance locus and investigated whether they had alleles linked to Sharka resistance. The PGS1.21-240 allele was observed in 3 accessions, while the PGS1.23-161 and PGS1.24-119 alleles in 15 accessions each. The ZP002-127 allele, located near the PPVres locus, was detected in 9 samples. 28 accessions had at least one of these four resistance-linked alleles. The resistant cultivars “Harlayne” and “SEO”, used as controls, also had resistance alleles (240, 161 and 119), as expected. May Natig cultivar of Agdash origin showed resistant alleles that were homozygous at one locus (PGS1.24) and heterozygous at two loci (PGS1.23 and ZP002), while Gaysi cultivar of Nakhchivan origin showed resistant alleles that was heterozygous at three loci (PGS1.21, PGS1.24 and ZP002). 19 out of 44 accessions (43.2%) of Nakhchivan origin, 2 out of 6 accessions (33.3%) of Tartar origin, all 3 accessions of Goranboy

origin (100%) and 4 out of 8 accessions (50%) of Agdash origin had at least one of the resistance alleles. Zeynebi and Yeni forma 1 accessions showed heterozygous resistant alleles at 4 loci (PGS1.21, PGS1.23, PGS1.24 and ZP002), while Ag Nabati, Turkiye sortu, Forma 1 (PGS1.24, ZP002) and Irandan gelme (PGS1.23, PGS1.24) accessions showed heterozygous resistant alleles at 2 loci. Maychicheyi, Mehmani, Haqverdi, Kurdeshi, Talibi, Limon erik 1, Ag erik Gejyetishen, Agja Nabad 2, Iran sortu, Forma 2 and Ag erik Elchin accessions had the 161 allele at only one resistant PGS1.23 locus. 7 accessions (Agjanabad 1, Ag erik Tezyetishen, Badam erik, Alcha erik, Mayovka 1, Girmiziyanag and Mayovka 2) had 119 alleles at the PGS1.24 locus, Ag badami and Esgerabat cultivars of Nakhchivan origin had 127 alleles at the ZP002 locus. Since we had no phenotypic information on apricot accessions from Azerbaijan, we could not check the validity of markers in advance. As a result of the research, among the studied accessions of Azerbaijan, 28 of them were found to have at least one of the PPVres alleles. In addition to the control cultivars "Harlayne" and "SEO", the genotypes Zeynabi of Aghdash origin and Yeni form 1 of Nakhchivan origin have alleles linked to resistance on four loci, which showed the feasibility of their use in breeding work related to resistance in the future. Thus, it was established that both genotypes under consideration were heterozygous for four loci and had alleles linked to *PPVres*.

Finally, PPV resistance of 61 apricot accessions of Azerbaijani origin with markers linked to the *PPVres* locus was studied. A certain proportion of accessions were susceptible to the virus, indicating the need to improve virus control and disease resistance in the area. About half (46%) of the 61 apricot genotypes studied by us had PPV resistance alleles, and 28 had at least one resistance allele. In 1st linked group, 9 unique allelic variants of the ZP002 locus linked to the major PPV resistance locus were identified. The findings showed that Azerbaijani apricots can serve as an important source for the cultivation of PPV-resistant apricots. Considering that the risk of disease penetration into the territory of Azerbaijan can be realized at any moment, we consider it relevant to study the disease resistance of cultivars and forms of apricots cultivated in Azerbaijan,

as well as to select resistant cultivars and forms and use them in breeding. On the other hand, accessions with resistance genes detected in the genome may be involved in breeding activities towards PPV resistance in the future, and can also be used as genetic donors in the creation of resistant cultivars in other countries.

