TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2022) 46: 204-213

doi:10.3906/tar-2109-99

Morphological, biochemical, and bioactive characterization of naturally grown European cranberrybush genotypes

Ayşen Melda ÇOLAK[1,*], Fatma ALAN[2], Kerem MERTOĞLU[3], İbrahim BULDUK[4]

[1]Department of Horticulture, Faculty of Agriculture, Usak University, Usak, Turkey
[2]Department of Horticulture, Faculty of Agriculture, Erciyes University, Kayseri
[3]Department of Horticulture, Faculty of Agriculture, Eskisehir Osmangazi University, Eskisehir, Turkey
[4]Department of Occupational Health and Safety, School of Health, Uşak University, Uşak, Turkey

Received: 28.09.2021 • **Accepted/Published Online:** 24.02.2022 • **Final Version:** 25.04.2022

Abstract: Turkey has very rich potential in terms of wild edible fruit species, especially berries that grow naturally in different parts of the country as population or scattered shrubs. One of the most important wild edible fruits found in Turkey's flora is the European cranberrybush (EC). All EC plants are found as wild in nature and there are no commercial EC orchards in the country. In this study, a total of 22 seed propagated EC genotypes that naturally grown in the Akkışla district of Kayseri province located in the middle part of Turkey were assessed in terms of their morphological (fruit dimensions, fruit weight, the number of fruit per cluster, and cluster weight) and biochemical (soluble solids content, pH, and organic acids), bioactive (total phenolic content, total flavonoid content, and antioxidant activity) content in order to determine the superior ones. Fruit width, fruit length, fruit weight, cluster weight, and the number of fruit per cluster of 22 EC genotypes varied from 8.66 to 11.19 mm, 9.08 to 11.11 mm, 0.27 to 0.80 g, 8.50 to 55.20 g, and 23.00 to 81.40, respectively. Malic acid was the dominant organic acid in fruits of all investigated EC genotypes (12.177 g L^{-1} average of 22 genotypes), followed by citric acid (1.605 g L^{-1}), oxalic acid (0.776g L^{-1}), and ascorbic acid (65.18 mg 100 mL^{-1}), respectively. In terms of phenolic acids, a stable order could not be determined, while chlorogenic acid has come to the fore in fruits of 17 genotypes as main phenolic acid, while gallic acid was the highest in fruits of 4 genotypes and protocatechuic acid was the highest in the remained 1 genotype and average values of 22 EC genotypes of these phenolic acids were 27.05 mg L^{-1}, 23.50 mg L^{-1}, and 16.50 mg L^{-1}, respectively. Results indicated that seed propagated EC genotypes have variable morphological, biochemical, and bioactive traits that could be important to select cultivar candidates for future use at cultivation conditions. It is also important that characterization of 22 EC genotypes will add value for EC germplasm enhancement in Turkey.

Key words: Phenolic compounds, organic acids, *Viburnum opulus* L.

1. Introduction

In recent years, studies on wild edible fruit species all over the world have revealed how important these species are in human health and nutrition. Studies also indicate that these species can be used as insurance against climate change in the future in different parts of the world due to their high environmental plasticity (Garzon et al., 2010; Blando et al., 2016; Li et al., 2016). Around two billion people are reported to rely on wild-harvested products for nutrition and income and the "invisible" trade in wild resources is estimated to reach $300 billion/annually. In fact, around the world, wild edible fruits have played a significantly vital part in supplementing the diet of people since ancient times (Roslan et al., 2019; Suwardi et al., 2019; Navia et al., 2020). In particular, many local people living in rural areas still use them as a supplement to their basic needs of food. European cranberrybush (EC) is one of the remarkable species in these contexts (Ersoy et al., 2018; Ozkan et al., 2019; Ozrenk et al., 2020; Bhattacharjya et al., 2021). Previous studies conducted on different wild edible fruits exhibited that those species are very rich in terms of bioactive and biochemical content (phenolic acids, flavonoids, organic acids, antioxidants, etc.) and significant differences found in total phenolic content, phenolic acids, total flavonoid, and organic acid content among different genotypes within the same species. All wild edible fruits are also traditionally used for medicinal applications as for food supplements or functional foods contexts (Cosmulescu et al., 2019; Bayram and Ozturkcan, 2020; Engin and Mert, 2020; Gecer et al., 2020; Bozhuyuk et al., 2021).

Phenolic and organic acids commonly found at higher levels in EC fruits show high antioxidant activity by

* Correspondence: e-mail:aysen_melda@yahoo.com

preventing oxidation and peroxidation reactions (Velioğlu et al., 2006; Cam et al., 2007; Perova et al., 2014; Dienaite et al., 2020). This antioxidative effect reduces the risk of many chronic diseases including cancer and cardiovascular diseases (Sarıözkan et al., 2017; Zaklos-Szyda et al., 2020; Kajszczak et al., 2020). Thus, the availability of these compounds in the daily diet is very important in terms of health, adequate and balanced nutrition. In addition, these compounds are important for the defense mechanisms of plants under different environmental stress conditions such as injury, infection, excessive light, or ultraviolet rays to respond for survival (Marchiosi et al., 2020). Furthermore, wild edible fruits populations should increase in areas with heavy industrial production and highways due to their tolerance to sulfur dioxide. Mazepa (1986), determined EC as a potential species that can be used in landscape areas in North America to reduce air pollution. Also, many in vitro researches were emphasized the antimicrobial effects of different parts of EC. It is stated that by processing these products in the industry, the activity of microorganisms in the products is limited. Thus microbial decays decrease and the stability of the product is maintained (Bubulica et al., 2012).

Although EC has been processed into several products such as jellies, marmalades, sauces, liquors, yogurt, desserts, mead, beer, and vinegar since Seljuks and Ottoman times, cultivation of this species is not developed. It is very important to choose the genotypes that show superior features in EC and bring them commercial orchards because the species has the potential for consumption as dried or fresh, used in the industry, in the alternative medicine, etc. In addition, selected superior or promising genotypes will be ready material for cross-breeding to obtain new EC cultivars.

