RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2024; 8(1): 88-93
DOI: https://doi.org/10.31015/jaefs.2024.1.10

Molecular characterization of Turkish hazelnut cultivars and genotypes using SSR markers

Muharrem YILMAZ[1] • **Ahsen Işık ÖZGÜVEN**[2] • **Yıldız AKA KAÇAR**[3]

[1] Department of Horticulture, Faculty of Agriculture, University of Ordu, 52200, Ordu, Türkiye
[2] Faculty of Agricultural Sciences and Technologies, Cyprus International University 99258 Nicosia, North Cyprus
[3] Department of Horticulture, Faculty of Agriculture, Çukurova University, Adana, Türkiye

Citation: Yilmaz, M., Ozguven, A.I., Aka Kacar, Y. (2024). Molecular characterization of Turkish hazelnut cultivars and genotypes using SSR markers. International Journal of Agriculture, Environment and Food Sciences, 8(1), 88-93

Received: *January 07, 2024*
Accepted: *March 04, 2024*
Published Online: *March 25, 2024*

Correspondence: Muharrem YILMAZ
E-mail: muharremyilmaz@gmail.com

Available online at
https://dergipark.org.tr/jaefs
https://jaefs.com/

Abstract
This study defines the genetic characterization of 16 hazelnut varieties and 64 genotypes. SSR method was used in molecular characterization studies. 18 SSR primers were used. In the SSR method, 118 bands were obtained, and 115 were polymorphic. The similarity coefficient in overall genotypes were between 0.12 and 0.98 in SSR a n d the polymorphism rate of the primer pairs used was calculated as 98.0%. The lowest polymorphism information content value (0.542) was obtained from the CAC- C 028 locus, and the highest polymorphism information content value (0.987) was obtained from the CAC- B 113 locus. The average polymorphism information content value was 0.786. The total discrimination power values of SSR loci were determined as 17.98; the lowest (0.748) discrimination power value was obtained from the CAC- B 020 locus, while the highest (1.404) discrimination power value was obtained from the CAC- A 024 b locus. The genotypes'clustering positions across the dendograms were essentially correlated with their geographic origins. This result shows that genetic origin plays an important role in terms of classification of genotypes in hazelnut.

Keywords: Hazelnut, molecular characterization, SSR

INTRODUCTION

Hazelnuts belong to the genus *Corylus* of the subfamily Coryleae of the family Betulaceae of the order Fagales in the systematics and grow in temperate regions of the northern hemisphere. *Corylus* species are diploid and have a chromosome number of 2n = 2x = 22. More than 25 species have been described within the genus Corylus. Among these species, *C. avellana*, *C. colurna* and *C. maxima* Mill are commercially important (Özbek, 1978). Turkish hazelnut varieties are hybrids of *C. avellana* and *C. maxima* (Okay et al., 1986). It has been reported that Central Asia, Caucasus, and Anatolia are the homelands of hazelnut, and hazelnut was first cultivated in Anatolia in the Eastern Black Sea Region (Islam, 2000). Hazelnut plantations are found in the Black Sea and Marmara Regions of Turkey, in Tokat, Adana, and Mersin provinces, and in the Vangölü Basin, which includes Bitlis province (Islam and Özgüven, 1997). Turkey is among the richest gene sources of cultivated hazelnuts and has rich genetic material due to thousands of years of cultivation culture. Wild hazelnuts, which are formed as a result of natural hybridizations and are still found in the gardens of producers, increase this variation even more. Turkey has natural populations that are very important in breeding among hazelnut-growing countries. In Turkey, 20 standard hazelnut cultivars have been identified as 'Tombul', 'Palaz', 'Çakıldak', 'Foşa', 'Mincane', 'Uzunmusa', 'Cavcava', 'Kargalak', 'Kan', 'Kalınkara', 'İncekara', 'Sivri', 'Karafındık', 'Yassı Badem' and 'Yuvarlak Badem', Girusun Melezi, Okay 28, Allahverdi, Yomrali and Çetiner (Ayfer et al., 1986; Köksal, 2002; HRI,2024). It is important that the genetic material used in plant breeding and variety development studies is correct to its name and that the genetic relationships between them are determined. To obtain

