Turkish Journal of Agriculture - Food Science and Technology, 11(10): 1947-1952, 2023
DOI: https://doi.org/10.24925/turjaf.v11i10.1947-1952.6341

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

Detection of *in silico* SSR Markers Specific to Uzun and Kırmızı Cultivars in Pistachio

Harun Karcı[1,a,*]

[1]*Çukurova University, Agriculture Faculty, Horticulture Department, Adana, Türkiye*
[*]*Corresponding author*

ARTICLE INFO

Research Article

Received : 04-09-2023
Accepted : 05-10-2023

Keywords:
Pistachio
Cultivar-Specific
SSRs
Prevention of confusion
Farmers

ABSTRACT

In the current paper, it was aimed to detect the SSR markers that can be used in the prevention of confusion that may occur in breeding or nurseries, and directly genetically separating Uzun and Kırmızı pistachio cultivars from other commercial cultivars. A total of genotypes of 16 *Pistacia vera* species, one *P. atlantica*, one *P. eurycarpa* and two *P. terebinthus* species were obtained from the farmer's orchard in Nizip district of Gaziantep province for genetic characterization. Genetic diversity and clustering analyzes were performed with UPGMA (Unweighted Pair Group Method with Arithmetic Average) and STRUCTURE 2.3.4 programs using the scored SSR loci. Genetic relationship and population structure of genotypes were defined using common and distinct polymorphic PCR fragments. Cultivar-specific markers to be used in identifying and distinguishing the genetic structure of Uzun and Kırmızı cultivars were carried out in the current research. CUPOhBa2127 marker has the highest allele number (Na=10). In addition, 11 out of 25 SSR markers were explained as cultivar-specific SSRs that can distinguish Uzun and Kırmızı cultivars. These markers can be used directly by breeders and geneticists without any preliminary screnning of the markers. A quite serious providence will be achieved in the cost and time that will occur with the preliminary analysis, and thus, the confusion that may occur in large scale orchard establishments or nurseries will be reduced to pretty low levels with DNA analysis.

[a] *karciharun42@gmail.com* *https://orcid.org/0000-0002-7219-7135*

Introduction

Pistacia vera L. takes place in the genus *Pistacia* of the Anacardiaceae family, and there are at least 11 species in the genus *Pistacia* (Kafkas, 2006a). *P. vera* is the only edible *Pistacia* species and it has commercially importance. *Pistacia* species are pollinated by the wind and they have dioecious inflorescences nature, except for monoecious genotypes (Kafkas et al., 2000). The chromosome number of the pistachio is 2n=30 (Kafkas, 2022). The origin of the pistachio defines as from Central Asia and spread from Southern Europe to China, and it has existed in the United States, Australia and the Mediterranean region (Kafkas, 2006b). Among 30°-45° in the south-north parallels are pretty suitable for cultivation which are accepted as microclimate conditions such as in the Northern Hemisphere geographical regions (Tunalıoğlu and Taşkaya, 2003). The amount of world production of pistachio reached 1,375,770 tons in 2022, and the main producing countries were Iran, the USA, and Turkey (Faostat, 2022).

The previous molecular diversity papers in plants have been carried out in many plant species with different molecular methods. The molecular markers enabled the polymorphism to be detected effectively based on DNA at an early phase of plant growing for marker assisted selection in terms of agronomic important properties (Orman et al., 2020; Paizila et al., 2022). So far, they have been used in genetic diversity, mapping and marker assisted breeding. Several molecular markers have been developed and used in distinct plant species (Güney et al., 2018; Khodaeiaminjan et al., 2018; Karcı et al., 2022). Simple Sequence Repeats (SSRs) are the PCR-based method, and it is one of the most preferable technic by geneticist and breeders for characterization studies of cultivars and genotypes due to its codominant inheritance nature, spread throughout the whole genome, high polymorphism and reproducibility, and high transferability rate (Kafkas, 2019; Karcı et al., 2022). The performed papers in apricot (Hormaza, 2002), pear (Fan et al., 2013), almond (Esgandaripirmorad et al., 2022), pistachio (Karcı

et al., 2022) and walnut (Güney et al., 2022) were main examples. SSRs are pretty beneficial technic for evaluation of genetic relationships among the cultivars, and it is an essential tool for identification of the genetic diversity and genomic nature in pistachio breeding programs for characterization of the germplasm. The usage of SSRs to identify the genetic relationship between pistachio cultivars or landraces is pretty reliable tool, and they play a significant role in overcome to many issues.

