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Comprehensive Characterization of Cornelian Cherry (*Cornus mas* L.) Genotypes: Integrating Biochemical and Genetic Analyses for Breeding and Commercial Potential

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ABSTRACT

Cornelian cherry (*Cornus mas* L.) is utilized for food and traditional medicine due to its nutritional properties, bioactive compounds, and health benefits. This study aimed to investigate the biochemical and molecular characteristics of cornelian cherry (CC) genotypes grown under uniform ecological conditions in Malatya, Türkiye. Significant differences ($p \leq 0.05$) were observed among genotypes in physico-chemical parameters. Fruit weight ranged from 1.49 to 6.41 g, with genotype 44-01 exhibiting superior fruit size. Total flavonoid content varied between 29.33 and 77.68 mg QE/100 g FW, with genotype 44-04 showing the highest value, while genotype 44-06 had the highest ß-carotene content (0.167 mg/100 g FW). Total carotenoid content ranged from 0.15 to 0.45 mg/100 g FW. Nine ISSR primers generated 44 bands with a polymorphism rate of 57.35%, revealing moderate genetic diversity among genotypes. Notably, genotype 44-04 demonstrated both high bioactive compound content and distinct genetic clustering. The integration of biochemical and molecular analyses provides valuable insights for breeding programs and targeted commercial applications.

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Kızılcık (*Cornus mas* L.) Genotiplerinin Kapsamlı Karakterizasyonu: Islah ve Ticari Potansiyel için Biyokimyasal ve Genetik Analizlerin Entegrasyonu

ÖZET

Kızılcık (*Cornus mas* L.), besleyici özellikleri, biyoaktif bileşikleri ve sağlık yararları nedeniyle gıda ve geleneksel tıpta kullanılmaktadır. Bu çalışma, Türkiye'nin Malatya bölgesinde tek tip ekolojik koşullar altında yetiştirilen kızılcık genotiplerinin biyokimyasal ve moleküler özelliklerini araştırmayı amaçlamıştır. Fizikokimyasal parametrelerde genotipler arasında anlamlı farklılıklar ($p \leq 0,05$) gözlemlenmiştir. Meyve ağırlığı 1,49 ile 6,41 g arasında değişmekte olup, 44-01 genotipi daha büyük meyve boyutuna sahiptir. Toplam flavonoid içeriği 29,33 ile 77,68 mg QE/100 g FW arasında değişmekte olup, 44-04 genotipi en yüksek değeri gösterirken, 44-06 genotipi en yüksek ß-karoten içeriğine (0,167 mg/100 g FW) sahiptir. Toplam karotenoid içeriği 0,15 ila 0,45 mg/100 g taze ağırlık arasında değişmiştir. Dokuz ISSR primeri, %57,35'lik bir polimorfizm oranıyla 44 bant üretmiş ve genotipler arasında orta düzeyde genetik çeşitlilik ortaya koymuştur. Özellikle, 44-04 genotipi hem yüksek biyoaktif bileşik içeriği hem de belirgin genetik kümelenme göstermiştir. Biyokimyasal ve moleküler analizlerin entegrasyonu, ıslah programları ve hedeflenen ticari uygulamalar için değerli bilgiler sağlamaktadır.

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INTRODUCTION

The Cornelian cherry (*Cornus mas* L.), a deciduous shrub of the Cornaceae family, is an important yet underutilized fruit species with a long history of cultivation in Europe, the Caucasus, and Western Asia. Known for its high nutritional value and bioactive compounds, CC has gained increasing attention in recent years due to its potential applications in functional foods and natural medicine. This species is widely distributed across countries such as Türkiye, Bosnia and Herzegovina, Serbia, Ukraine, Georgia, Azerbaijan, Romania, the Czech Republic, and Slovakia, and thrives in temperate climates and diverse soil conditions (Kucharska et al., 2015). Its fruits are consumed both fresh and processed into syrups, jams, jellies, marmalades, compotes, dried fruits, and juices, owing to their distinctive tart-sweet flavor and rich biochemical composition.

CC has long been utilized in traditional medicine, and recent scientific studies confirm its richness in polyphenols, flavonoids, iridoids, and organic acids (Kucharska et al., 2015; Güzel & Elmastaş, 2020; Szczepaniak et al., 2021; Uğur, 2023). These bioactive compounds contribute to strong antioxidant, antimicrobial, and anti-inflammatory activities (Güzel et al., 2025; Ugur et al., 2021; Zengin et al., 2024, 2025). Moreover, CC has been associated with antidiabetic, cardioprotective, and potential anticancer effects (Bijelić et al., 2011; Martinović & Cavoski, 2020), further emphasizing its nutraceutical relevance.

Despite these recognized benefits, CC remains underexploited in commercial agriculture. Considerable variation in fruit morphology and biochemical composition exists among genotypes, influenced by genetic background and environmental conditions (Milošević et al., 2022; Skender et al., 2022). This variability underscores the importance of systematic genotype characterization to identify superior selections with enhanced nutritional, agronomic, and commercial traits.

Advances in molecular marker technologies have significantly improved plant genetic characterization and breeding strategies. Among various marker systems, Inter-Simple Sequence Repeat (ISSR) markers are particularly advantageous due to their reproducibility, cost-effectiveness, and ability to detect polymorphisms in species with limited genomic information (Farmanpour Kalalagh et al., 2017; Dönmez et al., 2016; Hassanpour et al., 2013). These markers have been successfully applied in assessing genetic diversity and population structure in CC and other fruit species.

Although previous studies have evaluated pomological traits, biochemical composition, or genetic diversity of Cornelian cherry separately, comprehensive investigations integrating molecular marker analysis with detailed biochemical profiling under uniform ecological conditions remain limited, especially within Türkiye's germplasm resources. Most available studies focus on regional characterization or single-approach assessments, making it difficult to distinguish environmentally induced variation from true genotypic effects. Furthermore, the potential overlap between biochemical superiority and genetic distinctness has not been systematically examined in CC populations.

In this context, the present study advances existing knowledge by integrating morphological, biochemical, and ISSR-based molecular analyses of ten genetically distinct CC genotypes cultivated under uniform ecological conditions in Malatya, Türkiye. By evaluating whether genetically differentiated genotypes also exhibit superior bioactive compound profiles, this research provides a structured framework for identifying elite selections within national germplasm resources. The integrative approach adopted here contributes to both breeding strategies and commercial cultivar development by linking molecular diversity with fruit quality traits in a controlled ecological background.

MATERIAL and METHOD

Plant material

This study was conducted on 10 genetically distinct CC genotypes cultivated under uniform ecological conditions in Malatya, Türkiye, located at 38°19′25″N latitude and 38°17′10″E longitude. These genotypes were selected from the National CC Collection Orchard of the Apricot Research Institute Directorate. Fruit and leaf samples were collected from each genotype at full maturity. The fruits were stored at +4°C before chemical analyses, while leaf samples were flash-frozen in liquid nitrogen and stored at -80°C until molecular analysis.