patent rights for new varieties and to have the sanctioning power of the laws, methods that can distinguish varieties from each other precisely should be used. Developments in molecular markers provide great convenience in this regard. Investigation of hazelnut gene resources using molecular markers, identification of species and cultivars, and studying of inheritance markers provide the emergence of agriculturally important characters. SSR or microsatellites consist of 2-6 nucleotide groups distributed throughout eukaryotic genomes and repeated consecutively. The DNA sequences surrounding microsatellites are usually conserved among individuals of the same species, allowing the selection of overlapping SSRs in different genotypes by amplification with PCR primers. The difference in the number of consecutive SSR repeats results in PCR amplification of fragments of different lengths. These repeats are highly polymorphic due to mutations that lead to changes in the number of repeated units, even for very close species and varieties. Different alleles at a locus can be detected by PCR using the conserved DNA sequences surrounding SSRs as primers. Since SSRs are highly polymorphic, they are highly informative in plants. SSRs are very abundant in plant genomes and have a uniform distribution. Due to these advantages, using the SSR technique in genetic mapping studies in plants is increasing daily (Özcan et al., 2001). In this study, it was aimed to identify the molecular characteristics of hazelnut varieties cultivated in Turkey, clones of some important varieties, and hazelnut types cultivated in the Vangölü basin by the SSR marker method.

MATERIALS AND METHODS

Plant materials

In this study, Turkish hazelnut varieties in the genetic resources orchard of Giresun Hazelnut Research Institute, Tombul, Palaz, Foşa, Çakıldak, Mincane, Cavcava, Uzunmusa, Sivri, Yassı Badem, Yuvarlak Badem, Kargalak, Kalınkara, İncekara, Kuş, Acı, Kan, varieties and 45 hazelnut genotypes in the same institute, which were determined by selection studies carried out in previous years, and in addition to these, 17 genotypes selected from the Vangölü basin and 2 genotypes taken from Çorum province were used. This study was conducted between 2005-2008. In addition, DNAs of 4 types of *C. colurna* species in Giresun Hazelnut Research Institute were also used. The tag numbers of the genotypes taken from the genetic resources parcel of Giresun Hazelnut Research Institute were used exactly based on the numbers in the institute records. SSR analyses were conducted in the Biotechnology laboratory of Çukurova University, Faculty of Agriculture, Department of Horticulture.

Microsatellite (SSR) Analyzes

Newly blooming young leaves of the cultivars were used for DNA isolation. The leaf samples were washed with 50% alcohol, dried, and placed in liquid nitrogen (-196 ºC), and kept at -80 ºC until DNA isolation. The "minipreparation" DNA isolation method was modified by Doyle and Doyle (1990). The primers used in SSR analysis were selected by Mehlenbacher et al. (2006) by considering the primers used for hazelnuts in different studies which gave positive results in these studies. The 18 SSR primers (Operon Technologies) were labeled with a fluorescent label for use in the Li-Cor imaging system. SSR analyses were performed according to the method modified by Kaçar (2001). The total volume was adjusted to 20 µl for each sample and consisted of 8 µl 2XPCR Master Mix (Fermantas), 1 µl Primer (forward + reverse), 0.5 µl $MgCl_2$ (25 mM), 0.5 µl M13 primer, 0.05 µl Taq DNA Polymerase, 5 µl ddH_2O, 5 µl genomic DNA (5ng). PCR cycling conditions were applied, as shown in Table 1.