The papers related to molecular genetic of pistachio have been published from last three decades to today with several marker systems such as RAPD, AFLP, ISSR and SSR, respectively (Hormaza et al., 1994; Kafkas et al., 2006; Kolahi-Zonoozi et al., 2014; Karcı et al., 2022). The first investigation in pistachio DNAs was performed using the RAPD technique (Hormaza et al., 1994). In the following years, Kafkas et al. (2006) published an article related to the genetic relationship of pistachio germplasm by using RAPD, AFLP, and ISSR markers. In addition, Khadavi et al., (2018) carried out genetic diversity analysis with SSR markers developed by Topçu et al. (2016) using only Iranian pistachio genotypes. In a recent report carried out by Karcı et al. (2022), the large-scale pistachio genetic resources were characterized by SSR markers.

Here, genetic diversity status of pistachio genotypes sampled from Nizip district of Gaziantep province were identified, and SSR markers specific to Uzun and Kırmızı cultivars were mined from polymorphic *in silico* SSR markers.

Materials and Methods

Plant material and DNA extraction

A total of 16 pistachios (*P. vera*) cultivars and genotypes, one *P. atlantica*, one *P. eurycarpa* genotypes and two *P. terebinthus* genotypes were used in the present research. Pistachio plant samples were taken from Nizip district in Gaziantep province. Samples of wild species were used to identify the genetic dissimilarities of *P. vera* samples to other *Pistacia* species most abundant in Turkey.

Total genomic DNA was isolated from fresh young leaves by the CTAB method described by Doyle and Doyle (1987) with some modifications (Kafkas et al. 2006). Qubit Fluorometer (Invitrogen) was used to quantify the isolated DNAs, followed by diluting them to 10 ng/µl for SSR-PCR reactions, and then the samples were stored at -20°C for further analysis.

SSR-PCR reactions

Totally, 52 most polymorphic SSR primer pairs were selected from developed by Khodaeiaminjan et al. (2018), and they were screened (Table 1). Of the 52 primers screened, 25 scoreable and polymorphic SSRs were selected according to their polymorphism levels.

All SSR-PCR reactions were done based on a three primer strategy according to Scheulke (2000) with minor modifications. A total volume of 12.5 µl containing 10 ng DNA, 20 mM $(NH_4)_2SO_4$, 75 mM Tris–HCl (pH 8.8), 0.01% Tween 20, 2.0 mM $MgCl_2$, 200 µM of each dNTP, 10 nM M13 tailed forward primer at the 5′ end, 200 nM reverse primer, 200 nM universal M13 tail primer (5′-TGTAAAACGACGGCCAGT-3′) labeled with FAM, VIC, NED or PET dye, and 0.6 U of Taq DNA polymerase were used for each reaction. PCR amplifications were done in two consecutive steps. The first step included initial denaturation at 94°C for 3 min, followed by 28 cycles of 94°C for 30 s, 58°C for 45 s, and 72°C for 60 s. The second step involved 10 cycles of 94°C for 30 s, 52°C for 45 s and 72°C for 60 s, and a final extension at 72°C for 5 min. When the PCRs were completed, the reactions were subjected to denaturation for capillary electrophoresis in an ABI 3130xl genetic analyzer [Applied Biosystems Inc., Foster City, CA, USA (ABI)] using a 36-cm capillary array with POP7 as the matrix (ABI). Then samples were denatured by mixing 1.0 µl (in 6-FAM and VIC labeled primers) or 1.5 µl (in NED and PET labeled primers) of the amplified product, 0.3 µl of the size standard and 9.7 µl of Hi-Di formamide. The ABI data collection software 3.0 was used for resolving the fragments, and then SSR fragment analysis was done using the GeneMapper 4.0 (Applied Biosystems Inc.).

Data analysis

After capillary electrophoresis of the SSR loci, effective number of alleles (*Ne*), expected heterozygosity (*He*), the number of alleles per locus (*Na*), and observed heterozygosity (*Ho*) were calculated using the GenAlEx version 6.5 program (Peakall and Smouse, 2012). Polymorphism information contents (*PIC*) for the SSR loci were calculated using PowerMarker software version 3.25 (Liu and Muse, 2005).

