Gürcan *et al. BMC Plant Biology* (2024) 24:1158
https://doi.org/10.1186/s12870-024-05846-3

BMC Plant Biology

RESEARCH

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Evaluation of genetic diversity in some hybrid individuals of honeyberry (*Lonicera caerulea* L.) based on fruit characteristics, leaf morphology, vitamin C, antioxidant activity, and biochemical and nutritional contents

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Abstract

Background Genetic diversity is a prerequisite for breeding programs, and one of the main goals here is to obtain quality products. Therefore, this study aims to evaluate the genetic diversity in some hybrid individuals of honeyberry (*Lonicera caerulea* L.) based on fruit characteristics, leaf morphology, vitamin C, antioxidant activity, biochemical, and nutritional content. In this context, superior quality individuals have been identified based on the 42 variables examined in our study. These hybrid individuals can be economically incorporated into production after the registration stages, and their sustainability for use in breeding programs can also be ensured.

Results The fruit weight ranged from 0.71 ('H11') to 1.66 g ('H6'). The ascorbic acid varied between 17.13 ('H7') and 20.64 mg AAE/100 g ('H15'). The antioxidant activity changed between 12.59 ('Store') and 15.03 µmol Trolox g^{-1} ('Aurea'). The total anthocyanins were found to be highest in 'Borrel Beast' (163.79 mg cyn-3-gluc 100 g^{-1}), followed by 'H8' (163.20 mg cyn-3-gluc 100 g^{-1}). The highest nutrient levels in the fruits were found in the 'H10' individual, with calcium (2445.77 mg kg^{-1}), potassium (2274.36 mg kg^{-1}), phosphorus (2123.27 mg kg^{-1}), magnesium (1263.95 mg kg^{-1}), and sulfur (859.62 mg kg^{-1}), respectively. The highest nutrient levels in the leaves were found in the 'H14' individual for calcium (19,493.21 mg kg^{-1}), 'H5' for magnesium (5643.52 mg kg^{-1}), 'H8' for sulfur (2312.11 mg kg^{-1}), 'H6' for phosphorus (2007.51 mg kg^{-1}), and 'H6' for potassium (1099.32 mg kg^{-1}). In general, the nutrients in the fruit exhibited significant correlations among themselves at different levels (*, **, ***). Within the scope of principal component analysis, the first 8 principal components explained 80.69% of the total variance. According to the cluster and population analyses, it was determined that there was a high variation in subgroup B2. Additionally, although honeyberry is a relatively new fruit in Türkiye, efforts have begun to develop new cultivars through hybrid breeding.

Conclusions When 42 variables were evaluated together to determine genetic diversity, hybrid individuals 'H14', 'H5', 'H8', and 'H1' were identified as superior individuals, respectively.

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Keywords Biochemical content, Genetic diversity, Honeyberry, *Lonicera caerulea* L., Hybridization, Multivariate analysis, Mineral contents

Introduction

Berries have attracted considerable attention in agriculture because of their positive impact on human health and high economic value [1]. Amid global environmental and economic changes, there is a growing interest in innovative berry crops that can prolong production seasons, require less maintenance, and have the potential for organic cultivation [2].

Honeyberry (*Lonicera caerulea* L.) is a deciduous perennial shrub from the Caprifoliaceae family [3], with a chromosome structure of 2n = 36 [4]. The genus *Lonicera* includes approximately 180 species [5]. Its fruit is a small berry, varying in color from azure-blue to dark blue, and has a taste that ranges from sour to sweet. It is commonly referred to as 'honeysuckle', 'haskap', or 'honeysuckle berry' [6]. Honeyberry is widely cultivated in North America, Europe, Russia, Japan, and China [7]. It can withstand temperatures as low as -40°C, while its flowers can tolerate up to -7°C [8], and is highly resistant to many diseases and pests [9]. Additionally, it is increasingly regarded as an environmentally beneficial crop due to its perennial nature, which leads to reduced soil disturbance compared with other crops, as well as its less intensive cultivation system [2]. Its fruits are typically 1–2 cm in length and 1 cm in width [8]. The early ripening of honeyberry, occurring between May and June similar to strawberries and before all other fruits may be one of its significant advantages. The growing recognition of the taste, nutritional benefits, and versatility of berries has resulted in increased popularity in commercial growing regions and new breeding programs in Canada, Japan, Russia, Poland, and more recently, the UK [10]. Anthocyanins are widely distributed plant pigments that give fruits and flowers their red to blue colors. They are structurally composed of anthocyanidin and sugar, linked by a glycoside bond. The most common anthocyanidins petunidin, cyanidin, pelargonidin, delphinidin, malvidin, and peonidin are found in plants attached to glucose, galactose, arabinose, rhamnose, or xylose. Due to their high cyanidin-3-glucoside content, honeyberry may possess antioxidant, anti-inflammatory, antimicrobial, cardioprotective, and hepatoprotective properties [11, 12]. Research on the mineral nutrient content of honeyberry compared with other fruits like aronia, blueberries, and grapes is insufficient. However, several studies have reported that honeyberry is rich in potassium, calcium, and magnesium content [13]. As research on the antioxidant and biochemical properties of honeyberry has only recently begun, available information on the topic remains limited. Some studies suggest that honeyberry fruits may help protect against diseases triggered by inflammation and oxidation [14]. Furthermore, honeyberry was found to safeguard the liver from lipopolysaccharide-induced damage, highlighting its potential role in hepatitis prevention [15]. In an analysis of 30 different fruits, including oranges, apples, pineapples, bananas, grapes, and several other types, honeyberry berries exhibited the strongest inhibitory activity against carbohydrate-degrading enzymes, suggesting their possible benefit in reducing obesity and type-2 diabetes risks [16].

Recent studies have highlighted the presence of fruit characteristics, leaf morphology, vitamin C content, antioxidant activity, total anthocyanins, total phenolics, and mineral nutrient content in honeyberry fruits; however, there is a significant gap in the literature on this topic. To the best of our knowledge, the present study is the first in Türkiye to provide information on the fruit characteristics, leaf morphology, vitamin C content, antioxidant activity, biochemical, and nutritional composition of honeyberry. This study aims to fill the significant gap in the existing literature, provide valuable information for researchers working on similar topics, and offer guidance for different industries such as food, cosmetics, pharmaceuticals, etc. Thus, an important gap in the literature on the subject will be filled.

Materials and methods

Plant material

This study was conducted at the Fruit Farm of the Agricultural Research and Application Center of Erciyes University on 3 to 4-year-old hybrid honeyberry plants and some honeyberry cultivars, including the parents of these hybrids (Table 1).

Three repetitions were conducted, with each repetition involving 50 fruits and 50 leaves. The samples were harvested during the ripening period in June. The plants were cultivated outdoors in pots, utilizing a substrate composed of perlite, peat, and garden soil in a ratio of 1:1:1. Regular cultural practices, including irrigation, fertilization, and pest control, were systematically implemented to ensure optimal growth conditions. The climate in Kayseri is characterized by cold, snowy winters and hot, arid summers, which further influences the growth and development of the plants. The superior characteristics of the honeyberry cultivars used as parents are presented in Table 2.

