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Results of Genetic Studies Conducted on Pome Fruit Plants in the Northern Regions of Azerbaijan

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Abstract. *This work examines the genetic diversity of local and introduced fruit crop varieties common in the mountainous regions of Azerbaijan, analyzes their current status, examines their biomorphological features, identifies them using molecular markers, and determines their degree of genetic relatedness. The degree of genetic similarity between varieties was assessed using cluster analysis. Genotypic assessment of the gene pool was conducted using hybridological analysis, differential staining, and PCR analysis of chromosomes. Fruit plants collected from orchards were selected according to relevant regulations. In this study, we conducted a genetic evaluation of material including 14 apple and 15 pear varieties. Genetic diversity of the breeding samples was analyzed using six SSR markers. SSR marker polymorphism ranged from two to eight alleles per locus. The SSR markers used in the study proved to be a reliable genotyping tool for the studied crops. Furthermore, based on the high observed heterozygosity values of the selected varieties, it can be concluded that the parental pairs selected for the cross are genetically distinct. Thus, the study and genetic analysis of the tested genomes allows us to identify the most effective genes and preserve the genetic diversity of cultivated samples and avoid genetic depletion of the gene pool. A comprehensive study of a collection of apple and pear trees using six SSR markers revealed a high number of heterozygotes, indicating significant genetic diversity in the parental pairs. The analysis conducted in this study, in turn, makes it possible to establish kinship relationships within the sample under evaluation. Thus, genetic analysis using SSR is a reliable means for reliably assessing parental forms. Conserving the genetic diversity of cultivated pear and apple varieties, as well as introduced forms of these plants, is a key focus in the development of new varieties in modern horticulture. When developing a variety, selecting parental pairs that possess a combination of positive traits is crucial. Years of research on these traits have resulted in the identification of varieties of interest for use in breeding research.*

Keywords: *genotype, gene pool, genetic markers, selection, clustering, genetic diversity*

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Azərbaycanın şimal bölgələrində tumlu meyvə bitkiləri üzərində aparılmış genetik tədqiqatların nəticələri

Nəzakət Hüseynova

Xülasə. *Bu tədqiqat Azərbaycanda dağlıq bölgələrdə geniş yayılmış yerli və introduksiya olunmuş meyvə bitkisi sortlarının genetik müxtəlifliyinin öyrənilməsinə, onların mövcud vəziyyətinin təhlilinə, biomorfoloji xüsusiyyətlərinin araşdırılmasına, molekulyar markerlər vasitəsilə identifikasiyasına və*

genetik qohumluq dərəcəsinin müəyyən edilməsinə həsr edilmişdir. Sortlar arasında genetik oxşarlıq dərəcəsi klaster analizi əsasında qiymətləndirilmişdir. Genofondun genotipik qiymətləndirilməsi hibridoloji analiz, xromosomların differensial boyanması və PZR (PCR) analizi vasitəsilə həyata keçirilmişdir. Bağlardan toplanmış meyvə bitkiləri müvafiq normativ tələblərə uyğun olaraq seçilmişdir. Tədqiqat çərçivəsində 14 alma və 15 armud sortunu əhatə edən materialın genetik qiymətləndirilməsi aparılmışdır. Seleksiya materiallarının genetik müxtəlifliyi altı SSR markeri vasitəsilə təhlil edilmişdir. SSR markerlərinin polimorfizmi hər lokus üzrə iki ilə səkkiz allel arasında dəyişmişdir. Tədqiqatda istifadə olunan SSR markerləri öyrənilən bitki nümunələrinin genotipləşdirilməsi üçün etibarlı vasitə olduğunu təsdiq etmişdir. Bundan əlavə, seçilmiş sortlarda müşahidə olunan yüksək heteroziqotluq göstəricilərinə əsasən belə nəticəyə gəlmək olar ki, hibridləşdirmə üçün seçilmiş valideyn cütlükləri genetik baxımdan əhəmiyyətli dərəcədə fərqlənirlər. Beləliklə, tədqiq olunan genomların öyrənilməsi və genetik təhlili ən effektiv genlərin müəyyən edilməsinə, becərilən nümunələrin genetik müxtəlifliyinin qorunmasına və genofondun genetik tükənməsinin qarşısının alınmasına imkan verir. Alma və armud kolleksiya nümunələrinin altı SSR markeri əsasında aparılmış kompleks tədqiqatı yüksək heteroziqotluq səviyyəsini aşkar etmiş və bu da valideyn cütlüklərinin yüksək genetik müxtəlifliyə malik olduğunu göstərmişdir. Aparılmış analiz qiymətləndirilən nümunələr daxilində qohumluq əlaqələrinin müəyyən edilməsinə də imkan yaratmışdır. Beləliklə, SSR markerləri əsasında aparılan genetik analiz valideyn formalarının etibarlı qiymətləndirilməsi üçün etibarlı üsul hesab edilə bilər. Becərilən alma və armud sortlarının, eləcə də bu bitkilərin introduksiya olunmuş formalarının genetik müxtəlifliyinin qorunması müasir bağçılıqda yeni sortların yaradılmasının əsas istiqamətlərindən biridir. Yeni sortların yaradılması prosesində müsbət təsərrüfat əhəmiyyətli əlamətlərin optimal kombinasiyasına malik valideyn cütlüklərinin seçilməsi mühüm əhəmiyyət kəsb edir. Bu xüsusiyyətlər üzrə uzunmüddətli tədqiqatlar seleksiya işlərində istifadə üçün perspektivli sortların müəyyən edilməsinə imkan vermişdir.

