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Determination of Genetic Diversity in European Cranberrybush(*Viburnum opulus* L.) Genotypes Based on Morphological, Phytochemical and ISSR Markers

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Research Article

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Abstract

Turkey's plant diversity varies considerably. Many of these plants are native and commercially grown. European Cranberrybush, which is among the fruit species grown economically in the country, is also of interest in terms of health. In this study, it was aimed to determine genetic diversity with morphological, molecular, and phytochemical markers in 24 different genotypes from Kayseri province, which has an important place in the production of European Cranberrybush in Turkey. The results show that wide variations were detected between genotypes in the morphological parameters. While the genotype G13 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 range in fruit weights of genotypes varied between 0.16 g and 0.80 g. In ISSR marker analysis, a total of 73 scoreable bands were obtained from 11 different primers, and 44 of these bands were polymorphic bands. 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, total phenolic and total anthocyanin contents 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, 21.36 mg cyn-3-gluc /100 g to 16.48 mg cyn-3-gluc /100 g, respectively. It is thought that the results of the study may be useful to plant breeders in terms of its development and preservation, as well as giving an opinion to the researchers in new studies to be carried out in the European Cranberry.

Introduction

Turkey is a very important country in the world in terms of plant diversity. (**Gümüş and Avcı., 2020; Ozturk et al., 2020**). This genetic diversity has spread throughout the country, and most plant species are grown naturally and economically in Turkey. European Cranberyybush (*Viburnum opulus* L.) is one of the fruits that has increased in production in recent years, it is from the *Caprifoliaceae* family (**Al et al., 2017**), and Europe, Northwest Africa, Turkestan, and Canada are accepted as the homeland for European Cranberrybush(ECB) (**Richard and Pierre 1992; Ozrenk et al., 2020**).

ECB is grown in Tokat, Artvin, Samsun, Trabzon, Sivas, Erzurum, Bursa, Izmit, Sakarya, Istanbul, Izmir, Kirsehir, Ankara, Kahramanmaras provinces in Turkey, and it grows more especially in Kayseri than other provinces (**Yıldız and Ekici, 2009).** In Turkey, ECB is used as an ornamental plant in home gardens, fresh consumption (**Kajszczak et al., 2020**), fruits as beverage, dried and used as marmalade **(Kalyoncu et al., 2013**). In addition to these areas of use, it is a plant that is valued for its medicinal properties. ECB's juice is used for different purposes such as colds, kidney disease and especially diabetes. (**Eryılmaz et al., 2013**).

Different studies are carried out to determine genetic diversity in plants, both for breeding purposes and for the protection of the species. (**Hosseinpour et al., 2020**). These studies were generally made on morphological and phytochemical characters. However, it should be considered that morphological and phytochemical properties of plants can be affected by environmental conditions. (**Schneider et al., 2017**). Therefore, it is necessary to focus on molecular marker systems that will provide reliable results in

studies to be carried out to determine genetic diversity and where environmental conditions are not affected. (**Yu, 2020**). In addition, considering that some features such as yield, quality, resistance to diseases and pests in plants are controlled by multiple genes, there is a need for multidisciplinary studies in which morphological, biochemical, and molecular marker analyzes are combined. These studies, which were also carried out in the ECB species, were generally morphological (**Ozkan et al., 2020**), molecular (**Krupa-Małkiewicz et al., 2014**) and phytochemical (**Polka et al., 2019**), and multidisciplinary studies are almost nonexistent in the literature.

In this study, it was aimed to determine the genetic diversity of 24 different ECB genotypes with some morphological, phytochemical, and molecular markers in the Kayseri province, where the ECB population is dense in Turkey.

Material And Method

Material

24 different genotypes of ECB were used as material in this study. Genotypes were determined by selection 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). In these districts, ECB genotypes are generally grown in home gardens for landscaping and commercially (Figure 1). The leaves of the genotypes were taken in the middle of the summer season (July) and frozen in liquid nitrogen, brought to the laboratory and stored at -80 C until the analyzes were performed.

Table 1
Some information about the regions where ECB genotypes were taken.

