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Research Article

Determination of pomological, morphological, antioxidant activity, biochemical content and nutritional content values of kumquat (*Citrus japonica* Thunb.) accessions using multivariate analysis methods

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Abstract

This study determined the pomological, morphological, biochemical, antioxidant, and nutritional properties of 33 naturally grown kumquat (*Citrus japonica* Thunb.) accessions using multivariate statistical methods, revealing significant variation. Fruit weight ranged from 1.85 ('K2') to 13.23 g ('K1'), and fruit width varied between 10.43 ('K2') and 25.01 mm ('K1'). Pearson's correlation (r = 0.89**) indicated heavier fruits tend to be wider. Principal component analysis (PCA) explained 83.93% of the variation, with PC1 (17.25%) influenced by leaf area (0.36), petiole length (0.38), and fruit width (0.47). The biplot showed 'K18', 'K21', and 'K24' outside the 95% confidence ellipse, indicating distinct phenotypes. Heat map analysis associated 'K32', 'K24', and 'K21' with high fruit weight, leaf area, and maturity index, while 'K8', 'K6', and 'K20' had high mineral content, particularly iron, manganese, and zinc. Overall, 'K13', 'K12', 'K15', 'K10', and 'K9' showed the highest values, making them promising for breeding. These findings highlight significant genetic diversity and breeding potential. Future studies should explore the genetic basis of these variations and agronomic performance under different environments.

Keywords: Breeding, Genetic diversity, Multivariate analysis, *Citrus japonica*, Hatay

Kumkat (*Citrus japonica* Thunb.) aksesyonlarının pomolojik, morfolojik, antioksidan aktivite, biyokimyasal içerik ve besin içeriği değerlerinin çok değişkenli analiz yöntemleri kullanılarak belirlenmesi

Öz

Bu çalışma, çok değişkenli istatistiksel yöntemler kullanarak doğal olarak yetişen 33 kumkat (*Citrus japonica* Thunb.) genotipinin pomolojik, morfolojik, biyokimyasal, antioksidan ve besinsel özelliklerini belirlemiş ve önemli varyasyonlar ortaya koymuştur. Meyve ağırlığı 1.85 ('K2') ile 13.23 g ('K1') arasında, meyve çapı ise 10.43 ('K2') ile 25.01 mm ('K1') arasında değişmiştir. Pearson korelasyonu ($r = 0.89$**) daha ağır meyvelerin genellikle daha geniş olduğunu ortaya koymuştur. Temel bileşen analizi (PCA), toplam varyasyonun %83.93'ünü açıklamış; PC1 (%17.25) özellikle yaprak alanı (0.36), yaprak sapı uzunluğu (0.38) ve meyve çapı (0.47) tarafından etkilenmiştir. Biplot analizi, 'K18', 'K21' ve 'K24' genotiplerinin %95 güven aralığının dışında yer alarak belirgin fenotipik özelliklere sahip olduğunu göstermiştir. Isı haritası analizi, 'K32', 'K24' ve 'K21' genotiplerinin yüksek meyve ağırlığı, yaprak alanı ve olgunluk indeksi ile ilişkili olduğunu, 'K8', 'K6' ve 'K20' genotiplerinin ise özellikle demir, mangan ve çinko açısından zengin mineral içeriğine sahip olduğunu ortaya koymuştur. Tüm veri setleri bir arada değerlendirildiğinde, 'K13', 'K12', 'K15', 'K10' ve 'K9' en yüksek değerlere sahip olarak ıslah çalışmaları için umut verici bulunmuştur. Bu bulgular, kumkat genotipleri arasında önemli genetik çeşitlilik ve ıslah potansiyelini ortaya koymaktadır. Gelecek çalışmalar, bu varyasyonların genetik temeli ve farklı çevresel koşullardaki tarımsal performansı üzerine odaklanmalıdır.

Anahtar Kelimeler: Islah, Genetik çeşitlilik, Çok değişkenli analiz, *Citrus japonica*, Hatay

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1. Introduction

The *Citrus* genus holds great importance within the Rutaceae family as it encompasses economically valuable species, including oranges, mandarins, lemons, limes, and grapefruits [1]. Within the Rutaceae family, there are also species such as kumquats, whose fruits are primarily utilized for medicinal and culinary purposes. The term ‘‘kumquat’’ is believed to originate from the ancient Chinese phrase “chin kan,” translating to “gold orange.” Kumquats are characterized as cold-tolerant, vigorous, and highly productive small trees that yield round or oval-shaped fruits. Initially classified under the genus *Citrus*, kumquats were reassigned to the genus *Fortunella* about a century ago, which includes six species: *Fortunella japonica*, *F. margarita*, *F. obovata*, *F. crassifolia*, *F. polyandra*, and *F. hindsii* [2]. Recently, however, the genus *Fortunella* has been renamed as *Citrus japonica* [3]. The kumquat tree originates from Southeast China, especially Taiwan, where it has been grown since the 12th century. Its cultivation has since spread to various regions, including Japan, India, the Philippines, South Asia, Argentina, Brazil, Florida, California, the Mediterranean, Australia, and South Africa [2; 4].

Kumquat trees typically grow to a height of 2.5–4.5 meters and develop a low-set crown with dense branching, glossy dark green leaves, and sharp thorns located at the base of the leaves. Similar to most citrus species, the flowers of kumquat trees are small, featuring five petals, a white coloration, and a strong, pleasant fragrance. Optimal growth and fruit production in kumquat trees require relatively high temperatures during their vegetative period. The most vigorous growth and flowering occur at temperatures ranging from 25°C to 32°C, while flowering ceases when temperatures drop below 18°C [5]. Although kumquat trees are moderately sensitive to prolonged droughts, they are also vulnerable to excessive water conditions caused by heavy rainfall or flooding. Nevertheless, they demonstrate remarkable cold tolerance in winter, enduring short-term frosts as low as −10°C [4].