Açar sözlər: *genotip, genofond, genetik markerlər, seleksiya, klasterləşmə, genetik müxtəliflik*

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Introduction

Global changes in weather and climate conditions are prompting a new perspective on work carried out in the fields of genetics and breeding, leading to the creation of new varieties, the renewal of fruit plants, and the development of more resilient species. Taking into account the changing climatic factors of nature, new requirements are emerging for varieties cultivated in horticulture and fruit growing. Today, the species of fruit plants, including local and introduced samples, are expected to demonstrate high adaptability, complex resistance to major diseases, as well as increased productivity and production capacity (Gurbanov, 2014). Breeding is the main tool in creating new generations of fruit plants. Both classical and modern selection methods form the basis of breeding. By using these methods, it has become possible to obtain new elite forms, varieties, clones, and so on. Alongside classical selection, one of the methods for expanding the range of fruit plants has been introduction (Ərəbzadə, 2021). During the introduction process, the adaptation of plants of different ecological and geographical origin to specific conditions is determined. More adaptive varieties are observed to adapt more quickly to certain conditions. The genetic diversity of plants, especially the creation of resistant forms of pome fruit crops (apple, pear, quince), the comparative analysis of their genomes, and their adaptation to ecological changes are of great importance. Year by year, nature, climate, and soil cover change and undergo modification; the use and potency of pesticides increase, and their

application causes continuous and serious damage to the environment, human health, and biodiversity. From this point of view, it is highly relevant to carry out scientific research in several directions. First, the complete preservation of the gene pool of the studied plants is essential so that it can serve as a basis in the future for creating new species with more resilient genes. Second, diversity in natural habitats and cultivated areas is decreasing day by day. One of the main tasks is the purposeful collection of a significant part of plant genetic diversity from across the entire country, its comprehensive study using modern methods, and its reliable conservation. Characterization of the collected genetic material, identification of the potential of plants based on key biological and breeding traits, genetic passportization of each sample in the collection, evaluation according to quality indicators, and the investigation of the immunological and molecular-genetic bases of resistance all contribute to the formation of a genetic foundation. Research is also being carried out to measure genetic variability, in other words, the degree of polymorphism, for the study and use of plant diversity (Hüseynova, 2024).

At present, fruit plant varieties are identified according to morphological traits. This has certain shortcomings. Morphological features appear late during development stages and may be influenced by environmental conditions. They make it difficult to distinguish closely related genotypes and to control the varietal purity of planting material. The difficulties of morphological identification make it necessary to search for new, more convenient, and reliable identification methods. DNA identification methods are a justified choice because of their advantages over classical methods. Molecular methods make it possible to detect differences between genotypes at the DNA level, where such differences are represented most completely. They do not require phenotypic expression of a trait, can be used at any stage of plant development, and significantly reduce the time required for varietal identification, which is especially important for perennial plants. However, at present, universal DNA identification methods for fruit plants do not exist. This is partly due to the lack of knowledge about the genetic diversity of varieties cultivated in different countries and the level of polymorphism in individual regions of fruit crop genomes. The territory of the Republic of Azerbaijan is located in subtropical and partly temperate climatic zones. Of the 11 climate types existing on Earth, 9 and most soil types are found within the country.