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 solid 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 Doyle1992**). 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), 1.5µl 10x PCR Buffer, 0.2 µl Taq DNA polymerase (5u/µL), 1 µl dNTP (2.5mM), 1.5µl $MgCl_2$ (25 mM), 2 µl 10 mM ISSR primer, and 6.8 µl of H_2O. The amplification reactions using Thermal cycle (Sense Quest) Lab Cycle programmed for an initial denaturation step at 94°C for 3 minutes, followed by 35 cycles of 1 minute at 94°C, 35 cycles of 50 seconds at the specific annealing temperature at 53°C, 35 cycles of 2 minutes at 72°C and ended with a final extension step 7 minutes at 72°C. PCR products were electrophoresed on a 2% agarose gel prepared from 1X TAE buffer at 110 volts for 4 hours and visualized under UV light in the gel imaging (Kodak) unit after staining with ethidium bromide.

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 minutes 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 reportedd 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 Phenolic Compounds

Total phenolic 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 (Na_2CO_3) were added. After the prepared solution was incubated for 2 hours 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 hours 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.

ISSR markers were scored as presence (1) or absence (0) of bands. The sizes of bands were estimated by comparison with GENESTATM 100 bp DNA ladder. To evaluate the genetic diversity among the ECB genotypes, NTSYS-pc (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 Table 2 and Table 3. There were wide variations among genotypes in all parameters examined in leaves and fruits, and these differences are statistically significant. Leaf length values between 48.14 mm and 130.69 mm, while leaf width values ranged from 52.25 mm to 135.76 mm. For both values, the lowest results were G1, and the highest results were G13. In the petiole length values, the lowest value was determined in G4 with 15.94 mm, while the

highest value appeared in G11 with 25.09 mm. The average value of the genotypes was 1.62 mm in petiole thickness, which is one of the leaf parameters examined in the study.

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 ghi	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 ij	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 bcd
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

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 Width, **FWT**: Fruit Wight, **SSC**: Soluble Solid Content

Gen.	LL (mm)	LW (mm)	PL (mm)	PT (mm)	FL (mm)	FW (mm)	FWT (g)
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 Width, **FWT**: Fruit Wight, **SSC**: Soluble Solid Content							

In fruit analysis, G11 had the lowest fruit length value with 7.32 mm. On the other hand, G13 produced the highest result compared to other genotypes with 10.01 mm in this parameter, as in leaf width and leaf length values. The range in fruit width values varied between 4.64 mm and 9.52 mm. Fruit weight parameter is among the important fruit characteristics in ECB as in most fruit species. (**Asencio et al., 2018**). G1 with 0.16 g resulted in very low fruit weight compared to other fruit types. The highest fruit weight was detected in the G21 with 0.80 g. The fruit weight values obtained from G5, G6, G9, G10, G11, G13, G14, G16, G19 yielded results below the average fruit weight values of the study. There are various morphological studies about ECB in the literature. In one of these studies, fruit length and fruit width were determined to be 11.85 mm and 9.60 mm, respectively, in *Viburnum opulus* genotypes (**Konarska and Domaciuk, 2018**). In another 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**). The study conducted with genotypes taken from the same region but different district as in the current study, fruit length was determined between 7.65 mm and 8.81 mm, while 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., 2015**). 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. It was determined by **Taskin et al., 2019** that the L*, a*, b* values in ECB genotypes were 25.58, 35.39 and 24.60, respectively. While the average value of the genotypes in the pH value results was 3.08, the average value of the genotypes in the SSC values was 10.55%. SSC values were determined to be between 10.40% and 12.20% (**Ozrenk et al., 2020**), from 9.8% to 12.6% according to genotypes (**Ersoy et al., 2017),** while the pH value was determined as 2.9 in fresh fruits (**Taskin et al.., 2021**). The morphological data obtained from our study produced similar results with the studies in the literature. The reason for the partial differences can be explained by the fact that ecology and genotype differences can affect fruit characteristics. (**Bostan ve İşbakan, 2020**).

