Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 30 (2), 399-409
Mustafa Kemal University Journal of Agricultural Sciences, 30 (2), 399-409
https://dergipark.org.tr/tr/pub/mkutbd
e-ISSN: 2667-7733

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Detection of genetic diversity of Turkish pistachio (*Pistacia vera* L.) cultivars using SSR markers

Türk antepfıstığı (*Pistacia vera* L.) şeşitlerinin SSR markörleri kullanılarak genetik çeşitliliğinin belirlenmesi

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ARTICLE INFO

Article history:
Recieved / Geliş: 20.12.2024
Accepted / Kabul: 04.04.2025

Keywords:
Pistachio
P. vera
SSR markers
Ptms
Genetic diversity

Anahtar Kelimeler:
Antepfıstığı
P. vera
SSR markörler
Ptms
Genetik çeşitlilik

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ABSTRACT

The Southeastern Anatolian region of Türkiye contributes to nearly a quarter of the global pistachio production. This area also contains a diverse pistachio germplasm. In this study, the genetic diversity of four long-type standard local pistachio cultivars “Siirt”, “Uzun”, “Kırmızı” and “Akıncı” was investigated using 7 SSR markers. The seven SSR markers (Ptms-3, Ptms-9, Ptms-14, Ptms-31, Ptms-33, Ptms-40, and Ptms-41) used in the analyses revealed a total of seventeen polymorphic alleles and six of them (35.3 %) were identified between 2 to 3 (with an average of 2.42). Pairwise genetic similarity coefficients varied from 64 % to 79 %. Genetic similarity between the “Siirt” and “Akıncı” (78.6 %), “Siirt” and “Uzun”; “Kırmızı” and “Akıncı” cultivars (78.0 %), “Akıncı” and “Uzun” (71.4 %), “Siirt” and “Kırmızı”, (64.3 %) and “Kırmızı” and “Uzun” (64.0 %) were calculated. The average values of expexted (He) and observed (Ho) were calculated as 0.450 and 0.286, respectively.

ÖZET

Türkiye’nin Güneydoğu Anadolu Bölgesi dünya antepfıstığı üretiminin yaklaşık dörtte birine katkıda bulunmaktadır. Bu alan aynı zamanda çeşitli fıstık genetik materyallerini de içermektedir. Bu çalışmada *P. vera* türüne ait “Siirt”, “Uzun”, “Kırmızı” ve “Akıncı dört uzun meyve şekline sahip standart yerli antepfıstığı çeşitlerinin genetik çeşitliliği 7 SSR markörü kullanılarak araştırılmıştır. Analizlerde kullanılan yedi SSR markör (Ptms-3, Ptms-9, Ptms-14, Ptms-31, Ptms-33, Ptms-40 ve Ptms-41) toplam on yedi polimorfik alel ortaya çıkarmış ve bunlardan altısı (Çeşitler arasında % 35.3) polimorfik bulunmuştur. Polimorfik alellerin aralığı 2 ile 3 arasında belirlenmiştir (ortalama 2.42). Genetik benzerlik oranları (Pairwise) % 64 ile % 79 aralığında değişmiştir. “Siirt” ile “Akıncı” çeşitleri arasında genetik benzerlik oranı % 78.6, “Siirt” ile “Uzun” ve “Kırmızı” ile “Akıncı” çeşitleri arasında genetik benzerlik oranı % 78.0, “Akıncı” ile “Uzun” çeşitleri arasında genetik benzerlik oranı % 71.4, “Siirt” ile “Kırmızı” çeşitleri arasında genetik benzerlik oranı % 64.3, “Kırmızı” ile “Uzun” çeşitleri arasındaki genetik benzerlik oranı ise % 64.0 olarak gözlemlenmiştir. Ortalama beklenen ve gözlenen heterozigotluk değerleri sırasıyla 0.450 ve 0.286 olarak hesaplanmıştır.

Cite/Atıf Özdemir, B. (2025). Detection of genetic diversity of Turkish pistachio (*Pistacia vera* L.) cultivars using SSR markers. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 30* (2), 399-409. https://doi.org/10.37908/mkutbd.1604635

INTRODUCTION

Pistachio, called green gold, is cultivated in suitable microclimates between 30° and 45° south and north parallels. According to Vavilov's determination, the primary gene center of pistachio is the Central Asian Gene Center, which includes the north of India, Afghanistan, and Tajikistan (Anonymous, 1993). *Pistacia vera* L. stands as one of the earliest cultivated nut crops in the annals of human history and is the only comestible and commercially notable *Pistacia* species (Kafkas & Perl-Treves, 2002; Kafkas, 2006).

Pistachios, like other nuts, are high in fat, predominantly mono- and polyunsaturated fatty acids and great source of protein, fiber, and mineral composition (potassium, phosphorus, calcium, magnesium, iron, zinc, and copper). Total fat amounts were examined between 50.77 % and 58.31 % (Çınar, 2012). Saturated fatty acid composition of all examined cultivars consisted of myristic, palmitic, margaric, stearic, arachidic, behenic, and lignoceric acid. Unsaturated fatty acid composition comprised palmitoleic, margaoleic, oleic, linoleic, linolenic, and gadoleic acid (Çınar, 2012; Mandalari et al., 2022).

