

Research article

Genetic Diversity Analysis of Fruška Gora Quince Cultivars Based on SSR Markers

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ABSTRACT

Quince (*Cydonia oblonga* Mill.) is a well-known pome fruit species, primarily used for the production of jams and marmalades as well as for pear rootstocks. It has long been cultivated in Serbia at various locations and it possesses vast genetic variability. The aim of this study was to evaluate genetic diversity of 36 genotypes collected from the Fruška Gora locality. Seven SSR markers were used for genetic diversity analyses, revealing a total of 85.7 % polymorphic loci. Six of them were polymorphic, while marker Hi02d04 was homozygous. A total of 20 alleles were recorded, with an average number of alleles per marker of $N_a = 1.58$. The number of effective alleles was $N_e = 1.4$, observed heterozygosity was $H_o = 0.34$, while expected heterozygosity was $H_e = 0.22$. Principle coordinates analyses (PCoA) explained 74.60% of total diversity with its 3 coordinates and formed 4 different groups of genotypes. The dendrogram constructed with the UPGMA method placed genotypes in 3 different clusters, with variation observed within clusters. The use of SSR markers revealed significant differences between the tested genotypes, providing a closer insight into the state of the genetic resources of this pome species in our country.

Key words:

DNA markers, SSR, *Cydonia oblonga* Mill, PCA, UPGMA

Abbreviations:

BP – Bešenovački Prnjavor; J – Jazak; L – Ledinci; B – Bukovac; V – Vrdnik; K – Krčedin; SK – Sremski Karlovci; MR – Mala Remeta; G – Grgurevci; STD – standard varieties; SE – standard error

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INTRODUCTION

Quince (*Cydonia oblonga* Mill.) belongs to the Rosaceae family and the Maloideae subfamily, which contains around 1000 species and 30 genera, including the monotypic genus *Cydonia*. It is closely related to apples (*Malus*) and pears (*Pyrus*). Areas from Dagestan to Talysh in Transcaucasus and northern Iran are considered as the center of origin of this species. Quince has been cultivated there for more than 4000 years, and from this area it has spread across the world (Abdollahi, 2019). Persians brought it to the Minoan city-state of Cydonia (today Chania) on Crete, 600 years BC, where it got its name (Ferreira et al., 2022). World production of this pome fruit was 695,474.15 t and it is grown on 74,317 ha. The biggest producer is Turkey, with 29.2 % of total global production. Other countries with significant quince production are China (16.8%), Uzbekistan, Iran and Morocco (FAO, 2026). The average yield is 10-12 t/ha, while in Europe it reaches about 30t/ha (Umidov et al., 2023). In Serbia, it is grown on 2,030 ha, with a production of 12,422 t, representing about 3% of the total world production. The average yield of 6.2 t/ha is considerably lower than the European average (Radović et al., 2016). The presence of stone cells, tannins and pectin diminishes its use for fresh consumption. Therefore, the fruit is much more often used in the food industry for producing jams, marmalades and drinks (Babić et al., 2008). Production of quince in our country is still below satisfactory level, though considerable efforts have been made to improve varieties and the overall cultivation process (Mratinić, 2010). According to FAO (2026), quince production in Serbia reached 10,086 t in 2024. Recent years have shown that introducing foreign genetic variability by importing varieties from abroad is not always the best solution for improving production, comparing to the utilization of domestic genetic resources

(Keserović et al., 2017). Plant diseases pose great problems in production, especially fire blight (*Erwinia amylovora*), which causes significant yield losses. There are many other diseases, such as monilia caused by *Monilinia linhartiana*, and pests like the oriental fruit moth or peach moth (*Grapholita molesta* Busck), that also cause serious problems in cultivation of this fruit. In order to improve quince disease and pest resistance, it is crucial to search for genetic resources that contain desirable genes that could be used in quince breeding. Desirable resistance genes could be found in locally well-adapted landraces, which have managed to overcome these problems through years of adaptation to local environmental conditions. Besides resistance, utilization of these genotypes could also contribute to breeding varieties with improved morphological tree and fruit characteristics, as well as better fruit chemical properties.

Selection of plants containing important traits for breeding processes is not always an easy task, especially when it is phenotype-based. Phenomics is considered as the most common approach to evaluate plant traits. However, it has its disadvantages in the evaluation of quantitative traits, where the impact of environmental variation on phenotypic variance could complicate the classification of quince plants into categories by their fruit shape into 'apple-shaped' (maliformis Mill.-Schneid.) and 'pear-shaped' (pyriformis Dierb.- F. Zinn) types of quince (Halász et al., 2009). Nevertheless, new methods have been developed. DNA markers emerged as a new way of high-precision genotype identification, which is not influenced by environmental factors. The most common DNA markers are PCR-based markers with various forms, such as RAPD, AFLP, SNP, ISSR and finally SSR markers, which are used in most cases, including this investigation because of their co-dominant nature, suitability for automation, high polymorphism and repeatability (Barać, 2016). Molecular markers, particularly SSRs, remain integral to modern breeding due to their high polymorphism and co-dominance, and are now frequently integrated into automated, high-throughput workflows to streamline diversity analysis and the identification of complex quantitative traits (Panahi et al., 2024; Sahoo et al., 2025).

The aim of this study was the determination of genetic diversity, classification and relationship analysis between quince genotypes found in the Fruška Gora locality utilizing PCR-based Simple Sequence Repeat (SSR) markers.

