Morpho-Biochemical Diversity and Chemometric Evaluation of *Viburnum opulus* L. Genotypes from Central Anatolia

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Forty-eight naturally growing *Viburnum opulus* L. genotypes were collected from the Sarıoğlan, Felahiye, Melikgazi, and Kocasinan districts in Kayseri province, and characterized for morphological and horticultural traits. Fruit width (7.3 to 11.6 mm), fruit length (8.2 to 12 mm), fruit weight (0.3 to 0.8 g), number of fruits per cluster (18 to 111) and cluster weight (10 to 78 g) exhibited significant heterogeneity among the genotypes. Soluble solids content ranged from 7.5% to 11.6% and pH values ranged between 2.6 and 3.7. Oxalic acid content ranged from 221 to 779 mg/100 mL, malic acid from 8.6 × 10^3 to 1.4 × 10^4 mg/100 mL, citric acid from 8.4 × 10^2 to 2.7 × 10^3 mg/100 mL, and ascorbic acid from 544 to 919 mg/100 mL. Total phenolic content was 1.8 × 10^3 to 2.0 × 10^3 mg/L GAE, total flavonoid content 1.2 × 10^3 to 2.0 × 10^3 mg/L QUE, and antioxidant activity remained relatively stable, ranging from 83.00% to 85.03% with a mean of 84.42. Principal component analysis (PCA) and hierarchical clustering revealed relationships between morphological and biochemical traits. Correlation analyses indicated strong positive associations between fruit size and cluster characteristics. Phenolic compounds and vitamin C contents are the primary factors determining antioxidant capacity. The results highlight the importance of genetic diversity and provide a foundation for breeding, selection, and sustainable utilization efforts.

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INTRODUCTION

The increasing global population, urbanization, and climate change are leading to a reduction in arable land suitable for agricultural production, making the sustainable utilization of local plant species for food, health, and industrial applications a critical research focus (Hunter *et al*. 2019; Raudonė *et al*. 2021). In this context, *Viburnum opulus* L. (gilaburu), naturally occurring in the Turkish flora and widely used traditionally, is an important fruit species distinguished by its biological activity profile and nutritional composition. Commonly recognized for its beneficial effects on stomach and kidney health, gilaburu fruits are traditionally consumed, particularly in certain parts of the Central Anatolia region, through fermentation (Polat *et al*. 2021; Dönmez 2023). The biochemical richness of guelder rose fruits is attributed to components such as phenolic compounds, flavonoids, organic acids, and antioxidant capacity. Recent studies have shown that the phenolic profile and antioxidant activity of the fruit vary significantly depending on the ripening stage and processing techniques (Sevindik *et al*. 2022). Moreover, fruit

composition is influenced by environmental conditions, microclimatic factors, altitude, and soil characteristics, with genetic diversity and growth environment playing a key role in fruit quality, especially in wild and semi-cultivated forms (Dursun Capar *et al*. 2021; Kirazlı and Tunca 2022). Furthermore, gilaburu fruits are collected by local communities in Central Anatolia, frequently sold in local markets, and utilized in traditional medicinal practices, such as for kidney stone ailments. Although their applications remain relatively limited, this highlights both the cultural significance of these fruits and their potential use in food, health, and industrial sectors.

Kayseri province is among the important regions where guelder rose grows naturally, and various studies have been conducted in this area. For instance, Yaman (2022) investigated 24 genotypes collected from six districts using morphological, phytochemical, and ISSR markers; Çolak *et al*. (2022a) evaluated 22 genotypes from the Akkışla district for morphological and biochemical traits; Çolak *et al*. (2022b) analyzed a total of 15 genotypes from Özvatan, Bünyan, and Hacılar districts in terms of pomological traits, total phenolic, and flavonoid contents and antioxidant activity. Additionally, Polat *et al*. (2021) compared three different genotypes for physicochemical parameters, while Dal and Karacabey (2021) and Dönmez (2023) investigated the potential effects of different drying methods on phenolic profiles and antioxidant activity. Although these studies provide valuable insights into the genetic and biochemical diversities of guelder rose, they are mostly limited to specific genotypes or ecological regions. Thus, broader, more detailed data on phenolic profiles and organic acids could be beneficial.

The genus *Viburnum* comprises more than 200 species worldwide and is predominantly distributed in the Northern Hemisphere (Chen *et al*. 2021; Sharifi-Rad *et al*. 2021; Kajszczak *et al*. 2021). The fruits, flowers, bark, and leaves of these species have ethnopharmacological significance in traditional medicine and nutrition (Skrypnik *et al*. 2021; Kolosova *et al*. 2022). Literature indicates that *Viburnum* species exhibit antioxidant, antimicrobial, anticancer, neuroprotective, and anti-endometriosis activities (Bae *et al*. 2010; Barak *et al*. 2019). Phenolic acids, flavonoids, tannins, lignans, iridoid glycosides, and triterpenoids are the primary components responsible for these biological effects (Altun *et al*. 2008; Chen *et al*. 2021). However, despite prior studies focusing on fruits and bark, there has been limited research on the biochemical composition of *Viburnum* leaves and especially flowers. Existing evidence suggests that flowers may contain phenolic compounds and flavonoids at levels equal to or higher than fruits, contributing significantly to their biological activities (Polka *et al*. 2019; Kajszczak *et al*. 2021).

The aim of this study was to comprehensively evaluate the morphological, biochemical, and physicochemical characteristics of naturally growing *Viburnum opulus* L. individuals collected from different districts of Kayseri province. Special emphasis was placed on phenolic profiles, flavonoid content, and antioxidant activity, while considering the potential effects of environmental conditions, microclimatic factors, and altitude. This study also aimed to provide detailed insights into the variation among wild populations, which may support their sustainable utilization in food, health, and industrial applications.

This study represents one of the more comprehensive and integrated assessments of the morphological and biochemical characteristics of wild *Viburnum opulus* L. populations in Anatolia. Unlike the information provided by existing studies in the literature, the integration of morpho-biochemical analyses with principal component analysis and hierarchical clustering offers novel insights into the genetic and functional diversity of the species.