The UPGMA (Unweighted Pair Group Method with Arithmetic Average) based dendrogram analysis was conducted in MEGA*X* software (Kumar et al. 2018). Population structure and identification of admixed individuals was performed using the model-based software program, STRUCTURE 2.3.4 (Pritchard et al. 2000). In this model, a number of populations (K) are considered to be available, which each of them is characterized by a set of allele frequencies at each locus. Individuals in the sample are given to populations (clusters), or jointly to more populations if their cultivars indicate that they are admixed. Ln P (D) values (logarithm probability for each K) were applied to determine the Delta K indicating the probable population number. The term of Delta K is calculated by the change ratio of logarithm probability ($\Delta K=2$ to $\Delta K=5$). In the diagram, the highest K of Delta K confers the information about the probable population number.

Results and Discussion

Polymorphism degrees of SSR markers

Totally, 25 SSR markers were scored, and PCR fragments were recorded, and genetic diversity analysis was performed on a total of 20 genotypes (Table 1). The produced 125 alleles all genotypes were used in the current research, and the number of alleles were varied from 2.00 to 10.00 alleles, while the average number of alleles was 5.00. The highest number of alleles was calculated at the CUPOhBa2127 locus (Na=10). The number of effective alleles (Ne) was identified from 1.13 (CUPSiPa2066) to 3.86 (CUPOhBa2127 and CUPSiPa3487), and average effective number of alleles was 2.23. The mean observed heterozygosity (Ho) value was 0.46, and it ranged from to

0.00 to 1.00. The highest observed heterozygosity (Ho) was found at the CUPSiOh510 locus. The mean He value was explained as 0.49, and the highest expected heterozygosity (0.74) was detected from the CUPOhBa2127 and CUPSiPa3487 markers. The average of the polymorphism information content (PIC) was calculated as 0.45, and was found between 0.11 (CUPSiPa2066) and 0.70 (CUPOhBa2127 and CUPSiPa3487) (Table 1).

Various molecular papers have been performed related to genetic variation and relationships of pistachios and other *Pistacia* genotypes with SSRs (Zaloglu et al., 2015; Khodaeiaminjan et al., 2018; Karcı et al., 2022). The first SSR development research was reported by Ahmad et al. (2003, 2005) using a limited number of pistachio cultivars. Since there are not enough SSR markers to define genetic diversity of the pistachio cultivars, Kafkas et al. (2006) performed a research using AFLF, ISSR and RAPD markers in large scale pistachio cultivar collection. In addition, Iranian pistachio cultivars has recently been characterized developed by Topçu et al. (2016) (Khadivi et al., 2018).

Table 1. Forward and reverse sequences of the markers, SSR motifs, Na, Ne, Ho, He and PIC values of polymorphic *in silico* SSR markers