Table 1 Investigated honeyberry hybrids and cultivars

Hybrid	Parents
'H1'	'Aurea' (♀) × 'Borrel Beast' (♂)
'H2'	'Aurea' (♀) × 'Borrel Beast' (♂)
'H3'	'Aurea' (♀) × 'Borrel Beast' (♂)
'H4'	'Aurea' (♀) × 'Borrel Beast' (♂)
'H5'	'Aurea' (♀) × 'Borrel Beast' (♂)
'H6'	'Aurea' (♀) × 'Borrel Beast' (♂)
'H7'	'Aurea' (♀) × 'Borrel Beast' (♂)
'H8'	'Aurea' (♀) × 'Borrel Beast' (♂)
'H9'	'Aurea' (♀) × 'Borrel Beast' (♂)
'H10'	'Aurea' (♀) × 'Borrel Beast' (♂)
'H11'	'Aurea' (♀) × 'Borrel Beast' (♂)
'H12'	'Aurea' (♀) × 'Borrel Beast' (♂)
'H13'	'Aurea' (♀) × 'Borrel Beast' (♂)
'H14'	'Aurea' (♀) × 'Borrel Beast' (♂)
'H15'	'Aurea' (♀) × 'Borrel Beast' (♂)
'Aurea'	'Blue Moon' (♀) × 'Honey Bee' (♂)
'Bornel Beaty'	'Blue Belle' (♀) × 'Boreal' (♂)
'Borrel Beast'	'Boreal' (♀) × 'Blue Honey' (♂)
'C2 Kolenka'	'Kolenka' (♀) × 'Blue Moon' (♂)
'Store'	'Blue Honey' (♀) × 'Honeybee' (♂)

Evaluation of fruit characteristics and leaf morphology

Fruit width, fruit length, leaf width, leaf length, petiole length, and petiole thickness of the individuals, were measured using a digital caliper (Insize 1104 IP54) with 0.01 mm sensitivity and the results are expressed in mm. Fruit weight was determined using a digital scale with 0.01 g sensitivity (Precisa, XB 4200C). Fruit outer surface color measurement was determined in L*, a*, b* using a hand chronometer (Fru, WR10). L* represented the relative lightness of colors, with values ranging from 0 (black) to 100 (white). The a* and b* values ranged from -60 to 60, where: a* was negative for green and positive for red, while b* was negative for blue and positive for yellow [21].

Determination of vitamin C, antioxidant activity, and biochemical contents

Ascorbic acid

The ascorbic acid (vitamin C) content was determined according to Balta et al. [22]. Briefly, the fruit juice was diluted with distilled water at a 1:100 ratio, and 1 mL of this solution was mixed with five drops of TS-1 reagent and 10 mL of distilled water. A test strip (Cat. No. 116136, Reflectoquant, Total Sugar Test, Merck, Germany) was immersed in the prepared solution for 2 s, and after a 10-min wait to remove excess liquid, the strip was placed in the reflectometer's strip adapter (RQFlex Plus 10, Merck, Darmstadt, Germany) for measurement. The result was multiplied by the dilution factor and reported as grams of ascorbic acid equivalent (mg AAE/100 g fresh weight, FW) [22].

Sample preparations

The fresh fruits extracts were extracted by homogenizing them with a hand blender (Arçelik HB 6150, İstanbul, Türkiye). For this, 10 g of each sample was taken and 10 mL of 80% methanol was added. The samples were centrifuged at 6000 × g for 5 min at 4°C (Elektromag M615 E, İstanbul, Türkiye). The supernatant was collected. The supernatant was filtered through filter paper (Borox, 90 mm Ø) and used in the determination procedures of total antioxidant, phenolic and flavonoids [23]. The vitamin C, antioxidant activity, and biochemical contents of each individual were determined using a total of 60 fruits, with 3 replicates and 20 fruits in each replicate.

Antioxidant activity (AA)

The DPPH (2,2-diphenyl-1-picryl-hydrazyl) (Sigma Aldrich, St Louis, MO, USA) radical scavenging activity was measured following the method describe by Brand-Williams et al. [24]. Briefly, 10 µL of the supernatant was mixed with 40 µL of methanol, followed by 950 µL of DPPH solution. The mixture was shaken using a shaker (Biosan PSU-20i, Rīga, LV-1067, Latvia) at 250 rpm for 3 min at room temperature, and left in the dark for 10

Table 2 The superior characteristics of the honeyberry cultivars used as parents [17–20]

Cultivar	Characteristic
Blue Moon	Produces a large quantity of fruit, ideal for fresh consumption, and is resistant to low temperatures
Honey Bee	Rich in flavor and aroma, it is a resilient cultivar with good yield potential
Blue Belle	Has a distinct and pleasant fruit fragrance, is rich in antioxidants, and adapts well to different climatic conditions
Boreal	Produces attractive and large fruits, is rich in vitamins and minerals, and is resistant to pests
Kolenka	Produces delicious and sweet fruits suitable for fresh consumption, grows rapidly, and has high yield potential
Blue Honey	Has sweet and aromatic fruits, performs well under diverse conditions, and is suitable for both fresh and processed products
Honeybee	Stands out for its aroma and sweetness, provides a high fruit yield, and contains a high level of antioxidants

min. Absorbance was then measured at 515 nm using a spectrophotometer. The results were expressed as micromoles of Trolox 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) per gram of fresh weight (μmol Trolox g^{-1} FW).

Total anthocyanins (TAs)

The honeyberry, which contains various anthocyanins, has cyanidin-3-glucoside as its main anthocyanin source [25]. Li et al. [26] reported that the anthocyanin content in honeyberry is higher compared to species such as blackberry, blueberry, and cornelian cherry. The total anthocyanin content in honeyberry fruit has been determined according to Guo et al. [27]. Specifically, the absorbance of the extract was measured at 510 and 700 nm in buffers with pH 1.0 (hydrochloric acid–potassium chloride, 0.2 M) and 4.5 (acetic acid-sodium acetate, 1 M). The total anthocyanin content was calculated using the absorbance value (A) of the diluted sample according to the formula presented in Eq. 1 [28].

$$A = (A_{520} - A_{700})pH1.0 - (A_{520} - A_{700})pH4.5 \quad (1)$$

After determining the absorbance value, the total anthocyanin content was calculated using the formula provided in Eq. 2 [29].

$$Total\ anthocyanins(mgcyn - 3 - gluc100g^{-1}) = (A \times MW \times DF \times 1000)/(\varepsilon \times L) \quad (2)$$

ϵ: Molar extinction coefficient = 26,900, MW: Molecular weight = 484.83 g mol^{-1} for cyanidin-3-glucoside (C3G), DF: Dilution factor, L: cell path length = 1 cm.

Total phenolics (TPs)

To assess the total phenolics in the samples, 200 μL of extract was initially combined with 1800 μL of distilled water and 1 mL of 1/10 diluted Folin-Ciocalteu solution [30]. Then, 2 mL of 2% Na_2CO_3 was added, and the mixture was allowed to sit for 5 min. The samples were subsequently mixed at 250 rpm for 3 min and kept in the dark for 1 h prior to measuring the absorbance values using a spectrophotometer at a wavelength of 760 nm. The absorbance values obtained were converted to gallic acid equivalents based on a standard curve established with gallic acid, and the results were expressed in mg GAE 100 g^{-1} FW (fresh weight) [23].

Determination of mineral nutrients

The macro [carbon (C), calcium (Ca), potassium (K), magnesium (Mg), sodium (Na), phosphorus (P), and sulfur (S)] and micro [aluminum (Al), boron (B), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn)] nutrient element contents in honeyberry fruits and leaves have been determined. Nutrient element analysis was performed according to the method described by Mabotja et al. [31]. Briefly, for the examination of 0.5 g of fruit and leaf samples, digestion was conducted with microwave assistance using nitric acid (HNO_3) and hydrogen peroxide (H_2O_2). Dried and ground honeyberry fruit samples underwent digestion with an HNO_3-H_2O_2 acid mixture (2:3 v/v). The samples were subsequently processed in a microwave oven (Anton Paar, multiwave 7000) in three stages: 5 min at 145 °C and 75% relative humidity, followed by 10 min at 180 °C and 90% relative humidity, and finally 10 min at 100 °C and 40% relative humidity. The nutrient content of the samples was quantified as mg kg^{-1} using inductively coupled plasma optical emission spectrometry (ICP-OES) (ICP-AES-9820, Shimadzu Corporation, Kyoto, Japan).

Statistical analyses

The research was carried out in 2023 and 2024, using a two-year average for all data sets. The analysis of all data sets was performed with the JMP® Pro 17 [32] statistical software package (SAS Institute Inc., Cary, NC, USA), employing the TUKEY multiple comparison test. Results were reported at a 5% significance level ($p < 0.05$) [33]. Furthermore, multivariate analysis methods were utilized to identify genetic similarities and differences [34, 35]. Correlation between traits, principal component analysis, and cluster analysis based on unweighted pair group method (UPGMA) with arithmetic mean were performed using the Origin Pro® 2024b [36] statistical software package. The Pearson correlation coefficient was used for the correlation analysis. To enhance the visualization of hybrid individuals and cultivars distribution, a two-dimensional plot representing two principal components was created as part of the principal component analysis. For cluster and population analyses, the Ward method with Euclidean distance was applied. Prior to conducting the cluster analysis, each trait was averaged and normalized using Z-scores to minimize scale differences [37].