Table 3
Color, pH and SSC values of ECB genotypes

Gen.	L*	a*	b*	pH	SSC (%)
G1	29.77 b-e	24.64 de	10.76 d-g	3.03 def	15.38 a
G2	29.71 b-e	29.22 cd	11.88 c-g	3.04 def	10.24 c-f
G3	33.36 abc	27.93 cde	12.86 b-e	3.02 def	11.40 bcd
G4	30.73 b-e	23.78 de	11.72 c-g	2.93 fg	10.46 c-f
G5	26.04 e	46.82 a	16.67 a	3.14 bcd	9.28 efg
G6	29.69 b-e	31.66 c	11.09 c-g	2.96 efg	10.92 b-e
G7	31.57 bcd	40.74 b	15.65 ab	3.27 bc	12.16 bc
G8	28.51 cde	22.74 e	8.95 g	3.29 b	10.46 c-f
G9	31.19 bcd	24.70 de	11.26 c-g	2.91 fg	9.88 def
G10	29.10 b-e	23.88 de	9.45 fg	3.08 de	9.50 d-g
G11	29.97 b-e	24.99 de	10.22 d-g	3.28 bc	10.40 c-f
G12	31.97 a-d	29.60 cd	12.22 c-g	3.76 a	9.78 d-g
G13	27.65 de	47.24 a	17.15 a	3.14 bcd	10.96 b-e
G14	31.41 bcd	28.88 cd	9.85 e-g	3.13 cd	12.70 b
G15	32.17 a-d	26.30 cde	12.45 b-f	2.86 g	10.80 cde
G16	32.76 a-d	26.98 cde	12.62 b-f	2.90 fg	10.52 c-f
G17	33.98 ab	27.56 cde	12.61 b-f	2.90 fg	9.82 d-g
G18	33.11 abc	28.72 cd	13.25 bcd	3.10 de	10.66 cde
G19	29.59 b-e	24.68 de	10.83 d-g	3.09 de	10.58 cde
G20	33.58 abc	26.86 de	12.82 b-e	2.89 fg	9.72 d-g
G21	36.71 a	27.05 cde	14.22 abc	3.10 de	7.98 g
G22	32.10 a-d	27.05 cde	12.77 b-e	3.17 bcd	11.82 bc
G23	28.76 b-e	22.73 e	9.40 fg	2.90 fg	9.26 efg
G24	32.26a-d	26.97 cde	12.68 b-f	3.17 bcd	8.62 fg
Mean	**31.07**	**28.71**	**12.22**	**3.08**	**10.55**

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

ISSR Analysis

24 different ECB genotypes were evaluated in the study with ISSR markers. 20 different primers were used, and band formation was observed in 11 of these primers. A total of 73 scoreable bands were obtained, 44 of which were polymorphic. The base lengths of the primers ranged from 130 bp to 1400 bp. 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 band numbers of the primers varied between 1 ((AG)$_7$YC, (CA)$_6$AC, (GAA)$_6$) and 11 (VHV(GTG)$_7$. While the total number of bands per primer was 6.63, the average number of bands was determined as 4.0. As the lowest polymorphism values of the primers were 20%, the highest rate was obtained from (GT)$_8$YA and (GACA)$_4$ primers as 100%. The mean polymorphism value of the study was 60.27%. 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 study conducted in Viburnum, it was determined that the average number of bands per primer was 12.55 and the number of polymorphic bands per primer was 6.0 with SSR markers, while the average polymorphism percentage was 66.4% (**Senavaitytė, 2013**). In another SSR study, 8 different SSR primers were used in ECB genotypes and a total of 97 bands which ranging from 2 bands to 10 bands were obtained. (**Paulauskas, et al., 2014).** In addition to SSR markers, in the ISSR study, which is a different marker used in ECB genotypes, it was determined that the base lengths of the primers varied between 440 bp and 2650 bp, and the average polymorphism rate of the study was 55.5%. In the same study, the number of bands obtained from the primers was determined in the range of 8 to 20 (**Krupa- Małkiewicz et al., 2014**).