MATERIAL AND METHODS

Material

Plant material

In this study, 48 naturally growing *Viburnum opulus* L. individuals were collected from the districts of Sarıoğlan, Felahiye, Melikgazi, and Kocasinan in Kayseri province. Sampling was carried out in October 2023, targeting fruits that had reached consumption maturity. To increase the objectivity of the selection process, soluble solids content (SSC) measurements were performed. Visual observations were used to initially assess ripening stage, and SSC analysis confirmed the objectivity of ripeness and taste criteria. The collected *Viburnum opulus* L. individuals naturally grow in wild or semi-natural habitats, often intermixed with other plant species, and are not cultivated as a commercial crop. However, they can be propagated and planted under suitable conditions. These plants typically prefer partially shaded areas, well-drained soils, and moderate moisture levels, which were considered during sampling to ensure representative collection. Since the plants do not form dense natural populations and are generally sparsely distributed or located in home gardens, samples were collected from multiple locations to adequately represent each district. From each population, 5 to 10 individuals were randomly selected, resulting in a total of 48 samples. The individuals used in this study were not previously identified or registered genotypes, and no genetic authentication was performed; therefore, the term "genotype" refers to the individual plants collected from the field.

The fruit samples were transported to the laboratory under cold-chain conditions for analysis. Morphological evaluations were conducted using three independent biological replicates for each genotype. In each replicate, 5 to 10 clusters were randomly selected, and an average of 35 fruits per cluster were counted, resulting in approximately 175 to 350 fruits analyzed per genotype (Ozrenk et al. 2011). Cluster and berry weights were measured using an electronic balance with a precision of 0.001 g (Sartorius-CPA 16001S, Göttingen, Germany), while fruit length and width were measured using a digital caliper with an accuracy of 0.01 mm. After the morphological measurements were completed, the remaining fruits were juiced using a fruit extractor, filtered through coarse-pore filter paper, and stored at −20 °C. The obtained fruit juices were used for all biochemical analyses.

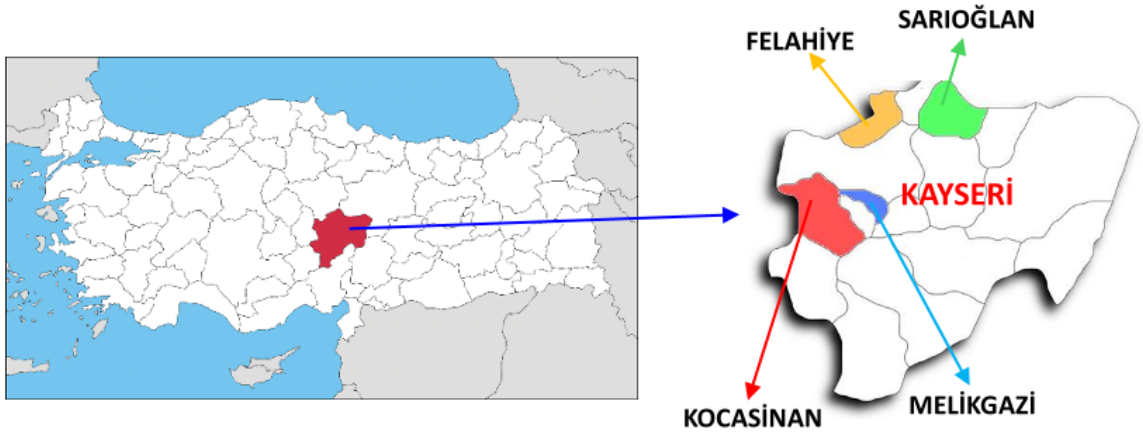

Fig. 1. Geographic distribution of the sampling locations of *V. opulus* L. genotypes studied in Kayseri province by district (Anonymous 2025a)

The samples were stored under cold conditions until biochemical analyses were conducted. The altitude of the study sites ranged from 1.085 to 1.501 m, with detailed geographic information presented in Table 1. According to current Turkish regulations, this species is neither cultivated nor protected and therefore, it can be collected from its natural habitats without official permits. Sampling was conducted following sustainability principles to ensure that local biodiversity was not harmed. The taxonomic identification of the *V. opulus* L. plant material used in this study was carried out by Dr. Alper Durmaz from the Department of Biology, Faculty of Science, Ondokuz Mayıs University. Species identity was confirmed using the Flora of Türkiye, Turkish Plants Database, Plants of the World Online (POWO), and up-to-date scientific literature.

Table 1. Geographic and Site Description of Sampling Locations for *V. opulus* L. Genotypes

Genotype No.	Location	Altitude	Coordinates	
			Latitude (N)	Longitude (E)
1-3	Sarıoğlan-Karaözü (N)	1148	39° 11′ 24″ N	35° 56′ 08″ E
4-6	Sarıoğlan-Palas (S)	1139	39° 01′ 25″ N	35° 51′ 25″ E
7-9	Sarıoğlan-Çiftlik (E)	1237	39º 01' 55" N	36º 02′ 31" E
10-12	Sarıoğlan-Üzerlik (W)	1151	39º 05' 26" N	35º 50′ 35" E
13-15	Felahiye-İsabey (N)	1408	39° 10′ 31″ N	35° 33′ 25″ E
16-18	Felahiye-Alabaş (S)	1420	39° 01′ 10″ N	35° 30′ 23″ E
19-21	Felahiye-Silahtar (E)	1497	39° 04′ 30″ N	35° 38′ 15″ E
22-24	Felahiye-Acırlı (W)	1424	39° 01′ 38″ N	35° 26′ 04″ E
25-27	Melikgazi-Ağırnas (N)	1298	38° 48′ 49″ N	35° 42′ 54″ E
28-30	Melikgazi-Kıranardı (S)	1444	38° 38′ 09″ N	35° 31′ 07″ E
31-33	Melikgazi-Büyükbürüngüz (E)	1468	38° 45′ 45″ N	35° 44′ 33″ E
34-36	Melikgazi-Eğribucak (W)	1085	38° 41′ 33″ N	35° 26′ 57″ E
37-39	Kocasinan-Amarat (N)	1501	38° 03′ 14″ N	35° 40′ 41″ E
40-42	Kocasinan-Erkilet (S)	1237	38° 49′ 12″ N	35° 26′ 59″ E
43-45	Kocasinan-Gömeç (E)	1123	38° 52′ 18″ N	35° 39′ 47″ E
46-48	Kocasinan-Himmetdede (W)	1198	38° 54′ 19″ N	35° 05′ 56″ E

