



SSR markers-based molecular characterization and genetic diversity in pomegranate (*Punica granatum* L.) genotypes

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Received: 18 December 2024 / Accepted: 8 January 2025 / Published online: 13 March 2025
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Abstract Pomegranate (*Punica granatum* L.) is the most important Lythraceae family species of the Myrtales order. It is one of the quite old fruit species and the pomegranate history goes back about five thousand years, is consumed by people for food and medicine. In the current study, 40 simple sequence repeat (SSR) primers were screened to determine the genetic relationships of 127 pomegranate genotypes sampled from distinct locations with genetic resources in Türkiye. In total, 34 SSRs generated a total of 177 alleles, the average number of effective alleles ($N_e=2.50$) and the average expected heterozygosity value ($H_e=0.53$) were estimated. The observed heterozygosity was measured for each marker with an average of 0.36. The average polymorphism information content (PIC) was estimated as 0.48. Phylogenetic tree was constructed using a total of 34 polymorphic markers based on unweighted pair group method with arithmetic mean (UPGMA) analysis of pomegranate genotypes, and genotypes

were clustered into two major groups with subgroups. STRUCTURE analysis was also carried out to calculate the possible population number according to Delta K value that has picked the point corresponding to population number. Delta K was picked in $K=2$, population was divided to two main clusters. The results of UPGMA and STRUCTURE analyses were examined, and both analyses were found to corroborate each other. The analysis of molecular variance exhibited 38 and 36% of the total variance between and within the populations. This is the first comprehensive molecular report enjoyed in Türkiye germplasm for pomegranate. These results can be utilized to establish novel breeding orchards or consist of the populations and to reveal the marker-trait association. Hence, it brings knowledge quality in pomegranate breeding programs.

Keywords Pomegranate · *Punica granatum* L. · Molecular characterization · SSR · Structure

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10722-025-02336-9>.

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Introduction

Punica granatum L. belongs to the Lythraceae family, and it is cultivated in subtropical, tropical and temperate climate regions. Pomegranate is spread over a wide geography and the pomegranate cultivation is performed in Iran, Türkiye, Tunisia, Spain, Morocco, Egypt, China, the United States, India, and East Asia (Valero-Mendoza et al. 2023).

Global pomegranate market size was worth around \$208.9 million in 2019 and Türkiye led global pomegranate exports with 155,000 tons, accounting for 23% of worldwide exports (626,336 tons) (Pienaar 2021). World pomegranate production was about 8 million tons in 2019 (Yılmaz 2023; Pienaar 2021). The main pomegranate producer countries were India and China, which recorded a production exceeding 3 and 1 million tons, respectively (Yuan and Zhao 2017; Pienaar, 2021), while pomegranate production of Türkiye has been reached 638,821 tons in 2023 according to Turkish Statistical Institute (TUIK 2024).

The increasing production of the pomegranate all over the world was owing to its higher nutrition values in the last decades. The increasing demand for pomegranate cultivation was linked to its use in the pharmaceutical industry, advancements in food processing technologies, and improvements in transportation and storage methods (Şen and Eroğul 2012). Recently, the demand for pomegranate production had many positive effects due to its benefits on human health with the therapeutic properties of pomegranate (Sharmila et al. 2014). Pomegranate juice is rich in natural sugars like fructose, sucrose, and glucose, along with organic acids such as ascorbic acid, citric acid, fumaric acid, and malic acid. It also contains small amounts of all amino acids, with notable levels of proline, methionine, and valine (Zarfeshany et al. 2014). The pomegranate is a good source of polyphenols. The main flavonoids include anthocyanins, luteolin, kaempferol and quercetin (Napoli et al. 2023). About 60% of pomegranate weight, primarily consist of mesocarp, endocarp and exocarp, however, these byproducts are a valuable source of bioactive polyphenols, including flavonoids, anthocyanins, tannins, and ellagitannins like punicalagin (Aviram et al. 2000; Marrone et al. 2024). Pomegranate provides significant health benefits by strengthening the immune system and preventing free radical formation, thanks to its high antioxidant and anti-radical properties (Zarfeshany et al. 2014; Marrone et al. 2024). Pomegranates can help prevent or treat various disease risk factors including high blood pressure, high cholesterol, oxidative stress, hyperglycemia, and inflammatory activities (Zarfeshany et al. 2014).

In recent decades, advancements in software and marker technologies, supported by various sequencing platforms, have significantly contributed to plant breeding. These innovations have enhanced our understanding of genomic domestication and diversity in agriculturally important species (Duminil and Di Michele 2009; Şimşek et al. 2017). Molecular methods, which are not influenced by environmental factors, can be applied at any stage of plant breeding. These methods offer high diversity, generate reliable data, and have become integral to modern plant breeding practices (Rafalski et al. 1996; Farooq and Azam 2002; Ribaut et al. 2010; Jonah et al. 2011; Nadeem et al. 2018; Hasan et al. 2021). In this context, several attempts at the construction of chromosome-scale genome sequences in pomegranate were carried out by distinct research teams using different domestic cultivars (Yuan et al. 2018; Luo et al. 2020; Sowjanya et al. 2022; Yan 2024). They highlighted the dissections of the fruit quality traits and their genomic responsibility thanks to high-quality reference genome assembly. Thus, the genic or genomic marker development and molecular investigations using these genome assemblies can be applied and more accurate and reliable results reveal for pomegranate breeding (Patil et al. 2021; Wang et al. 2023; Sagar et al. 2024; Pandey et al. 2024).

