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Explanation of morphological and biochemical diversity of autochthonous grapes grown in Türkiye (Kelkit Basin) using multivariate analysis

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ABSTRACT

Grapes are widely grown around the world thanks to their different uses and nutritional importance. The demand for grapes is changing day by day in line with consumer preferences. This situation reveals the importance of identifying and protecting autochthonous grape varieties. This study was carried out to evaluate the morphological and biochemical characteristics of a previously unexplored autochthonous grape (*Vitis vinifera*) population using multivariate analyses. Morphological and biochemical characteristics were evaluated using principal component analysis (PCA), correlation analysis and hierarchical clustering analysis based on Ward's method. In the study, bunch weight varied between 71.67 g and 554.17 g, berry weight varied between 1.54 g and 10.98 g, and the number of seeds in berries varied between 0.00 and 3.50. Among the biochemical properties, total antioxidant content varied between 10.12% and 91.75%, total phenolic content varied between 123.77 mg 100 g^{-1} and 664.58 mg 100 g^{-1}, total flavonoid content varied between 16.48 mg 100 g^{-1} and 270.92 mg 100 g^{-1} and total anthocyanin content varied between 3.35 mg 100 g^{-1} and 74.42 mg 100 g^{-1}. The coefficient of variation (CV) among the characteristics examined ranged from 5.16% to 102.58%. As a result of PCA, the first two components explained 43.43% of the variation. The autochthonous grapes examined were divided into two main groups with different sub-clusters as a result of hierarchical clustering analysis. As a result of multivariate analysis, was detected significant variation among autochthonous grapes. The variations obtained show that the germplasm examined will be a valuable genetic resource for future grape breeding.

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1. Introduction

Türkiye has a special position in the world in terms of plant genetic resources. The Mediterranean and Near Eastern Centers which are the centers of plant diversity and origin defined by Vavilov (1951), intersect on Türkiye. Türkiye, located at the intersection of two different gene and diversity centers, is the gene center of grapes, as well as many fruit species (Sümbül et al., 2024). Türkiye, geographically located between 36º - 42° north latitude, is in the most suitable climate zone for viticulture in the world (Sabır, 2008). Türkiye is very rich in local grape genotypes resulting from natural hybridization, mutation and selection (Aradhya et al., 2003). The genetic resources of Türkiye, one of the homelands of grapevine, are gradually disappearing as a result of adverse events such as global climate change, urbanization, low number of varieties used in trade, natural disasters and various stress factors. Breeders using grapevine genetic resources can develop new varieties that are resistant to various stress factors and have high quality. In addition, grapevine genetic resources provide a valuable resource to breeders in solving problems that may be encountered. As a result, collecting, identifying, and protecting local grape resources is important for the future of grape growing (Sümbül et al., 2024).

Grapevines differ from each other in terms of bunch and berry characteristics depending on the region where they are grown. In the characterization of grapevine genotypes, ampelographic methods based on morphological and pomological characteristics were first used. Ampelographic methods are widely used in the characterization of grapevine genotypes based on their phenotypic characteristics. Morphological characteristics increase diversity in terms of desired agricultural characteristics (Iezzoni and Pritts, 1991; Khadivi-Khub and Anjam, 2014).

Grape fruit, which is rich in vitamins and minerals, is also rich in phytochemicals with antioxidant properties. Phenolic compounds are the most important phytochemical group that is a powerful defense tool in the fight against free radicals for humans and show antioxidant properties (Yang and Xiao, 2013). Phenolic compounds, which are abundant in grape fruit, have anticancer, anti-inflammatory, anti-aging and antimicrobial effects (Xia et al., 2010) as well as protective and preventive properties against cancer, cardiovascular diseases, cataracts, diabetes, Alzheimer's and eye diseases (Yahia, 2017). Consumer preferences in grapes are generally in the direction of physical properties such as bunch size, berry size, berry colour and berry shape. However, with the discovery of the health effects of grapes, consumer preferences have changed towards products that are beneficial for health (Filimon et al., 2017). Consumer demand for products with rich biochemical content such as grapes has revealed that biochemical content is also an important criterion in the characterization of genotypes. As a result, biochemical properties have been used in many studies on the characterization of grapes (Eyduran et al., 2015; Küpe et al., 2020; Özden and Deveci, 2023).

Grapes are one of the important commercial products of regions with temperate and tropical climates due to their high adaptability to different climatic and soil conditions, variety of usage areas and high nutritional value (Çelik, 2006). The global economic importance of the grapevine has led to a large proportion of clonally propagated genetic resources (Boz et al., 2011). The heterozygotic genetic structure of grapevines and the preservation of heterozygosity by clonal propagation can lead to the emergence of different types (Arroyo-Garcia et al., 2006). Thanks to this diversity in grapes, there are still many undefined genotypes in the gene centres of grapes (Magris et al., 2021). In recent years, with the understanding of the importance of genetic resources, many studies have been carried out both in Türkiye (Eyduran et al., 2015; Keskin, 2017; Küpe et al., 2020; Özden and Deveci, 2023; Güler and Karadeniz, 2023; Sümbül et al., 2023) and in the world (Khadivi-Khub et al., 2014; Vafaee et al., 2017; Abiri et al., 2020) to define the morphological and biochemical contents of local grapes.