Results and discussion

Fruit characteristics and leaf morphology of the assessed honeyberry hybrid individuals are presented in detail in Table 3. One-way ANOVA ($p < 0.05$) revealed significant variations among the assessed honeyberry cultivars and hybrid individuals. Fruit width varied between 8.16 ('H15') and 11.84 mm ('H4'), fruit length changed between 13.89 ('H5') and 24.50 mm ('H1'), fruit weight ranged from 0.71 ('H11') to 1.66 g ('H6'). Our findings are supported by the results of other researchers [1]. In a

Table 3 Fruit characteristics and leaf morphology of the assessed honeyberry individuals

Genotype No	FWi	FL	FWe	L*	a*	b*	LWi	LLe	PLe	PTh
'H1'	9.39 a-c	24.50 a	1.04 c-f	25.00 cd	24.41 de	1.74 b-d	9.39 a-c	67.92 b	4.00 a-d	1.61 ab
'H2'	11.90 a	20.62 c-f	1.55 ab	24.02 ab	18.94 f–h	1.27 c-h	11.90 a	60.59 c	3.61 b-e	1.50 ab
'H3'	11.37 ab	19.03 c-g	1.36 a-c	23.79 a-c	17.60 h-j	1.16 e–h	11.37 ab	53.89 e–g	3.59 b-e	1.49 ab
'H4'	11.84 a	18.79 d-g	1.29 a-c	23.73 a-c	18.17 g-i	1.14 e–h	11.84 a	47.00 i	4.58 a-c	1.39 ab
'H5'	9.67 a-c	13.89 i	0.96 c-f	23.23 a-d	14.63 j	1.13 e–h	9.67 a-c	55.53 de	2.52 de	1.39 ab
'H6'	11.79 a	23.72 ab	1.66 a	22.06 a-d	22.90 de	0.74 gh	11.79 a	51.10 gh	3.14 c-e	1.36 ab
'H7'	10.38 a-c	21.00 a-e	1.27 a-c	21.41 de	34.42 a	1.30 c-g	10.38 a-c	55.26 d-f	4.70 a-c	1.30 ab
'H8'	9.81 a-c	17.99 e–h	1.17 b-e	21.37 a-d	22.49 d-f	1.78 bc	9.81 a-c	48.96 hi	3.36 b-e	1.24 ab
'H9'	10.97 a-c	15.11 hi	1.10 c-f	18.88 f	24.51 b-d	1.23 c-h	10.97 a-c	52.90 e–g	3.16 c-e	1.20 ab
'H10'	9.71 a-c	19.55 c-f	1.25 b-d	24.85 ab	18.67 f-i	1.21 d-h	9.71 a-c	46.31 i	4.87 ab	1.59 ab
'H11'	8.35 bc	13.93 i	0.71 f	24.81 ab	17.73 hi	1.32 c-f	8.35 bc	43.12 j	4.82 ab	1.59 ab
'H12'	10.52 a-c	18.73 c-g	1.23 b-d	24.80 a-c	17.90 hi	0.75 gh	10.52 a-c	46.45 i	3.74 b-e	1.59 ab
'H13'	8.84 a-c	20.60 c-f	1.16 b-e	24.74 a	21.02 e–g	0.73 h	8.84 a-c	58.17 cd	3.85 a-e	1.51 ab
'H14'	9.31 a-c	17.67 f–h	1.07 c-f	24.46 b-d	26.71 bc	1.23 c-h	9.31 a-c	52.30 fg	2.30 e	1.51 ab
'H15'	8.16 c	20.89 b-e	0.86 d-f	24.45 de	24.38 cd	1.60 c-e	8.16 c	41.63 j	3.43 b-e	1.50 ab
'Aurea'	9.93 a-c	20.69 b-f	1.18 b-e	26.73 a-c	16.00 h-j	1.06 e–h	9.93 a-c	60.66 c	5.45 a	1.71 a
'Bornel Beaty'	10.81 a-c	14.09 i	1.27 a-c	25.71 a-d	17.70 h-j	2.43 a	10.81 a-c	66.04 b	3.37 b-e	1.70 ab
'Borrel Beast'	10.37 a-c	21.72 a-d	1.14 c-e	25.67 a-c	15.59 ij	1.01 f–h	10.37 a-c	73.27 a	3.47 b-e	1.62 ab
'C2 Kolenka'	10.00 a-c	21.27 a-c	1.19 b-d	25.67 ef	27.52 b	1.30 c-g	10.00 a-c	50.95 gh	3.80 b-e	1.62 ab
'Store'	8.59 bc	16.01 g-i	0.78 ef	17.06 a-d	22.36 de	2.28 ab	8.59 bc	51.78 gh	2.54 de	1.08 b
Mean	*10.09*	*18.99*	*1.16*	*23.62*	*21.08*	*1.32*	*10.09*	*54.24*	*3.72*	*1.48*
Sd	*± 1.39*	*± 3.13*	*± 0.25*	*± 2.50*	*± 4.83*	*± 0.53*	*± 6.04*	*± 8.19*	*± 1.15*	*± 0.27*

FWi Fruit width, *FL* Fruit length, *FWe* Fruit weight, *LWi* Leaf width, *LLe* Leaf length, *PLe* Petiole length, *PTh* Petiole thickness. *Sd* Standard deviation. The differences between the means indicated by different letters in the same column are significant at the $p < 0.05$ level

study performed in Canada, it was found that fruit width varied between 8.78 and 14.38 mm, fruit length ranged from 14.76 to 26.41 mm, and fruit weight varied between 0.61 and 2.18 g [38]. Holubec et al. [39] conducted a study in which they found that fruit width ranged from 9.7 to 14.3 mm, fruit length varied between 21 and 23.8 mm, and fruit weight changed between 1.01 and 2.20 g. In a study carried out in Ukraine, it was reported that fruit width ranged from 4.92 to 15.50 mm, fruit length varied between 8.47 and 35.97 mm, and fruit weight changed between from 0.73 and 1.60 g [40]. Thompson and Barney [41] found that fruit weight varied between 0.50 and 2.70 g. Honeyberry fruits can reach approximately 20 mm in length and 10 mm in width, and their weights generally change between 0.3 and 2 g [8]. L* color value varied between 18.88 ('H9') and 24.74 ('H13'), a* value changed between 14.63 ('H5') and 34.42 ('H7'), b* value ranged from 0.73 ('H13') to 2.43 ('Bornel Beaty'). Due to the lack of literature on the L*, a*, and b* color values of honeyberry, the assessment was carried out independently within itself. However, Gołba et al. [13] reported that the fruits are dark purple in color and have a waxy coating on their surfaces. This statement of the researchers supports our finding. Leaf width varied between 8.16 ('H15') and 11.90 mm ('H2'), leaf length changed between 43.12 ('H11') and 73.72 mm ('Borrel Beast'), petiole length ranged from 2.30 ('H14') to 5.45 mm ('Aurea'), petiole thickness varied between 1.08 ('Store') and 1.71 mm ('Aurea'). Holubec et al. [39] reported that leaf width and leaf length ranged from 17.4 to 41.00 mm and from 42.8 to 75.00 mm, respectively. Our findings are in line with the findings of the research. With this study, L*, a*, and b* values and petiole length and petiole thickness values of honeyberry fruits will enter the literature.