Among these, simple sequence repeat (SSR) markers have gained prominence as a codominant marker system. They are widely distributed across the genome and are species-specific, with high transferability to closely related species within the same genus (Karcı et al. 2022). Due to their reliability in providing insights into genetic diversity and heredity, SSR markers are widely utilized for characterizing and identifying species, cultivars, and populations (Kafkas 2019). Thus, SSR marker technique prefers to perform the genetic diversity or molecular characterization research due to its codominant inheritance, adaptation to automation, and in particular, a quite reliable system, as the results cannot change in the repetition of the reactions. Research employing SSR markers for DNA fingerprinting, genetic diversity and characterization, detection of relationship among the plant genetic resources, transferability of the SSRs to closely related species and identification of species or variety specific markers have been extensively reported in numerous species, including citrus (Ruiz

et al. 2000), apricot (Zhebentyayeva et al. 2003; Sánchez-Pérez et al. 2005), almond (Xu et al. 2004; Shiran et al. 2007; Zeinalabedini et al. 2010), apple (Zhang et al. 2012), pear (Kimura et al. 2002; Fan et al. 2013), walnut (Chen et al. 2014; Bernard et al. 2018), pistachio (Karcı et al. 2022), and pomegranate (Liu et al. 2020; Gunnaiah et al. 2021). These investigations underscore the critical role of SSR markers in advancing genetic research and breeding strategies.

Constructing segregating populations and identifying genomic and genic loci linked to agronomic traits are fundamental steps in plant breeding. These processes are essential for developing new lines and identifying promising genotypes with desirable characteristics. In pomegranate, the characterization of the entire germplasm, including naturally cultivated genotypes, is crucial to capturing the wide genetic variability present among individuals. Historically, breeding efforts in pomegranate have primarily focused on pomological traits, with less emphasis on fruit quality traits, which have been prioritized more recently (Alighourchi et al. 2008; Sarkhosh et al. 2009). While several investigations have explored genetic diversity and characterized local pomegranate germplasm using various marker techniques (Liu et al. 2020; Gunnaiah et al. 2021; Koş and Erdem 2024), comprehensive molecular diversity and phylogenetic analyses specifically involving pomegranate samples from Türkiye remain limited. Although Türkiye has extremely important pomegranate genetic resources, approximately fifty pomegranate domestic cultivars were registered in Türkiye. The registered pomegranate cultivars have a wide genetic diversity in terms of the size of the fruits and several fruit quality traits such as sweetness, peel and flesh color of the fruit. Thus, this highlights the need for further research to understand this important crop's genetic relationships and diversity. Hence, we investigated a total of 127 pomegranate genotypes gathered from Türkiye using the 34 polymorphic SSR markers for the first time. The objectives of the present report were (i) to detect the genetic diversity of SSR markers using the pomegranate germplasm, (ii) to identify population structure and relationships of the pomegranate plants sampled from Türkiye, and (iii) to provide the basis for future pomegranate breeding.

Materials and methods

Plant material and DNA extraction

The local genotypes (125) and two (2) commercial cultivars Wonderful and Hicaz were used as plant materials. The flesh leaves of plants were sampled from Birecik, Halfeti and Suruç districts of Şanlıurfa province, located in the Southeastern Anatolia Region, where the pomegranate population is increasingly widespread in Türkiye (Supplementary Table 1).

DNA extraction of frozen leaves stored in -80 °C refrigerator was carried out using the CTAB method (Doyle and Doyle 1987), with minor modifications performed by Kafkas et al. (2006). The quality and yield of DNAs were evaluated using Qubit Fluorometer (Invitrogen) with standard protocols. The clear extracted DNAs were diluted to 10 ng/μl to perform the PCR analysis and they were stored at -20 °C.

PCR analysis and electrophoresis

In total, 40 SSR primers developed by several researches for pomegranate were screened to detect polymorphism in eight pomegranate genotypes. A set of 34 polymorphic SSRs were selected for the characterization of 127 pomegranate genotypes based on scorable and clear bands (Table 1). A three primer strategy according to Scheulke (2000) was followed for SSR-PCR reactions. SSR-PCR reactions and capillary electrophoresis for each primer were performed according to Karcı et al. (2022). The capillary electrophoresis was carried out in an ABI 3130xl genetic analyzer machine that has a branded Applied Biosystems Inc., Foster City, CA, USA (ABI). The data collection of samples in ABI genetic analyzer evaluated the fragments. Then fragments of SSR markers was scored using the GeneMapper v.4.0 (Applied Biosystems Inc.).

