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Araştırma Makale/ Research article

Molecular Characterization of Some Local Watermelon Genotypes of Türkiye with SSR Markers

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ABSTRACT
This study investigates the genetic diversity and relationships among some of the Turkish watermelon landraces and commercial varieties using Simple Sequence Repeat markers. Watermelon genotypes primarily from the Marmara, Thrace, and Central Anatolia regions were analyzed to assess their genetic structure and potential value for breeding programs. The research employed 13 SSR markers, of which 10 were found to be polymorphic. Results revealed two main genetic clusters among the studied genotypes, reflecting their genetic relationships and potential origins. The genetic similarity analysis showed that the genotypes were grouped under two main branches, with specific genotypes exhibiting close genetic similarities. The study found an average number of alleles of 1.32 and an effective number of alleles of 1.18 across the analysed SSR markers. The observed heterozygosity (Ho) was 0.135, while the expected heterozygosity (He) was 0.158. The Shannon's information index averaged 0.203, indicating moderate genetic diversity among the studied genotypes. These results align with previous studies suggest a relatively narrow genetic base in cultivated watermelon. However, the identification of unique alleles in some Turkish landraces highlights their potential value for broadening the genetic base in watermelon breeding program.

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Introduction

Watermelon stands as a cornerstone crop within the vast *Cucurbitaceae* family, which also encompasses squash, cucumbers, and gourds. This genus is particularly cherished globally for its large, sweet, and exceptionally refreshing fruit, which is botanically a pepo, a type of berry with a hard rind [1, 2]. Its cultivation spans tropical and subtropical regions worldwide, underscoring its significant role in global agriculture and diverse dietary patterns [3].

Historically, evidence suggests watermelon cultivation originated in Northeastern Africa, possibly near the Kalahari Desert, with ancient Egyptians using it both as a source of water and food; tomb paintings dating back to around 1500 BC depict its presence [4]. Today, major production hubs include China, which consistently accounts for the largest share of global output, followed by countries like Türkiye, Iran, and Brazil [5].The economic importance of watermelon is substantial, driven by high yields per hectare and strong consumer demand, particularly during summer months. Commercially, watermelons are categorised based on rind pattern (e.g., striped or solid dark green), flesh colour (most commonly red, but also yellow or orange varieties), and seed presence (seeded or seedless, the latter often achieved through triploidy). For instance, the Crimson Sweet variety remains a popular commercial standard due to its excellent shipping qualities and consistent sweetness. Furthermore, the fruit is nutritionally valuable, being over 90% water, low in calories, and a rich source of the antioxidant lycopene, especially in the red-fleshed cultivars, which has been linked to cardiovascular health benefits [6].

Watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is an important crop in the *Cucurbitaceae* family, valued for its sweet, refreshing fruit. It is cultivated worldwide and plays a significant role in global agriculture. Türkiye, with its diverse geographical and climatic conditions, has a rich variety of watermelon landraces that represent valuable genetic resources for breeding programs [7].

The genetic diversity of crop plants is crucial for their improvement and adaptation to changing environmental conditions [8]. Despite high morphological diversity, watermelon has a narrow genetic base within the species [9]. This paradox presents both challenges and opportunities for watermelon breeders and researchers.

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Molecular markers, particularly Simple Sequence Repeats (SSRs), have become invaluable tools for assessing genetic diversity and relationships among plant genotypes. SSR markers are highly polymorphic, co-dominant, and distributed throughout the genome, making them ideal for genetic diversity studies [10]. The inherent robustness of SSR data renders them foundational for numerous pivotal agricultural applications. They enable breeders to quantify genetic distances between germplasm collections, thereby identifying unique parental lines for crossing programs [11]. SSR profiles serve also as distinctive "fingerprints" for individual accessions within gene banks, facilitating the management, identification, and prevention of duplicate storage, which is crucial for the conservation of genetic resources [12]. By comparing SSR profiles, researchers can construct phylogenetic trees or dendrograms that accurately depict evolutionary relationships among cultivars or wild relatives, thereby informing crop improvement strategies [13].

This study aims to evaluate the genetic diversity and relationships among Turkish watermelon landraces and some commercial varieties using SSR markers. The objectives of this research are to assess the level of genetic diversity among selected watermelon genotypes, to determine the genetic relationships and population structure of the studied genotypes, to compare the molecular diversity with previously observed morphological diversity, and to identify potential genetic resources for future watermelon breeding programs.