The biochemical content of the assessed honeyberry hybrid individuals is shown in detail in Table 4. Ascorbic acid content varied between 17.13 ('H7') and 20.64 mg AAE/100 g ('H15'), antioxidant activity changed between 12.59 ('Store') and 15.03 μmol Trolox g^{-1} ('Aurea'), total anthocyanins content ranged from 99.56 ('Aurea') to 163.79 mg cyn-3-gluc 100 g^{-1} ('Borrel Beast'), total phenolics content varied between 153.85 ('H14') and 381.53 mg GAE 100 g^{-1} ('H10'). In a study conducted in Slovakia, the amount of ascorbic acid in honeyberry fruits was reported to range between 9.17 and 46.67 mg AAE/100 g [38]. In studies conducted in Spain and the Czech Republic, the antioxidant activity was found to

Table 4 Biochemical content of the assessed honeyberry individuals

Genotype No	A.acid	AA	TAs	TPs
'H1'	19.88 a-c	13.50 ab	134.07 e	355.85 a-c
'H2'	18.19 a-c	12.98 ab	121.56 fg	286.88 f–h
'H3'	17.84 a-c	12.95 ab	115.01 ij	316.21 c-f
'H4'	18.80 a-c	12.95 ab	117.88 hi	361.40 ab
'H5'	20.04 a-c	12.89 ab	124.57 f	310.18 d-g
'H6'	19.84 a-c	12.89 ab	117.80 hi	248.03 hi
'H7'	17.13 c	12.86 ab	120.53 gh	303.37 d-g
'H8'	19.64 a-c	12.77 ab	163.20 a	226.75 i
'H9'	19.88 a-c	12.77 ab	147.75 bc	336.03 b-e
'H10'	17.28 bc	13.50 ab	144.81 c	381.53 a
'H11'	19.97 a-c	13.47 ab	150.84 b	295.91 e–g
'H12'	19.36 a-c	13.47 ab	116.04 ij	337.46 b-d
'H13'	17.75 a-c	13.42 ab	107.50 k	290.68 fg
'H14'	19.46 a-c	13.42 ab	122.59 fg	153.85 j
'H15'	20.64 a	13.09 ab	137.38 d	274.19 gh
'Aurea'	19.28 a-c	15.03 a	99.56 l	286.56 f–h
'Bornel Beaty'	19.97 a-c	14.44 ab	150.69 b	342.37 a-d
'Borrel Beast'	20.29 ab	13.83 ab	163.79 a	315.10 d-f
'C2 Kolenka'	18.05 a-c	13.50 ab	116.41 ij	274.03 gh
'Store'	19.07 a-c	12.59 b	114.42 j	302.10 d-g
Mean	*19.12*	*13.32*	*129.32*	*301.59*
Sd	*± 1.32*	*± 1.01*	*± 18.23*	*± 51.50*

A.acid Ascorbic acid, *AA* Antioxidant activity, *TAs* Total anthocyanins, *TPs* Total phenolics. *Sd* Standard deviations. The differences between the means indicated by different letters in the same column are significant at the $p < 0.05$ level

change between 6.59 and 10.17 μmol Trolox g^{-1} [42]. Gołba et al. [13] detected that the total anthocyanin content in their study conducted in Poland varied between 86 and 655 mg cyn-3-gluc 100 g^{-1}. Celli et al. [7] determined that the total phenolic content in honeyberry fruits ranged from 140.5 to 1142.0 in their study conducted in Canada. Our findings for ascorbic acid, total anthocyanins, and total phenolics are consistent with those of other researchers. However, our finding for antioxidant activity is significantly higher compared to researchers' results. This difference is thought to be due to the different cultivars used, the hybrid individual and different growing environments.

The nutrient content in the fruits of some hybrid individuals is given in detail in Table 5. Accordingly, aluminum varied between 21.25 ('H15') and 424.08 mg kg^{-1} ('Bornel Beaty'), boron changed between 10.59 ('Borrel Beast') and 15.28 mg kg^{-1} ('H5'), calcium ranged from 1267.53 ('Store') to 2445,77 mg kg^{-1} ('H10'), carbon varied between 4.40 ('Bornel Beaty') and 8.52 mg kg^{-1} ('H10'), iron changed between 9.99 ('Borrel Beast') and 54.77 mg kg^{-1} ('Aurea'), potassium ranged from 868.73 ('H15') to 2274.36 mg kg^{-1} ('H10'), magnesium varied between 577.06 ('Borrel Beast') and 1263.95 mg kg^{-1} ('H10'), manganese changed between 8.08 ('H7') and 16.45 mg kg^{-1} ('H5'), sodium ranged from 138.51 ('H15') to 236.62 mg kg^{-1} ('H5'), nickel varied between 0.17 ('H7') and 1.71 mg kg^{-1} ('H9'), phosphorus changed between 819.26 ('H15') and 2123.27 mg kg^{-1} ('H10'), lead ranged from 0.13 ('H15') to 0.37 mg kg^{-1} ('H10'), sulfur varied between 312.78 ('H7') and 859.62 mg kg^{-1} ('H10'), zinc changed between 6.34 ('H7') and 17.87 mg kg^{-1} ('H10'). The research on the mineral content of honeyberry fruits is quite limited. Kusznierewicz et al. [43] found that honeyberry fruits contain similar amounts of calcium, magnesium, and potassium compared to wild fruits in their study conducted in Poland. Sochor et al. [44] reported that potassium levels in honeyberry fruits reached up to 5000 mg kg^{-1} in their study conducted in the Czech Republic. In a study by Pokorná-Juríková and Matuškovič [45], the average magnesium content of honeyberry fruits was determined to be 711 mg kg^{-1}. Additionally, a study conducted in Slovakia indicated that the most abundant mineral nutrients in honeyberry fruits are potassium, phosphorus, magnesium, and calcium [45]. Overall, our findings are parallel to those of previous researchers. Furthermore, among the few existing studies, the amounts of calcium, magnesium, potassium, phosphorus, and sodium have generally been investigated. In our study, however, we identified the quantities of a total of 14 mineral nutrients, categorized as macro and micronutrients, in the fruits.

The nutrient content in the leaves of some hybrid individuals is given in detail in Table 6. Accordingly, aluminum varied between 16.74 ('Borrel Beast') and 52.12 mg kg^{-1} ('H12'), boron changed between 27.22 ('Bornel Beaty') and 57.45 mg kg^{-1} ('H14'), calcium ranged from 9072.73 ('H13') to 19,493.21 mg kg^{-1} ('H10'), carbon varied between 4.59 ('Bornel Beaty') and 16.04 mg kg^{-1} ('H12'), iron changed between 26.37 ('Borrel Beast') and 106.38 mg kg^{-1} ('H6'), potassium ranged from 193.80 ('H13') to 1099.32 mg kg^{-1} ('H6'), magnesium varied between 2637.44 ('H4') and 5643.52 mg kg^{-1} ('H5'), manganese changed between 9.83 ('Bornel Beaty') and 65.05 mg kg^{-1} ('H3'), sodium ranged from 169.49 ('H7') to 327.89 mg kg^{-1} ('H10'), nickel varied between 0.23 ('Aurea') and 2.17 mg kg^{-1} ('Borrel Beast'), phosphorus changed between 887.46 ('H13') and 2007.51 mg kg^{-1} ('H6'), lead ranged from 0.35 ('H2') to 1.01 mg kg^{-1} ('H12'), sulfur varied between 827.21 ('Aurea') and 2312.11 mg kg^{-1} ('H8'), zinc changed between 11.75 ('Aurea') and 42.07 mg kg^{-1} ('H10'). The average nutrient content in honeyberry leaves ranged from nickel (0.56 mg kg^{-1}) to calcium (15,117.38 mg kg^{-1}). Calcium was followed by magnesium (4016.69 mg kg^{-1}), phosphorus (1351.80 mg kg^{-1}), sulfur (1251.43 mg kg^{-1}), and

