Determination of genetic diversity in European cranberrybush (Viburnum opulus L.) genotypes based on morphological, phytochemical and ISSR markers

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Abstract Turkey has a high diversity of native and commercially grown plants. European cranberrybush, a fruit species grown commercially in the country, is of interest because of its health benefits. This study aimed to determine genetic diversity using morphological, molecular, and phytochemical markers in twenty-four different genotypes of European cranberrybush from Kayseri province, which is an important region in the production of plant in Turkey. The results show wide variations among genotypes in the morphological parameters. The G13 genotype was the prominent genotype compared to other genotypes in leaf length (130.69 mm), leaf width (135.76 mm) and fruit length (10.01 mm). The fruit weights of genotypes varied between 0.16 g and 0.80 g. In ISSR marker analysis, out of 73 scoreable bands obtained from 11 different primer sets, 44 were polymorphic. The average polymorphism rate in the study was 60.27%, and the similarity index of the genotypes varied between 0.77 and 0.95. Total flavonoid, phenolic and anthocyanin content ranged from 106.28 mg CAE/100 g to 318.87 mg CAE/100 g, 451.23 mg GAE/100 g to 679.57 mg GAE/100 g, and 16.48 mg cyn-3-gluc/100 g to 21.36 mg cyn-3-gluc/100 g, respectively. The results of this study can be useful to plant breeders interested in developing and conserving the species and provide a baseline for new studies on European cranberry.

Keywords European cranberrybush · Genetic diversity · Morphology · ISSR · Phytochemical

Introduction

Turkey is a very important country in the world in terms of plant diversity (Gümüş and Avci 2020; Ozturk et al. 2020). This plant diversity has spread throughout the country, and most economically important plant species grow naturally in the country. European cranberrybush (ECB) (Viburnum opulus L. Family: Caprifoliaceae) is one of the fruits that has increased in production in recent years (Al et al. 2017). It is native to Europe, Northwest Africa, Turkestan, and Canada (Richard and Pierre 1992; Ozrenk et al. 2020).

ECB grows in Tokat, Artvin, Samsun, Trabzon, Sivas, Erzurum, Bursa, Izmit, Sakarya, Istanbul, Izmir, Kirsehir, Ankara and Kahramanmaras provinces in Turkey, and it grows more in Kayseri than in other provinces (Yıldız and Ekici 2019). In Turkey, ECB is used as an ornamental plant in home gardens, for fresh consumption (Sagdic et al. 2014), as a beverage, and as marmalade (Kalyoncu et al. 2013). In addition, it is valued for its medicinal properties. ECB juice is used for controlling diseases such as colds, kidney disease and diabetes (Eryılmaz et al. 2013).
ECB is also used in the treatment of hemorrhoids, obesity, and urinary tract disorders as well as an antimicrobial (Kajsyczak et al. 2020).

Several studies have been carried out to determine genetic diversity in plants both for breeding and conservation purposes (Hosseinpour et al. 2020), using morphological and phytochemical characters. However, morphological and phytochemical properties of plants can be affected by environmental conditions (Schneider et al. 2017). Therefore, it is necessary to use molecular markers to provide reliable results in genetic diversity studies where environmental conditions fluctuate (Yu 2020; Yildiz et al. 2021). Moreover, considering that plant characteristics such as yield, quality, and resistance to diseases and pests are controlled by multiple genes, there is need to combine morphological, biochemical, and molecular marker analyses. Studies carried out on the ECB, so far, were generally morphological (Ozkan et al. 2020), molecular (Krupa-Małkiewicz et al. 2014) or phytochemical (Polka et al. 2019) but there is no evidence that studies combining all the three markers have been done before.

This study aimed to determine the genetic diversity of twenty-four different ECB genotypes using morphological, phytochemical, and molecular marker analyses. Studies carried out on the ECB, so far, were generally morphological (Ozkan et al. 2020), molecular (Krupa-Małkiewicz et al. 2014) or phytochemical (Polka et al. 2019) but there is no evidence that studies combining all the three markers have been done before.