Table 4
Some data of ISSR primers that formed bands in the study

Primer	Bp	TBN	PBN	PR %	p	q	Ne	I	He	uHe
$(CA)_8R$	200-1100	7	4	57.14	0.705	0.295	1.505	0.428	0.293	0.299
$(GT)_6GG$	350-1050	8	6	75	0.339	0.661	1.205	0.246	0.145	0.148
$(AG)_7YC$	250-1000	5	1	20	0.853	0.147	1.127	0.115	0.078	0.079
$(CA)_6AC$	370-950	5	1	20	0.859	0.141	1.141	0.121	0.083	0.085
$(GT)_8YA$	500-800	3	3	100	0.525	0.475	1.542	0.517	0.338	0.345
$(AGC)_6G$	130-1000	10	7	70	0.552	0.448	1.569	0.439	0.307	0.314
$BDB(CA)_7C$	200-900	7	3	42.85	0.636	0.364	1.149	0.178	0.108	0.111
$(GAA)_6$	350-870	4	1	25	0.790	0.210	1.091	0.109	0.067	0.068
$VHV(GTG)_7$	310-1400	13	11	84.61	0.367	0.633	1.399	0.362	0.236	0.241
$(TCC)_5RY$	210-600	6	2	33.33	0.905	0.095	1.225	0.194	0.132	0.135
$(GACA)_4$	250-750	5	5	100	0.833	0.167	1.394	0.410	0.264	0.270
Mean	**130-1400**	**6.63**	**4.0**	**60.27**	**0.620**	**0.380**	**1.325**	**0.279**	**0.195**	**0.200**
Total	**-**	**73**	**44**	**-**	**-**	**-**	**-**	**-**	**-**	**-**

Bp: Base pair, **TBN**: Total band number, **PBN**: Polymorphic band number, **PR**: Polymorphism rate, **q** and **p**: Allele frequency, **Ne**: Number of effective alleles, **I**: Shannon's information index, **He**: Expected heterozygosity, **uHe**: Unbiased expected heterozygosity,

The expected and observed allelic frequency values (p,q) depending on the ISSR primers ranged from 0.339 ($(GT)_6GG$) to 0.905($(TCC)_5RY$) and from 0.095($(TCC)_5RY$) to 0.661($(GT)_6GG$), respectively. Number of effective alleles (Ne) ranged from 1.091 $(GAA)_6$ to 1.569 $(AGC)_6G$ (average 1.325), Shannon's information index (I) values ranged from 0.109 $(GAA)_6$ to 0.517 $(GT)_8YA$, expected heterozygosity (He)

values from 0.083 ($(CA)_6$AC) to 0.338 ($(GT)_8$YA) and unbiased expected heterozygosity (uHe) values 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 BIn 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. (Figure 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 between 0.7 – 0.9 indicate a well-correlation between similarity indices and dendrogram (**Uzun et al., 2017)**. Because present value of this study showed that there was a high correlation between the similarity indices and the dendrogram, present dendrogram well represented the similarity index. 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 viburnum species, it was determined that ISSR and RAPD marker systems can be used to determine variations between genotypes. (**Moura et al., 2013**). These studies in the literature and the results of the current studies are similar, and the reason for the differences can be related to the difference in the used marker systems and genotypes.

Phytochemical Content

The result of all phytochemicals analyzes examined in ECB genotypes were found to be statistically significant. In the inhibition percentages of antioxidant activity, G2 produced a very high result with a value of 53.78% compared to other genotypes. The lowest value was determined in G6 with a value of 19.07%. Studies have reported that antioxidant content in ECB genotypes varies considerably depending on genotypes (**Kraujalyte et al., 2013; Ozdal et al., 2014**). Total flavonoids content values ranged from 106.28 mg CAE/100 g (G22) to 318.87 mg CAE/100 g (G10). (Table 5). ECB's fruits contain different flavonoids such as hyperoside, rutin, querticin, luteolin (**Yurkiv and Grytsyk, 2017**). It was determined in the study conducted by Velioğlu et al., 2006 that the content of querticin is 26.1 mg/ 100g. Different studies have found that the flavonoids contained in the ECB are effective in the regulation of blood flow as well as the anti-aging effect. **(Ersoy et al., 2017**).