Genetic diversity and population structure analyses

The scored fragments of the SSR markers in capillary electrophoresis were used to calculate the genetic diversity properties such as the number of alleles per locus (Na), the effective number of alleles (Ne),

Table 1 List of the polymorphic simple sequence repeat (SSR) markers used in the present study

No	Markers	Forward (3'-5')	Reverse (5'-3')	Annealing Temp °C	References
1	RiM036	AGCAACCACCACCTCAAC TAAT	CTAGCAGAATCACCTGAG GCTT	52	Castillo et al. (2010)
2	RhM003	CCATCTCCAATTCAGTTCTTCC	AGCAGAATCGGTTCTTAC AAGC	52	
3	RhM001	GGTTCGGATAGTTAATCCTCCC	CCAAGTGTGTAAATGCA GGAA	52	
4	GAPU103	TGAATTTAACTTTAAACCCAC ACA	GCATCGCTCGATTTTATCC	52	Carriero et al. (2002)
5	GD147	TCCCGCCATTTCTCTGC	AAACCGCTGCTGCTGAAC	52	Hokanson et al. (1998)
6	UDO24	GGATTTATTAAGCAAAACA TACAAA	CAATAACAAATGAGCATGATA AGACA	52	Cipriani et al. (2002)
7	PG080	CTGACTGTTGCAGAGAGTAGG CTG	AGGAGGTGAAACAACGAATAG CTG	55	Liu et al. (2020)
8	PG093	CGTCAATAGGACGTCCCTGAG ATA	GATGACGTGGCAGAGTAAGAG AGC	55	
9	PG090	ATTCTTTTATACTAACCAAAA TTTGCGA	ATGTCATGAGAGGACCCA CAAA	55	
10	pg18	TCTAAGGGCAGAATGGCACT	TGGCACTAGATCCGTAAATCTC	59	Parvaresh (2012)
11	M03	CCCAGTAATCACCCATTTG	GTGGACATAAATAATTGCTGA AGA	56	Luo et al. (2018)
12	PGKVR026	TAACCTGGCCACTTCCTTTG	GGAGAGTCCGTCCCTTTAG	55	Ravishankar et al. (2015)
13	PGKVR027	CGATAAAAGCCCAACACGAT	ACCAATGGAGACGAAACTGG	55	
14	PGKVR028	TCGATCGTTTCATTCTCATTG	CCGACGTTAGATATGGGCTTA	55	
15	PGKVR029	TTCGATTCACCTTCCTTTCG	GGCCTAGGAAGCTGACACAC	55	
16	PGKVR030	TGTGTCCTAGTTTAGGAGAAC AATCA	CATCCAAATGGCCTCAGATAA	55	
17	PGKVR031	CCCTAACAATTATCCCAT TTGGT	GTGCGTCCAAAAGCTTTCAT	55	
18	PGKVR064	GCTGAGGAACAAGTCGAAGG	TTCGCTGCCAATAATTTTCC	55	
19	PGKVR065	CTGCTTCACAGTCGTGGAAG	TTTTATTTTCTTGCAGTCAC AAA	55	
20	PGKVR078	TAATGCAGGAAGAGCCCATT	TTCAGGCTCCTCTTCCTCAA	55	
21	PGKVR098	CGGTCGAAGGCTCACATATT	CCCCCTAAAAATATCCACTACA	55	
22	PGKVR099	CCCTACCCCACTAGGAGAG	GGATCTTATGCGACGGGATA	55	
23	PGKVR100	CCCATCGATCATCTTTCGTT	CGTGTGGAAAGTCATCGACA	55	
24	PGKVR105	GGAAGAGTTGGACTGGGAGA	CCGCTCCTTCTACTCCTCCT	55	
25	PGKVR111	ACAAGCTAAGCCGCTACTG	CTCCCGACGTCAAAGTTCAT	55	
26	PGKVR114	AGGGAGATTATGCGGGAGTT	CCGAGCTCTACTCCCTCTCC	55	
27	PGKVR127	TGTCACGGTTTTAGGTGCAA	GGGAGTGTGATGGAAGAGGA	55	
28	PGKVR129	ACATTGTCAACGACCGGATT	CGTGCGATACTGCTACCTGA	55	
29	PGKVR131	TTTGTTCGCGATAGCACAAG	AAGGAACCTGGGCATAAGGT	55	
30	PGKVR135	ACCTGAGCCACATCGATAC	TCGGGATATCGTAATCCTCTTG	55	
31	PGKVR136	GGTTCGGGAATTGTAAACG	GCTGCGAGAGAACGAAGAAC	55	
32	PGKVR153	TTTGGAAGTTTGACTGGAGGA	GCATTCTGGGATTGGAAGAA	55	
33	PGKVR154	GGCAATCAGTGCCATGAATA	CCAATCGAATTGAATGCTCA	55	
34	PGKVR165	TGGGCCATTATAGGCAAAG	CTTTTCCCGGTTTCTCCTC	55	

observed heterozygosity (H_o) and expected heterozygosity (H_e) in the GenAIEx v.6.5 program based on Excel Microsoft Office (Peakall and Smouse 2012). And, PowerMarker software v.3.25 for the calculation of the polymorphism information contents (PIC) value of each SSR markers in this study (Liu and Muse 2005).

