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Antioxidant capacity, biochemical composition, and mineral composition of leaves in two apple species (*Malus domestica* Borkh. and *M. kirghisorum* Al. Fed. & Fed.)

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Abstract

Background Apple leaves are a rich source of bioactive compounds such as phenolics, flavonoids, and essential minerals, which exhibit significant antioxidant and therapeutic properties. This study focuses on comparing the biochemical composition, antioxidant capacity, and mineral contents of *Malus domestica* Borkh. cultivars and *M. kirghisorum* Al. Fed. & Fed. genotypes. The goal is to identify potential health-promoting compounds and establish a basis for utilizing apple leaves as a sustainable resource in the food, pharmaceutical, and cosmetic industries.

Results The study revealed significant biochemical and nutritional variation among the genotypes. Total antioxidant capacity ranged from 36.00 in 'A12' to 59.50% in 'Starking Delicious'. Total phenolic content varied between 70.42 in 'A28' and 147.45 mg GAE/100 g in 'Granny Smith', while total flavonoid content ranged from 15.43 in 'A28' to 38.66 mg QE/100 g in 'A16', demonstrating considerable variability in bioactive compound composition. Correlation matrix analysis identified several significant relationships. Total phenolics and total flavonoids showed a positive correlation ($r = 0.52$**), while calcium strongly correlated with magnesium ($r = 0.79$**), potassium ($r = 0.52$**), and phosphorus ($r = 0.52$**), underscoring their physiological interconnections. Multiple regression analysis clarified key traits. Total phenolic content was positively influenced by total flavonoids ($\beta = 0.52$, $p < 0.00$). Calcium was strongly associated with magnesium ($\beta = 0.52$, $p < 0.00$) and sodium ($\beta = 0.46$, $p < 0.00$), reflecting their synergistic roles in cellular and metabolic functions. Principal component analysis revealed that the first three components explained 57.80% of the total variation. PC1 (30.56% variance) was predominantly associated with calcium, potassium, phosphorus, and magnesium. PC2 (14.16%) highlighted the relationship between manganese and total antioxidant capacity, while PC3 (13.08%) reflected the influence of lead, total phenolics, and total flavonoids. Heat map analysis indicated that

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the calcium, phosphorus, sulfur, phenolic compounds, and antioxidant activities in subgroup A1 suggest that the genotypes may be beneficial for health. Additionally, the accumulation of heavy metals such as lead, nickel, and aluminum in subgroup B1 could pose a health risk; however, the genotypes 'A18', 'A21', 'A21-1', and 'A22' possess the capacity to reduce this accumulation.

Conclusions The results highlight the nutritional and therapeutic potential of apple leaves as a natural source of antioxidants and essential minerals. In particular, the genotypes 'A21-1' and 'A16' stand out due to their high content of bioactive compounds and nutrients, offering promising prospects for further research and applications. These findings contribute to the conservation of wild apple genetic resources and their potential for industrial use.

Clinical trial number Not applicable.

Keywords Functional foods, Multivariate analysis, Nutraceutical potential, Sustainable resources, Wild apple genotypes

Introduction

Apple is one of the most widely cultivated fruit crops globally, with an annual production exceeding 97 million tons [21]. The domesticated apple (*Malus domestica* Borkh.) is grown across a vast geographic range, from the cold regions of Siberia to the temperate zones of Ecuador. Its broad adaptability and rich diversity of species and cultivars have contributed to its widespread cultivation. Apple cultivation originated in East Asia, Central Asia, West Asia, Europe, and North America [78], with Central Asia being a primary center of diversity. Kyrgyzstan, located in Central Asia, is a key region for apple cultivation [77]. In addition to *M. domestica*, the wild species *M. kirghisorum* Al. Fed. & Fed. is naturally found in Kyrgyzstan. Wild apple populations in this region show variation in fruit size, color, and ripening time, with some genotypes exhibiting fruit quality comparable to that of cultivated apples [80]. The fruits of *M. kirghisorum* are diverse in size, shape, color, and taste, ranging from sweet-sour to bitter, and typically contain 10–12% sugar. This species thrives in nutrient-rich, moist soils on northern slopes at altitudes between 1,200 and 1,800 m [18].

The increasing awareness of the health benefits associated with apples has led to a steady rise in global production. Apples, with their rich chemical composition, help reduce the risks of oxidative stress-related diseases such as cardiovascular diseases, asthma, and diabetes [30]. They also contain phenolic compounds with potent antioxidant capacity that inhibit cancer cell proliferation, reduce lipid oxidation, and lower cholesterol levels [46].

Phenolic compounds in plants play a critical role in their defense mechanisms [31]. Plants accumulate polyphenols across all organs as a protective measure against pests and UV radiation. However, the concentration of these compounds varies among morphological parts, with leaves being the primary site of accumulation [11, 73]. The biochemical composition of leaves often surpasses that of fruits due to exposure to biotic and abiotic stressors, as well as sunlight [32].

Apple leaves are a rich source of phenolic compounds with potent antiradical and antimicrobial activities [70]. The growing demand for natural products with high bioactive compound content highlights the potential of using easily accessible leaves for isolating bioactive compounds. These compounds have significant potential for improving human health, preventing infections, and aiding in disease recovery [2]. Apple leaves can serve as a cost-effective antioxidant, either independently or with other antioxidants, for use in the pharmaceutical, cosmetic, and food industries [51]. In China, they have traditionally been used as a remedy for menoxenia [73]. Moreover, apple leaves, with their rich bioactive composition, can be considered a potential functional food ingredient. Their high levels of phenolic compounds, minerals, and antioxidants suggest their applicability in functional food formulations aimed at promoting health and preventing diseases. The use of apple leaves as a functional food could contribute to the development of novel nutraceutical products, particularly in the context of natural antioxidant sources. Apple leaves are increasingly recognized as an alternative source of antioxidant polyphenols. Moreover, they contain a higher concentration of bioactive and nutritional compounds compared to other fruit tree leaves, with essential minerals like calcium (Ca), magnesium (Mg), iron (Fe), and potassium (K). Mineral levels, particularly K and Ca, are reported to be 3–6 times greater in apple leaves than in the fruit itself [83]. The rising global interest in dietary minerals is driven by their role in disease prevention [27]. Apple leaves, abundant in bioactive compounds, can be used for disease prevention and as valuable resources in the pharmaceutical and cosmetic industries [83]. The rising global interest in dietary minerals is driven by their role in disease prevention [27]. Apple leaves, abundant in bioactive compounds, can be used for disease prevention and as valuable resources in the pharmaceutical and cosmetic industries [83].

The phenolic and mineral contents of *M. domestica* fruits have been extensively studied. Recently, there has been growing interest in investigating the phenolic and mineral compositions of apple leaves. However, no studies have been identified in the literature focusing on the leaves of *M. kirghisorum*. The wild apple genetic resources of Central Asia are particularly important, as intensive selection for desirable traits in commercial apple species has led to a narrowing of genetic diversity [78]. Despite the significant potential of wild species, there is a lack of comprehensive research on their biochemical properties, especially concerning the leaves. This study aims to fill this research gap by evaluating the macro- and micronutrient contents, total phenolic compounds, total flavonoids, and antioxidant capacities of leaves from genotypes of *M. kirghisorum*, a species widely distributed in Central Asia. Additionally, it seeks to perform a comparative analysis with the leaves of *M. domestica* cultivars, highlighting the biochemical differences between these two species. This research will not only contribute to the conservation of Central Asia's wild apple genetic resources but also facilitate the identification of potentially valuable bioactive compounds for applications in nutrition, health, and the cosmetic industry.

Materials and methods

Plant material

The study was conducted in the "Apple Adaptation Parcel" located in the Talas district of Kayseri province (38°42'45" N 35°32'28" E), affiliated with the Agricultural Research and Application Center of Erciyes University, Republic of Türkiye, during the years 2022 and 2023 (Fig. 1). The Talas district of Kayseri has a continental climate. Summers are hot and dry, while winters are cold and snowy. The region experiences significant temperature differences between day and night. Additionally, the average annual precipitation is approximately 564 mm. The plant material for the study consisted of 28 apple genotypes ('A1', 'A2', 'A4', 'A5', 'A6', 'A8', 'A12', 'A13', 'A14', 'A15', 'A16', 'A17', 'A18', 'A21', 'A21-1', 'A22', 'A26', 'A28', 'A29', 'A35', 'A42', 'A44', 'A45', 'A46', 'A47', 'A48', 'A53', and 'A57') belonging to the species *M. kirghisorum*, collected from different regions of Kyrgyzstan, and commercial apple cultivars (*M. domestica*), including 'Fuji', 'Starking Delicious', 'Granny Smith', 'Red Chief', and 'Royal Gala'. The genotypes, aged between 8 and 10 years, were cultivated at 3 × 3 m planting intervals and were in good health, exhibiting full fruit development. Standard cultural