Therefore, the main objective of this work was (1) to screen and compare 22 EC genotypes that naturally grown in the Akkışla district belongs to Kayseri province in Turkey with regards to physico-chemical characteristics, (2) to make factor analysis to determine the various sources and interrelations among the investigated characteristics in order to provide useful information for EC.

2. Material and methods

2.1. Materials

Fruit samples belonging to naturally grown seed originated 22 EC genotypes which collected from different locations of Akkışla district of Kayseri province were used as material in the study. As indicated in Table 1, the altitude of locations was ranged from 1267 m a.s.l to 1616 m a.s.l and other geographical descriptions are given in Table 1 (Anonymous 2021a). Fruits were harvested in October in 2016 at the commercial maturity stage of EC genotypes according to taste and colour. Fruits were picked from all sides of the shrubs and they were harvested by a single person to maintain consistency of maturity degree.

Akkışla has a harsh continental climate. Observed climatic characteristics at the selection site during the study period are given in Table 2. When long term (average of 1990–2008) and 2016 year were compared, it was observed that the average temperatures of the long term were higher than 2016 except April. Humidity was higher in summer in 2016 while towards winter it was measured higher in long term, possibly due to larger amounts of precipitation in these months. Also, irregular rainfall and dry periods were observed in the study year (2016) compared to the long term.

2.2. Methods

2.2.1. Morphological traits

Harvested fruits are quickly put into a cold chain without losing time and transported to the laboratory. Fruit weight and cluster weight were determined using an electronic scale susceptible to 0.001 g and a digital caliper was used to measure fruit dimensions (fruit width and fruit length). The number of fruits per cluster was obtained by counting the fruits one by one (Ozrenk et al., 2020).

2.2.2. Biochemical content

Soluble Solid Content (SSC) was measured by a digital refractometer (Atago PR-32, Japan) and the results were given in percentile values and a pH meter was used for pH measurement (Karaçalı, 2012).

2.2.2.1. Determination of organic acids

The samples were homogenized with Heidolph Silent Crusher M (Heidolph, Germany). The samples were mixed 1 h with a shaker (Heidolph Unimax 1010, Germany) and then centrifuged at 14.000 rpm for 15 min. The supernatant was passed through a 0.45 µm membrane filter (Millipore Millex-HV Hydrophilic PVDF; Millipore, USA). This paration was carried out using reverse-phase ACE-C18 column (4 mm x150 mm, 5 µm). The mobile phase was a 10 mM potassium dihydrogen phosphate aqueous solution adjusted top with ortho-phosphoric acid. The mobile phase was vacuum-filtered through a 0.45 µm nylon filter and degassed on-line by a micro vacuum degasser. The chromatographic separation of these compounds was performed at room temperature. Analysis was run at a flow rate of 1 mL min^{-1}. Run time was 10 min. The detector was set at λ= 245 nm for ascorbic acid and the detector was set at λ=210 nm for the other organic acids. The injection volume was10 µL (Büyüktuncel et al., 2017). Each experiment was run in triplicate. Results are expressed as g L^{-1} fresh weight base.

2.2.3. Bioactive content

2.2.3.1. Total phenolic content

Total phenol content (TPC) was determined using the Folin-Ciocalteu method (Kahkönen et. al. 1999). Two

Table 1. Site description for EC genotypes.

Genotypes	Location	Altitude	Coordinates	
			Latitude	Longitude
1	Akkışla-Kululu-Kale	1423 m	38° 58’ 33”N	36° 08’2 0”E
2	Akkışla-Gümüşsu-Bağ yeri	1293 m	39° 00’ 24”N	36° 07’ 30”E
3	Akkışla-Gümüşsu	1293 m	39° 00’ 24”N	36° 07’ 30”E
4	Akkışla-Merkez-Yukarı	1370 m	39° 00’ 08”N	36° 10’ 25”E
5	Akkışla-Merkez-Güney	1370 m	39° 00’ 08”N	36° 10’ 25”E
6	Akkışla-Kululu-Güney	1423 m	38° 58’ 33”N	36° 08’2 0”E
7	Akkışla-Alevkışla	1267 m	39° 00’ 45”N	36° 06’ 03”E
8	Akkışla-Uğurlu-Güney	1454 m	39° 00’ 01”N	36° 12’ 48”E
9	Akkışla-Uğurlu-Batı	1454 m	39° 00’ 01”N	36° 12’ 48”E
10	Akkışla-Akin-Batı	1322 m	38° 59’ 33”N	36° 07’ 24”E
11	Akkışla-Merkez-Akbayır	1370 m	39° 00’ 08”N	36° 10’ 25”E
12	Akkışla-Akin-Güney	1322 m	38° 59’ 33”N	36° 07’ 24”E
13	Akkışla-Akin-Doğu	1322 m	38° 59’ 33”N	36° 07’ 24”E
14	Akkışla-Uğurlu-Güney	1454 m	39° 00’ 01”N	36° 12’ 48”E
15	Akkışla-Uğurlu-Doğu	1454 m	39° 00’ 01”N	36° 12’ 48”E
16	Akkışla-Kululu-Doğu	1423 m	38° 58’ 33”N	36° 08’2 0”E
17	Akkışla-Kululu-Batı	1423 m	38° 58’ 33”N	36° 08’2 0”E
18	Akkışla-Girinci-Batı	1616 m	38° 55’ 38”N	36° 06’ 50”E
19	Akkışla-Girinci-Kuzey	1616 m	38° 55’ 38”N	36° 06’ 50”E
20	Akkışla-Girinci-Güney	1616 m	38° 55’ 38”N	36° 06’ 50”E
21	Akkışla-Girinci-Doğu	1616 m	38° 55’ 38”N	36° 06’ 50”E
22	Akkışla-Gömürgen	1477 m	39° 01’ 58”N	36° 13’ 01”E

Table 2. Climatic data of Akkışla district on related months in 2016 and long term (1990–2008).