Leaf, bunch and berry characteristics and biochemical contents of grapes are widely used in characterization studies of grapevine genotypes. This study is the first study to identify autochthonous grapes (*Vitis vinifera*) in the Kelkit Basin, Türkiye. The aim of the study is to determine the morphological and biochemical diversity of the grapes in the region by multivariate analysis.

2. Material and methods

2.1. Plant materials

The material of the study consisted of 60 autochthonous grape (*Vitis vinifera*) genotypes. The leaf characteristics of grape genotypes were determined in healthy leaves above the middle part of the shoots. Grape fruit were collected on harvest date specific to the genotypes. Collected fruit and leaves were placed in labeled plastic transport containers and transported to the laboratory in ice boxes. Pomological analyzes were carried out on the fruits transported to the laboratory, and necessary measurements were carried out on the leaves. Samples were taken from the fruits for biochemical analysis and stored at -20°C until analysis. The names of the genotypes and some descriptive characteristics are given in Table 1. Descriptive information of the genotypes was selected from the "Descriptors for Grapevine (Vitis spp.)" (Anonymous, 1997) list of descriptives fit for purpose.

Table 1. Some descriptive characteristics and local names of genotypes

Genotype	Code	OIV 204	OIV 225	OIV 241	Genotype	Code	OIV 204	OIV 225	OIV 241
Kokulu	U1	Medium	Green-Yellow	Seeded	Karadeniz	U31	Dense	Blue-Black	Seeded
Siyah üzüm 1	U2	Medium	Dark red-Violet	Seeded	Müşkü	U32	Dense	Green-Yellow	Seeded
Adıyaman	U3	Very Loose	Green-Yellow	Seeded	Beyaz üzüm 2	U33	Dense	Green-Yellow	Seeded
Mor üzüm 1	U4	Medium	Dark red-Violet	Seeded	Mor üzüm 3	U34	Very Loose	Dark red-Violet	Seeded
Beyaz üzüm 1	U5	Loose	Green-Yellow	Seeded	Ağ üzüm	U35	Dense	Green-Yellow	Seeded
Alyanak	U6	Medium	Green-Yellow	Seeded	Çavuş 1	U36	Medium	Green-Yellow	Seeded
Cemin	U7	Loose	Dark red-Violet	Seeded	Müşküle	U37	Medium	Green-Yellow	Seeded
İstanbul	U8	Loose	Green-Yellow	Seeded	Emcoğlu	U38	Very Loose	Green-Yellow	Seeded
Gazova 1	U9	Dense	Green-Yellow	Seeded	Dağ üzümü	U39	Loose	Dark red-Violet	Seeded
Mor üzüm 2	U10	Medium	Dark red-Violet	Seeded	Danagözü	U40	Medium	Green-Yellow	Seeded
Uzun üzüm	U11	Loose	Green-Yellow	Seeded	Çavuş 2	U41	Medium	Green-Yellow	Seeded
Parmak üzümü	U12	Loose	Green-Yellow	Seeded	Çekirdeksiz 1	U42	Loose	Red	Seedless
Dökülen	U13	Very Loose	Green-Yellow	Seeded	Siyah çekirdeksiz	U43	Loose	Red-Grey	Seedless
Kara üzüm 1	U14	Medium	Blue-Black	Seeded	Tokat üzümü	U44	Dense	Green-Yellow	Seeded
Gazova 2	U15	Dense	Green-Yellow	Seeded	Mor üzüm 4	U45	Loose	Red	Seeded
Siyah üzüm 2	U16	Medium	Blue-Black	Seeded	Bursa üzümü	U46	Medium	Green-Yellow	Seeded
Sarı üzüm 1	U17	Medium	Green-Yellow	Seeded	Sarı yanak	U47	Medium	Green-Yellow	Seeded
Gazova 3	U18	Loose	Green-Yellow	Seeded	Beyaz üzüm 3	U48	Loose	Green-Yellow	Seeded
Siyah gazova	U19	Very Loose	Blue-Black	Seeded	Güççük	U49	Loose	Green-Yellow	Seeded
Şirelik	U20	Loose	Green-Yellow	Seeded	Kara Salkım	U50	Very Dense	Dark red-Violet	Seeded
Pembe üzüm 1	U21	Loose	Dark red-Violet	Seeded	Keribar	U51	Dense	Green-Yellow	Seeded
Kara üzüm 2	U22	Medium	Blue-Black	Seeded	Ak üzüm	U52	Medium	Green-Yellow	Seeded
Pembe üzüm 2	U23	Loose	Dark red-Violet	Seeded	Davut üzümü	U53	Dense	Green-Yellow	Seeded
Dedem	U24	Loose	Green-Yellow	Seeded	Işıklar	U54	Very Dense	Green-Yellow	Seeded
Sık üzüm	U25	Very Dense	Green-Yellow	Seeded	İri mor	U55	Medium	Dark red-Violet	Seeded
Gevrek	U26	Very Dense	Green-Yellow	Seeded	Yeşil üzüm	U56	Dense	Green-Yellow	Seeded
Siyah Gevrek	U27	Dense	Dark red-Violet	Seeded	Geçci	U57	Very Dense	Green-Yellow	Seeded
Sarı üzüm 2	U28	Loose	Green-Yellow	Seeded	Mor üzüm 5	U58	Medium	Red	Seeded
Keçi memesi	U29	Medium	Green-Yellow	Seeded	Uzun kara	U59	Loose	Dark red-Violet	Seeded
Tatlı kara	U30	Medium	Blue-Black	Seeded	Çekirdeksiz 2	U60	Medium	Green-Yellow	Seedless