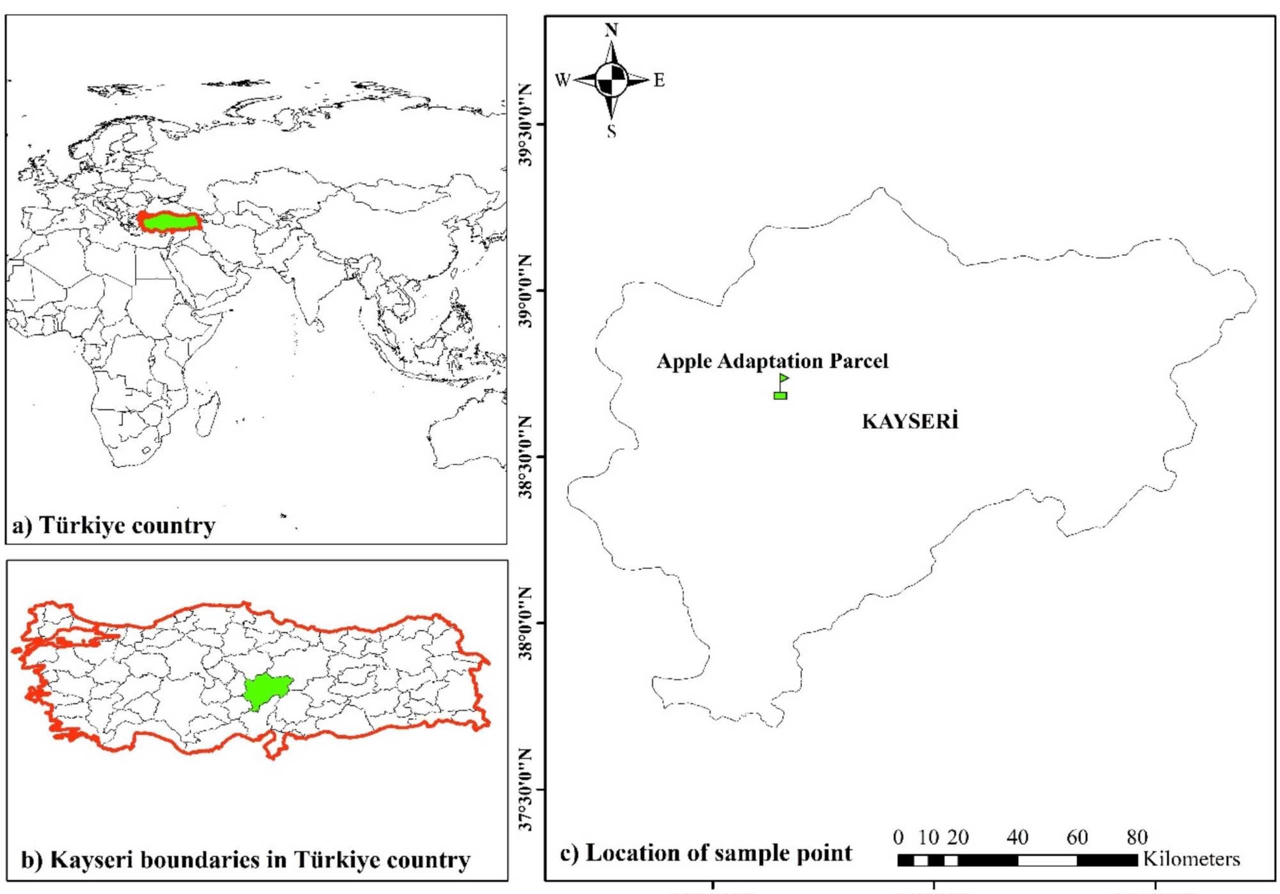

Fig. 1 Geographic location of the studied apple genotypes and cultivars

practices, including fertilization, irrigation, pest management, and disease control, were meticulously performed throughout the study. All genotypes and cultivars used as material in the study were grafted onto the MM106 rootstock.

Chemical characteristics

Sample preparation

Biochemical analyses were performed with three replications, each consisting of 50 leaves. The sample preparation followed the method described by Hannachi et al. [26]. Biochemical components were extracted using a maceration technique with continuous agitation for 24 h at a temperature of 25 °C, employing methanol as the solvent. The resulting extract was filtered and then centrifuged (Hettich H-1650R, Tuttlingen, Germany) at 11,200×g for 15 min. The methanolic extracts obtained from apple leaves were then used to evaluate the biochemical content.

Total antioxidant capacity (TAC)

The total antioxidant capacity (TAC) of apple leaves was evaluated using the method described by Sharma and Bhat [69]. A 0.26 mM DPPH (1,1-diphenyl-2-picrylhydrazyl) solution was used for the assay. For each apple genotype and cultivar, 100 µL of leaf extract was combined with 2900 µL of ethanol and 1 mL of the DPPH solution. The mixture was agitated and incubated in darkness for 30 min. After incubation, the absorbance of the sample was measured at 517 nm using a spectrophotometer (Systonic 119 UV-VIS, Haryana, India), and the absorbance of the leaf extract [$X_{(sample)}$] was recorded. A blank solution, which did not contain any apple extract, was used as a control, and its absorbance [$X_{(blank)}$] was also measured [15]. The antioxidant capacity was calculated using the formula provided in Eq. 1 [8].

$$\%\,inhibition\,of\,DPPH\,=$$

$$[(X\,(blank)\,-\,X\,(sample))\,/\,X(blank)]\,\times\,100 \quad (1)$$

Total phenolics (TPs)

The total phenolics (TPs) in apple leaves were assessed using the Folin-Ciocalteu method, as outlined by Lamuela-Raventós [47]. A 500 µL aliquot of fresh leaf extract was combined with 4.1 mL of distilled water. To this mixture, 100 µL of Folin-Ciocalteu reagent and 2% sodium carbonate (Na_2CO_3) were added. The resulting solution was kept in the dark for two hours, after which the absorbance was measured at 760 nm using a spectrophotometer. The absorbance readings were then converted into gallic acid equivalents using a standard calibration curve made with gallic acid [Hussain et al., 2025], and the results were expressed as mg/100 g of fresh weight (FW).

Total flavonoids (TFs)

The total flavonoids (TFs) in apple leaves were determined following the procedure outlined by Demir et al. [16]. A 1000 µL sample from each genotype was combined with 3.3 mL of methanol. To this mixture, 0.1 mL of a 10% solution of aluminum chloride hexahydrate ($AlCl_3{\cdot}6H_2O$) and potassium acetate (CH_3COOK) was added. The resulting mixtures were then analyzed using a spectrophotometer at 415 nm. The flavonoid content was calculated as quercetin equivalents (QE) and reported in mg/100 g [16].

Mineral composition

The mineral analysis of apple leaves [Aluminum (Al), Calcium (Ca), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Nickel (Ni), Phosphorus (P), Lead (Pb), Sulfur (S)] was performed with three replicates, using 100 leaves for each replicate [57]. The nutrient evaluation involved healthy leaves selected as representative of varietal traits from four different sections of the tree. The samples were initially ground, and 0.25 g of this ground plant material was digested using 0.5 ml of nitric acid (HNO_3) and 2 ml of hydrogen peroxide (H_2O_2) according to the method described by Miller [55]. Following digestion, the leaf samples were dried at 68 °C for 48 h in an oven (Nükleon NKD1000, Ankara, Türkiye) as outlined by Çoban et al. [14]. Subsequently, the dried and digested samples underwent a three-step heating process in an oven (Refsan, RK55, Kütahya, Türkiye) as described by Mertens [54]: (1) 5 min at 145 °C with 75% relative humidity, (2) 10 min at 180 °C with 90% relative humidity, and (3) 10 min at 100 °C with 40% relative humidity. The concentrations of minerals in the processed samples were then quantified in mg/kg dry weight (DW) using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Perkin-Elmer, Optima 2100 DV, Shelton, CT, USA) as detailed by Kızılkaya et al. [44].

Statistical analysis

To increase the reliability of the results and minimize the effects of seasonal variations, the average data from both years were used for statistical analysis. The statistical assessments for antioxidant capacity, biochemical composition, and mineral composition of the apple leaves were performed using the JMP® Pro 17 software (SAS Institute Inc., Cary, NC, USA) [34]. The significance of the results was evaluated using the TUKEY multiple comparison test with a 5% significance level ($p<0.05$), as described by Savaşlı et al. [66]. All data are presented as mean ± standard deviation [3].

For multivariate analysis, the following methods were employed using Origin Pro® 2025 software [62]: correlation matrix analysis, principal component analysis, and heat map analysis. The correlation matrix analysis utilized the Pearson correlation coefficient (r) to assess the strength and direction of relationships between variables. Principal component analysis was conducted with the Varimax rotation method and Kaiser Normalization to clarify component structure. A two-dimensional biplot was constructed to display the first and second principal components (PC1/PC2) to visualize genotype distribution and their associated variables. Ward's method, coupled with heat map analysis based on Euclidean distance, was used for clustering the genotypes and variables, providing a deeper insight into their interrelationships. Additionally, multiple regression analysis was performed to identify the traits that most significantly influence key leaf characteristics, with selected leaf traits considered as dependent variables. Multiple regression analysis was conducted using the "stepwise" method under the "linear regression analysis" option in SPSS® software (SPSS Inc., Chicago, IL, USA) [19, 60].

In summary, the application of these multivariate statistical techniques allowed for a thorough examination of the relationships among the antioxidant capacity, biochemical composition, and mineral composition of apple leaves, providing valuable insights into the categorization and classification of apple genotypes and cultivars.

Results and discussion

Descriptive statistics among genotypes

To assess the biochemical and mineral composition variations of the evaluated characteristics, the minimum, maximum, mean, standard deviation (SD), and coefficient of variation (CV%) values were computed and presented in Table 1. In this context, one-way ANOVA ($p < 0.05$) revealed significant variations among the assessed genotypes and cultivars. The highest variation was observed in phosphorus (75.65), lead (61.76), magnesium (56.07), potassium (44.53), and calcium (44.13). In contrast, the lowest variation was recorded in aluminum (23.53), total phenolics (22.58), total flavonoids (20.43), sulfur (20.14), and total antioxidant capacity (15.10). Notably, 12 out of 13 variables (representing 92.31% in total) had coefficients of variation (CVs) greater than 20.00%. This value indicated a notable degree of variation among the cultivars studied [43]. Traits with more than 20.00% variation are more distinguishable among specimens and act as dependable markers for differentiating various genotypes and cultivars [42]. Conversely, characteristics with a wider quantitative range exhibit a higher coefficient of variation (CV%), which suggests a greater potential for the selection of these characteristics [58]. On the other hand, characteristics with lower CVs are more consistent and can be considered stable characteristics among accessions [42].

