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Microsatellite analysis in some watermelon (*Citrullus lanatus*) genotypes

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Abstract
Conservation of genetic resources is essential for the continuation of future crop production. Watermelon (Citrul-lus lanatus), a member of Cucurbitaceae, is widely distributed in tropical and subtropical regions. The aim of this study was to reveal the genetic relationships with the help of microsatellite markers in a watermelon collection free of unnecessary repetitions, and to determine the success of SSR (Simple Sequence Repeats) primers developed in cucurbits. In this study, 96 watermelon genotypes with good agronomic characteristics were used among the geno types collected from different regions of Turkey and purified up to the S4-S6 (self-pollination) stage. In the study, 33 SSR primer pairs were used to determine the genetic relationship between watermelon genotypes. In the study, a total of 67 bands were obtained with SSR primers. As a result of UPGMA (Unweighted Pair Group Method Using Arithmetic Averages) analysis, genotypes showed similarity at the level of 0.84-1.00. The number of alleles detected per primer varied between 1 and 6. In terms of the total number of alleles obtained, CMCT44 (5 units) and Cgb4767 (6 units) loci produced the most alleles. Primers with high polymorphism rate and allele excess were determined, and the possibilities for use in genetic stability analyses, variety differentiation and other genetic analyses were determined.

Keywords
Citrullus lanatus, watermelon, SSR, microsatellite, genetic characterization

Introduction
Watermelon is a member of the Cucurbitaceae family, which includes many commercial species such as melon, cucumber, squash, gourd, and pumpkins. Watermelon originating from South Africa is widespread in subtropical and tropical regions (Düzyaman, 2013). Watermelon is an important species with economic value in the Cucurbitaceae family, and its fruits differ considerably in terms of size and shape. Watermelons, which have seeded and seedless varieties, are the species whose leaves are highly fragmented (Solmaz et al., 2010). Watermelon cultivation is carried out in a very wide area in the world. As of 2019, watermelon production in the world is 100.4 million tons (Anonymous, 2021). China and Turkey lead the world in watermelon production.

Watermelon contains various vitamins (A, B, C and E), carotenoids (lycopene and beta-carotene), amino acids and some phenolic compounds (Tlili et al., 2009). Due to the lycopene, it contains, watermelon is a good antioxidant and is known to reduce the risk of prostate, stomach, and pancreatic cancer in humans (Collins et al., 2006). It has been stated that the lycopene content (23-72 µg/g/fresh weight) in watermelon is higher than other vegetables and fruits. It is very important to genetically improve and protect vegetables that stand out with their nutritional value and agronomic properties. Conservation of plant genetic resources is very important for future breeding studies. Plant gene resources are faced with the threat of decrease and loss due to environmental and other effects in the regions where they are located. Conservation of genetic

resources is essential for the continuity of plant production. Since the number of varieties in agricultural products is constantly increasing, morphological markers are insufficient to detect the differences between varieties. Therefore, molecular markers should also be used to protect genetic resources (Lombard et al., 2001).

In-plant genetics and breeding, genetic markers are generally used in selection, variety identification and genome mapping. After the discovery of the PCR reaction, a wide variety of molecular marker techniques have been developed for mapping, genetic labelling, detection of different gene regions, phylogenetic analysis, genetic diversity studies and Marker Assisted Selection (MAS) studies. Microsatellites or SSRs, consist of sequentially repeated 2-6 nucleotide groups scattered throughout eukaryotic genomes. Among the markers, SSR markers are preferred because of their cost, simplicity, and efficiency (Powel et al., 1996). Since SSRs are highly polymorphic, they give a lot of information to plants. In addition, it is widely used because it gives a codominant marker and has the ease of PCR (Röder et al., 1995). Genetic studies have been successfully carried out in plant species using different molecular marker techniques (Coskun et al., 2017; Karaman et al., 2018; Uzun et al., 2020; Aslan et al., 2021; Morilipınar et al., 2021; Kırac et al., 2022). Genetic characterization studies were performed using SSR markers in different watermelon genotypes (Guerra-Sanz, 2002; Solmaz, 2010; Zhang et al., 2012; Gama et al., 2013; Kwon, 2013; Nantoume et al., 2013; Kong et al., 2014; Lu et al., 2018).