A distinctive feature of kumquat fruit, unlike most *Citrus* species, is that it is consumed whole, offering a unique sensory profile with an intensely sweet initial taste followed by a slightly bitter aftertaste. The overall sensory experience of kumquat, particularly its flavor and aroma, is significantly influenced by the content and composition of essential oils present in its rind [6]. Kumquats are primarily consumed fresh or processed into various products such as liqueurs, marmalades, sauces, and juices, and they can also be preserved in sugar syrup. In addition to their culinary uses, kumquats hold a notable place in traditional medicine. The bioactive compounds isolated from the fruit rind, known for their therapeutic, antimicrobial, and antioxidant properties, have garnered increasing interest from the scientific community [7].

In recent years, kumquat has increasingly become the focus of both scientific and commercial interest. However, comprehensive literature reviews reveal that there are no studies focusing on naturally occurring kumquat accessions. In particular, no research has been conducted on basic parameters such as morphological and pomological characteristics, antioxidant activities, biochemical composition and nutrient content using multivariate analysis methods. This gap in the literature highlights an important area of deficiency, which the current study aims to address, thus enhancing its originality. The findings presented in this research not only contribute

valuable data to the scientific community but also serve as a guiding reference for future studies in this field. In this context, the results of this research offer valuable insights for both fundamental and applied sciences, providing critical contributions to the conservation and sustainable use of natural genetic resources.

2. Material and Methods

2.1. Plant Materials

This study was conducted on 33 kumquat accessions naturally grown in the Hassa district of Hatay province during the 2022-2023 period. Hassa district is under the influence of the Mediterranean climate, characterized by hot and dry summers and mild and rainy winters. Fruit and leaf materials exhibiting healthy growth were harvested from the four cardinal directions of the tree during the harvest period in January and February. The soils of Hassa district generally exhibit a light-textured structure due to their high sand content. However, since more than 50% of the soil volume consists of gravel, they are classified as gravelly sandy loam. The average organic matter content of the soils is approximately 1.7% [38].

2.2. Pomological and Morphological Characteristics

A total of 50 adult leaves and 50 mature fruits were randomly sampled and collected from each accession. Quantitative traits, including the length and width of leaves, petioles, and fruits, were measured using a digital caliper with an accuracy of 0.01 mm (Insize brand). The fruit weight was determined using a digital scale with a precision of 0.01 g (Swock brand, model JD602). The leaf samples were scanned at a resolution of 300 dpi using a scanner (Brother, DCP-L3551CDW), and the obtained images were processed using the ImageJ software [8]. The leaf area was analyzed and calculated in square centimeters (cm^2) through this software. The indices of fruit, leaf, and petiole were calculated by dividing their length measurements by their width measurements. The peel color of 50 fruits from each genotype was measured using a handheld colorimeter (WR10 FRU) at three different regions of each fruit, and the L*, a*, and b* color values were determined [9].

For pH and titratable acidity measurements, 5 g of kumquat fruits were accurately weighed, and 95 ml of distilled water was added to achieve a total weight of 100 g. The pH values of the samples were measured using a Hanna HI98190 digital pH meter to ensure accuracy and reliability. The total titratable acidity was determined by titration with a 0.1 N sodium hydroxide solution, and the results were expressed as the percentage of citric acid content in accordance with established analytical standards. To assess the total soluble solids, a Hanna HI96801 refractometer was employed. The total soluble solids values were reported in terms of Brix degrees, measured at a standardized temperature of 25 °C to ensure consistency and accuracy. The maturity index was calculated by dividing the total soluble solids by the total titratable acidity [10]. These methods provided accurate quantification of key quality parameters, offering valuable insights into the compositional characteristics of the samples.

2.3. Chemical Characteristics

2.3.1. Sample preparation: The extraction process was conducted following the protocol outlined by Xu and Chen [11]. A 10 g fruit sample was homogenized with 25 ml of absolute methanol at 1000 rpm for 5 minutes using a vortex mixer (DLAB MX-S Vortex, 100–3000 rpm). The resulting homogenates were stored at 4 °C for 12 h and then centrifuged at 6000 rpm for 20 min using a Hettich H-1650R centrifuge. The supernatants were carefully separated, and the extraction procedure for the residue was repeated three times under identical conditions. The supernatants obtained from each extraction were pooled together and stored at -20 °C until further analysis.

2.3.2. Total antioxidant activity: For the determination of standard Trolox equivalent antioxidant capacity, ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) was dissolved in acetate buffer, and potassium persulfate was prepared following the method described by Özgen et al. [12]. To ensure the stability of the mixture over an extended period, a 20 mM sodium acetate buffer solution was used in an acidic environment (pH 4.5) and diluted to achieve an absorbance of 0.700 at 734 nm. For spectrophotometric measurement, 2.97 ml of the ABTS+ solution was mixed with 30 µl of pulp-free fruit juice, which was obtained after centrifugation. The mixture was incubated for 10 minutes, and the absorbance was then measured at 734 nm. The results were expressed as micromoles of Trolox equivalents per gram of fresh weight (µmol TE/100 g FW).