Since there is no study conducted on apples using a similar statistical method, the findings were compared with studies conducted on other fruit species. In a study conducted on pomegranate, it was reported that 90% of the CV values were greater than 20.00% [39], this rate was 92% in olives [41] and 90% in *Prunus scoparia* Spach [40]. The findings of our study are consistent with the results of these studies.

Detailed TUKEY results of the statistical descriptive parameters for biochemical traits in apple genotypes and cultivars are presented in Table 2, highlighting significant differences in total antioxidant capacity, total phenolic content, and total flavonoid content (One-way ANOVA, $p < 0.05$). Total antioxidant capacity ranged from 36.00 ('A12') to 59.50% ('Starking Delicious'). A similar study conducted in Estonia reported that the total antioxidant capacity in apple leaves ranged from 28.9 to 48.4% [9]. Wojdyło et al. [83] examined the total antioxidant capacity in apple leaves using ABTS, FRAP, and ORAC methods and found that it ranged from 43.1 to 55.8 mmol

Table 1 Statistical descriptive parameters of biochemical and mineral composition traits used in the evaluation of apple genotypes and cultivars

Characteristics	Abb.	Unit	Min.	Max.	Mean	±SD	CV
Total antioxidant capacity	TAC	%	36.00	59.50	47.61	± 7.19	15.10
Total phenolics	TPs	mg GAE/100 g	70.42	147.45	111.58	± 25.20	22.58
Total flavonoids	TFs	mg QE/100 g	15.43	38.66	23.74	± 4.85	20.43
Aluminum	Al	mg/kg DW	0.16	0.99	0.68	± 0.16	23.53
Calcium	Ca	mg/kg DW	134.81	753.58	350.24	± 154.56	44.13
Potassium	K	mg/kg DW	30.03	139.15	56.14	± 25.00	44.53
Magnesium	Mg	mg/kg DW	2.32	19.37	7.49	± 4.20	56.07
Manganese	Mn	mg/kg DW	0.020	0.083	0.047	± 0.017	36.17
Sodium	Na	mg/kg DW	3.37	18.11	8.68	± 2.66	30.65
Nickel	Ni	mg/kg DW	0.10	0.30	0.19	± 0.05	26.32
Phosphorus	P	mg/kg DW	42.84	348.35	99.11	± 74.98	75.65
Lead	Pb	mg/kg DW	0.01	0.86	0.34	± 0.21	61.76
Sulfur	S	mg/kg DW	82.27	229.29	130.95	± 26.37	20.14

Abb: Abbreviations, Max: Maximum, Min: Minimum, ±SD: Standard Deviation, CV: Coefficient of Variation

Table 2 Detailed TUKEY results of statistical descriptive parameters for biochemical traits used to study apple genotypes and cultivars

Genotypes/Cultivars	Total antioxidant capacity (% inhibition)	Total phenolics (mg GAE/100 g)	Total flavonoids (mg QE/100 g)
'A1'	48.00 $^{g–i}$	75.29 ^{m}	15.98 ^{uv}
'A2'	47.50 ^{hi}	141.35 ^{b}	27.73 $^{c–f}$
'A4'	50.50 $^{f–h}$	143.59 ^{ab}	26.54 $^{e–h}$
'A5'	43.00 ^{jk}	111.68 ^{f}	23.69 $^{i–m}$
'A6'	37.50 $^{m–o}$	145.42 ^{ab}	28.65 $^{b–e}$
'A8'	41.00 $^{k–m}$	134.64 ^{c}	23.23 $^{i–n}$
'A12'	36.00 ^{o}	75.70 ^{m}	21.03 $^{n–r}$
'A13'	48.50 $^{g–i}$	120.21 ^{e}	25.62 $^{f–i}$
'A14'	48.50 $^{g–i}$	116.96 ^{e}	21.30 $^{m–q}$
'A15'	39.00 $^{l–o}$	136.07 ^{c}	25.16 $^{g–j}$
'A16'	46.50 ^{ij}	142.16 ^{b}	38.66 ^{a}
'A17'	51.50 $^{e–g}$	112.08 ^{f}	18.36 $^{s–u}$
'A18'	57.50 $^{a–}$c	142.77 ^{b}	22.96 $^{j–n}$
'A21'	46.50 ^{ij}	99.28 ^{g}	22.31 $^{k–p}$
'A21-1'	55.00 $^{c–e}$	94.40 ^{hi}	30.67 ^{b}
'A22'	55.50 $^{b–d}$	134.44 ^{c}	21.30 $^{m–q}$
'A26'	49.5 $^{f–i}$	134.24 ^{c}	21.85 $^{l–p}$
'A28'	42.00 ^{kl}	70.42 ^{n}	15.43 ^{v}
'A29'	50.00 $^{f–i}$	97.86 ^{gh}	16.99 $^{t–u}$
'A35'	55.00 $^{c–e}$	86.68 $^{j–l}$	23.97 $^{i–l}$
'A42'	59.00 ^{ab}	88.10 ^{jk}	20.29 $^{o–s}$
'A44'	55.00 $^{c–e}$	90.54 ^{ij}	23.05 $^{j–n}$
'A45'	38.00 $^{m–o}$	72.04 ^{mn}	20.20 $^{p–s}$
'A46'	38 $^{m–o}$	135.66 ^{c}	30.12 ^{bc}
'A47'	40.00 $^{k–n}$	124.89 ^{d}	19.01 $^{q–t}$
'A48'	37.50 $^{m–o}$	93.99 ^{hi}	22.68 $^{k–o}$
'A53'	57.00 $^{a–c}$	101.31 ^{g}	29.02 $^{b–d}$
'A57'	36.50 ^{no}	135.86 ^{c}	29.93 ^{bc}
'Fuji'	47.5 ^{hi}	147.45 ^{a}	27.27 $^{d–g}$
'Granny Smith'	53.00 $^{d–f}$	84.03 ^{kl}	25.25 $^{g–j}$
'Red Chief'	53.00 $^{d–f}$	93.59 ^{hi}	18.82 $^{r–t}$
'Royal Gala'	48.50 $^{a–i}$	82.81 ^{l}	24.33 $^{h–k}$
'Starking Delicious'	59.50 ^{a}	116.55 ^{e}	21.95 $^{k–p}$

Levels not connected by the same letters are significantly different ($p < 0.05$)

Trolox/100 g, 17.1 to 20.7 mmol Trolox/100 g, and 364.8 to 492.3 mmol Trolox/100 g, respectively. These studies highlight the impact of measurement methods on the results and suggest that total antioxidant capacity in apple leaves can exhibit significant variation depending on the apple cultivar, climatic conditions, and cultivation practices. Our findings are generally consistent with the results obtained by Ben-Othman et al. [9]. Total phenolics ranged from 70.42 ('A28') to 147.45 mg GAE/100 g ('Granny Smith'). In similar studies conducted on apple leaves, total phenolic content ranged from 98.81 to 163.35 mg GAE/100 g in Lithuania [49], from 12.50 to 30.38 mg GAE/100 g in India [65], and varied between 257.8 and 320.4 mg GAE/100 g in Poland [70], while another study in Poland reported values ranging from 92.30 to 191.70 mg GAE/100 g [83]. In the Czech Republic, total phenolics ranged from 54.68 to 106.81 mg GAE/100 g [2]. The findings of our study are consistent with the results obtained by Wojdyło et al. [83]. Total flavonoids ranged from 15.43 ('A28') to 38.66 mg QE/100 g ('A16'). The total flavonoid content in apple leaves has been reported in varying ranges across different studies. Liaudanskas et al. [49] determined the total flavonoid content in apple leaves to range from 21.59 mg QE/100 g to 45.02 mg QE/100 g. In contrast, Rana et al. [65] reported this value to range from 10.95 mg QE/100 g to 20.92 mg QE/100 g. Ben-Othman et al. [9] found the flavonoid content in apple leaves to range between 7.47 mg QE/100 g and 12.23 mg QE/100 g. Finally, Wojdyło et al. [83] reported the flavonoid content in apple leaves to range from 16.32 mg QE/100 g to 34.55 mg QE/100 g. Our findings for total flavonoids are generally similar to those of Wojdyło et al. [83]. The detected flavonoid contents in these studies highlight the significant impact of genetic diversity, cultivation conditions, and climatic factors on the concentration of flavonoid compounds in apple leaves.

From a health perspective, these compounds collectively offer remarkable benefits. Antioxidants play a crucial role in neutralizing free radicals, thereby reducing oxidative stress, which is a key factor in the onset of chronic diseases such as cardiovascular disorders, diabetes, and cancer [68]. Phenolics and flavonoids, as primary antioxidants, contribute significantly to these protective effects due to their strong free radical scavenging capacity and anti-inflammatory properties [52, 89, 91].

Genotypes and cultivars with high antioxidant capacity, high phenol, and high flavonoid in their leaves indicate the potential of these leaves as a valuable natural source of bioactive compounds. These leaves could be incorporated into functional foods, dietary supplements, or nutraceuticals to contribute to human health. Further research into the bioavailability, clinical efficacy, and therapeutic applications of these compounds could open up new opportunities for the use of apple leaves in health-focused products and therapies.