This study, it is aimed to reveal the molecular characterization of watermelon, which has a rich genetic pool, and the genetic relationship between different watermelon genotypes. The main purpose of this study is to perform microsatellite marker analysis in the core watermelon collection free of unnecessary repetitions. This study, it was aimed to optimize the primers and determine the allele sizes on the watermelon lines in the Turkish watermelon seed collection by using the SSR marker technique.

Materials and Methods

In this study, genotypes collected from different regions of Turkey by Çukurova University Faculty of Agriculture, Department of Horticulture and purified up to S4-S6 grade were used. Among 250 watermelon lines with good agronomic characteristics, 96 genotypes selected from the previous project were used. These genotypes were selected from among those that were found to be the most genetically different from each other. Some of these genotypes are commercial varieties (35 Sugar baby, 235 Charleston Gray Seminis USA, 238 Dixilee North caroline USA, 365 China, G11 DIMA 4B Hungary and G12 Gyulavari Hungary). Other genotypes originate from Turkey.

DNA isolations were made, and PCR studies were carried out. Equal amounts of formamide loading buffer containing 10 mM EDTA (pH 8.0), 95% formamide, 0.025% bromophenol blue and 0.025% xylene cyanol were added to each tube containing the amplification product. PCR products were loaded on a 30% polyacrylamide gel (Long Ranger, FMC Biozym, Hessisch Oldendorf, Germany) and visualized on the 4300 DNA Analyzer (Li-Cor). M13 reverse (GGATAACAATTTCACACGG) or M13 forward (CACGACGTTGTAAAACGAC) primers were added to the 5' end of the synthetically prepared SSR Forward primers (700 or 800 nm wavelength). Data were analyzed using NTSYS program, UPGMA dendrogram was produced and PCA analyses were performed.

Results and Discussion

In this study, watermelon genotypes belonging to *C. lanatus* var. *lanatus* species, most of which have different geographical origins in Turkey and commercial cultivars were used. In this study, genetic characterization studies were performed on 96 watermelon genotypes with 33 SSR primers showing amplification. A total of 67 bands were obtained and 16 primers (including primers CMTA170a, Cgb4767, CSJCT641 and CSJCT435) showed high polymorphism. In terms of the total number of alleles obtained, CMCT44 (5 units) and Cgb4767 (6 units) loci produced the most alleles (Table 1). In terms of allele sizes detected with the 33 SSR primers used in this study, which produced scoreable bands, the largest allele was obtained from the CSJCT781 locus (330 bp), while the smallest allele was obtained from the CMTA170a (77 bp) and CSCTTT15a (80 bp) loci.

In the study conducted by Solmaz (2010), the highest locus was found in the Cgb4767 locus with 7 alleles. Also, a study examined by Guerra-Sanz (2002) to determine the allele numbers, the number of alleles obtained from 19 microsatellite primers in *C. lanatus* genotypes was between 1 and 8, while the number of alleles was between 1 and 6. In another studies carried out by Zhang et al. (2012) and number of alleles per locus was found to be between 2 and 7. Nantoume et al. (2013) investigated the genetic differentiation of 134 watermelon genotypes in their study and a total of 397 plants were analyzed with 24 SSR primer pairs and a total of 129 alleles were obtained. In our study, a similar number of alleles per primer was obtained. Considering the obtained polymorphism (100%) rate and when compared with studies investigating genetic diversity in watermelons with SSR markers (Danin-Poleg et al., 2001; Tzitzikas et al., 2009; Solmaz, 2010). It is seen that the number of SSR primers used is sufficient.