Physico-chemical analyses

The physical and chemical properties of the fruits were assessed using standardized analytical methods.

Fruit weight, length, and width: Measured using an analytical balance (Shimadzu, Japan) and digital caliper with

0.01 mm precision.
Color analysis: Performed using a Minolta CR 300 (Japan) reflectance colorimeter, recording L* (brightness), a* (red-green), and b* (yellow-blue) parameters.
pH and acidity: Determined using an Orion Star pH meter (Thermo, USA) and titration with 0.1 N NaOH, expressed as malic acid percentage.
Soluble Solids Content (SSC): Measured with a digital refractometer (ATAGO, Japan), representing the total dissolved sugars.

Spectrophotometric analysis

Total flavonoid content (TFC) was determined using the aluminum nitrate colorimetric method as described by Cosmulescu et al. (2015). To achieve this, 2 g of homogenized fruit pulp was extracted using 20 mL of a methanol:water:HCl (70:29.9:0.1 v/v/v) solvent mixture. The extraction was carried out at room temperature in an ultrasonic water bath for 1 hour, and the resulting extract was filtered. The filtrate was then subjected to the aluminum nitrate colorimetric method to quantify flavonoid content. Absorbance was recorded at 415 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The results were expressed as mg quercetin equivalents per 100 g fresh weight (mg QE/100 g FW).
Total carotenoid content (TCC) was determined as described by Wang et al. (2008). A 5 g homogenized fruit sample was mixed with 50 mL of a hexane:acetone:ethanol (50:25:25 v/v/v) solvent mixture in an Erlenmeyer flask. The extraction was performed on a shaker at 200 rpm for 10 min at 25°C, after which the mixture was centrifuged at 6500 rpm. The supernatant was collected, and absorbance was measured at 450 nm using a UV-Vis spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). The results were expressed as mg ß-carotene equivalents per 100 g fresh weight (mg/100 g FW).

Chromatographic analysis

The ß-carotene content was determined using ultra-fast liquid chromatography (UFLC) following the method described by Çuhacı et al. (2021). A 1 g fruit sample was placed in a Falcon tube, and 30 mL of a methanol:tetrahydrofuran (50:50 v/v) solvent mixture was added. The mixture was vortexed and then centrifuged at 9000 rpm, after which the upper phase was filtered through a 0.45 µm cellulose filter to remove particulates.
Chromatographic analysis was performed using a Shimadzu UFLC system (Kyoto, Japan) equipped with a 20 ADXR solvent pump, a DGU-20A5 model vacuum degasser, and an SPDM-20A photodiode array detector (PDA). Separation was carried out using a Phenomenex C18 column (150 mm × 4.6 mm, 5 µm particle size). The isocratic elution method was applied wiat flow rate of 1 mL/min at 30°C, and 20 µL of the extract was injected into the system. The mobile phase consisted of methanol, and detection was performed at a wavelength of 450 nm.
DNA extraction and ISSR analyses
Genomic DNA was extracted from leaf samples following the miniprep protocol described by Edwards et al. (1991). The quality and concentration of extracted DNA were assessed using a NanoDrop ND-100 spectrophotometer (Thermo Fisher Scientific, USA). DNA integrity was additionally verified by electrophoresis on 1% agarose gel prior to PCR amplification. A total of nine ISSR primers were utilized to assess the genetic relationships among the ten CC genotypes.
Polymerase chain reaction (PCR) amplifications were conducted in a 25 µL reaction mixture, containing 10 ng of CC DNA template, 2X PCR Master Mix (Fermentas K0171, USA), 10 µM primer, and 1 U of Taq DNA polymerase (Fermentas EP0402). The amplification reactions were performed using a Veriti thermal cycler (Applied Biosystems, USA) under the following conditions: an initial denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 45 sec, annealing at 55°C for 1 min, and extension at 72°C for 45 sec. A final elongation step at 72°C for 7 min was included. After completion, PCR products were stored at 4°C until further analysis.
All PCR amplifications were performed at least twice to ensure reproducibility of banding patterns. Only clear and consistently reproducible bands were scored across independent reactions.

Electrophoresis stage

Fragments obtained using nine ISSR primers were separated on 1.5% agarose gel. Fragment patterns were photographed by a gel documentation system (Genesys) under UV light for further analysis.

ISSR data analysis

ISSR data were recorded to generate a binary matrix, where band presence was scored as 1 and absence as 0. Only clear and reproducible bands were considered for scoring across all genotypes. Faint or ambiguous bands were excluded from analysis to minimize scoring errors. Band scoring was conducted manually and verified independently to ensure consistency. Genetic relationships among CC genotypes were determined using the unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis in the PAST software package (Hammer & Harper, 2001). The robustness of the dendrogram was evaluated by calculating the cophenetic

correlation coefficient (r) according to Mantel (1967). Principal coordinate analysis (PCoA) was performed to further assess genetic relationships. The similarity matrix for PCoA was constructed using Jaccard coefficients, and analyses were conducted using PAST. Additionally, polymorphism information content (PIC), effective multiplex ratio (EMR), and marker index (MI) values of ISSR primers were calculated to evaluate marker informativeness and efficiency, following Anderson et al. (1993) and Powell et al. (1996).

Statistical Analyses

All analyses were performed in triplicate, and the obtained data were evaluated using SPSS software. The Shapiro-Wilk test was used to check the normality of the data distribution. Differences among genotypes were determined using one-way analysis of variance (ANOVA), and significantly different groups were compared using Tukey's HSD test ($p \leq 0.05$). For genetic relationship analysis, the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering method and Principal Coordinate Analysis (PCoA) were applied. Genetic diversity was assessed using the Jaccard similarity coefficient, and the reliability of ISSR data was tested using the Cophenetic Correlation Coefficient (r). To determine the relationships between chemical components and ISSR data, Spearman correlation analysis was conducted. Correlation coefficients were considered significant at $p \leq 0.05$. Given the exploratory nature of the analysis, no multiple testing correction was applied. All statistical computations were performed using SPSS and PAST (Paleontological Statistics Software Package for Education and Data Analysis) v4.03 software.

RESULTS and DISCUSSION

Fruit physical properties

The pomological properties of CC genotypes, including fruit weight, width, length, and color parameters (L*, a*, and b*), differed significantly ($p \leq 0.05$; Table 1). These variations highlight the genetic diversity among the studied genotypes and their potential for selection in breeding programs. Fruit weight ranged from 1.49 g (genotype 44-04) to 6.41 g (44-01). Among the evaluated genotypes, 44-01 (6.41 g, 24.70 mm) displayed the highest values in terms of fruit weight and length, which are considered key commercial characteristics and quality criteria. Conversely, 44-04 (1.49 g, 15.89 mm) and 44-07 (1.60 g, 15.10 mm) had the lowest values. Fruit width was highest in 44-01 (18.43 mm) and 44-29 (18.45 mm), whereas 44-04 (11.19 mm) and 44-07 (10.83 mm) had the lowest values (Table 1). Previous studies have reported considerable variation in fruit weight across different geographical regions (Okatan, 2016; Taş et al., 2023; Tural & Koca, 2008; Milošević et al., 2022; Cosmulescu & Cornescu, 2020; Brindza et al., 2006). The values obtained in the present study fall within previously reported ranges; however, because all genotypes were cultivated under uniform ecological conditions, the observed differences are more likely attributable to genetic background rather than environmental variation. This strengthens the reliability of genotype-based comparisons and enhances their relevance for breeding selection.