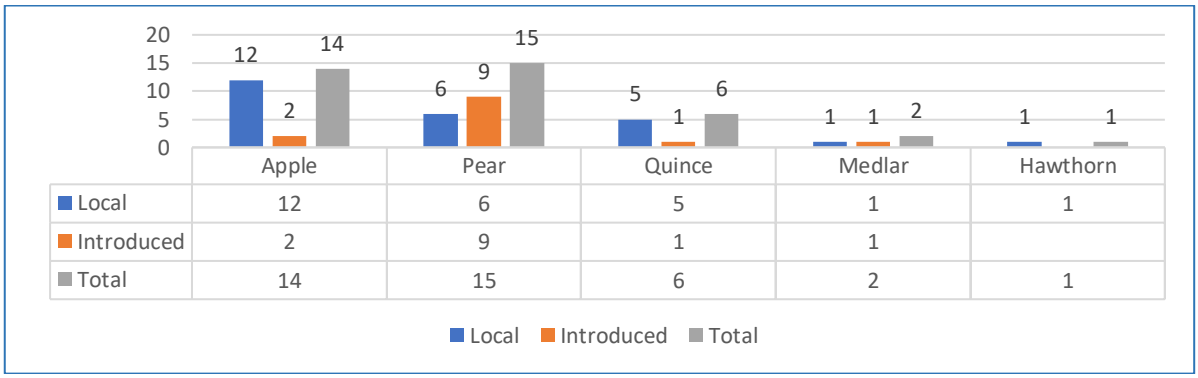
The main soil types in our country include mountain-meadow, brown mountain-forest, chestnut mountain-forest, yellow, chestnut, gray and gray-brown soils, saline soils, and solonchaks, among others. Fruit growing is a major branch of agriculture in Azerbaijan and plays a key role in supplying the population with fruit products and industry with raw materials. Agriculture accounts for 35–40% of the gross domestic product (Sadigov, 2019, Sadigov, 2023). The research presents the results of a comprehensive evaluation of the primary material. The main aim of the work was to increase the efficiency of breeding by incorporating genetic methods into the breeding process, evaluating the gene pool and its influence on the genotype, and significantly improving varietal parameters in terms of fruit adaptability, productivity, and commercial quality. The genotypic evaluation of the gene pool was carried out using hybridological analysis, differential staining, and PCR analysis of chromosomes. Given the considerable value of pome fruit plants, it is important to systematically study and conserve the rich gene pool of both wild and cultivated forms of these fruit trees. Genetic diversity is an important component of the genetic characteristics of a population, a group of populations, or species. Assessing genetic diversity is important for monitoring genetic variability in both wild and cultivated plant species and populations; the genetic data obtained provide information for developing strategies for their conservation and sustainable use.

The study of genetic diversity is based on morphological, biochemical, and molecular markers. Molecular markers are genetic markers analyzed at the DNA level. Classical genetic terms such as locus, allele, and dominant and codominant inheritance patterns apply to them. The alleles of marker loci are different forms of the same marker located at the same positions (loci) on homologous chromosomes, differing in nucleotide length and/or nucleotide substitutions. The main objectives in

selecting fruit plants collected from orchards are as follows: the creation of varieties with high universal commercial quality, frost resistance, drought resistance, and high productivity. The main methods of classical breeding of fruit plants are intraspecific and distant hybridization, which make it possible to obtain new genotypes in generative generations as a result of gene recombination (Urbanovich, 2015). The fruit plants used in the study are presented in Diagram 1. This article presents the results of the genetic analyses of two species—apple and pear. The genetic results of other plants will be presented in the next article.

Diagram 1

Species of the fruit plants studied



apple – 14, pear – 15, quince – 6, medlar – 2, hawthorn - 1.
The numbers shown along the x-axis represent the number of varieties

Materials and Methods

Starting from 2015, the soil and climatic conditions of the Guba–Gusar, Khachmaz, and Sheki–Zagatala regions of the republic were analyzed, and it was noted that they are fully suitable for the cultivation of the studied fruit crops—apple and pear. Under the conditions of the northern region of our republic, the economic and biological characteristics of a number of previously known and newly introduced varieties of two fruit species (apple and pear) were studied under different cultivation practices, planting schemes, and types of orchards (Huseynova & Asadova, 2024). To identify genetic diversity and kinship relationships among fruit plant samples, their DNA was extracted according to the CTAB protocol proposed by Rogers. PCR reactions were performed, and the obtained amplification products were analyzed by electrophoresis in agarose gels (Rogers,1985). PCR products were analyzed using an ABI 3500 capillary electrophoresis system.

Nuclear DNA extraction from apple and pear genotypes was carried out in the Biotechnology Laboratory. 2 grams of leaf samples were taken from each genotype, ground into powder using liquid nitrogen, and collected in 2 ml Eppendorf tubes for DNA extraction. DNA extraction was carried out according to the CTAB (cetyltrimethylammonium bromide) protocol proposed by S.O. Rogers (1985), in the following sequence (Rogers, 1985).