No	Markers	Forward 5'-3'/Reverse 3'-5'	The motifs of the repeats	Na	Ne	Ho	He	PIC
1	CUPOhBa2127	F:TGGAAGAACAAGTGAGGAGCA R:GTTGAGGAAGGAATGGAGGTC	-	10	3.86	0.8	0.74	0.7
2	CUPSiOh3876	F:CCTATTTCCCCTTACTTCTTCCA R:TCAAACCTTAGTGAAGGGCATT	$(TC)_{21}$	9	3.4	0.6	0.71	0.67
3	CUPSiOh3348	F:CTTGAAAATTGTTTTGCAGT R:CAGACTGAAAGTTTAAGATTGA	-	8	2.22	0.44	0.55	0.53
4	CUPSiOh3646	F:ATACACCCGTGTGAAATGCAA R:GGCTGGTAGTCTGGTGCTTT	$(TC)_{15}$	4	3.23	0.59	0.69	0.64
5	CUPSiPa694	F:TGTTGTATGCAATACCCTAGATT R:TCATGTGTATTCATTGGTCGAT	$(AAT)_{10}$	7	1.93	0.3	0.48	0.46
6	CUPBaPa992	F:CGCAAAGAGTTTTTCAAAGAGG R:TGGTTTCAAATACCGAAAAACA	$(CT)_{12}$	6	1.73	0.25	0.42	0.4
7	CUPSiPa3487	F:TGAGAGTCGTGTAAGGGCTTC R:CTGTTTAAGGAACGGAAAGG	-	7	3.86	0.8	0.74	0.7
8	CUPSiOh2834	F:GCGCTGTAAATCCAAGAAAAC R:TGTTCGTTGTTGCCTTTCTTT	$(AG)_{16}$	3	1.59	0	0.37	0.34
9	CUPSiOh4004	F:TGGGGCTAAAAATCACTTCAC R:TTGCAAAATGAGTTTGGAGGT	$(TG)_{13}$	3	2.15	0.69	0.54	0.43
10	CUPSiOh2178	F:CCAGAATTTGTTGGAAGTTGC R:TTATCTCACATGAGGCAAAAT	-	6	1.92	0.18	0.48	0.46
11	CUPSiPa1583	F:GAGAAGTAAAAGAAGGGACGGTTA R:TTTCTTCCATAATCAATCCGACT	$(GA)_{11}$	7	1.79	0.32	0.44	0.42
12	CUPSiOh3920	F:GAAGGGAAGGAGAGAACGATG R:GAAAAACAACAAAGCGACGAC	$(GAT)_4CTATTTATCGCAG(AGGCTC)_4$	6	3.8	0.53	0.74	0.69
13	CUPSiPa1904	F:ATCCAGAATCCAAGGGAAGAA R:CAATATGGCCAGACTCAGCAT	-	5	1.39	0.19	0.28	0.27
14	CUPSiOh4419	F:CAAACTAAATGTGGTGCATTATCAA R:GCTTTGCTAGATAAAAATACCCAGA	$(TAT)_{10}$	3	1.95	0.73	0.49	0.4
15	CUPSiOh2995	F:TGCATTTTCAGCTTCAATGTC R:TGACCCCTCTTCTCTTTCACC	$(AAG)_8$	4	1.21	0.19	0.18	0.17
16	CUPBaPa2462	F:TCAAGCTTTCTTGTCATCACCT R:GCTCAACACTTGGTTTTGTCTTT	$(TAA)_9$	4	1.37	0.3	0.27	0.26
17	CUPSiOh3903	F:GAATATACATGGTGGACCCTCA R:GAATAGGGTTCGTACCTGCAA	$(AAT)_{10}$	3	2.11	0.44	0.53	0.44
18	CUPSiOh3994	F:AGGTCGCAGATAACGAGTTGA R:CACCTGTTGAACAATAGGCTCA	$(AG)_{14}$	5	2.84	0.93	0.65	0.58
19	CUPSiOh510	F:CATTTTTCATTTTGGAGCTGAA R:ATTGCAGGAAAACAAGCAAAG	-	5	2.91	1	0.66	0.6
20	CUPOhBa2087	F:CTGCAATTTATGAAAAGTTGTTCTC R:GCTTTGAGCTTCTTTCACAACTC	$(ATT)_8$	4	1.46	0.21	0.31	0.3
21	CUPOhBa2356	F:GCATGCGTGCTGGATTTATAC R:TTTGACGACTTTCCCACACTT	$(AG)_{18}$	3	1.46	0.18	0.31	0.29
22	CUPSiPa2066	F:CAGCTCCCACTAGGTTTGTGT R:ACAATCTCACAACAAACAAAGAACA	$(TTGTTA)_6$	2	1.13	0.13	0.12	0.11
23	CUPSiOh2340	F:GACTACTGTGCCCACATGACA R:GGATCGTCAGAGAAGACGTTG	-	3	1.66	0	0.4	0.35
24	CUPSiOh1325	F:TTTTCTTTGATCTTTCTACCGCTAC R:TGAGCAAACAATACAGTTGAATCC	$(ATTT)_8$	6	2.75	0.92	0.64	0.57
25	CUPSiPa912	F:TGCAGTGAGTAGGAAGTTTGGA R:AGCGAACAAGAGAACGAACAC	$(TGAGTG)_5$	2	1.95	0.83	0.49	0.37
Mean				5	2.23	0.46	0.49	0.45

Table 2. The fragments and frequencies of SSR markers specific to Uzun and Kırmızı pistachio cultivars

No	Markers	PCR fragments	The frequencies of the markers	Cultivars
1	CUPSiOh3876	142	0.275	Uzun
2	CUPSiOh3646	182	0.265	Uzun
3	CUPSiPa3487	113	0.025	Kırmızı
		115	0.250	Uzun
4	CUPSiOh2834	164	0.111	Kırmızı
		166	0.778	Uzun
5	CUPSiOh4419	159	0.033	Kırmızı
		192	0.333	Uzun
6	CUPSiOh2995	161	0.031	Kırmızı
7	CUPSiOh3903	145	0.344	Uzun

The mean allele values reported in previous SSR studies were 3.30 (Ahmad et al., 2003), 2.75 (Baghizadeh et al., 2010), 2.80 (Arabnezhad et al., 2011), 3.60 (Zaloglu et al., 2015), 4.2 (Khodaeiaminjan et al., 2018) and 2.73 (Karcı et al., 2020). In this paper, totally, 125 alleles were created with an average of 5.00 alleles per locus derived from 25 SSRs. In previous findings, the reasons of the differentiation of allele counts are low number of cultivars or genotypes, insufficient number of markers, and limited genetic variation among the individuals (Karcı et al., 2022). Despite the limited number of SSRs of present paper, the high average number of alleles was identified due to the high variation among the genotypes.