Total world production of *P. vera* has reached 1,026,802 tonnes in 2022 and the USA, Iran, and Türkiye are the main countries, respectively. Approximately 95 % of Türkiye's production comes from the Southeastern Anatolia region (Anonymous, 2022), providing almost a quarter of the world pistachio production (228,283 tonnes). The *P. vera* species was cultivated and cultured in Türkiye. To date, the cultivars within this species obtained have mostly emerged through natural selection. Pistachio cultivar breeding studies through hybridization are quite limited (Khodaeiaminjan, 2017).

Pistachio cultivars commonly are cultivated in Türkiye are "Siirt", "Uzun", "Kırmızı", "Halebi" and "Ohadi" cultivars. Although the long-type standard local pistachio cultivars ("Uzun", "Kırmızı" and "Halebi"), which are mostly cultivated commercially in Türkiye, are delicious and have green kernel, they have disadvantages with their unpretentious nuts, low splitting rates and tendency to alternate bearing. On the other hand, cultivars with large nuts and round grains with high splitting rates are weak in terms of green inner color, good taste, and aroma. While the pistachio cultivars are grown in Türkiye, which mostly have a green interior, and appeal to the cake, ice cream, and dessert industry, the pistachio cultivars ("Siirt" and "Ohadi") in the round kernel group are mostly used as snacks. Considering these aspects of pistachio, it is necessary to develop cultivars and rootstocks that are suitable for domestic and foreign market purposes and demands, are also suitable for processing, have low tendency to alternate bearing, and give high yield (Anonymous, 1993).

Pistachio breeding programs encounter several challenges since it is a dioecious species and its juvenility period is quite long. In this prospect, molecular markers could facilitate breeding processes and allow early selection in seedlings, saving time and economic resources (Vendramin et al., 2009; Alhajjar & Muzher, 2019). To date, genetic diversity in pistachio has been analyzed via various methods related to the plant's physical characteristics, functions, and biochemistry. Isozymes were firstly utilized to differentiate between *P. vera* species and cultivars (Barone et al., 1993). The initial investigation of pistachio DNAs was conducted using the RAPD technique (Hormaza et al., 1994). RAPD, ISSR, AFLP and SSR analysis techniques have been widely employed for various purposes such as identifying different cultivars, developing markers linked to sex, studying genetic diversity, genetic mapping, fingerprinting, examining phylogenetic relationships, and characterizing germplasm in *Pistacia* species. SSR, a PCR-based technique, is widely preferred by geneticists and breeders for characterizing cultivars and genotypes. Because it has codominant inheritance, widespread presence along with the genome, high polymorphism and reproducibility, as well as a high transferability rate (Kafkas, 2019).

In this investigation, SSR analyses were performed using four *P. vera* cultivars, and the genetic relationship between these commercial cultivars was investigated with 7 SSR markers and determined the level of genetic variability of local Turkish pistachio cultivars (*P. vera* L.) in UPGMA analysis by the SSR markers.

MATERIALS and METHODS

Plant material

Young leaves of four *P. vera* cultivars ("Siirt", "Uzun", "Kırmızı" and "Akıncı") were used as plant material and sampled from the collection parcel located in the Pistachio Research Institute (Gaziantep, Türkiye). Leaf samples were gathered from healthy growing trees of the cultivars and brought to Ankara University Biotechnology Institute where the experiment took place without losing their moisture and kept at -80 °C.

DNA exraction

DNAs were isolated from recently collected, young leaves employing the methodology established by Lefort et al. (1998). The quality of the DNA was evaluated through electrophoresis on 1% w/v agarose gels and spectrophotometric (NanoDrop Technologies, Wilmington, DE, USA) analysis at 260 nm. Isolated DNA samples were stored at -20 °C until PCR reactions were performed.

PCR reactions and capillary electrophoresis

A total of 7 SSR markers namely Ptms-3, Ptms-9, Ptms-14, Ptms-31, Ptms-33, Ptms-40, and Ptms-41 (Ahmad et al., 2003; Vendramin et al., 2009; Baghizadeh et al., 2010; Alhajjar & Muzher, 2019; Choulak et al., 2019) were used for genetic analysis. Information of the 7 SSR markers utilized (marker name, marker sequence, annealing Tm (C°), repeat motif) is given in Table 1.

Table 1. SSR marker information
Çizelge 1. SSR markör bilgisi

SSR Marker	Marker sequence (5'….3')	Annealing Tm (C°)	Repeat motif
Ptms-3	**F*:**TGATGAACAAGTCCAAAAGGG	55	(CA)16
	R:AAAACAGCACAGCATGCATC		
Ptms-9	**F*:**TTGACCGTGGACTTGAAGC	55	(CA)7
	R:AACCTCCTCTCTTCTCTTTGCC		
Ptms-14	**F*:**GGGAAACACAAACATGCAAA	55	(CA)46
	R:GGCCTCTGGAGAACATGGTA		
Ptms-31	**F*:**CTGATCATGGGGCCTTTG	60	(CT)20
	R:GGAAGCACACACATGCAAAC		
Ptms-33	**F*:**TTCTGCTGGTCATGGGGC	55	(CA)12
	R:TGCCATTTAACCCAAAGGAG		
Ptms-40	**F*:**CAGCTCTCACTGATCCGATTC	55	(CTT)4
	R:TTCGAAAGCCAGTCTCAGGT		
Ptms-41	**F*:**AGAAGAGGGGAACAGGGAGA	55	(CT)11
	R:CTGAGGACTGGGCAGAATGT		

* fluorescent-labeled.