2.2. Morphological and physico-chemical characterization

Morphological characteristics consisted of mature leaf, bunch and berry characteristics. Leaf width, leaf length, main vein length and petiole length were measured with a ruler, while petiole thickness was measured with a digital caliper with a precision of 0.01 mm. The leaf area was calculated in cm^2 in the Image J package program on the leaf images photographed from an equal distance. Bunch weight, berry weight and 100 seed weight of the genotypes were determined with a precision balance with an accuracy of 0.01 g. Bunch width and bunch length were measured with a ruler, while berry width and berry length were measured with a digital caliper with a precision of 0.01 mm. The skin color of the berries was measured in terms of L*, a* and b* with a color measuring device (PCE-XXM 30, UK).

Measurements were made bidirectionally from the middle parts of the berries. Chroma (Chroma = $(a^2+b^2)^{1/2}$) and hue angle values (Hue angle = arctan(b/a)) were calculated using a* and b* values (McGuire, 1992). Fruit juice was obtained from 100 berries taken from the central parts of the bunches. From the obtained fruit juice, amount of total soluble solids (TSS) was determined in % Brix with a digital hand refractometer (PAL-1 Atago, USA), pH level was determined with a pH meter, and titratable acidity (TA) was determined in tartaric acid according to the titration method with a pH meter (Hanna pH212; USA) (Cemeroğlu, 2007). Maturity index was determined by the ratio of TSS to TA.

2.3. Biochemical characterization

Extraction: For biochemical analyses, 100 berries taken from the central part of the bunches were homogenized using a blender. 1 g of the homogenized sample was weighed and homogenized by adding 10 mL of 80% methanol. The samples were kept in the refrigerator (+4ºC) in the dark during a day. Then, the samples were shaken at 200 rpm for 30 min at room temperature and filtered with filter paper.

Total Phenolic Content: Total phenolic content was determined using Folin-Ciocalteu solution according to the method described by Slinkard and Singleton (1977). The absorbance of the resulting solution was read in a UV-Vis spectrophotometer (Shimadzu, Japan) at a wavelength of 765 nm. The values were calculated as mg gallic acid (GAE) 100 g^{-1} fresh weight (fw).

Total Flavonoid Content: Total flavonoid content was determined according to the method described by Karadeniz et al. (2005). The absorbance of the resulting solution was read in a UV-Vis spectrophotometer (Shimadzu, Japan) at a wavelength of 510 nm. The values were calculated as mg catechin (QE) 100 g^{-1} fw.

Total Anthocyanin Content: Total anthocyanin content was determined according to the pH-differential method described by Giusti et al. (1999). In this method, total monomeric anthocyanin content was determined at two different wavelengths (520 and 700 nm) and two different pH values (1.0 and 4.5). The values were calculated as mg malvidin 3-glycoside 100 g^{-1} fw.

Total Antioxidant Content: Total antioxidant content was determined according to the DPPH (1.1-diphenyl-2-picryl-hydrazyl) antioxidant activity method described by Brand-Williams et al. (1995). The absorbance values of the samples were determined in a UV-Vis spectrophotometer (Shimadzu, Japan) at a wavelength of 517 nm and the values were calculated according to the control and expressed as % inhibition.

2.4. Statistical analysis

In order to evaluate morphological and biochemical characteristics, minimum, maximum and mean values and CV showing the variation between the data were calculated. Analyzes based on morphological and biochemical characteristics were carried out in the JMP Pro 17.0 (SAS Institute Inc., Cary, NC, USA) statistical package program. PCA was used to determine the degree of influence of the examined characteristics and the relationship between the genotypes, hierarchical clustering analysis was used to group the examined characteristics and genotypes and correlation analysis based on Ward's method was used to determine the relationship between the examined characteristics.

3. Results and discussion

3.1. Morphological and Biochemical Analysis

Bunch and berry characteristics of grapevines are generally preferred in variety identification studies because they provide variety-specific information. Among the genotypes examined, bunch frequency was generally classified as loose and medium. In terms of berry skin color, more than half of the genotypes (37 genotypes) have green-yellow skin color. While three of the genotypes were seedlessness, the presence of seeds was detected in the other genotypes (57 genotypes) (Table 1). Grape genotypes examined within the scope of the study are generally in the table grape class. However, there are also grape genotypes with potential for wine grapes (U9, U15, U25, U26, U27, U31, U32, U33, U35, U44, U50, U51, U53, U54, U56, U57). In table grapes, berry skin color (Vafee et al., 2017; Somogyi et al., 2020) and seedlessness (Reisch et al., 2012) are important criteria affecting consumer preferences.