1. 100 mg of the obtained plant powder was poured into a 2 ml tube. 1000 µl of CTABx2 (2% CTAB, 0.1 M Tris HCl (pH=8.0), 1.4 M NaCl, 20 mM EDTA) solution and 1% β-mercaptoethanol (pH=8.0) pre-heated at 65^0C are added to 100 mg of plant powder poured into Epperdorf tubes and mixed well using a vortex until a homogeneous mass is formed;
2. The resulting suspension is placed in a water bath (65^0C) for 20 minutes, inverting every 5 minutes;
3. After removing the tubes from the water bath and cooling them at room temperature for 5-10 minutes, 700 µl of chloroform (isoamyl alcohol (24:1) (XIS)) is added to the suspension, inverted 20-25 times, and then kept on ice for 30 minutes. In this way, all remaining protein and phenol-containing compounds, except for nucleic acids, are dissolved;

4. The mixture is centrifuged at 14,000 rpm for 5 minutes, and then the supernatant obtained is poured into a new 2 ml tube;
5. XIS is added again to repeat this step;
6. In order to precipitate the DNA, 800-850 µl of cold isopropanol is added to the supernatant obtained, and the Eppendorf tube is covered with parafilm, mixed thoroughly, and placed in a refrigerator to remain at -20^{0}C for 1 day;
7. Samples from -20^{0}C are centrifuged at 14000 rpm for 1 minute; 8. After pouring the supernatant into a new tube, cold washing solution (76% ethanol and 10 mM ammonium acetate) is added to it twice and placed in the centrifuge again;
8. The obtained DNA should be kept at room temperature for 30 minutes to dry and 100 µl of TE (ph:8) should be added to it. This DNA solution is used as a stock solution;
9. After measuring the amount of DNA in a spectrophotometer, 1 µl of RNAse is added and kept at 37^{0}C for 30 minutes.

The quality of the extracted DNA was checked on a 1% agarose gel prepared in TBEx1 (18 mM Tris-HCL, 18 mM Boric acid, 100 mM EDTA, pH 8.0) buffer. The mixture prepared for quality checking consisted of 2 µl of extracted DNA, 4 µl of dye, and 1.5 µl of distilled water. The gel was stained with 0.5 µg/ml ethidium bromide solution, viewed under ultraviolet light, and images were visualized using the Bio-Rad Gel Documentation system. The total volume of the PCR mixture was 20 µl. The volume of each reaction mixture was 2 µl of DNA, 2 µl of 10X buffer [10 mM Tris–HCl pH 8.0, 50 mM KCl, 1.5 mM MgCl2], 2 µl of dNTP (at 5 mM), 0.5 µl of enzyme. O3 to H3.O3-, 0.2 µl of Taq polymerase were used. The polymerase chain reaction was initiated by denaturing the DNA at 94^{0}C for 5 minutes in the amplification apparatus and was performed in 3 conditions– 1 minute at 94^{0}C for denaturation, 45 seconds at 40-60^{0}C (and 5 minutes) for annealing the used primer to the DNA.

After the reaction was prepared, the Eppendorf tubes were placed in a PCR apparatus (Gene Amp 9700 Applied Biosystems thermocycler). The polymerase chain reaction began with DNA denaturation at 94^{0}C for 3 minutes after placement in the amplifier and continued in three stages. These three stages were repeated one after the other for 35 cycles. It ended with incubation at 72^{0}C for 5 minutes. Genotyping based on SSRs with fluorescent labeling, determination of allele sizes, and characterization of polymorphic loci were carried out according to the method proposed by Schuelke (Edwards, Johnstone, Thompson, 1991; Karaca, 2008). PCRs were conducted using dyes 6-FAM, NED, PET, and VIC (Bavykin, 2017).

The study of genetic parameters using SSR markers was carried out using the PowerMarker V3.25 software (Schuelke, 2000). The number and frequency of alleles, expected heterozygosity, observed heterozygosity, genetic diversity, and polymorphism information content (PIC) were calculated based on genetic parameters amplified with SSR primers.

Using ISSR primers, statistical parameters such as polymorphism information content (PIC) (Garkava-Gustavsson, 2008), effective multiplex ratio, marker index (MI) (Powell, 1996), resolving power (RP), and mean resolving power (MRP) were calculated to assess the efficiency of genetic analysis in fruit samples. Genetic parameters based on ISSR markers were analyzed using the DARwin 6.0 software (Reales, 2010). Cluster analysis was performed using ISSR and SSR primers. The Jaccard genetic similarity index was determined using UPGMA, and genetic distance indices were analyzed using the NTSYS software.