		April	May	June	July	August	September	October	Mean of year
Temperature (°C)	2016	11.6	12.4	17.6	20.0	22.6	15.0	11.2	10.77
	Long term	10.7	15.1	19.3	22.7	22.5	17.9	12.1	11.5
Humidity (%)	2016	48.3	67.7	61.3	50.7	43.9	50.3	48.2	58.60
	Long term	58.7	59.5	54.2	45.0	45.8	50.7	61.2	60.8
Precipitation ($mm.m^{-2}$)	2016	24.5	106.9	35.0	8.5	0.1	9.2	9.9	32.50
	Long term	47.2	59.0	39.5	12.9	9.4	15.0	31.7	33.9

hundred µL of freshly squeezed and filtered juice and 500 µL of Folin-Ciocalteu reagent (diluted 10 times with water) were added to the 10 mL of the test tube. The solution was then kept in the dark for 5 min and then 1000 µL of sodium carbonate solution (7.5% w/v in water) was added. The tubes were capped, shaken, and kept in the dark again for 1 h. UV spectrophotometric analyzes were performed in a dual-beam spectrophotometer device at 765 nm using 1.0 cm quartz cells for all absorbance measurements. It was compared to the gallic acid calibration curve. Results are expressed in mg GAE L^{-1}. Each experiment was run in triplicate.

2.2.3.2. Total flavonoid content

The total flavonoid content of fruit juices was determined by the aluminum chloride colorimetric method (Chang et al., 2002). Briefly, 50 µL of freshly squeezed and filtered

juice was taken into the 10 mL of the test tube. It was mixed with 950 µL of methanol and 4 mL of distilled water and then with 300 µL of sodium nitrite solution (5% in water). After the incubation, 5 mL of 300 µL of aluminium chloride solution (10% in water) was added and the mixture was allowed to stand for 6 min. Next, 2 mL of sodium hydroxide solution (1 M, in water) was added and the final volume of the mixture was made up to 10 mL with distilled water. The mixture was left to stand for 15 min. UV spectrophotometric analyzes were carried out in a double beam spectrophotometer device at 510 nm using 1.0 cm quartz cells for all absorbance measurements. The total flavonoid content was calculated from the quercetin calibration curve and the result was expressed as mg quercetin equivalent per liter.

2.2.3.3. Determination of phenolic compounds

Phenolic compounds were determined by an HPLC method using an Agilent 1260 Series liquid chromatography (USA) equipped with a UV/DAD detector. Chromatographic separation was performed on an ACE-C18 (4.6 mm ×150 mm, 5 µm) column. The solvent system had a constant flow rate of 1.0 mL min^{-}. The mobile phase was distilled water with 0.1% glacial acetic acid (solvent A) and acetonitrile with 0.1% glacial acetic acid (solvent B). The gradient was as follows: 0–3.25 min, 8%–10% B; 3.25–8 min, 10%–12% B; 8–15, 12%–25% B; 15–15.8 min, 25%–30% B; 15.8–25 min, 30%–90% B; 25–25.4 min, 90%–100% B; and 25.4–30 min, 100% B. The injection volume was 10 µL, and the temperature was kept constant at 25 °C. Detection wavelengths were chosen considering the absorption maximums of UV spectra of the selected phenolic compounds. Gallic acid, catechin, and syringic acid were detected on 280 nm, vanillic on 225 nm, coumaric acid on 305 nm, and chlorogenic and caffeic acid on 330 nm, while quercetin and kaempferol were monitored on 360 nm (Krstonosic et al., 2020). The result was expressed as mg per liter.

2.2.3.4. Antioxidant activity (DPPH)

The DPPH (2.2-diphenyl-1-picrylhydrazyl) assay was performed with small modifications from Thaipong et al. (2006). DPPH Stock solution; 24 mg DPPH was weighed and it was dissolved in some methanol. It was transferred to a 100 mL flask; the volume was completed with 100 mL of methanol. It was stored at −18 °C until use. The working solution was obtained by mixing 20 mL of the stock solution with 90 mL of methanol to obtain an absorbance value of 1.1 +/- 0.02 at 515 nm using a spectrophotometer. Three hundred µL of freshly squeezed and filtered juice was taken into a tube and 5700 µL of DPPH working solution was added and mixed. It was allowed to react for one h in a dark place. Then, the absorbance of this solution was measured at 515 nm wavelength in the spectrophotometer.

Antioxidant activity was calculated as a decrease in absorbance value using the formula:

Antioxidant activity (%) = $(A - A_1) / A_0 \times 100$

Where A_0 is the absorbance value of the control solution without sample.

A_1: Absorbance of the mixture containing the sample.

2.4. Statistical analysis

The study was designed in accordance with the randomized plot experimental design. Minitab-17 statistical package program was used in the statistical analyses. Linear correlation analysis was used to determine the relations between the characteristics and expressed with Pearson correlation coefficients. Principal component analysis (PCA) was used to examine the interrelations among the observed set of variables to identify the similarities and differences of characteristics. In addition, a scatter plot based on the first two principal components (PC1 and PC2) was generated. The first component describes most of the variation in the data. The second principal component is orthogonal and covers much of the remaining variation. Hierarchical cluster analysis (HCA) was performed for classifying the genotypes into groups by using Ward's method and Euclidean distance (Zar, 2013).

3. Results and discussion

Studies in the world on the morphological, biochemical, and bioactive characterization of EC fruits are limited to a few countries. In addition, studies have generally focused on cultivars belonging to the EC species, and there is a limited number of studies on wild-grown genotypes. This situation reveals the importance of the present study.

The data obtained in the study regarding the statistical analysis values of 22 EC genotypes grown in different locations are given in Table 3. Investigated morphological, biochemical, and bioactive characteristics showed high variation among the genotypes. Previous studies conducted on wild grown EC plants revealed high variation in morphological, biochemical, and bioactive content and our finding is comparable with previous studies conducted on *Viburnum opulus* L. (Ersoy et al., 2018; Özkan et al., 2020; Zarifikhosroshahi et al., 2020).