The results of this study will contribute to our understanding of the genetic structure of Turkish watermelon landraces and provide valuable information for conservation strategies and breeding efforts aimed at improving watermelon cultivars.

Materials and methods

The genetic materials utilised in this study underwent evaluation prior to the assessment of their seed characteristics, morphological attributes, and genitor capabilities [14-16]. Three commercial standard varieties are used as a control provided from the local seed market; seventeen of them were provided by Dr. Polat's (Tekirdağ Namık Kemal University) personal collection, and three of them, which are originally from Türkiye, were supplied by the United States Department of Agriculture (USDA) (Table 1).

Table 1 Accession numbers and origins of the plant materials were used in the study

Accession No/Cultivar	Province/Country	Donor
Washington	-	Standard variety
Galaxy	-	Standard variety
Crimson Sweet	-	Standard variety
94004	Çanakkale/Türkiye	Dr. Polat/ TNKU
94005	Mersin/Türkiye	Dr. Polat/TNKU
94006	Sakarya/Türkiye	Dr. Polat/ TNKU
94017	Uşak/Türkiye	Dr. Polat/ TNKU
94027	Unknown/Türkiye	Dr. Polat/ TNKU
94047	Unknown /Türkiye	Dr. Polat/ TNKU
94051	Unknown /Türkiye	Dr. Polat/ TNKU
94054	Sakarya/Türkiye	Dr. Polat/ TNKU
94058	Tekirdağ/Türkiye	Dr. Polat/ TNKU
94064	Tekirdağ/Türkiye	Dr. Polat/ TNKU
94081	Unknown /Türkiye	Dr. Polat/ TNKU
94100	Rize/Türkiye	Dr. Polat/ TNKU
94120	Unknown /Türkiye	Dr. Polat/ TNKU
94123	Unknown /Türkiye	Dr. Polat/ TNKU
94127	Unknown /Türkiye	Dr. Polat/ TNKU
94128	Unknown /Türkiye	Dr. Polat/ TNKU
94131	Unknown /Türkiye	Dr. Polat/ TNKU
PI169240 / cv. Kaymakam	Istanbul/Türkiye	USDA
PI169264 / cv. Yenidunya	Bursa/Türkiye	USDA
PI169294 / cv. Cinilikiz	Manisa/Türkiye	USDA

Fresh leaf samples were used for the study that were collected from 5 sample plants of each heirloom or variety and then bulked together. DNA extractions were carried out according to Doyle and Doyle [17]. DNA quantities were measured using the NanoDrop 1000 spectrophotometer V3.7 (ThermoFisher Scientific, Delaware, USA), and DNA qualities were assessed through electrophoresis and visualised with 1 % 1X TBE agarose gel. For PCR, 10 µg sample DNA, 1.25 U Taq polymerase enzyme (DreamTaq, Thermo Fisher), and 1X reaction buffer (DREAMTaq Buffer, Thermo Fisher) were used, along with a reaction mixture of dNTPmix

(dATP, dCTP, dGTP, and dTTP), including 0.2 µM of each nucleotide, and primers including 1 µM from both directions, and ddH_2O. PCR reactions were carried out in 35 cycles after 5 minutes of initial denaturation at 94 °C for 30 seconds of denaturing at 94 °C, attachment 30 seconds at 50-65 °C, and extension 1 minute at 72 °C, with all cycles ending with 10 minutes of last extension at 72 °C. The products were run in vertical electrophoresis to 15 V/cm in 6 % polyacrylamide gel for 3 hours. After electrophoresis, polyacrylamide gels were dyed with silver dye and documented. Documented gels were evaluated by GelAnalyzer 19.1 to calculate fragment sizes [18]. Fragments evaluated as absent or present and scored as 0 and 1 respectively.
A total of 13 SSR markers were evaluated in the study (Table 2) [19-25]. Ten of all markers (which were polymorphic) were used for calculating polymorphic information contents by POPGENE v. 1.32, and GENALEX v. 6.3 [26] to assess genetic diversity.