Likewise, fruit width and length values in this study align with previous findings, where fruit width ranged between 7.40 and 15.20 mm, and fruit length varied from 12.00 to 23.80 mm (Brindza et al., 2006; Milošević et al., 2022; Okatan, 2016; Tural & Koca, 2008). Variability in fruit weight, width, and length among genotypes can be attributed to genetic factors, ecological conditions, and plant age. Additionally, color parameters (L*, a*, and b*), which significantly influence product quality and consumer preference, also varied among the genotypes. The L values*, representing lightness, ranged from 23.58 (44-04) to 30.09 (44-06, 44-07). The a values*, indicating the red-green spectrum, varied between 19.48 (44-20) and 30.56 (44-07). The b values*, representing the yellow-blue spectrum, were between 5.01 (44-20) and 11.62 (44-07) (Table 1). These results emphasize the morphological diversity within CC genotypes, supporting their potential use in breeding programs aimed at improving fruit quality traits for both fresh consumption and processing applications.

Table 1. Comparative Analysis of Pomological Traits and Colorimetric Properties in Different CC Genotypes
Çizelge 1. Farklı CC Genotiplerinin Pomolojik ve Kolorimetrik Özelliklerinin Karşılaştırmalı Analizi

Genotypes	Weight (g)	Length (mm)	Width (mm)	L*	a*	b*
44-01	6.41±0.20^{a}	24.70±0.18^{a}	18.43±0.41^{a}	27.66±0.79^{bc}	29.06±1.85^{a}	9.72±0.74^{b}
44-04	1.49±0.08^{g}	15.89±0.48^{e}	11.19±0.33^{e}	23.58±0.73^{f}	20.81±2.49^{cd}	5.12±0.89^{ef}
44-06	3.17±0.28^{e}	17.05±0.55^{d}	15.17±0.41^{c}	30.09±1.25^{a}	26.04±0.80^{b}	10.30±0.98^{b}
44-07	1.60±0.13^{g}	15.10±0.47^{e}	10.83±0.76^{e}	30.09±0.86^{a}	30.56±1.17^{a}	11.62±0.89^{a}
44-09	4.25±0.16^{d}	21.07±0.57^{c}	15.95±0.63^{b}	24.66±0.13^{ef}	20.57±1.23^{cd}	5.67±0.30^{def}
44-12	3.36±0.23^{e}	22.97±0.52^{b}	14.45±0.54^{c}	25.55±0.36^{de}	23.08±1.40^{c}	6.57±0.59^{cd}
44-13	4.44±0.26^{cd}	22.42±0.41^{b}	16.24±0.34^{b}	28.66±1.26^{b}	28.72±2.01^{a}	9.66±1.10^{b}
44-20	4.78±0.13^{c}	23.12±0.62^{b}	16.67±0.22^{b}	24.08±0.31^{f}	19.48±0.23^{d}	5.01±0.13^{f}
44-29	5.71±0.37^{b}	22.85±0.63^{b}	18.45±0.32^{a}	26.53±0.86^{cd}	22.74±1.44^{c}	7.08±0.65^{c}
44-30	2.41±0.06^{f}	20.30±0.22^{c}	12.62±0.27^{d}	24.43±0.54^{ef}	21.44±1.54^{cd}	6.35±0.81^{cde}

Data are the mean ± standard deviation (n=10). Means followed by the same letter(s) in each column are not significantly different ($p < 0.05$)

Fruit chemical properties

The chemical composition of CC fruits exhibited significant variability among the evaluated genotypes ($p < 0.05$, Table 2). The measured pH, acidity, and soluble solids content (SSC) ranged from 2.59 to 2.95, 1.67% to 3.84%, and 15.00% to 22.57%, respectively (Table 2). Among the investigated genotypes, 44-07, 44-20, and 44-04 were characterized by significantly higher pH, acidity, and SSC, whereas 44-13, 44-09, and 44-07 had the lowest values for these parameters. CC fruits are known for their tart and sour taste, which is influenced primarily by pH, acidity, and SSC levels (Martinović & Cavoski, 2020). Previous studies have reported pH values ranging from 2.44 to 4.95 (Milošević et al., 2022; Taş et al., 2023) and acidity values between 0.91% and 4.02% (Okatan, 2016; Skender et al., 2022), aligning with the results of this study. SSC is a key determinant of soluble solids in fruits, including sugars, salts, organic acids, and vitamins, making it an important quality parameter affecting fruitiness and consumer acceptance (Milošević et al., 2022). The SSC values in this study were slightly higher than those reported by Taş et al. (2023), Okatan (2016), and Milošević et al. (2022), but comparable to those reported by Cosmulescu and Cornescu (2020) and Martinović and Cavoski (2020). However, the SSC values were lower than those documented by Bijelić et al. (2011). These wide variations in SSC can be attributed to genotypic differences, fruit maturity stage, and ecological conditions.

CC fruits are known for their high antioxidant activity due to their rich content of bioactive compounds. In this study, total flavonoid content (TFC), total carotenoid content (TCC), and *β*-carotene levels were analyzed, revealing statistically significant differences among the genotypes ($p < 0.05$). The TFC values ranged from 29.33 to 77.68 mg QE/100 g, while TCC varied between 0.15 and 0.45 mg/100 g. *β*-carotene content was recorded between 0.045 and 0.167 mg/100 g (Table 2). Among the genotypes, 44-04, 44-30, and 44-06 were identified as promising selections due to their high TFC, TCC, and *β*-carotene contents, respectively. Milošević et al. (2022) reported TFC values for CC fruits ranging from 4.51 to 6.09 mg QE/100 g, which were lower than the results obtained in this study. Demir et al. (2020), however, documented TFC levels between 28.64 and 288.28 mg QE/100 g, indicating a broader variability. Similarly, Cosmulescu et al. (2019) found TFC values ranging from 12.14 to 64.48 mg QE/100 g, which are comparable to the findings of the present study. Studies on carotenoid content in CC fruits are relatively limited. In Poland, Szczepaniak et al. (2021) reported TCC values ranging from 0.16 to 1.42 mg/100 g, suggesting that the results obtained in this study fall within the expected range. Variability in the biochemical composition of CC fruits is influenced by multiple factors, including genetic structure, maturity stage, ecological conditions, and solvent types used in extraction. Karaaslan et al. (2018) demonstrated that extraction methods significantly affect flavonoid content, with TFC values reported as 255.75 mg QE/100 g (ethanol), 126.20 mg QE/100 g (methanol), 76.01 mg QE/100 g (acetone), 80.54 mg QE/100 g (water), and 28.57 mg QE/100 g (acetonitrile). These results underscore the nutritional and functional potential of CC genotypes, particularly in terms of bioactive compounds and antioxidant properties, making them valuable for breeding programs and functional food applications.