PCR optimization reactions were utilized as indicated by Yılmaz et al. (2020). According to procedures, fluorescent-labeled D2 (black), D3 (green), and D4 (blue) and forward markers belonging to each SSR marker were used. The presence of possible contamination in PCR reactions was checked using a negative control. In the negative control

sample sterile distilled water was used instead of DNA and possible amplification after PCR was checked by agarose gel electrophoresis.The samples underwent PCR with the following conditions: 3 min. at 94 °C, followed by 35 cycles of 1 min. at 94 °C, 1 min. at 48-66 °C (depending on marker binding), and 2 min. at 72 °C, 10 min. at 72 °C with a total of 35 cycles. The PCR steps were completed for the SSR marker products, followed by 2 % agarose gel electrophoresis. PCR products were then compared to a 100-bp DNA ladder (Invitrogen, Carlsbad, CA, USA) and were stored at -20 °C until the capillary electrophoresis step. Sample loading solution (SLS, Beckman Coulter, Fullerton, CA, USA) was used for PCR products diluting, Genomelab DNA Size-Standard Kit 400 (0.4 µL, Beckman Coulter, Fullerton, CA, USA) was added to the mixture and capillary electrophoresis was performed with the CEQ 8800XL capillary DNA analysis system (Beckman Coulter). It was used to determine peak sizes (bp) using the Beckman Coulter'system's fragment analysis specific software. The reactions were conducted at least twice to maintain the correctness of the data. Following capillary electrophoresis, PCR fragments were identified as heterozygous or homozygous based on the types and fluorescent-labeled colors of each SSR marker peak.

Genetic analyses

In this study, number of alleles (Na), allele frequency (alf), expected (He) and observed heterozygosities (Ho), and probability of identity (PI) were calculated for each marker using the program "IDENTITY 1.0" (Wagner & Sefc, 1999) according to Paetkau et al. (1995). Proportion of shared alleles was calculated by using ps (option 1-(ps) (Bowcock et al., 1994) as genetic dissimilarity in the Microsat (version 1.5) program (Minch et al., 1995). All observed data were converted to a similarity matrix and a genetic similarity dendrogram was constructed with UPGMA (unweighted pair-group method with arithmetic mean) (Sneath & Sokal, 1973), using the software NTSYS-pc (Numerical Taxonomy and Multiware Analysis System) (version 2.0) (Rohlf, 1988).

RESULTS and DISCUSSIONS

SSR analyses

A total of 17 alleles were generated across all studied cultivars. The number of alleles per marker varied from one (1) for marker Ptms-3 to three (3) for Ptms-14, Ptms-31, Ptms-40, and Ptms-41, yielding an average value of 2.42. While Ptms-3 was detected as monomorphic, all the other used markers revealed different levels of polymorphisms. A heterozygote excess was recorded for all Ptms markers except Ptms-3. Ptms-31 created the maximum expected heterozygosity value. The observed heterozygosity (Ho) ranged from 0 to 1 and the mean Ho was 0.286. The expected heterozygosity (He) value varied from 0 to 0.593 (Ptms-31), with an average value of 0.450. To assess the effectiveness of SSR markers for detecting polymorphism, Ho and He were calculated. Ho value was calculated in 2 of 7 SSR markers, as 0.75 (Table 2).

The lowest number of alleles (Na) was 1, detected in Ptms-3 while the highest number of alleles (Na) was 3 in the Ptms-14, Ptms-31, Ptms-40, and Ptms-41 markers. The lowest PI value of 0.208 was determined in Ptms-41 with the highest discrimination power (Table 2). Allele size for each SSR marker for four Turkish pistachio cultivars was deteched similar with Table 1 (data not shown).

It has been observed that the allele frequencies (alf) of the 6 SSR markers (except Ptsm-3) are not homogeneous (Table 3). Alleles with the highest allele frequency of the SSR marker (except Ptsm-3) were determined as follows: allele 128 (alf: 0.625) at Ptms-3, allele 100 (alf: 0.625) at Ptms-14, allele 202 (alf: 0.625) at Ptms-40, allele 236 (alf: 0.625) at Ptms-41 (Table 3).

Table 2. Genetic parameters of Gaziantep region pistachio cultivars: allele number (Na), He, Ho, and identity probability value (PI)

Çizelge 2. Gaziantep bölgesi antepfıstığı çeşitlerinin genetik parametreleri: alel sayısı (Na), He, Ho ve tanımlama olasılık değeri (PI)

No	**SSR markers**	***Na***	***He***	***Ho***	***PI***
1	Ptms-3	1	0.000	0.000	1.000
2	Ptms-9	2	0.468	0.750	0.611
3	Ptms-14	3	0.531	0.500	0.408
4	Ptms-31	3	0.593	1.000	0.412
5	Ptms-33	2	0.500	1.000	0.625
6	Ptms-40	3	0.531	0.750	0.408
7	Ptms-41	3	0.532	0.740	0.208
Total		17	-	-	-
Average		2.42	0.450	0.286	**0.524**

Table 3. Allele frequencies of 7 SSR markers (N: number, alf: allel frequency)