Table 2 Marker information of the molecular markers were employed for the genetic characterisation of selected watermelon genotypes

Marker Name	Reverse Primer Sequence	Forward Primer Sequence	Expected Product Size (bp)	Marker Location on Reference Genome
BVWS00358	CATTTCCGTTTCCATTTTCTTCAC	AAGTAACATCAAGCAGTTCGCCAT	160	Chr 7
BVWS01708	GGGTTTGTTTCCATTTCCCT	GGAAGGGTTCTGCATGTGTT	279	Chr 4
BVWS00711	TGCGACTTGAATTTTCTTGC	CATCTGACAGGTCCAAGAATGA	134	Chr 10
BVWS00681	TCTTGTCGTGCAATCTCTGC	TTCAAGAAGAAAATTGGTCACCT	222	Chr 2
BVWS02309	AATCTCCACTACAATCCACCAG	TTCCTCCAAACTCATCATTACC	149	Chr1
BVWS01133	CATCCACCTCAAACTTTAGAAACA	TTCTATTCCCGTCATTTCATTG	260	Chr 9
BVWS02333	GGGGGTTTTGGTTTCTTGAT	ATGATGTCACCATTACGGGG	103	Chr 10
BVWS02441	GCAAAATGCAACTGTTTATCG	CCATTATGATTTCAATCAATCTCC	232	Chr 8
MCPI_05	ATTTCTGGCCCCAGTGTAAG	GAACAACGCAACCACGTATG		Chr 6
MCPI_16	TGCTCAATCCACCCTTTCTC	AAAAACAGCAACTCTCCCATC		Chr 6
MCPI-11	GAGCAGGGGAGAAGGAAAAC	CCAGTAGCTTTTTCCGATGC	241	Chr 2
CYSTSIN	ATTTTCTTGCTTCAAATGGA	ATAAGCAAAAGCATCGAAAG	118	Chr 2
SNP02	TGCATGATGAGCCTTCTTTGAA	TAGACGGGGCTCACAAGTCA		Chr 11

Results

Ten of thirteen markers were used to assess the molecular characterisation of watermelon landraces were showed polymorphism and they were employed for calculation of genetic diversity (Table 3). Three of thirteen markers were showed monomorphic bands (BVWS02309, BVWS02333, SNP02) since that they were not used for further evaluations. A total of 22 polymorphic alleles were documented across 10 SSR markers. Among the analysed genotypes, the average effective allele count was (N_e) 1.732, the average observed heterozygotes (H_o) 0.307, the expected average heterozygotes (H_e) 0.391, the average self-sufficiency ratio (F_{it}) 0.215, the average total genetic variance (F_{st}) 0.608, the average polymorphic information content (PIC) 0.869 and the average Shannon information index (I) 0.593.
Across ten polymorphic markers, the highest number of alleles, specifically three alleles, was identified in the BVWS02441 and MCPI_16 markers. The highest effective allele count was once again observed in the BVWS02441 marker (2.60), while the lowest active allele number was recorded in the BVWS00681 marker (1.09). The observed heterozygotes ranged from 0 (BVWS00711, BVWS00681) to 0.696 (CYSTSIN, MCPI_11), whereas the expected heterozygotes varied from 0.085 (VVWS0681) to 0.625 (VBWS02441). The indicator varies between the PIC values of 0.912 (BVWS02441) and 0.796 (VVWS00681). Shannon information index values vary between 1.007 (BVWS02441) and 0.179 (VVWS00681).
UPGMA cluster analysis based on genetic similarity values was used to construct a dendrogram, which is presented in Fig 1. According to the dendrogram, the genotypes were grouped under two main branches. The first branch, which is quite narrower than the other, included Crimson Sweet, Galaxy. The genotypes 94120, Washington, PI169240, 94058, 94006, 94017, 94047, 94064, 94004, 94131, PI169294, 94005, 94127, 94100, PI169264, 94123, 94027, 94081, 94054, 94051, and 94128 were placed in the second branch. It was determined that the genotypes 94027, 94081, 94100, and 94123 were genetically similar according to the ten markers used. Similarly, as a group PI169264 and PI169294, and as another group 94051 and 94128 were determined to be identical.