The mean polymorphism information content was calculated as 0.45, and it varied between and 0.11 and 070. The explained mean PIC in this study was found higher than 0.33 (Kolahi-Zonoozi et al., 2014) and 0.44 (Baghizadeh et al., 2010) and below 0.64 (Khadivi et al., 2018), respectively. The mean He and Ho values were determined as 0.49 and 0.46, respectively, although Khadivi et al. (2018) stated that these values were 0.22 and 0.44. And, Kolahi-Zonoozi et al. (2014) defined that those were 0.35 and 0.49. Khodaeiaminjan et al. (2018) reported that the average PIC, Ho and He values obtained from a total of 18 *P. vera* cultivars were 0.51, 0.53 and 0.56, respectively. A similar result of the He, Ho and PIC was calculated from 51 genic SSR markers designed from *P. vera*, and they were 0.40, 0.38 and 0.34, respectively (Karcı et al., 2020). On the other hand, Arabnezhad et al. (2011) calculated the mean Ho value as 0.64 from18 SSR markers fragments, while Baghizadeh et al. (2010) calculated the mean Ho value as 0.52 from four SSR markers. In addition, the mean He values performed by previous papers were found as 0.45 and 0.75, respectively (Arabnezhad et al., 2011; Baghizadeh et al., 2010). The waves of the genetic diversity results in the previous researches were based on the genetic variation of the populations, the scale of the characterized genotypes or cultivars and the number of screened primer pairs and their polymorphism levels. Thus, the polymorphism information content and the observed and expected heterozygosities resulted from a greater or lesser abundance of polymorphic SSRs, or a higher and less narrow rate of genetic variation in the population.

Identification of cultivar-specific SSR markers

A total of seven (7) primer pairs were generated 10 cultivar-specific genomic fragments in Uzun and Kırmızı pistachio cultivars, and allele frequencies ranged from 0.031 to 0.778. Of the cultivar-specific markers, CUPSiOh3876, CUPSiOh3646, CUPSiPa3487, CUPSiOh2834, CUPSiOh4419, CUPSiOh2995, and CUPSiOh3903 produced cultivar-specific alleles for Uzun pistachio cv., while CUPSiPa3487, CUPSiOh2834, CUPSiOh4419 and CUPSiOh2995 produced cultivar-specific alleles for Kırmızı pistachio cultivar.

The characterization of the accessions using SSR markers is a quite effective tool for early selection in different breeding programs (Ashkenazi et al., 2001). The identified cultivar-marker specific to Uzun and Kırmızı cultivars can be used by many breeders, geneticists and even farmers in pistachio breeding programs and nurseries. In addition, several SSR markers have been used in many previous studies; for example; detection of disease resistance alleles in rice (Melaku et al., 2018), triploid apples (Mazeikiene et al., 2019) and the sex determination of sugarcane (Pan et al., 2006). Therefore, a total of 10 cultivar specific markers that can separate Uzun and Kırmızı cultivars without any preliminary screening of the primer pairs can be beneficial for future pistachio breeding programs.

Detection of genetic similarities of the pistachio accessions

Phylogenetic analysis of 20 cultivars and genotypes was performed using 25 SSR primers amplified in all *Pistacia* accessions. *Pistacia* individuals were clustered in two main groups by UPGMA analysis (Figure 1). *P. vera* cultivars and genotypes were included in the first group in the dendrogram, while wild types of *Pistacia* species were included in the second group. Genetic dissimilarity coefficients among all cultivars ranged from 0.00 to 1.00. The highest genetic distance was detected between *P. vera* genotypes and wild accessions. According to the UPGMA analysis, Uzun cultivar was clustered with Kırmızı-1, Kırmızı-2, Pv-1, Pv-2, Pv-4 and Pv-10 genotypes, and the genetic difference coefficient was 0.00. Thus, these genotypes may be same genetic structure with Uzun cultivar. On the other hand, they separated from Kırmızı pistachio cultivar in early step.