Descriptive statistics for the investigated characters in EC genotypes are given in Table 3. According to obtained results; cluster weight (CV = 48.58%) and total flavonoid content (CV = 43.10%) showed the largest variability, while the lowest CVs were obtained from pH and fruit length with 4.72% and 5.61%, respectively. Previously, the lowest and the highest CV values in European cranberrybush were reported as 8.6% for dry matter and 55.5% for sucrose (Cesoniene et al., 2010). In general, higher variations were seen in biochemical in particular bioactive content than morphological characters in *Viburnum* species (Cesoniene et al., 2010).

Table 3. Descriptive statistics for the investigated characters in EC Genotypes.

Abbreviation		Unit	Minimum	Maximum	Mean ± St. Dev	CV (%)
Morphological characters						
Fruit width	FrWi	mm	8.66	11.19	9.85 ± 0.65*	6.56
Fruit length	FrH	mm	9.08	11.11	10.03 ± 0.56*	5.61
Fruit weight	FrWe	g	0.27	0.80	0.58 ± 0.12*	20.38
The number of fruit per cluster	FrN	number	23.00	81.40	45.20 ± 17.44*	38.58
Cluster weight	CLWe	g	8.50	55.20	26.76 ± 13.00*	48.58
Organic acids						
Malic acid	MalA	g L^{-1}	6.316	21.625	12.177 ± 3.768*	30.95
Citric acid	CitA	g L^{-1}	0.764	2.303	1.605 ± 0.420*	26.19
Oxalic acid	OxaA	g L^{-1}	0.322	1.497	0.776 ± 0.265*	34.24
Ascorbic acid	AscA	mg100 mL^{-1}	2.70	117.6	65.18 ± 22.22*	34.11
Phenolic compounds						
Chlorogenic acid	ChloA	mg L^{-1}	23.43	31.08	27.05 ± 2.38*	8.80
Gallic acid	GallA	mg L^{-1}	21.76	26.11	23.50 ± 1.71*	7.28
Caffeic acid	CafA	mg L^{-1}	14.58	19.27	16.59 ± 1.53*	9.24
Protocatechuic acid	PrKA	mg L^{-1}	11.62	28.51	16.50 ± 4.48*	27.15
Vanillic acid	VanA	mg L^{-1}	11.01	15.44	13.00 ± 1.19*	9.19
Syringic acid	SyrA	mg L^{-1}	6.14	11.32	8.54 ± 1.33*	15.30
Coumaric acid	CoumA	mg L^{-1}	6.09	10.08	8.14 ± 1.13*	13.83
Biochemical and Bioactive characters						
Soluble solid content	SSC	%	7.26	18.14	11.35 ± 2.86	25.20
pH	pH	-	2.82	3.34	3.05 ± 0.14	4.72
Total phenol content	TPC	mg GAE L^{-1}	2866	3557	3160 ± 125	6.23
Total flavonoid content	TPFlvC	mg Quercetin L^{-1}	974	3663	1682 ± 130	43.10
Antioxidant activity	AntAc	%	72.36	89.57	83.16 ± 4.86	10.53

StDev: Standart deviation; CV: Coefficient of variation; *: Means statistical difference among genotypes

Differences of all investigated morphological characteristics among genotypes were found to be statistically significant (Table 3). Fruit width, fruit length, fruit weight, cluster weight, and the number of fruits per cluster were varied within the limits of 8.66–11.19 mm, 9.08–11.11 mm, 0.27–0.80 g, 8.50–55.20 g, and 23.00–81.40, respectively. In previous studies conducted with different European cranberrybush genotypes, average fruit width, fruit length, and fruit weight were reported respectively as 9.60 mm; 11.85 mm; 0.43 g (Konarska and Domaciuk, 2018) and 10.25 mm; 10.60 mm; 0.77 g (Özrenk et al., 2011), respectively. Higher ranges were reported for cluster weight and the number of fruit per cluster which is in agreement with our study and change intervals for these characteristics were reported between 16.7–37.6 g and 29–71 per raceme, respectively (Özrenk et al., 2011; Gündoğar 2013; Ersoy et al., 2018; Ozkan et al., 2020; Ozrenk et al., 2020).

Malic acid was the dominant organic acid in fruits of all investigated EC genotypes with 12.177g L^{-1} average. This finding is comparable with Cam et al. (2007) and Perova et al. (2014) but in contrast with Özrenk et al. (2011) and Ozrenk et al. (2020) who reported tartaric acid as the dominant organic acid of European cranberrybush. On the other hand, Ersoy et al. (2018) found that the main organic acid is not certain and depends on genotype in EC. They identified malic acid as dominant in four of the ten genotypes they examined and tartaric acid in the other six. Ranking of the other organic acids average values were found in descending order citric acid (1.605g L^{-1}) > oxalic acid (0.776g L^{-1}) > ascorbic acid (65.18 mg 100 mL^{-1}).

In terms of phenolic acids, a stable order could not be determined, while chlorogenic acid has come to the fore in 17 genotypes as main phenolic acid, while gallic acid was the highest in 4 genotypes and protocatechuic acid was the highest in the remained 1 genotype and considering

average values of 22 genotypes, these phenolics were found average 27.05 mg L^{-1}, 23.50 mg L^{-1} and 16.50 mg L^{-1}, respectively. Also, quantities of caffeic acid (16.59 mg L^{-1}) and vanillic acid (13.00 mg L^{-1}) generally were found higher than syringic acid (8.54 mg L^{-1}) and coumaric acid (8.14 mg L^{-1}) in the genotypes (Table 3). The obtained results were found parallel with literature (Velioğlu et al., 2006; Dienaite et al., 2020; Kajszczak et al., 2020).

The previously soluble solid content was reported between 9.80%–12.60% (Ersoy et al., 2017) and 10.96–12.65 (Özkan et al., 2020) in EC fruits and indicated similarities with the average values of our 22 genotypes (11.35%). pH varied from 2.82 to 3.34 and this finding is in agreement with Soylak et al. (2002) and Gündoğar (2013) who reported the limits between 2.80–3.10 and 2.83–3.17 in a number of EC genotypes, respectively. Total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity were found in the range of 2866–3557 mg GAE L^{-1}, 974–3663 mg Quercetin L^{-1}, and 72.36%–89.57%, respectively. Previous research have shown that European cranberrybush is a valuable source of health-promoting compounds. Obtained results are largely in line with previous studies. Although the differences are thought to be mainly caused by the variation of the investigated genotypes, climate and soil characteristics, altitude, time of harvest, maturity stage, storage or processing of the crop cause significant differences in the final form of the phytochemical composition (Tiwari and Cummins, 2013; Çolak et al., 2019; Mertoğlu et al., 2020; Polat et al., 2020).