*N: North; S: South; E: East; W: West

A continental climate predominates in the districts of Sarıoğlan, Felahiye, Kocasinan, and Melikgazi, from which the study material was collected. Winters are cold and snowy, while summers are hot and dry. Precipitation is irregular throughout the year, with distinct dry periods occurring, especially during the summer months. As shown in Table 2, summer temperatures in Melikgazi are lower compared to the other districts, whereas Sarıoğlan and Kocasinan experience higher summer temperatures. Similarly, relative humidity is higher in Melikgazi, with a marked increase in precipitation particularly between April and June. In contrast, humidity and precipitation in Sarıoğlan and Felahiye are more variable and generally lower. Although relative humidity is generally low, limited variations are observed among districts due to local microclimatic differences. Therefore, the variation observed in the biochemical and physicochemical traits of the genotypes cannot be explained solely by differences in altitude. While biochemical analyses in this study were conducted on samples from 2023, climate data from 2022, 2023, and long-term records (1931-2024) presented in Table 2 were also considered to assess potential impacts of inter-annual variability and ecological conditions on genotype characteristics.

Table 2. Temperature, Humidity, and Precipitation Data for 2022, 2023, and the Long-term Period (1931-2024) for the Districts of Sarıoğlan, Felahiye, Melikgazi, and Kocasinan (Anonymous 2025b)

	District	Year	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.
Temperature (°C)	Sarıoğlan	2022	12.7	13.0	18.6	19.9	24.5	19.0	12.4
		2023	9.1	12.7	17.4	21.0	24.4	18.2	13.2
		1931-2024	11.4	15.2	19.8	22.8	23.5	19.6	13.5
	Felahiye	2022	12.0	12.7	18.1	19.4	24.6	19.1	12.1
		2023	8.7	12.8	17.0	20.8	24.9	18.5	13.7
		1931-2024	10.3	13.8	18.2	21.5	22.7	18.8	13.1
	Melikgazi	2022	5.1	6.7	12.3	13.8	17.9	12.9	7.2
		2023	2.6	7.2	11.1	14.8	18.6	12.6	8.0
		1931-2024	3.5	7.9	11.9	15.2	16.0	12.1	6.9
	Kocasinan	2022	13.9	14.8	20.6	21.6	26.3	20.7	13.5
		2023	11.0	15.1	19.4	23.0	26.6	19.8	15.2
		1931-2024	12.0	15.8	20.2	22.9	23.6	20.1	14.0
Humidity (%)	Sarıoğlan	2022	40.8	51.9	54.4	46.9	38.8	42.4	53.3
		2023	61.4	59.7	58.9	42.5	35.0	43.2	52.5
		1931-2024	55.4	60.9	57.2	45.9	43.5	44.5	53.1
	Felahiye	2022	45.6	61.7	64.0	53.1	37.9	43.8	61.7
		2023	77.6	81.8	99.5	46.9	35.0	45.5	54.7
		1931-2024	59.6	62.8	59.0	47.2	43.5	44.6	53.2
	Melikgazi	2022	53.2	61.9	61.0	52.7	43.7	46.2	58.0
		2023	67.1	61.2	66.8	49.2	37.6	46.7	56.5
		1931-2024	65.3	65.0	64.4	54.4	49.5	52.4	59.2
	Kocasinan	2022	44.1	55.4	53.2	46.2	35.7	42.4	58.7
		2023	59.7	57.6	57.4	40.4	32.6	43.5	53.9
		1931-2024	57.2	58.5	56.3	49.0	46.9	47.8	55.8
Precipitation (mm/m)	Sarıoğlan	2022	11.0	42.2	65.4	0.2	0.0	38.6	14.5
		2023	74.7	32.2	56.9	3.9	2.9	23.4	22.5
		1931-2024	24.73	56.75	48.30	5.62	7.86	18.93	16.21
	Felahiye	2022	12.5	49.0	99.7	0.0	0.4	43.4	18.5
		2023	83.9	97.6	39.0	1.5	0.0	23.6	69.8
		1931-2024	34.14	63.34	51.32	4.69	10.35	20.06	24.71
	Melikgazi	2022	33.9	97.5	105.1	4.7	2.2	90.8	60.6
		2023	176.3	86.0	190.0	56.8	41.8	51.6	123.3
		1931-2024	68.86	84.30	80.96	13.62	23.86	30.00	49.88
	Kocasinan	2022	20.1	66.9	62.0	0.0	0.0	17.7	22.8
		2023	66.5	46.8	24.1	14.0	0.0	42.7	14.7
		1931-2024	28.73	47.18	42.49	4.35	5.46	16.14	17.97

*The biochemical traits of the genotypes examined in this study were obtained exclusively from 2023 samples. The 2022 and long-term climate data are provided for the purpose of comparing inter-annual variations.
*April: Apr.; May: May; June: Jun.; July: Jul.; August: Aug.; September: Sep.; October: Oct.

Determination of morphological traits and sample preparation

Fruit samples were transported to the laboratory under cold chain conditions for analysis and morphological assessments. The samples were conducted with three independent biological replicates for each genotype. In each replicate, 5 to 10 clusters were randomly selected and an average of 35 fruits per cluster were counted, resulting in approximately 175 to 350 fruits analyzed per genotype (Ozrenk *et al*. 2011). Cluster and fruit weights were measured using an electronic balance with 0.001 g accuracy (Sartorius-CPA 16001S, Göttingen, Germany), while fruit length and width were determined using a digital caliper with 0.01 mm precision. After completing morphological measurements, the remaining fruits were juiced, filtered through coarse-pored filter paper and stored at -20 °C. The obtained fruit juices were subsequently used for all biochemical analyses.