Cluster analysis of 20 cultivars and genotypes was performed using 25 SSR loci with the STRUCTURE and STRUCTURE HARVESTER programs. The highest Delta K (ΔK) value was obtained at $\Delta K=2$ (Figure 2). $\Delta K=2$ indicates the possible population number of cultivars and genotypes. Therefore, accessions were divided into two main clusters, similar to the UPGMA analysis results. The genetic relationships of the genotypes were detected by both UPGMA and STRUCTURE analysis, and the results of both programs were found compatible with each other (Figure 2).

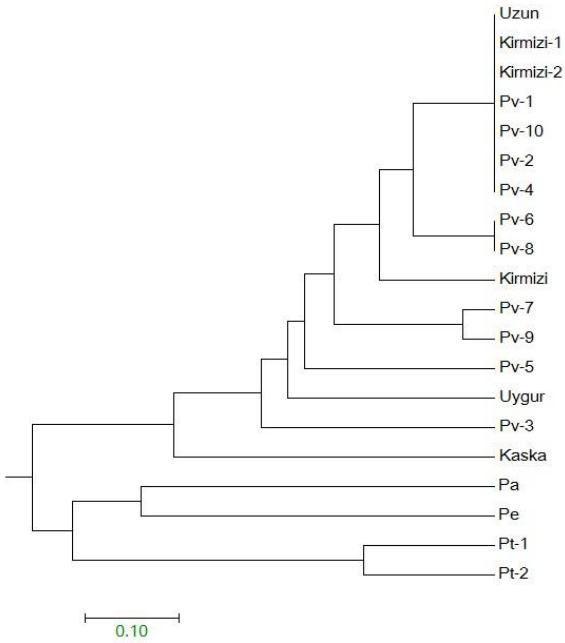

Figure 1. Dendrogram of UPGMA analysis of pistachio genotypes

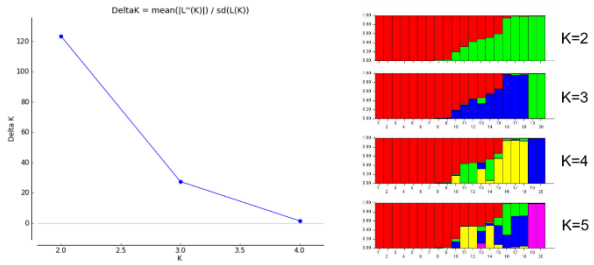

Figure 2. ΔK graph and K values of pistachio individuals consisted of the structure analysis

According to the SSR-based structural and UPGMA analysis clustering results, the original Kırmızı cultivar was clearly distinguished from the other Kırmızı-1 and Kırmızı-2 genotypes. However, many studies have reported that Kırmızı and Uzun cultivars were genetically close varieties (Khodaeiaminjan et al., 2018; Karcı et al., 2022). Similarly, Kaska and Uygur cultivars were reported by the same researchers to be close to wild *Pistacia* species, although they were included in *P. vera* cultivars. Similar results were obtained in both UPGMA and STRUCTURE programs in the current paper. The previous study performed by Kafkas et al. (2006) was carried out using ISSR, AFLP and RAPD markers, however these techniques have many disadvantages such as automation applicability, less reliability and reproducibility compared to the SSR marker technique. Therefore, although the results of the clustering were similar to their research; dominant marker systems are not practical for getting rapid results in the labs. In conclusion, the detected polymorphic and cultivar specific SSR markers may be preferred for geneticists and breeders to identify cultivars in the early stages of nursery and pistachio breeding studies. Thus, it can be saved for time and costs derived from extra laboratory processes, and prevention of mistakes based on the density of markers in the populations. Cultivar-specific SSRs can be used more accurately and quickly by geneticists and breeders studying *Pistacia* breeding.

Conclusion

DNA fingerprints of 20 genotypes belonging to *Pistacia* were identified by using 25 SSR markers and the accessions were characterized by two different genetic analysis methods. It has been proven once again that the SSR marker system is a pretty useful and efficient marker system in terms of the practical. In addition, it is quite significant to identify cultivars in breeding programs and nurseries in the early phase. In particular, the fact that Uzun and Kırmızı are the two most important cultivars preferred in the pistachio dessert industry due to their high aroma and green kernels, and the fact that these varieties can be genetically identified from other commercial cultivars or selections without the need for a preliminary screening analysis were important findings in terms of breeding. Cultivar-specific SSR markers presented can be also used in the identification of these two cultivars, without the need for long-term screening with many previously published SSR markers.