Berry skin color, berry size and bunch shape are important quality criteria for table grapes (Harindra Champa, 2015). While large-berry and loose bunches are preferred for table grapes, medium and small-berry and dense bunches are preferred for wine grapes (Melo et al., 2015).

Statistical information on the morphological and biochemical characteristics of grape genotypes are presented in Table 2. Wide variations have been observed among the characteristics examined. The coefficient of variation showing the change of the properties was determined at the lowest pH value (CV: 5.16%) and the highest a* value (CV: 102.58%). In addition, CV value of 19 of the 28 characteristics analyzed was found more than 20%. Characteristics with a CV value of more than 20% show more significant differences between genotypes and these characteristics can be used to distinguish genotypes. As a matter of fact, characteristics with high CV values have a wider selection range, while characteristics with low CV values are more stable among genotypes (Khadivi-Khub and Etemadi-Khah, 2015). In studies on morphological characteristics of grapes, CV varied between 0.00 % and 258.46 % (Khadivi-Khub et al., 2014; Vafaee et al., 2017; Akhram et al., 2019; Abiri et al., 2020; Güler and Karadeniz, 2023).

Table 2. Descriptive statistics of morphological and biochemical characteristics of genotypes

No	Traits	Abbreviation	Unit	Min.	Max.	Mean	SD	CV (%)
1	Leaf Width	LW	cm	9.15	16.45	12.33	1.67	13.54
2	Main Vein Length	MVL	cm	6.80	13.33	9.75	1.54	15.76
3	Leaf Length	LL	cm	8.87	17.38	13.15	1.93	14.70
4	Leaf Area	LA	cm^2	51.38	220.30	109.41	30.56	27.93
5	Petiole Length	PL	cm	3.72	9.90	6.05	1.37	22.71
6	Petiole Thickness	PT	mm	1.34	2.83	1.89	0.28	14.62
7	Number of Lobes	NL	Number	3.00	11.00	5.15	1.16	22.56
8	Bunch Weight	BW	g	71.67	554.17	238.23	111.09	46.63
9	Bunch Width	BWi	cm	7.18	15.83	10.69	2.04	19.08
10	Bunch Length	BL	cm	8.53	24.82	17.32	3.53	20.38
11	Berry Weight	BrW	g	1.54	10.98	3.78	1.74	45.97
12	Berry Width	BrWi	mm	12.31	24.63	16.82	2.31	13.74
13	Berry Length	BrL	mm	13.13	28.45	18.40	3.66	19.91
14	100 Seed Weight	SW	g	0.00	9.65	6.13	1.93	31.46
15	Number of Seed	NS	Number	0.00	3.50	2.30	0.76	32.97
16	L*	L	Code	23.85	50.80	37.66	10.15	26.96
17	a*	a	Code	-19.39	96.56	23.81	24.42	102.58
18	b*	b	Code	4.26	39.39	19.25	11.95	62.08
19	C*	C	Code	17.89	96.85	37.68	15.78	41.88
20	Hue*	H	Code	3.57	125.85	46.08	35.50	77.03
21	TSS	TSS	%	12.80	20.30	15.53	1.88	12.11
22	pH	Ph	Code	3.03	4.02	3.44	0.18	5.16
23	TA	TA	mg/L	0.33	1.47	0.76	0.22	28.68
24	TSS/TA	TSS/TA	Code	10.74	51.31	22.34	7.55	33.79
25	Total Antioxidant	TAnt	%	10.12	91.75	40.13	17.77	44.28
26	Total Phenolic	TP	mg GAE 100 g^{-1}	123.77	664.58	359.08	104.80	29.19
27	Total Flavonoid	TF	mg QE 100 g^{-1}	16.48	270.92	81.52	50.83	62.35
28	Total Anthocyanin	TAnth	mg malvidin 3-glycoside 100 g^{-1}	3.35	74.42	23.29	21.71	93.21

As a result of the study, leaf characteristics (leaf width, leaf length, main vein length, leaf area, petiole length and petiole thickness) showed wide variations among genotypes. Among leaf characteristics, the largest CV was determined in leaf area (27.93%), petiole length (22.71%) and number of lobes (22.56%). Leaf width varied between 9.15 cm and 16.45 cm, main vein length varied between 6.80 cm and 13.33 cm, leaf length varied between 8.87 cm and 17.38 cm, leaf area varied between 51.38 cm^2 and 220.30 cm^2, petiole length varied between 3.72 cm and 9.90 cm, and petiole thickness varied between 1.34 cm and 2.83 cm. Leaf characteristics of the genotypes in the study coincide with the research findings of Sümbül et al. (2023). Leaf lobe numbers of genotypes varied between 3 and 11. Although the number of lobes of the leaves varied according to genotypes, our study findings were found compatible with the literature (Vafaee et al., 2017; Abiri et al., 2020). Leaf characteristics, which provide objective information about genotypes, are of great importance in grapevine genotypes identification studies (Santiago et al., 2007).