2.3.3. Total phenolics: The total phenolic content of kumquat fruits was determined using the method described by Singleton and Rossi [13]. For this analysis, 2 g of supernatant was weighed, and a buffer solution composed of acetone, distilled water, and acetic acid was added. The mixture was kept in the dark for 24 hours to allow sufficient interaction. Subsequently, 0.5 ml of the extract was mixed with 0.5 ml of Folin-Ciocalteu phenol reagent and 9 ml of distilled water. After an 8-minute reaction time, a 7% sodium carbonate solution was added, and the mixture was incubated for 2 hours. Absorbance readings were then recorded at 750 nm using a spectrophotometer. Gallic acid equivalents (GAE) were used as the standard, and the results were expressed as milligrams of gallic acid equivalents per 100 grams of fresh weight (mg GAE/100 g FW).

2.3.4. Total flavonoids: The total flavonoid content was determined according to the method described by Zhishen et al. [14]. In this procedure, 1 mL of fruit juice was mixed with pure water to a final volume of 6 mL. Subsequently, 0.3 mL of a 5% sodium nitrite ($NaNO_2$) solution was added to the mixture, and it was allowed to react for 5 minutes. Then, 0.3 mL of a 10% aluminum chloride ($AlCl_3$) solution was added, and the mixture was left to react for another 5 minutes. In the final step, 2 mL of 1 M sodium hydroxide (NaOH) was added, and the total volume was adjusted to 10 mL with pure water after 1 minute. The absorbance of the resulting solution was measured at a wavelength of 510 nm. The results were expressed as milligrams of quercetin equivalent (mg QE/100 g FW) per 100 grams of fresh weight.

2.3.5. Vitamin C: The ascorbic acid content in the fruit extracts was determined using a titration method with 2,6-dichlorophenolindophenol (DPIP) as described by AOAC [15]. For this

analysis, 1 g of supernatant was combined with 40 mL of a buffer solution containing 1 g/L oxalic acid and 4 g/L anhydrous sodium acetate. The resulting mixture was titrated against a DPIP solution prepared with 295 mg/L DPIP and 100 mg/L sodium bicarbonate. A standard curve was generated using L-ascorbic acid (AnalaR, BDH, Buffalo, NY, USA) as the reference standard. The ascorbic acid content was expressed as milligrams of ascorbic acid equivalent (AAE) per liter of fresh weight (FW).

2.4. Nutritional Content Characteristics

For the analysis of nutrient elements, kumquat fruit samples were initially dried in a laboratory oven (Binder FD 56) at 65 °C until weight loss stabilized (~48 hours) to obtain dry weight (DW). During the drying process, the samples were weighed every 24 hours, and the drying was considered complete when the weight difference between consecutive measurements fell below 0.01 g. The dried samples were then ground into a fine powder to prepare them for analysis. The elemental analysis was performed using the microwave digestion method. For the digestion process, approximately 0.5 g of dry fruit sample was weighed on a precision analytical balance and placed into Teflon digestion vessels. Subsequently, 6 mL of concentrated nitric acid (HNO_3, 65%) and 2 mL of hydrogen peroxide (H_2O_2, 30%) were added to the vessels. The microwave digestion was carried out following an appropriate protocol, with the temperature ramped to 180–200 °C within 10 minutes and held constant for 20 minutes [16; 17]. After digestion, the resulting solutions were diluted with deionized water to a final volume of 50 mL and analyzed for nutrient elements using an Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) device (Agilent Technologies 5100 ICP-OES) [18; 19]. The analyzed elements included aluminum, calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium, sulfur, and zinc. The concentrations of the elements were expressed in milligrams per gram of dry weight (mg/g DW). Each genotype was processed in triplicate during the analysis. Additionally, to ensure the accuracy of the device, certified reference material (NIST standard) was used for quality control after every 10 samples [20].

2.5. Statistical Analysis

To enhance the reliability of the findings and mitigate the influence of seasonal fluctuations, the statistical evaluations were based on the average data from both years. Relevant statistical analyses were performed using JMP® Pro 17 software (SAS Institute Inc., Cary, NC, USA, [21]). The significance of differences among means was determined using Tukey's multiple comparison test at a 5% significance level ($p < 0.05$), following the methodology outlined by Savaşlı et al. [22]. Data are reported as mean ± standard deviation [23]. For multivariate statistical assessments, Origin Pro® 2024b software [24] was employed to perform correlation matrix analysis (CMA), principal component analysis (PCA), and heat map analysis (HMA). Pearson's correlation coefficient (r) was used to measure the strength and direction of associations between variables. PCA was performed with Varimax rotation and Kaiser Normalization to optimize the interpretation of component structure. A two-dimensional biplot was generated to illustrate the distribution of accessions along the first and second principal components (PC1/PC2) and their related variables. Ward's hierarchical clustering method,

based on Euclidean distance, was applied to group accessions and variables, providing a comprehensive overview of their interconnections.

3. Results and Disscussion

3.1. Variation of Accessions

To evaluate the variations of the examined traits, the minimum, maximum, mean, standard deviation (SD), and coefficient of variation (CV%) values were calculated and summarized in Table 1. In this regard, a one-way ANOVA ($p<0.05$) demonstrated significant differences among the analyzed accessions.