Results and Discussion

The similarity of fruit genotypes cultivated under natural mountainous conditions was evaluated based on their quantitative traits. In addition, the application of SSR and ISSR molecular marker technologies has proven to be more effective in studying polymorphism and genetic diversity within fruit collections. SSR and ISSR molecular markers are independent of environmental factors and are

highly important because they allow evaluation at any stage of plant development. Thus, the analysis of morphological traits and molecular analyses of fruit genotypes makes it possible to identify genetically similar genotypes and avoid ineffective combinations in the breeding process. At the same time, apple varieties such as Golden Ahmed, Shirvan Gozali, Sini, Rajabi, Karbalayi Jafar, Vahidi, Sari Tursh, Cir Haji, Aq Alma, Arazbeyi, Sari Sinab, Gusar Alma, Papirovka, Slava Pobedidelyam, which were selected for their fruit taste and durability, can be used in the creation of new varieties on farms. For genetic analysis, leaf samples were collected from varieties and forms of fruit plants characterized by their biodiversity and biomorphological traits across different regions. Information on allele combinations of SSR markers in the studied apple varieties is presented in Table 1. The sizes of the amplified fragments are given in base pairs.

Table 1
Allelic polymorphism of SSR markers in apple varieties

No.	Variety / locus	CH03d04	CH582493	CH04a03	CH03f02	Hi16e02	CH02b03a
1	Qizil Ahmədi	97/99	200/202	189	143	134/154	157
2	Shirvan gozeli	97/103	190/200	190	84	141/158	164
3	Sini	118/120	202/218	86	125/126	145	168
4	Recebi	102/103	200/208	192	143	143	169
5	Kerbalayi Cafar	106	194/212	200	140	141	165
6	Vahidi	105	202/204	189	142	144	166
7	Sari tursh	114/118	192/208	184	127/128	143	165
8	Cir Haci	95/105	200	185	130	144/166	165
9	Ag alma	103	192/202	184	123	143	173
10	Arazbeyi	95/100	190/196	196	140	141	164
11	Sari sinab	105/116	208	193	128	145	166
12	Qusar alma	96/102	208	190	125	142	165
13	Papirovka	104	193/203	185	123	145	170
14	Slava pobeditelyam	95/100	192/196	198	143	143	168

It can be observed from the table that the values of heterozygosity, characterized by the simultaneous presence of two amplification fragments of different sizes, vary significantly. The maximum level of heterozygosity was detected at the CH03d04 and CN582493-SSR loci. In contrast, low levels of heterozygosity were observed at the CH03f02, Hi16e02, and CH02b03a loci. At the CH04a03 locus, a homozygous state was identified in all studied varieties. Based on comprehensive information on the sizes of amplification sequences and allele frequency, the degree of genetic similarity among the studied apple varieties was evaluated. For this purpose, cluster analysis was applied, and the results of clustering were reflected in a dendrogram.

Based on the second direction of analysis of genetic diversity of apple varieties and species, an optimal set of SSR markers was developed, allowing for reliable identification of individual samples. The information content of each marker, the frequency of alleles among varieties, and the ease of analysis of amplification fragments were taken into account (Table 2).

Table 2
Allele characteristics calculated for apple species and hybrid samples

Marker name	Number of alleles A_s	Observed heterozygosity H_e	Expected heterozygosity H_g	Effective number of alleles A_{ef}	Wright's index R	Quantity of unique genotypes	Proportion of unique genotypes	Marker Assisted Selection MAS
CH03d04	15	0.82	0.49	5.2	0.42	37	0.336	0.9022
CH582493	13	0.85	0.74	5.9	0.14	35	0.308	0.9283
CH04a03	9	0.77	0.62	4.4	0.32	20	0.178	0.8702
CH03f02	16	0.82	0.58	5.2	0.32	38	0.345	0.9220
Hi16e02	42	0.92	0.72	16.68	0.28	84	0.770	0.9844
CH02b03a	19	0.90	0.46	9.08	0.51	37	0.335	0.9412
Average	19	0.85	0.60	7.7	0.33	41.8	0.378	0.9248

As – number of calculated alleles, He – observed heterozygosity, Hg – expected heterozygosity, Aef – effective number of alleles, R – Wright's index, number and proportion of unique genotypes, and marker discriminating power (MAS). Using the proposed set of 6 SSR markers, the average number of detected alleles among the studied samples was 19, while the average number of unique genotypes was 41.8. The proportion of unique genotypes averaged 0.378, and MAS was 0.9248.