Table 5 Fruit nutrient content in fruits of the assessed honeyberry individuals

Genotype No	F.Al	F.B	F.Ca	F.C	F.Fe	F.K	F.Mg	F.Mn	F.Na	F.Ni	F.P	F.Pb	F.S	F.Zn
'H1'	152.17 j	11.81 b-e	2016.26 c	6.15 a-c	22.48 de	1677.22 c	639.74 j	11.63 c-f	190.03 g	0.54 c-e	1259.43 p	0.31 ab	362.93 p	7.70 f–h
'H2'	271.95 d	11.95 b-e	1623.46 l	5.51 a-c	16.35 gh	1155.25 fg	591.34 l	11.82 b-e	155.70 l	0.45 d-g	1376.19 k	0.21 ab	502.84 h	8.99 d-f
'H3'	151.01 j	12.09 b-e	1862.00 f	6.88 a-c	19.40 e–g	1583.35 cd	779.20 e	13.08 b-d	212.64 c	0.79 bc	1583.23 f	0.23 ab	575.54 e	10.37 cd
'H4'	382.06 b	14.03 a-c	2428.06 b	5.50 a-c	19.96 ef	1554.18 de	646.39 i	11.94 b-e	180.41 i	0.42 d-g	1613.84 e	0.21 ab	494.73 i	9.88 c-e
'H5'	151.81 j	15.28 a	1955.04 d	8.01 ab	24.48 cd	1855.7 b	1035.03 b	16.45 a	236.62 a	0.95 b	1914.34 c	0.29 ab	712.75 c	12.64 b
'H6'	96.43 n	12.00 b-e	1398.27 q	4.80 c	19.38 fg	1645.42 cd	577.35 m	9.82 e–g	190.36 g	0.45 d-g	1486.02 h	0.17 ab	461.02 l	9.75 c-e
'H7'	125.05 m	13.21 a-e	1353.06 r	4.52 c	14.71 hi	1118.08 f–h	598.19 k	8.08 g	194.97 e	0.17 g	1264.73 o	0.17 ab	312.78 q	6.34 h
'H8'	198.69 e	11.79 b-e	1730.72 j	4.90 c	19.89 ef	1597.86 cd	578.80 m	8.63 fg	194.30 ef	0.31 d-g	1644.31 d	0.33 ab	573.64 e	7.49 f–h
'H9'	137.86 l	12.57 a-e	1642.67 k	6.53 a-c	16.96 f–h	971.21 i-j	663.84 h	9.56 e–g	158.19 l	1.71 a	1092.89 r	0.31 ab	546.99 f	10.51 cd
'H10'	185.57 g	14.88 ab	2445.77 a	8.52 a	23.45 cd	2274.36 a	1263.95 a	14.91 ab	236.15 a	1.43 a	2123.27 a	0.37 a	859.62 a	17.87 a
'H11'	81.25 o	13.10 a-e	1869.03 e	6.86 a-c	16.31 gh	1201.37 f	716.56 f	13.44 a-c	235.75 a	0.89 b	1453.52 i	0.14 ab	773.77 b	11.34 bc
'H12'	50.48 p	13.43 a-e	1563.92 m	5.37 bc	12.38 ij	1123.37 f–h	637.17 j	10.55 c-g	185.03 h	0.36 d-g	1209.00 q	0.14 ab	466.34 k	7.66 f–h
'H13'	147.73 k	12.86 a-e	1530.86 n	5.80 a-c	33.85 b	1056.46 g-i	841.87 d	10.06 d-g	167.98 k	0.51 c-f	1291.87 m	0.25 ab	507.11 g	9.56 de
'H14'	347.66 c	12.49 a-e	1760.22 h	6.42 a-c	36.30 b	1475.6 e	638.93 j	10.38 c-g	191.79 fg	0.34 d-g	1484.12 h	0.23 ab	455.04 m	8.34 e–g
'H15'	21.25 q	13.81 a-d	1643.19 k	5.13 bc	10.28 j	868.73 j	716.66 f	8.38 g	138.51 m	0.24 e–g	819.26 s	0.13 b	368.23 o	6.66 h
'Aurea'	174.40 i	10.89 de	1525.93 o	5.96 a-c	54.77 a	1038.25 hi	662.06 h	9.82 e–g	200.85 d	0.55 cd	1274.92 n	0.19 ab	547.73 f	9.52 de
'Bornel Beaty'	424.08 a	10.94 c-e	1740.08 i	4.40 c	25.15 cd	1213.09 f	702.75 g	10.88 c-g	214.39 c	0.79 bc	1553.82 g	0.29 ab	495.62 i	9.03 d-f
'Borrel Beast'	181.42 h	10.59 e	1520.14 p	4.45 c	9.99 j	1150.59 fg	577.06 m	8.34 g	172.34 j	0.22 fg	1302.46 l	0.16 ab	410.16 n	6.99 gh
'C2 Kolenka'	190.79 f	13.13 a-e	1817.40 g	6.47 a-c	25.08 cd	1656.81 cd	852.11 c	11.97 b-e	221.90 b	0.53 c-e	2070.78 b	0.35 ab	681.95 d	12.93 b
'Store'	185.25 g	12.29 a-e	1267.53 s	4.63 c	25.58 c	1029.78 h-i	589.76 l	8.52 g	193.86 ef	0.27 d-g	1393.43 j	0.27 ab	480.72 j	6.40 h
Mean	*182.85*	*12.66*	*1734.68*	*5.84*	*22.34*	*1366.83*	*715.44*	*10.91*	*193.59*	*0.60*	*1460.57*	*0.25*	*529.53*	*9.50*
Sd	*±101.93*	*±1.48*	*±304.43*	*±1.40*	*±10.11*	*±354.37*	*±170.50*	*±2.39*	*±26.88*	*±0.40*	*±309.82*	*±0.10*	*±136.29*	*±2.82*

F Fruit. *Sd* Standard deviations. The differences between the means indicated by different letters in the same column are significant at the $p < 0.05$ level

Table 6 Leaf nutrient content in leaves of the assessed honeyberry individuals

Genotype No	L.Al	L.B	L.Ca	L.C	L.Fe	L.K	L.Mg	L.Mn	L.Na	L.Ni	L.P	L.Pb	L.S	L.Zn
'H1'	41.69 bc	50.92 b	16,974.46 g	7.92 b	59.14 e	1049.53 b	4288.78 f	36.87 d	275.20 c	1.45 ab	1882.96 b	0.69 b	1558.33 c	25.30 de
'H2'	21.50 hi	33.37 k	13,702.65 o	5.42 bc	36.67 h	408.22 l	3290.77 n	46.62 c	196.96 jk	0.28 d	1042.32 r	0.35 c	919.19 p	26.22 d
'H3'	39.60 cd	34.69 k	15,140.86 j	5.68 bc	52.63 f	641.74 e	3179.33 o	65.05 a	209.19 i	0.26 d	1203.42 l	0.48 bc	1141.39 j	25.06 de
'H4'	27.55 g	41.92 hi	17,394.21 d	6.60 bc	44.16 g	985.74 c	2637.44 q	35.16 d	267.01 d	0.38 d	1879.27 c	0.61 bc	1028.84 m	24.36 de
'H5'	28.00 g	47.12 c-e	16,691.53 h	6.87 bc	68.26 d	591.83 f	5643.52 a	54.53 b	264.90 d	0.36 d	1390.56 i	0.54 bc	1286.20 i	38.27 b
'H6'	43.98 b	41.49 hi	17,016.85 f	6.01 bc	106.38 a	1099.32 a	3349.75 m	48.21 c	272.89 c	0.43 d	2007.51 a	0.60 bc	849.06 r	21.22 fg
'H7'	35.51 ef	42.16 g-i	12,605.48 r	5.52 bc	79.09 b	503.43 i	3724.96 k	27.86 e	169.49 o	0.29 d	958.91 s	0.56 bc	987.77 n	22.48 ef
'H8'	28.02 g	38.06 j	17,544.73 c	4.66 c	42.58 g	559.60 g	3963.30 ij	17.68 g	260.59 e	0.31 d	1518.29 f	0.56 bc	2312.11 a	15.62 h
'H9'	28.37 g	48.73 b-d	14,227.60 n	5.93 bc	54.24 f	282.18 q	4213.21 g	22.39 f	195.18 k	0.45 d	1054.21 q	0.43 bc	1741.87 b	33.64 c
'H10'	24.14 h	45.14 e–g	14,326.46 m	6.45 bc	37.60 h	417.59 k	4902.20 c	49.26 c	327.89 a	0.50 d	1546.78 e	0.45 bc	1284.59 i	42.07 a
'H11'	37.19 de	46.24 d-f	15,136.87 k	6.09 bc	60.36 e	309.59 p	4486.56 e	34.59 d	185.66 m	0.53 d	1106.35 n	0.49 bc	1521.69 e	25.83 d
'H12'	52.12 a	50.78 b	12,899.21 q	16.04 a	72.14 c	328.28 n	4857.19 d	35.29 d	215.99 h	1.36 bc	1095.52 o	1.01 a	1433.97 f	37.99 b
'H13'	22.95 hi	40.36 ij	9072.73 t	4.96 bc	35.10 h	193.80 r	3995.18 i	14.54 hi	174.53 n	0.26 d	887.46 t	0.40 bc	1114.37 k	15.77 h
'H14'	40.78 c	57.45 a	19,493.21 a	6.79 bc	54.07 f	664.60 d	5223.44 b	33.81 d	285.61 b	0.61 cd	1703.26 d	0.53 bc	1526.82 d	32.93 c
'H15'	44.68 b	48.03 b-e	13,148.20 p	6.05 bc	66.55 d	311.46 op	4938.71 c	20.41 fg	258.13 e	0.45 d	1059.73 p	0.56 bc	1352.99 g	26.04 d
'Aurea'	19.92 i	43.72 f–h	15,375.87 i	4.74 c	27.19 i	314.52 o	4041.13 h	11.29 jk	199.27 j	0.23 d	1187.81 m	0.38 c	827.21 s	11.75 i
'Bornel Beaty'	23.60 h	27.22 l	11,562.91 s	4.59 c	37.06 h	339.49 m	2742.43 p	9.83 k	190.83 l	0.25 d	1221.45 k	0.44 bc	868.38 q	12.15 i
'Borrel Beast'	16.74 j	41.75 hi	14,864.09 l	4.62 c	26.37 i	415.81 k	3321.37 mn	22.04 f	196.67 jk	2.17 a	1293.37 j	0.40 bc	1305.17 h	18.45 gh
'C2 Kolenka'	36.17 ef	55.73 a	18,037.69 b	6.01 bc	54.06 f	540.44 h	3554.51 l	17.40 gh	239.30 g	0.38 d	1513.28 g	0.64 bc	924.99 o	20.08 fg
'Store'	33.16 f	49.85 bc	17,131.94 e	4.80 c	44.31 g	445.61 j	3946.60 j	13.13 ij	252.38 f	0.28 d	1480.59 h	0.62 bc	1043.57 l	15.48 h
Mean	*32.28*	*44.24*	*15,117.38*	*6.29*	*52.90*	*520.14*	*4016.69*	*30.80*	*231.88*	*0.56*	*1351.80*	*0.54*	*1251.43*	*24.54*
Sd	*±9.50*	*±7.35*	*±2471.97*	*±2.55*	*±10.05*	*±254.90*	*±805.78*	*±15.55*	*±43.16*	*±0.54*	*±323.54*	*±0.16*	*±360.52*	*±8.67*