Methods

Chemical characteristics

Determination of soluble solids content and pH

The soluble solid content (SSC) of the fruit juices was measured using a digital refractometer (Atago PR-32, Tokyo, Japan) and the results were reported as a percentage (%). The pH values of the fruit juices were determined using a pH meter (Hach Co., Loveland, CO, USA) (Karacalı 2012). All analyses were conducted with three independent replicates for each genotype and separate fruit samples were used for each replicate, ensuring the repeatability of the measurements.

Spectrophotometric analyses

The total phenolic content (TPC) of the fruit juices was determined using the Folin-Ciocalteu method (Singleton and Rossi 1965). For this purpose, 200 mL of freshly crushed and filtered fruit juice was transferred to a 10 mL test tube. Then, 500 µL of Folin-Ciocalteu reagent, diluted tenfold with distilled water, was added. After incubating the mixture in the dark for 5 min, 1,000 µL of 7.5% sodium carbonate solution was added. The tubes were capped, shaken and incubated in the dark for 1 hour. Absorbance values were measured at 765 nm using a spectrophotometer. The corresponding mg GAE L^{-1} values were calculated from a calibration curve prepared with gallic acid standards.

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method (Chang *et al*. 2002). In brief, 50 µL of fruit juice, 950 µL of methanol, and 6,400 µL of deionized water were transferred into 10 mL test tubes, followed by the addition of 300 µL of 5% sodium nitrite solution. Subsequently, 300 µL of 10% aluminum chloride solution was added and the mixture was incubated for 5 min. Then, 2,000 µL of 4% sodium hydroxide solution was added and the tubes were allowed to stand for 15 min. Absorbance was measured at 510 nm using a spectrophotometer. Total flavonoid content was calculated as mg QUE equivalent L^{-1} based on a calibration curve prepared with quercetin standards.

Antioxidant activity of the genotypes was determined with minor modifications using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay (Elshafie *et al*. 2020). In this assay, 300 µL of fruit juice was mixed with 5,700 µL of 40 mg/L DPPH working solution in 10 mL test tubes. The tubes were incubated in the dark for 1.0 h to allow the reaction to complete, after which the absorbance of the solution was measured at 515 nm using a spectrophotometer. Antioxidant activity was calculated based on the decrease in absorbance using the following formula:

$$\text{Antioxidant activity (\%)} = (A_0 - A_1) / A_0 \times 100 \quad (1)$$

Here, A_1 represents the absorbance of the mixture containing the sample, while A_0 corresponds to the absorbance of the control solution without the sample.

Organic acid and vitamin C analyses

Organic acid and vitamin C contents were determined from homogenized fruit samples prepared for sugar analysis using the HPLC method developed by Bozan *et al*. (1997). For the analysis, 1.0 g of sample was weighed and 4 mL of 3% metaphosphoric acid was added. After shaking for 1.0 h, the samples were centrifuged at 14,000 rpm for 15 min. The resulting supernatant was filtered through a 0.45 µm membrane and prepared for HPLC analysis.

Determinations of organic acids and vitamin C contents were performed in triplicate using a Shimadzu Prominence LC-20A HPLC system equipped with a UV detector and a Transgenomic 87H column (7.8 × 300 mm). For organic acid analysis, an Agilent 1260 HPLC system equipped with Chemstation software, a quaternary pump, an autosampler and a UV detector, along with an ACE-C18 column (4 × 150 mm, 5 µm), was also used. The mobile phase consisted of 10 mM potassium phosphate solution, adjusted to pH 2.2 with ortho-phosphoric acid and was delivered at a flow rate of 1 mL/min. The detector wavelength was set to 245 nm for ascorbic acid and 210 nm for other organic acids (Fu *et al*. 2015).

Calibration curves ranging from 0.5 to 15 ppm were prepared using appropriate standard solutions for each compound, achieving high linearity (R^2 = 0.9999). Sample contents were calculated based on these curves and expressed as mg/100 g fresh weight (FW). Method validation included determination of LOD (Limit of Detection) and LOQ (Limit of Quantification) values. Repeatability was evaluated by performing five injections at each concentration level, with all RSD% values below 1% across three injection sets. Additionally, recovery studies were conducted to confirm the accuracy of the method.

Determination of phenolic compounds

Phenolic compounds were analyzed using an Agilent 1260 HPLC system equipped with a UV detector. Chromatographic separation was performed on an ACE-C18 column (4.6 mm × 150 mm, 5 µm). The mobile phase flow rate was maintained at 1.0 mL/min. Mobile phase A consisted of ultrapure water containing 0.1% acetic acid, while mobile phase B consisted of acetonitrile with 0.1% acetic acid. The gradient conditions were applied as follows: 0-3.25 min, 8%-10% B; 3.25-8 min, 10%-12% B; 8-15 min, 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 column temperature was maintained at 25 °C. Detection of phenolic compounds was based on their maximum absorption wavelengths: syringic acid, protocatechuic acid, and gallic acid at 280 nm; vanillic acid at 225 nm; *p*-coumaric acid at 305 nm; and caffeic acid and chlorogenic acid at 330 nm (Wen *et al*. 2005).

Statistical Analysis

The experiment was conducted according to a randomized complete block design. The obtained data were analyzed using IBM SPSS Statistics 21 (IBM Corp., Armonk, NY, USA). Descriptive statistics, including mean, standard deviation and minimum-maximum values, were calculated for physicochemical and morphological traits. Multivariate

differences among genotypes and relationships between traits were examined using Pearson's correlation and Principal component analysis (PCA). Prior to PCA, data were standardized using z-scores to eliminate scale differences among variables. A heatmap based on the PCA results was generated in Microsoft Excel using conditional formatting.