This study aimed to determine the genetic diversity of twenty-four different ECB genotypes using morphological, phytochemical, and molecular marker analyses. Studies carried out on the ECB, so far, were generally morphological (Ozkan et al. 2020), molecular (Krupa-Małkiewicz et al. 2014) or phytochemical (Polka et al. 2019) but there is no evidence that studies combining all the three markers have been done before.

### Materials and methods

#### Materials

Twenty-four different genotypes of ECB were used as material in this study). In the selection of genotypes, plant habitus, size and shape differences in leaves and fruits were taken into account. Genotypes were taken from “Talas, Bünyan, Develi, Sarıoğlan, Yahyalı” and “Melikgazi” districts of Kayseri province, which has an important place in ECB production in Turkey and is in the center of Central Anatolia. (Table 1). Climate conditions in Kayseri are cold and snowy in winters, and hot and dry in summers. ECB genotypes are generally grown in home gardens for landscaping and commercially (Fig. 1). The leaves were taken in the middle of the summer season (July), frozen in liquid nitrogen, brought to the laboratory and stored at -80 C until the analyzes were performed.

| Genotype | Coordinate | Altitude (m) | District |
|----------|------------|-------------|----------|
| G1       | 39°11'29"N 35°56'10"E | 1147 | Sarıoğlan |
| G2       | 39°11'27"N 35°56'10"E | 1145 | Sarıoğlan |
| G3       | 39°11'15"N 35°56'02"E | 1144 | Sarıoğlan |
| G4       | 39°10'43"N 35°55'40"E | 1127 | Sarıoğlan |
| G5       | 39°11'56"N 35°56'09"E | 1157 | Sarıoğlan |
| G6       | 38°42'31"N 35°32'30"E | 1108 | Talas |
| G7       | 38°42'17"N 35°32'19"E | 1110 | Talas |
| G8       | 38°42'16"N 35°33'51"E | 1136 | Talas |
| G9       | 38°47'25"N 35°39'21"E | 1208 | Melikgazi |
| G10      | 38°47'38"N 35°41'51"E | 1317 | Melikgazi |
| G11      | 38°48'48"N 35°43'00"E | 1299 | Melikgazi |
| G12      | 38°47'51"N 35°42'12"E | 1325 | Melikgazi |
| G13      | 38°03'25"N 35°23'31"E | 1276 | Yahyalı |
| G14      | 38°39'30"N 35°28'51"E | 1230 | Melikgazi |
| G15      | 38°50'51"N 35°51'28"E | 1330 | Bünyan |
| G16      | 38°50'33"N 35°51'36"E | 1360 | Bünyan |
| G17      | 38°50'43"N 35°51'14"E | 1393 | Bünyan |
| G18      | 38°11'18"N 35°21'03"E | 1089 | Yahyalı |
| G19      | 38°10'58"N 35°21'24"E | 1092 | Yahyalı |
| G20      | 38°22'01"N 35°25'53"E | 1150 | Develi |
| G21      | 38°22'51"N 35°27'21"E | 1199 | Develi |
| G22      | 38°23'11"N 35°29'51"E | 1256 | Develi |
| G23      | 38°23'09"N 35°29'51"E | 1259 | Develi |
| G24      | 38°22'59"N 35°28'44"E | 1221 | Develi |

#### Methods

##### Leaf and fruit analysis

While determining the leaf and fruit characteristics, 20 leaves and 20 fruits randomly taken from different parts of the plant were used for each genotype. Leaf width, leaf length, petiole length, petiole thickness, fruit width and fruit length values were determined with a digital caliper (Mitutoyo lp67) with 0.01 mm sensitivity. The fruit weight value was determined with a precision balance with a sensitivity of 0.01 g, and the pH value was determined using a pH meter. Soluble solids content (SSC) of the genotypes were determined with the help of a handheld refractometer, and the color measurements (L*, a*, b*) were made with the Minolta CM-700d spectrophotometers.
ISSR marker analysis