Table 5
Pyhtochemical 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

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

Gen	Antioxidant activity (% inhibition)	Total flavonoids (mg CAE/100 g)	Total phenolics (mg GAE/100 g)	Total Antosiyanin (mg cyn-3-gluc /100 g)
Mean	**30.18**	**232,21**	**611,05**	**18,11**
Different lower case letters show statistically significant differences between genotypes in column ($p < 0.05$).				

There were differences in total phenolic values depending on genotypes. The highest value was determined in G7 with 679.57 mg GAE/100 g, while the lowest value was in G23 with 451.23 mg GAE/100 g. It has been reported that the total phenolic contents of Viburnum opulus vary between 680 and 831 mg/100 g depending on the cultivars (Rop et al., 2010), and 373 mg/100 g in another study. (**Polka et al., 2019).** The total anthocyanin content, which is the last phytochemical parameter examined, was found to vary from 21.36 mg cyn-3-gluc /100 g to 16.48 mg cyn-3-gluc /100 g in genotypes. (Table 5). The anthocyanin content in fresh fruits of ECB is in the range of 22mg/100g –29mg/100g (**Moldovan et al., 2012**) and varied between 15mg/100g and 48mg/100g depending on the genotypes (**Ersoy et al., 2017**). The phytochemical results of the study are generally similar to the studies in the literature. However, there are slight differences. There may be various reasons for these differences. It should be considered that the different methods and plant material used may affect the results.

In summary, the study was conducted to determine genetic diversity by using different marker techniques in 24 different genotypes collected from the districts of Kayseri region, which has a very important position in the ECB population. Wide variations in morphology and phytochemicals were observed among genotypes. It has been determined that combining it with ISSR molecular marker technique can give more reliable results in distinguishing genotypes from each other rather than using these methods alone. In addition, it is foreseen that the results of the study will provide information that will give an opinion for new research to be carried out especially on the protection and development of this species.

Declarations

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Conflicts of interest/Competing interests (no conflict of interest)

Availability of data and material (Available)