According to the obtained UPGMA dendrogram, similarity levels were determined between 0.84 and 1.00. In the dendrogram, two main groups were formed at the 0.85 similarity level between 96 genotypes. Genotypes 313 and 182 in the first main group were found to be 99% similar. In the second main group, both 97- 90 genotypes and 147- 194 genotypes are similar (Figure 1). As a result, in the dendrogram obtained from the SSR analysis data, it was revealed that the watermelons belonging to the *C. lanatus* var. *lanatus* subspecies collected from different regions of Turkey are genetically different from each other. In the second group obtained in the dendrogram, genotypes 2 and 9 were located in a single branch, while the others were clustered in a large group (Figure 1). It was determined that the genetic similarity ratios of the studied watermelon genotypes were divided into different subgroups varying between 0.86 and 1.00, and that neither the origin of the watermelon was collected, nor the morphological characteristics had any effect on the

formation of these subgroups. In the study of Sarı et al. (2007), in which the genetic diversity of watermelons collected from different regions of Turkey was investigated with RAPD markers, they determined that

Table 1. Polymorphism rates of SSR primers

Locus Name	Sequence Information	Total Number of Bands	Number of Polymorphic Bands	Polymorphism Rate
CMCT44F CMCT44R	TCAACTGTCCATTTCTCGCTG CCGTAAAGACGAAAACCCTTC	5	5	%100
CMTA170aF CMTA170aR	TTAAATCCCAAAGACATGGCG AGACGAAGGACGGTTAGCTTT	2	2	%100
CMCT160aF CMCT160aR	GTCTCTCTCCCTTATCTTCCA ACGGTGTTTGGTGTGAGAAG	1	1	%100
CSTCC813F CSTCC813R	GTTGTGCTCCCCAATAGTTG CACCACTTCTTCCACCGAA	2	1	%50
CSAT425F CSAT425R	TAGGGCAGGTATTATTTCAG ACGGACTGATTTAGTATAGGC	2	1	%50
CMGA104F CMGA104F	TTACTGGGTTTTGCCGATTT AATTCCGTATTCAACTCTCC	1	0	%0
CMCCA145F CMCCA145R	GAGGGAAGGCAGAAACCAAAG GCTACTTTTGTGGTGGTGG	2	2	%100