RESULTS AND DISCUSSION

Descriptive statistics of the morphological, organic acid, phenolic, and bioactive traits of the genotypes examined in this study are presented in Table 3. Morphological characteristics such as fruit width and length showed a relatively narrow distribution among genotypes. Genotypes from Sarıoğlan, Kocasinan, and Melikgazi exhibited similar fruit sizes, whereas the Felahiye genotype produced smaller and more variable fruits. These differences indicate that genotype-specific morphological variation exists in natural populations and may provide potential targets for selection studies (Ozrenk *et al*. 2020; Çolak *et al*. 2022).

Fruit weight and the number of fruits per cluster exhibited higher variability, reflecting natural heterogeneity and potential yield differences among genotypes. In particular, cluster weight was more variable in the Felahiye and Kocasinan genotypes, highlighting differences in fruit traits and reproductive distribution and demonstrating the interaction between genetic and environmental conditions.

Regarding organic acids, oxalic and citric acids were more variable among genotypes, whereas malic and ascorbic acids showed a more balanced distribution. The Felahiye genotype had higher oxalic and ascorbic acid contents compared to other genotypes, with ascorbic acid contributing significantly to antioxidant capacity. Variations in malic and citric acid levels were influenced by genotype, collection site and microclimatic conditions, consistent with previous studies showing environmental effects on phenolic accumulation and organic acid profiles (Kraujalytė *et al*. 2013; Vdovina *et al*. 2025).

For phenolic compounds, the TPC, TFC, and antioxidant activity measured by DPPH showed a relatively balanced distribution among genotypes. Chlorogenic acid and protocatechuic acid were the dominant phenolics, whereas minor phenolics such as syringic and *p*-coumaric acids were more variable, particularly in the Melikgazi genotype. Despite variations in phenolic compounds, the stability of antioxidant activity was attributed to interactions between phenolics and ascorbic acid. Previous studies have demonstrated the effects of processing conditions and environmental factors on phenolic accumulation and antioxidant capacity (Zakłos-Szyda *et al*. 2020a,b; Dal and Karacabey 2021; Goławska *et al*. 2023; Juhnevica-Radenkova *et al*. 2024). In this study, the preservation of antioxidant activity across genotypes represents a novel observation, highlighting genotypes capable of tolerating high oxidative stress.

Soluble solids content and pH values showed limited variation among genotypes, indicating homogeneity for these parameters. Vitamin C content varied among genotypes, with Felahiye and Kocasinan genotypes exhibiting higher values than Sarıoğlan and Melikgazi. These findings suggest that morphological traits are sensitive to genotype, whereas biochemical and functional traits remain relatively stable among genotypes (Ozrenk *et al*. 2020; Yurteri *et al*. 2021; Giritlioglu *et al*. 2025).

Overall, the results indicate that morphological and yield-related traits vary among genotypes, while biochemical and functional traits are more consistent. Relationships

among organic acids, phenolic compounds, and antioxidant capacity provide important insights into the functional quality of *V. opulus* fruits. Moreover, ecological and physiological factors such as altitude, climate, and light conditions influence the observed variation, emphasizing the importance of evaluating genetic potential in conjunction with environmental conditions (Dal and Karacabey 2021; Polat *et al*. 2021; Goławska *et al*. 2023). In this context, despite variations in phenolic content, the preservation of antioxidant activity and the resilience of bioactive traits among genotypes provide a biologically meaningful criterion for selecting functional fruits.

Table 3. Descriptive Statistics for the Physicochemical Properties of *V. opulus* L.

Abbreviation		Unit	Min.	Max.	Mean ± StDev	CV (%)
Morphological Characteristics						
Fruit Width	FrWi	mm	7.33	11.55	10.03±1.05	10.46
Fruit Length	FrL	mm	8.15	11.97	10.40±0.76	7.30
Fruit Weight	FrWe	g	0.32	0.77	0.58±0.12	20.68
Fruits per Cluster	FrC	number	18.00	111.00	53.50±24.26	45.34
Cluster Weight	CLWe	g	10.00	78.00	34.27±19.57	57.10
Organic Acids						
Oxalic Acid	OxaA	mg 100 mL^{-1}	221.44	778.48	490.04±178.11	36.34
Malic Acid	MalA	mg 100 mL^{-1}	8586.27	13862.43	10750.81±1880.71	17.79
Citric Acid	CitA	mg 100 mL^{-1}	843.51	2675.22	1958.66±736.40	37.59
Ascorbic Acid	AscA	mg 100 mL^{-1}	544.00	919.00	718.64±103.51	14.40
Phenolic Compounds						
Gallic Acid	GallA	mg 100 mL^{-1}	18	25	21.35±1.52	7.11
Protocatechuic Acid	PrKA	mg 100 mL^{-1}	10	17	13.79±1.79	12.98
Chlorogenic Acid	ChloA	mg 100 mL^{-1}	24	30	26.96±1.63	6.04
Syringic Acid	SyrA	mg 100 mL^{-1}	4	9	6.81±1.46	21.43
Caffeic Acid	CafA	mg 100 mL^{-1}	14	18	16.17±1.31	8.10
Vanillic Acid	VanA	mg 100 mL^{-1}	10	14	11.90±1.24	10.42
Coumaric Acid	CoumA	mg 100 mL^{-1}	6	10	7.73±1.28	16.55
Biochemical and Bioactive Characteristics						
Soluble Solid Content	SSC	%	7.56	11.55	9.48±1.05	11.07
pH	pH	-	2.59	3.66	3.20± 0.24	7.5
Total Phenolic Content	TPC	mg GAE L^{-1}	1816.52	1987.24	1879.61±48.12	2.56
Total Flavonoid Content	TFC	mg QUE L^{-1}	1815.52	1986.24	1878.61±48.12	2.56
Antioxidant Activity (DPPH)	AntAc	%	83.00	85.03	84.42±0.38	0.45
Vitamin C	VitC	mg/100 g FW	310.72	741.84	541.00±160.04	29.58