Aluminum ranged from 0.16 ('A8') to 0.99 mg/kg ('A47') (Table 3). Although aluminum is not essential for human health, excessive exposure has been linked to neurotoxicity and other health concerns. Therefore, monitoring aluminum intake is important, as it could potentially accumulate in the body over time [59]. Calcium varied between 134.81 ('A35') and 753.58 mg/kg ('A21-1'). Calcium is a crucial mineral for maintaining healthy bones and teeth, as well as supporting muscle function and nerve transmission. Adequate calcium intake is essential for bone health and preventing conditions like osteoporosis [74]. Potassium changed between 30.03 ('A5') and

Table 3 Detailed TUKEY results of statistical descriptive parameters for mineral compositions used to study apple genotypes and cultivars (mg/kg)

Genotypes/Cultivars	Aluminum	Calcium	Potassium	Magnesium	Manganese	Sodium	Nickel	Phosphorus	Lead	Sulfur
'A1'	0.84 ^{bc}	209.07 ^{p}	42.55 ^{o}	4.69 $^{m–o}$	0.033 $^{e–g}$	7.48 $^{k–n}$	0.18 $^{e–j}$	46.12st	0.46 $^{e–g}$	101.84 ^{l}
'A2'	0.49 ^{m}	244.97 ^{o}	48.31 $^{k–m}$	6.16 $^{i–k}$	0.050 ^{de}	7.27 $^{l–n}$	0.22 $^{d–f}$	65.27 $^{n–p}$	0.59 ^{cd}	123.42 ^{hi}
'A4'	0.57 $^{k–m}$	601.52 ^{b}	43.56 ^{no}	9.38 ^{fg}	0.047 ^{de}	11.39 ^{cd}	0.20 $^{d–h}$	61.90 ^{p}	0.10 ^{qr}	113.36 ^{jk}
'A5'	0.84 ^{bc}	438.47 ^{e}	30.03 ^{t}	6.21 $^{i–k}$	0.063 $^{a–d}$	9.11 ^{fg}	0.16 $^{f–l}$	61.58 ^{pq}	0.33 $^{i–l}$	113.03 ^{jk}
'A6'	0.75 $^{d–f}$	587.82 ^{c}	126.78 ^{b}	14.82 ^{c}	0.053 $^{c–e}$	13.12 ^{b}	0.29 ^{ab}	318.09 ^{b}	0.01 ^{s}	149.25 ^{cd}
'A8'	0.16 ^{o}	346.28 ^{h}	44.39 $^{m–o}$	6.47 $^{h–k}$	0.033 $^{e–g}$	6.87 $^{m–o}$	0.11 ^{lm}	75.51 ^{k}	0.43 $^{f–h}$	116.87 ^{j}
'A12'	0.56 ^{lm}	241.81 ^{o}	37.16 $^{r–s}$	6.06 $^{i–l}$	0.060 $^{b–d}$	5.99 ^{o}	0.28 $^{a–c}$	48.88 ^{s}	0.16 $^{o–q}$	126.04 ^{gh}
'A13'	0.71 $^{e–g}$	218.24 ^{p}	58.87 ^{h}	5.75 $^{j–m}$	0.043 $^{d–f}$	6.90 $^{m–o}$	0.23 $^{c–e}$	83.40 ^{j}	0.39 $^{g–i}$	115.44 ^{jk}
'A14'	0.78 $^{c–e}$	385.16 ^{g}	50.90 $^{j–l}$	9.25 ^{fg}	0.050 ^{de}	6.98 $^{m–o}$	0.19 $^{d–i}$	60.72 $^{p–r}$	0.86 ^{a}	130.89 ^{g}
'A15'	0.68 $^{f–h}$	567.42 ^{d}	50.20 $^{j–l}$	15.11 ^{c}	0.063 $^{a–d}$	8.70 $^{g–j}$	0.21 $^{d–g}$	76.11 ^{k}	0.49 ^{ef}	130.42 ^{g}
'A16'	0.54 ^{lm}	249.52 ^{no}	66.76 ^{fg}	5.51 $^{k–n}$	0.033 $^{e–g}$	7.18 ^{mn}	0.24 $^{b–d}$	70.17 $^{l–n}$	0.07 ^{rs}	145.32 ^{de}
'A17'	0.82 $^{b–d}$	397.48 ^{f}	33.16st	7.38 ^{h}	0.053 $^{c–e}$	7.42 $^{l–n}$	0.12 $^{j–m}$	67.31 $^{m–o}$	0.64 ^{bc}	141.63 ^{ef}
'A18'	0.67 $^{f–i}$	396.23 ^{f}	51.65 ^{jk}	2.91 ^{q}	0.060 $^{b–d}$	13.03 ^{b}	0.21 $^{d–f}$	106.95 ^{g}	0.33 $^{i–l}$	137.68 ^{f}
'A21'	0.84 ^{bc}	378.75 ^{g}	51.84 ^{jk}	5.04 $^{l–o}$	0.043 $^{d–f}$	7.39 $^{l–n}$	0.14 $^{h–m}$	64.71 ^{op}	0.12 $^{p–r}$	229.29 ^{a}
'A21-1'	0.64 $^{g–k}$	753.58 ^{a}	139.15 ^{a}	19.37 ^{a}	0.033 $^{e–g}$	18.11 ^{a}	0.13 $^{i–m}$	348.35 ^{a}	0.67 ^{b}	151.43 ^{cd}
'A22'	0.68 $^{f–h}$	750.32 ^{a}	101.79 ^{c}	12.5 ^{d}	0.033 $^{e–g}$	12.44 ^{bc}	0.17 $^{f–k}$	198.64 ^{e}	0.11 ^{qr}	140.49 ^{ef}
'A26'	0.67 $^{f–i}$	323.38 ^{ij}	40.81 $^{a–q}$	6.33 $^{h–k}$	0.080 ^{ab}	9.30 $^{e–g}$	0.17 $^{f–k}$	86.62 ^{j}	0.19 $^{n–p}$	150.46 ^{cd}
'A28'	0.59 $^{i–l}$	440.61 ^{e}	52.74 ^{ij}	10.92 ^{e}	0.053 $^{c–e}$	9.85 ^{ef}	0.19 $^{d–i}$	100.94 ^{h}	0.34 $^{i–k}$	162.63 ^{b}
'A29'	0.61 $^{h–l}$	256.09 ^{mn}	57.77 ^{h}	6.75 $^{h–j}$	0.023 ^{fg}	9.03 $^{f–h}$	0.12 $^{j–m}$	50.62 ^{s}	0.17 $^{o–q}$	110.62 ^{k}
'A35'	0.86 ^{b}c	134.81 ^{r}	41.41 ^{op}	2.32 ^{q}	0.023 ^{fg}	3.37 ^{p}	0.28 $^{a–c}$	47.33st	0.08 ^{rs}	82.27 ^{n}
'A42'	0.38 ^{n}	286.06 ^{l}	64.27 ^{g}	8.98 ^{g}	0.053 $^{c–e}$	7.97 $^{h–m}$	0.17 $^{f–k}$	85.42 ^{j}	0.58 ^{cd}	140.75 ^{ef}
'A44'	0.72 $^{e–g}$	332.60 ^{i}	56.48 ^{hi}	4.48 ^{no}	0.050 ^{de}	8.61 $^{g–k}$	0.21 $^{d–g}$	74.09 ^{kl}	0.31 $^{j–l}$	128.02 ^{gh}
'A45'	0.58 $^{j–l}$	250.51 $^{m–o}$	35.79 ^{rs}	6.09 $^{i–l}$	0.073 $^{a–c}$	9.11 ^{fg}	0.30 ^{a}	71.87 $^{k–m}$	0.57 ^{cd}	93.42 ^{m}
'A46'	0.68 $^{f–h}$	315.88 ^{jk}	38.35 $^{p–r}$	10.18 ^{ef}	0.023 ^{fg}	6.72 ^{no}	0.28 $^{a–c}$	56.18 ^{r}	0.11 ^{qr}	118.19 ^{ij}
'A47'	0.99 ^{a}	287.73 ^{l}	34.91 ^{rs}	7.34 ^{h}	0.083 ^{a}	7.86 $^{i–m}$	0.17 $^{f–k}$	65.83 $^{n–p}$	0.40 $^{g–i}$	118.62 ^{ij}
'A48'	0.58 $^{j–l}$	240.71 ^{o}	47.52 ^{ln}	5.68 $^{k–m}$	0.033 $^{e–g}$	7.97 $^{h–m}$	0.19 $^{d–h}$	71.52 $^{k–m}$	0.27 $^{k–m}$	113.71 ^{jk}
'A53'	0.62 $^{h–l}$	331.95 ^{i}	40.65 $^{o–q}$	7.09 ^{hi}	0.053 $^{c–e}$	6.67 ^{no}	0.17 $^{f–k}$	56.44 ^{qr}	0.44 $^{f–h}$	127.52 ^{gh}
'A57'	0.58 $^{j–l}$	443.77 ^{e}	43.37 ^{o}	16.76 ^{b}	0.080 ^{ab}	10.26 ^{de}	0.15 $^{g–l}$	63.93 ^{op}	0.53 ^{de}	131.37 ^{g}
'Fuji'	0.81 $^{b–d}$	260.99 ^{m}	70.77 ^{ef}	3.33 ^{pq}	0.023 ^{fg}	7.76 $^{j–n}$	0.17 $^{f–k}$	206.54 ^{d}	0.45 $^{f–h}$	140.15 ^{ef}
'Granny Smith'	0.86 ^{bc}	211.68 ^{p}	37.70 ^{pr}	4.42 ^{o}	0.020 ^{g}	5.94 ^{o}	0.10 ^{m}	42.84 ^{t}	0.21 $^{m–o}$	94.36 ^{m}
'Red Chief'	0.65 $^{g–j}$	306.72 ^{k}	57.83 ^{h}	3.22 ^{pq}	0.043 $^{d–f}$	9.46 $^{e–g}$	0.20 $^{d–h}$	93.42 ^{i}	0.38 $^{h–j}$	165.45 ^{b}
'Royal Gala'	0.67 $^{f–i}$	158.14 ^{q}	74.01 ^{e}	2.57 ^{q}	0.033 $^{e–g}$	8.99 $^{f–i}$	0.13 $^{i–m}$	117.13 ^{f}	0.19 $^{n–p}$	125.58 ^{gh}
'Starking Delicious'	0.88 ^{b}	209.54 ^{p}	81.29 ^{d}	4.11 ^{op}	0.043 $^{d–f}$	8.35 $^{g–l}$	0.22 $^{d–f}$	226.21 ^{c}	0.26 $^{l–n}$	151.76 ^{c}