Table 2. Comparative Assessment of pH, Acidity, Soluble Solids, Total Flavonoid, Total Carotenoid, and *β*-Carotene Content in CC Genotypes

Çizelge 2. CC Genotiplerinin pH, Asitlik, Suda Çözünebilir Kuru Madde, Toplam Flavonoid, Toplam Karotenoid ve β-Karoten İçeriklerinin Karşılaştırmalı Değerlendirmesi

Genotypes	pH	Acidity (%)	SSC (%)	TFC (mg QE/100 g FW)	TCC (mg /100 g FW)	*β*-carotene (mg/100 g FW)
44-01	2.71±0.03^{cd}	2.32±0.09^{d}	19.63±0.06 ^{c}	58.59±1.13^{c}	0.27±0.01^{de}	0.047±0.004^{f}
44-04	2.88±0.02^{b}	2.55±0.04^{c}	22.57±0.15 ^{a}	77.68±2.74^{a}	0.44±0.02^{ab}	0.118±0.007^{b}
44-06	2.68±0.03^{de}	2.70±0.03^{b}	19.07±0.06^{d}	39.07±0.57^{e}	0.40±0.02^{ab}	0.167±0.012^{a}
44-07	2.95±0.03^{a}	2.17±0.07^{e}	15.00±0.26^{h}	39.78±1.48^{e}	0.36±0.02^{bc}	0.126±0.004^{b}
44-09	2.85±0.03^{b}	1.67±0.05^{f}	18.50±0.15^{ef}	69.22±1.30^{b}	0.38±0.01^{abc}	0.059±0.001^{e}
44-12	2.63±0.03^{fg}	2.33±0.07^{d}	18.27±0.06^{f}	49.02±3.12^{d}	0.32±0.00^{cd}	0.081±0.003^{d}
44-13	2.59±0.01^{g}	2.60±0.05^{c}	18.67±0.32^{e}	56.49±2.84^{c}	0.24±0.01^{e}	0.049±0.002^{f}
44-20	2.65±0.03^{ef}	3.84±0.07^{a}	22.03±0.15^{b}	70.78±0.95^{b}	0.15±0.01^{f}	0.081±0.003^{d}
44-29	2.72±0.03^{cd}	2.12±0.03^{e}	15.83±0.12^{g}	29.33±0.56^{f}	0.20±0.01^{ef}	0.045±0.002^{f}
44-30	2.75±0.04^{c}	2.39±0.07^{d}	19.03±0.12^{d}	37.08±2.56^{e}	0.45±0.02^{a}	0.106±0.001^{c}

Data are the mean ± standard deviation (n=3). Means followed by the same letter(s) in each column are not significantly different ($p < 0.05$)

The dendrogram obtained from the chemical analysis is presented in Figure 1. The degree of similarity among the evaluated CC genotypes ranged from 0.04 to 0.30. Based on this analysis, the genotypes were classified into four main groups, indicating significant chemical variability within the population. Among the studied genotypes, 44-01, 44-12, and 44-13 exhibited the greatest chemical divergence, suggesting substantial differences in their biochemical composition. Conversely, 44-04 and 44-07 were found to be highly similar, indicating a close

relationship in terms of their chemical profiles. These findings highlight the diversity in bioactive compounds and chemical traits among CC genotypes, which may be attributed to genotypic differences and environmental influences.

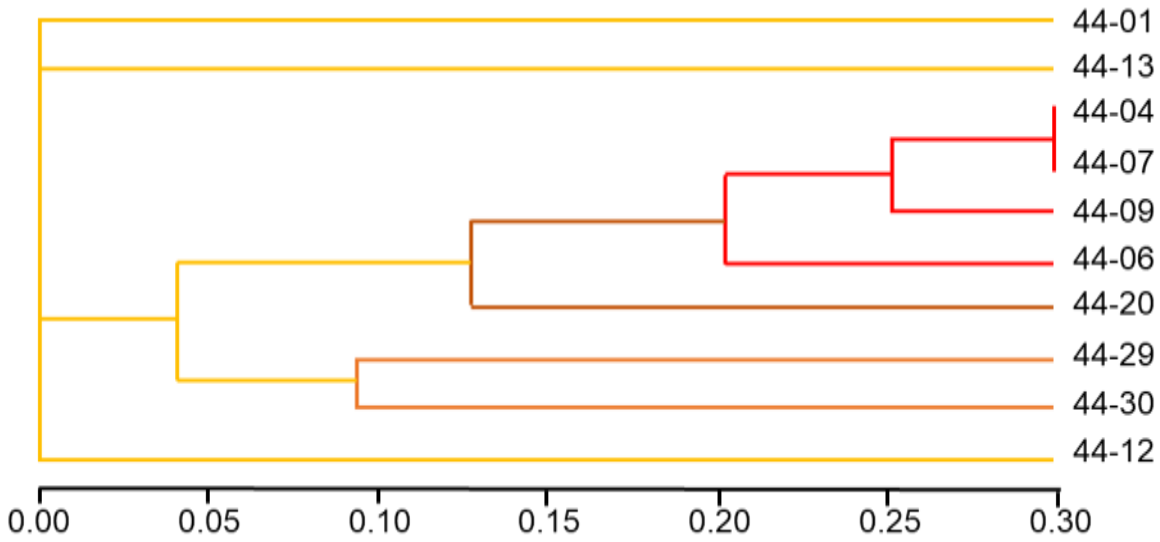

Figure 1. Cluster Analysis of CC Genotypes Based on Chemical Composition Using UPGMA Dendrogram
Şekil 1. CC Genotiplerinin Kimyasal Bileşimlerine Dayalı UPGMA Dendrogramı ile Kümeleme Analizi

Genetic diversity

A total of nine ISSR primers generated 44 bands across the 10 CC accessions, of which 25 were polymorphic, yielding an average polymorphism rate of 57.35%. The number of amplified fragments varied between 4 (UBC807, UBC812, UBC813, and UBC825) and 7 (UBC824) across the accessions. The average number of polymorphic bands per primer was 2.77. Among the primers, UBC815 exhibited the highest polymorphism level (83.33%), whereas the lowest polymorphism rate (40%) was observed with UBC808 and UBC828 (Table 3). The mean polymorphism information content (PIC) value for the primers was 0.55, ranging from 0.29 (UBC824) to 0.72 (UBC825). The effective multiplex ratio (EMR) mean value for all primers was 1.70, with individual values ranging from 0.80 (UBC808 and UBC828) to 4.16 (UBC815). Additionally, the marker index (MI) mean value was 1.10, with variation between 0.33 (UBC828) and 3.83 (UBC815) (Table 4). These findings indicate a moderate level of genetic diversity among the CC accessions, with primer UBC815 demonstrating the highest efficiency in detecting polymorphism.