5.2. Determination of *S*-genotype profiles of apricot germplasm cultivated in Azerbaijan

One of our research focuses was to determine the *S*-genotype profiles of different apricot germplasms cultivated in Azerbaijan using polymerase chain reaction (PCR) based on specific primer pairs. The detection of *S*-genotypes of 61 apricot genotypes was amplified using SRc-F and SRcR consensus primers for the first intron and EM-PC2consFD/EM-PC3consRD primers for the second intron analysis of the S-RNase gene. F and R primers of AprFBC8 were used for discrimination of $SFB_{C/8}$ allele[18]. Bands could not be obtained for the second intron of only 20 accessions. In one accession (Iravan apricot), no PCR product was detected at all. In EM-PC2consFD/EM-PC3consRD primer, 280, 310, 370, 820, 900, 1250, 1300 and 1700-bp bands were synthesized in 10, 3, 3, 7, 4, 27, 4 and 8 accessions, respectively.

Previous studies have found that using EM-PC2consFD/EM-PC3consRD primer, 310, 370, 500, 820, 900, 1250, 1300 and 1700-bp bands are specific to S_3, S_{12}, S_9, S_7, S_2, S_{13}, S_6 and S_{11} alleles, respectively[19]. 280-bp band produced by Zeynebi, Yeni forma 1, Gaysi, Maychicheyi, Yeni forma 2, Shalakh 1, Abu Talibi, Teberze 2, Shalakh 3 and Girmiziyanag was not defined for any known *S*

[18] Yılmaz, K. U. S-Genotype Profiles of Turkish Apricot Germplasm /K. U. Yılmaz, B., Basbug, K. Gürcan, [et al] //Notulae Botanicae Horti Agrobotanici Cluj-Napoca, – 2016. 44(1), – p.67-71.

[19] Gürcan, K. Genotyping by Sequencing (GBS) in Apricots and Genetic Diversity Assessment with GBS-Derived Single-Nucleotide Polymorphisms (SNPs) /K. Gürcan, S. Teber, S. Ercisli [et al] //Biochemical Genetics, – 2016. 54(6), – p.854–885.

locus. In 4 cultivars (Jir Zeferani, Gaysi, Mayovka 1, Mayovka 2) a fragment with a length of 900 bp was detected, which is the indicator of the presence of the S_2 allele. In 3 cultivars (Shemsi, Agja Nabad 2, Goyje Nabad) a fragment with a length of 310 bp was formed, and this confirmed that allele in them was S_3. In 4 cultivars (Hampa, Yay Sherefi, Gejyetishen, Ordubad Sherefi) a fragment with a length of 1300 bp was obtained and they are labeled with the S_6 allele. In 7 cultivars (Maychicheyi, Yeni forma 2, Teberze 1, Agja Nabad 2, Hagverdi 2, Alcha erik, Abu Talibi) fragment with a length of 820 bp appreared and it was labeled with the S_7 allele. In 8 cultivars (Ag erik Gulnar, Ag erik, Badam erik 1, Heydari, Shalakh 2, May Goranboy, Badami 2, Limon erik 2), fragment with a length of 1700 bp was appeared and this proved the presence of S_{11} allele in them. The fragment size specific to the S_{12} allele was observed in 3 cultivars (Hampa, Jir Nakhchivan, Yay Sherefi). 1250-bp one fragment appeared in 27 cultivars (Ag erik Gulnar, Yeni forma 1, Balyarim, Shalakh 1, Teberze 1, Gejyetishen, Badami 1, Helena, Ag badami, Forma 1, Ag erik Tezyetishen, Ordubad erik, Ag erik, Shemsi, Goyje Nabad, Haqverdi 2, Heydari, Shalakh 2, May Goranboy, Badami 2, Limon erik 2), indicating that the S_{13} allele was common to those cultivars.

A total of 9 different *S* genotypes (S_2, S_3, S_6, S_7, S_8, S_{11}, S_{12}, S_{13} and S_c) were identified in 61 apricot genotypes. Among the apricot accessions used in the study, with the exception of Mayovka 1 and Forma 2, the remaining accessions showed self-incompatibility, nonspecific for the S_c haplotype. 9 *S* alleles were identified among Azerbaijani cultivars. It was established that the S_{13} allele is the most widespread among apricot germplasms of Azerbaijan and it was found in 27 cultivars. S_{11} allele was found in 8, S_7 in 7, S_2 in 4, S_6 in 4, S_{12} in 3, S_3 in 3, S_8 in 3 and S_c in 2 genotypes.

