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Molecular-Genetic Characterization of Common Quince (*Cydonia oblonga* Mill.)

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Abstract. *Studies were conducted on seven quince cultivars in the northern and northwestern regions of Azerbaijan. The investigation into the intraspecific genetic diversity and structure of the quince species was based on the analysis of seven quince genotypes across eight ISSR loci. A total of 69 bands were generated using ISSR markers; of these, 47 (69.4%) were polymorphic, while 22 (30.6%) were monomorphic. The UBC868 primer yielded 9 bands, 8 of which were polymorphic, resulting in a polymorphism rate of 89%. The number of amplified fragments per locus ranged from 7 to 10. Comprehensive analysis of the quince collection using these eight ISSR markers revealed a high level of heterozygosity in hybrid seedlings compared to expected values, indicating significant genetic distance between the parental pairs selected for hybridization. It is recommended to use the UBC 868, UBC 807, and UBC 825 ISSR primers for the assessment of polymorphism and genetic diversity in the quince collection, as they are more effective.*

Keywords: *quince, DNA markers, genetic diversity, genome, gene pool*

Introduction

Quince is highly valued as a source of iron and as a means of improving mood and boosting vitality. Among pome fruits, quince ranks third after apples and pears. Autumn and winter varieties and forms of quince are distinguished. The average lifespan of the plant is 60–80 years. A single tree yields between 50 and 160 kg of fruit. Quince is cultivated in all regions of Azerbaijan (Bayramov, 2018; Bayramov, 2021; Muradova, 2018).

The objectives of this study included the selection of high-yielding, high-quality, and cold-resistant forms adapted to local soil and climatic conditions. The primary research material was sourced from promising varieties found in the Guba, Gusar, and Khachmaz districts, as well as the Sheki and Balaken districts of Azerbaijan. Their agrobiological and pomological characteristics were investigated through phenological observations (Guseinova & Asadova, 2024).

The aim of this study is to analyze the genotypic structure of quince varieties and forms distributed across the mountainous regions of Azerbaijan—specifically in the Guba, Gusar, Khachmaz, Sheki, and Balaken districts. The research objectives also included determining the level of genetic diversity and elucidating the genetic relationships between parental forms and their progeny. The aim of the study is to assess genetic diversity among quince genotypes using ISSR molecular markers, as well as to evaluate polymorphism and its information content.

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Research conducted in the northern and northwestern regions of Azerbaijan revealed numerous quince forms cultivated over various periods, many of which are valuable for fruit breeding. Table 1 presents the agrobiological characteristics of the quince plant.

Table 1
Agrobiological characteristics of the quince plant (as of 2023)

Variety	Origin	Area of one sheet (cm^2)	Fruit weight, g		Fruit diameter (cm)	Fruit length (cm)	Quality indicators				
			medium	maximum			Dry matter, in %	Total sugar content, in %	Vitamin C, mg %	Yield	Shelf life, days
Jardam	Folk selection	55	518	575	8	8.6	12.3	11.1	5.1	69	150
Pensar		63	283	320	5	5.5	11.5	12.3	5.1	71	161
Bardag quince		63	455	545	6	8.3	10.2	13.5	6.4	73	158
Sari ayva		59	350	405	7	6.3	10.2	11.4	5.2	74	145
Velechin		62	230	255	5	6.5	11.4	12.1	5.5	72	142
Karaman ayva		63	333	380	6	7.5	12.0	12.3	5.8	85	161
Ege-22	Introduction	55	518	575	8	8.6	12.3	11.1	5.1	69	150

The range of quince in Azerbaijan comprises over a hundred local forms and varieties. The quince collection in the studied regions represents a rich gene pool—encompassing a large number of varieties, forms, and hybrids primarily from the northern and northwestern regions of Azerbaijan—that is actively utilized in breeding programs (Bayramov, 2018; Baskakova, 2019).

The significance of this work lies in the utilization of a valuable and highly promising breeding gene pool that was developed and evaluated in Azerbaijan based on phenotypic and genetic traits. For medicinal purposes, a thick, mucilaginous aqueous extract derived from the seeds has been used in clinical medicine as a coating, emollient, and soothing agent for gastrointestinal disorders and colitis in children. Experiments have demonstrated that preparations derived from quince leaves exert a beneficial effect on the cardiovascular system (Aliyeva, 2018).

It should also be noted that an aqueous infusion made from the leaves and seeds of the common quince has the ability to alleviate and suppress attacks of coughing and bronchial asthma. In Azerbaijani folk medicine, quince fruit juice was used as a choleretic and diuretic agent (Sadıqov, 2023).