L Leaf. *Sd* Standard deviations. The differences between the means indicated by different letters in the same column are significant at the $p < 0.05$ level potassium (520.14 mg kg^{-1}), respectively. There have been no studies found in the literature regarding honeyberry leaves. Therefore, the results have been evaluated independently among themselves. In this comprehensive study, we have contributed a total of 14 macro and micronutrients from honeyberry leaves to the literature.

The differences in nutrient levels can be attributed to a combination of factors, including genotype, environmental conditions, and soil characteristics. Firstly, the genotype of the plants plays a significant role in determining nutrient uptake and allocation, as different genotypes exhibit inherent variations in their capacity to absorb and utilize nutrients. Research by López-Bucio et al. [46] highlights the influence of genetic factors on nutrient requirements and absorption capabilities. Secondly, environmental conditions such as temperature, humidity, and light exposure can also impact nutrient content in plant tissues [47]. As noted by Thepbandit and Athinuwat [48], environmental stressors can alter physiological and biochemical processes, thereby affecting the accumulation of nutrients in leaves. Additionally, soil composition is critical in determining the nutrient availability for plants. Factors such as soil type, pH, and organic matter content significantly influence the bioavailability of essential nutrients. Brady and Weil [49] emphasize that soils rich in organic matter enhance nutrient availability and uptake by plants. Therefore, the observed variability in nutrient content among the hybrid individuals reflects a complex interplay between genetic makeup, environmental influences, and soil characteristics. This multifaceted relationship underscores the importance of considering these variables in future studies.

Pearson correlation analysis

The correlation between variables is shown in detail in Fig. 1. In general, the nutrients in the fruit exhibited significant correlations among themselves at different levels (*, **, ***). Similarly, significant correlations were observed among the nutrients in the leaves and with the nutrients in the fruit at various levels (*, **, ***). In addition, these statistically significant correlations were found between fruit weight with fruit width (***) and fruit length (*), a* color value and L* color values (**), petiole thickness with leaf width (*) and leaf length (**).

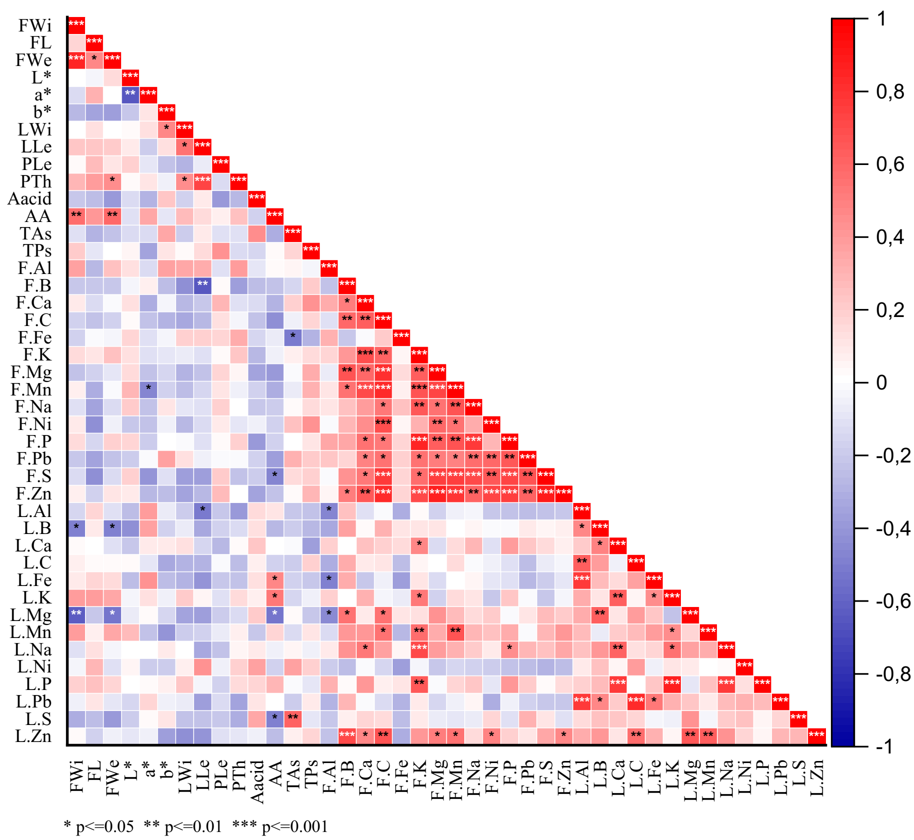

Fig. 1 Pearson correlation matrix between different variables in honeyberry cultivars and hybrid individuals Abbreviations are as in Tables 3, 4, 5 and 6

Principal component analysis (PCA)

The PCA results offer valuable insights into the positioning of hybrid individuals and their associated traits, revealing intricate relationships that can inform breeding strategies. The eigenvectors of nineteen principal component axes from PCA analysis in honeyberry cultivars and hybrid individuals are presented in detail in Table 7.

PC1, accounting for 23.06% of the total variance, clearly indicates that fruit carbon, zinc, manganese, sulfur, and magnesium content are pivotal for distinguishing these hybrids. This clustering suggests that hybrids exhibiting higher levels of these nutrients are likely to possess not only enhanced fruit quality but also improved health benefits for consumers. For instance, the strong correlation between carbon content and overall fruit quality implies that optimizing these nutrients could lead to hybrids that stand out in both marketability and nutritional value.

Moving to PC2, which explains 14.59% of the variance, the inclusion of leaf lead and boron content further emphasizes the significance of leaf health. Hybrids that cluster together in this component may benefit from superior nutrient uptake and photosynthetic efficiency. This highlights the importance of leaf nutrient status, as it can directly impact fruit development and yield. A hybrid with robust leaf nutrition could exhibit not only higher fruit yield but also better resistance to environmental stressors.

PC3, accounting for 12.43% of the total variance, underscores the importance of traits such as leaf potassium and phosphorus, along with fruit weight and antioxidant activity. The positive relationships observed here suggest that hybrids with greater fruit weight also exhibit higher antioxidant levels. This relationship is crucial, as it implies that selecting for increased fruit size could concurrently enhance the health-promoting properties of the fruit. Thus, a dual focus on both weight and antioxidant activity may yield hybrids that appeal to health-conscious consumers.