The highest variation was observed in leaf area (46.98), fruit weight (39.29), petiole length (36.87), maturity index (35.49), and titratable acidity (34.21). In contrast, the lowest variation was recorded in potassium (6.98), calcium (6.85), fruit peel L* (5.98) pH (3.27), and vitamin C (2.54). Notably, 16 out of 33 variables (representing 48.48% in total) had coefficients of variation (CVs) greater than 20.00%. This value exhibited considerable variation among the accessions studied [25]. Traits with more than 20.00% variation tend to be more distinct between specimens, making them reliable indicators for differentiating accessions/genotypes/cultivars [26]. Conversely, traits that span a wider quantitative range show a higher CV, indicating their greater potential for selection [27]. In contrast, morphological traits with lower CVs exhibit more consistency and can be considered stable characteristics across accessions [26].

The fruit weight ranged from 1.85 (‘K2’) to 13.23 g (‘K1’), with a mean value of 7.05 g. Fruit length varied between 21.06 (‘K10’) and 42.26 mm (‘K1’), while fruit width ranged from 10.43 (‘K2’) to 25.01 mm (‘K1’), with mean values of 29.98 and 19.28 mm, respectively. The fruit index, calculated as the length-to-width ratio, had a minimum of 1.06 (‘K32’), a maximum of 2.60 (‘K17’), and an average of 1.59. In a study conducted in Taiwan, fruit weight varied between 4.6-14.0 g, fruit length 24.4-32.6 mm, fruit width 16.9-24.0, and fruit index 1.36-1.45 [28]. The fruit peel color parameters also showed notable differences. The L* value, indicating lightness, ranged from 51.02 (‘K18’) to 65.21 (‘K11’), with a mean of 58.49. The a* value, representing the red-green spectrum, varied between 10.85 (‘K9’) and 37.83 (‘K22’), with an average of 28.29, while the b* value, associated with the yellow-blue spectrum, ranged from 56.73 (‘K18’) to 80.89 (‘K28’), with a mean of 68.45. In a study conducted in Türkiye, the fruit peel L*, a*, and b* values were reported to range between 58.14–62.54, 19.18–25.70, and 52.71–57.29, respectively [9].

Regarding leaf traits, leaf length varied from 32.95 (‘K24’) to 99.48 mm (‘K11’) (mean: 66.60 mm), while leaf width ranged between 11.00 (‘K24’) and 33.28 mm (‘K11’) (mean: 23.71 mm). The leaf index, defined as the length-to-width ratio, had a minimum of 1.93 (‘K31’), a maximum of 3.83 (‘K19’), and an average of 2.83. The leaf area exhibited considerable variation, ranging from 2.74 (‘K24’) to 22.83 cm² (‘K11’), with an average of 11.60 cm². For petiole characteristics, petiole length ranged from 2.88 (‘K21’) to 14.02 mm (‘K11’), with a mean of 8.19 mm, while petiole thickness varied between 0.86 (‘K24’) and 1.81 mm (‘K30’),

with an average of 1.45 mm. The petiole index (length-to-thickness ratio) ranged from 2.40 ('K29') to 9.10 ('K31'), with a mean value of 5.60. Due to the absence of literature on the size of kumquat leaves in previous studies, the results have been compared independently within the study itself.

The pH ranged from 3.19 ('K13') to 3.55 ('K23'), with a mean value of 3.36. The total soluble solid content was between 11.48 ('K6') and 15.84 °Brix ('K15'), averaging 13.66 °Brix. Titratable acidity varied from 1.05 ('K15') to 2.97% ('K12') (mean: 1.90%), while the maturity index ranged between 4.26 ('K32') and 15.09 ('K15'), with an average of 8.03. The ascorbic acid (Vitamin C) content was found to be between 299.76 ('K25') and 324.67 mg/L ('K3'), with an average of 313.41 mg/L. Total antioxidant activity varied from 2.55 ('K7') to 3.33 µmol TE/100 g FW ('K8'), with a mean of 2.98 µmol TE/100 g FW. The total phenolic content ranged from 1587.25 ('K30') to 2096.66 mg GAE/100 g FW ('K32'), with an average of 1798.39 mg GAE/100 g FW. Similarly, total flavonoid content varied between 125.75 ('K3') and 179.79 mg QE/100 g FW ('K11'), with a mean value of 150.49 mg QE/100 g FW. The study by Gül et al. [9] found that the pH, total soluble solids, and titratable acidity of kumquat fruits ranged from 3.20 to 3.52, 12.80 to 13.20 ˚Brix, and 1.46% to 1.49%, respectively. In contrast, Chang and Lin [28] reported average values for total soluble solids, titratable acidity, and the maturity index as 12.07 ˚Brix, 3.88%, and 3.14, respectively. Similarly, Pérez [10] reported that the pH of kumquat fruits ranged from 3.18 to 3.47, while titratable acidity varied from 1.55% to 1.95%. The total phenolic content was found to range from 1622.5 to 2039.2 mg GAE/100 g FW, and the total flavonoid content ranged from 131.5 to 165.5 mg QE/100 g FW.