The cluster analysis was performed using the UPGMA (Unweighted Pair Group Method with Arithmetic Average) algorithm in MEGAX software (Kumar et al. 2018). While population structure study was performed using model-based software program STRUCTURE v.2.3.4 (Pritchard et al. 2000), the results were outputted with printed graphs that a number of populations (K) from STRUCTURE HARVESTER. A set of allele frequencies calculated for each locus that described the $\ln P$ (D) values (logarithm probability for each K) were used for determination of the Delta K values. The change ratio of logarithm probability was set from $\Delta K=2$ to $\Delta K=10$. The highest K of Delta K refers to the probable population number. Arlequin v.3.5 software was used to determine the pairwise genetic distance (F_{ST}) for subpopulations, to calculate genetic variation within and between populations, and geographic groups by molecular analysis of variance (AMOVA) (Excoffier and Lischer 2010). An F_{ST} value of 0 means that there is no genetic variation between subpopulations, while an F_{ST} value of 1 indicates extreme fragmentation in the whole population. Genetic differentiation is considered insignificant at a mean value of 0.05, while 0.25 to 1 indicates strong fragmentation, 0.05 to 0.15 and 0.15 to 0.25 indicate low and moderate differentiation, respectively (Mohammadi and Prasanna 2003).

Results

Genetic diversity of SSR markers among Türkiye pomegranate genotypes

Initially, a total of 40 SSR primer pairs were screened to detect polymorphism-level in pomegranate using eight genotypes. Then, the selected polymorphic 34 SSR markers were used for molecular characterization, the remaining markers from 40 SSRs were eliminated due to their monomorphic bands. Amplification fragments of 34 SSR markers

were subsequently utilized as input to perform characterization of the pomegranate genotypes (Table 2).

In total, 177 alleles were produced for all genotypes, and the average number of alleles was calculated 5.21 and the highest number of alleles was obtained from the PGKVR031, RhM003 and M03 locus ($N_a=9$). The number of effective alleles (N_e) ranged from 1.04 (PGKVR026) to 5.51 (PGKVR078) with an average of 2.50. The observed heterozygosity (H_o) was calculated with a mean of 0.36. The observed heterozygosity (H_o) was highest in the PGKVR028, RhM001, GD147, M03 and PGKVR065 markers. The average value of expected heterozygosity (H_e) was 0.53 and the highest value (0.82) was created by PGKVR078 marker. The highest Polymorphism information content (PIC) was recorded to be 0.79 for PGKVR078 marker with an overall average of 0.48 (Table 2). The average number of polymorphic markers per primer was calculated as 5.21. A wide allelic range was detected in the GAPU103 marker and the allelic range was ranged from 352 to 509 bp.

Molecular characterization of Türkiye pomegranate genotypes

The dendrogram was created using the UPGMA algorithm, and detected the relationships among the genotypes and results are shared in Fig. 1. In the cluster analysis, 127 pomegranate genotypes were basically divided into two main groups (Group I and Group II). Both main groups were separated to two subgroups within themselves. Group I was subdivided into A and B and Group II was subdivided into C and D, respectively. Group I contain the samples from Suruç and Halfeti districts including several plants sampled from Birecik district. In addition, Group II has the plants sampled from Suruç and Birecik districts of the Şanlıurfa province. In this population, the closest genetic similarity (0.010) in the Group I was created among 63SUR61 and 63SUR62 pomegranate genotypes, while the furthest genetic similarity (0.909) was calculated between 63HAL06 and 63BIR100, 63BIR101, 63BIR102 genotypes. The highest genetic dissimilarity coefficient (0.906) in the Group IA was obtained between 63SUR122 and 63SUR49 accessions. On the other hand, the lowest genetic dissimilarity coefficient (0.010) in the Group IB was detected

Table 2 Allele range, number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), and polymorphism information content (PIC) of simple sequence repeat (SSR) markers in pomegranate germplasm

No	SSR loci	Allele range	Na	Ne	Ho	He	PIC
1	PGKVR026	165–167	3	1.04	0.01	0.04	0.04
2	PGKVR027	103–110	7	3.64	0.00	0.73	0.69
3	PGKVR028	122–244	8	4.01	1.00	0.75	0.71
4	PGKVR029	139–146	8	4.65	1.00	0.78	0.75
5	PGKVR030	179–180	2	1.16	0.00	0.14	0.13
6	PGKVR031	185–322	9	2.63	0.13	0.62	0.60
7	PGKVR064	258–260	2	1.43	0.37	0.30	0.26
8	PGKVR078	136–145	8	5.51	0.59	0.82	0.79
9	PGKVR098	218–219	2	1.17	0.00	0.14	0.13
10	PGKVR099	225–303	6	2.19	0.00	0.54	0.47
11	PGKVR100	302–304	3	1.61	0.00	0.38	0.32
12	PGKVR105	136–191	4	1.67	0.00	0.40	0.37
13	PGKVR111	157–284	6	3.37	1.00	0.70	0.65
14	PGKVR114	204–224	3	2.13	0.93	0.53	0.42
15	PGKVR127	169–267	3	1.93	0.32	0.48	0.43
16	PGKVR129	153–164	6	2.46	0.00	0.59	0.55
17	PGKVR131	245–264	4	2.08	0.00	0.52	0.41
18	PGKVR135	207–225	3	3.00	0.67	0.67	0.59
19	PGKVR136	138–173	5	1.74	0.00	0.43	0.40
20	PGKVR153	172–188	5	1.81	0.00	0.45	0.41
21	pg18	185–221	8	3.22	0.49	0.69	0.67
22	PG093	148–218	5	2.76	0.11	0.64	0.59
23	RhM003	136–372	9	4.09	0.15	0.76	0.72
24	RhM001	169–188	3	2.59	1.00	0.61	0.54
25	GAPU103	352–509	5	2.28	0.00	0.56	0.51
26	UDO24	142–145	4	3.47	0.00	0.71	0.66
27	PG080	158–218	6	2.50	0.00	0.60	0.55
28	PGKVR165	141–321	7	1.29	0.24	0.22	0.22
29	RIM036	133–193	5	1.29	0.14	0.23	0.22
30	GD147	191–303	4	2.72	1.00	0.63	0.57
31	PG090	143–251	8	1.70	0.20	0.41	0.39
32	M03	258–290	9	3.11	1.00	0.68	0.63
33	PGKVR065	275–285	3	2.37	1.00	0.58	0.49
34	PGKVR154	119–182	4	2.40	0.87	0.58	0.52
	Total	–	177	–	–	–	–
	Mean	–	5.21	2.50	0.36	0.53	0.48