Principal component analysis (PCA) was conducted to obtain a broad view on the dispersion of genotypes according to characteristics, determination of variation sources, determination of correlations between morphological and biochemical characters, etc. This method was adjusted in lots of minor berry species such as mulberry (Gecer et al., 2016), goji berry (Polat et al., 2020), barberry (Safari-Khuzani et al., 2020), and including European cranberrybush (Cesoniene et al., 2010; Özrenk et al., 2020).

According to PCA results, observed variability was explained by the first three components at 55.6%. The PC1 accounted for 24.1% of the total variance and was significantly correlated with soluble solids content, total flavonoid content, oxalic acid, malic acid, and ascorbic acid. Five characters, including fruit length, fruit height, fruit weight, cluster weight, and fruit number per cluster were placed into PC2 and explained 20.3% of the total variance. This also means that identified properties that were found in PC1 and PC2 are the most prominent properties that distinguish genotypes from each other. Bigger fruit sizes along with high biochemical accumulation are the main breeding purpose for European cranberrybush. In this context, it can be said that in addition to the pomological properties selection of genotypes that are rich in terms of organic acids will bring success inbreeding (Table 4). Similar observations and findings were reported by Özrenk et al. (2020) in *Viburnum opulus* L.

PCA method is a powerful multivariate statistical method to evaluate the relations among the characters. On the bi-plot platform, smaller angles than 90 among the characters mean that there is a positive correlation between those features, and close to 0 makes stronger the relation (Polat et al., 2020). In this respect, highly positive relationships were obtained between morphological characters (Figure 1A). The correlation coefficient between fruit width and fruit length was found as r = 76*** (data not shown). In fruit species, after fertilisation, an increase in the number of cells is observed, and this stage is followed by cell expansion. During the cell expansion phase, the combination of horizontal and longitudinal development in the cell explains the strong relationship between these two characters. The increase in the volume of the cells that make up the fruit weight increase. In this context, a strong positive relationship was determined between fruit weight and both fruit dimensions (fruit width and fruit length) 0.85*** and 0.66**, respectively. Contrary to expectations, the increase in the number of fruits per cluster caused an increase in morphological properties. This may be due to the increase in the number of seeds. As well known, the seed is one of the organs where auxin, cytokine, and gibberellic acid are produced in abundance, which has positive effects on fruit size by increasing cell number and expansion. It is also noted that these hormones also increase the transport of plant nutrients from leaves etc. to fruit (Musacchi and Serra, 2018). An increase in the pH value occurs as a result of the breakdown of organic acids that carry the H^+ ion. Thus negative correlations were detected between pH and organic acids, especially with malic acid (r = –0.45**) and ascorbic acid (r = –0.50**). The highest contribution to the total phenolic content was made by protocatechuic, coumaric, and chlorogenic acids. Interestingly, total phenolic content was found in a negative relationship with gallic acid, vanillic acid, and total flavonoid content. This case may be due to the fact that these compounds transform into each other when needed. Similar tendencies were reported in different fruit species (Gunduz et al., 2015; Çolak, 2018; Eskimez et al., 2020).

The distribution of genotypes according to investigated characters is given in Figure 1B. All investigated characters can be dispersed to genotypes visually by using PCA. Obtained results show that genotypes can be used for different purposes due to their diverse properties which distinguish them from each other. For example, Genotype 8 and Genotype 22 are suitable for industrial product processing thanks to their higher organic acids and soluble

Table 4. Eigenvalues and total variability proportion of principal component (PC) axes for investigated characteristics.

	PC1	PC2	PC3	PC4	PC5
Fruit width	0.003	–0.400	0.225	0.026	0.062
Fruit length	0.108	–0.392	0.016	–0.132	–0.051
Fruit weight	0.043	–0.389	0.244	–0.047	0.106
The number of fruit per cluster	0.091	–0.335	–0.180	–0.108	–0.228
Cluster weight	0.083	–0.436	–0.021	–0.083	–0.111
Soluble solid content	0.431	0.067	–0.041	0.044	–0.039
Ph	–0.165	0.107	–0.291	–0.406	–0.147
Total phenolic content	–0.053	–0.207	–0.116	0.464	–0.265
Total flavonoid content	0.330	0.188	–0.173	–0.008	–0.153
Antioxidant activity	0.013	0.127	0.363	0.040	–0.464
Gallic acid	–0.005	0.109	0.178	0.492	-0.084
Protocatechuic acid	–0.048	–0.125	–0.109	0.008	-0.446
Chlorogenic acid	–0.192	–0.077	–0.270	0.095	-0.341
Syringic acid	–0.195	0.080	0.262	–0.232	-0.329
Caffeic acid	–0.047	0.004	0.527	–0.202	-0.111
Vanillic acid	0.104	0.084	0.348	0.030	-0.020
Coumaric acid	–0.040	–0.035	0.024	0.461	0.073
Oxalic acid	0.413	0.065	0.086	–0.077	0.076
Malic acid	0.434	–0.022	–0.033	–0.088	-0.014
Ascorbic acid	0.415	–0.074	–0.042	0.066	-0.141
Citric acid	0.156	0.253	0.012	–0.013	–0.334
Cumulative variance (%)	24.1	44.4	55.6	65.9	75.1