Table 1. PCR Cycle Conditions in SSR Analysis

Process	Temperature (ºC)	Duration	Cycle
Pre-Denaturation	94ºC	5 min	1
Denaturation	94ºC	1 min	
Annealing	55-60ºC	30 sn	35
Elongation	72ºC	1 min	
Final elongation	72ºC	4 min	1

Polymorphism rates of the primers were calculated by dividing the number of polymorphic bands by the total number of bands and multiplying by 100. The polymorphism information content (PII) of the SSR primers used was determined using the formula "PII = 1 - Σ Pi2" according to Smith et al. (1997). According to this method, the total number of present (1) and absent (0) bands in polymorphic bands was determined and frequency values were calculated for each of these bands (Pi: frequency of band i). The discrimination power of SSR primers was calculated according to the formula developed by Prevost and Wilkinson (1999); discrimination power = Σ Ib (Ib = 1 - (2 X $\left|0.5 - p\right|$)). p in the formula is the ratio of I band in the total genotype.

The PCR products obtained were electrophoresed in polyacrylamide gel using a Li-Cor imaging system, and band images were obtained. For this purpose, 6.5% polyacrylamide gel was prepared. The amplification products obtained from PCR cycles were separated by agarose gel electrophoresis. The bands formed were evaluated as present (1) or

absent (0). The numerical information obtained was analyzed using the computer program NTSYSpc 2.0V (Numerical Taxonomy and Multivarious Analysis System, Version 2.0V) (Rohlf, 2004). The genetic similarity index between genotypes was calculated using the Jaccard coefficient, and pedigree analysis was obtained using the UPGMA method.

RESULTS AND DISCUSSION

Total allele numbers, polymorphic allele numbers, polymorphism rate, polymorphism information content and discrimination power values obtained as a result of PCR and electrophoresis applications are presented in Table 2. A total of 118 alleles were obtained from 18 SSR loci, and 115 were polymorphic. The total number of alleles per primer varied between 2 and 11 (mean 6.55). The number of polymorphic bands per locus varied between 2 and 11 (mean 6.38). The differences in the number of alleles are due to the different genotypes used in the study. Regarding the total number of alleles, the CAC- B 110 locus produced the least number of alleles (2), and the CAC- A 014 b locus produced the highest number of alleles (11). The polymorphism rate of the primer pairs used was determined as 98.0%. The lowest (0.542) polymorphism information content value was obtained from CAC- C 028 locus and the highest (0.987) polymorphism information content value was obtained from CAC- B 113 locus. The average polymorphism information content value was 0.786. Total discrimination power values of SSR loci were determined as 17.98; the lowest (0.748) discrimination power value was obtained from CAC- B 020 locus, whereas the highest (1.404) discrimination power value was obtained from CAC- A 024 b locus. The gel image obtained as a result of PCR amplification of CAC- B 109 primer with 84 hazelnut genotypes is presented in Figure 1. In this image, 9 out of 10 alleles were polymorphic. Bassil et al. (2003) tested 12 SSR primers in a study with 19 hazelnut genotypes and determined that the number of alleles per microsatellite locus varied between 4 and 7 and the heterozygosity ratio varied between 0.58 and 0.87. Boccacci et al. (2006), using 78 hazelnut cultivars and 16 SSR loci, found that the number of alleles per locus was 9.4, with high allele frequencies up to 78% and a discrimination power of 91%. Gökırmak et al. (2008) used 21 SSR primers to analyze the genetic profiles of 270 clones of *C. avellena* species. It was reported that the average number of alleles per locus was 9.81 and heterozygosity rate was 0.67 in 198 clones showing different genetic profiles. In a study conducted by Yang et al. (2021) in China, 27 SSR primers were used to identify 57 Ping'ou hazelnut hybrids. The results showed heterozygosity (Ho) of 0.84, expected heterozygosity (He) of 0.80, and polymorphism information content (PIC) of 0.78. A total of 301 alleles were detected and the number of effective alleles ranged from 11.2 to 18 alleles per locus on average. Polymorphism rates of the loci used in the study were higher than in previous studies. The presence of *C. avellana* and *C. colurna* species among the genotypes used in the study can be considered among the reasons for the high polymorphism rates.