Principal component analysis was performed to summarize the variation in the morphological and biochemical properties of the *Viburnum opulus* L. genotypes and to reveal the relationships among the genotypes. Results of the analysis are presented in Table 4. Prior to analysis, all variables were normalized using z-scores (mean = 0, standard deviation = 1) due to differences in measurement scales. A critical matrix loading value of |0.40| was adopted and variables with loadings above this threshold were considered significant for the biological interpretation of components. The first four principal components (PC1 to PC4) explained a total of 73.61% of the variance. The first principal component (PC1) summarized the variation through variables representing fruit width, fruit weight, oxalic acid, malic acid, and the total phenolic and flavonoid contents. The presence of both positive and negative loading values indicated that genotypes with larger fruits may not directly coincide with those exhibiting higher phenolic and flavonoid contents. This finding suggests that environmental and genetic interactions play a significant role in shaping the relationship between fruit size and bioactive compounds (Table 4). The second principal component (PC2) was primarily associated with cluster traits (number of fruits per cluster and cluster weight) and citric acid content. Genotypes with high PC2 scores exhibited larger clusters and higher citric acid levels, indicating adaptive differences among genotypes in terms of cluster structure and acid profile. In particular, microclimatic factors (such as sunlight duration and light intensity) were observed to influence citric acid accumulation. The third principal component (PC3) was found to be related to the phenolic compound profile and antioxidant activity, including syringic acid, caffeic acid, vanillic acid, and DPPH. A portion of the variation in phenolic compounds likely resulted from genotype-specific responses to environmental stresses (*e.g.*, water stress and high light exposure). In this context, phenolic compounds are understood to be key contributors to antioxidant capacity, and higher accumulations were observed in genotypes from certain regions. The fourth principal component (PC4) was associated with pH and chlorogenic acid; however, vitamin C had low factor loadings and therefore did not contribute significantly to this component. Nonetheless, synergistic interactions between vitamin C and phenolic compounds are considered to be a potential mechanism supporting the stability of antioxidant activity. This could explain why DPPH activity remained relatively stable despite variations in phenolic content.

The PCA results not only summarized the statistical variation but also reflected the biological effects of genotype × environment interactions. For instance, it was observed that low temperatures at higher altitudes slowed the degradation of malic acid and contributed to the preservation of organic acid content, while water stress and high light conditions enhanced the accumulation of phenolic compounds. These findings provide valuable insights into the genetic potential and adaptive responses of *V. opulus* genotypes to environmental conditions.

When compared with the literature, the present PCA findings are consistent with previously reported relationships between fruit morphology and phenolic content in both Turkish and international studies (Oszmiański *et al*. 2015; Yaman *et al*. 2024; Singh *et al*. 2025). Additionally, the data revealed a potentially novel biological pattern, namely the stability of antioxidant activity despite variations in phenolic compounds. This phenomenon was explained by the dominance of ascorbic acid and matrix interactions mong phenolic compounds.

Overall, the PCA analysis elucidated the complex interactions between morphological and biochemical traits, offering both scientific and practical value for genotype selection and the assessment of bioactive compounds.

Table 4. Principal Component Analysis of Morphological and Biochemical Traits of Gilaburu (*V. opulus* L.) Fruits

	PC1	PC2	PC3	PC4
Fruit Width	-0.88	0.17	0.10	-0.06
Fruit Length	-0.52	0.04	0.22	-0.36
Fruit Weight	-0.84	0.43	-0.03	-0.09
Fruits per Cluster	-0.02	0.87	-0.09	0.07
Cluster Weight	-0.19	0.83	-0.07	-0.07
Oxalic Acid	0.85	0.19	0.21	-0.03
Malic Acid	0.80	-0.46	0.27	-0.03
Citric Acid	0.02	0.91	-0.30	-0.03
Ascorbic Acid	-0.65	-0.35	-0.45	0.27
Gallic Acid	-0.11	-0.56	-0.33	0.25
Protocatechuic Acid	0.47	0.28	-0.44	0.11
Chlorogenic Acid	-0.31	0.12	-0.39	0.61
Syringic Acid	-0.12	-0.36	0.77	-0.02
Caffeic Acid	0.16	-0.49	0.59	0.06
Vanillic Acid	-0.24	0.30	0.70	0.26
Coumaric Acid	-0.05	-0.81	0.11	-0.05
Soluble Solid Content	0.83	0.32	-0.17	-0.09
pH	0.19	-0.11	0.14	0.72
Total Phenolic Content	0.93	-0.12	0.01	0.01
Total Flavonoid Content	0.93	-0.12	0.01	0.01
Antioxidant Activity (DPPH)	-0.17	0.09	-0.55	0.15
Vitamin C	0.80	0.50	0.06	-0.07
Eigenvalue	7.26	5.15	2.59	1.17
% of Variance	33.03	23.44	11.80	5.35
Cumulative variance (%)	33.03	56.47	68.27	73.61

The dendrogram provided a visual representation of the similarities and differences among the examined genotypes (Fig. 2a). The results of the hierarchical clustering analysis were largely consistent with the PCA findings, demonstrating that the multidimensional relationships among genotypes were reliably revealed by both approaches. The population was grouped into three main clusters. The first cluster included genotypes collected from the Melikgazi and Kocasinan districts, which exhibited similar characteristics in terms of fruit width, fruit weight and total phenolic and flavonoid contents. The second cluster consisted of genotypes from the Sarıoğlan district and showed a homogeneous structure with respect to cluster weight and citric acid content. The third cluster comprised genotypes from the Felahiye district, characterized by fruit number and antioxidant activity. The branching distances in the dendrogram reflected the magnitude of differences among genotypes, where shorter branches indicated higher similarity and longer branches indicated lower similarity.