Regarding mineral composition, aluminum content ranged from 0.35 ('K8') to 0.87 mg/g DW ('K27'), averaging 0.60 mg/g DW. Calcium levels varied between 51.70 ('K4') and 68.88 mg/g DW ('K8'), with a mean of 63.18 mg/g DW. Copper concentrations ranged from 0.06 ('K4', 'K13', 'K22', and 'K28') to 0.12 mg/g DW ('K5', 'K14', 'K29', and 'K31') (mean: 0.09 mg/g DW), while iron content varied between 0.32 ('K22') and 0.86 mg/g DW ('K8'), with an average of 0.57 mg/g DW. Magnesium levels were between 16.09 ('K20') and 20.72 mg/g DW ('K8'), with an average of 18.66 mg/g DW. Manganese content ranged from 0.08 ('K22') to 0.17 mg/g DW ('K8'), with a mean of 0.12 mg/g DW. Phosphorus concentrations varied between 16.00 ('K30') and 19.98 mg/g DW ('K8') (mean: 18.16 mg/g DW), while potassium content ranged from 155.35 ('K1') to 193.29 mg/g DW ('K8'), with an average of 173.68 mg/g DW. Sodium levels showed considerable variation, ranging from 6.89 ('K9') to 16.63 mg/g DW ('K13'), with a mean of 11.68 mg/g DW. Sulfur content was between 11.24 ('K33') and 18.84 mg/g DW ('K18'), averaging 15.14 mg/g DW. Lastly, zinc concentrations ranged from 12.30 ('K11') to 24.04 mg/g DW ('K8'), with an average of 18.85 mg/g DW. Studies conducted in China [3] and Poland [4] have reported partially similar results for kumquat fruits.

Overall, when comparing our findings with studies conducted in different countries, the results have shown a broader range of variation compared to previous research. This difference is likely attributed to both the material used and the environmental factors [29]. The variations in the examined kumquat accessions are shown in Fig. 1.

Table 1. Statistical descriptive parameters for characteristics used to study kumquat accessions.

Characteristics	Abb.	Unit	Min.	Max.	Mean	±SD	CV
Fruit weight	V1	g	1.85	13.23	7.05	±2.77	39.29
Fruit length	V2	mm	21.06	42.26	29.98	±4.94	16.48
Fruit width	V3	mm	10.43	25.01	19.28	±3.86	20.02
Fruit index	V4	Ratio	1.06	2.60	1.59	±0.32	20.13
Fruit peel L*	V5	-	51.02	65.21	58.49	±3.50	5.98
Fruit peel a*	V6	-	10.85	37.83	28.29	±6.16	21.77
Fruit peel b*	V7	-	56.73	80.89	68.45	±5.76	8.41
Leaf length	V8	mm	32.95	99.48	66.60	±17.06	25.62
Leaf width	V9	mm	11.00	33.28	23.71	±5.89	24.84
Leaf index	V10	Ratio	1.93	3.83	2.83	±0.35	12.37
Leaf area	V11	cm^2	2.74	22.83	11.60	±5.45	46.98
Petiole length	V12	mm	2.88	14.02	8.19	±3.02	36.87
Petiole thickness	V13	mm	0.86	1.81	1.45	±0.26	17.93
Petiole index	V14	Ratio	2.40	9.10	5.60	±1.74	31.07
pH	V15	-	3.19	3.55	3.36	±0.11	3.27
Total soluble solid	V16	˚Brix	11.48	15.84	13.66	±1.40	10.25
Titratable acidity	V17	%	1.05	2.97	1.90	±0.65	34.21
Maturity index	V18	Ratio	4.26	15.09	8.03	±2.85	35.49
Vitamin C	V19	mg/L	299.76	324.67	313.41	±7.96	2.54
Total antioxidant activity	V20	µmol TE/100 g FW	2.55	3.33	2.98	±0.25	8.39
Total phenolics	V21	mg GAE/100 g FW	1587.25	2096.66	1798.39	±165.22	9.19
Total flavonoids	V22	mg QE/100 g FW	125.75	179.79	150.49	±15.24	10.13
Aluminum	V23	mg/g DW	0.35	0.87	0.60	±0.16	26.67
Calcium	V24	mg/g DW	51.70	68.88	63.18	±4.33	6.85
Copper	V25	mg/g DW	0.06	0.12	0.09	±0.02	22.22
Iron	V26	mg/g DW	0.32	0.86	0.57	±0.16	28.07
Magnesium	V27	mg/g DW	16.09	20.72	18.66	±1.34	7.18
Manganese	V28	mg/g DW	0.08	0.17	0.12	±0.02	16.67
Phosphorus	V29	mg/g DW	16.00	19.98	18.16	±1.34	7.38
Potassium	V30	mg/g DW	155.35	193.29	173.68	±12.12	6.98
Sodium	V31	mg/g DW	6.89	16.63	11.68	±2.90	24.83
Sulfur	V32	mg/g DW	11.24	18.84	15.14	±2.30	15.19
Zinc	V33	mg/g DW	12.30	24.04	18.85	±3.77	20.00

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

Fig. 1. Images of the fruits and leaves of the examined kumquat accessions.

3.2. Correlation Matrix Analysis (CMA)

Correlation matrix analysis is a statistical method used to examine the relationships between multiple variables simultaneously. It presents correlation coefficients in a matrix format, indicating the strength and direction (positive or negative) of associations between variables. This analysis helps identify patterns, multicollinearity, and potential dependencies within a dataset [30]. The correlation matrix analysis provided valuable insights into the relationships