in a pairs of genotypes 63HAL01, 63HAL02, 63HAL03 and 63HAL04 genotypes. In the Group IIC, the closest relationship (0.083) was among 63SUR123 and 63SUR122 genotypes and the furthest genetic distance (0.906) was between 63HAL23 and 63SUR121, 63SUR122 genotypes. And, the highest genetic dissimilarity value was computed as 0.100 among the 63BIR102 and 63BIR101 (Supplementary Table 1; Fig. 1).

Structure analysis was performed in 127 pomegranate genotypes using 34 amplified polymorphic SSR marker alleles by using STRUCTURE program and the results were printed by STRUCTURE HARVESTER online web interface. Pomegranate genotypes were basically divided into two main groups [Group I (A and B) and Group II (C and D)]. The highest value of Delta K (ΔK) and possible population number was picked at $K=2$ (Fig. 2). In

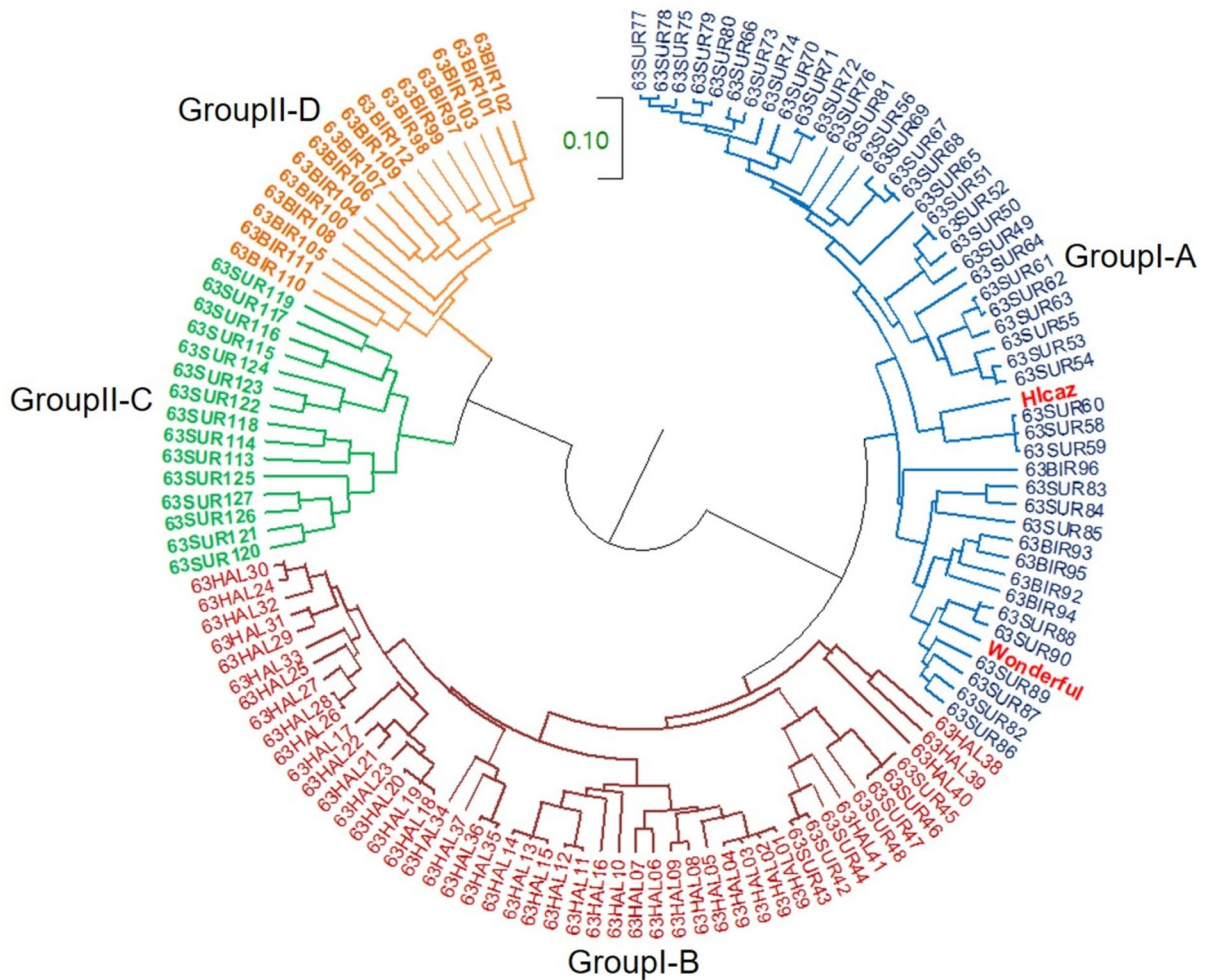
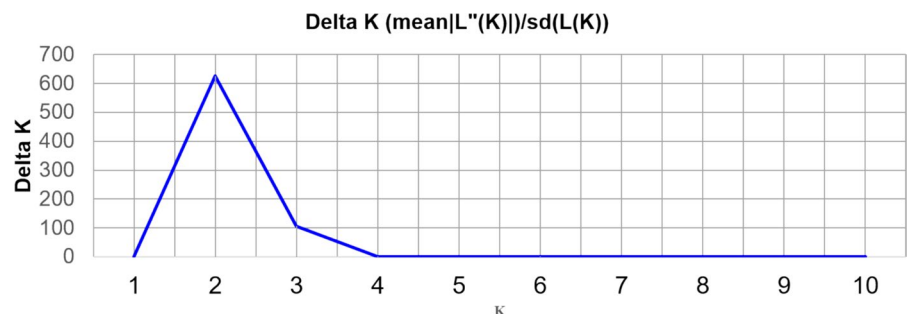