DNA isolation from young leaves taken from genotypes was made according to the CTAB method (Doyle and Doyle 1992). DNA concentrations were measured by spectrophotometer (BioTek Instruments, Inc., Winooski, VT, United States) and DNA samples were prepared using TE (10 mMTris-HCl, 0.1 mM EDTA, pH 8.0) solution. DNA samples were stored at -20 °C. PCR components were prepared in a total volume of 15 µl. PCR components consist of 2 µl DNA (20 ng), 1.5 µl 10× PCR Buffer, 0.2 µl Taq DNA polymerase (5u/µL), 1 µl dNTP (2.5 mM), 1.5 µl MgCl₂ (25 mM), 2 µl 10 mM ISSR primer, and 6.8 µl of H₂O. The amplification reactions using Thermal cycle (Sense Quest) Lab Cycle programmed for an initial denaturation step at 94 °C for 3 min, followed by 35 cycles of 1 min at 94 °C, 35 cycles of 50 s at the specific annealing temperature at 53 °C, 35 cycles of 2 min at 72 °C and ended with a final extension step 7 min at 72 °C. PCR products were electrophoresed on a 2% agarose gel prepared from 1X TAE buffer at 110 V for 4 h and visualized under UV light in the gel imaging (Kodak) unit after staining with ethidium bromide.

Fig. 1 Some images of ECB genotypes

Phytochemical analysis

Phytochemical analyzes were performed with 3 replications and 20 fruits in each replication. While the fruits are being prepared for analysis, they are first removed from the seeds with a stainless-steel knife and homogenized in a food blender. Homogenized fruit samples were placed in falcon tubes and stored at -20 °C until phytochemical analysis.

**DPPH antioxidant activity (Free radical scavenging activity)**

DPPH antioxidant activity of ECB was determined updating the method reported by Brand-Williams et al. (1995). 0.26 mM DPPH (1,1-diphenyl-2-picryl-hydrazil) solution was used in the analysis. 2900 μL of ethyl alcohol and 1 ml of DPPH solution were added to 100 μL of ECB extracts. After mixing with the help of vortex, the mixture was incubated for 30 min in the dark. The absorbance values of the samples were read in the spectrophotometer at a wavelength of 517 nm and results are given as % according to Garcia et al. (2012).
Total Flavonoids Content

The total flavonoids content of genotypes was determined by reference to a method reported by Chang et al. (2002). 3.3 ml of methanol was added to 1000 μL sample taken from fruit extract, and then 0.1 ml of 10% AlCl₃·6H₂O and CH₃COOK were added to the mixture. The measurements of the samples were made at a wavelength of 415 nm in a spectrophotometer, and the total flavonoids content was presented mg/100 g fresh weight as catechin equivalent (CAE).

Total Phenolics Compounds

Total phenolics compounds were determined with the help of Folin-Ciocalteu’s chemical. 4.1 mL of distilled water was added to 500 μL of fresh fruit extract, and then 100 μL of Folin-Ciocalteu’s reagent and 2% sodium carbonate (Na₂CO₃) were added. After the prepared solution was incubated for 2 h in the dark, the solution was bluish in color and was analyzed at a wavelength of 760 nm in a spectrophotometer. Absorbance values were calculated as gallic acid and presented as mg/100 g (fresh weight) (Eyduran et al. 2014).

Total anthocyanin content

Different pH methods were used to determine the total anthocyanin content of the ECB genotypes, and the samples were incubated for 2 h in a buffer medium. Following, readings were made at 527 and 700 nm wavelengths. The results are given in mg/100 g after converting the values to 26.900 (Gil et al. 2000).

Data analysis

SPSS 23.0 statistical package program was used in the evaluation of morphological (including fruit and leaf characteristics) and phytochemical characteristics. Duncan’s multiple comparison method was used to compare the difference between the means at the 5% significance level. Results are given as mean. Correlation analysis of morphological and biochemical properties of fruits was performed with XLSTAT 2014.5.03 software (Addinsoft, NY, United States).

ISSR markers were scored as presence (1) or absence (0) of bands (Yaman et al. 2020). The sizes of bands were estimated by comparison with GENESTATM 100 bp DNA ladder. To evaluate the genetic diversity among the ECB genotypes, NTSYSpc (Version 2.11X, Rohlf 2000) software (Numerical Taxonomy and Multi-variation Analysis System) was used to constitute the similarity index and UPGMA (Unweighted Pair Group Method with Arithmetic Mean of Cluster analysis) dendrogram (Sneath and Sokal 1973).