MATERIAL AND METHODS

Plant material

In total, 36 quince genotypes from nine locations (Krčedin, Sremski Karlovci, Bukovac, Ledinci, Vrdnik, Jazak, Mala Remeta, Bešenovački Prnjavor and Grgurevci) in the Fruška Gora locality ((DD): 45°09'20.2"N 19°42'36.0"E) were used for the purpose of genetic diversity analysis (Figure 1).



Figure 1. Locations from which the examined genotypes were collected

Most of the analyzed genotypes (32 out of 36) represent accessions found in the wild. The remaining four genotypes represent bred varieties (the domestic cultivars Vranjska i.e., Dunjac and Leskovačka, as well as the Provencal quince clone BA29 and Champion, which originated in the United States in 1870s, here labeled in Serbian as Šampion). All genotypes were labeled according to the abbreviations of the locations where they were collected (Table 1).

Table 1. List of 36 quince genotypes collected from Fruška Gora (Serbia) and analyzed using SSR molecular markers. Genotype labels were assigned according to their locations

Genotype	Location	Genotype	Location	Genotype labels	Location
BP1	Bešenovački Prnjavor	SK2	Sremski Karlovci	G1	Grgurevci
BP2	Bešenovački Prnjavor	MR1	Mala Remeta	G2	Grgurevci
BP3	Bešenovački Prnjavor	Leskovačka	Standard varieties	G3	Grgurevci
J1	Jazak	BA29	Standard varieties	G4	Grgurevci
J2	Jazak	Šampion	Standard varieties	G5	Grgurevci
J3	Jazak	Vranjska	Standard varieties	G6	Grgurevci
J4	Jazak	B1	Bukovac	G7	Grgurevci
JB1	Jazak	B2	Bukovac	G8	Grgurevci
K1	Krčedin	B3	Bukovac	G9	Grgurevci
K2	Krčedin	V1	Vrdnik	G10	Grgurevci
K3	Krčedin	V2	Vrdnik	G11	Grgurevci
SK1	Sremski Karlovci	V3	Vrdnik	L1	Ledinci

DNA extraction

Entire DNA used for SSR analysis was isolated from fresh healthy leaves using a modified CTAB method (Padmalatha and Prasad, 2006). Samples were then diluted to a working concentration of 10 ng/ml.

SSR Analysis

Twenty SSR markers originally developed for apples and pears and suggested by Yamamoto et al. (2004) were analyzed for their ability to amplify and produce polymorphic products in quince genotypes. Seven out of these twenty SSR markers (Hi02d04, Hi01c11, Hi03a03, Hi04a05, Hi04a08, AJ000761b and CN444542) showed promising results and were selected for use in further research. SSR marker labels and their sequences in both forward and reverse directions are given in Table 2.

Polymerase chain reactions (PCR) were performed in a total volume of 20 µl containing 100 ng of genomic DNA, 20mM MgCl₂ as PCR buffer, 0.2 mM dNTPs, 0.15µM both forward and reverse primers and 0.2 µl of Taq DNA polymerase (Thermo Fisher Scientific Inc., Massachusetts, USA). The PCR amplification procedure consisted of five consecutive stages, initial denaturation for 2 min and 30 sec at 94°C, followed by 35 cycles at 94°C for 30 sec, primer annealing at 60°C for 30 seconds, elongation at 72°C for 1 min and final extension at 72°C for 5 min. All PCR amplifications were performed in T Personal and T Gradient PCR thermocyclers (Biometra®, Thuringia, Germany).

Table 2. SSR marker labels and sequences in 5' and 3' direction used for the examination of 36 quince genotypes collected from Fruška Gora (Serbia)

Marker number	SSR marker label	DNA Sequence (5' - 3')	
		Primer forward	Primer reverse
1.	Hi02d04	TGCTGAGTTGGCTAGAAGAGC	GTTTAAGTTTCGCCAACATCGTCTC
2.	Hi01c11	TTGGGCCACTTCACAACAG	GTTTGAGTTTGATCTCCAACATTAC
3.	Hi03a03	ACACTTCCGGATTCTGCTC	GTTTGTTGCTGTTGGATTATGCC
4.	Hi04a05	GGCAGCAGGGATGTATTCTG	GTTTCATGTCAAATCCGATCATCAC
5.	AJ000761b	CCCTAAACACACAGCCTCCT	GTTTCAGCATCGCAGAGAACTGAG
6.	Hi04a08	TTGAAGGAGTTTCCGGTTTG	GTTTCACTCTGTGCTGGATTATGC
7.	CN444542	ATAAGCCAGGCCACCAAATC	GTTTGCACTGGATTGATGTTCC

After the end of the amplification process in the PCR device, products were separated using polyacrylamide gel electrophoresis – PAGE (Sambrook and Russel, 2001). In this study, a 6% polyacrylamide gel was used, consisting of: a) urea (240g), b) 10x TBE buffer and c) A+B mix consisting of acrylamide and bis-acrylamide. Once the process of electrophoresis was finished, amplification products were visualized using the silver staining method. The number of alleles and polymorphism were recorded and translated into binary inscription for further analysis.