Levels not connected by the same letters are significantly different ($p < 0.05$)

139.15 mg/kg ('A21-1'). Potassium plays a key role in maintaining proper heart function, muscle contractions, and fluid balance [76]. Low potassium levels can lead to muscle weakness, arrhythmias, and other cardiovascular issues, making it essential for overall health [79]. Magnesium ranged from 2.32 ('A35') to 19.37 mg/kg ('A21-1'). Magnesium is involved in hundreds of biochemical reactions in the body, including energy production and muscle function. Insufficient magnesium intake is associated with muscle cramps, fatigue, and an increased risk of cardiovascular disease [7]. Manganese varied between 0.020 ('Granny Smith') and 0.083 mg/kg ('A47'). Manganese is essential for bone formation, metabolism, and antioxidant defense. Although the body requires only small amounts, deficiencies or excess intake of manganese can lead to health issues such as bone and joint problems or neurological effects [20]. Sodium changed between 3.37 ('A35') and 18.11 mg/kg ('A21-1'). Sodium is vital for fluid balance, nerve function, and muscle contractions [64]. However, excessive sodium intake can lead to hypertension, heart disease, and stroke, making it important to maintain a balanced intake [24]. Nickel ranged from 0.10 ('Granny Smith') to 0.30 mg/kg ('A45'). Nickel, though not essential in large amounts, plays a role in certain enzyme functions and metabolic processes. However, excess nickel can be toxic, particularly for individuals with allergies or sensitivities to the metal [88]. Phosphorus varied between 42.84 ('Granny Smith') and 348.35 mg/kg ('A21-1'). Phosphorus is vital for bone health, energy production, and cellular function [10]. A proper balance of phosphorus is necessary for maintaining bone density and supporting various metabolic processes, though excessive phosphorus intake can negatively affect kidney function [67]. Lead changed between

0.01 ('A6') and 0.86 mg/kg ('A14'). Lead is a toxic metal with no known beneficial role in human health. Even low levels of lead exposure can lead to serious health issues, including cognitive impairments and developmental delays, particularly in children, underscoring the importance of minimizing exposure [6]. Sulfur ranged from 82.27 ('A35') to 229.29 mg/kg ('A21'). Sulfur is crucial for protein synthesis, enzyme activity, and detoxification processes. It also plays a key role in the formation of connective tissues. Adequate sulfur levels support overall metabolic health and may help reduce the risk of certain chronic conditions [28].

Our findings partially align with the results of previous studies on the mineral composition of apple leaves [5, 50, 56, 71, 85–87]. The observed differences are thought to arise from ecological variations, the genetic diversity of the studied genotypes [75], and agricultural practices [27].

Correlation matrix analysis (CMA)

The Pearson correlation coefficient (*r*) is a statistical method used to measure the linear relationship between two variables. This coefficient takes a value between − 1 and + 1. A positive value indicates a positive relationship between the two variables, meaning that as one variable increases, the other also increases. A negative value, on the other hand, indicates an inverse relationship, meaning that as one variable increases, the other decreases. If the coefficient is close to 0, it suggests that there is no linear relationship between the two variables [72]. The calculation of the Pearson correlation coefficient involves the product of the deviations of each variable from their respective means, divided by the product of their standard deviations, which normalizes the value [63]. As a result, the Pearson correlation coefficient is unitless, unaffected by the measurement scales of the variables, and is applies to quantitative data [48]. Simple correlations between examined traits in the studied apple genotypes and cultivars are presented in Fig. 2.

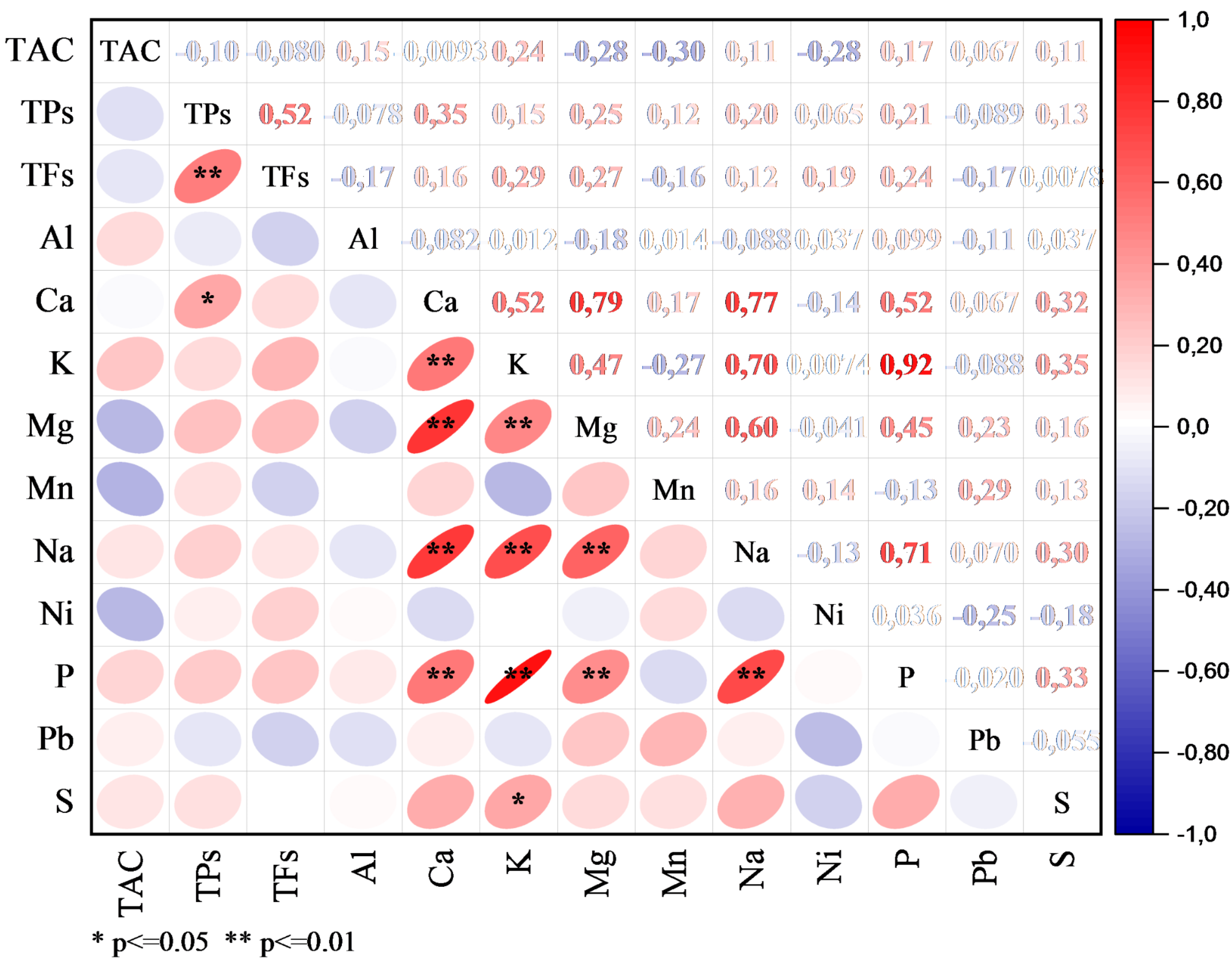

Fig. 2 Simple correlations between examined traits in the studied apple genotypes and cultivars. For an explanation of the abbreviations, see Table 1

Significant positive correlations were observed between total phenolics and total flavonoids ($r = 0.52^{**}$), as well as calcium ($r = 0.35^{*}$), indicating that higher levels of phenolic compounds may be associated with elevated flavonoid and calcium contents. Similarly, calcium showed statistically significant positive correlations with potassium ($r = 0.52^{**}$), magnesium ($r = 0.79^{**}$), sodium ($r = 0.77^{**}$), and phosphorus ($r = 0.52^{**}$), suggesting interconnected roles of these minerals in the physiological processes of the plant. Potassium exhibited significant positive correlations with magnesium ($r = 0.47^{**}$), sodium ($r = 0.70^{**}$), phosphorus ($r = 0.92^{**}$), and sulfur ($r = 0.35^{*}$), indicating its close association with the transport and accumulation of these nutrients. Magnesium was also positively and significantly correlated with sodium ($r = 0.60^{**}$) and phosphorus ($r = 0.45^{**}$). Lastly, a strong positive correlation was observed between sodium and phosphorus ($r = 0.71^{**}$), highlighting their potential co-regulation in plant metabolic pathways.

From a human health perspective, these findings emphasize the nutritional and therapeutic potential of apple leaves, as the observed positive correlations between phenolic compounds and essential minerals suggest that certain genotypes could serve as rich sources of both bioactive compounds and vital nutrients [61]. Phenolics and flavonoids are well-documented antioxidants with anti-inflammatory and anticancer properties, contributing to the neutralization of free radicals and the prevention of oxidative stress-related diseases [22]. Minerals such as calcium, magnesium, potassium, and phosphorus are critical for bone health, cardiovascular function, and cellular metabolism, while sodium, despite its potential health risks in excess, plays a vital role in maintaining fluid balance and nerve function [4, 84].