Results and discussion

Leaf and fruit characteristics

Leaf and fruit characteristics of ECB genotypes are given in Tables 2 and 3. There were statistically significant differences among genotypes in all the leaf and fruit parameters examined. Leaf length values ranged from 48.14 mm and 130.69 mm, while leaf width values ranged from 52.25 mm to 135.76 mm. For both leaves and fruits, the lowest values were in the G1, and the highest values were in the G13. In the petiole length values, the lowest value was recorded in G4 with 15.94 mm, whereas the highest value occurred in G11 with 25.09 mm. The average value of the genotypes was 1.62 mm in petiole thickness.

In fruit analysis, G11 had the lowest fruit length value with 7.32 mm whereas G13, as for leaf width and leaf length, had the highest value with 10.01 mm. Fruit width values varied between 4.64 mm and 9.52 mm. Fruit weight parameter is among the most important fruit characteristics in ECB as in most fruit species (Asencio et al. 2018). G1 with 0.16 g had the lowest fruit weight and G21 with 0.80 g had the highest fruit weight. The fruit weight values recorded in G5, G6, G9, G10, G11, G13, G14, G16, and G19 were below the average fruit weight values of the study. In a previous study, fruit length and fruit width of 11.85 mm and 9.60 mm, respectively, were reported in ECB genotypes (Konarska and Domaciuk, 2018). In another previous study, it was reported that the fruit length value ranged from 1.04 mm to 11.85 mm, and the fruit weight values were between 0.40 g and 1.80 g (Ozrenk et al. 2011). In a study conducted with genotypes taken from the same region but different district as in the current study, fruit length was between
and 8.81 mm, whereas fruit weight ranged from 0.30 g to 0.37 g (Polat et al. 2021). Color characteristics of fruits affect most parameters including phytochemical structures (Šamec et al. 2016). In the color properties evaluated in the study, the parameters ranged from 26.04 (G5) to 36.71 (G21) for L* value, 22.73 (G23) to 47.24 (G13) for a* value, and 8.95 (G8) to 17.15 (G13) for b* value. Taskin et al. (2019) reported L*, a*, and b* values in ECB genotypes of 25.58, 35.39 and 24.60, respectively. An average pH value of 3.08 and an average SSC value of 10.55% were recorded for the genotypes, and in previous studies, SSC values between 10.40% and 12.20% were recorded by Ozrenk et al. (2020), and those ranging from 9.8% to 12.6% were reported by Ersoy et al. (2017). A pH value of 2.9 in fresh fruits was reported by Taskin et al. (2021). The morphological data obtained from our study were similar to those of previous studies. The slight differences observed can be explained by ecological and genotypic differences (Bostan and İşbakan, 2020).