RESULTS

1. Among 61 apricot genotypes cultivated in different environmental conditions, statistically significant high genetic diversity was found according to individual morphological

quantitative traits, as a result of cluster analysis, apricot genotypes were divided into 3 main groups based on 10 biomorphological, phenological and pomological parameters. According to the results of the cluster analysis, the closest genotypes were Agja Nabad 2 (Nakhchivan) and Goyje Nabad (Nakhchivan) (0.100), and the most distant genotypes were Ag erik Elchin of Agdash origin and Ordubad erik of Tartar origin (116.518).

2. In total, 140 alleles were synthesized in 61 local apricot accessions with 17 SSR and 1 SSLP markers, the average polymorphism information content (PIC) for microsatellite loci in the studied collection was 0.65, and the expected heterozygosity (He) was 0.69 units. Genotypes from Nakhchivan and Agdash had the highest genetic diversity (PIC=0.65), and accessions from Tartar had the lowest genetic diversity (PIC=0.45).
3. Based on the molecular data identified by means of cluster analysis, the genetic distance index varied between 0-0.97. t was found that 4 apricot samples included in the collection are synonyms, and 10 samples are homonyms. In the **NJ** dendrogram, 61 genotypes were grouped into 4 clusters, and 4 subpopulations were identified in the collection as a result of **STRUCTURE** analysis.
4. As a result of the screening of apricot collection accessions through 3 SSR and 1 SSLP markers linked to the *PPVres* locus, which provides resistance to Sharka disease, PPV resistance PGS1.21-240 allele was found in 3 accessions, PGS1.23-161 and PGS1.24-119 alleles in 15 accessions respectively, ZP002-127 allele in 9 accessions. It was found that 28 accessions had at least one, 4 accessions had two, 2 accessions had three and other 2 accessions (Zeynabi, Yeni forma 1) had 4 resistance alleles.
5. As a result of the characterization of the 61 studied apricot samples according to the self-incompatibility phenotype, in total, 9 different *S* genotypes (S_2, S_3, S_6, S_7, S_8, S_{11}, S_{12}, S_{13} and S_c) were identified: in the most (27) samples genotype S_{13} is the

highest, and genotypes S_{12}, S_3, S_8 and S_c are characterized by the lowest frequency (3, 3, 3 and 2, respectively) in the sample.

RECOMMENDATION

Accessions (Zeynabi, May Natig, Yeni forma 1, Gaysi, Maychicheyi, Mehmani, Hagverdi 1, Ag Nabati, Kurdeshi, Talibi, Turkiye sortu, Ag badami, Agjanabad 1, Limon erik 1, Forma 1, Ag erik Gejyetishen, Ag erik Tezyetishen, Badam erik 1, Agja Nabad 2, Iran sortu, Forma 2, Irandan gelme, Alcha erik, Ag erik Elchin, Mayovka 1, Girmiziyanag, Mayovka 2, Esgerabat) with identified resistance gene to Sharka disease are recommended to be planted on farms as well as to be used as a source material in apricot breeding programs.

PUBLISHED WORKS ON THE TOPIC OF THE DISSERTATION

1. Rəkidə, Ə. M. Initial characterization of genetic diversity in local apricot cultivars of Azerbaijan // Azərbaycan Respublikası Təhsil Nazirliyi Bakı Dövlət Universiteti Azərbaycan xalqının böyük oğlu,Ulu Öndər Heydər Əliyevin anadan olmasının 94-cü ildönümünə həsr olunmuş Gənc Alimlərin və Tədqiqatçıların "Müasir Biologiyanın İnnovasiya Problemləri" mövzusunda VII Beynəlxalq Elmi Konfransının materialı, – Bakı: –2017,– s.107-108.
2. Rakida A.M. Genetic diversity and Plum pox virus resistance of apricot (*Prunus armeniaca* L.) in Azerbaijan // Conference of Young Scientists and Students "Innovations in Biology and Agriculture to Solve Global Challenges" Dedicated to the 90th Anniversary of Academician Jalal A.Aliyev, – Baku: –2018, – p. 150.