Data analysis

Genetic diversity parameters such as the number of alleles (Na), number of effective alleles (Ne), information index (I), observed heterozygosity (Ho), expected heterozygosity (He), unbiased expected heterozygosity (uHe) and Wright's F-statistics fixation index (FST) were calculated using the DARwin program (Perrier et al., 2003). This

software was also used for constructing an unweighted pair-group method with arithmetic averages (UPGMA) dendrogram, which grouped the quince genotypes. Principal coordinates analysis (PCoA) was performed using the GenAlex software GenALEX (Peakall and Smouse, 2006). The dissimilarity index – DIS (d_{ij}) was calculated using the simple matching method, according to the following formula:

$$d_{ij} = 1 - \frac{1}{L} \sum_{i=1}^L \frac{m_{ij}}{n}$$

Where:

d_{ij} – measures the dissimilarity between two entities

L – total number of attributes

m_{ij} – matched characteristics between two items

n – normalizing factor related to the total observations (Kosman and Leonard, 2005).

RESULTS AND DISCUSSION

SSR markers polymorphism level analysis

A total of 20 SSR markers were initially tested for their ability to produce polymorphism in the 36 quince genotypes included in this study. Seven of these markers were chosen for further research based on preliminary testing. Six markers produced polymorphic products, while marker Hi02d04 was found to be monomorphic. The most important genetic diversity parameters were calculated for all SSR markers (Table 3).

Table 3. Mean values of genetic diversity parameters for all SSR markers selected for further testing of 36 quince genotypes collected from Fruška Gora (Serbia)

SSR loci	Na	Ne	I	Ho	He	uHe	F	Diversity	Usability
Hi02d04	1.000	1.000	0.000	0.000	0.000	0.000	-	Lack	Full homozygous – no use
Hi01c11	1.800	1.411	0.375	0.331	0.238	0.276	-0.318	Moderate	Basic genetic differentiation
Hi03a03	1.700	1.478	0.402	0.436	0.273	0.324	-0.518	Higher	Identification of genetic variation
Hi04a05	2.000	1.897	0.655	0.808	0.465	0.635	-0.703	High	Most suitable for genetic structure analysis
AJ000761b	1.500	1.371	0.298	0.244	0.205	0.285	-0.198	Moderate	Moderate genetic polymorphism
Hi04a08	1.300	1.129	0.141	0.111	0.089	0.108	-0.230	Lower	Identification of uniform populations
CN444542	1.800	1.535	0.410	0.435	0.264	0.360	-0.572	Higher	Population differentiation and genetic mapping
Min	1.000	1.000	0.000	0.000	0.000	0.000	-0.703		
Max	2.000	1.897	0.655	0.808	0.465	0.635	-0.198		
Grand Mean	1.586	1.403	0.326	0.338	0.219	0.284	-0.474		

Legend: Na - total the number of alleles; Ne - the number of effective alleles; I - the information index; Ho - observed heterozygosity; He - the average value of expected heterozygosity; uHe - the unbiased expected heterozygosity mean; F - the fixation index

Total number of alleles (Na) was 11, with values ranging from a minimum of 1 (Hi02d04) to a maximum of 2 (Hi04a05), with an average value of 1.586 alleles per marker. These values are comparable to those reported by Halász et al. (2009), where most loci exhibited 2 or 3 alleles. However, they differed significantly from the findings of Guney et al. (2019), who reported up to 10 alleles per marker. The lower allele count might be due to the genetic background of quince in the examined regions. Furthermore, Khoramdel Azad et al. (2013) recorded an average of 5.36 alleles, further supporting the notion that regions from which quince originates have greater genetic diversity of this pome species. The number of effective alleles (Ne) was the highest in marker Hi04a05 (Ne = 1.897), followed

by CN444542 ($N_e = 1.535$). The marker CN444542 ranked among the highest N_e values, which correlates with its effective allele count. The marker with the lowest N_e value was Hi02d04 ($N_e = 1.000$), while the average number of effective alleles for all SSR markers was 1.403, suggesting a relatively low level of effective allele diversity across the studied quince genotypes. The Information index (I), a measure of genetic diversity, was the highest for marker Hi04a05 ($I = 0.655$), while Hi02d04 had the lowest I value ($I = 0.000$). This result aligns with expectations, as Hi02d04 is homozygous and does not combine significantly to genetic variation. The observed heterozygosity (H_o) varied across markers, ranging from $H_o = 0.000$ (Hi02d04) to $H_o = 0.808$ (Hi04a05), showing substantial variability across SSR markers. This highlights Hi04a05 as an effective marker for distinguishing quince genotypes, making it a strong candidate for future genetic studies. The average observed heterozygosity was $H_o = 0.338$, slightly lower than the value reported by Guney et al. (2019), where H_o was 0.58 and significantly lower than $H_o = 0.77$ recorded by Khoramdel Azad et al. (2013). Notably, Hi02d04 exhibited the lowest H_o values in this study ($H_o = 0.000$), whereas Guney et al. (2019) recorded the highest H_o value ($H_o = 1.000$) for the same marker, possibly due to differences in genotypes or reaction conditions. The expected heterozygosity (H_e) averaged $H_e = 0.219$ with Hi04a05 having the highest H_e value ($H_e = 0.465$) and Hi02d04 the lowest ($H_e = 0.000$). The relatively low heterozygosity values suggest genetic similarity among genotypes, likely due to their common origin (G1–G11) and the generally narrow genetic base of quince in Serbia, which is believed to originate from a limited number of traditional cultivars. The mean unbiased expected heterozygosity (uH_e) was $uH_e = 0.284$, further supporting the findings of genetic similarity. Once again, Hi04a05 showed the highest uH_e value ($uH_e = 0.635$), while Hi02d04 had the lowest $uH_e = 0.000$, mirroring its trends in other parameters. The fixation index (F), which measures population genetic structure, had a mean value of -0.474 . The markers Hi04a05 and CN444542 showed the most negative F values (-0.703 and -0.572 , respectively), indicating an excess of heterozygosity, suggesting low inbreeding and good genetic diversity within these populations.