Table 3 Genetic diversity parameters of the molecular markers were employed for the genetic characterisation of selected watermelon genotypes

Marker Name	**OFS (bp)**	**N_a**	**N_e**	**H_o**	**H_e**	**F_{is}**	**F_{st}**	**PIC**	**I**
BVWS00358	149-160	2	1.830	0.174	0.464	0.617	0.808	0.912	0.646
BVWS01708	279-320	2	1.482	0.045	0.333	0.886	0.943	0.814	0.507
BVWS00711	90-134	2	1.967	0.000	0.502	1.000	1.000	0.870	0.685
BVWS00681	203-222	2	1.091	0.000	0.085	1.000	1.000	0.796	0.179
BVWS02441	232-260	3	2.574	0.521	0.625	0.147	0.573	0.914	1.007
BVWS01133	251-260	2	1.240	0.130	0.198	0.327	0.663	0.879	0.344
MCPI_16	250-259	3	2.156	0.591	0.549	0.018	0.509	0.845	0.901
MCPI_11	260-300	2	1.910	0.696	0.487	-0.460	0.270	0.863	0.669
MCPI_05	204-198	2	1.240	0.217	0.198	-0.122	0.439	0.898	0.344
CYSTSIN	118-126	2	1.830	0.696	0.464	-0.533	0.233	0.898	0.646
Mean		2.2	1.732	0.307	0.391	0.215	0.608	0.869	0.593
Standard Deviation		0.422	0.466	0.287	0.177				0.256

OFS; Observed Fragment Size, N_a; Observed Number of Allele, N_e; Effective Number of Allele, H_o; Observed Heterozygosity, H_e; Expected Heterozygosity, F_{it}; inbreeding coefficient, F_{st}; total genetic variance, PIC; Polymorphic Information Content, I; Shannon Information Index

Solmaz, Sarı [27] conducted molecular characterizations of different local watermelon genotypes using RAPD markers in one of their studies. As a result of the study, they reported that genetic diversity among the investigated genotypes was low. Although cucurbits exhibit high morphological diversity when examined, they share a narrow genetic base within the species [28]. For this reason, when using molecular markers to determine diversity, the problem of low polymorphism is encountered [29]. To overcome this difficulty, specific amplification markers such as EST (Expressed Sequence Tags), HFO-TAG (High Frequency Oligonucleotides: Targeting Active Gene), and SSR (Simple Sequence Repeats) should be preferred instead of random amplification molecular markers like AFLP (Amplified Fragment Length Polymorphism) and RAPD [30]. SSR markers are highly suitable for this purpose, providing amplification of specific regions of the genome.