Among the bunch and berry characteristics, the highest CV was found in bunch weight (46.93%) and berry weight (45.97%). These characteristics were followed by number of seeds (CV= 32.97%) and 100 seed weight (CV= 31.46%). While the bunch weights of the genotypes varied between 71.67 and 554.17 g, the average bunch weight was determined as 238.33 g. The bunch width varied between 7.18 and 15.83 cm, and the bunch length varied between 8.53 and 24.82 cm. Among the genotypes, berry weights varied between 1.54 and 10.98 g, berry widths varied between 12.31 and 24.63 mm, and berry lengths varied between 13.13 and 28.45 mm. According to the literature, the bunch and berry weights of grapes vary according to genotypes and growing regions. The bunch and berry weights obtained as a result of the study are similar to the previous studies. Bunch weights of grapes were reported to vary between 62.75 - 214.99 g (Vafee et al., 2017) and 33.65 - 890.70 g (Razi et al., 2019) in a study conducted in Iran, 71.00 - 872.00 g (El Oualkadi and Hajjaj, 2019) in a study conducted in Morocco, 77.70 - 583.55 g (Akhram et al., 2019) in a study conducted in Pakistan and 195.60 - 272.70 g (Habib et al., 2020) in a study conducted in Tunisia. In studies conducted in different regions of Türkiye, bunch weight varied between 60.57 and 876.38 g (Keskin, 2017; Serhat et al., 2017; Özden and Deveci, 2023; Sümbül et al., 2023; Güler and Karadeniz, 2023). It has been stated that the berry weight of grapes varies between 1.50 and 5.94 g by Khadivi-Khub et al. (2014), between 0.64 and 3.74 g by Vafee et al. (2017), betweeen 0.64 and 3.47 g by Razi et al. (2019) and between 2.35 and 4.97 g by Habib et al. (2020). In the studies conducted in Türkiye, berry weights of grapes were determined between 3.10 - 5.40 g by Keskin (2017), between 1.20 - 6.70 g by Serhat et al. (2017), between 1.53 - 7.44 g by Sümbül et al. (2023) and between 1.29 - 9.48 g by Güler and Karadeniz (2023).

Seed characteristics are a frequently used distinguishing feature in the identification of diversity of grape genotypes (Benito et al., 2017). In the study, number of seeds per berry and 100 seed weight showed great variation among genotypes. The number of seeds per berry varied between 0.00 and 3.50, while the 100 seed weight varied between 0.00 and 9.65 g. The number of seeds per berry in grapes was reported to vary between 0.00 and 4.00 by Khadivi-Khub et al. (2014), between 0.00 and 3.00 by Vafaee et al. (2017), between 0.00 and 4.00 by Abiri et al. (2020) and between 0.88 and 5.50 by Güler and Karadeniz (2023). The seedlessness of varieties is a desirable characteristic in table grape growing. This situation was revealed in a study on grape production projection (Sümbül and Yıldız, 2022).

The skin color values of berries showed wide variations among genotypes. Among the color values, a* value had the highest CV (CV= 102.58%), while L* value had the lowest CV (CV= 26.96%). The average L*, a*, b*, C and Hue color values were determined as 37.66, 23.81, 19.25, 37.68, 46.08, respectively. Although berry skin color in grapes is specific to genotypes, berry skin color intensity is affected by factors such as the location, sunlight utilization and training method of grapes. There may be color differences even in bunches on the same grapevine (Kılıç et al., 2011). Differences in berry skin color of the grapes are important in determining harvest dates and consumer preferences (Vafaee et al., 2017; Somogyi et al., 2020).

Among the fruit must characteristics of the genotypes (TSS, pH, TA and TSS/TA), the highest CV was found in TSS/TA (33.70%). Among the genotypes, TSS varied between 12.80% and 20.30%, pH varied between 3.03 and 4.02, TA varied between 0.33 and 1.47, and TSS/TA varied between 10.74 and 51.31. The must characteristics of grapes depend on genetics but are also affected by climate and environmental conditions. TSS in grapes was reported between 15.00 and 25.40 in Iran (Khadivi-Khub et al., 2014) and between 17.00 and 21.03 in Tunisia (Habib et al., 2020). In studies conducted in Türkiye, TSS of grapes was reported between 7.83 - 26.39 in Bolu (Güler and Karadeniz, 2023) and between 14.00 - 24.13 in Kayseri (Sümbül et al., 2023). In order to ensure unity on the maturity criterion in grapes, the International Organization of Vine and Wine (OIV) has reported that TSS will be considered ripe between 12.5 and 16 °Brix (OIV, 2008). In addition, according to the Turkish Standards Institute Table Grape Standard, it is stated that the TSS value of table grapes should be at least 13% for seeded varieties and at least 14% for seedless varieties (Polat, 2016). In this regard, the genotypes within the scope of the study are generally suitable for table consumption.

Grapes are known as a natural and rich source of antioxidants due to their phenolic compound and anothcyanin contents. Among the biochemical properties examined in the study, the highest CV was detected in the total anthocyanin content (93.21%) and the lowest CV was detected in the total phenolic content (29.19%).