### Table 2  Leaf and fruit characteristics of ECB genotypes

| Gen | LL (mm) | LW (mm) | PL (mm) | PT (mm) | FL (mm) | FW (mm) | FWT (g) |
|-----|---------|---------|---------|---------|---------|---------|---------|
| G1  | 48.14 l | 52.25 k | 16.29 hi | 1.02 ij | 7.46 ij | 4.64 g  | 0.16 j  |
| G2  | 71.86 h–k | 65.47 j | 20.6 a–i | 1.32 f–j | 8.78 c–g | 8.54 a–f | 0.70 ab |
| G3  | 61.15 jk | 73.64 f–j | 20.03 c–i | 1.52 d–h | 9.22 a–d | 9.40 ab  | 0.57 d–h|
| G4  | 68.42 ijk | 71.38 hij | 15.94 i  | 1.42 e–j | 8.86 c–f | 9.26 ab  | 0.58 c–g|
| G5  | 82.21 e–h | 85.57 c–g | 21.19 a–h | 1.80 c–f | 8.22 e–j | 8.00 ef  | 0.44 i  |
| G6  | 71.29 h–k | 71.71 hij | 18.46 f–i | 1.21 hij | 7.96 f–j | 8.06 c–f | 0.55 d–h|
| G7  | 77.45 g–i | 75.85 f–j | 17.98 g  | 1.94 bcd | 8.44 c–h | 8.64 a–e | 0.60 b–e|
| G8  | 76.59 g–i | 73.50 f–j | 25.27 a  | 1.27 g–j | 8.32 d–I | 8.32 b–f | 0.64 b–e|
| G9  | 66.30 ijk | 68.72 iij | 24.08 a–e | 1.57 d–h | 7.96 f–j | 7.88 ef  | 0.53 e–i|
| G10 | 82.92 d–h | 86.44 c–f | 19.73 d–i | 2.58 a  | 7.70 hij | 7.88 ef  | 0.47 f–i|
| G11 | 59.95 k  | 63.36 jk | 25.09 a  | 1.15 hij | 7.32 j  | 7.46 f  | 0.46 ghi|
| G12 | 78.72 f–i | 72.70 g–j | 20.66 a–i | 1.75 c–g | 8.34 c–I | 8.04 def | 0.58 d–g|
| G13 | 130.69 a | 135.76 a | 24.86 abc | 2.34 ab | 10.01 a | 9.17 ab  | 0.56 d–h|
| G14 | 89.52 c–g | 83.12 d–h | 21.16 a–h | 1.62 d–h | 7.84 g–j | 7.66 ef  | 0.45 hi |
| G15 | 67.96 ijk | 68.38 ij | 19.60 e–i | 0.95 j  | 8.88 c–f | 9.52 a  | 0.59 b–f|
| G16 | 73.50 hij | 81.48 e–i | 22.23 a–g | 1.45 e–i | 8.78 c–g | 9.22 ab  | 0.56 d–h|
| G17 | 83.65 d–h | 97.26 ab | 24.59 a–d | 1.27 g–j | 9.32 abc | 9.54 a  | 0.57 d–h|
| G18 | 95.79 cd | 90.26 cde | 20.09 b–i | 1.60 d–h | 8.00 f–j | 8.52 a–f | 0.70 abc|
| G19 | 94.74 cde | 91.35 cde | 25.03 ab | 1.36 f–j | 8.10 e–j | 7.80 ef  | 0.56 d–h|
| G20 | 91.71 c–f | 96.82 ab | 21.10 a–h | 2.17 abc | 8.54 c–h | 9.14 abc | 0.66 cde|
| G21 | 90.77 c–f | 90.60 cde | 23.13 a–f | 1.86 cde | 9.24 a–d | 9.42 ab  | 0.80 a  |
| G22 | 73.97 hij | 76.36 f–j | 16.66 hi  | 1.60 d–h | 9.26 a–d | 9.10 a–d | 0.61 b–e|
| G23 | 114.36 b | 106.32 b | 20.88 a–i | 2.4 a  | 9.84 ab  | 9.44 ab  | 0.67 bcd|
| G24 | 98.79 c | 94.97 abc | 16.81 hi  | 1.63 d–h | 9.02 b–e | 9.46 a  | 0.63 b–e|
| Mean| 81.27 | 82.22 | 20.90 | 1.62 | 8.55 | 8.50 | 0.57 |

Different lower case letters show statistically significant differences between genotypes in column ($p < 0.05$)

**LL** Leaf Length, **LW** Leaf Width, **PL** Petiole Length, **PT** Petiole Thickness, **FL** Fruit Length, **FW** Fruit Weight, **SSC** Soluble Solids Content

ISSR analysis

A total of 24 different ECB genotypes were evaluated in the study with ISSR markers. Twenty different primers pairs, ranging in length from 130 to 1400 bp, were used, and band formation was observed for 11 of these primers. A total of 73 scoreable bands were obtained, 44 of which were polymorphic. In terms of band numbers, VHV(GTG)$_7$ primer (13 bands) produced the highest number of bands, and the lowest band number was obtained from (GT)$_8$YA primer (3 bands). In the number of polymorphic bands, the number of bands of the primers ranged from 1 for (AG)$_7$YC, (CA)$_8$AC, and (GAA)$_6$ to 11 for (VHV(GTG)$_7$). While the total number of bands per primer was 6.63, the average number of bands was 4.0. The lowest polymorphism values for the primers...
were 20%, and the highest rate of 100% was obtained for (GT)$_8$YA and (GACA)$_4$ primers. The mean polymorphism value recorded in the study was 60.27% (Fig. 2). In addition, no primer producing a completely monomorphic band was found in the study (Table 4).