The significance of the eigenvalues highlights the importance of these traits in differentiating hybrid individuals. The **strong significance of PC1, PC5, and PC6 at $p < 0.001$ suggests that these traits are not just correlated but are fundamental to understanding the genetic diversity present in the hybrids. Meanwhile, the moderate significance of **PC3, PC4, and PC8 at $p < 0.01$ indicates that while these traits are important, they may also be influenced by environmental factors, adding a layer of complexity to their relationships.

Overall, the intricate interplay among these traits suggests that breeding programs should prioritize hybrids that excel in multiple dimensions—nutritional content, fruit size, and leaf health. By doing so, they can develop cultivars that not only thrive under varying environmental conditions but also meet consumer demand for high-quality, healthful fruits. This comprehensive approach may lead to significant advancements in honeyberry cultivation, emphasizing the economic and ecological benefits of these hybrids.

The 2D principal component analysis biplot of honeyberry hybrid individuals and cultivars is presented in detail in Fig. 2. The biplot of the first two principal components constituted 37.64% of the total variation. Hybrid individuals and cultivars were scattered all over the plot. Cluster 1 includes the samples 'H12', 'H15', 'H1', 'H6', 'H9', 'H7', 'H8', and 'Store', with leaf aluminum (L.Al) and leaf nickel (L.Ni) present in this cluster. Additionally, ascorbic acid (A.acid) and the a* color value are also located in Cluster 1. This indicates that these samples exhibit similarities in both nutrient content and physical characteristics. Cluster 2 comprises the samples 'H14', 'H11', 'H5', and 'C2 Kolenka', with all other leaf nutrient contents grouped within this cluster. Parameters such as antioxidant activity (AA), b* color value, leaf width (LWi), fruit width (FWi), and fruit weight (FWe) are also included in this cluster. These findings suggest that these samples possess a higher diversity in nutrient content. Cluster 3 consists of the samples 'H13', 'H2', 'Borrel Beast', 'Aurea', and 'Bornel Beaty'. Leaf aluminum (F.Al) is present in this cluster, while all other leaf nutrient contents are clustered in Cluster 4. This distinction reveals a notable difference in nutrient content and other physical characteristics among these samples. Finally, Cluster 4 includes the samples 'H3', 'H4', and 'H10', with petiole length (PLe), L* color value, and total phenolic compounds (TPs) also found in this cluster. Total anthocyanins (TAs) are positioned in the center. This distribution indicates that the nutrient contents and physical characteristics of the samples form a specific structure among different groups, showing significant relationships between them. All these findings emphasize the complex nature of interactions between plant nutrient contents and physical characteristics, highlighting their important role in understanding the overall health and productivity of plants. Such statistical analyses provide a guiding foundation for future studies. In addition, 'H5', 'H10' and 'Bornel Beaty' remained outside the %95 confidence ellipse, while all other individuals were inside. In a biplot, a variable falling outside the 95% confidence ellipse indicates a significant deviation from the overall distribution of the dataset. This situation may encompass potential anomalies or interesting variations, necessitating further investigation by the researcher into these observations. Additionally, points outside the ellipse may also highlight errors in the data collection process or anomaly situations. Therefore, such observations provide an important reference for understanding the structure of the data [50].

Table 7 Eigenvectors of nineteen principal component axes from PCA analysis in honeyberry cultivars and hybrid individuals

Eigenvectors	Component							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
FWi			0.27*					
FL								
FWe			0.30*					
L*							-0.43*	
a*					-0.30*		0.35*	
b*								
LWi								
LLe								
PLe								
PTh						0.29*	0.34*	
A.acid					0.27*			
AA			0.30*					
TAs					0.38*			
TPs					0.28*			0.46*
F.Al								
F.B								
F.Ca								
F.C	0.29*							
F.Fe					-0.35*			
F.K								
F.Mg	0.27*							
F.Mn	0.28*							
F.Na								
F.Ni								
F.P								
F.Pb								
F.S	0.28*							
F.Zn	0.29*							
L.Al								
L.B		0.27*						
L.Ca				0.33*				
L.C								
L.Fe								
L.K			0.38*					
L.Mg								
L.Mn								
L.Na								
L.Ni					0.37*	0.35*		
L.P			0.31*					
L.Pb		0.29*						0.32*
L.S								
L.Zn								
Eigenvalue	*9.68*	*6.13*	*5.22*	*3.95*	*2.89*	*2.33*	*1.94*	*1.75*
Eigenvalue degree of significance	***	*	**	**	***	***	*	**
Variance	*23.06*	*14.59*	*12.43*	*9.41*	*6.89*	*5.54*	*4.61*	*4.16*
∑ variance (%)	*23.06*	*37.65*	*50.07*	*59.48*	*66.37*	*71.91*	*76.52*	*80.69*

Abbreviations are as in Tables 3, 4, 5, and 6. Components degree of significance *$p <= 0.05$ **$p <= 0.01$ ***$p <= 0.001$. *Eigenvectors degree of significance ≥ 0.27

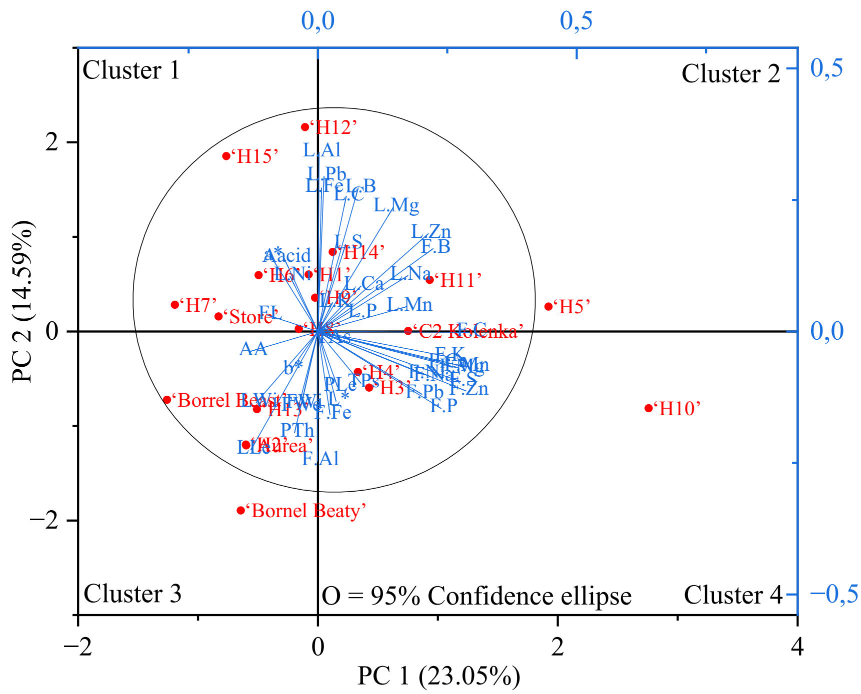

Fig. 2 2D principal component analysis biplot of honeyberry hybrid individuals and cultivars. Abbreviations are as in Tables 3, 4, 5 and 6

Hierarchical cluster analysis (HCA)

The results of the genetic diversity analysis highlight the distinct clustering patterns among hybrid individuals and cultivars. The dendrogram created using the Ward method and Euclidean distance reveals significant genetic differences within the examined population. The initial division into two main groups, A and B, indicates that the hybrids and cultivars possess distinct genetic characteristics.

Subsequent subdivisions into A1 and A2, as well as B1 and B2 subgroups, provide further insight into the genetic relationships among the accessions. The presence of only 'H13' and 'H10' in subgroup A1 suggests a close genetic relationship between these individuals, likely due to shared ancestry or similar selective pressures during cultivation. This close clustering may be attributed to specific traits such as flowering time, disease resistance, or stress tolerance, which have been favored in their respective breeding programs.

In contrast, the hybrids in subgroup A2, which include 'H15', 'H12', 'H11', 'H9', 'H7', and 'H5', exhibit a broader genetic diversity. This diversity can be a result of diverse parental lines used in their development, leading to variations in traits such as fruit size, yield, or adaptability to different environmental conditions.

The high variation observed in subgroup B2 signifies a rich genetic resource, indicating potential for future breeding programs to select for desirable traits. The diverse genetic backgrounds in this subgroup might reflect a range of phenotypic traits, which can enhance resilience against pests and diseases, as well as adaptability to changing climates.