Declarations

Ethical Approval: This manuscript does not contain any studies with human participants or animals performed by the author.

Consent to Participate and Publish: The author reviewed and approved the final version for publication.

Acknowledgements

Special thanks to Prof. Dr. Salih KAFKAS for technical and scientific support. I also thank to for the material support of Kasım Boler.

References

Ahmad R, Ferguson L, Southwick S. M. 2003. Identification of pistachio (Pistacia vera L.) nuts with microsatellite markers. Journal of the American Society for Horticultural Science, 128(6): 898-903.

Ahmad R, Ferguson L, Southwick SM. 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.

Arabnezhad H, Bahar M, Pour AT. 2011. Evaluation of genetic relationships among Iranian pistachios using microsatellite markers developed from Pistacia khinjuk Stocks. Scientia horticulturae, 128(3): 249-254.

Ashkenazi V, Chani E, Lavi U, Levy D, Hillel J, Veilleux RE. 2001. Development of microsatellite markers in potato and their use in phylogenetic and fingerprinting analyses. Genome, 44(1): 50-62.

Baghizadeh A, Noroozi S, Javaran MJ. 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, 9(45): 7632-7640.

Caruso T, Iannini C, Barone E, Marra FP, Sottile F, Greco CI, Laghezali M. 1997. Genetic and phenotypic diversity in pistachio (P. vera L.) germplasm collected in Mediterranean countries. In II International Symposium on Pistachios and Almonds 470 (pp. 168-180).

Dollo L, Hormaza JI, Polito VS. 1995. RAPD Polymorphisms Among Pistachio (Pistacia vera L.) Cultivars.

Doyle JJ. 1987. A rapid isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull., 19: 11-15.

Fan L, Zhang MY, Liu QZ, Li LT, Song Y, Wang LF, Wu J. 2013. Transferability of newly developed pear SSR markers to other Rosaceae species. Plant Molecular Biology Reporter, 31(6): 1271-1282.

Güney M, Kafkas S, Koc A, Aras S, Keles H, Karci H. 2019. Characterization of quince (Cydonia oblonga Mill.) accessions by simple sequence repeat markers. Turkish Journal of Agriculture and Forestry, 43(1): 69-79.

Güney M, Kafkas S, Keles H, Aras S, Ercişli S. 2018. Characterization of hawthorn (Crataegus spp.) genotypes by SSR markers. Physiology and molecular biology of plants, 24(6): 1221-1230.

Hasanbegovic J, Hadziabulic S, Kurtovic M, Gasi F, Lazovic B, Dorbic B, Skender A. 2021. Genetic characterization of almond (Prunus amygdalus L) using microsatellite markersin the area of Adriatic Sea. Turkish Journal of Agriculture and Forestry, 45(6): 797-806.

Hormaza JI. 2002. Molecular characterization and similarity relationships among apricot (Prunus armeniaca L.) genotypes using simple sequence repeats. Theoretical and Applied Genetics, 104(2): 321-328.

Hormaza JI, Dollo L, Polito VS. 1994. Determination of relatedness and geographical movements ofPistacia vera (Pistachio; Anacardiaceae) germplasm by RAPD analysis. Economic Botany, 48(4): 349-358.

Kafkas S. 2006. Phylogenetic analysis of the genus Pistacia by AFLP markers. Plant Systematics and Evolution, 262(1): 113-124.

Kafkas S. 2006b. Phylogeny, Evolution and Biodiversity in the Genus Pistacia (Anacardiaceae). (A.K. SHARMA and A. SHARMA, Editor). Plant Genome, Biodiversity and Evolution. Volume 1, part C, Phanerogams (Angiosperm Dicotyledons), Science Publishers, Enfield(NH), Jersey, Plymouth, USA, pp 526-557.

Kafkas S. 2019. Advances in breeding of pistachio. Chapter. Burleigh Dodds Science Publishing Limited. Doi.10.19103/AS.2018.0042.17

Kafkas S, Ozkan H, Ak BE, Acar I, Atli HS, Koyuncu S. 2006. Detecting DNA polymorphism and genetic diversity in a wide pistachio germplasm: Comparison of AFLP, ISSR, and RAPD markers. Journal of the American Society for Horticultural Science, 131(4): 522-529.