Molecular marker analysis studies on ECB are very limited in the literature. In a previous study on ECB, an average number of bands per primer of 12.55, a number of polymorphic bands per primer of 6.0 with SSR markers, and an average polymorphism of 66.4% were recorded (Senavaitytė 2013). In another SSR study, 8 different SSR primers were used in ECB genotypes and a total of 97 bands, with 2 bands to 10 bands per primer pair, were obtained (Paulauskas et al. 2014). In an ISSR study, which is a different marker used in ECB genotypes, the average polymorphism rate recorded was 55.5% and the number of bands obtained ranged from 8 to 20 (Krupa-Małkiewicz et al. 2014).

The expected and observed allelic frequency values (p,q) ranged from 0.339 ((GT)$_6$GG) to 0.905 ((TCC)$_5$RY) and from 0.095 ((TCC)$_5$RY) to 0.661 ((GT)$_6$GG), respectively. Number of effective alleles (Ne) ranged from 1.091 (GAA)$_6$ to 1.569 (AGC)$_6$G (average 1.325), Shannon’s information index (I) values ranged from 0.109 (GAA)$_6$ to 0.517 (GT)$_8$YA, expected heterozygosity (He) values ranged from 0.083 ((CA)$_6$AC) to 0.338 ((GT)$_8$YA), and unbiased expected heterozygosity (uHe) values ranged from 0.079 ((AG)$_7$YC) to 0.345 ((GT)$_8$YA) (average 0.200) (Table 4).

According to the UPGMA dendrogram of the genotypes, the similarity index varied between 0.77 and 0.95, and 2 main groups were formed between the genotypes in the dendrogram. While only the G5 was in group A, the other 23 genotypes were in group B. In group B, two subgroups were formed, and in subgroup B-I the genotype G8 was grouped alone. According to the dendrogram, the closest genotypes to each other are G10 and G24 with a similarity index of 0.95. In the molecular marker analysis results of the study, genotypes were randomly distributed and grouped in general, and an intense grouping of the regions from which they were taken did not emerge. In addition, all genotypes were separated from each other in the dendrogram (Fig. 3). Cophenetic correlation between ultra-metric similarity tree and similarity matrix was found to be relatively high ($r = 0.73, P < 0.01$). Values of 0.7–0.9 indicate a high correlation between similarity indices and dendrogram (Uzun et al. 2017). The high correlation between the similarity indices and the dendrogram indicate that the dendrogram represented the similarity index well.

It was determined wide variations among the dendrogram genotypes created according to the SSR marker analysis performed in Viburnum rufulum (Dean et al. 2015). In another study conducted in ECB, it was reported that ISSR and RAPD marker systems can be used to determine variations between genotypes (Moura et al. 2013). The results in this study are similar to those of previous studies. The minor differences observed can be attributed to the differences in the type of marker systems and genotypes used.
Phytochemical content

The result of all phytochemical analyses examined in ECB genotypes were found to be statistically significant. For antioxidant activity, G2 had the highest value of 53.78% and the lowest value was recorded in G6 with a value of 19.07%. Previous studies have reported that antioxidant content in ECB genotypes varies considerably with genotypes (Kraujalyte et al. 2013; Ozdal et al. 2014). In this study, total flavonoid content values ranged from 106.28 mg CAE/100 g (G22) to 318.87 mg CAE/100 g (G10) (Table 5). ECB fruits contain different flavonoids such as hyperoside, rutin, quercitin, and luteolin (Yurkiv and Grytsyk 2017). Veloğlu et al. (2006) reported a querticitin content of 26.1 mg/100 g in ECB. Different studies have found that the flavonoids contained in ECB are effective in the regulation of blood flow and have an antiaging effect (Ersoy et al. 2017).