CMTC160a+bF CMTC160a+bR	GTCTCTCTCCCTTATCTTCCA GATGGTGCCTTAGTTGTTCCG	2	1	%50
CSCT335F CSCT335R	CCTTCACTTCCATCTTCATC CGGTCCTTCATTTCATAGAC	2	2	%100
CMACC146F CMACC146R	CAACCACCGACTACTAAGTC CGACCAAACCCATCCGATAA	2	2	%100
CMTC168F CMTC168R	ATCATTGGATGTGGGATTCTC ACAGATGGATGAAACCTTAGG	3	3	%100
CMGA165F CMGA165R	CTTGTTTCGAGACTATGGTG TTCAACTACAGCAAGGTCAGC	2	2	%100
CMCT505F CMCT505R	GACAGTAATCACCTCATCAAC GGGAATGTAAATTGGATATG	2	2	%100
CSTA050F CSTA050R	GAATTATGCAGATGGGTCTT CAAGAAGATCAAATGATAGC	1	0	%0
CMCTT144F CMCTT144R	CAAAAGGTTTCGATTGGTGGG AAATGGTGGGGGTTGAATAGG	3	2	%66
CMGA172F CMGA172R	CAATCGCAGATACTTCCACG TGCTTGTCCCAACGGTGTCAT	1	0	%0
CSCTTT15aF CSCTTT15aR	GTTTGATAATGGCGGATTGT GTAGAAATGAAGGTATGGTGG	1	0	%0
CMTC51F CMTC51R	ATTGGGGTTTCTTTGAGGTGA CCATGTCTAAAAACTCATGTGG	2	2	%100
CSJCT 674F CSJCT 674R	TGAAAGGAAGGGATGTGATTAGG ACAGGTGGTTAGAGGTTAGAGCTG	2	1	%50
Cgb4767F Cgb4767R	TTCTCTTCATTCCCCCAAAATC ACGGGTGAGGGAAAACGAG	6	6	%100
CSJCT 641F CSJCT 641R	GAACAACCCTCCAATTTTGCTC GCCACTTCCATGTCCAAATTC	3	3	%100
CSJCT 904F CSJCT 904R	GTAGGCCTGAATTTAGGCATGAGA ATATCACACGCTAACTTTGGGTCA	3	2	%66
ASUW2F ASUW2R	GCTTCGTTGTTGCTGCCGTTG GCATAAAATCACACTCAAAC	2	2	%100
CI.2-23F CI.2-23R	GAGGCGGAGGAGTTGAGAG ACAAAACAACGAAACCCATAGC	3	3	%100
CSJCT 662F CSJCT 662R	ACGTCGTAAAACCATCGGAGTC GCTTCCAAGCGTCAAAGGTATC	1	1	%100
CSJCT 775F CSJCT 775R	TAGGCCTGAATTTAGGCATAGGAGA TTGGGTCATTTGGTGTATCTAACAC	2	1	%50
ASUW19F ASUW19R	GTGTGTTTTTGCGTGTG GGGCAAATCCAATAATCCAG	3	3	%100
C.I.2-140F C.I.2-140R	CTTTTTCTTCTGATTTGACTGG ACTGTTTATCCCGACTTCACTA	1	0	%0
CSJCT 602F CSJCT 602R	GAGCTGAGCCAAGTTATCGTTTTG CAATTGAGGAAGAGGAGTTGGTTC	1	0	%0
CSJCT 664F CSJCT 664R	AAGTGGGCTCGATTGGAAGA CCGTCGCCTTTCTCAAGTTC	1	0	%0
CSJCT 781F CSJCT 781R	AAAGAAGATAGGCCTAGAATTTAG GCCCACATATGTCTAAATTGTCA	1	0	%0
CLG7992F CLG7992R	CTAACGCAATTTGAATCACTCAAA GGTAAAATGAAATCAATTGTGGAA	1	0	%0
Cgb5009F Cgb5009R	CAGTGGCACCGTCATCTAAAG AGTGGGGGATTCTCTTCCTAAG	1	0	%0
TOTAL		67	53	%79