Çizelge 3. 7 SSR markörlerinin allel frekansları

N	**Ptms-3**	***alf***	**Ptms-9**	***alf***	**Ptms-14**	***alf***	**Ptms-31**	***alf***	**Ptms-33**	***alf***	**Ptms-40**	***alf***	**Ptms-41**	***alf***
1	144	1.000	94	0.375	108	0.125	84	0.500	163	0.500	148	0.250	222	0.125
2			128	0.625	110	0.625	124	0.125	173	0.500	156	0.125	232	0.250
3					126	0.250	130	0.375			202	0.625	236	0.625

Genetic similarity

In all studied *P. vera* cultivars, genetic similarity ranged from 64 % to 79 %. Genetic similarity between "Siirt" and "Akıncı" was found 78.6 %; "Siirt" and "Uzun" was 78.0 %; "Kırmızı" and "Akıncı" was 78.0 %; "Akıncı" and "Uzun" was 71.4 %; "Siirt" and "Kırmızı" was 64.3 %, "Kırmızı" and "Uzun" was 64.0 % (Figure 1).

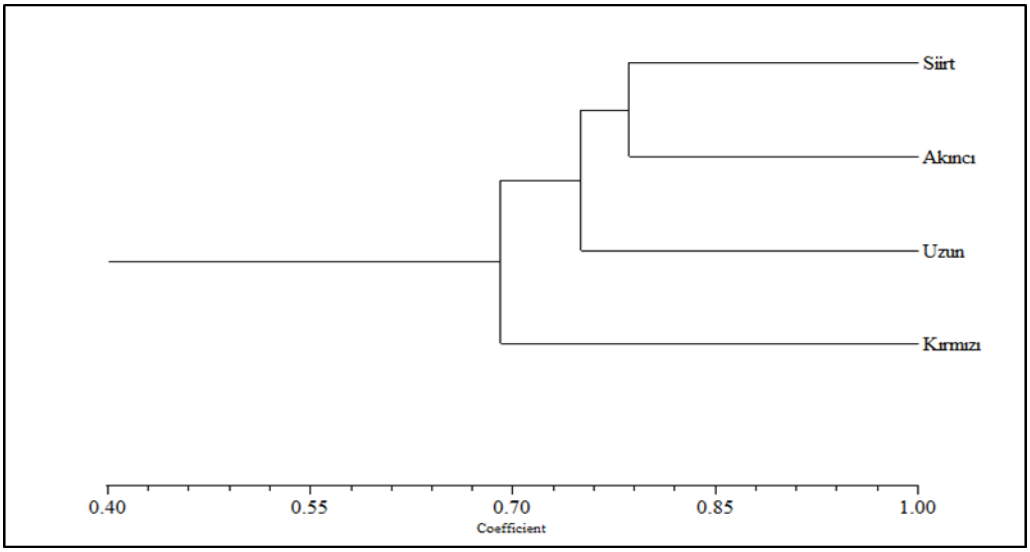

Figure 1. Genetic similarities based on SSR alleles between pistachio cultivars clustered in the UPGMA dendrogram

Şekil 1. UPGMA dendrogramında kümelenen antepfıstığı çeşitleri arasındaki SSR alellerine dayanan genetik benzerlikler

SSR marker analysis

First SSR studies on pistachio cultivars were conducted by Ahmad et al. (2003, 2005). Following this, Kafkas et al. (2006) explored the polymorphism of a wide range of pistachio germplasm using various molecular markers, including AFLP, ISSR, and RAPD. This research was prompted by the limited availability of SSR markers, and it also involved a comparison of the efficacy of these markers.

To date a lot of genetic diversity studies with SSR markers were carried out in *Pistacia* (Vendramin et al., 2009; Kolahi-Zonoozi et al., 2014; Zaloğlu et al., 2015; Khadivi, 2018; Alhajjar & Muzher, 2019; Choulak et al., 2019; Karcı et al., 2022; Karcı et al., 2023). However, seven SSR marker pairs developed by Ahmad et al. (2003, 2005) were firstly utilized in different pistachio cultivars. SSR markers were moderate polymorphism degree and they produced a total of 17 alleles in all cultivars. On average, there were 2.42 alleles per polymorphic marker (14 %).

In the present study, it is found that there are 17 different alleles for 7 SSR, with a range of 1 to 3 alleles per marker. Notably, the Ptms-14, Ptms-31, Ptms-40, and Ptms-41 loci exhibited high allele numbers. This study has generated similar results with previous studies that have highlighted the Ptms-31 marker (Choulak et al., 2019) and Ptms-14 marker (Ahmad et al., 2005) were among the most informative loci. When we compare the average number of alleles in studies, it has been identified that it varies between 1.96 (Pazouki et al., 2010) and 4.50 (Motalebipour et al., 2016). In the study conducted by Karcı et al. (2022), large-scale pistachio cultivar germplasm was qualified by 74 genomic and 18 SSR markers and the average number of alleles was established as 6.26. They stated that the low or high average number of alleles in genetic diversity researches was attributed to lack of an adequate number of markers, only a few for many cultivars, and higher or lower genetic diversity within individuals. It was emphasized that SSR marker efficiency may be more effective in a high number of pistachio populations of different origins (Topçu et al., 2015; Khodaieminjan et al., 2018). Also, in another study aiming to develop new SSR (eSSR) markers and to examine the phylogenetic relationship between *Pistacia* species, transcriptome sequencing was carried out in different tissues of “Siirt” and “Atlı”pistachio cultivars. In the study, 55 polymorphic eSSR loci and an average of 7.89 alleles per marker in 10 *Pistacia* species were identified (Karcı et al., 2020).