DNA identification using SSR markers enables effective and reliable identification of apple genotypes obtained through cross-pollination. However, clonal mutants of apple trees are also known, which differ phenotypically from the original forms and from each other in fruit size and color, crown shape and size, branching patterns, etc. Some of these can be classified as independent varieties, which is of great practical importance in identifying clonal mutants of apple trees. In modern intensive horticulture, the mobilization and conservation of genetic diversity of cultivated pear varieties and their wild relatives is one of the key aspects in the development of new varieties. Parent selection—choosing pairs with a combination of desirable traits—is very important in breeding diversity (Plugatar et al., 2018).

In the studied regions, 15 pear species were investigated. Of these, 6 are local varieties—Abbasbeyi, Chirnadiri, Aday pear, Nar pear, Ahmad Ghazi, and White Ordubadi; while 9 are introduced varieties—Williams, Red Williams, Bere Bosc, Kure, Clapp's Favorite, Bere Ardanpon, Pass Crassan, Forest Beauty, and Devechi (Table 3). The best rootstocks for pear varieties are wild pear species, quince, hawthorn, and bird pear. Pear varieties in Azerbaijan, including the Caucasian wild pear, are derived from wild pear species. In arid mountainous areas, willow-leaved wild pear develops well and serves as a good rootstock for cultivated varieties. The fruits of cultivated pears are larger, juicier, and more delicate than those of wild pears.

Studies demonstrate a high level of genetic diversity, confirming that pear is a valuable breeding resource.

Table 3
Some agrobiological characteristics of pear plant species

Plant	Variety	Origin	Ripening period	Leaf area (cm²)	Fruit weight (g, avg)		Fruit diameter (cm)	Fruit height (cm)	Quality indicators				
					average	maximum			Dry matter (%)	Total sugar %	Vitamin C (mg%)	Marketable yield (%)	Storage period (days)
1	2	3	4	5	6	7	8	9	10	11	12	13	14
PEAR	1. Abbasbeyi	Local selection	Summer	22.4	6	7	5.2	7.5	14.5	7.7	4.7	70	23
	2. Chirnadiri		Summer	21.5	110	115	5.3	7.4	13.6	7.6	5.3	75	25
	3. Aday pear		Summer	22.0	75	83	4.8	6.3	12.5	6.5	6.3	70	22
	4. Nar pear		Autumn	24.4	53	64	11.4	11.3	13.3	8.4	5.5	75	56
	5. Ahmad Ghazi		Autumn	24.5	125	153	10.4	11.3	14.3	7.6	5.5	79	30
	6.White Ordubadi		Summer	22.0	134	156	5.6	7.4	12.3	8.3	5.7	83	27
	7.Williams	Introduced variety	Summer	21.3	115	121	6.9	7.4	10.1	8.3	8.2	76	30
	8. Red Williams		Summer	24.0	124	130	6.6	7.3	10.3	8.5	8.7	77	28
	9. Bere Bosc		Winter	22.5	120	128	6.1	7.5	12.4	8.3	7.3	70	60
	10. Cure		Winter	21.2	130	135	6.1	7.5	13.1	7.6	7.5	70	65
	11. Clapp's Favorite		Summer	26.2	108	119	7.3	9.5	12.1	8.4	6.6	75	23
	12. Bere Ardanpon		Winter	23.5	120	133	7.2	8.5	12.2	7.5	7.3	75	60
	13. Pass Crassan		Autumn	27.7	113	121	8.5	12.9	12.2	8.3	5.6	73	32
	14. Forest Beauty		Summer	26.5	135	139	6.8	10.5	14.1	7.7	4.6	75	34
	15. Devechi		Autumn	23.0	115	125	6.4	7.1	12.6	8.1	4.5	77	63

The average observed heterozygosity (He) for pear varieties across the studied loci was 0.78, while for apple varieties it was 0.85. Pear varieties, like apple varieties, are characterized by a relatively high level of heterozygosity (Hg = 0.60, F = 0.33), which is facilitated by cross-pollination typical for both pear and apple. The average number of informative alleles among pear varieties was 6.49, which is slightly lower than that of apple (7.5). Among pear samples, the maximum values for this indicator were obtained using the CH582493 and CH03f02 markers—11.09 and 5.26, respectively; for apple, the maximum value was obtained using the CH04h04 marker—16.68.

The genetic distance among samples ranged from 0.11 to 0.30. Similarity was detected in the genomes of some pear varieties, indicating that these varieties form a separate group. A more detailed explanation of these findings will be provided in the next article.