These correlations also highlight the importance of selecting appropriate genotypes to maximize the health-promoting properties of apple leaves. The co-occurrence of phenolic compounds and minerals may enhance their bioavailability and synergistic effects, offering an avenue for the development of nutraceuticals and functional foods. Further research on the bioaccessibility and metabolic pathways of these compounds in humans is necessary to fully exploit their health benefits.

Multiple regression analysis (MRA)

In this study, multiple regression analysis (MRA) was performed to model the relationship between a dependent variable and multiple independent variables, with the "stepwise" method employed to determine the inclusion of independent variables in the model. The stepwise approach sequentially added or removed independent variables based on their statistical significance, guided by their p-values, with variables having $p \leq 0.05$ being retained in the model. This method provides an efficient framework for identifying the most significant predictors and optimizing model accuracy [17]. The correlation coefficient (r) was used to indicate the strength and direction of the linear relationship between independent and dependent variables, while the coefficient of determination (r^2) measured the proportion of variance in the dependent variable explained by the independent variables. Standardized beta coefficients (β) were calculated to evaluate the relative impact of each independent variable. To test the statistical significance of individual predictors, t-tests were conducted, and the corresponding p-values determined their inclusion in the model [13]. The application of the stepwise method ensured that the model included only statistically significant variables, enhancing its explanatory power and reliability. This approach, supported by established multivariate and regression analysis principles, offered a robust framework for assessing the contributions of independent variables to the dependent variable [25].

Initially, after calculating the simple correlation coefficients, total phenolics, calcium, magnesium, and potassium were considered as dependent variables, and the direct and indirect effects of the independent variables on these key traits were analyzed. The MRA results indicated that total phenolics were associated with one trait, calcium with two traits, magnesium with one trait, and potassium with two traits (Table 4).

In the study, a positive standardized beta coefficient ($\beta = 0.52$, $p < 0.00$) was observed between total phenolics

Table 4 The variables associated with biochemical and mineral composition-related traits in apple genotypes and cultivars as revealed using MRA and coefficients

Dependent character	Independent character	r	r^2	β	t-value	p-value
Total phenolics	Total flavonoids	.518[a]	0.27	0.52	3.38	**0.00***
Calcium	Magnesium	.793[a]	0.63	0.52	4.66	**0.00***
	Sodium	.874[b]	0.76	0.46	4.15	**0.00***
Magnesium	Total antioxidant capacity	.838[a]	0.70	-0.27	-2.73	0.01
Potassium	Phosphorus	.924[a]	0.85	0.90	13.96	**0.00***
	Manganese	.936[b]	0.88	-0.15	-2.31	0.03

* The bold values are supported by the statistically significant correlations from the correlation matrix analysis

r Correlation coefficient, r^2 Coefficient of determination, β Standardized beta coefficients and total flavonoids. This indicates that phenolic compounds and flavonoids tend to increase together and potentially support each other. Both phenolic and flavonoid compounds exhibit strong antioxidant properties, reducing oxidative stress by neutralizing free radicals. This finding is significant for human health, as foods rich in these compounds may help prevent chronic diseases. For instance, a high intake of these compounds has been associated with reduced risks of cardiovascular diseases, cancer, and neurodegenerative disorders.

Significant positive standardized beta coefficients were also identified between calcium and magnesium ($\beta = 0.52$, $p < 0.00$) and calcium and sodium ($\beta = 0.46$, $p < 0.00$). These results suggest that calcium interacts biochemically with other minerals and that their regulation is interconnected. Magnesium and calcium are essential for human physiology, playing critical roles in muscle and nerve function, bone health, and energy metabolism. The relationship with sodium highlights the importance of cellular balance and electrolyte regulation. A balanced intake of these minerals can help prevent health problems associated with mineral imbalances, such as hypertension.

Although a negative standardized beta coefficient was found between magnesium and total antioxidant capacity ($\beta = -0.27$, $p < 0.01$), the relationship was statistically significant. This suggests that an increase in magnesium levels may be associated with a decrease in total antioxidant capacity. However, the underlying mechanism of this relationship requires further investigation. While magnesium is known to play a role in energy production at the cellular level, its potential negative impact on antioxidant capacity may depend on dietary composition and the presence of other nutrients.

A positive standardized beta coefficient was observed between potassium and phosphorus ($\beta = 0.90$, $p < 0.00$), while a negative coefficient was found between potassium and manganese ($\beta = -0.15$, $p < 0.03$). The strong positive relationship with phosphorus indicates the role of potassium in phosphorus metabolism. Potassium is a key electrolyte in cellular functions, including muscle contraction, nerve transmission, and fluid balance, while phosphorus is critical for energy metabolism, particularly in ATP production. These findings highlight the functional synergy between these two minerals, which is essential for human health. On the other hand, the negative relationship between potassium and manganese suggests potential antagonistic interactions. Manganese is an important trace element involved in enzymatic activities and antioxidant systems, and potassium intake may influence its bioavailability. The bold values are supported by the statistically significant correlations from the correlation matrix analysis.

These findings emphasize the interrelations between total phenolic compounds, minerals, and antioxidant properties, which should be considered both for the improvement of plant characteristics and the optimization of food composition for human health. Balanced mineral intake and sufficient consumption of bioactive compounds can have positive effects on human health.

Principal component analysis (PCA)

In this study, principal component analysis (PCA) was applied to identify the underlying patterns in the dataset and to better understand the relationships among variables. PCA, a widely used multivariate statistical technique, reduces the variables in a dataset to a smaller number of components that best explain the total variance [25, 36]. During the analysis, Kaiser Normalization was employed to balance the contribution of components to the variance and to improve comparisons among them [38]. To enhance the interpretability of the obtained components, the Varimax rotation method was utilized. This rotation method optimizes the component structure by ensuring that each component is associated with a limited number of variables with high loadings, making the results clearer and more interpretable [37]. The combined application of these methods improved the reliability and interpretability of the analysis results. The eigenvalue 1.00 criterion is a widely used method in factor analysis and principal component analysis. According to Kaiser's criterion, factors with an eigenvalue greater than 1.00 are included in the analysis because they explain as much variance as a single variable, while factors with an eigenvalue less than 1.00 are excluded due to insufficient explanatory power. This approach is employed to identify meaningful factors in the data and to select components that account for a significant portion of the total variance [1].

In this study, the first 5 principal components accounted for 74.67% of the total variation in the dataset. The degree of significance for the components was as follows: PC1 and PC3 were significant at $^{**}p < 0.01$, while PC2 was significant at $^{*}p < 0.05$ (Table 5).

The analysis revealed that PC1 accounted for 30.56% of the total variance, with the highest contributions from sodium (0.44), calcium (0.42), potassium (0.42), phosphorus (0.42), and magnesium (0.38). These elements are essential macronutrients, playing a critical role in physiological and biochemical processes, which may explain their strong representation in PC1. PC2 explained 14.16% of the total variance and was primarily influenced by manganese (0.48), magnesium (0.31), and total antioxidant capacity (-0.53). The negative association of total antioxidant capacity with PC2 suggests an inverse relationship with the elements contributing positively, potentially highlighting distinct metabolic

Table 5 Eigenvalues of the principal component axes from the PCA of the examined traits in the studied apple genotypes and cultivars

Eigenvectors	**Components**				
	1	**2**	**3**	**4**	**5**
Total antioxidant capacity	0.04	**-0.53**	0.13	-0.12	0.25
Total phenolics	0.20	0.25	**-0.32**	-0.11	**0.58**
Total flavonoids	0.18	0.14	**-0.51**	-0.26	0.21
Aluminum	-0.04	-0.24	0.01	**0.65**	0.20
Calcium	**0.42**	0.13	0.14	-0.01	0.02
Potassium	**0.42**	-0.28	-0.12	0.02	-0.22
Magnesium	**0.38**	**0.31**	0.12	-0.07	-0.20
Manganese	0.03	**0.48**	**0.30**	**0.40**	0.21
Sodium	**0.44**	-0.02	0.15	0.05	-0.12
Nickel	-0.03	0.22	**-0.42**	**0.44**	**-0.33**
Phosphorus	**0.42**	-0.23	-0.08	0.14	-0.18
Lead	0.02	0.17	**0.51**	-0.22	-0.02
Sulfur	0.22	-0.13	0.13	0.22	**0.48**
Eigenvalue	*3.97*	*1.84*	*1.70*	*1.19*	*1.01*
Component degree of significance	**	*	**	*	*
Variance (%)	*30.56*	*14.16*	*13.08*	*9.13*	*7.74*
∑ variance (%)	*30.56*	*44.72*	*57.80*	*66.93*	*74.67*

Bold values indicate the characteristics that most influence each PC (Eigenvalues ≥ 0.30). Component degree of significance: *$p < 0.05$, **$p < 0.01$

or minor environmental interactions. PC3 represented 13.08% of the total variance, with lead (0.51), manganese (0.30), total phenolics (-0.32), nickel (-0.42), and total flavonoids (-0.51) as the main contributors. The significant positive loading of lead and negative loadings of phenolics and flavonoids in PC3 may indicate the influence of heavy metal stress on secondary metabolite production. Altogether, the first three principal components cumulatively explained 57.80% of the total variance, underscoring their importance in capturing the primary patterns of variation within the dataset and reflecting complex interactions between nutrient elements, heavy metals, and bioactive compounds.