Ptms SSR markers are widely used in SSR researches archieved on the determination of genetic relationships in pistachio and have high discrimination power (Khadivi, 2018; Choulak et al., 2019). On the other hand, Ptms markers have never been used in Turkish pistachio genotypes so far, and in this study, they were used for the first time in Turkish genotypes. The discrimination power of Ptms markers (except Ptms-3) was found to be high although the population number was small.

Similarly, in the study by Ahmad et al. (2003), 17 pistachio cultivars from different origins and 9 other genotypes gathered from the market were tested with Ptms SSR markers for their efficiency in detecting polymorphism and all markers (except Ptms-11) showed sufficient polymorphism. On the other hand, Vendramin et al. (2009) detected that Ptms-14, Ptms-33, and Ptms-40 markers are monomorphic in 82 pistachio genotypes that (*P. vera*, *P. terebinthus, P. atlantica* subsp. *mutica*, *P. mutica x P. khinjuk* and *P. integerrima*), are different from samples of Ahmad et al. (2003).

Alhajjar and Muzher (2019) identified that some Ptms markers such as Ptms-11, Ptms-14, Ptms-42 were monomorphic. Also, Choulak et al. (2019) reported that two markers (Ptms-3 and Ptms-7) were generated specific alleles in *P. atlantica*. The Ptms-3 marker showed monomorphic nature in Tunisian pistachio (42 accessions) accessions. Similarly, the Ptms-3 marker, which has no sufficient polymorphic power, was monomorphic in the study, which is different from the conclusion of Alhajjar and Muzher (2019). In SSR-based genetic diversity studies, several problems may encounter during development of SSR marker, primer design and PCR optimisation. Thus,monomorphic SSR markers may reveal due to several devepment methods. On the other hand, SSR markers can be developed from not more conserved genomic regions (Viera et al., 2016; Karcı et al., 2022). However, the Ptms-3 marker was successfully amplified in this study, this marker should be tested in different population types in pistachio germplasm to identify the polymorphism of the Ptms-3 marker.

This situation may be associated with a small number of populations. The variability in observed and expected heterozygosities is owing to using more or less polymorphic SSR markers, which affects the overall genetic diversity in the population (Karcı et al., 2022). In this study, the expected heterozygosity (He) for individual loci varied from 0.0 to 0.59, and the observed heterozygosity (Ho) varied from 0.00 to 1.0 (Table 2). These results and several previously performed studies have been stated similar conclusions (Mirzaei et al., 2006; Arabnezhad et al., 2011; Choulak et al., 2019). As known, the Ho value symbolizes the proportion of heterozygotic individuals in the population. He or genetic diversity indicates the percentage of the population that would be heterozygous if an random cross between individuals were to occur (Arslan et al., 2023). Also, in the SSR marker-based genetic diversity study among 15 different male genotypes of *P. vera* L., total expected heterozygosity (He) was deteched as 0.507, similar to our study (Alhajjar & Muzher, 2019). The highest Ho value was reported in marker Ptms-31 and Ptms-33, reflecting high genetic diversity in these cultivars. The findings of these studies support low genetic variation in cultivated pistachio using various molecular markers, including SSR markers. While Khadivi et al. (2018) reported average He and Ho values as 0.22 and 0.44, respectively, Kolahi-Zonoozi et al. (2014) reported these values as 0.35 and 0.49, respectively. In this study, He was identified slightly higher than Ho in the Ptms-14. A high He value may indicate the presence of a null allele, and investigation of the null allele frequencies (r) of these loci revealed that the values were either positive or negative (data not shown). However, the small values of the positive values reduce the risk of null alleles at the loci, as stated by some grape SSR researches (Santana et al., 2007). Moreover, the average He and Ho values in this study are 0.45 and 0.28, respectively. Otherwise Khodaeiaminjan et al. (2018) detected these values as 0.56 and 0.53. The probability of identity (PI) value is described as the probability with which two randomly taken genotypes expose the same SSR profile (Doulati-Baneh et al., 2013). In this study, the probability of detection (PI) value was higher than the value of 0.05 stated by Sefc et al. (2000) in all markers. The PI values were calculated between 0.20 and 1.000 with an average of 0.52. The average PIC (Polymorphic Information Contents) value in the study was higher than 0.44 and 0.33, respectively (Baghizadeh et al., 2010; Kolahi-Zonoozi et al., 2014), while it was lower than 0.64 (Khadivi et al., 2018).