Genetics is currently one of the most popular scientific fields and one that is open to innovation. Plant genetics is one such area, and the molecular marker technologies employed within it serve as a significant tool. Thanks to research conducted on molecular markers to date, it has become possible to rapidly obtain significant information regarding the genetic characteristics and heredity of economically important plant species, much like with many other living organisms. This information has led to a reduction in the breeding cycle—particularly for fruit species that require long cultivation periods—thereby resulting in labor savings. The application of data generated by researchers using molecular markers has enabled the development of higher-quality and more productive fruit varieties (Huseynova, 2025).

A molecular marker represents a specific gene region or a DNA segment associated with a gene region within the genome. Consequently, molecular markers have gained widespread use in plant genetics research, enabling the achievement of more efficient and rapid scientific results. A molecular marker reflects variation in a specific DNA segment within the genome; such variations can arise from events such as insertions, deletions, substitutions, or duplications. Data derived from molecular markers facilitate the selection of samples for preservation and the management of collections (Guseynova, 2023). Evaluating samples using molecular markers allows for their identification and the elimination of redundant duplicates (Sarwat & Nabi, 2014; Sarwat & Yamdagni, 2016).

Scientific Novelty

Currently, the identification of quince varieties in Azerbaijan is primarily based on fruit composition and morphological characteristics. However, experimental evidence suggests that morphological and pomological indicators alone are insufficient for variety identification, as environmental factors influence both the morphological traits of the trees and the composition of the fruit. Consequently, identifying quince varieties using molecular markers is a more effective method. To date, the comparative characterization of quince varieties in the country using molecular methods has not been investigated. We have conducted an identification of quince varieties and forms collected from various regions of Azerbaijan using both morphological and molecular-genetic methods.

Materials and Methods

Seven quince cultivars from the collection of the Research Institute of Fruit Growing and Tea Cultivation (Guba District)—part of the Sheki Branch of the Institute of Genetic Resources of Azerbaijan—were selected for study, as they are of particular interest for regional breeding programs. One objective of the research was to determine the relationships between the cultivars, given that their morphological and organoleptic similarities might indicate genetic kinship. Another objective was to assess the influence of parental genotypes on the similarities and differences observed in the progeny. DNA extraction from leaf tissue at the bud-break stage was performed using the CTAB method (Rogers & Bendich, 1985; Murray & Thompson, 1980). Eight ISSR markers developed for Japanese quince (*Cydonia japonica*) were selected for genotyping: UBC807, UBC810, UBC811, UBC815, UBC818, UBC825, UBC840, and UBC868.

The selection of these markers for quince was driven by the goal of expanding the existing set of ISSR markers by testing markers originally developed for closely related species. To perform the PCR, optimal parameters—such as component concentrations and reaction temperature conditions—were determined. The resulting optimal protocol involved a total PCR mixture volume of 25 μL containing 50 ng of DNA, 0.25 mM dNTPs, 0.2 μM of each primer, 2.5 μL of 10× buffer, and 1 U of Taq polymerase. PCR was performed using the following program: initial denaturation at 94 °C for 3 minutes, followed by 35 cycles of denaturation at 94 °C (45 s), annealing at 58 °C (45 s), and elongation at 72 °C (45 s); the final step was elongation at 72 °C for 4 minutes 30 seconds. The sizes of the PCR products were analyzed using an ABI Prism 3130 instrument. The obtained results were processed using GeneMapper 4.1 software. Both the quality and concentration of the extracted DNA were measured using a NanoDrop 2000 instrument. Thus, after verifying the suitability of the DNA for the polymerase chain reaction, genetic analysis was conducted using ISSR markers.

Table 2
Characteristics of the 7 selected quince samples

Samples	NS	NA	NEA	I	OH	EH	FI
Jardam	7	3.273	2.014	0.782	0.364	0.422	0.098
Pensar	6	3.455	2.429	0.960	0.682	0.542	−0.262
Bardag quince	10	4.273	2.719	1.085	0.727	0.574	–0.266
Sari ayva	5	3.455	2.265	0.893	0.509	0.484	−0.056
Velechin	28	5.000	2.945	1.167	0.588	0.598	−0.124
Karaman ayva	17	3.355	2.827	1.123	0.489	0.567	−0.229
Ege-22	26	4.434	2.016	1.109	0.466	0.535	−0.139

NS – number of samples, NA – number of alleles, NEA – number of effective alleles, I – Shannon index, OH – observed heterozygosity, EH – expected heterozygosity, FI – Fixation index

Results and Discussion

In our study, eight ISSR primers characteristic of the *Cydonia* genus were used to investigate the genetic diversity of seven quince genotypes. ISSR (Inter-Simple Sequence Repeat) marker analysis involves the amplification of genomic segments located between two microsatellite regions using primers composed of di-, tri-, tetra-, and penta-nucleotide repeats. In this process, genomic loci are detected as amplicons of varying sizes, and the primers—consisting of 15–30 nucleotides that extend into the microsatellite regions at the 5' and 3' ends—exhibit high levels of polymorphism. These markers are widely used in genetic diversity analyses. A set of seven quince genotypes was analyzed using eight ISSR markers (Table 3). One of the study's objectives was to evaluate these markers on a selection of common quince cultivars in order to identify the most polymorphic ones for future use.