Allele frequencies were calculated, revealing variability within SSR loci, except for Hi02d04, which was homozygous and exhibited no allele diversity. The most frequent allele for each locus was identified as follows: Allele 215 in Hi01C11 ranged from 0.600 to 1.000, Allele 175 in Hi03a03 was the most represented with values from 0.500 to 1.000, Allele 230 in Hi04a05 varied between 0.500 and 0.833, Allele 250 in AJ000761b ranged from 0.500 to 1.000, Allele 215 in Hi04a08 had the highest frequency values (0.750 to 1.000), and Allele 160 in CN444542 exhibited a frequency between 0.500 and 1.000. The studied quince populations contained alleles with base pair sizes ranging from 155 bp to 285 bp, and each population possessed between five and nine different alleles in its genetic constitution. The lowest allele count (5 alleles) was observed in populations MR, L and K, indicating lower genetic diversity. Conversely, the highest allele diversity was recorded in STD (9 alleles) and SK (8 alleles) populations. This high allele count in STD is expected, as it represents standard varieties where breeding efforts have significantly enhanced genetic diversity. Additionally, SK's high polymorphism levels suggest its strong potential for breeding programs aimed at developing new quince varieties. Due to its rich genetic variation, SK could serve as an important genetic resource for enhancing traits such as fruit quality, disease resistance and adaptability in future quince breeding efforts (Figure 2).

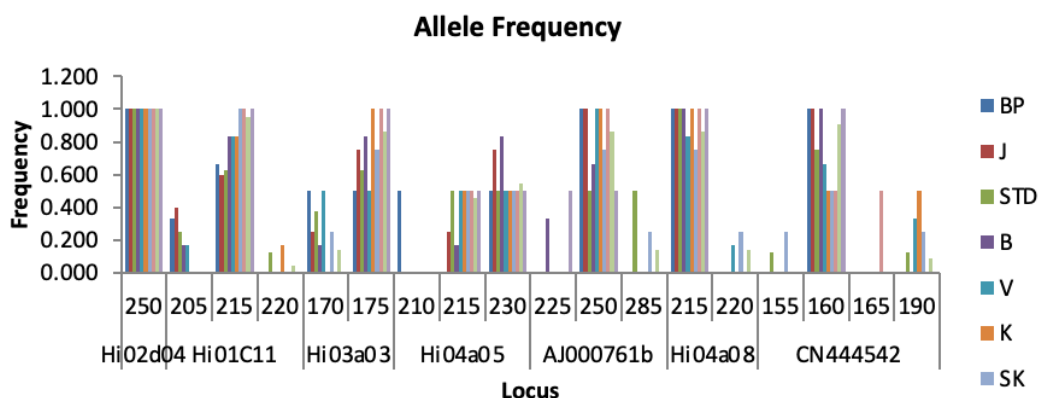


Figure 2. Allele frequency for all SSR markers and populations of 36 quince genotypes collected from Fruška Gora (Serbia) (BP – Bešenovački Prnjavor; J – Jazak; STD – standard varieties; B – Bukovac; V – Vrdnik; K – Krčedin; SK – Sremski Karlovci)

The graph generally confirms the findings of the SSR table. However, minor deviations were observed in markers Hi01c11, Hi04a05, AJ000761b and CN444542, where more alleles were observed than those reported in the calculation. Consequently, the total number of alleles increased from 11 to 14, suggesting hidden genetic variability within the studied quince population. One potential reason for this discrepancy is the influence of population genetic structure, where alleles with low frequencies or localized distribution may contribute to genetic diversity, but remain underrepresented in SSR diversity parameter calculations. These estimates are constrained by statistical thresholds, which often consider only the most prominent alleles per marker. Markers such as Hi04a05 and CN444542, which show three or four alleles in the graph, might be simplified to thresholds, meaning that if an allele appears below a certain percentage, it may not be included in N_a calculations despite being present in raw observations, leading to only two alleles in SSR calculations to align with population-wide diversity trends. For instance, certain alleles appear in only a few genotypes, making them detectable in direct counting but excluded from statistical diversity estimates due to their minimal impact on overall heterozygosity measures. In the case of Hi01c11, the presence of alleles 205 and 215 across localities is far more pronounced than allele 220, which appeared rarely, in only a few localities at low frequency. The same pattern was observed for Hi04a05, with the very rare allele 210 and predominant alleles 215 and 230. Allele 210 appears in only one population, reducing its impact on N_a and N_e calculations and potentially leading to its exclusion in SSR table estimates despite appearing in the graph. Conversely, allele 230 (Hi04a05) is present across all localities, with its frequency peaking in J and BP. While frequency spikes are evident in the graph, SSR calculations consider its overall balanced presence, explaining why N_e values remain moderate despite visual variations. Similar rare-allele patterns were observed in other markers where discrepancies arose between statistical SSR estimates and direct allele observations. The graph representation captures direct allele observations, including those appearing in only one or two populations. SSR estimation applies statistical calculations that may exclude rare alleles with minimal contribution to genetic diversity indices. This variation stems from methodological differences, including statistical allele filtering, frequency thresholds, experimental bias in genotyping efficiency and duplication effects across SSR loci. Understanding these factors highlights the importance of combining statistical SSR diversity parameters with direct allele visualization to improve genetic assessment and breeding strategies. Rare alleles may reflect local adaptation and could harbor a valuable variability for breeding (Figure 2, Table 3).