Genetic relations among pistachio cultivars

Siirt pistachio (*P. vera* cv. “Siirt”) belong to the Anacardiaceae family and are native to Western Asia, extending from this region to the Middle East, Mediterranean countries, and Europe (Tomaino et al., 2010). The “Siirt” pistachio product is traded globally, especially in Türkiye. It is an agricultural product grown in regions such as Gaziantep and Siirt, making significant contributions to Türkiye’s economy. This genotype has also been popularly used in some genomic DNA-based SSR marker development studies (Zaloğlu et al., 2015; Motalebipour et al., 2016; Khodaeiaminjan et al., 2018). Kafkas et al. (2006) did not find any polymorphism among Siirt genotypes (“Siirt-85”, “Siirt-88”, “Siirt-91”) in the analyses performed by RAPD, ISSR and AFLP markers. Similarly, Ahmad et al. (2003) could not make a clear distinction between the two “Siirt” genotypes (“Siirt a” and “Siirt b”) with SSR markers. Although the origin of the “Siirt” genotype is not clear, it is stated that it may be a genotype originating from Iran (Kafkas et al., 2006). In this study, the "Siirt” genotype showed at least 64 % similarity (between “Siirt” and “Kırmızı”) to the other 3 genotypes and showed a clear distinction between these genotypes.

Kafkas et al. (2006) also reported that RAPD, ISSR, and AFLP markers did not find any difference in the “Kırmızı” genotypes from Adana and Gaziantep provinces, while the “Kırmızı” genotype from Şanlıurfa showed difference. Some studies have indicated that the “Kırmızı” and “Uzun” genotypes are closely related varieties at a genetic level (Khodaeiaminjan et al., 2018; Karcı et al., 2022). Karcı, (2023) found that the "Uzun” genotype was clustered with “Kırmızı-1”, and "Kırmızı-2” genotypes, and the genetic difference coefficient was 0.00. Thus, it was determined these genotypes might have the same genetic structure as the “Uzun” genotype. Similarly, a high genetic similarity rate (64 %) was determined between the "Kırmızı” and “Uzun” genotypes in this study.

The valuation of genetic diversity in pistachio germplasm is crucial for enhancing desirable traits and contribution to economy. SSRs are highly effective markers in genotype diversity, germplasm characterization, and phylogenetic studies. In this study, clear distinction was observed between the genotypes in terms of genetic relationship and similarity rates. However, the differentiation of pistachio genotypes, which are important for the Turkish economy, was determined with SSR-based 'Ptms' markers. The SSR data and genetic relationships identified in this study are supposed to play a constructive role to progress the genetic characterization, protection, and enhancement of pistachio germplasms, offering valuable insights for the development of innovative breeding programs.

ACKNOWLEDGEMENTS

This study was supported by the Ankara University Biotechnology Institute.

STATEMENT OF CONFLICTS OF INTEREST

The author declares that there is no conflict of interest in the publication.

STATEMENT OF ETHICS CONSENT

Ethical approval is not required as this article does not contain any studies with human or animal subjects.

REFERENCES

Ahmad, R., Ferguson, L., & Southwick, S. (2003). Identification of pistachio (*Pistacia vera* L.) nuts with microsatellite markers. *Journal of the American Society for Horticultural Science, 128* (6), 898-903. https://doi.org/10.21273/JASHS.128.6.0898

Ahmad, R., Ferguson, L., & Southwick, S.M. (2005). Molecular marker analyses of pistachio rootstocks by simple sequence repeats and sequence-related amplified polymorphisms. *The Journal of Horticultural Science and Biotechnology, 80* (3), 382-386. https://doi.org/10.1080/14620316.2005.11511948

Alhajjar, N.M., & Muzher, B.M. (2019). Genetic relationships among *Pistacia vera* L. F1 hybrids and their parents (*P. vera* x Hermaphrodite Genotpyes of *P. atlantica*) using SSR markers. *AGROFOR International Journal, 4* (2), 28-34. https://doi.org/10.7251/AGRENG1902028A

Anonymous (1993). Antepfıstığı Çeşit Kataloğu. Tarım ve Köyişleri Bakanlığı, Ankara, Türkiye. 361 (20), 6-29.

Anonymous (2022). Faostat. Agriculture data [online]. Available form: http://faostat.fao.org/ (Erişim tarihi: 17.12.2024)

Arabnezhad, H., Bahar, M., & Pour, A.T. (2011). Evaluation of genetic relationships among Iranian pistachios using microsatellite markers developed from *Pistacia khinjuk* Stocks. *Scientia Horticulturae, 128* (3), 249-254. https://doi.org/10.1016/j.scienta.2011.01.028

Arslan, N., Yılmaz Baydu, F., Hazrati, N., Yüksel Özmen, C., Ergönül, O., Uysal, T., Yaşasın, A.S., Özer, C., Boz, Y., Kuleyin, Y.S., & Ergül, A. (2023). Genetic diversity and population structure analysis of Anatolian Kara grapevine (*Vitis vinifera* L.) germplasm using simple sequence repeats. *Horticulturae, 9* (7), 743. https://doi.org/10.3390/horticulturae9070743

Baghizadeh, A., Noroozi, S., & Javaran, M.J. (2010). Study on genetic diversity of some Iranian pistachio (*Pistacia vera* L.) cultivars using random amplified polymorphic DNA (RAPD), inter sequence repeat (ISSR) and simple sequence repeat (SSR) markers: A comparative study. *African Journal of Biotechnology, 45,* 7632-7640. https://doi.org/10.5897/AJB10.701

Barone, E., Marco, L.D., Marra, F.P., & Sidari, M. (1993). Isozymes and multivariate analysis to discriminate male and female Sicilian germplasm of pistachio. IX G.R.E.M.P.A. meeting Pistachio, Agrigento, Italy. *IXth Groupe de Recherches et d'Etudes Mediterraneen pour le Pistachier et l'Amandier,* Agrigento (1993): 73-79.