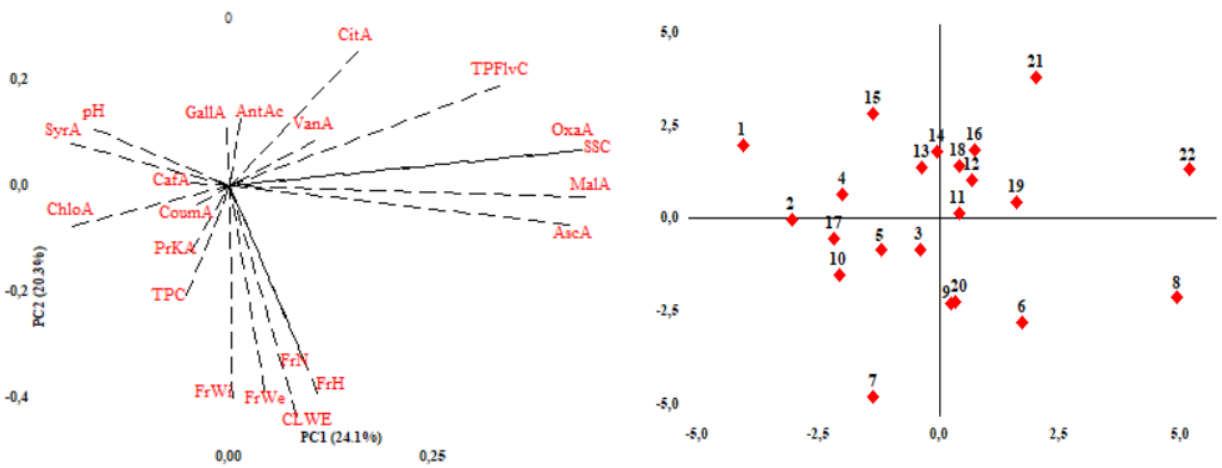

Figure 1A. Biplot with first two principal components for quality attributes and **B.** Score plot of EC genotypes according to biplot.

solids content. Because organic acids ensure the stability of the products and limit the activity of microorganisms that cause decays in products. On the other hand, higher SSC increases productivity during processing. Genotype 7 was found to be superior to the others in terms of all investigated morphological characters and has the potential to be used in breeding programs as a parent in order to obtain new EC genotypes. Genotypes 21 and 22 could be promising in terms of both taste and biochemical content in fermented products such as wine and vinegar,

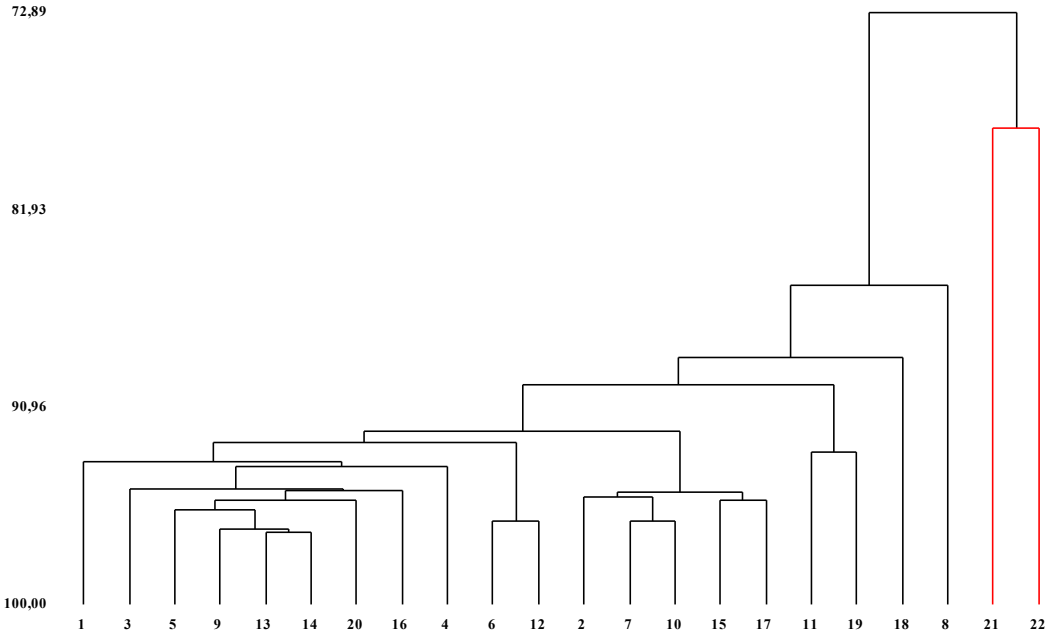

Figure 2. Cluster analysis of the investigated EC genotypes based on morphological and biochemical traits using Euclidean distances.

thanks to their high flavonoid content which gave bitter and astringency taste.

The number of EC cultivars throughout the world is very limited and a few studies conducted on EC genotypes for suitability to local climatic conditions. The results indicated that the region is particularly rich for EC genotypes and it can be accepted for ready breeding material.

Determination of seed propagated EC diversity and conservation of these genotypes is imperative as they are increasingly threatened. Genetic resource identification's main objective is to reveal trait-specific germplasm such as early or late ripening, high fruit weight, low chilling, biotic and abiotic stress resistance, and conservation for crop improvement utilization.

Previous studies also showed that high variation was evident among EC genotypes and they have the potential for use of diverse purposes in European cranberrybush breeding and cultivation (Özrenk et al., 2020; Zarifikhosroshahi et al., 2020).

The population was grouped under two main clusters and these are shown with different colours (black and red) in Figure 2. Phenolic compounds and organic acids were the main contributors to the formation of groups and it is also clearly seen in bi-plot segregation with taking the opposite place of these characters on the platform (Figure 1A). Cluster 1 consisted of Genotype 21 and 22. These genotypes were distinctly separated from the rest of the genotypes due to their higher organic acid and total flavonoid contents. Genotype 8, formed one branch of cluster 2 alone and takes place between the two groups. In other words, the members of the cluster 2 come to the front with their phenolics. In this context, it can be said that the morphological properties are ineffective to contribute cluster characteristics. This is thought to be due to the fact that fruits of these species have a minor size with rich biochemical compounds. Cesoniene et al. (2010), distinguished 13 accessions of EC on dendrogram into 2 main clusters according to their morphological and biochemical traits.