Table 3. Genetic Variation Analysis of CC Genotypes Based on ISSR Markers: Total and Polymorphic Band Distribution
Çizelge 3. CC Genotiplerinin ISSR Markörlerine Dayalı Genetik Varyasyon Analizi: Toplam ve Polimorfik Bant Dağılımı

No	Primer code	Size range (bp)	Total band	Monomorphic band	Polymorphic band	Polymorphism rate (%)
1	UBC807	120-280	4	1	3	75.00
2	UBC808	120-390	5	3	2	40.00
3	UBC812	120-280	4	2	2	50.00
4	UBC813	200-430	4	2	2	50.00
5	UBC815	350-530	6	1	5	83.33
6	UBC824	160-480	7	4	3	42.86
7	UBC825	130-280	4	1	3	75.00
8	UBC827	180-320	5	2	3	60.00
9	UBC828	120-390	5	3	2	40.00
	Total		44	19	25	
	Mean		4.88	2.11	2.77	57.35

Table 4. Polymorphism Information Content (PIC), Effective Multiplex Ratio (EMR), and Marker Index (MI) Values of ISSR Primers Used in the Genetic Characterization of CC Genotypes

Çizelge 4. CC Genotiplerinin Genetik Karakterizasyonunda Kullanılan ISSR Primerlerinin Polimorfizm Bilgi İçeriği (PIC), Efektif Multipleks Oranı (EMR) ve Markör İndeksi (MI) Değerleri

No	Primer code	PIC	EMR	MI
1	UBC807	0.69	2.25	1.55
2	UBC808	0.51	0.80	0.40
3	UBC812	0.44	1.00	0.43
4	UBC813	0.44	1.00	0.43
5	UBC815	0.92	4.16	3.83
6	UBC824	0.29	1.28	0.37
7	UBC825	0.72	2.25	1.62
8	UBC827	0.56	1.80	1.01
9	UBC828	0.42	0.80	0.33
	Mean	0.55	1.70	1.10

The dendrogram constructed using ISSR marker data is presented in Figure 2. The cophenetic correlation coefficient (r) was determined as 0.96, indicating a high degree of reliability in the dendrogram structure. The genetic similarity coefficients among the analyzed CC genotypes ranged from 0.64 to 0.96, demonstrating a moderate to high genetic diversity within the studied population. According to the cluster analysis, all genotypes were grouped into two main clusters (Figure 2). Notably, the 44-04 genotype was placed alone in the first major branch, distinguishing it from all other genotypes with a similarity value of 0.64. In the second major cluster, the 44-01 genotype separated distinctly from the rest of the accessions. When these genetic findings are compared with biochemical characteristics, certain patterns emerge. For instance, genotype 44-04, which clustered separately, exhibited the highest total flavonoid content (77.68 mg QE/100 g) and total carotenoid content (0.44 mg/100 g), suggesting a potential correlation between genetic uniqueness and bioactive compound richness (Table 2). Similarly, the genetic proximity of genotypes 44-12, 44-13, and 44-29 (Figure 2) aligns with their similar biochemical profiles, particularly in terms of pH and acidity (Table 2). These findings suggest that certain patterns of genetic similarity detected by ISSR markers may partially correspond to similarities in biochemical composition; however, further validation using sequence-based or co-dominant markers would be required to confirm functional relationships. Furthermore, Principal Coordinate Analysis (PCoA) (Figure 3) reveals a strong association between genetic and biochemical clustering, suggesting that molecular variation detected at the ISSR level may be associated with biochemical trait variability. Figure 4 illustrates statistically significant correlations between ISSR markers and certain biochemical fruit traits; however, these relationships should be considered indicative and require further validation through advanced genomic approaches. The presence of high polymorphism in ISSR markers (e.g., UBC815, UBC825) aligns with variations in flavonoid and carotenoid content, reinforcing the importance of genetic screening in biochemical trait selection. The correlation matrix presented in Table 6 further supports this observation, highlighting significant relationships between genetic markers and fruit biochemical properties. For instance, ISSR primer UBC815, which exhibited the highest polymorphism level (83.33%), showed a strong negative correlation with fruit weight (-0.48) and a positive correlation with total flavonoid content (0.56), suggesting a possible association between the genetic variation detected by this marker and flavonoid-related traits. However, as ISSR markers are dominant and generally located in non-coding genomic regions, these findings should be interpreted as exploratory rather than indicative of direct genetic regulation.

Hassanpour et al. (2013) evaluated the genetic variation of 40 CC varieties using 20 ISSR markers, identifying 171 polymorphic bands with a polymorphism rate ranging from 80% to 100%. Their study reported mean PIC, EMR, and MI values of 0.46, 8.0, and 3.75, respectively. Similarly, Farmanpour Kalalagh et al. (2017) investigated the phylogenetic relationships, genetic variation, and population structure of CC genotypes from the Arasbaran region using ISSR markers. Their study detected 153 total bands, of which 98 were polymorphic, yielding a 64% polymorphism rate. The Jaccard similarity coefficient was determined as 0.777, while the mean PIC and MI values were 0.230 and 1.769, respectively. In the present study, nine ISSR primers were used, all of which proved to be highly informative. The PIC, EMR, and MI values obtained from ISSR markers ranged from 0.29 to 0.72, 0.80 to 4.16, and 0.33 to 3.83, respectively. These findings indicate that while the genetic diversity observed in this study was moderate, the applied ISSR markers were effective in distinguishing the genetic relationships among the studied CC genotypes.

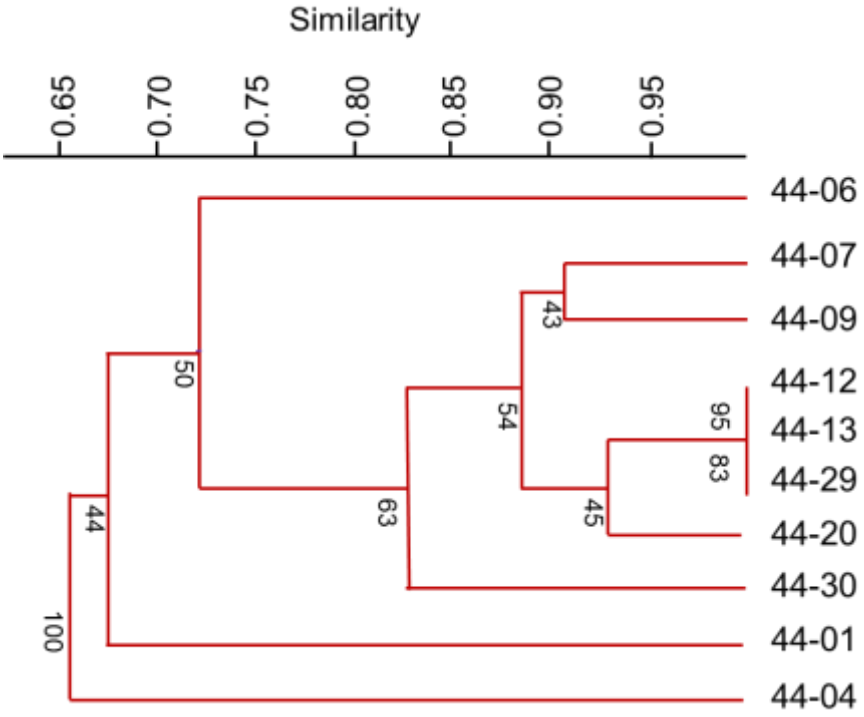

Figure 2. UPGMA Dendrogram Depicting Genetic Relationships Among CC Genotypes Based on ISSR Marker Data