In the genotypes, total antioxidants varied between 10.12% and 91.75%, total phenolics varied between 123.77 and 664.58 mg GAE 100 g^{-1}, total flavonoids varied between 16.48 and 270.92 mg QE 100 g^{-1} and total anthocyanins varied between 3.35 and 74.42 mg malvidin 3-glycoside 100 g^{-1}. In studies conducted on grapes, it has been reported that total phenolics vary between 237.12 and 4680.00 mg GAE kg^{-1} (Revilla et al., 2010; Aydın, 2015; Oktay, 2022; Özden and Deveci, 2023), total flavonoids vary between 96.26 and 1440.00 mg QE kg^{-1} (Aydın, 2015; Soltekin, 2019; Küpe et al., 2021; Oktay, 2022; Özden and Deveci, 2023) total antioxidants vary between %19.79 and %81.46 (Özden and Özden, 2014; Soltekin, 2019; Balbaba and Bağcı, 2021; Küpe et al., 2021), and total anthocyanin vary between 24.00 and 1914.00 mg kg^{-1} (Crupi et al., 2012; Gervasi et al., 2016; Özden and Deveci, 2023). It is known that genotypic effects, climatic conditions, growing conditions, soil structure and harvest and post-harvest storage conditions are effective on the biochemical content of fruits (Yahia, 2017). Recently, it has been claimed that the biochemical contents of grapes can be used in studies on the identification of grape genotypes (Laurentiu and Popa, 2018). In addition, it is thought that determining the biochemical content of grapes may be important in the introduction and consumption of new varieties (Özden and Deveci, 2023).

3.2. Principal Component Analysis (PCA)

PCA is widely used to explain the degree of influence of the studied characteristics or patterns of variation among genotypes. The first three basic components provide significant savings in time in the characterization of genotypes (Iezzoni and Pritts, 1991). Within the scope of the study, PCA of 28 characteristics was performed. In order to reveal the components explaining the largest variation as a result of PCA, components with eigenvalues greater than 1 were evaluated. As a result of the analysis, there are 7 components with eigenvalues greater than 1. These 7 components explain 83.09% of the total variation. However, the first three principal components explained 57.06% of the total variation. PCA1 explained 23.43% of the variation, PCA2 explained 20.00% of the variation, and PCA3 explained 13.63% of the variation. While our findings are similar to the study results of Khadivi-Khub et al. (2014) (PCA3 53.98%), they were found higher than the results of Vafaee et al. (2017) and Abiri et al. (2020) (PCA3 32.41%, PCA3 25.82%, respectively). According to the PCA, the contribution of each characteristic to the principal components varied. L* and b* values from berry skin color values, total antioxidant content, total flavonoid content and total anthocyanin content from biochemical properties, bunch weight from bunch properties showed the highest effect on PC1. Leaf characteristics (leaf width, leaf length, leaf area, leaf main vein length, petiole thickness and petiole length) showed the highest correlation with PC2, while bunch weight, berry width, berry length, berry weight and 100 seed weight and TSS showed the highest correlation with PC3 (Table 3; Figure 1).

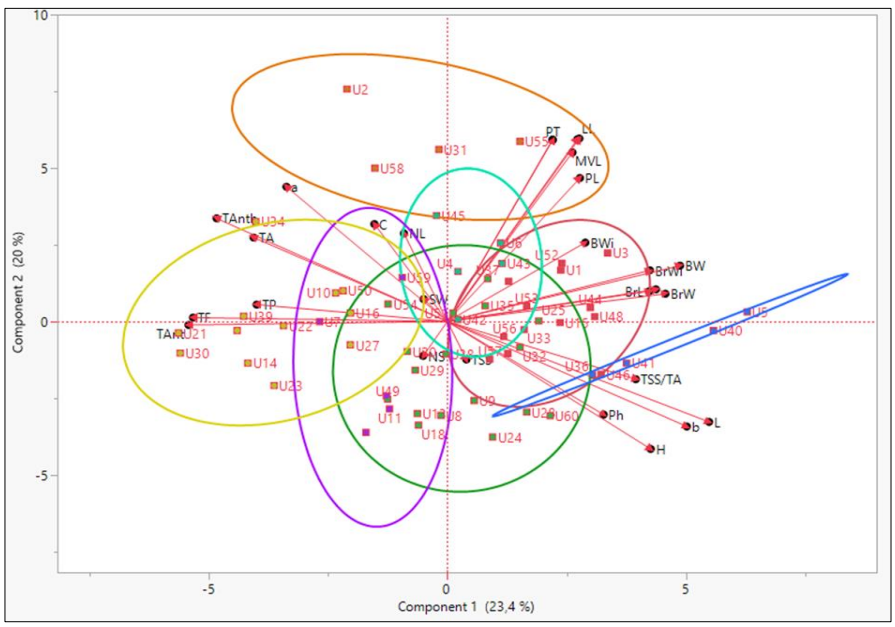

Figure 1. Biplot graph of the first two principal components in the investigated grape genotypes

Table 3. Principal component analysis and contribution rates of the investigated characteristics