The simplest parameter reflecting marker polymorphism is the number of detected alleles. In the conducted research, a total of 69 bands were generated across 8 primers for the quince genotypes; of these, 47 (69.4%) were polymorphic and 22 (30.6%) were monomorphic. The number of amplified fragments per locus ranged from 7 to 10. The length of the obtained fragments ranged from 100 to 1200 bp. The average number of bands per primer was 8.6.

Table 3
Polymorphism and key parameters of genetic diversity in cherry genotypes determined using ISSR primers

Primers	Sequence 5'–3'	Synthesized dam	Polymorphic dam	Polymorphism %	Rp	PIC	EMR	MI	MRp	GM
UBC807	$(AG)_8T$	10	6	70	5.56	0.33	6.75	2.2	0.02	0.96
UBC810	$(GA)_8T$	7	4	57	7.46	0.41	4.1	1.6	0.02	0.85
UBC811	$(GA)_8AC$	8	6	75	5.0	0.33	5.6	1.7	0.03	0.94
UBC815	$(CT)_8G$	8	5	62.5	6.30	0.41	5.6	2.3	0.02	0.91
UBC818	$(CA)_8G$	7	5	71.4	5.16	0.43	4.2	1.7	0.03	0.93
UBC825	$(AC)_8T$	10	8	80	7.34	0.45	8.0	3.6	0.01	0.97
UBC840	$(GA)_8YT$	10	5	50	8.34	0.41	8.0	3.33	0.01	0.93
UBC868	$(GAA)_6$	9	8	89	4.46	0.32	6.8	2.0	0.03	0.89

General	8	69	47							
Average price		8.6	5.9	69.4	6.20	0.38	6.13	2.30	0.02	0.92

PIC – capacity of polymorphic information, EMR – effective multiplexing index, MI – marker index, Rp – imaging capability, MRp – average imaging capability, GM – genetic diversity. The highest number of amplicons (10) was synthesized using the UBC807, UBC825, and UBC840 primers. Of the amplicons synthesized with the UBC825 primer, 8 were polymorphic. With the UBC840 primer, 5 of the synthesized bands were polymorphic, while the other 5 were monomorphic. The lowest number of amplicons (7) was recorded with the UBC810 and UBC818 primers. Four of the amplicons generated by the UBC810 primer were polymorphic, whereas 5 of the bands synthesized by the UBC818 primer were polymorphic. The number of polymorphic bands ranged from 4 to 8, with an average of 5.9. The UBC 868 ISSR primer exhibited the highest level of polymorphism among the cherry genotypes; out of the 9 recorded amplicons, 8 were polymorphic, representing a polymorphism rate of 89%. The amplicon lengths ranged from 400 to 1200 bp.

Conclusion

All ISSR markers employed in the study yielded polymorphic products during PCR. The average number of alleles detected for these markers in the common quince (*Cydonia oblonga*) samples (5 alleles per locus) is comparable to the average number of alleles identified in the Japanese quince (*Chaenomeles japonica*) sample set (5.75 alleles per locus). This finding indicates the potential for adopting ISSR markers developed for Japanese quince in genetic studies of common quince diversity. The data obtained from the genetic analysis of quince breeding material using SSR markers provide insight into the genetic diversity of the genotypes under study. Thus, an approach based on crossing breeding material of contrasting origins makes it possible to preserve the genetic diversity of the cultivated species and avoid genetic depletion of the gene pool.

A comprehensive study of a quince collection using 8 ISSR markers revealed a high frequency of heterozygotes among hybrid seedlings compared to expected values, indicating significant genetic divergence between the parental pairs selected for crossing. Furthermore, the analysis conducted in this study makes it possible to determine kinship relationships within the evaluated sample. Thus, İSSR-based genetic analysis serves as a reliable tool for accurately identifying the parental forms of the resulting seedlings and for cultivar identification in cases where the varietal identity of a sample is in doubt. Overall, for a number of seedling groups, their origin from known parents was confirmed.

Declaration of Competing Interests

The author declares that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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