Şekil 2. ISSR Markör Verilerine Dayalı Olarak CC Genotipleri Arasındaki Genetik İlişkileri Gösteren UPGMA Dendrogramı

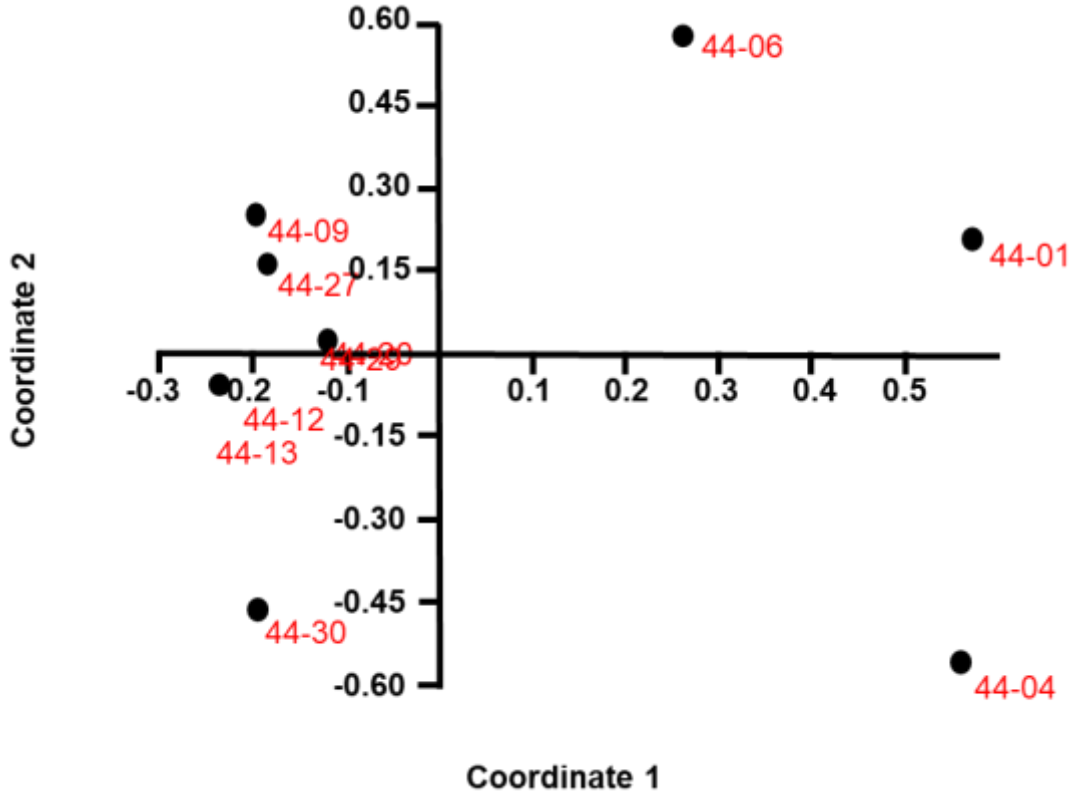

Figure 3. Principal Coordinate Analysis (PCoA) Plot Illustrating Genetic Relationships Among CC Genotypes Based on ISSR Marker Data

Şekil 3. ISSR Markör Verilerine Dayalı Olarak CC Genotipleri Arasındaki Genetik İlişkileri Gösteren Temel Koordinat Analizi (PCoA) Grafiği

Table 5. Genetic Similarity Matrix of CC Genotypes Based on ISSR Marker Data
Çizelge 5. CC Genotiplerinin ISSR Markör Verilerine Dayalı Genetik Benzerlik Matrisi

	44-01	44-04	44-06	44-07	44-09	44-12	44-13	44-20	44-29	44-30
44-01	1									
44-04	0.64	1								
44-06	0.69	0.63	1							
44-07	0.66	0.65	0.78	1						
44-09	0.65	0.63	0.8	0.90	1					
44-12	0.67	0.65	0.71	0.90	0.87	1				
44-13	0.67	0.65	0.71	0.90	0.87	1	1			
44-20	0.72	0.66	0.71	0.90	0.87	0.93	0.93	1		
44-29	0.67	0.65	0.71	0.90	0.87	1	1	0.93	1	
44-30	0.63	0.70	0.62	0.78	0.76	0.86	0.86	0.81	0.86	1

Integration of Chemical and Molecular Analyses

Several studies have combined chemical and molecular analyses to determine genetic and phenotypic relationships among plant species. Serçe et al. (2011) characterized three different hawthorn species using chemical analyses and RAPD markers, reporting that the dendrograms obom both datasets were highly compatible, successfully clustering genotypes by species. Similarly, Li et al. (2020) conducted a study on two different hawthorn species grown in China, utilizing 47 chemical composition parameters. Their findings demonstrated that chemical characterization, though somewhat limited, was effective in distinguishing species differences.

Conversely, Kaczmarska et al. (2015) evaluated the genetic and chemical similarity of 10 honeysuckle genotypes using ISSR markers and chemical analyses. Their study found discrepancies between DNA-based and chemical-based similarity results, attributing these differences to non-coding genomic regions that do not directly contribute to phenotypic expression, as well as variations in genome coverage by ISSR markers. Figure 4 illustrates the relationships among various fruit quality traits, including fruit weight, color parameters (L, a, b), soluble solid content (SSC%), pH, acidity, flavonoid content, carotenoid content, and β-carotene levels. The Spearman correlation test was employed to evaluate the ordinal relationships between these traits and ISSR marker data. Correlation coefficients, which range from -1 to 1, were used to determine the strength and direction of these associations, with values close to 1 indicating strong positive correlations, values near -1 indicating strong negative correlations, and values around 0 suggesting weak or no linear relationships.