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Figures

Figure 1

Some images of ECB genotypes

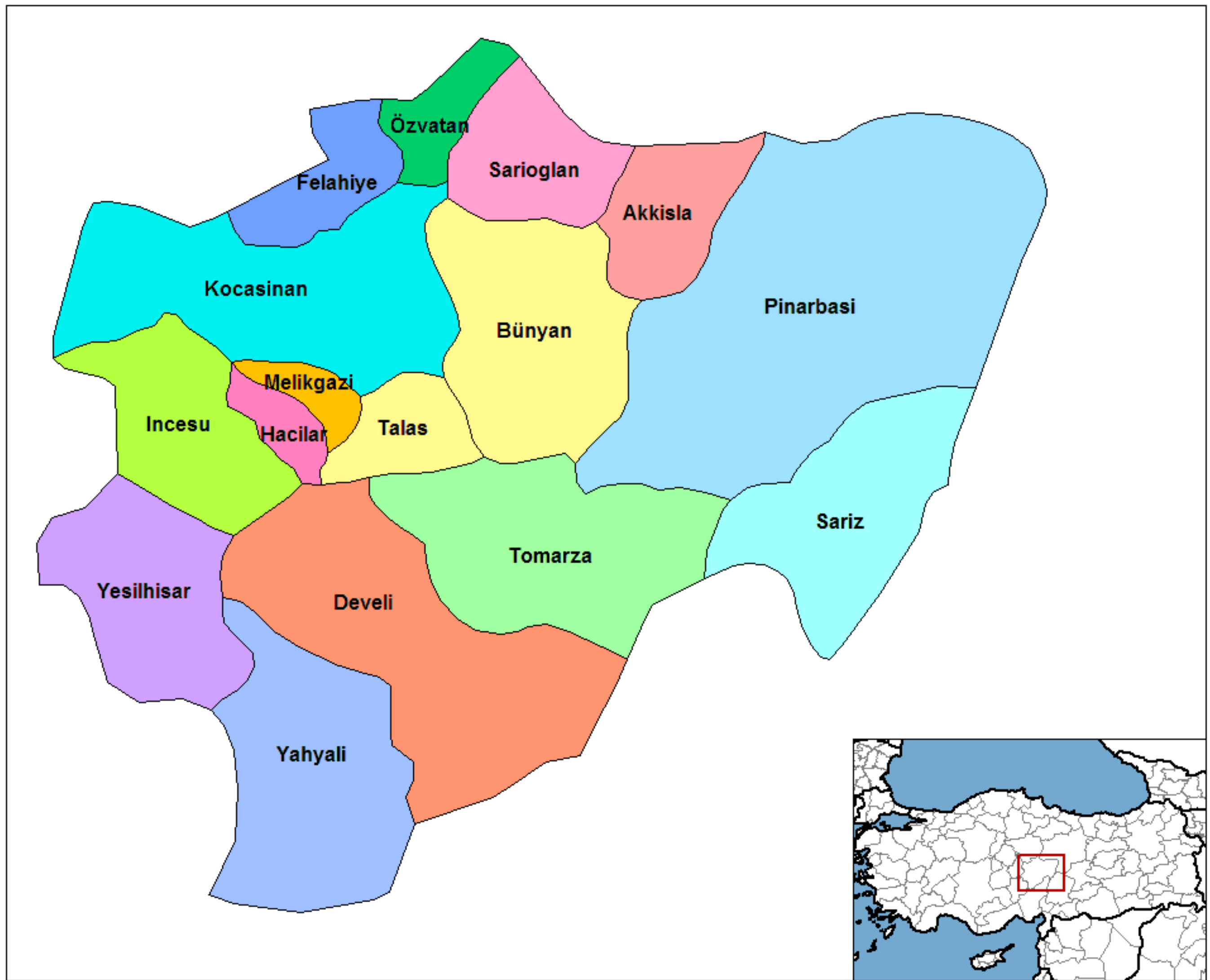

Figure 2

Map of districts where ECB genotypes were collected

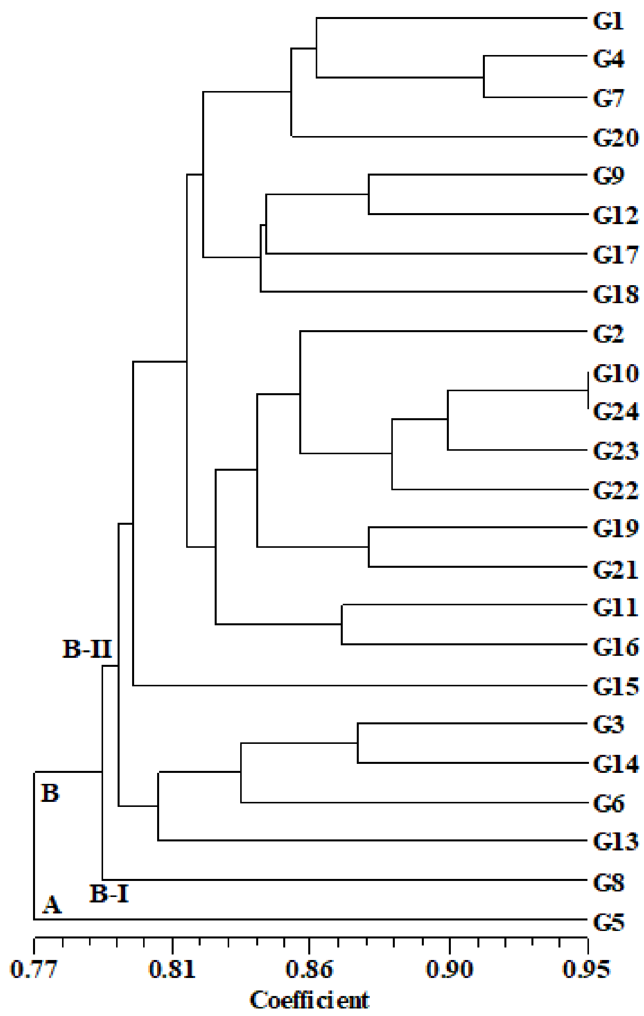

Figure 3

UPGMA dendrogram of ECB genotypes