Genetic diversity and relatedness of the populations included in the study were further analyzed using Wright's fixation index (F_{ST}), which quantifies population differentiation due to genetic structure. By measuring genetic divergence between populations i.e., how much of the genetic variation is explained by population structure, the F_{ST} index provides a clearer insight into the degree of inbreeding within a subpopulation relative to the total population. If the F_{ST} value is greater than 0.15, the populations can be considered genetically distinct based on allelic frequency variation (Frankham et al., 2002). Analyzing the results, three populations appeared to be the most genetically distant from one another. Highly divergent populations were Bešenovački Prnjavor (BP) vs. Mala Remeta (MR) with $F_{ST} = 0.267$ (indicating strong genetic separation), BP vs. Ledinci (L) with $F_{ST} = 0.267$ (showing the same degree of differentiation as BP vs. MR) and BP vs. Krčedin (K) with $F_{ST} = 0.243$, showing significant divergence. A slightly higher F_{ST} value (0.291) was recorded by Barać (2016). These values suggest that BP has the highest genetic distinction across multiple comparisons. These findings suggest that certain populations maintain localized genetic traits, possibly due to selection pressures or environmental adaptation, indicating substantial differentiation that could be valuable for breeding purposes. The lowest F_{ST} values and thus the closest genetic similarity, was observed between the populations Sremski Karlovci (SK) and Grgurevci (G), where F_{ST} was 0.038. This indicates high genetic similarity, suggesting frequent gene flow and minimal population divergence between these two regions. Overall, these findings indicate diversity among the populations examined in this study, suggesting that they could be a promising gene pool for mining desirable genes and broadening genetic variability of the existing quince breeding programs (Figure 3).

BP	J	STD	A	V	K	SK	MR	G	L	
0.000										BP
0.083	0.000									J
0.133	0.108	0.000								STD
0.141	0.072	0.099	0.000							A
0.106	0.098	0.086	0.123	0.000						V
0.243	0.170	0.147	0.159	0.100	0.000					K
0.164	0.139	0.074	0.117	0.059	0.095	0.000				SK
0.267	0.198	0.179	0.168	0.137	0.097	0.114	0.000			MR
0.115	0.077	0.077	0.065	0.062	0.077	0.038	0.081	0.000		G
0.267	0.198	0.141	0.084	0.175	0.189	0.139	0.222	0.072	0.000	L

Figure 3. Fixation index (FST) values of the populations of 36 quince genotypes collected from Fruška Gora (Serbia) (BP – Bešenovački Prnjavor; J – Jazak; STD – standard varieties; V – Vrdnik; K – Krčedin; SK – Sremski Karlovci; MR – Mala Remeta; G – Grgurevci; L – Ledinci).

Interpretation of FST values: Low FST (0–0.05) – populations are genetically similar, Moderate FST (0.05–0.15) – some genetic differentiation exists, High FST (0.15–0.25) – significant genetic divergence, Very High FST (>0.25) – Populations are highly structured and isolated.

PCoA analysis

The Principal Coordinate Analysis (PCoA) successfully explained 74.60% of the total genetic variance among quince populations in this study. The application of Principal Coordinate Analysis (PCoA) in this study is fundamental to assessing the adaptive potential and genetic landscape of Serbian quince populations. PCoA is uniquely suited for this purpose as it translates complex, non-linear genetic distances into a multi-dimensional space. Coordinate 1 (PC1) accounted for 37.88% of the total variance, Coordinate 2 (PC2) for 23.21% and Coordinate 3 for 13.50%. The first two principal coordinates, which represent the majority of the variance, were used to construct the PCoA biplot, allowing visualization of genetic relationships among genotypes (Figure 4).