The PCA biplot facilitates the visualization of the components obtained from PCA, thereby making the complexity of the dataset more comprehensible [23]. Variables are represented as vectors in the biplot, and the length of each vector indicates the contribution of the respective variable to the explained variance in the analysis. The angle between vectors represents the correlation between variables; small angles are considered to represent a positive correlation, while larger angles indicate a negative correlation. Observations are represented as points, and the proximity of points indicates similarity in characteristics, while greater distances indicate differences [12]. Since the biplot visualizes only two components, it reflects only a portion of the total variance; therefore, the proportion of explained variance should be carefully specified, and interpretations should consider this limitation. This visualization aids in simplifying multidimensional datasets and provides a clearer understanding of complex relationships [45].

The PC1/PC2 distribution biplot, illustrating the relationships among the studied apple genotypes, cultivars, and variables, is presented in detail in Fig. 3. Therefore, the first two components, PC1 and PC2, explain 30.56% and 14.16% of the total variation in the dataset, respectively. Together, these two components account for 44.72% of the variation cumulatively, indicating that almost half of the variability in the data can be effectively captured and summarized within these two dimensions.

The biplot analysis reveals distinct clustering patterns among the genotypes and cultivars, which can provide valuable insights into the biochemical and nutritional characteristics that may impact human health. In Cluster 1, genotypes 'A2', 'A5', 'A8', 'A12', 'A14', 'A26', 'A45', 'A46', 'A47', and 'A48' were associated with the Nickel variable. Nickel plays a role in several biological processes, including enzymatic activity and cellular metabolism, but excessive exposure to nickel can be detrimental to human health. Understanding the distribution of genotypes concerning Nickel could help in identifying varieties that either accumulate or tolerate this element, providing a foundation for selecting cultivars that contribute to balanced nutrition while minimizing the risks of heavy metal accumulation in the human diet.

In Cluster 2, genotypes such as 'A4', 'A6', 'A15', 'A16', 'A28', and 'A57' were linked to a range of elements and compounds, including calcium, lead, magnesium, manganese, total flavonoids, and total phenolics. Calcium and magnesium are essential for bone health and cellular function, while manganese and phenolic compounds are known for their antioxidant properties. On the other hand, lead is a toxic heavy metal, and its presence, even in small amounts, can pose significant health risks. This cluster's diversity in biochemical traits may suggest that these genotypes are rich in beneficial nutrients, but caution should be taken regarding the presence of lead. It is crucial to ensure that these genotypes do not accumulate excessive lead, which could be harmful to human health.

Cluster 3, which includes genotypes 'A1', 'A13', 'A17', 'A29', 'A35', 'A42', 'A44', and 'A53', as well as cultivars 'Granny Smith', 'Red Chief', and 'Royal Gala', exhibited a strong association with aluminum. Aluminum is an element that, when accumulated in excess, has been linked to various health concerns, including neurological and cognitive disorders. However, some genotypes may have developed mechanisms for aluminum tolerance, which could be beneficial for cultivation in soils with high aluminum content. These genotypes may be of particular interest for breeding programs that aim to improve crop resilience while ensuring that aluminum accumulation does not pose a risk to human health.

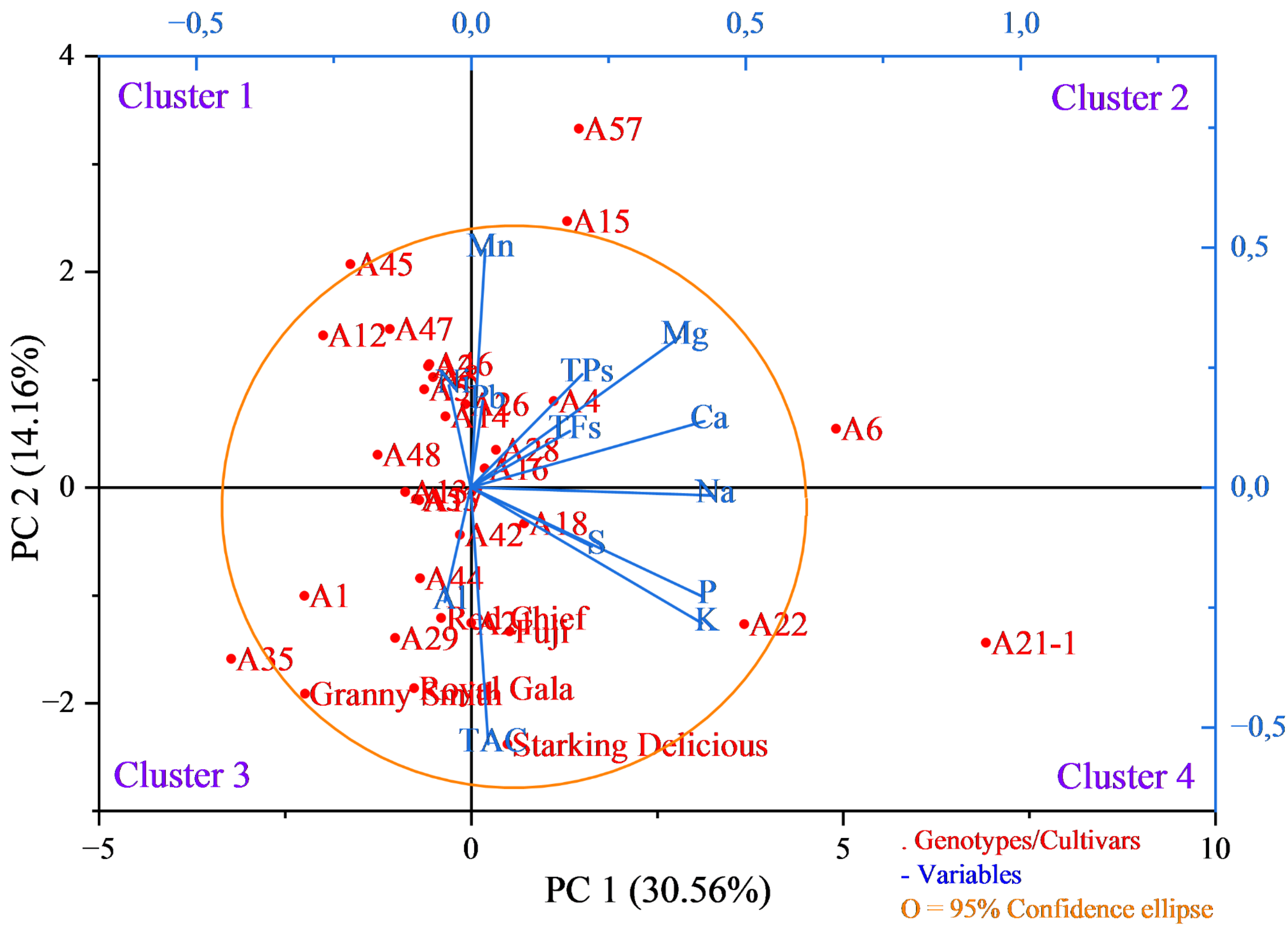

Fig. 3 PC1/PC2 distribution biplot of studied apple genotypes, cultivars, and variables. For an explanation of the abbreviations, see Table 1

Cluster 4 contained genotypes 'A18', 'A21', 'A21-1', and 'A22', along with cultivars 'Fuji' and 'Starking Delicious', which were primarily associated with variables related to mineral composition and antioxidant capacity, including potassium, phosphorus, sulfur, sodium, and total antioxidant capacity. Potassium, phosphorus, and sulfur are essential nutrients that play critical roles in maintaining fluid balance, energy production, and cellular function. Sodium, while necessary in small quantities, can be harmful when consumed in excess, leading to cardiovascular diseases and hypertension. Antioxidants, such as those found in these genotypes, are vital for neutralizing free radicals and protecting cells from oxidative stress, thereby supporting overall health and reducing the risk of chronic diseases such as cancer and heart disease.

Notably, the genotypes 'A6', 'A15', 'A21-1', 'A35', 'A45', and 'A57' were found to fall outside the 95% confidence ellipse, indicating that they possess unique biochemical characteristics that differentiate them from the other genotypes. This deviation could indicate novel genetic traits or metabolic pathways that may offer new opportunities for developing cultivars with enhanced nutritional profiles or health benefits. These genotypes warrant further investigation, as they may have the potential to provide health-promoting compounds or better nutrient utilization, benefiting human health through improved dietary intake.

A 95% confidence ellipse is an estimate that indicates the range within which the true value of a statistical parameter is expected to lie with 95% probability, based on the sample data. Values that fall outside this range are considered statistically "outliers" or "excluded". If an observation lies outside the 95% confidence ellipse, it suggests that the observation deviates significantly from the general trend and is likely to exhibit a noteworthy difference or characteristic [29, 35, 53].

In conclusion, the clustering analysis provides valuable insights into the relationship between genotype and biochemical traits in apple genotypes and cultivars, with implications for human health. While many of the genotypes show promise in terms of mineral composition and antioxidant capacity, careful consideration must be given to the presence of potentially harmful elements such as lead and aluminum. These findings underscore the importance of selecting genotypes that not only offer nutritional benefits but also minimize health risks. Future research should focus on further characterizing the genetic mechanisms underlying these traits and assessing their impact on human health, especially in terms of bioavailability, antioxidant capacity, and the accumulation of potentially harmful elements.

Heat map analysis (HMA)

Ward's method, a hierarchical clustering technique, minimizes the total within-cluster variance at each step of the clustering process [81]. This is achieved by merging clusters in a way that results in the smallest possible increase in the total sum of squared deviations from the cluster centroids. It is particularly effective in producing compact, spherical clusters, making it well-suited for applications where clear group separation is desired [33].