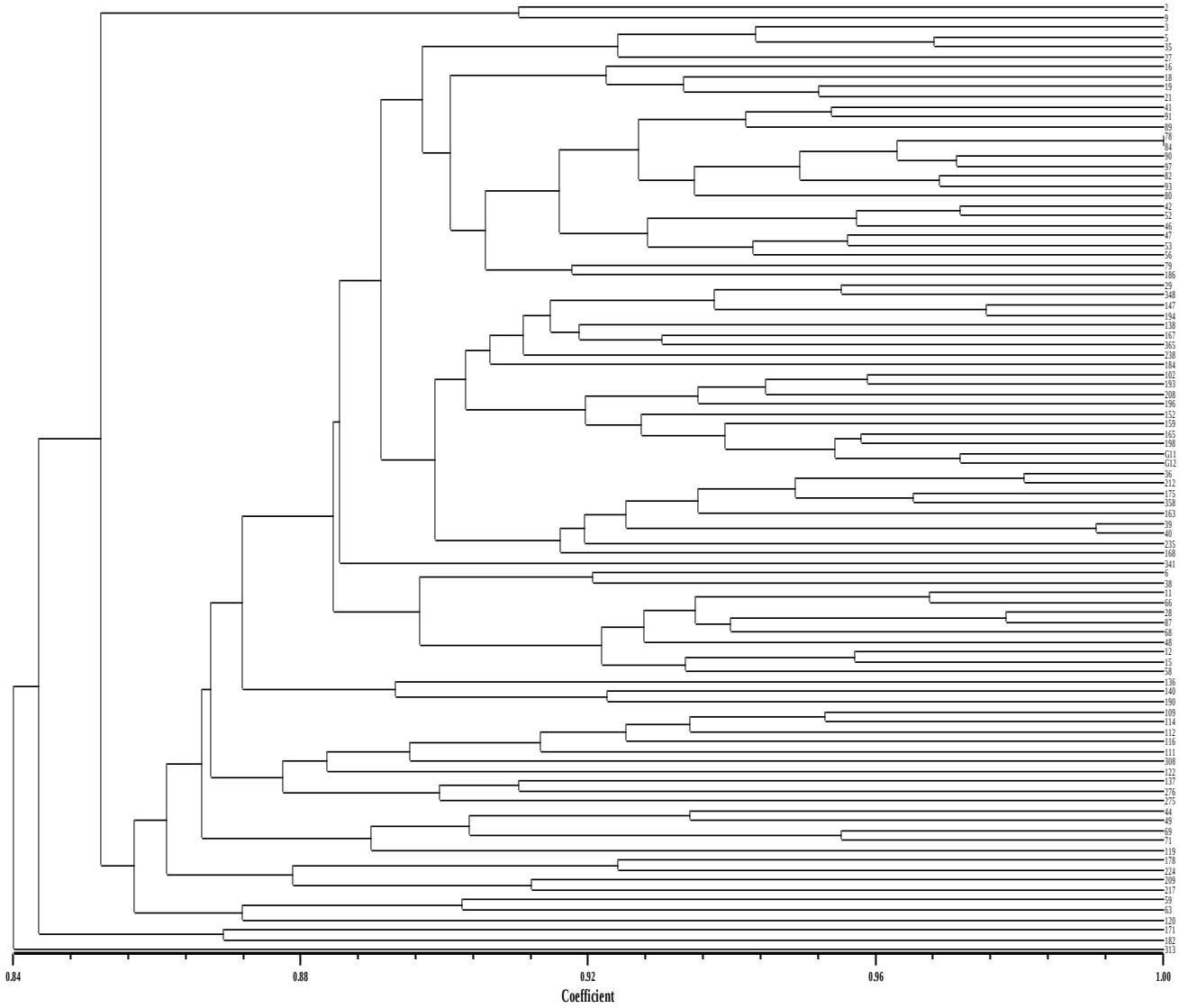

Figure 1. UPGMA dendrogram based on DICE similarity matrix of 96 genotypes

the similarity ratios of genotypes ranged between 0.93-1.00 and these watermelons were extremely close to each other in terms of genetic structure. In a study by Hwang et al. (2011), 6 watermelon varieties were found to be closely related to each other with a similarity ratio of 0.91-0.97. In our study, the similarity rate between watermelons was 0.84-1.00. Although cultivated watermelon genotypes and varieties (*C. lanatus* var. *lanatus*) are highly variable in terms of morphological characters such as skin color and thickness, fruit shape and size, flesh structure and color, sugar content, seed shape and color, it has been reported that the reason why they have limited polymorphism at the DNA level (Navot & Zamir, 1987) may be that the watermelon was cultured outside of its center of origin. As a result of the findings, it was concluded that SSR markers are effective in investigating the genetic diversity of cultured watermelons, which are not very rich in genetic structure. SSR markers have been successfully used to determine genetic diversity among genotypes of different species in watermelons. When the distribution of the markers on the two-dimensional graph is examined, it is observed that some markers are very close to each other, and some are quite far from each other (Figure 2). The closely related markers probably originate from the same chromosome region.

Therefore, the contribution of markers that are close to each other will be lower. This may mean repeating the marker sampling. As a result of this analysis, according to the graph in Figure 2., CMCT505 and CMCT505 were far from each other. According to principal components analysis, the first three eigenvalues were found to be approximately 27. This shows that the first three characters explain only 27% of the total variation. According to the literature, PCA analysis gives meaningful results if the sum of the first three eigenvalues is 25% or more. Therefore, it can be said that PCA results are important in our study.