Table 2. Total band numbers, polymorphic band numbers, polymorphism rate, polymorphism information content and discrimination power values obtained from SSR primers

No	Primer	Total band	Polymorphic band	Polymorphism rate (%)	Polymorphism information content	Discrimination power values
1	CAC- A 014 a	10	9	90	0.742	1.154
2	CAC- A 014 b	11	11	100	0.724	0.845
3	CAC- A 024 b	5	5	100	0.671	1.404
4	CAC- A 36	5	5	100	0.784	0.822
5	CAC- A 105	7	6	86	0.741	1.451
6	CAC- B 001	6	6	100	0.776	1.282
7	CAC- B 020	8	8	100	0.674	0.748
8	CAC- B 028	7	7	100	0.747	0.815
9	CAC- B 029 b	10	10	100	0.771	0.774
10	CAC- B 109	10	9	90	0.582	0.825
11	CAC- B 110	2	2	100	0.728	0.852
12	CAC- B 111	5	5	100	0.787	0.958
13	CAC- B 113	8	8	100	0.987	0.752
14	CAC- B 114	5	5	100	0.784	0.958
15	CAC- C 003	5	5	100	0.775	1.225
16	CAC- C 111	2	2	100	0.672	0.958
17	CAC- C 118	6	6	100	0.714	1.242
18	CAC- C 028	6	6	100	0.542	0.817
	Total	118	115	--	--	17.98
	Average	6.55	6.38	98	0.786	