Several strong positive correlations were observed. Notably, fruit weight showed a robust correlation with width ($r = 0.98$), suggesting that as fruit width increases, weight also increases, indicating a consistent size-to-weight relationship. Similarly, color parameters L and a ($r = 0.97$) and L and b ($r = 0.89$) were strongly correlated, suggesting that fruits with higher lightness values also exhibit higher red and yellow color components. Conversely, several negative correlations were detected. For instance, fruit length correlated negatively with pH ($r = -0.70$), indicating that taller fruits tend to have lower pH values, which could be linked to higher acidity. A moderate negative correlation was observed between fruit weight and total carotenoid content ($r = -0.76$) and between fruit weight and β-carotene content ($r = -0.76$), suggesting that heavier fruits may contain lower concentrations of these bioactive compounds. Additionally, SSC% and L values ($r = -0.59$) showed an inverse relationship, implying that fruits with higher brightness levels tend to have lower soluble solids content. Some moderate positive correlations were also identified, such as SSC% and total flavonoid content ($r = 0.77$), suggesting that fruits with higher SSC levels may also have increased flavonoid concentrations. This association is particularly relevant for breeding programs aimed at enhancing both flavor and nutritional quality. However, some variables showed weak or no correlation, such as a and pH ($r = 0.02$), indicating no significant relationship between these parameters. These correlation results provide valuable insights into the complex interactions among fruit quality traits, supporting fruit breeding and quality control programs. The findings indicate that easily measurable traits such as color and weight may serve as predictors for more complex biochemical traits, potentially simplifying the selection process in breeding programs. Additionally, recognizing trade-offs—such as the negative relationship between fruit weight and carotenoid content—is crucial for breeding efforts aimed at balancing market appeal with nutritional benefits. The strong associations between visual traits and chemical composition suggest that fruit selection based on observable characteristics could enhance efficiency in breeding and commercial production (Table 6).

Several studies have linked ISSR markers with morphological and biochemical traits in different fruit species. While no such studies have been conducted on CC genotypes, similar research exists for other fruits. For instance,

Kar et al. (2008) investigated associations between ISSR marker profiles and biochemical traits in mulberry (Morus spp.) genetic resources and reported that four ISSR markers were positively associated with protein content, thereby supporting the potential use of ISSR markers in marker-assisted selection. Similarly, Dönmez et al. (2023) evaluated fruit characteristics and genetic relationships among 68 mulberry genotypes grown in Adıyaman, Türkiye, using ISSR primers for molecular characterization. By applying the Spearman correlation test, they determined associations between fruit quality data and genetic marker information, ultimately identifying potential markers for early selection. These studies suggest that ISSR markers can be valuable tools for linking genetic data with agronomic and biochemical traits, which could be applied to future CC breeding programs to enhance fruit quality and genetic selection strategies.

It is important to emphasize that ISSR markers are dominant markers and typically amplify anonymous genomic regions. Therefore, the correlations identified in this study should be interpreted as preliminary associations rather than evidence of direct genetic control. Functional validation through co-dominant markers (e.g., SSR, SNP) or sequence-based approaches would be necessary to establish causal relationships between molecular markers and biochemical traits.

In addition to genetic and biochemical characterization, the results of this study provide practical implications for commercial utilization of CC genotypes. Fruit size is a primary determinant of fresh market value; therefore, genotype 44-01, which exhibited the highest fruit weight and size parameters, may be considered a promising candidate for table consumption and fresh market commercialization. Similarly, genotypes with high soluble solids content, such as 44-04 and 44-20, demonstrate potential suitability for processing industries, including juice and syrup production. From a functional food perspective, genotype 44-04, characterized by the highest total flavonoid and carotenoid content, and genotype 44-06, with superior ß-carotene levels, may represent valuable raw materials for nutraceutical and health-oriented products. The observed variability among genotypes enables targeted selection depending on market segment, whether prioritizing fruit size, processing efficiency, or bioactive compound richness. Thus, integrating molecular and biochemical data provides not only breeding advantages but also a strategic framework for selecting genotypes with defined commercial applications.

Table 6. Correlation Analysis Between Fruit Quality Traits and ISSR Marker Data in CC Genotypes
Çizelge 6. CC Genotiplerinin Meyve Kalite Özellikleri ile ISSR Markör Verileri Arasındaki Korelasyon Analizi

Primer	Weight	Width	Length	L	a	b	SSC	pH	Acidity	Total Flavonoid	Total Carotenoid	*β*-Carotene
807-120	-0.49	-0.48	-0.53	-0.40	-0.32	-0.38	0.56	0.43	0.01	0.55	0.46	0.31
807-180	-0.48	-0.49	-0.49	-0.42	-0.30	-0.37	0.56	0.41	0.04	0.53	0.40	0.26
807-250	-0.44	-0.45	-0.48	-0.45	-0.24	-0.31	0.58	0.38	0.06	0.52	0.42	0.30
807-280	-0.52	-0.53	-0.47	-0.48	-0.36	-0.45	0.52	0.43	0.04	0.53	0.37	0.21
808-120	-0.31	-0.26	-0.57	0.48	0.25	0.43	-0.39	0.51	-0.35	-0.14	0.41	0.50
808-390	0.06	-0.04	-0.04	-0.20	-0.10	-0.06	0.50	0.23	-0.03	0.50	0.16	-0.07
812-220	-0.48	-0.49	-0.49	-0.42	-0.30	-0.38	0.56	0.41	0.05	0.53	0.40	0.26
812-280	0.48	0.49	0.49	0.42	0.30	0.38	-0.56	-0.41	-0.05	-0.53	-0.40	-0.26
813-200	0.60	0.51	0.52	-0.11	0.02	-0.06	0.43	-0.28	0.57	0.41	-0.60	-0.31
813-290	0.58	0.49	0.53	-0.14	0.002	-0.08	0.45	-0.29	0.57	0.38	-0.57	-0.31
813-420	0.58	0.49	0.53	-0.14	0.002	-0.08	0.45	-0.29	0.57	0.38	-0.57	-0.31
813-430	0.55	0.46	0.49	-0.18	-0.02	-0.11	0.50	-0.25	0.58	0.43	-0.54	-0.29
815-420	-0.03	-0.02	-0.27	0.16	0.18	0.19	0.46	0.08	0.06	0.24	0.32	0.39
815-500	-0.12	0.02	-0.36	0.51	0.16	0.38	0.03	-0.18	0.15	-0.29	0.28	0.68
815-510	0.56	0.44	0.43	0.16	0.41	0.29	0.11	-0.10	-0.09	0.13	-0.18	-0.35
815-520	-0.12	0.02	-0.36	0.51	0.16	0.38	0.03	-0.18	0.14	-0.29	0.28	0.68
815-530	-0.48	-0.49	-0.48	-0.42	-0.30	-0.38	0.56	0.41	0.04	0.53	0.40	0.26
824-410	0.48	0.49	0.48	0.42	0.30	0.38	-0.56	-0.41	-0.05	-0.53	-0.40	-0.26
824-480	-0.56	-0.44	-0.43	-0.16	-0.42	-0.29	-0.12	0.10	0.09	-0.13	0.18	0.35
824-490	0.56	0.44	0.43	0.16	0.42	0.29	0.12	-0.10	-0.09	0.13	-0.18	-0.35
827-180	-0.69	-0.66	-0.93	0.39	0.27	0.38	0.01	0.56	0.004	-0.02	0.54	0.83
827-220	0.58	0.60	0.38	0.54	0.41	0.43	-0.44	-0.33	0.003	-0.15	-0.63	-0.31
827-230	-0.58	-0.60	-0.38	-0.54	-0.41	-0.43	0.44	0.33	-0.003	0.15	0.63	0.31
834-130	-0.12	0.03	-0.36	0.48	0.12	0.35	0.03	-0.15	0.09	-0.25	0.30	0.66
834-180	-0.19	-0.03	-0.41	0.48	0.10	0.34	0.02	-0.17	0.15	-0.30	0.29	0.71
834-185	-0.06	0.07	-0.32	0.53	0.20	0.42	0.04	-0.19	0.14	-0.28	0.26	0.65
834-280	-0.12	0.02	-0.36	0.51	0.16	0.38	0.3	-0.18	0.15	-0.29	0.28	0.68
835-120	0.18	0.16	-0.11	0.33	0.23	0.33	-0.01	0.24	0.14	0.18	-0.08	0.21
835-390	-0.14	-0.23	-0.05	-0.37	-0.08	-0.19	0.45	0.22	-0.06	0.21	0.43	0.04