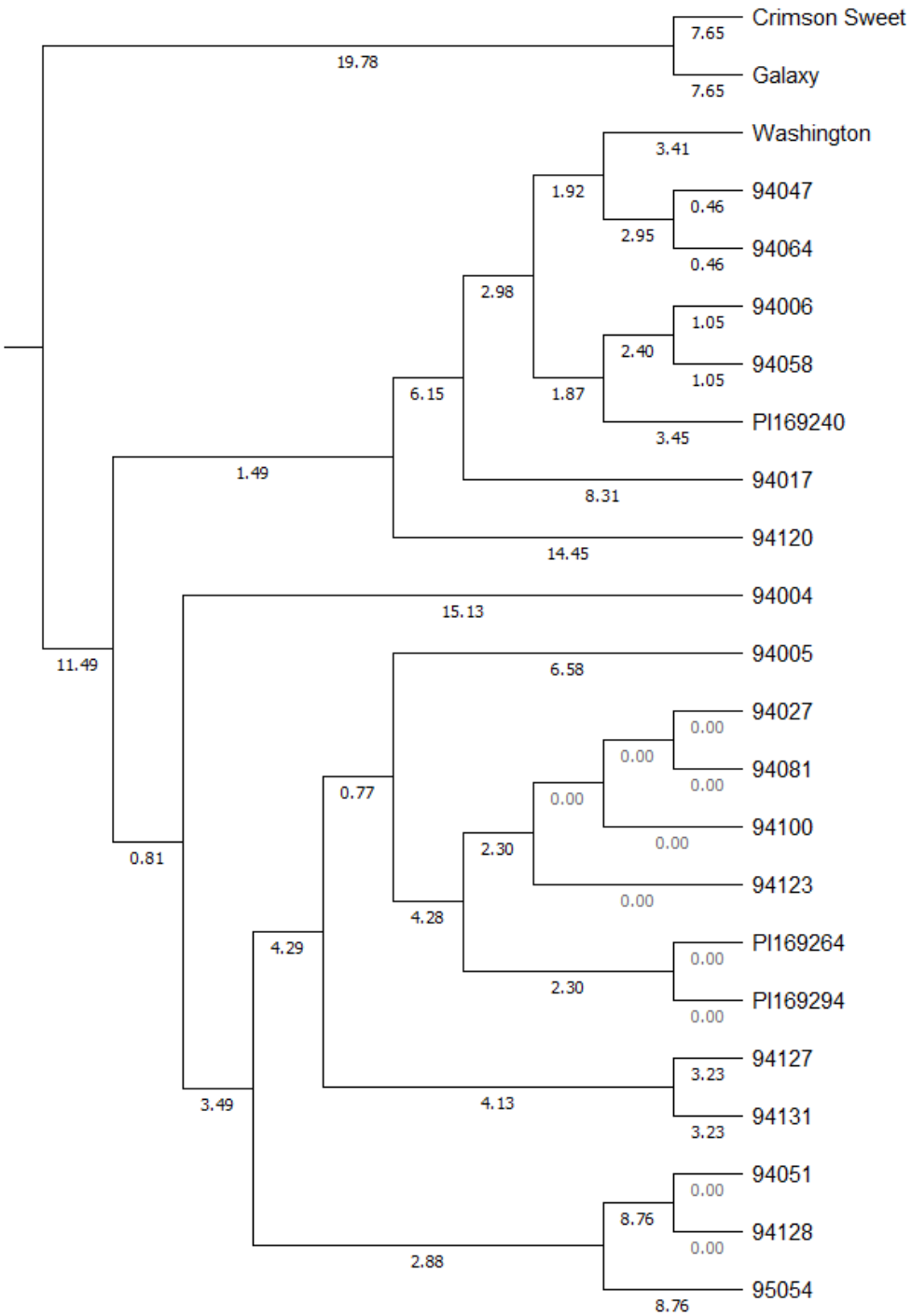

Fig 1 Dendrogram depicting the classification of 23 genotypes constructed using UPGMA and based on SSR markers

Discussion

Researchers [31] evaluated the genetic diversity among a total of 93 genotypes in the watermelon genetic resource collection of Çukurova University's Department of Horticulture using SSR and SRAP markers. In the study, 14 SSR primers and 31 SRAP primer combinations were used. The highest polymorphism rate was obtained from SSR markers with 100%, followed by SRAP markers with 97.3%. According to the cluster analysis performed with the data obtained from molecular characterization, they reported that watermelon genotypes belonging to the subspecies *Citrullus lanatus* var. *lanatus* collected from different regions of Türkiye were genetically close to each other and clustered together.

In early studies [32], reported that only 7 out of 8 SSR markers developed in watermelon were polymorphic. Similarly, in another study, Guerra-Sanz [24] reported that 18 out of 19 SSR markers showed polymorphism. Consistent with these studies, it was determined that 10 out of 13 SSR markers used in this study were polymorphic for this sample group.

Angui, Koffi [33] reported in their study using SSR markers on *Citrullus* samples collected from 36 different countries that the average number of alleles was 1.243, the effective number of alleles was 1.153, the Shannon index was 0.191, and the observed and expected heterozygosity were 0.124 and 0.149, respectively. They attributed this to the narrow genetic base of watermelon. Similar results were obtained in this study.

The SSR markers employed in this investigation demonstrate that the assessed genotypes exhibit predominantly low to moderate genetic diversity. The average number of alleles per locus (Na = 2.2) and the generally low expected heterozygosity (He = 0.391) signify constrained allelic richness, while the mean Shannon Index (I = 0.593) further corroborates limited variability, with only one marker (BVWS02441) attaining the high-diversity threshold ($I \geq 1.0$). Several loci display very low diversity ($I < 0.5$), and the persistently elevated fixation index (F_{is}), frequently nearing 1.0, indicates pronounced homozygosity and minimal observed heterozygosity among genotypes. Although the markers are moderately informative based on their PIC values (mean PIC = 0.608), they collectively capture only modest genetic differentiation within the evaluated genotypes. In summary, these metrics suggest a narrow genetic base, implying that the genotypes are closely related or share a common ancestry, resulting in limited genetic divergence across the population.

A prior investigation utilising identical genotypes as in this study demonstrated that [15]; the genotypes exhibited significant morphological diversity. However, it was observed that the molecular diversity was comparatively lower than the morphological diversity. This result can be attributed to the narrow genetic base, which has been frequently encountered and reported in previous studies on *Cucurbitaceae*. With this study, some watermelon genetic resources that can be used by researchers for various breeding purposes in future studies have been identified.

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Compliance with ethical standards / Etik standartlara uyum
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The author declares no conflict of interest.

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The study is proper with ethical standards.

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