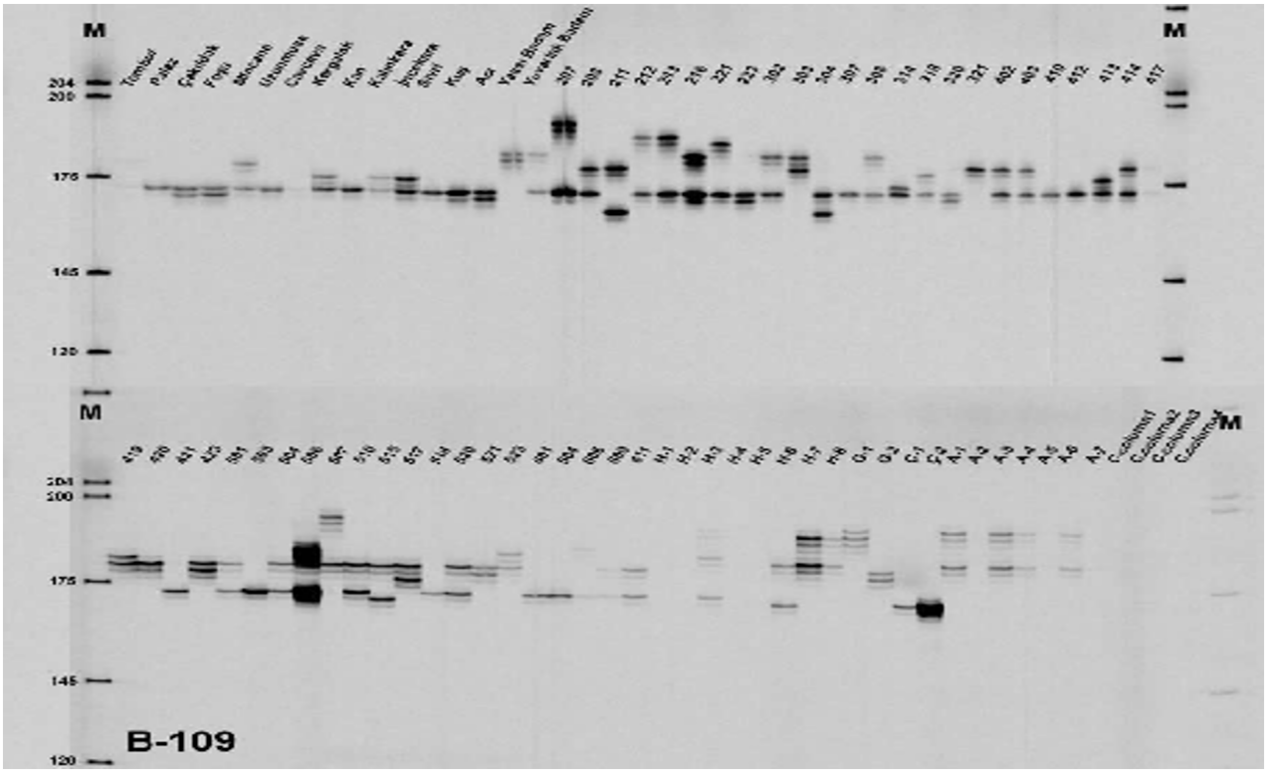

Figure 1. Vertical electrophoresis gel image of SSR primer CAC-B 109

In the dendrogram created as a result of SSR analyses, the similarity ratio for all genotypes ranged between 0.250 and 0.983. This dendrogram obtained on the basis of SSR analyses was first divided into two main branches numbered 1 and 2 (Figure 2). In the main branch numbered 1, four genotypes belonging to *C. colurna* species were included. *C. colurna* species were found to be 25% close to and approximately 75% distant from all other genotypes used in the study. *C. colurna* types formed two branches among themselves and the similarity ratio between these branches was 0.580. According to SSR results, this species, which has pomologically small fruits and different morphological characteristics, also differed genetically from the other hazelnut genotypes in the study. The main branch numbered as 2 on the dendrogram was again divided into 2 subgroups. These two subgroups were numbered 2.1 and 2.2 on the dendrogram. These two subgroups were genetically close to each other by about 28%. Genotypes Ç1 and Ç2 were included in the subgroup numbered 2.1. According to the SSR results, the similarity index between the genotypes Ç1 and Ç2 taken from Çorum province within the scope of the experiment and the *C. colurna* genotypes examined in the SSR analysis was determined as 0.250. The similarity index of Ç1 and Ç2 genotypes with genotypes other than *C. colurna* types was 0.280. The subgroup numbered 2.2 on the dendrogram was divided into two branches. These two branches are numbered 2.2.1 and 2.2.2 on the dendrogram. These two branches were found to be 42% close to each other. Among the hazelnut genotypes taken from Vangölü Basin, all genotypes except G-2 were collected in the branch numbered 2.2.1 on the dendrogram. It is noteworthy that the Cavcava variety is also located in this branch. Within the scope of the study, 15 standard varieties and 46 genotypes were clustered in the group numbered 2.2.2. Fiore et al. (2022), in their study conducted with 9 SSR primers in 75 hazelnut genotypes selected from the Sicily region.Authors reported that they distinguished between local genotypes and commercial varieties and that the Nebrodi genotypes examined showed significant differences from Northern Italian, Iberian, and Turkish genotypes.

CONCLUSION

Standard Turkish hazelnut varieties, genotypes from the genetic resources parcel of Giresun Hazelnut Research Institute, and hazelnut genotypes from Vangölü Basin were separated from each other on the pedigree tree obtained by molecular methods. This result shows that genetic origin plays an important role in the classification of genotypes. It has been reported that in the Black Sea region where hazelnut is intensively cultivated in our country, our important standard varieties have synonyms. This is an important source of confusion for researchers in a variety of identification studies. Using the primer and molecular techniques used in this study, similarity, and dissimilarity rates between the varieties and genotypes reported to be synonymous can be determined.