Bowcock, A.M., Ruiz-Linares, A., Tomfohrde, J., Minch, E., Kidd, J.R., & Cavalli-Sforza, L.L. (1994). High resolution of human evolutionary trees with polymorphic microsatellites. *Nature, 368,* 455-457. https://doi.org/10.1038/368455a0

Choulak, S., Chatti, K., Rhouma-Chatti, S., Guenni, K., Salhi-Hannachi, A., Said, K., & Chatti, N. (2019). Microsatellite (SSR) markers reveal genetic diversity and population structure in Tunusian pistachio. *Fruits, 74* (2), 73-81. https://doi.org/10.17660/th2019/74.2.3

Çınar, B. (2012). Researches on vitamin, mineral compound, fat and fatty acid compositions of Turkish pistachio nut cultivars. (Publication No. 323229) [Master dissertation, Ankara University]. PQDT Open. https://tez.yok.gov.tr/UlusalTezMerkezi/tezDetay.jsp?id=iNPmzcQ3OfkkQzI3S9tQ-g&no=d68Rs78GQ3w7fD2ygt73mA

Doulati-Baneh, H., Mohammadi, S.A., & Labra, M. (2013). Genetic structure and diversity analysis in *Vitis vinifera* L. cultivars from Iran using SSR markers. *Scientia Horticulturae, 160,* 29-36. https://doi.org/10.1016/j.scienta.2013.05.029

Hormaza, J.I., Dollo, L., & Polito, V.S. (1994). Determination of relatedness and geographic movements of *Pistacia vera* (Pistachio; Anacardiaceae) germplasm by RAPD analysis. *Economic Botany, 48,* 349-358. https://www.jstor.org/stable/4255662

Kafkas, S., Kaska, N., Wassimi, A.N., & Padulosi, S. (2006). Molecular characterization of Afghan pistachio accessions by amplified fragment length polymorphisms (AFLPs). *The Journal of Horticultural Science and Biotechnology, 81,* 864-868. https://doi.org/10.1080/14620316.2006.11512151

Kafkas, S., & Perl-Treves, R. (2002). Interspecific relationships in *Pistacia* based on RAPD Fingerprinting. *Hortscience, 37* (1), 168-171. https://doi.org/10.21273/HORTSCI.37.1.168

Kafkas, S. (2006). Phylogenetic analysis of the genus Pistacia by AFLP markers. *Plant Systematics and Evolution, 262* (1), 113-124. https://doi.org/10.1007/s00606-006-0460-7

Kafkas, S., Dogan, Y., & Zaloglu, S. (2009). Phylogenetic analysis in the genus *Pistacia* by simple sequence repeat markers. *5th International Symposium on Pistachios and Almonds,* ISHS- Şanlıurfa-Türkiye, 06-10 October 2009 Şanlıurfa. Abstract, pp. 84.

Kafkas, S. (2019). Advances in breeding of pistachio. Chapter. Burleigh Dodds Science Publishing Limited. https://doi.org/10.19103/AS.2018.0042.17

Karcı, H., Paizila, A., Topçu, H., Ilikçioğlu, E., & Kafkas, S. (2020). Transcriptome sequencing and development of novel genic SSR markers from *Pistacia vera* L. *Frontiers in Genetics, 11,* 1021. https://doi.org/10.3389/fgene.2020.01021

Karcı, H., Paizila, A., Güney, M., Zhaanbaev, M., & Kafkas, S. (2022). Revealing genetic diversity and population structure in pistachio (*Pistacia vera* L.) by SSR markers. *Genetic Resources and Crop Evolution, 69,* 2875-2887. https://doi.org/10.1007/s10722-022-01410-w

Karcı, H. (2023). Detection of in silicoSSR markers specific to Uzun and Kırmızı cultivars in pistachio. *Turkish Journal of Agriculture-Food Science and Technology, 11* (10), 1947-1952. https://doi.org/10.24925/turjaf.v11i10.1947-1952.6341

Khadivi, A. (2018). Assessment of genetic variability in pistachio (*Pistacia vera* L.) with nuclear SSR molecular markers. *Erwerbs-Obstbau, 60* (4), 289-294. https://doi.org/10.1007/s10341-018-0372-z

Khodaeiaminjan, M. (2017). Development of novel SSR markers by next generation sequencing and construction of the first SSR linkage reference maps in pistachio. [Doctoral dissertation, Çukurova University].

Khodaeiaminjan, M., Kafkas, S., Motalebipour, E.Z. & Coban, N. (2018). In silico polymorphic novel SSR marker development and the first SSR-based genetic linkage map in pistachio. *Tree Genetics and Genomes, 14* (4), 1-14. https://doi.org/10.1007/s11295-018-1259-8

Kolahi-Zonoozi, S.H., Mardi, M., Zeinalabedini, M., Pirseydi, S.M., Mahmoodi, P., Tabatabaei, I., Mosavi-Derazmahalleh, S.M., Farsi, M., Ebrahimi, M.A., Khayam-Nekoui, S.M., & Ahmadi, K. (2014). Development of 12 new SSR markers for genetic diversity and structure analysis in pistachio (*Pistacia vera* L.). *Journal of Horticultural Science and Biotechnology, 89* (6), 707-711. https://doi.org/10.1080/14620316.2014.11513141

Lefort, F., Lally, M., Thompson, D., & Douglas, G C. (1998). Morphological traits microsatellite fingerprinting and genetic relatedness of a stand of elite oaks (*Q. robur* L.) at Tuallynally, Ireland. *Silvae Genetica, 47,* 5-6.