3. Rəkidə Ə.M. Ərik bitkisinin şarka xəstəliyinə davamlılığının ssr markerlə analizi // Azərbaycan Milli Elmlər Akademiyası Mikrobiologiya İnstitutu Gənclər Gününə Həsr Olunmuş Müasir Biologiyanın Aktual Problemləri mövzusunda elmi-praktiki konfransın materialları, – Bakı: – 2019,– s.50-51.
4. Rakida A.M. Genetic diversity of apricot (Prunus armeniaca L.) cultivars spread in Azerbaijan using SSR markers // Международная научная конференция студентов, аспирантов и молодых учёных «Ломоносов-2019», –Москва: – 2019.
5. Rakida A.M. Assessment of variability in morphological and pomological characters of apricot germplasm of Nakhchivan, Azerbaijan // Karabakh II International congress of applied sciences Azerbaıjan national academy of sciences / – Azerbaijan: – 2020.– p.12
6. Rakida A.M. Morphological and pomological diversity among apricot (*Prunus armeniaca*) genotypes grown in Azerbaijan // 4th International conference on advanced engineering technologies / – Turkey: – 2022. – p. 869.
7. Rəkidə Ə.M. Azərbaycanın müxtəlif rayonlarından toplanılmış ərik genotiplərinin şarka (*Plum Pox Virus*) xəstəliyinə davamlılığının tədqiqi // – Bakı: Əkinçilik Elmi Tədqiqat İstitutunun elmi əsərləri məcmuəsi, – 2018. C. XXIX, – s. 163-165.
8. Rakida A.M. Investigation of variability of apricot (*Prunus armeniaca* L.) using morphological, pomological traits and microsatellite markers // Khazar Journal of Science and Technology, – 2021. 5(2), – p. 34-48.
9. Ракида А.М. Оценка генетического разнообразия генотипов абрикоса (Prunus armeniaca L.) Азербайджана с помощъю

SSR маркеров // Успехи современного естествознания. – 2020. 7, – с. 37-42.
10. Rəkidə Ə.M. Azərbaycanda ərik (*Prunus armeniaca* L.) genotiplərində şarka (*Plum Pox*) xəstəliyinə qarşı davamlılıq allellərinin tədqiqi // – Bakı: Genetik Ehtiyatlar Institutunun elmi əsərləri, – 2022. XI(2), – s.17-24.
11. Rakida A.M. Analysis of morphological and pomological features of apricot in the Nakhchivan Autonomous Republic of Azerbaijan // – Turkey: Turkish journal of agriculture and forestry, – 2023. 47(1), – p. 23-30.
12. Rakida A.M., Akparov Z. S-Genotype Profiles of Azerbaijan Apricot Germplasm // Khazar Journal of Science and Technology, – 2023. 7(1), – p. 20-31.
13. Rakida A, Çetinsağ N, Akparov Z, Bayramov L, Babayeva S, Izzatullayeva V, Abbasov M, Ercisli S, Gürcan K. Assessment of genetic diversity and search for *Plum pox virus* resistance alleles of apricot (*Prunus armeniaca* L.) genotypes spread in Azerbaijan using SSR markers // – Turkey: Turkish journal agriculture and forestry, – 2023. 47(4), – p.529-540.

The defense of dissertation will be held on 12th of January 2024 at 11^{00} at the meeting of the FD 1.37 Dissertation Council operating at the Institute of Genetic Resources of the Ministry of Science and Education of the Republic of Azerbaijan.

Address: Ave. 155 Azadlig, AZ1106, Baku.

Dissertation is accessible at the Library of the Institute of Genetic Resources of Ministry of Science and Education of the Republic of Azerbaijan.

Electronic versions of dissertation and its abstract are available on the official website of the Institute of Genetic Resources of the Ministry of Science and Education of the Republic of Azerbaijan (https://www.genres.az).

Abstract was sent to the required addresses on 7th of December 2023.

Signed for print: 06.12.2023
Paper format: A5
Volume: 37337
Number of hard copies: 20