It was determined that the variation among genotypes represented not only statistical differences but also the underlying genetic and environmental interactions. For example, it was observed that low temperatures at higher altitudes slowed the degradation of malic acid, thereby contributing to the preservation of organic acid content. Similarly, microclimatic factors such as sunlight duration and light intensity stimulated the phenylpropanoid metabolism, leading to an increase in phenolic compound accumulation. In this context, the high phenolic content and antioxidant activity observed in genotypes from the Felahiye district were attributed to these ecological conditions. From a physiological perspective, organic acids are utilized in respiration during ripening, whereas phenolic compounds are synthesized as part of the plant's stress response. The increase in phenolic compounds under water stress and high light conditions, coupled with the

limitation of fruit size under the same conditions, indicated that the observed variation resulted from genotype × environment interactions. This finding helps explain some of the contrasting trends observed in the dendrogram and PCA results. Despite variations in phenolic compounds, the stability of antioxidant activity emerged as a potentially novel finding. This phenomenon may be explained by the dominance of ascorbic acid, synergistic effects among phenolic compounds, or matrix interactions. Similar observations have been reported in fruits such as wild blackberry (*Rubus* spp.) and *Aegle marmelos*, suggesting that antioxidant activity is determined not only by total phenolic content but also by the composition and interactions of bioactive compounds (Oszmiański *et al*. 2015; Singh *et al*. 2025). The influence of processing and fermentation on biochemical properties was also notable. For instance, fermented *Viburnum opulus* samples were reported to have higher total phenolic content and antioxidant capacity, while ultrasonic treatment increased phenolic constituents and free amino acids (Erdal *et al*. 2022; Kılıçkaya Selvi 2022). These findings highlight the significant impact of processing methods on the phenolic profile and antioxidant potential of the genotypes.

Overall, the dendrogram and PCA analyses systematically revealed the variation in morphological and biochemical traits of *V. opulus* genotypes, providing valuable insights for the effective utilization of genetic resources and quality optimization. These results align with both local and international literature and offer a strong foundation for understanding the adaptive responses of genotypes and their biochemical reactions to processing conditions.

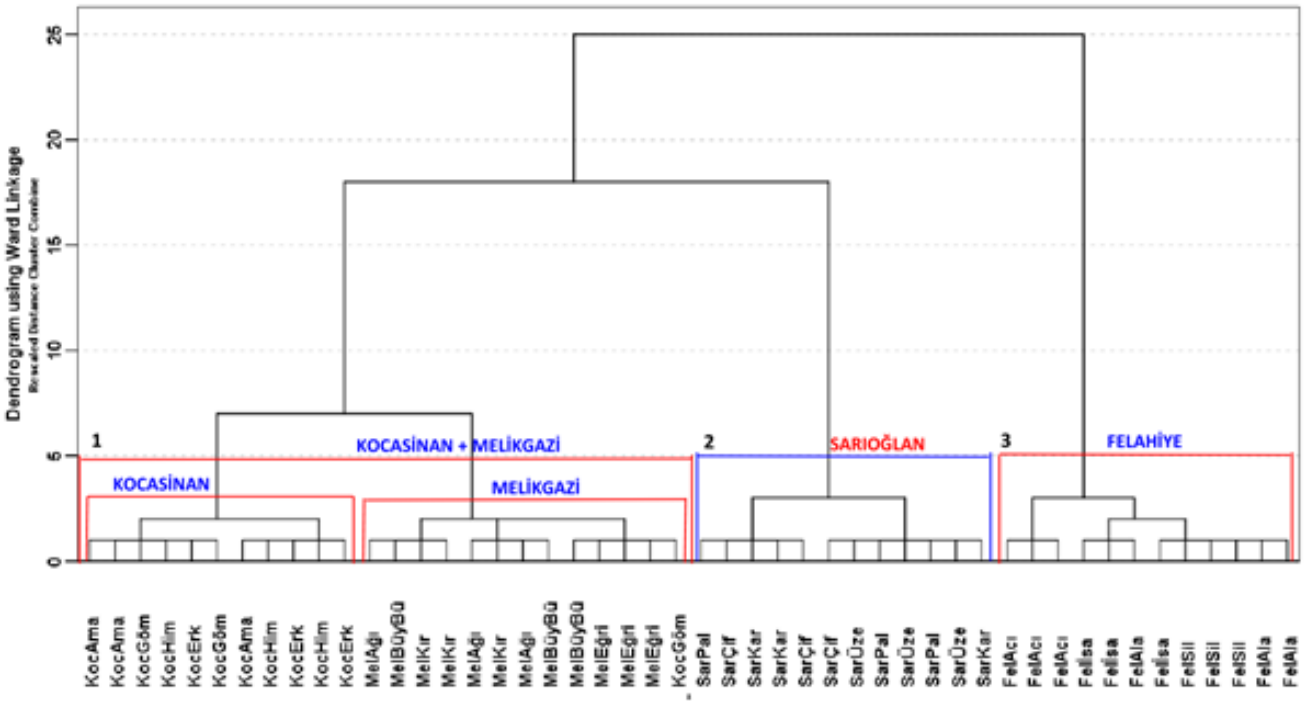

Fig. 2a. Hierarchical clustering analysis of *V. opulus* L. genotypes (Sarıoğlan-Karaözü: SarKar; Sarıoğlan-Palas: SarPal; Sarıoğlan-Çiftlik: SarÇif; Sarıoğlan-Üzerlik: SarÜze; Felahiye-İsabey: FelİsaFelahiye-Alabaş: FelAla; Felahiye-Silahtar: FelSil; Felahiye-Acırlı: FelAcı; Melikgazi-Ağırnas: MelAğı; Melikgazi-Kıranardı: MelKır; Melikgazi-Büyükbürüngüz: MelBüyBü; Melikgazi-Eğribucak: MelEğri; Kocasinan-Amarat: KocAma; Kocasinan-Erkilet: KocErk; Kocasinan-Gömeç: KocGöm; Kocasinan-Himmetdede: KocHim)

Variable	Component 1	Component 2	Component 3	Component 4
Fruit Width	-0.88	0.17	0.10	-0.06
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pH	0.19	-0.11	0.14	0.72
Total Phenolic Content	0.93	-0.12	0.01	0.01
Total Flavonoid Content	0.93	-0.12	0.01	0.01
Antioxidant Activity (DPPH)	-0.17	0.09	-0.55	0.15
Vitamin C	0.80	0.50	0.06	-0.07

Fig. 2b. Heatmap of *V. opulus* L. genotypes based on rotated component loadings of variables (Red indicates high negative loadings, blue indicates high positive loadings; the intensity of the color reflects the magnitude of the loading.)