Kafkas S, Perl-Treves R, Kaska N. 2000. Unusual Pistacia atlantica Desf.(Anacardiaceae) monoecious sex type in the Yunt Mountains of the Manisa Province of Turkey. Israel Journal of Plant Sciences, 48(4): 277-280.

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, 1-13.

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.

Kashaninejad M, Tabil LG. 2011. Pistachio (Pistacia vera L.). In Postharvest biology and technology of tropical and subtropical fruits (pp. 218-247e). Woodhead Publishing.

Khadivi A. 2018. Assessment of genetic variability in pistachio (Pistacia vera L.) with nuclear SSR molecular markers. Erwerbs-Obstbau, 60(4): 289-294.

Khodaeiaminjan M, Kafkas S, Motalebipour EZ, Coban N. 2018. In silico polymorphic novel SSR marker development and the first SSR-based genetic linkage map in pistachio. Tree Genetics & Genomes, 14(4): 1-14.

Kolahi-Zonoozi SH, Mardi M, Zeinalabedini M, Pirseyedi SM, Mahmoodi P, Tabatabaei I, Ahmadi K. 2014. Development of 12 new SSR markers for genetic diversity and structure analysis in pistachio (Pistacia vera L.). The Journal of Horticultural Science and Biotechnology, 89(6): 707-711.

Lui K. 2005. PowerMarker: integrated analysis environment for genetic marker data. Bioinformatics, 21: 2128-2129.

Mažeikienė I, Šikšnianienė JB, Baniulis D, Gelvonauskienė D, Frercks B, Starkus A, Stanys V. 2019. SSR analysis based on molecular characterisation of apple germplasm in Lithuania. Zemdirbyste-Agriculture, 106(2): 159-166.

Melaku G, Zhang S, Haileselassie T. 2018. Comparative evaluation of rice SSR markers on different Oryza species. J Rice Res Dev, 1(1): 38-48.

Orman E, Ateş D, Özkuru E, Hepaksoy S, Kafkas S, Tanyolac MB. 2020. Association mapping of several nut characters in walnut (Juglans regia L.). Turkish Journal of Agriculture and Forestry, 44(2): 208-227.

Pan YB. 2006. Highly polymorphic microsatellite DNA markers for sugarcane germplasm evaluation and variety identity testing. Sugar Tech, 8(4): 246-256.

Pazouki L, Mardi M, Shanjani PS, Hagidimitriou M, Pirseyedi SM, Naghavi MR, Khayam Nekoui SM. 2010. Genetic diversity and relationships among Pistacia species and cultivars. Conservation genetics, 11(1): 311-318.

Peakall, R, Smouse, PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular ecology notes, 6(1): 288-295.

Potts SM, Han Y, Khan MA, Kushad MM, Rayburn AL, Korban SS. 2012. Genetic diversity and characterization of a core collection of Malus germplasm using simple sequence repeats (SSRs). Plant Molecular Biology Reporter, 30(4): 827-837.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics, 155(2): 945-959.

S Kumar, G Stecher, M Li, C Knyaz, K Tamura. 2018. MEGA, X. molecular evolutionary genetics analysis across computing platforms; Molecular Biology and Evolution, 35(1): 1547-1549.

Schuelke M. 2000. An economic method for the fluorescent labeling of PCR fragments. Nature biotechnology, 18(2): 233-234.

Topcu H, Kafkas S, Dogan A, Akcay ME, Ercisli S. 2015. Genetic relatedness among quince (Cydonia oblonga Miller) accessions from Turkey using amplified fragment length polymorphisms. Journal of Applied Botany and Food Quality, 88(1).

Tunalıoglu R, Taskaya B. 2003 Antepfıstıgı Tarımsal Ekonomi Araştırma Enstitüsü T.E.A.E Bakıs. 5(2): 1-4.

Vendramin E, Dettori MT, Verde I, Micali S, Giovinazzi J, Mardi M, Quarta R. 2007. Molecular characterization of Pistacia genus by microsatellite markers. In I Balkan Symposium on Fruit Growing 825 (pp. 55-62).

Zaloğlu S, Kafkas S, Doğan Y, Güney 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.

Ziya 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(1): 1-14.

Zohary M. 1952. A monographical study of the genus Pistacia. Palestine Journal of Botany (Jerusalem Series), 5(4): 187-228