4. Conclusion

Within the scope of this study, it was emphasized that EC fruits have great potential with regards to be used in many products. Investigated genotypes were showed high variability both for morphological and biochemical traits. Some genotypes showed diverse traits that indicated richness of seed propagated EC germplasm in Turkey. Considering the biochemical compounds of EC fruits, organic acids and phenolic compounds were abundant. All EC genotypes were found to be used in sustainable fruit production to obtain more healthy and functional new food products.

Author contributions: The design of the research was done by AMÇ. AMÇ and FA contributed equally to the field and pomological measurements. IB made the HPLC analyses. KM made the statistical analyses and interpretation of the results. AMÇ and KM wrote the original draft. All authors read and approved the final draft of the manuscript.

References

Bayram HM, Ozturkcan SA (2020) Bioactive components and biological properties of cornelian cherry (*Cornus mas* L.): A comprehensive review. Journal of Functional Foods 75: 104252.

Bhattacharjya D, Sadat A, Dam P, Buccini DF, Mondal R et al. (2021). Current concepts and prospects of mulberry fruits for nutraceutical and medicinal benefits. Current Opinion in Food Science 40: 121-135.

Blando F, Albano C, Liu YZ, Nicoletti I, Corradini D et al. (2016). Polyphenolic composition and antioxidant activity of the under-utilised *Prunus mahaleb* L. fruit. Journal of the Science of Food and Agriculture 96: 2641–2649.

Bozhuyuk, MR, Ercisli S, Karatas N, Ekiert H, Elansary HO et al. (2021). Morphological and biochemical diversity in fruits of unsprayed *Rosa canina* and *Rosa dumalis* ecotypes found in different agroecological conditions. Sustainability 13: 14-8060.

Bubulica MV, Anghel I, Grumezescu AM, Saviuc C, Anghel GA et al. (2012). In vitro evaluation of bactericidal and antibiofilm activity of *Lonicera tatarica* and *Viburnum opulus* plant extracts on Staphylococcus strains. Farmacia 60 (1): 80-91.

Büyüktuncel E, Kalkan Ö, Şahin E (2017). Determination of organic acids in natural and commercial orange juices by HPLC/DAD. Hacettepe Journal of Biology and Chemistry 45 (3): 411–416.

Cam M, Hışıl Y, Kuşcu A (2007). Organic acid, phenolic content and antioxidant capacity of fruit flesh and seed of *Viburnum opulus*. Chemistry of Natural Compounds 43 (4): 460-461.

Cesoniene L, Daubaras R, Venclovienė J, Viškelis P (2010). Biochemical and agro- biological diversity of *Viburnum Opulus* genotypes. Central European Journal of Biology 6: 864-871.

Chang C, Yang M, Wen H, Chern J (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 10: 178-182

Cosmulescu S, Scrieciu F, Iordanescu O, Manda M (2019) Some pomological characteristics of medlar (*Mespilus germanica* L.) genotypes. Acta Horticulturae 1259: 43-46.

Çolak AM (2018). Effect of melatonin and gibberellic acid foliar application on the yield and quality of Jumbo blackberry species. Saudi Journal of Biological Sciences 25 (6): 1242-1246.

Çolak AM, Okatan V, Güçlü SF, Polat M (2019). **Different harvest times affect marketquality of** *Lycium barbarum L***. berries.** Turkish Journal of Agriculture and Forestry 43 (3): 326-333.

Dienaite L, Pukalskienė M, Pereira CV, Matias AA, Venskutonis PR (2020). Valorization of European cranberrybush (*Viburnum opulus* L.) berry pomace extracts isolated with pressurized ethanol and water by assessing their phytochemical composition, antioxidant, and antiproliferative activities. Foods 9 (10): 1413.

Engin SP, Mert C (2020). The effects of harvesting time on the physicochemical components of aronia berry. Turkish Journal of Agriculture and Forestry 44 (4): 361-370.

Ersoy N, Ercisli S, Akin M, Gundogdu M, Colak AM et al. (2018). Agro- morphological and biochemical characteristics of European cranberrybush (*Viburnum opulus* L.). Comptes Rendusde I' Academie Bulgare desSciences 71: 491-499.

Ersoy N, Ercisli S, Gundogdu M (2017). Evaluation of European Cranberrybush (*Viburnum opulus* L.) genotypes for agro-morphological, biochemical and bioactive characteristics in Turkey. Folia Horticulturae 29 (2): 181-188.

Eskimez İ, Polat M, Mertoğlu K (2020). M9 Anacı Üzerine aşılı arapkızı, jonagold ve fuji kiku elma (*Malus domestica* Borkh.) çeşitlerinin ısparta ekolojik koşullarında fenolojik ve fiziko-kimyasal Özellikleri. Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi 6 (2): 152-159 (in Turkish).

Garzon GA, Narvaez CE, Riedl KM, Schwartz SJ (2010). Chemical composition, anthocyanins, non-anthocyanin phenolics and antioxidant activity of wild bilberry (*Vaccinium meridionale* Swartz) from Colombia. Food Chemistry 122: 980-986.

Gecer M.K, Akin M, Gundogdu M, Eyduran S.P, Ercisli S et al. (2016). Organic acids, sugars, phenolic compounds and some horticultural characteristics of black and white mulberry accessions from Eastern Anatolia. Canadian Journal of PlantScience 96 (1): 27-33.

Gecer MK, Kan T, Gundogdu M, Ercisli S, Ilhan G et al. (2020). Physicochemical characteristics of wild and cultivated apricots (*Prunus armeniaca* L.) from Aras valley in Turkey. Genetic Resources and Crop Evolution 67: 935-945.

Gunduz K, Serçe S, Hancock JF (2015). Variation among highbush and rabbiteye cultivars of blueberry for fruit quality and phytochemical characteristics. Journal of Food Composition and Analysis 38: 69-79.

Gündoğar R (2013). Kayseri yöresinde yetişen gilebor (*Viburnum opulus* L.) genotiplerinin belirlenmesi. Ordu Üniversitesi Fen Bilimleri Enstitüsü Doktora Tezi, 61p (in Turkish).