When combined with heat map analysis (HMA) based on Euclidean distance, this method becomes a powerful tool for visualizing and interpreting clustering results. Euclidean distance measures the straight-line distance between data points in a multidimensional space, providing a quantitative basis for grouping similar observations. The integration of HMA allows the clustered data to be represented as a color-coded matrix, where the rows and columns correspond to samples and variables, respectively. The color intensity reflects the magnitude of the data values, providing a clear visual summary of patterns, relationships, and group memberships. This approach enhances interpretability by highlighting similarities and differences across clusters, making it widely used in various fields such as genomics, metabolomics, and environmental studies [82].

First, the variables were divided into two main groups: A and B. Subsequently, each group was further subdivided into two subgroups: A1, A2, and B1, B2. In subgroup A1, the variables calcium, phosphorus, sulfur, total phenolics, total flavonoids, potassium, and total antioxidant capacity were included, while subgroup A2 contained the variables sodium and magnesium. Subgroup B1 included the variables lead, nickel, and aluminum, while subgroup B2 only contained the variable manganese.

Similarly, the genotypes and cultivars were initially divided into two main groups: C and D. These groups were then further subdivided into two subgroups each: C1, C2, and D1, D2. In subgroup C1, the genotypes 'A5', 'A47', 'A26', 'A18', 'A44', and the cultivar 'Red Chief' were included, while subgroup C2 contained the genotypes 'A2', 'A13', 'A53', 'A45', 'A14', 'A17', 'A28', 'A42', 'A15', 'A57', and 'A8'. Subgroup D1 comprised the cultivars 'Royal Gala', 'Starking Delicious', and 'Fuji', along with the genotypes 'A22', 'A21-1', and 'A6', while subgroup D2 included the genotypes 'A12', 'A46', 'A16', 'A21', 'A4', 'A35', 'A48', 'A29', and 'A1', along with the cultivar 'Granny Smith'.

The division of these variables and genotypes into subgroups provides valuable insights into the potential impacts of these genotypes and cultivars on human health, particularly concerning their biochemical and nutritional characteristics (Fig. 4). Specifically, the variables in subgroup A1, such as calcium, phosphorus, sulfur, total phenolic compounds, total flavonoids, potassium, and total antioxidant capacity, suggest that the current genotypes may be beneficial for health in terms of their antioxidant and anti-inflammatory potential. On the other hand, the A2 subgroup, which includes variables like sodium and magnesium, may have different effects on cellular functions; while sodium plays a role in maintaining fluid balance, magnesium is necessary for numerous enzymatic processes.

The presence of heavy metals, such as lead, nickel, and aluminum, in subgroup B1 raises concerns about the accumulation of potentially toxic elements. However, identifying genotypes that possess mechanisms to reduce the accumulation of these metals ('A18', 'A21', 'A21-1', and 'A22') could be crucial. This would offer benefits for both human health and environmental sustainability.

On the other hand, the manganese-rich subgroup B2 suggests that the genotypes in this group may contribute to essential metabolic functions, as manganese is a vital trace mineral involved in many biochemical processes. The classification of genotypes into the C1, C2, D1, and D2 subgroups further highlights the complex biochemical relationships among these plants. The genotypes in subgroup C1, with their higher antioxidant capacity and essential mineral composition, may be of interest for breeding programs focused on enhancing the nutritional profile and disease resistance of apple varieties. In contrast, genotypes in D1, which include renowned cultivars like 'Royal Gala' and 'Fuji', could be valuable for their established quality traits but may require further investigation to assess their heavy metal content and overall suitability for health-related purposes.

In conclusion, the systematic classification of genotypes and variables into distinct subgroups allows for a deeper understanding of the biochemical and nutritional diversity within the apple genotypes studied. These findings have implications for both breeding strategies and human health, particularly regarding the potential for enhancing mineral composition and minimizing exposure to harmful elements. Future research should continue to explore the relationships between genotype, biochemical traits, and human health outcomes, focusing on the bioavailability of essential nutrients and the risk of toxic accumulation.

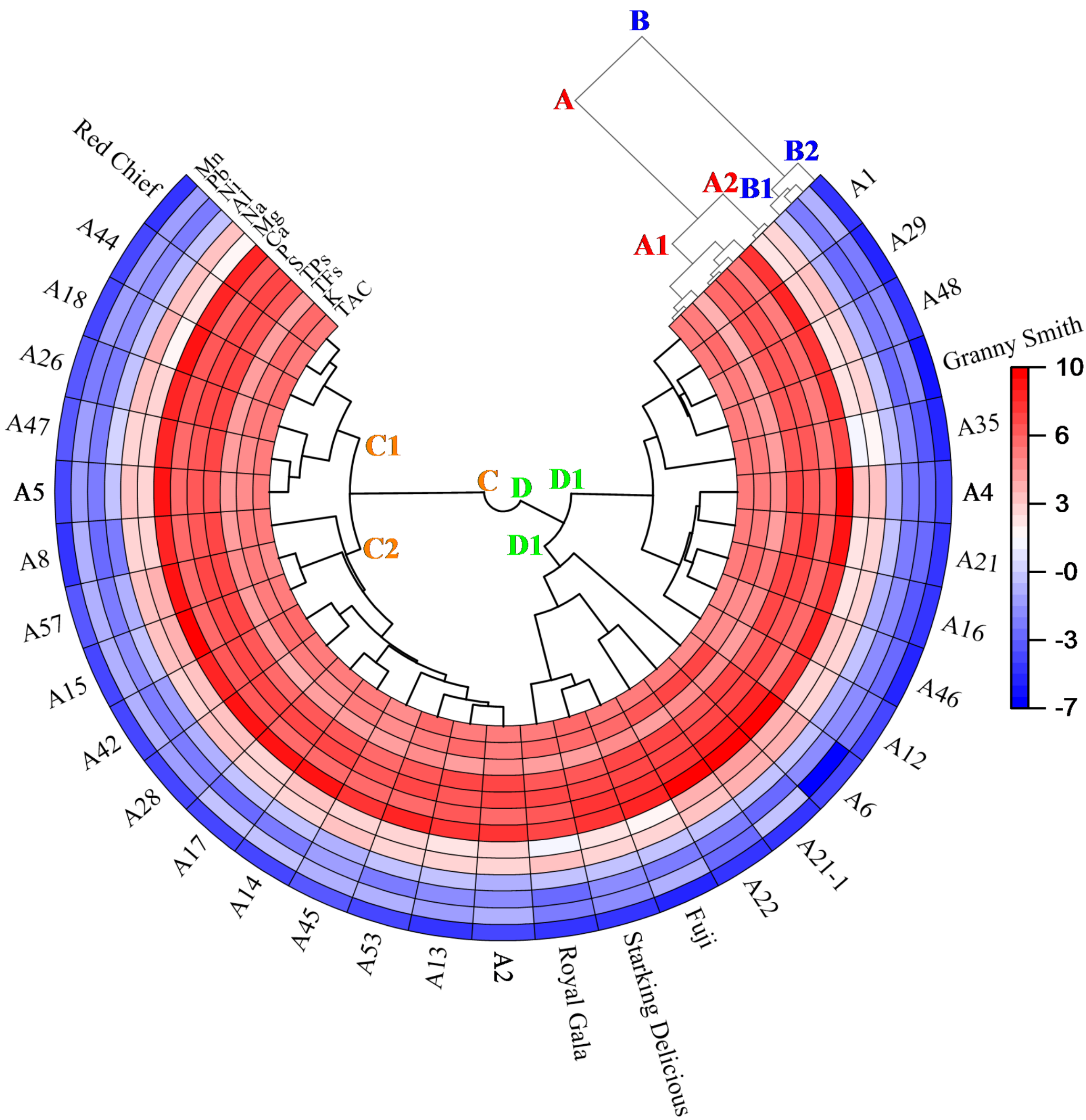

Fig. 4 Visualization of clustering patterns of apple genotypes, cultivars, and variables using heat map. For an explanation of the abbreviations, see Table 1

Conclusions

The findings of this study underscore the significant biochemical and nutritional diversity among the studied *M. kirghisorum* genotypes and *M. domestica* cultivars. Apple leaves were identified as a rich source of total phenolics, total flavonoids, and essential minerals, demonstrating high antioxidant capacity that holds the potential for various health benefits.

Genotypes such as 'A21-1', with high calcium and potassium levels, and 'A16', with superior phenolic and flavonoid content, emerged as promising candidates for functional food and nutraceutical applications. Additionally, the study highlights the importance of selecting genotypes with balanced nutrient profiles and minimal heavy metal accumulation, such as lead and aluminum, to ensure safety and efficacy in human health applications.

The use of advanced multivariate statistical techniques, including PCA and HMA, provided critical insights into the relationships among bioactive compounds, mineral contents, and antioxidant properties. These methods revealed distinct clusters of genotypes with unique biochemical traits, paving the way for targeted breeding programs to enhance the nutritional and therapeutic potential of apple leaves.

From a conservation perspective, the study contributes to the sustainable utilization of wild apple genetic resources, particularly *M. kirghisorum*, which exhibits remarkable variability in bioactive compounds and mineral composition. This not only aids in preserving biodiversity but also promotes the development of innovative applications in the food, pharmaceutical, and cosmetic industries. Future research should focus on elucidating the bioavailability and clinical efficacy of these compounds, as well as assessing the long-term health impacts of apple leaf-derived products.

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Author contributions
All authors contributed to the study's conception and design. AS, AU, EY, KUY, AG, YMK, and KT experimented. MY collected data. YT analyzed data and wrote and edited the manuscript. AK edited the manuscript. All authors approved the final manuscript.

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The data that support the findings of this study are available from the co-corresponding authors upon reasonable request.

Declarations

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The authors declare no competing interests.

Research involving human participants and, or animals
Not applicable.

Informed consent
Not applicable.

Statement specifying permissions
For this study, we acquired permission to study apple issued by the Agricultural and Forestry Ministry of the Republic 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.

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