The distribution of genotypes on the three-dimensional graph was determined by the eigenvector (Figure 3). It was observed that the genotypes 82 to 18, 167 to 147 were close to each other. A distribution compatible with the dendrogram was observed. In a study by Solmaz et al. (2010) genetic diversity in watermelons collected from Turkey was investigated with RAPD markers and the molecular data obtained were evaluated by PCA, and Praecitrullus fistulosus genotypes were grouped separately from other Citrullus

species. It has been determined that the genotypes of the *Citrullus lanatus* species collected from Turkey are densely clustered together (Solmaz et al., 2010). Similarly, in a study by Nantoume et al. (2013), the genetic differentiation of 134 watermelon genotypes was analyzed with 24 SSR primers. As a result, molecular analysis of variance explained 51% of the total variation within populations, while inter-population variation explained 14%

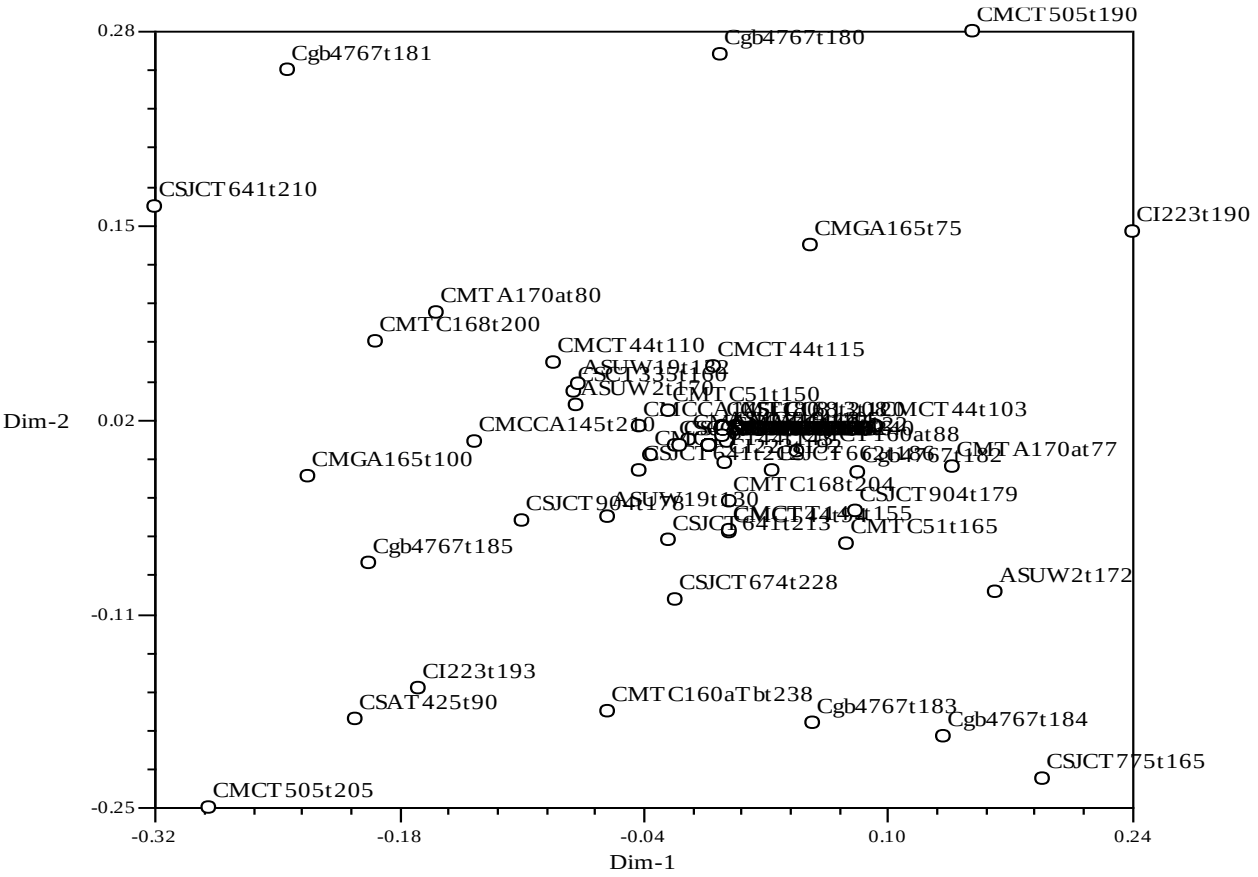

Figure 2. Distribution of markers on a two-dimensional graph

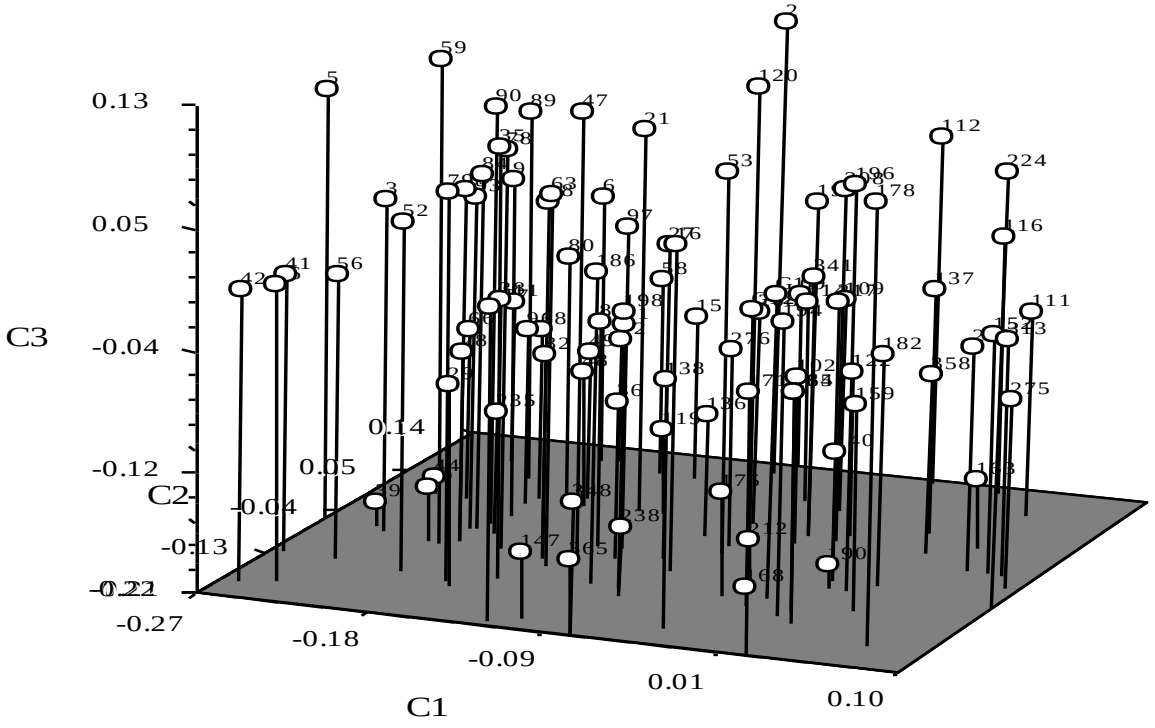

Figure 3. Distribution of genotypes on a three-dimensional graph

Conclusion

Turkey is a very rich country in terms of plant gene resources due to its geographical location. Although the origin of watermelon is not Turkey, it has valuable varieties in many regions. However, these valuable varieties have come to the point of extinction due to environmental conditions and other pressures. As a result, although they are very different in terms of morphological features, it was determined that these genotypes in the cultured *C. lanatus* species do not genetically have a high level of polymorphism. It is thought that this situation is because Turkey is far from the gene center of watermelon and that wild forms do not grow in our country. Within the scope of this study, very important loci that can be used to determine the purity tests and purification levels among watermelon seeds were determined and presented to the use of breeders.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential, or perceived conflict of interest.

Author contribution

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

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Consent for publication

Not applicable.

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