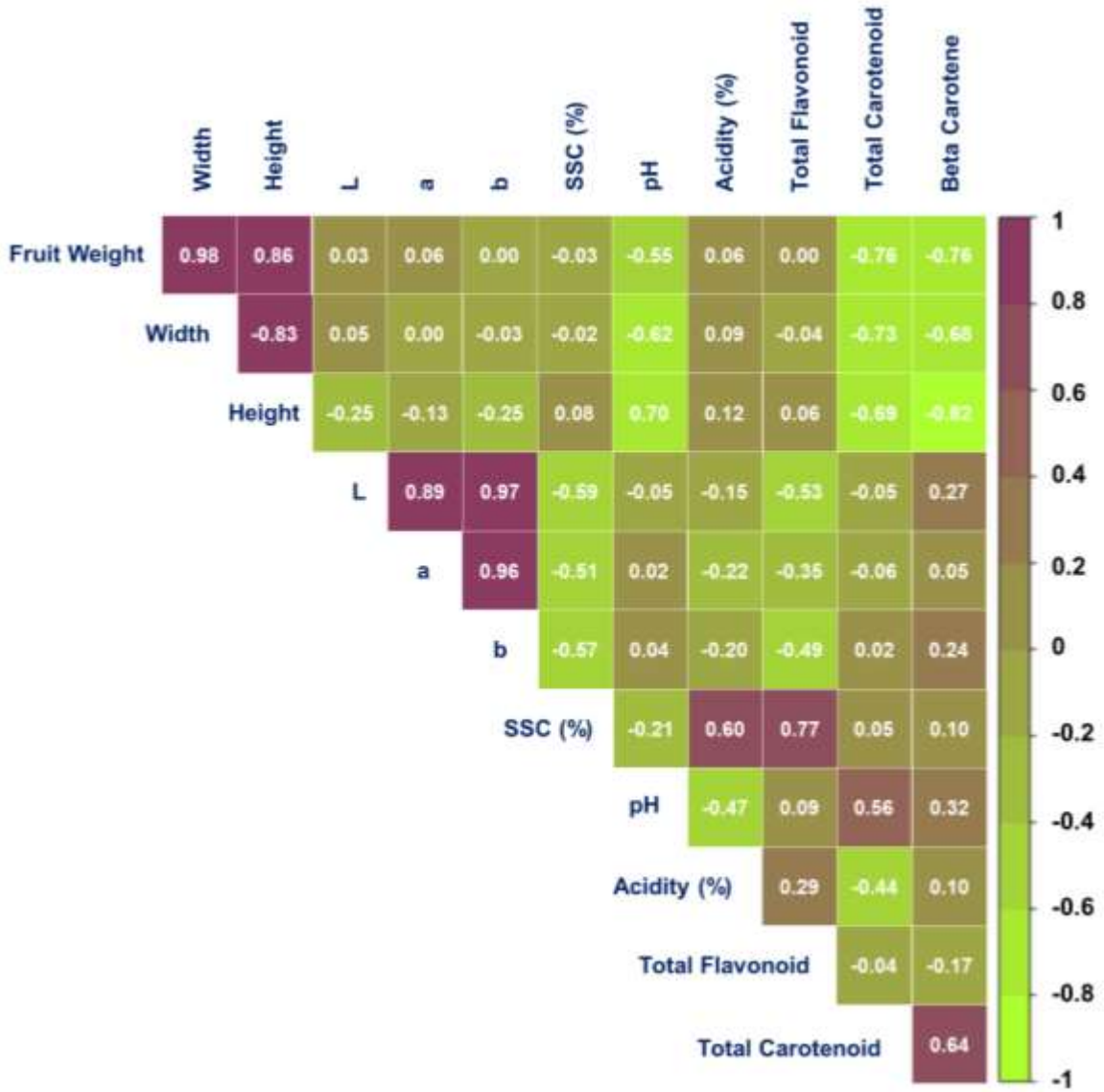

Figure 4. Correlation Network of Fruit Quality Traits in CC Genotypes: Associations Between Morphological and Biochemical Parameters

Şekil 4. CC Genotiplerinde Meyve Kalite Özelliklerinin Korelasyon Ağı: Morfolojik ve Biyokimyasal Parametreler Arasındaki İlişkiler

Study Limitations

This study has certain limitations that should be acknowledged. The evaluation was conducted on a limited number of genotypes (n = 10) under a single ecological location, and therefore genotype x environment interactions were not assessed. In addition, as ISSR markers are dominant and amplify anonymous genomic regions, the detected marker–trait associations should be considered preliminary. Future studies using larger populations, multi-location trials, and co-dominant or sequence-based markers would provide stronger validation of these findings.

CONCLUSION

By integrating morphological, biochemical, and ISSR-based molecular analyses under uniform ecological conditions, this study provides a structured framework for identifying genetically distinct and biochemically superior CC genotypes within Türkiye's germplasm resources. Significant variability in fruit size, SSC, acidity, and bioactive compound composition confirms the importance of genotype-based selection in breeding programs. Notably, genotype 44-04 combined high flavonoid and carotenoid content with clear genetic differentiation, illustrating the value of integrative evaluation. ISSR analysis revealed moderate genetic diversity and reliable clustering among genotypes, while correlation analysis indicated potential associations between morphological traits and biochemical composition. However, these relationships should be considered exploratory and require

further validation through advanced genomic approaches. Overall, the integration of molecular and biochemical profiling enhances genotype selection strategies and supports the development of nutritionally enriched and commercially competitive cultivars. Future research incorporating multi-location trials and sequence-based molecular tools will further refine marker–trait associations and improve breeding precision.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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