Mandalari, G., Barreca, D., Gervasi, T., Roussell, M.A., Klein, B., Feeney, M.J., & Carughi, A. (2022). Pistachio nuts (*Pistacia vera* L.): Production, nutrients, bioactives and novel health effects. *Plants, 11* (1), 18. https://doi.org/10.3390/plants11010018

Minch, E., Ruiz-Linares, A., Goldstein, D.B., Feldman, M., & Cavalli-Sforza, L.L. (1995). MICROSAT (Version 1.4d): A computer program for calculating various statistics on microsatellite allele data. Stanford, CA, USA: Stanford University.

Mirzaei, S., Bahar, M., & Sharifnabi, B. (2006). A phylogenetic study of Iranian wild pistachio species and some cultivars using RAPD markers. *Acta Horticulture, 726,* 30-43. https://doi.org/10.17660/ActaHortic.2006.726

Motalebipour, E., Kafkas, S., Khodaeiaminjan, M., Çoban, N., & Gözel, H. (2016). Genome survey of pistachio (*Pistacia vera* L.) by next generation sequencing: development of novel SSR markers and genetic diversity in *Pistacia* species. *BMC Genomics, 17,* 998. https://doi.org/10.1186/s12864-016-3359-x

Pazouki, L., Mardi, M., Shanjani, P.S., Hagidimitriou, M., Pirseyedi, S.M., Naghavi, M.R., Avanzatto, D., Vendramin, E., Kafkas, S., Ghareyazie, B., Ghaffari, M. R., & Khayam Nekoui, S.M. (2010). Genetic diversity and relationships among *Pistacia* species and cultivars. *Conservation Genetics, 11,* 311-318. https://doi.org/10.1007/s10592-009-9812-5

Rohlf, F. (1988). NTSYS-PC Numerical Taxonomy and Multivariate Analysis System, Version 2.0. Setauket, NY, USA: Exeter Publishing, Ltd.

Santana, J.C., Lucas, A.I., Arranz, C., Rubio, J.A., Yuste, J., et al. (2007). Genetic characterization of grapevine varieties from Castilla y León (Spain) using 23 microsatellite markers. *In: Proceedings of the 30th OIV World Congress;* Budapest, Hungary.

Sneath, P.H.A., & Sokal, R.R. (1973). Numerical taxonomy. Freeman WH and Company (eds) San Francisco, USA.

Sefc, K.M., Lopes, M.S., Lefort, F., Botta, R., Angelakis, K.A.R., Ibanez, J., Pejic, I., Wagner, H.W., Glossl, J., & Steinkellner, H. (2000). Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars. *Theoretical and Applied Genetics, 199* (3-4), 498-505. https://doi.org/10.1007/s001220050065

Tomaino, A., Martorana, M., Arcoraci, T., Monteleone, D., Giovinazzo, C., & Saija, A. (2010). Antioxidant activity and phenolic profle of pistachio (*Pistacia vera* L., variety Bronte) seeds and skins. *Biochimie, 92* (9), 1115-1122. https://doi.org/10.1016/j.biochi.2010.03.027

Topçu, H., Kafkas, S., Doğan, A., Akcay, M.E., & Ercişli, S. (2015). Genetic relatedness among quince (*Cydonia oblonga* Miller) accessions from Turkey using amplifed fragment length polymorphisms. *Journal of Applied Botany and Food Quality, 88,* 197-201. https://doi.org/10.5073/JABFQ.2015.088.028

Wagner, H.W., & Sefc, K.M. (1999). IDENTITY 1.0. Vienna, Austria: Centre for Applied Genetics, University of Agricultural Science.

Vendramin, E., Dettori, M.T., Verde, I., Micali, S., Giovinazzi, J., Mardi, M., Avanzato, D., & Quarta, R. (2009). Molecular characterization of genus *Pistacia* by microsatellite markers. *Acta Horticulture, 825,* 55-62. https://doi.org/10.17660/ActaHortic.2009.825.5

Vieira, M.L.C., Santini, L., Diniz, A.L., & Munhoz, C.D.F. (2016). Microsatellite markers: What they mean and why they are so useful. *Genetics and Molecular Biology, 39,* 312-328. https://doi.org/10.1590/1678-4685-GMB-2016-0027

Yılmaz, F., Shidfar, M., Hazrati, N., Kazan, K. & Yüksel Özmen, C., Uysal, T., Özer, C., Yaşasın, A.S., Söylemezoğlu, G., Boz, Y., Çelik, H., & Ergül, A. (2020). Genetic analysis of Central Anatolian grapevine (*Vitis vinifera* L.) germplasm by simple sequence repeats. *Tree Genetics & Genomes, 16,* 55. https://doi.org/10.1007/s11295-020-01429-z

Zaloglu, S., Kafkas, S., Dogan, Y., & Guney, M. (2015). Development and characterization of SSR markers from pistachio (*Pistacia vera* L.) and their transferability to eight *Pistacia* species. *Scientia Horticulturae, 189,* 94-103. https://doi.org/10.1016/j.scienta.2015.04.006