Furthermore, the similarity index, which ranges from 0.58 to 1.00, indicates the highest similarity between the 'Aurea' cultivar and the 'H14' hybrid (Fig. 3). This close genetic relationship offers significant opportunities for optimizing desired characteristics in future breeding efforts. It suggests that traits associated with the 'Aurea' cultivar could be effectively transferred to the 'H14' hybrid, thereby enhancing its performance.

Overall, this analysis emphasizes the critical role of hybrid genetic diversity in shaping adaptability and resilience in response to environmental challenges. The identification of specific traits driving clustering can inform breeding strategies aimed at improving crop performance and sustainability [51].

Conclusions

The evaluation of 42 variables has identified hybrids 'H14,' 'H5,' 'H8,' and 'H1' as promising candidates for enhancing genetic diversity in honeyberry cultivation. These hybrids not only show considerable potential for broad industry applications—including food, health, cosmetics, and personal care—but also demonstrate strong adaptability,

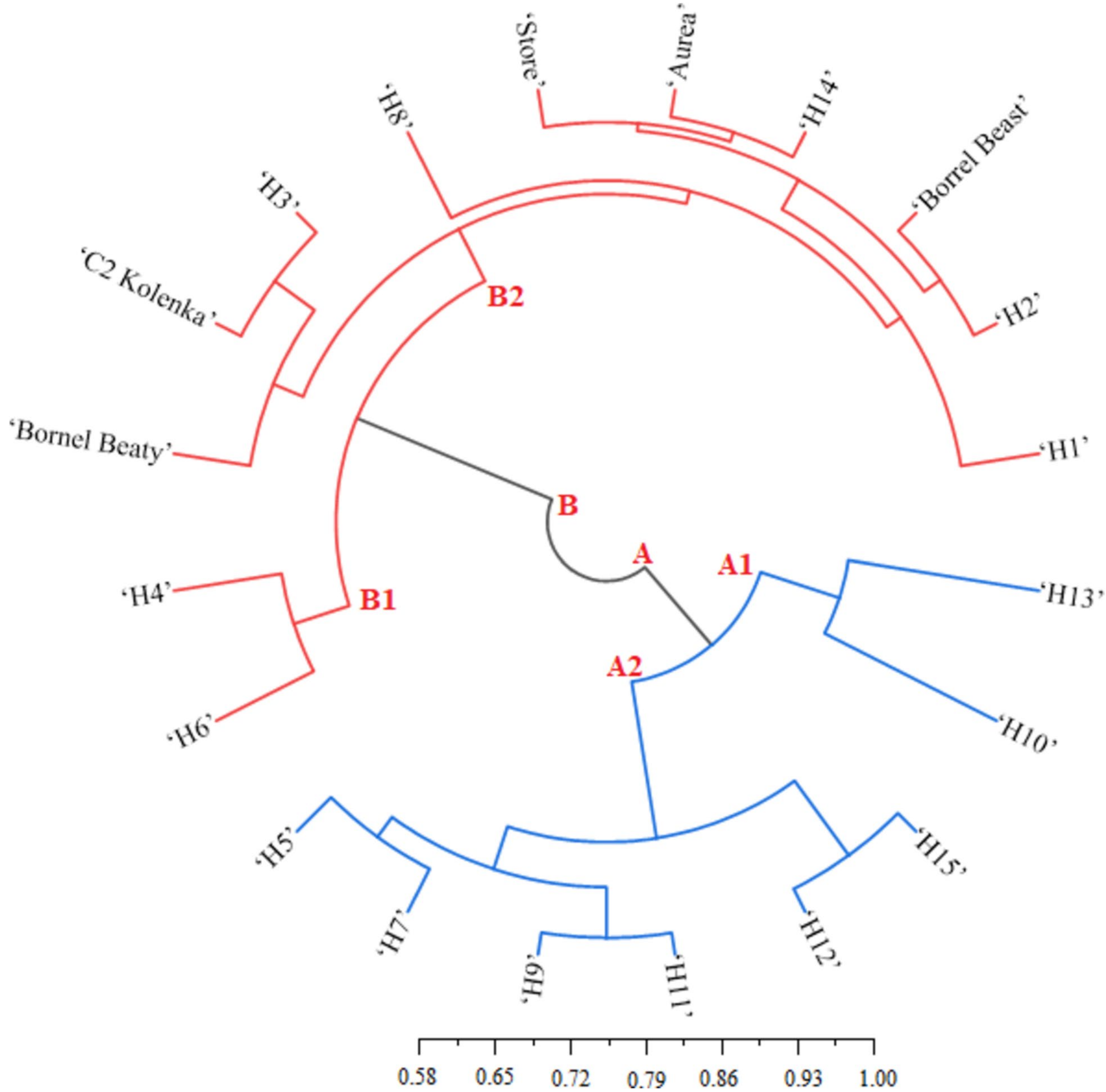

Fig. 3 UPGMA dendrogram of honeyberry hybrid individuals according to Ward's method and Euclidean distance

with low maintenance requirements and exceptional cold resistance, making them suitable for cultivation across various regions. Honeyberry's rich antioxidant profile and nutritional value are increasingly capturing attention, particularly within Türkiye, where its potential for fresh consumption, processed products, natural sweeteners, and health supplements is gaining popularity.

The fruit characteristics of these hybrids vary widely, with fruit weights ranging from 0.71 g ('H11') to 1.66 g ('H6'), ascorbic acid content between 17.13 mg AAE/100 g ('H7') and 20.64 mg AAE/100 g ('H15'), and antioxidant activity from 12.59 μmol Trolox g^{-1} ('Store') to 15.03 μmol Trolox g–1 ('Aurea'). The highest total anthocyanin levels were found in 'Borrel Beast' (163.79 mg cyn-3-gluc 100 g^{-1}) and 'H8' (163.20 mg cyn-3-gluc 100 g^{-1}), indicating significant health-promoting properties. Nutritional analyses highlight 'H10' as particularly nutrient-dense, with high levels of calcium (2445.77 mg kg^{-1}), potassium (2274.36 mg kg^{-1}), phosphorus (2123.27 mg kg^{-1}), magnesium (1263.95 mg kg–1), and sulfur (859.62 mg kg^{-1}) in the fruit. Meanwhile, leaf analyses revealed 'H14' as highest in calcium (19,493.21 mg kg^{-1}), 'H5' in magnesium (5643.52 mg kg^{-1}), 'H8' in sulfur (2312.11 mg kg^{-1}), and 'H6' in both phosphorus (2007.51 mg kg^{-1}) and potassium (1099.32 mg kg^{-1}).

Principal component analysis (PCA) of the data revealed that the first eight principal components explained 80.69% of the total variance, underscoring substantial phenotypic variation within the study group. Cluster and population analyses indicated particularly high variation within subgroup B2, suggesting that these hybrids could be pivotal for breeding programs aimed at cultivar development. Such programs are essential to expand genetic resources, improve productivity, and foster sustainable agricultural practices in honeyberry cultivation.

As honeyberry is an emerging crop in Türkiye, this research provides a crucial foundation for future studies. By offering a comprehensive overview of honeyberry's genetic diversity and nutritional profile, this study highlights the species' potential to support biodiversity, ecotourism, and local ecosystem protection, underscoring its economic and ecological value across multiple industries. These findings contribute valuable insights that address a significant gap in honeyberry research, reinforcing its importance as a resilient, nutritious crop with diverse applications.

Acknowledgements
None.

Research involving Human Participants and, or Animals
Not applicable.

Clinical Trial Study
Not applicable.

Informed consent
Not applicable.

Statement specifying permissions
For this study, we acquired permission to study honeyberry issued by the Ministry of Agriculture and Forestry of Türkiye.

Statement on experimental research and field studies on plants
The either cultivated or wild-growing plants sampled comply with relevant institutional, national, and international guidelines and domestic legislation of Türkiye.

Authors' contributions
All authors contributed to the study's conception and design. KG, FD, and KUY: Conceptualization, Data curation, Formal analysis, and Funding acquisition. YT and MY: Investigation, Methodology, and Project administration. AG, SD, and EY: Resources, Software, Supervision, and Validation. YT and AK: Visualization, Writing – original draft, and Writing – review & editing. The author read and approved the final manuscript.

Funding
Not applicable.

Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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Received: 30 September 2024 Accepted: 18 November 2024
Published online: 02 December 2024

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