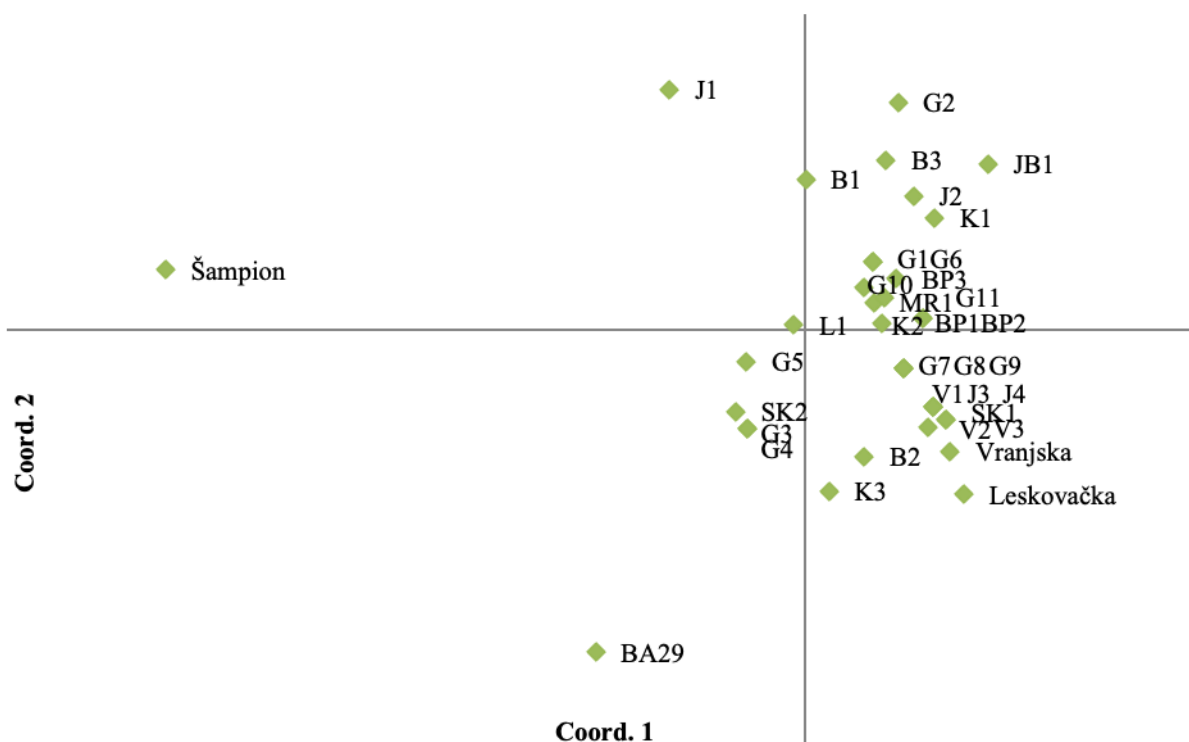


Figure 4. Principal Coordinate Analysis (PCoA) of genetic diversity among 36 quince populations collected from Fruška Gora (Serbia)

The Principal Coordinate Analysis (PCoA) explained 74.60% of the total variance among quince populations included in this study, providing insight into genetic diversity and population structuring. A similar approach was applied by [Coutinho et al. \(2019\)](#), who used multivariate principal component analysis (PCA) in combination with the unweighted pair group method with arithmetic means (UPGMA), based on Gower distance and Pearson correlation coefficients, to evaluate genetic divergence. PC1 (37.88%) accounted for the largest portion of genetic variance, followed by PC2 (23.21%) and PC3 (13.50%). Since PC1 and PC2 represent the majority of the variance, their graphical display in the PCoA biplot ([Figure 4](#)) reveals clustering patterns and genetic relationships among the analyzed genotypes. The application of PCoA is particularly important in assessing the adaptive potential of quince, as it enables visualization of genetic relationships and identification of genetically diverse genotypes that may serve as valuable resources for breeding and adaptation to changing environmental conditions. The sources of variation in PC1 (37.88%) primarily reflect major genetic differentiation driven by population structuring, historical cultivar lineage and broad genetic separation among highly distinct quince populations. PC2 (23.21%) likely captures regional environmental adaptations, influencing genetic divergence among local quince genotypes and reflecting mutation-driven diversity. PC3 (13.50%) contributes additional variation, though it does not significantly impact major genotypic clustering patterns, and therefore was omitted from further discussion. Eigenvalues represent the amount of total variance explained by each principal coordinate. Since PC1 (37.88%) and PC2 (23.21%) account for most of the variation, the spread of genotypes along these axes visually reflects their eigenvalue contributions. Genotypes located further from the center (the origin point) generally have higher eigenvalues, meaning they contribute more to the diversity structure observed in the analysis. Genotypes from all the populations included in this research formed four categories according to their positions in the biplot quadrants. Group 1 represents the largest and most diverse cluster. It consists of five Grgurevci genotypes (G1, G2, G6, G10, G11), two Bukovac genotypes (B1, B3), two Jazak genotypes (JB1, J2), two Krušedol genotypes (K1, K2), three Bešenovački Prnjavor genotypes (BP1, BP2, BP3) and one Mala Remeta genotype (MR1). BP1 and BP2 share identical eigenvalues, confirming clonal propagation, similarly to G1 and G6. Group 2 is the second largest cluster with strong synonymy. It includes Bukovac (B2), Krušedol (K3), Sremski Karlovci (SK1) and the standard varieties Vranjska and Leskovačka. This group also contains Jazak genotypes (J3, J4), three Grgurevci accessions (G7, G8, G9), and the entire Vrdnik population (V1, V2, V3). Strong synonymy was observed, with G7, G8 and G9 forming a clonal subgroup, as well as J3, J4 and V1. Similar findings were reported by [Xuan et al. \(2013\)](#), who identified identical values for ‘Seker Geurek’ and ‘Elemek’ cultivars. Group 3 is the smallest but genetically unique cluster, with significant differences observed among the genotypes. It includes Ledinci (L1), Jazak (J1) and the standard variety Šampion. Šampion displayed the highest negative eigenvalues, confirming its strong genetic distinction from other quince varieties. Group 4 appears to be a moderate cluster with high genetic similarity, composed of three Grgurevci accessions (G3, G4, G5), one Sremski Karlovci genotype (SK2) and the standard variety BA29. Genetic similarity within this group suggests shared ancestry or historical propagation through clonal selection. Highly divergent populations (BP × Jazak) may represent useful genetic resources for future breeding programs aimed at increasing variability; however, their practical value should be further evaluated based on pomological traits and agronomic performance. Genetically similar populations (SK × Grgurevci) maintain traditional quince traits while ensuring breeding stability. Clonal accessions (BP1–BP2, G1–G6, J3–J4–V1) provide insights into propagation methods and clonal selection strategies. Rare allele contributors and high PC1 influencers (Šampion, Ledinci) highlight potential breeding targets for variety improvement. Observing the genetic similarity between all analyzed accessions, it can be observed that most of them are genetically similar to the Vranjska and Leskovačka standard varieties and represent their clones or close descendants, which is expected considering the fact that these are old and long-cultivated Serbian varieties. However, some transitional genotypes, such as B1, G5, SK2, G3 and G4, could be observed at the borders of the groups. They might reflect recent mutational shifts that have not fully separated them from the main quince lineage but have made them genetically distinct enough to be noticeable in the biplot. SSR markers might reveal slight allele variations that are not fully expressed in core populations but appear frequently in transitional genotypes. Transitional genotypes bridge genetic gaps, making them excellent material for breeding programs aimed at broadening genetic diversity without disrupting key quince traits. These transitional genotypes may be products of localized ecological pressures that forced minor genetic differentiation while retaining core quince ancestry. If some accessions came from slightly different soil compositions, climate zones or elevation, small physiological or genetic differences associated with survival strategies could be expected. Furthermore, if these transitional genotypes show consistent trait expression patterns in further analysis, this could indicate the presence of regulatory alleles or small-effect mutations influencing quince characteristics. These findings establish a comprehensive framework for selecting quince genotypes based on genetic diversity and breeding optimization strategies ([Figure 4](#)).