Fig. 1 The phylogenetic tree of pomegranate genotypes created using unweighted pair group method with arithmetic mean (UPGMA) dendrogram

Fig. 2 The graph of Delta K estimated for the structure analysis of pomegranate genotypes (Delta K = 2)



the dendrogram and structure analyses from $\Delta K=7$ to $\Delta K=10$, genotypes of group C and D (Suruç and Birecik group genotypes) were clearly separated from the each other because of the absence of gene flow

and therefore common alleles. Thus, all genotypes were classified into two major clusters, and STRUCTURE and UPGMA analyses were verified each

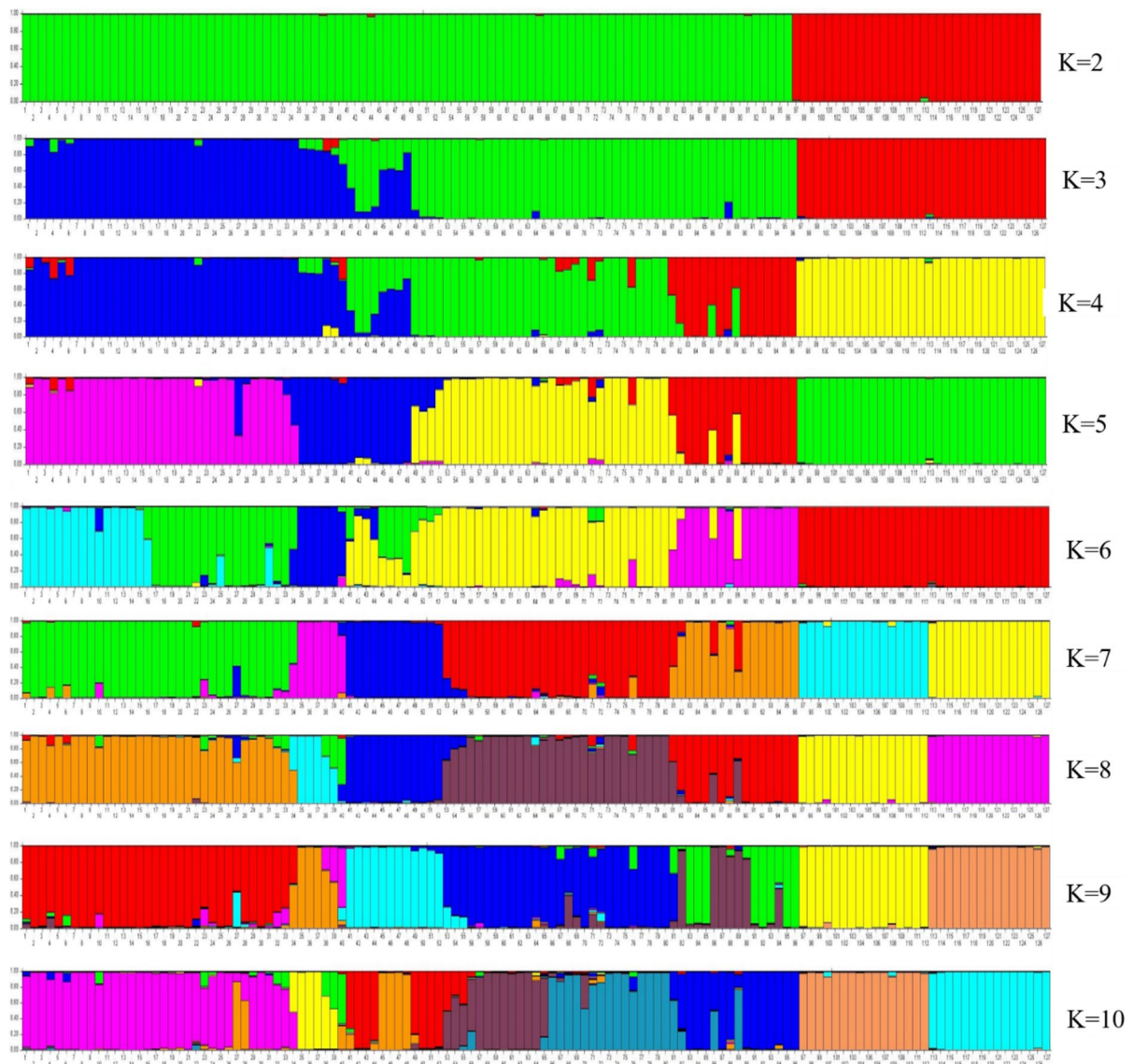


Fig. 3 The colored figures refer to the number of possible populations from $\Delta K = 2-10$

Table 3 Molecular analysis of variance (AMOVA) of pomegranate genotypes

Mean values in the column are not significantly different at $\alpha = 0.001$

Variation source	Degrees of freedom	Total squares	Squares mean	Variance%
Between Populations	3	732.816	244.272	38
Between Individuals	123	1398.321	11.368	36
Intra-individuals	127	385	3.031	26
Total	253	2516.138		100

other according to clustering branches or with shared common alleles of the genotypes (Fig. 3).

AMOVA analysis of population

The distribution of genetic variation among the 127 pomegranate individuals and geographic groups used in the study is shown in Table 3. According to the results of the analysis of variance, 38% of the total genetic variation occurred between populations, 36% between individuals within populations and 26% intra individuals (Fig. 2). The highest genetic variation was realized between populations and the least genetic variation was realized between individuals within populations. According to the results of molecular analysis of variance, the differences between all populations were significant ($p < 0.001$) (Fig. 4, Table 3).

Discussion

Polymorphism degree of SSR markers in pomegranate