among the measured traits in the studied kumquat accessions (Fig. 2). The strong positive correlations observed between fruit weight and both fruit length (*r=0.80***) and fruit width (*r=0.89***) indicate that larger fruits tend to be both longer and wider, which is expected in fruit morphology. Similarly, the positive correlation between fruit length and fruit width (*r=0.62***) suggests that these dimensions increase proportionally, further reinforcing the interdependence of fruit size parameters. For color traits, fruit peel L* exhibited a significant positive correlation with fruit peel a* (*r=0.49***) and fruit peel b* (*r=0.81***), suggesting that lighter-colored fruits also exhibit stronger red and yellow pigmentation. Additionally, the strong correlation between fruit peel a* and fruit peel b* (*r=0.71***) highlights the close relationship between these two colorimetric parameters, which jointly contribute to the visual appearance of the fruit peel. Among leaf traits, leaf length was strongly correlated with leaf width (*r=0.92***) and leaf area (*r=0.82***), while leaf width and leaf area were also highly correlated (*r=0.90***). These findings indicate that larger leaves tend to have proportional increases in both dimensions, reflecting a coordinated growth pattern in leaf morphology. The strength of these correlations suggests that these traits may be influenced by shared genetic or environmental factors regulating leaf development. A significant negative correlation was detected between maturity index and titratable acidity (*r=-0.90***), which aligns with the general trend of decreasing acidity as fruits mature. This relationship is consistent with the biochemical changes occurring during fruit ripening, where organic acids are metabolized or diluted as sugars accumulate. Total soluble solids exhibited a moderate positive correlation with both vitamin C (*r=0.36**) and total antioxidant activity (*r=0.36**), indicating that higher sugar content is associated with increased levels of these beneficial compounds. Additionally, titratable acidity was positively correlated with total phenolics (*r=0.38**) and total flavonoids (*r=0.37**), suggesting that more acidic fruits tend to have higher concentrations of these bioactive compounds, which are important for fruit quality and potential health benefits. Overall, the correlations among nutrient elements were found to be statistically insignificant, indicating that their variations within the dataset are independent of one another. These findings underscore the complexity of biochemical interactions in kumquat accessions and suggest that different physiological and genetic mechanisms regulate nutrient composition. The observed correlations provide useful information for selection strategies in breeding programs and for understanding the phenotypic relationships among key fruit and leaf traits.

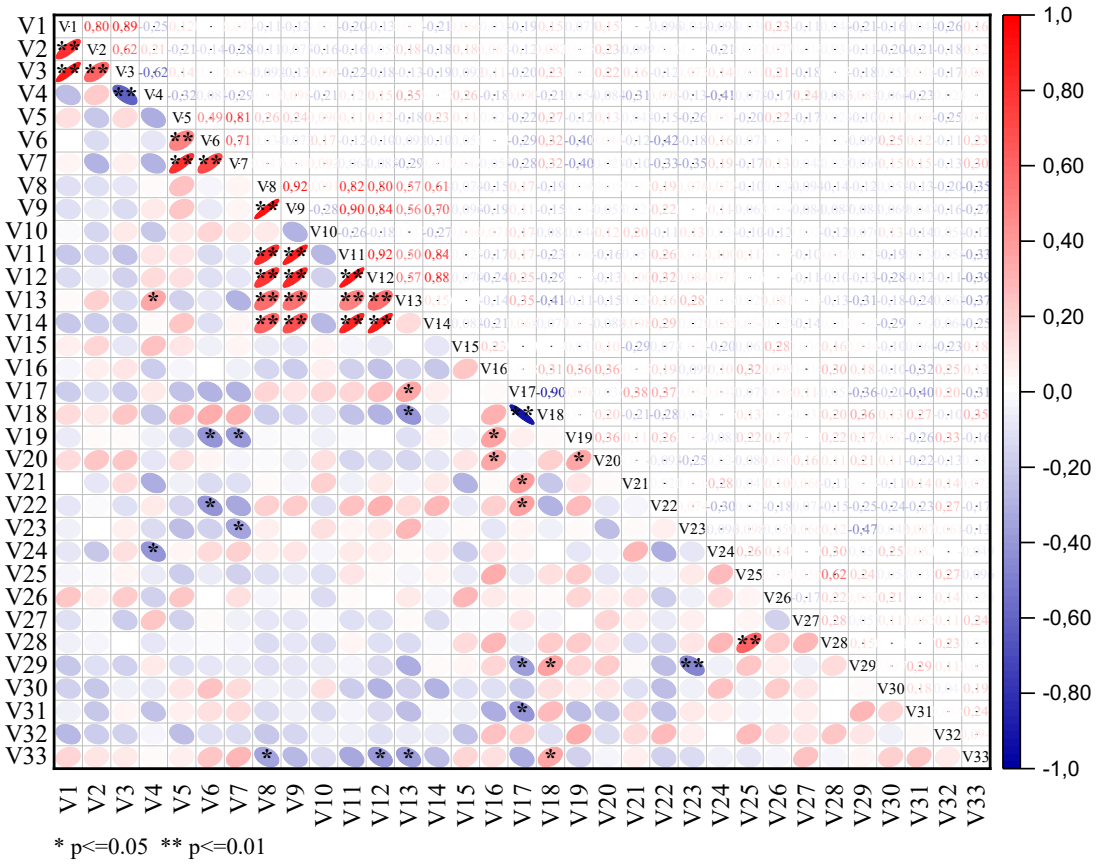

Fig. 2. Simple correlations between the variables utilized in the studied kumquat accessions. For abbreviations, please see Table 1.

3.3. Principal Component Analysis (PCA)

Principal component analysis is a statistical method used for dimensionality reduction and pattern identification by transforming correlated variables into uncorrelated principal components. The first component captures the most variance, with subsequent components orthogonal to the previous ones [31]. In this study, components with eigenvalues ≥1 were retained [32]. PCA was applied with Varimax rotation and Kaiser Normalization, improving interpretability [33], and the rotation process converged in 12 iterations (Table 2).

The PCA conducted in this study effectively captured the majority of the variability within the dataset, with the first 12 principal components explaining 83.93% of the total variation. This high proportion suggests that these components provide a comprehensive representation of the underlying structure of the data. Among them, PC1 accounted for 17.25% of the total variation, with petiole length (0.38), leaf area (0.36), leaf width (0.34), and leaf length (0.33) contributing most significantly. The strong loadings of these leaf-related traits indicate that they play a dominant role in defining the primary axis of variation, likely reflecting differences in vegetative growth patterns across the accessions.