Genetic relatedness analysis based on UPGMA cluster analysis

Cluster analysis using the UPGMA algorithm provided valuable insights into the genetic relationships among the 36 quince cultivars. The resulting dendrogram consists of three major clusters, where some genotypes are grouped according to their geographic locations, whereas many others show clustering that does not strictly align with their place of mapping. The observed genetic structure of the studied quince accessions is likely influenced by genetic lineage and historical propagation practices, while the effect of spatial distribution could not be clearly distinguished within the relatively small and geographically limited area of Fruška Gora. The first cluster, medium-sized compared to the remaining two, comprises genotypes from seven different populations, forming two subclusters. The larger subcluster includes accessions from Grgurevci (G3, G4 and G5), Sremski Karlovci (SK2), Ledinci (L1), Bukovac (B1) and two standard varieties (Šampion and BA29). Within this subcluster, genotypes G3 and G4 occupy identical positions, strongly indicating clonal propagation, a finding that should be further validated through allelic frequency analysis. Standard varieties Šampion and BA29, despite their geographical and historical differences, cluster closely together, suggesting possible shared breeding influences rather than direct genetic relatedness. Šampion, an old American variety created in the 1800s, and BA29, a widely used French rootstock from the 1900s (Kafkas et al., 2018), might exhibit convergent selection for specific quince traits, such as adaptability and resilience. The smaller subcluster within Cluster 1 consists of accessions from Krčedin (K2 and K3) along with one genotype from Mala Remeta (MR1). Although diverse, this subcluster does not show strong alignment with the broader quince lineage seen in traditional cultivars, indicating moderate genetic differentiation within local populations. The second cluster, the largest in the dendrogram, is structured into two subclusters containing ten accessions that branch into four closely related groups. The first subcluster includes Jazak quince genotypes (J3 and J4), positioned identically with accession V1 from Vrdnik, reinforcing high genetic similarity across distinct geographical locations. Similarly, the grouping of Bešenovački Prnjavor accessions (BP1 and BP2) with three Grgurevci accessions (G7, G8 and G9) confirms the presence of genetically identical cultivars mislabeled under different names, an issue requiring correction in germplasm collections. The second subcluster contains accessions from Sremski Karlovci (SK1) and Vrdnik (V3), both of which share identical eigenvalues, further validating their clonal status. The inclusion of another cultivar from Vrdnik (V2) and two standard varieties, Leskovačka and Vranjska, suggests that most genotypes in this cluster are genetically descended from these historically significant quince cultivars. Interestingly, Vranjska appears to exhibit greater genetic diversity compared to Leskovačka, aligning well with the findings of the PCoA analysis, where Vranjska played a crucial role in shaping quince lineage across multiple local populations. The third and smallest cluster contains genotypic misidentifications, with cultivars displaying identical placement across subclusters. Jazak accessions JB1 and J2 coincide within the first subcluster, along with J1 from the same population and Bukovac accession B3. While structurally grouped together, these accessions show higher genetic divergence than core cultivars, suggesting minor allelic variations that may affect adaptability traits. Genotypes K1 from Krčedin and G11 from Grgurevci appear more genetically distant than their respective population counterparts, possibly due to mutation-driven differentiation or historical introgression events. The second subcluster consists entirely of Grgurevci accessions positioned extremely close to one another, particularly G6 and G1, further reinforcing the high genetic relationship among individuals from this population. This clustering suggests either natural selection for distinct quince traits or historic propagation through regional cultivation practices. By comparing the dendrogram to the PCoA biplot, clear genetic relationships among quince genotypes can be observed, particularly in how the second cluster confirms the lineage continuity of Vranjska and Leskovačka, solidifying their status as foundational cultivars. However, while PCoA revealed transitional genotypes, UPGMA clustering provides greater clarity in detecting clonal duplicates and minor allele shifts, validating misidentified cultivars in germplasm collections. These findings support breeding strategies aimed at preserving core quince genetics while introducing structured diversity into new cultivar development. Further confirmation of genetic stability among hybrid crosses would require fixation index analysis, allele tracking through SSR markers, and longitudinal stability testing in controlled breeding trials (Figure 5).