There were differences in total phenolic values among the genotypes. The highest value was recorded in G7 with 679.57 mg GAE/100 g, whereas the
lowest value was in G23 with 451.23 mg GAE/100 g. Total phenolic content of *Viburnum opulus* varying between 680 and 831 mg/100 g depending on the cultivars was previously reported (Rop et al. 2010), and a value of 373 mg/100 g was also recorded another study (Polka et al. 2019). The total anthocyanin content varied from 16.48 mg cyn-3-gluc /100 g to 21.36 mg cyn-3-gluc /100 g in the genotypes (Table 5). Previous studies have reported an anthocyanin content ranging from 2 mg/100 g to 29 mg/100 g (Moldovan et al. 2012) and from 15 mg/100 g to 48 smg/100 g depending on the genotypes (Ersoy et al. 2017) in fresh fruits of ECB. The findings of this study on phytochemical content are generally similar to those of the previous studies. The slight differences observed could be due to different methods and plant material used.

Correlation analysis of morphological and biochemical properties of fruits

The results of the correlation analysis between the morphological and biochemical properties of fruit characteristics in the study are shown in Table 6. Fruit weight showed significant positive correlation with fruit width (0.70*** and fruit length (0.35***). Increasing cell number and cell enlargement with development in fruits might explain the strong relationship between these three features. However, fruit width (−0.52*** and fruit weight (−0.49*** showed a negative correlation with SSC. SSC per unit area tends to decrease with an increase in weight and volume in fruits. Weight, width, and length characteristics of fruits showed a negative correlation with total phenolic content, total anthocyanin content, and total flavonoid content. Although total phenolics content (0.33*** and total flavonoid content (0.30*** showed a positive correlation with pH, the correlation between SSC and biochemical properties was was not significant. Biochemical properties showed a positive correlation among themselves. Whereas total phenolic content showed a significant positive correlation with total flavonoid content (0.64*** and total anthocyanin content (0.63***), total flavonoid content showed a significant positive correlation with antioxidant content (0.49*** and total anthocyanin content (0.37**). The correlation results in the study were similar to those recorded for different fruit species in the literature (Cesoniene et al. 2010; Caliskan et al. 2012; Sarıdaş et al. 2017; Polat et al. 2021).

In summary, this study was conducted to determine the genetic diversity of ECB by using different marker techniques in 24 different genotypes collected from the districts of Kayseri province, which has high population density of the plant. Wide variations in morphology and phytochemical content were observed among the genotypes. It has been concluded that combining morphology and phytochemical data with ISSR molecular marker analyses can give more reliable results in distinguishing genotypes from each other rather than using these methods alone. In addition, the findings of this study provide a baseline for future research on the protection and development of this species.
Table 5  Phytochemical content of ECB genotypes