PC2 explained 11.38% of the total variation and was predominantly influenced by fruit peel b* (0.41), fruit peel L* (0.40), and fruit peel a* (0.32), highlighting the importance of fruit peel color in shaping the second axis of variation. The clustering of these colorimetric parameters in PC2 suggests that peel pigmentation is a key distinguishing feature among the accessions, possibly linked to genetic or environmental factors affecting fruit appearance.

PC3 accounted for 8.98% of the total variation, with fruit weight (0.52), fruit width (0.47), and fruit length (0.46) exerting the strongest influence. The high loadings of these fruit morphological traits indicate that PC3 primarily captures variation in fruit size, an essential characteristic in breeding and selection programs. Given that the first three principal components together explained 37.61% of the total variation, they provide a meaningful summary of the dataset's structure, reflecting major sources of phenotypic differentiation.

The statistical significance of these components ($p<0.01$) further underscores their relevance, confirming that the observed patterns are not due to random variation. The results demonstrate that PCA effectively reduces data dimensionality while preserving critical information, making it a valuable tool for exploring trait relationships and identifying key contributors to phenotypic diversity. The clear separation of leaf, color, and fruit size attributes into distinct principal components suggests that these traits vary independently, which may have implications for trait selection in breeding programs or ecological adaptations among accessions.

Table 2. Eigenvalues of the principal component axes from the PCA of the examined traits in the studied kumquat accessions.

Eigenvectors	Components											
	1	2	3	4	5	6	7	8	9	10	11	12
Fruit weight	-0.12	0.03	**0.52**	0.05	-0.03	-0.11	0.02	0.04	0.13	0.06	-0.02	-0.04
Fruit length	-0.06	-0.12	**0.46**	0.03	-0.24	-0.07	0.08	0.13	0.08	0.14	-0.04	-0.08
Fruit width	-0.15	0.05	**0.47**	0.17	0.15	-0.16	-0.05	0.01	-0.01	0.05	0.01	-0.02
Fruit index	0.11	-0.15	-0.12	-0.23	**-0.42**	0.11	0.16	0.16	0.08	0.07	-0.04	-0.08
Fruit peel L*	-0.03	**0.40**	0.06	0.05	0.06	0.26	-0.05	-0.17	0.09	-0.03	0.11	0.03
Fruit peel a*	-0.13	**0.32**	-0.06	-0.11	0.05	0.14	0.20	-0.10	-0.01	0.19	-0.10	-0.12
Fruit peel b*	-0.12	**0.41**	-0.05	-0.06	0.08	0.19	0.04	-0.18	0.19	0.08	-0.04	-0.04
Leaf length	**0.33**	0.20	0.08	0.06	0.01	0.09	0.02	0.07	-0.20	0.07	0.11	0.06
Leaf width	**0.34**	0.21	0.05	0.11	-0.08	-0.01	0.02	0.09	-0.02	0.08	0.14	-0.07
Leaf index	-0.07	-0.01	0.04	-0.12	0.30	0.25	0.01	-0.03	**-0.39**	-0.02	-0.05	**0.33**
Leaf area	**0.36**	0.16	-0.02	0.15	-0.09	-0.06	0.04	0.00	0.03	0.01	0.02	0.04
Petiole length	**0.38**	0.14	0.05	0.10	-0.04	-0.03	-0.01	0.01	0.03	0.06	0.00	0.07
Petiole thickness	0.28	-0.05	0.15	-0.08	-0.01	-0.01	0.31	0.10	-0.07	0.00	-0.01	-0.07
Petiole index	0.30	0.19	-0.04	0.17	-0.08	-0.07	-0.17	-0.05	0.09	0.07	-0.01	0.13
pH	-0.04	-0.02	0.07	-0.01	-0.26	0.27	0.24	-0.09	0.17	-0.31	0.10	**0.49**
Total soluble solid	-0.11	-0.13	-0.01	**0.33**	-0.01	0.30	0.00	-0.23	-0.01	0.14	-0.05	0.07
Titratable acidity	0.20	-0.24	0.01	-0.09	**0.32**	0.24	0.07	0.06	0.25	0.02	-0.08	0.07
Maturity index	-0.23	0.21	-0.02	0.21	-0.24	-0.11	-0.07	-0.14	-0.23	0.11	0.10	0.03
Vitamin C	0.02	-0.22	-0.02	**0.34**	0.00	0.16	-0.18	0.06	-0.13	-0.05	**0.32**	-0.12
Total antioxidant activity	-0.09	-0.02	0.12	0.20	-0.07	**0.37**	-0.09	0.30	-0.22	0.21	0.10	0.06
Total phenolics	0.02	-0.05	0.02	0.07	**0.40**	-0.08	-0.20	0.29	0.20	0.16	0.03	**0.33**
Total flavonoids	0.19	-0.19	0.03	0.03	-0.01	0.08	-0.28	**-0.35**	0.10	0.13	0.20	-0.14
Aluminum	0.07	-0.13	0.07	-0.05	0.13	-0.29	0.25	-0.31	-0.30	-0.08	**0.35**	0.22
Calcium	-0.01	0.14	-0.08	0.21	**0.32**	-0.09	0.24	0.20	0.04	0.05	-0.22	-0.15