Table 4
Allelic characteristics calculated for pear species and hybrid samples

Marker name	Number of alleles A_s	Observed heterozygosity H_e	Effective number of alleles A_{ef}	Expected heterozygosity H_g	Wright's index R	Quantity of unique genotypes	Proportion of unique genotypes	Marker Assisted Selection MAS
CH03d04	8	0.70	3.50	0.77	0.07	18	0.397	0.8740
CH582493	20	0.90	11.09	0.47	0.45	34	0.814	0.9682
CH04a03	10	0.76	4.35	0.61	0.24	17	0.373	0.8710
CH03f02	12	0.79	5.26	0.46	0.43	20	0.444	0.8958
Hi16e02	15	0.68	3.33	0.52	0.29	16	0.396	0.7573
CH02b03a	16	0.88	9.09	0.76	0.18	26	0.654	0.9342
Average	13	0.78	6.10	0.59	0.27	22.0	0.513	0.8835

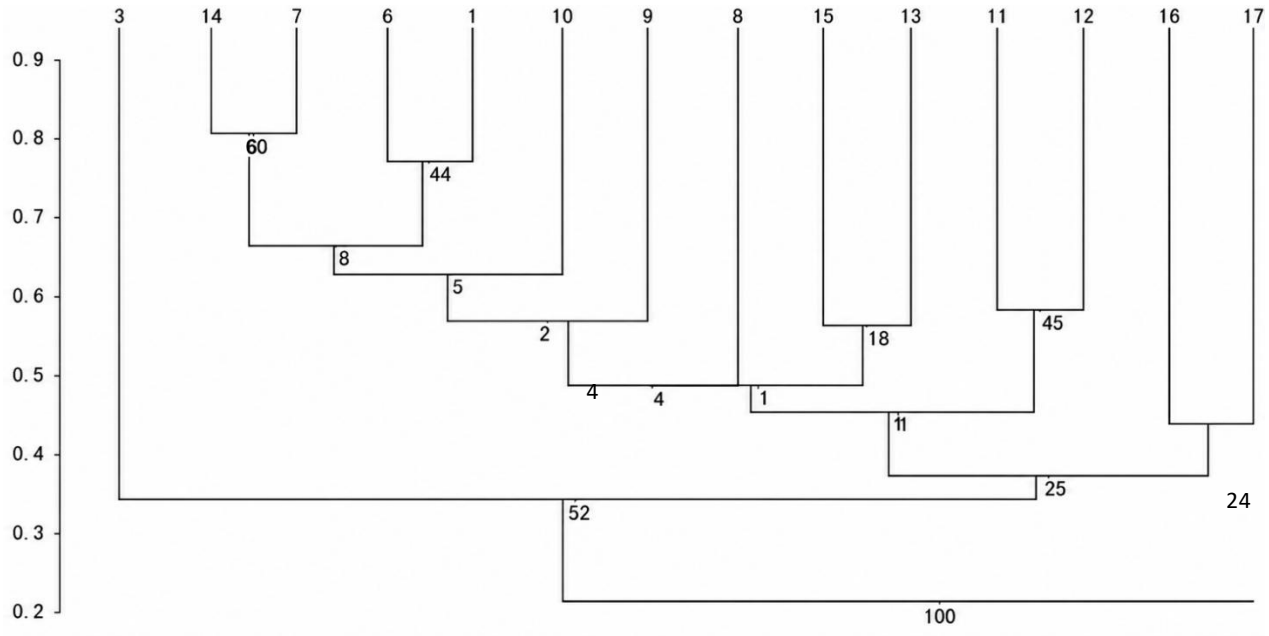
As – number of calculated alleles, He – observed heterozygosity, Hg – expected heterozygosity, Aef – effective number of alleles, R – Wright's index, number and proportion of unique genotypes, marker discriminating power (MAS).

The analysis of marker informativeness for DNA identification of pear varieties resulted in the selection of six highly polymorphic SSR markers that demonstrated successful performance in identifying apple varieties. This marker set revealed a high proportion of unique genotypes among pear samples (0.513) (Table 4). The discriminating power (MAS) of the markers was high across all loci, ranging from 0.7573 for the Hi16e02 marker to 0.9682 for the CH582493 marker, with an average value of 0.8835. Indicators characterizing the informativeness of individual markers in pear and apple genomes showed slight differences. The calculations presented in the table indicate that the marker set CH03d04, CH582493, CH04a03, CH03f02, Hi16e02, CH02b03a is effective for identifying pear genotypes.

Based on the polymorphism data of SSR markers, an analysis of the degree of genetic similarity among the studied pear varieties was carried out, allowing the evaluation of genetic relationships within the studied genotype samples (Khlestkina, 2013). To analyze genetic polymorphism in the studied group consisting of 15 varieties, six microsatellite DNA markers were used. SSR markers showed significant variation in the level of polymorphism: from 3 alleles (for the CH582493 marker) up to 11 alleles per locus. Cultivated apple varieties are genetically highly diverse. They form different groups, including both modern and traditional varieties, as well as varieties bred abroad.