| Gen  | Antioxidant activity (% inhibition) | Total flavonoids (mg CAE/100 g) | Total phenolics (mg GAE/100 g) | Total Antosiyanin (mg cyn-3-gluc /100 g) |
|------|------------------------------------|----------------------------------|-------------------------------|----------------------------------|
| G1   | 20.21 p                            | 212.58 n                         | 674.16 ab                     | 18.34 d                          |
| G2   | 53.78 a                            | 232.21 lm                        | 514.96 fg                     | 17.33 ijk                        |
| G3   | 50.09 b                            | 259.98 g                         | 612.55 d                      | 17.48 f-j                        |
| G4   | 31.20 h                            | 239.24 ij                        | 669.65 ab                     | 18.25 d                          |
| G5   | 31.81 g                            | 293.69 d                         | 667.34 ab                     | 18.45 d                          |
| G6   | 19.07 r                            | 242.58 i                         | 675.49 ab                     | 18.13 de                         |
| G7   | 38.93 f                            | 251.46 h                         | 647.18 c                      | 17.78 efg                        |
| G8   | 31.81 g                            | 271.09 e                         | 667.91 ab                     | 19.17 c                          |
| G9   | 31.55 g                            | 235.54 kl                        | 679.57 a                      | 21.36 a                          |
| G10  | 24.87 k                            | 318.87 a                         | 658.91 bc                     | 19.00 c                          |
| G11  | 23.46 m                            | 251.46 h                         | 669.99 ab                     | 19.89 b                          |
| G12  | 24.34 l                            | 294.43 d                         | 671.88 ab                     | 17.77 e–h                        |
| G13  | 44.99 e                            | 263.69 f                         | 579.45 e                      | 16.90 l                          |
| G14  | 46.92 c                            | 309.61 b                         | 668.09 ab                     | 19.85 b                          |
| G15  | 46.05 d                            | 299.24 c                         | 641.61 c                      | 17.22 jkl                        |
| G16  | 20.30 op                           | 193.32 o                         | 505.12 g                      | 17.20 jkl                        |
| G17  | 20.56 op                           | 230.72 m                         | 523.50 f                      | 17.40 h–k                        |
| G18  | 26.19 j                            | 237.39 jk                        | 657.28 bc                     | 18.47 d                          |
| G19  | 26.36 j                            | 161.46 r                         | 641.07 c                      | 18.12 de                         |
| G20  | 20.65 o                            | 111.09 t                         | 527.55 f                      | 17.03 kl                         |
| G21  | 22.76 n                            | 174.06 p                         | 466.47 h                      | 16.48 m                          |
| G22  | 20.56 op                           | 106.28 u                         | 525.39 f                      | 17.68 f-i                        |
| G23  | 20.65 o                            | 152.21 s                         | 451.07 h                      | 17.42 g–j                        |
| G24  | 27.24 i                            | 230.72 m                         | 668.91 ab                     | 17.83 ef                         |
| Mean | 30.18                              | 232.21                           | 611.05                        | 18.11                            |

Different lower case letters show statistically significant differences between genotypes in column ($p < 0.05$)

Table 6  Correlation analysis of morphological and biochemical properties of fruits

| Var | FW | FL | FWT | SSC | L | a | b | pH | TP | TF | AA | TA |
|-----|----|----|-----|-----|---|---|---|----|----|----|----|----|
| FW  | 1  |    |     |     |   |   |   |    |    |    |    |    |
| FL  | 0.58*** | 1  |     |     |   |   |   |    |    |    |    |    |
| FWT | 0.70*** | 0.35** | 1  |     |   |   |   |    |    |    |    |    |
| SSC | -0.52*** | -0.13 | -0.49*** | 1  |   |   |   |    |    |    |    |    |
| L   | 0.28* | 0.09 | 0.36** | -0.17 | 1 |   |   |    |    |    |    |    |
| a   | -0.03 | 0.05 | -0.01 | -0.06 | -0.02 | 1 |   |    |    |    |    |    |
| b   | 0.24* | 0.20 | 0.18 | -0.17 | 0.39*** | 0.36** | 1 |    |    |    |    |    |
| pH  | -0.22 | -0.19 | 0.01 | -0.02 | -0.09 | 0.43*** | 0.06 | 1 |    |    |    |    |
| TP  | -0.50*** | -0.58*** | -0.45*** | 0.23 | -0.26* | 0.14 | -0.12 | 0.33** | 1 |    |    |    |
| TF  | -0.17 | -0.24* | -0.28* | 0.03 | -0.25* | 0.25* | -0.03 | 0.30** | 0.64*** | 1 |    |    |
| AA  | 0.15 | 0.16 | 0.11 | 0.16 | -0.06 | 0.10 | 0.21 | 0.00 | 0.14 | 0.49*** | 1 |    |
| TA  | -0.49*** | -0.49*** | -0.38*** | 0.11 | -0.19 | -0.12 | -0.36** | 0.09 | 0.63*** | 0.37** | 0.08 | 1 |

*p < 0.5, **p < 0.01, ***p < 0.001

Var Variable, FW Fruit Width, FL Fruit Length, FWT Fruit Wight, SSC Soluble Solids Content, TP Total phenolics, TF Total flavonoids, AA Antioxidant activity, TA Total Antosiyanin
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Declarations

Conflict of interest  The author declare that 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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