Traits	PCA 1	% Cont	PCA 2	% Cont	PCA 3	% Cont	PCA 4	PCA 5	PCA 6	PCA 7
LW	0.11	1.29	**0.33**	**10.89**	-0.19	3.47	0.08	0.09	-0.12	0.10
MVL	0.14	1.82	**0.31**	**9.45**	-0.18	3.29	0.15	0.07	0.01	-0.05
LL	0.14	2.03	**0.33**	**11.07**	-0.18	3.26	0.12	0.10	0.01	-0.03
LA	0.14	1.97	**0.33**	**10.97**	-0.22	4.91	0.06	0.00	-0.02	-0.06
PL	0.14	2.05	**0.26**	**6.81**	-0.09	0.87	0.08	0.14	-0.20	-0.08
PT	0.11	1.29	**0.33**	**10.95**	-0.15	2.40	0.03	-0.05	-0.01	-0.05
NL	-0.05	0.22	0.16	2.56	0.07	0.44	0.17	0.05	0.03	0.08
BW	**0.25**	**6.32**	0.10	1.03	**0.26**	**6.71**	-0.02	-0.03	0.26	-0.21
BWi	0.15	2.22	0.14	2.06	0.23	5.18	-0.03	-0.07	0.42	-0.18
BL	0.23	5.08	0.06	0.34	0.17	2.87	0.00	0.04	0.41	0.13
BrW	0.24	5.55	0.05	0.25	**0.37**	**13.35**	0.00	0.04	-0.14	-0.06
BrWi	0.22	4.79	0.09	0.87	**0.38**	**14.55**	0.04	0.05	-0.11	0.01
BrL	0.22	4.75	0.05	0.30	**0.32**	**10.54**	-0.12	-0.05	-0.11	-0.08
SW	-0.03	0.07	0.04	0.16	**0.28**	**8.06**	0.33	0.06	-0.32	0.16
NS	-0.03	0.07	-0.06	0.39	0.11	1.30	0.35	0.19	-0.27	0.17
L	**0.28**	**8.00**	-0.18	3.34	-0.07	0.54	0.26	-0.04	0.08	0.15
a	-0.17	3.03	0.24	6.00	0.18	3.11	-0.23	0.10	0.04	0.36
b	**0.26**	**6.69**	-0.19	3.65	-0.12	1.39	0.26	-0.05	0.11	0.17
C	-0.08	0.62	0.18	3.14	0.11	1.24	-0.13	0.07	0.17	0.68
H	0.22	4.83	-0.23	5.38	-0.14	2.07	0.26	-0.08	0.07	0.05
TSS	0.02	0.04	-0.07	0.48	**-0.26**	**6.64**	-0.14	0.32	0.29	0.13
Ph	0.17	2.85	-0.17	2.87	0.01	0.00	-0.22	0.33	-0.20	0.07
TA	-0.21	4.39	0.15	2.33	0.03	0.07	0.14	-0.41	0.06	0.15
TSS/TA	0.20	4.14	-0.11	1.10	-0.06	0.36	-0.18	0.51	0.00	-0.03
TAnt	**-0.28**	**7.84**	-0.01	0.00	0.12	1.47	0.26	0.28	0.13	-0.09
TP	-0.21	4.25	0.03	0.09	0.11	1.30	0.31	0.23	0.29	-0.13
TF	**-0.27**	**7.56**	0.01	0.00	0.07	0.42	0.26	0.29	0.15	-0.13
TAnth	**-0.25**	**6.23**	0.19	3.52	0.04	0.19	-0.19	0.11	-0.09	-0.28
Eigenvalue	6.56		5.60		3.82		2.59	1.99	1.56	1.13
Variance	23.43		20.00		13.63		9.27	7.12	5.59	4.05
Cumulative Variance	23.43		43.43		57.06		66.33	73.45	79.04	83.09

* Cont: Contribution

3.3 Heatmap analysis and hierarchical clustering analysis

As a result of heatmap and hierarchical clustering analysis, genotypes were divided into two main groups (Figure 2). Each group was again divided into two subgroups within itself. In group A, U5, U36, U41 and U40 genotypes constitute the A2 subgroup, while the A1 subgroup is divided into two subgroups within itself. Group B is divided into two subgroups. These subgroups were again divided into two subgroups. Group A consists of genotypes with white skin color, while group B generally consists of genotypes with colored skin color. However, subgroups B1-2 and B2-1 consisted of genotypes with both white and colored skins. The genotypes of A1/1 subgroup in A1 group have high values of LW, MVL, LL, BWi, BL, L*, b*, Hue and TSS characteristics, while the genotypes of A1/2 subgroup have high values of L*, b*, Hue and TSS characteristics. BW, BL, BrW, BrW, BrWi, BrL, L* and SW traits of genotypes in A2 group had high values. Genotypes in B1 group showed high values for LW, LA, MVL, LL, PT, PL, BW, BWi, A*, Chroma and TAnth content characteristics, while genotypes in B2 group showed high values for TAnth, NS, TAnt and TF characteristics. Heatmap analysis can classify genotypes based on morphological characteristics. As a matter of fact, heatmap analysis has been used to group genotypes in many studies (Gündeşli et al., 2023; Yaman et al., 2023; Say et al., 2024). Identification of highly distinctive characteristics is important for the morphological characterization of genotypes. Because the correct selection of morphological characteristics that distinguish genotypes saves both time and cost. These characteristics are important for grapes where homonym and synonyms states are observed and have a wide variety richness.