Kahkönen MP, Hopia AI, Heikki JV, Rauha JP, Pihlaja K et al. (1999). Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry 47: 3954-3962.

Kajszczak D, Zakłos-Szyda M, Podsędek A (2020). *Viburnum opulus* L. A review of phytochemistry and biological effects. Nutrients 12: 3398.

Karaçalı İ (2012). Bahçe ürünlerinin muhafazası ve pazarlanması. Hasat öncesi dönemde gelişmeyi etkileyen faktörler. Ege Üniversitesi Yayınları, Yayın No: 494, İzmir (in Turkish).

Konarska A, Domaciuk M (2018). Differences in the fruit structure and the location and content of bioactive substances in *Viburnum opulus* and *Viburnum lantana* fruits. Protoplasma 255 (1): 25-41.

Krstonosic MA, Hogervorst JC, Mikulić M, Gojković-Bukarica L (2020). Development of HPLC method for determination of phenolic compounds on a core shell column by direct injection of wine samples. Acta Chromatographica 32 (2): 134–138.

Li Y, Zhang JJ, Xu DP, Zhou T, Zhou Y et al. (2016). Bioactivities and health benefits of wild fruits. International Journal of Molecular Sciences 17: 1258.

Marchiosi R, Dos Santos WD, Constantin RP, De Lima RB, Soares AR et al. (2020). Biosynthesis and metabolic actions of simple phenolic acids in plants. Phytochemistry Reviews 19: 865-906.

Mazepa VG (1986). Sensitivity of tree and shrub species to different concentration of SO_2. Lesovodsvo-i-Agrolesomelioratsiya 72: 33-37.

Mertoğlu K, Gülbandılar A, Bulduk İ (2020). Growing conditions effect on fruit phytochemical composition and anti-microbial activity of plum (cv. Black Diamond). International Journal of Agriculture Forestry and Life Sciences 4 (1): 56-61.

Musacchi S, Serra S (2018). Apple fruit quality: Overview on pre-harvest factors. Scientia Horticulturae 234: 409-430.

Navia ZI, Suwardi AB, Harmawan T, Syamsuardi, Mukhtar E (2020). The diversity and contribution of indigenous edible fruit plants to the rural community in the Gayo Highlands, Indonesia. Journal of Agriculture and Rural Development in the Tropics and Subtropics 121 (1): 89-98

Ozkan G, Ercisli S, Zeb A, Agar G, Sagbas HI et al. (2019). Some morphological and biochemical characteristics of wild grown Caucasian Whortleberry (*Vaccinium arctostaphylos* L.) genotypes from Northeastern Turkey. Notulae Botanicae Horti Agrobotanici Cluj Napoca 47 (2): 378-383.

Ozkan G, Ercisli S, Sagbas HI, Ilhan G (2020). Diversity on fruits of wild grown European cranberrybush from Coruh valley in Turkey. Erwerbs-Obstbau 62: 275-279.

Ozrenk K, Gündoğdu M, Keskin N, Kaya K (2011). Some physical and chemical characteristics of gilaburu (*Viburnum opulus L.*) fruits in Erzincan region. Institution of Science and Technology 14: 9-14.

Ozrenk K, Ilhan G, Sagbas HI, Karatas N, Ercisli S et al. (2020). Characterization of European cranberrybush (*Viburnum opulus* L.) genetic resources in Turkey. Scientia Horticulturae 273: 109611.

Perova IB, Zhogova AA, Cherkashin AV, Éller KI, Ramenskaya GV et al. (2014). Biologically active substances from European guelder berry fruits. Pharmaceutical Chemistry Journal 48: 332-339.

Polat M, Mertoglu K, Eskimez I, Okatan V (2020). Effects of the fruiting period and growing seasons on market quality in gojiberry (*Lycium barbarum* L.). Folia Horticulturae 32 (2): 229-239.

Roslan NSA, Zareen S, Zamri N, Akhtar MN (2019). Preliminary phytochemical screening, GC-MS profiling and in vitro evaluation of biological activities of *Garcinia atroviridis* root extracts. International Journal of Advanced Research 7 (1): 53-61.

Safari-Khuzani A, Rezaei M, Khadivi A (2020). Screening of the superior F1 segregating populations of barberry (*Berberiss* pp.) for cultivation based on phenotypic characterizations. Industrial Crops and Products 158: 113054.

Sarıözkan S, Türk G, Eken A, Bayram LÇ, Baldemir A et al. (2017). Gilaburu (*Viburnum opulus* L.) fruit extract alleviates testis and sperm damages induced by taxane-based chemotherapeutics. Biomedicine Pharmacotherapy 95: 1284-1294.

Soylak M, Elci L, Saracoğlu S, Divrikli U (2002). Chemical analysis of fruit juice of European cranberrybush (*Viburnum opulus*) from Kayseri-Turkey. Asian Journal of Chemistry 14 (1): 135-138.

Suwardi AB, Navia ZI, Harmawan T, Syamsuardi, Mukhtar E (2019). The diversity of wild edible fruit plants and traditional knowledge in West Aceh region, Indonesia. Journal of Medicinal Plants 7 (4): 285-290.

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Byrne DH (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19: 669-675.

Velioğlu YS, Ekici L, Poyrazoğlu ES (2006). Phenolic composition of European cranberrybush (*Viburnum opulus L.*) berries and astringency removal of its commercial juice, International Journal of Food Science and Technology 41: 1011- 1015.

Zaklos-Szyda M, Kowalska-Baron A, Pietrzyk N, Drzazga A, Podsędek A (2020). Evaluation of *Viburnum opulus* L. fruit phenolics cytoprotective potential on insulinoma MIN6 cells relevant for diabetes mellitus and obesity. Antioxidants 9 (5): 433.

Zar JH (2013). Biostatistical analysis: Pearson Prentice-Hall, Upper Saddle River, New Jersey.

Zarifikhosroshahi M, Murathan ZT, Kafkas E, Okatan V (2020). Variation in volatile and fatty acid contents among *Viburnum opulus* L. fruits growing different locations. Scientia Horticulturae 264: 109160.