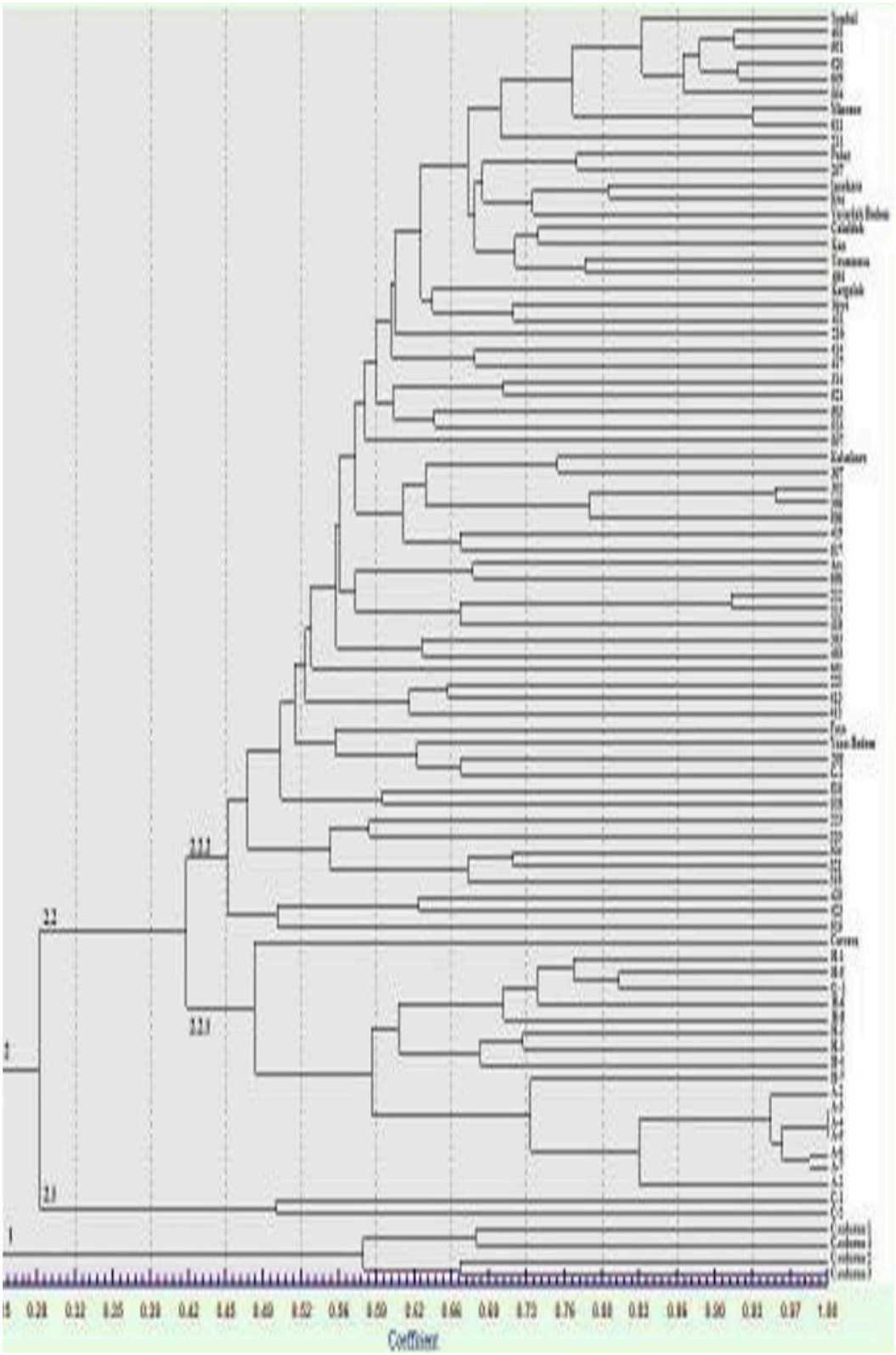

Figure 2. Pedigree obtained according to SSR analysis of hazelnut genotypes

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review
Externally peer-reviewed.

Conflict of interest
The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution
The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original and that they have not been published before.

Ethics committee approval
Ethics committee approval is not required.

Funding
No financial support was received for this study.

Data availability
Not applicable.

Consent to participate
Not applicable.

Consent for publication
Not applicable.

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