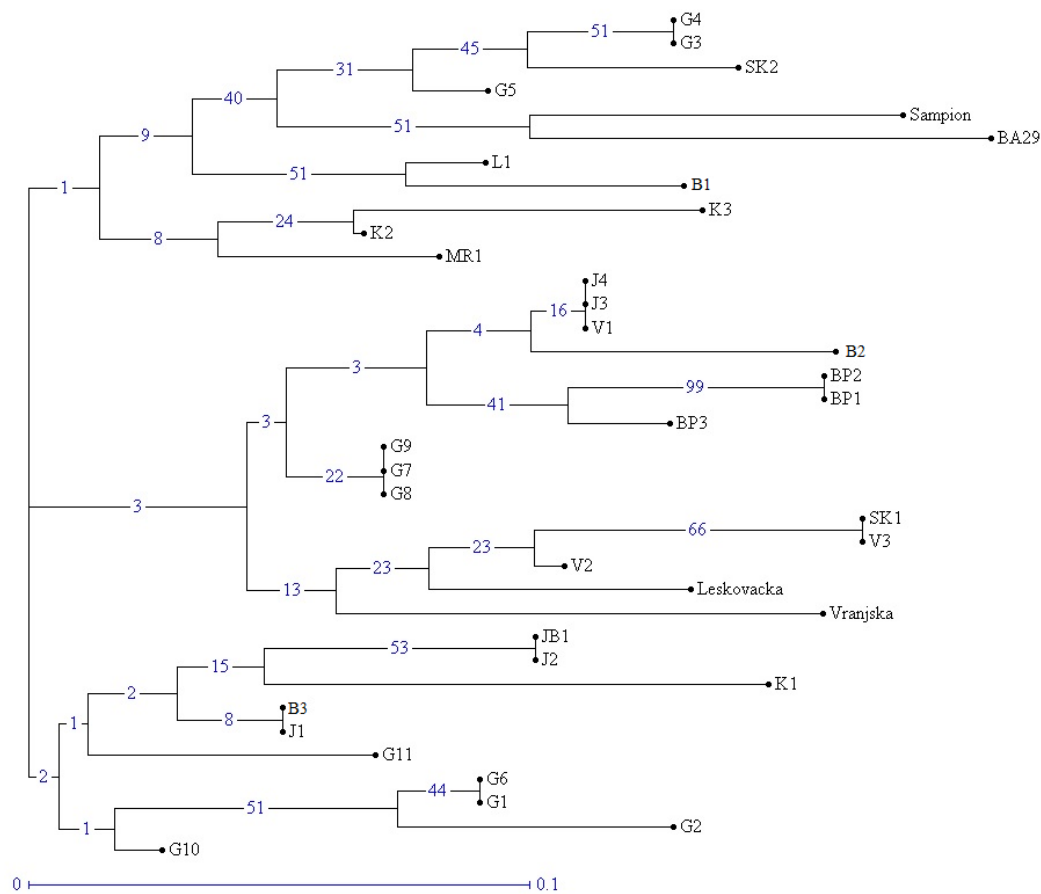


Figure 5. UPGMA cluster analysis of 36 quince cultivars collected from Fruška gora (Serbia)

CONCLUSION

This study underscores the significance of SSR markers in accurately evaluating the genetic diversity of quince cultivars, particularly considering their codominance, repeatability and polymorphism. Given the absence of quince-specific SSR markers, existing markers from apples and pears were successfully transferred. Among these, Hi04a05, CN444542 and Hi01c11 demonstrated strong transferability and reliable genetic parameter results, making them viable candidates for future quince polymorphism analyses. The genetic diversity parameters calculated provided a detailed view of quince population polymorphism, with the standard varieties population (STD) exhibiting the highest genetic variability, as expected due to prior breeding and selection efforts.

Several populations stood out due to their genetic distinctiveness, particularly Bešenovački Prnjavor (BP), Mala Remeta (MR) and Ledinci (L), which exhibited high genetic divergence compared to other populations. Insights from principal coordinate analysis (PCoA) aligned populations according to their genetic relationships, revealing that most accessions found in nature are descendants of two foundational quince varieties – Leskovacka and Vranjska – which originated in Serbia. The UPGMA dendrogram further reinforced this finding, highlighting the importance of these standard varieties as primary genetic sources for numerous quince populations. Additionally, the clustering pattern identified a substantial number of misidentified genotypes, confirming that many labeled accessions are actually genetic duplicates. This discovery is crucial for eliminating synonyms and improving the accuracy of germplasm collections, ensuring a more systematic approach to quince genetic conservation.

In conclusion, SSR markers have proven invaluable for quince genetic analysis, but the development of quince-specific SSR markers remains a priority to enhance precision in future studies. Serbia's quince populations exhibit remarkable genetic variability, necessitating further evaluation, preservation, classification and systematization. These efforts will not only safeguard quince genetic diversity but also provide essential resources for future breeding programs aimed at improving quince adaptability, disease resistance and fruit quality.

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