SSR markers are widely recognized as a fast and reliable DNA-based marker technique. Their extensive utilization in recent years can be attributed to their versatility, as SSR markers developed for one species are often transferable to related species. Numerous investigations have investigated the genetic diversity and relationships in pomegranate using various molecular marker methods, including RAPDs, AFLPs, ISSRs, SSRs, and SRAP (Zamani et al. 2007; Ercisli et al. 2011; Attanayake et al. 2017; Bengül 2019; Gunnaiah et al. 2021). A total of 127 pomegranate genotypes were used in the

study and the 34 SSR markers produced a total of 177 alleles. An average number of alleles ($N_a = 5.21$) were determined from a total of 34 SSR markers. The average number of allele value detected from previous research were 3.16 (Parvaresh et al. 2012), 5.56 (Ravishankar et al. 2015), 12.19 (Çetinkaya et al. 2019), 3.14 (Patil et al. 2020). The current study of the average effective number of allele was 2.50, although this value was examined as 1.87, 4.44 and 2.47 by Parvaresh et al. (2012), Çetinkaya et al. (2019), Liu et al. (2020) respectively. Although PIC value in this study was identified with average of 0.48, the average PIC value of this study was higher than 0.35, 0.45 and 0.33, respectively (Parvaresh et al. 2012; Liu et al. 2020; Gunnaiah et al. 2021), while it was lower than 0.54 and 0.64 values identified by previous reports (Ravishankar et al. 2015; Çetinkaya et al. 2019). An average expected heterozygosity (H_e) was 0.53, while average H_o value were 0.36 in this paper. These values (H_e and H_o) have been reported distinct ranges, for instance; average H_e and H_o were calculated as 0.24 and 0.24 (Hasnaoui et al. 2012), as 0.72 and 0.64 (Çetinkaya et al. 2019), as 0.55 and 0.41 (Liu et al. 2020). The differentiation of the genetic diversity values was due to more or less polymorphic SSR markers. That means monomorphic SSR markers affect the mean allele counts. Especially, if there is a limited genetic association within the genetic resources, it can be reasoned the decreasing allelic diversity values. On the other hand, narrow genetic diversity rate within the population cannot reflect the polymorphism degree of the SSRs (Zhao et al. 2023; Zhang et al. 2023).