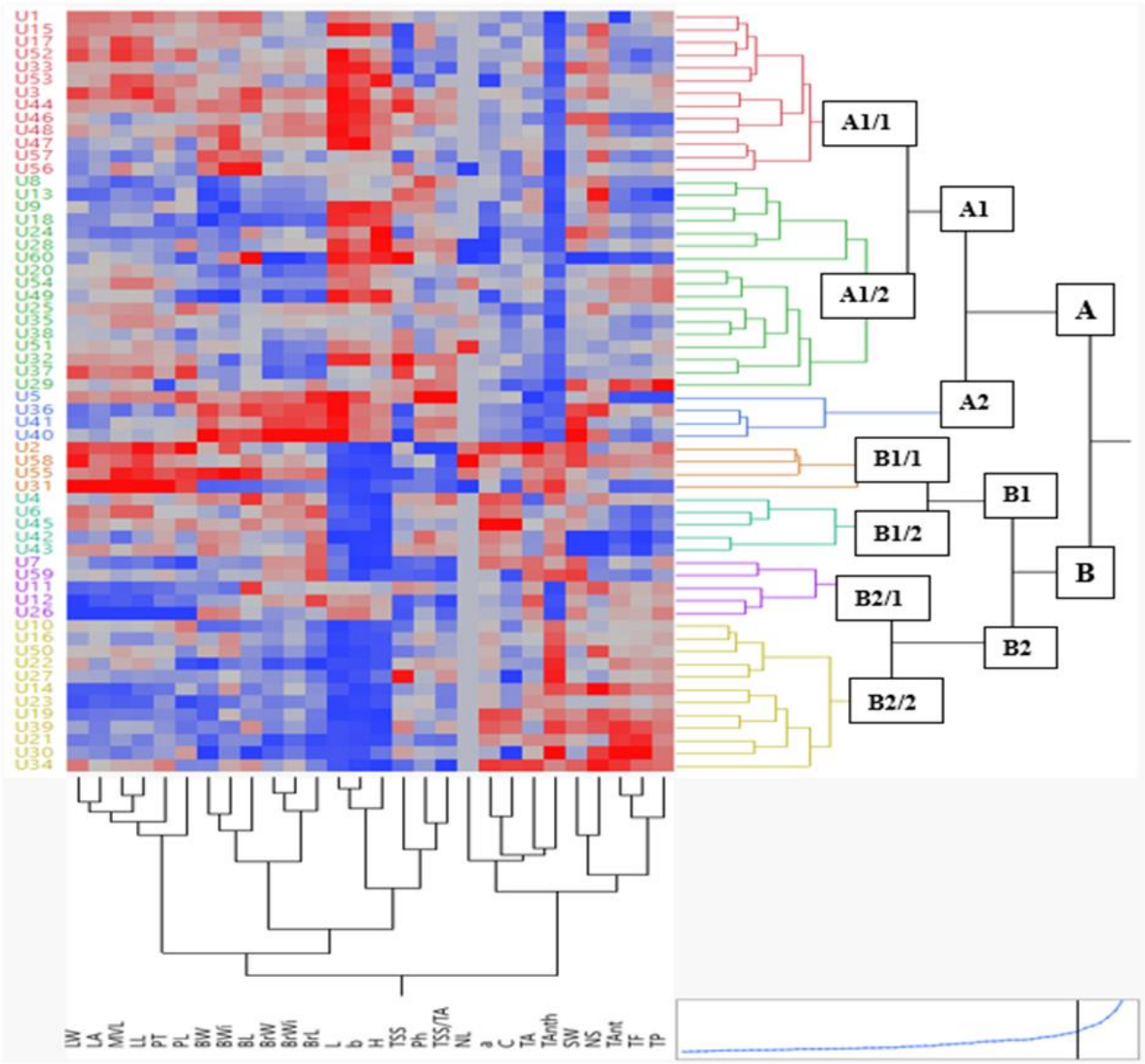

Figure 2. Heatmap analysis and hierarchical clustering of investigated grape genotypes based on morphological and biochemical characteristics

This study is the first study to describe the diversity of autochthonous grapes in the Kelkit Basin of Türkiye. The results of the study showed that there were significant differences between individuals in terms of morphological and biochemical characteristics. These differences are valuable genetic resources for the development of new grape varieties suitable for various usage purposes. While U5, U36, U40, U41 genotypes stand out with their cluster and berry characteristics, U42, U43 and U60 genotypes stand out with their seedless characteristics. These genotypes are candidates for becoming varieties. U34, U30, U21, U39, U19 genotypes stand out with their biochemical contents. The inclusion of these genotypes in breeding programs may enable the development of high-quality grape varieties. In addition, multifaceted statistical approaches were used to evaluate the diversity among autochthonous grapes in the region. Multivariate statistical approaches have divided individuals into different groups. As a result of the study, it was concluded that multivariate statistical approaches are a useful method that can be used in evaluating inter-individual variability.

Compliance with Ethical Standards

Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

Ahmet SÜMBÜL: Methodology, Investigation, Conceptualization, Validation, Data curation, Formal analysis, Writing - original draft, Visualization. **Ercan YILDIZ**: Methodology, Conceptualization, Validation, Review and editing.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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