The relationships among different morphological and biochemical traits were evaluated through correlation analysis (Fig. 2b). Strong positive correlations were observed among morphological characteristics. In particular, high correlation coefficients were detected among fruit width, fruit length, and fruit weight. This finding demonstrates the direct influence of fruit dimensions on fruit weight and reflects the consistency among pomological traits of the genotypes. Similarly, the strong positive relationship between cluster weight and the number of fruits per cluster indicated that cluster structure plays a critical role in determining fruit production potential.

Regarding biochemical components, a strong correlation was observed between TPC and TFC, highlighting the dominant role of flavonoids within the phenolic compound pool and their importance in determining antioxidant capacity. Significant positive correlations between TPC/TFC and antioxidant activity confirmed that phenolic compounds are the primary determinants of antioxidant potential. Additionally, the strong positive correlation between vitamin C content and DPPH activity indicated that ascorbic acid synergistically enhances antioxidant capacity alongside phenolic compounds. Moderate positive relationships among organic acids (*e.g.*, malic and citric acids) and the weak or negative correlation of oxalic acid suggested that organic acid profiles may vary independently across genotypes. Although a positive correlation was observed between gallic and protocatechuic acids, the overall low correlations among phenolic acids indicated that these compounds accumulate through different metabolic pathways. These results demonstrate that key biochemical components such as phenolic compounds and vitamin C determine antioxidant capacity, whereas morphological traits show strong interrelationships particularly through fruit size and cluster architecture. Similar findings have been reported in wild blackberry (*Rubus* spp.) and jackfruit (*Artocarpus heterophyllus*) genotypes, supporting the positive relationships between phenolic content and antioxidant activity (Oszmiański *et al*. 2015; Hazarika *et al*. 2025; Jagtap *et al*. 2010).

Moreover, metabolomic analyses have revealed associations between specific metabolites and biological activities, further confirming the link between phenolic compounds and antioxidant capacity (Lee *et al*. 2024).

The observed variation reflects not only genotypic differences but also the influence of ecological and physiological factors. At higher altitudes, lower temperatures reduced the rate of malic acid degradation, contributing to the preservation of organic acid content. Microclimatic conditions, particularly light intensity and sunlight duration, stimulated phenylpropanoid metabolism, leading to increased phenolic accumulation, which could be associated with the high TPC and antioxidant activity observed in certain genotypes. Physiologically, organic acids are consumed during respiration in the ripening process, whereas phenolic compounds are synthesized as part of stress responses. The increase in phenolic compounds under water stress and high light conditions, coupled with the limitation in fruit size under the same conditions, underscores the contribution of genotype × environment interactions to the observed variability. Furthermore, the stability of antioxidant activity despite changes in phenolic content could be explained by mechanisms such as ascorbic acid dominance, synergistic effects among phenolic compounds and matrix interactions. These findings, supported by both PCA and correlation analyses, enhance the understanding of the relationships between morphological and biochemical traits of *Viburnum opulus* genotypes and offer practical insights for genotype selection and the evaluation of bioactive compounds. Additionally, the consistency with international literature indicates that the results of this study hold validity not only in a local context but also at the global level.

These findings suggest that the biochemical variations determined by genetic factors have a strong potential for functional food applications. In particular, these genotypes could be promising candidates for the development of supplements, functional beverages, and value-added food ingredients due to their high phenolic and flavonoid contents and strong antioxidant capacity. Likewise, genotypes characterized by larger fruit size and higher cluster yield appear advantageous for commercial production because of their efficiency and processing suitability. Differences in organic acids and vitamin C contents indicate a meaningful link between fruit quality and functional value, highlighting their potential use as natural additives and quality enhancers in industrial applications. Furthermore, beyond contributing to the biochemical characterization of local genotypes, these results provide strategic insights for functional product development and genetic resource management. They demonstrate that *V. opulus* genotypes could be utilized in selection and breeding programs, aid in the optimization of production strategies, and hold considerable potential for application in the functional food sector.

CONCLUSIONS

This study systematically compared the morphological, physicochemical, and bioactive properties of *Viburnum opulus* L. genotypes collected from four different regions of Kayseri Province, thereby evaluating the effects of genetic diversity and ecological factors on fruit composition.

1. The analyses revealed statistically significant differences among genotypes in terms of fruit size, weight, and certain organic acid contents ($P < 0.05$). For example, the Felahiye genotype produced smaller and more variable fruits, whereas the Sarıoğlan,

Kocasinan, and Melikgazi genotypes showed similar fruit sizes. Significant variation was also observed among genotypes for yield-related traits, such as cluster weight and the number of fruits per cluster. These findings indicate that genetic diversity strongly influences morphological traits and production potential.

2. Soluble solid content, pH, TPC, TFC, and antioxidant capacity (DPPH) remained relatively uniform among genotypes. This indicates that bioactive traits are less influenced by environmental conditions and show notable genetic stability. However, variations in organic acid profiles (particularly oxalic and citric acids) and vitamin C contents were observed among genotypes, suggesting potential diversity in fruit quality and functional value.
3. Genotypes with high phenolic and flavonoid contents, together with strong antioxidant capacity, emerged as promising candidates for the development of supplements, functional beverages, and value-added food ingredients. Similarly, genotypes with larger fruit size and higher cluster yield offer advantages in commercial production efficiency and processing suitability.
4. The results indicate that both genetic diversity and ecological conditions (*e.g.*, altitude, climate, microclimate) influence fruit morphology and chemical composition. However, the genetic stability of bioactive traits supports their reliable use in selection and breeding programs.
5. Overall, these findings not only reveal the morphological and bioactive diversity of *V. opulus* genotypes but also provide strategic insights for functional food development, genetic resource management, and production optimization. Future studies, particularly those examining the molecular mechanisms of genotype × environment interactions, could further enhance their functional and commercial potential.

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