Identification of cultivars and genotypes using SSR markers is a pretty effective tool, particularly for genetic diversity and molecular characterization research and early-stage selection in plant breeding programs (Ashkenazi et al. 2001). However, there was a limited number of SSR markers developed by previous reports in pomegranate. In other words, there were no more options to select more polymorphic SSRs of earlier findings. Even if, SSRs haven't developed in a wide genetic resource, polymorphism degree cannot be guessed more accurately. Thus, SSR markers exhibited as polymorphic in the literature may not be able to create polymorphism in another population (Zhao et al. 2023; Zhang et al. 2023). In the present paper, the selected SSR markers from distinct references have not generated a quite

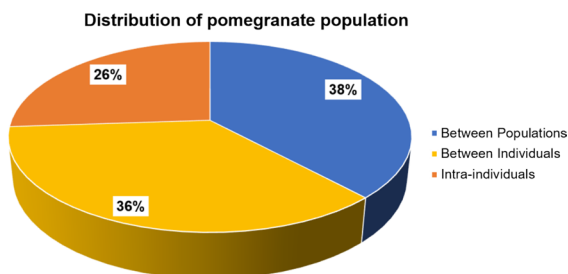


Fig. 4 Distribution of pomegranate genotypes according to molecular variance analysis

polymorphism, although the most polymorphic SSRs were selected. As a result, more SSR markers with higher polymorphism degrees should be used for diversity and molecular characterization research to reveal the variation among the germplasm and individuals. In addition to used genomic SSR markers, recently developed genic simple sequence repeats (eSSRs) can be preferred to identify the distribution and variation in whole pomegranate genome. The genic or genomic SSRs in terms of polymorphism can be compared using pomegranate germplasm sampled from distinct countries' trees. Thus, the polymorphism levels in both markers and genotypes can be detected and used for future pomegranate breeding.

Genetic characterization among pomegranate genotypes

The SSR-based genetic characterization, cluster analysis based on the UPGMA approach produced similar results with structure analysis on the genetic relationships of the pomegranate genotypes. In cluster analysis, two main groups (Group I and Group II) were consisted of the genotyped individuals and both main groups formed two (2) subgroups within themselves. Even though plants were collected from distinct locations, some genotypes were included in clusters with other locations. Wonderful and Hicaz commercial cultivars were evaluated to reveal a relationship of them among the all genotypes. And, 63SUR58, 63SUR59 and 63SUR60 sampled from Süruç districts were the closest to Hicaz, while 63SUR88 and 63SUR90 genotypes were identified the closest to Wonderful pomegranate cultivar.

Gene flow in plant breeding refers to the transfer of genetic material among quite close locations for grafting or through pollination, hybridization, or seed dispersal with birds. Gene flow can sometimes introduce new traits such as disease resistance, enhanced fruit quality, or environmental adaptability in breeding programs. However, uncontrolled gene flow can also lead to the spread of unwanted traits or reduce genetic diversity within the species, potentially affecting conservation efforts (Chagné et al. 2008; Evans and Campbell 2014). Thus, gene flow can lead to a process in which the genetic identity of a local population is negatively affected by the influx of genes from another population. Then, this can

result in reduced genetic diversity and the potential loss of locally adapted traits, which are crucial for the population's survival in specific environmental conditions (Allendorf et al. 2001). In this study, samples from distinct locations were grouped into different clusters in the cluster analysis. However, some accessions collected from geographically close locations were grouped into the same subgroup. This indicates a high rate of gene flow among individuals through various mechanisms.

According to the results, it is seen that all studied populations differ significantly from each other in the present paper. Similar results were obtained in research using other molecular markers. Noormohammadi et al. (2012) performed AMOVA analysis on 36 pomegranate genotypes in Iran to determine genetic diversity using ISSR, SSR and RAPD markers and found that only 2% of the total molecular variation was due to differences between populations, while 98% of the variation was due to within-population differences. Madadi et al. (2017) determined the genetic diversity and phylogenetic relationships of 39 pomegranate genotypes in Iran using 13 ISSR and 15 SSR primers. The researchers found that 17% of the total genetic diversity of pomegranate genotypes grown in 4 different regions was between populations and 83% was within populations.

Conclusion

This study underscores the utility of SSR markers for the molecular characterization of pomegranate genetic resources in Turkey, focusing on the Şanlıurfa province. The application of 34 SSR markers on 127 genotypes revealed notable polymorphism and geographical clustering. The promising and the most polymorphic SSR marker was identified as the PGKVR078 marker with nine (9) alleles, 5.51 effective alleles, and 0.79 polymorphism information content (PIC) values, which can be suggested for molecular research. On the other hand, PGKVR026 and PGKVR098 SSR markers generated the least allelic diversity values despite a wide pomegranate germplasm. Despite some limitations inherent to SSR markers, they proved to be an effective and reliable method for fingerprinting, germplasm evaluation,

and identifying specific markers for breeding purposes. These findings contribute valuable insights into genetic diversity and can inform parent selection for future cultivar breeding programs.

Acknowledgements The authors thank the Van Yüzüncü Yıl University, Scientific Research Projects Unit (Project No: 9622) for financial support.

Author contributions Y.P., H.K. Conceptualization, Methodology, Investigation, Supervision, Experiment, Writing – original draft. F.C: Investigation, Experiment, Resources. N.E.K, S.K: Conceptualization, Methodology, Formal analysis, Supervision, Writing-review & editing.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK). Van Yüzüncü Yıl University Scientific Research Projects Unit, Project No: 9622, Project No: 9622.

Data availability No datasets were generated or analysed during the current study.

Declarations

Conflict of interest The authors declare no competing interests.

Consent to participate Not applicable.

Ethics approval This article does not contain any research with human participants or animals performed by the authors.

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