According to the results of cluster analysis, two main groups (clusters) can be identified among the studied varieties. Cluster 1 includes Aday pear, White Ordubadi, Nar pear, Chirnadiri, and Ahmad Ghazi pear varieties. Cluster 2 includes Williams, Red Williams, Bere Bosc, Cure, Clapp's Favorite, Bere Ardanpon, Pass Crassan, Forest Beauty, and Devechi pear varieties. The Abbasbey pear variety forms a separate branch in the dendrogram.

According to the results of cluster analysis, although not completely, there is a relationship between the grouping of samples within subclusters and the regions where they were collected. The microsatellite markers used in this study can be applied in the future for the identification and differentiation of fruit tree genotypes.



Picture 1

Clustering of pear varieties

Based on the results of the cluster analysis, it can be noted that although not completely at the cluster level, a relationship is observed between the grouping of samples within subclusters and the districts where they were collected. The microsatellite markers used in our study can be applied in the future not only for distinguishing apple and pear genotypes but also for other pome fruit species. Genetically highly distinct forms that were isolated in the dendrogram and formed independent groups were identified. The varieties Williams and Forest Beauty, located in Cluster 2, possess rare alleles and can be used as important varieties in future breeding and genetic studies. Within each geographical region, both genetically close and distant genotypes were identified. The Bere Bosc and Pass Crassan varieties collected from the Guba region were placed in the same group, showing a higher degree of similarity. The Abbasbey pear variety was separated into an independent branch of the cluster and is distinguished by its rare alleles.

The expected heterozygosity level of pear varieties reached a minimum value of 0.46 at the CH03f02 locus. The highest heterozygosity level was observed for the CH02b03a locus (0.76). The average heterozygosity level for the studied genotypes was 0.52. In the studied apple samples, the heterozygosity level at the same locus was, on average, 0.58. In this study, a marker-assisted selection strategy was applied to obtain hybrid apple seedlings with комплекс resistance to scab, powdery mildew, fire blight, and red gall aphid, as well as to identify genotypes associated with extended storage life of apples.

Conclusion

1. Gene blocks of fruit plant varieties responsible for certain biologically valuable traits were identified using DNA and PCR analysis, partially passportized, and genotyped.
2. Approaches for the development, identification (using marker traits), and preservation of valuable genotypes used in the creation of new fruit plant varieties, pure lines, and hybrids characterized by high morphological and biological uniformity, productivity, and marketability were examined.
3. Modern molecular genetic and biotechnological approaches, along with the acceleration of hybridization and selection processes, were demonstrated. Additionally, a system for genotype identification and DNA certification of the studied apple and pear varieties was developed. The applied method is based on the use of SSR markers detected via polymerase chain reaction (PCR).

This method is suitable for performing genetic analysis of any plant organ at various stages of ontogenesis.

4. For developing DNA identification methods for fruit plant varieties, an SSR-based approach is recommended. The choice of identification method is based on criteria such as efficiency, reliability, stability, accuracy, high reproducibility, and ease of result documentation. Apple species, varieties, and hybrids cultivated in the studied regions of Azerbaijan are characterized by high genetic diversity at microsatellite loci observed heterozygosity (He) = 0.85, expected heterozygosity (Hg) = 0.60, effective number of alleles (A_{ef})= 7.7, Wright's index (R) = 0.3, number of alleles (As) = 19. Rare and wild species contribute significantly to the overall allelic diversity.

5. A set of 20 SSR markers, experimentally validated for effectiveness, is recommended for clarifying the pedigree and origin of apple varieties. A DNA identification system for apple varieties and species genotypes has been developed. The method is based on six highly informative SSR markers: CH03d04 (MAS = 0.9022), CH582493 (MAS = 0.9283), CH04a03 (MAS = 0.8702), CH03f02 (MAS = 0.9220), Hi6e02 (MAS=0.9844), and CH02b03a (MAS = 0.9412). This method allows the creation of a unique molecular genetic profile for apple genotypes and enables the identification of apple varieties, interspecific hybrids, and wild species. The proposed method also allows verification of apple varieties for compliance with DUS (Distinctness, Uniformity, Stability) criteria.

6. Pear varieties cultivated in Azerbaijan are characterized by high genetic diversity of SSR loci alleles (He = 0.78, Hg = 0.59, Aef = 6.10, R = 0.27, Ae = 13). Both closely related and genetically distant varieties, as well as interspecific hybrids, were identified.

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