Copper	-0.01	-0.10	-0.11	**0.38**	-0.01	-0.17	0.25	-0.14	-0.04	0.04	-0.29	-0.03
Iron	-0.03	0.06	0.11	0.22	0.07	0.11	0.18	0.16	**0.34**	**-0.49**	0.26	-0.06
Magnesium	-0.04	-0.10	-0.14	-0.04	-0.03	0.00	0.30	0.21	0.04	0.54	0.24	0.18
Manganese	-0.06	-0.06	-0.12	**0.38**	-0.03	-0.04	**0.40**	-0.09	0.03	0.03	-0.06	0.12
Phosphorus	-0.09	0.02	-0.23	0.23	-0.24	-0.02	-0.25	**0.34**	-0.03	-0.13	-0.21	0.18
Potassium	-0.12	0.11	-0.15	-0.03	0.10	0.05	0.16	0.29	-0.20	-0.14	0.41	**-0.38**
Sodium	-0.10	0.17	-0.15	-0.03	0.01	-0.43	-0.10	0.12	0.06	-0.09	0.21	0.28
Sulfur	-0.01	-0.20	-0.23	0.17	0.13	-0.05	-0.05	-0.17	0.27	0.07	0.18	-0.19
Zinc	-0.22	0.07	-0.04	-0.07	-0.12	-0.06	0.01	-0.01	**0.35**	0.30	0.29	0.10
Eigenvalue	*5.69*	*3.76*	*2.96*	*2.75*	*2.39*	*1.97*	*1.76*	*1.43*	*1.39*	*1.30*	*1.20*	*1.09*
Components degree of significance	**	**	**	**	**	*	*	*	**	*	*	**
Variance (%)	*17.25*	*11.38*	*8.98*	*8.33*	*7.24*	*5.97*	*5.34*	*4.34*	*4.22*	*3.94*	*3.64*	*3.31*
∑ variance (%)	*17.25*	*28.63*	*37.61*	*45.94*	*53.18*	*59.15*	*64.49*	*68.83*	*73.05*	*76.98*	*80.62*	*83.93*

Bold values indicate the characteristics that most influence each PC (Eigenvalues ≥ 0.32). Component degree of significance: *$p < 0.05$, **$p < 0.01$.

Biplot is a technique used to visualize multivariate datasets. It represents both observations (e.g., accessions) and variables (e.g., traits) in a two-dimensional plane [Gower et al., 2011]. The biplot displaying the PC1/PC2 distribution, which highlights the relationships between the kumquat accessions and the variables analyzed, is shown in detail in Fig. 3.

Cluster 1 includes 'K1', 'K3', 'K8', 'K15', 'K19', 'K25', 'K29', 'K32', and 'K33' accessions, which are primarily associated with fruit-related traits such as fruit weight, fruit peel L*, fruit peel a*, fruit peel b*, and maturity index. Additionally, this cluster is characterized by high levels of mineral elements, including calcium, iron, potassium, sodium, zinc, and phosphorus, as well as fruit width. The strong association between these accessions and fruit quality parameters suggests that this group may represent accessions with superior fruit characteristics in terms of size, color, and mineral content. Cluster 2 comprises 'K4', 'K6', 'K10', 'K11', 'K13', 'K17', 'K20', 'K22', 'K26', 'K27', and 'K30' accessions, which are predominantly linked to vegetative traits, including leaf length, leaf width, leaf area, petiole length, and petiole index. The concentration of these variables in a distinct cluster indicates that these accessions may exhibit a robust vegetative growth pattern, potentially influencing overall plant vigor and adaptability. This clustering pattern suggests that leaf morphology and petiole-related traits are tightly correlated, distinguishing this group from others. Cluster 3 consists of 'K5', 'K14', 'K16', 'K18', 'K21', and 'K24' accessions, which show strong associations with biochemical and physiological traits such as total antioxidant activity, leaf index, pH, and total soluble solids. Additionally, this cluster is linked to essential micronutrients, including manganese, copper, and magnesium, as well as fruit length and sulfur content. The co-localization of these variables implies that these accessions may possess enhanced biochemical properties and nutritional composition, potentially contributing to their functional and health-related attributes. Cluster 4 contains 'K2', 'K7', 'K9', 'K12', 'K23', and 'K31' accessions, which are primarily associated with fruit index, petiole thickness, titratable acidity, total phenolics, total flavonoids, and aluminum. The clustering of these variables suggests that these accessions exhibit distinctive biochemical and structural properties, particularly in terms of secondary metabolite accumulation and acidity regulation. The high phenolic and flavonoid content in this group indicates that these accessions may have a greater potential for antioxidant activity and stress tolerance, which could be valuable for breeding programs focused on functional fruit quality.

In addition, the accessions 'K18', 'K21', and 'K24' were found to be outside the 95% confidence ellipse. This suggests that these accessions exhibit traits that significantly differ from the main cluster of the data, highlighting their unique characteristics. The positioning of these accessions outside the confidence ellipse indicates potential outliers, which may possess distinct phenotypic or biochemical properties that are not well-represented by the majority of the other accessions [35]. Such deviations could be valuable for breeding programs aimed at introducing novel traits or improving the genetic diversity of kumquat cultivars. Further investigation into these accessions may provide deeper insights into their unique attributes and their potential role in enhancing the overall kumquat germplasm.

Overall, the observed clustering pattern highlights the underlying phenotypic and biochemical diversity among the studied kumquat accessions. These distinct groupings provide a structured

understanding of trait relationships, which can be instrumental in breeding efforts, germplasm characterization, and selection strategies aimed at improving fruit quality, plant vigor, and nutritional composition.

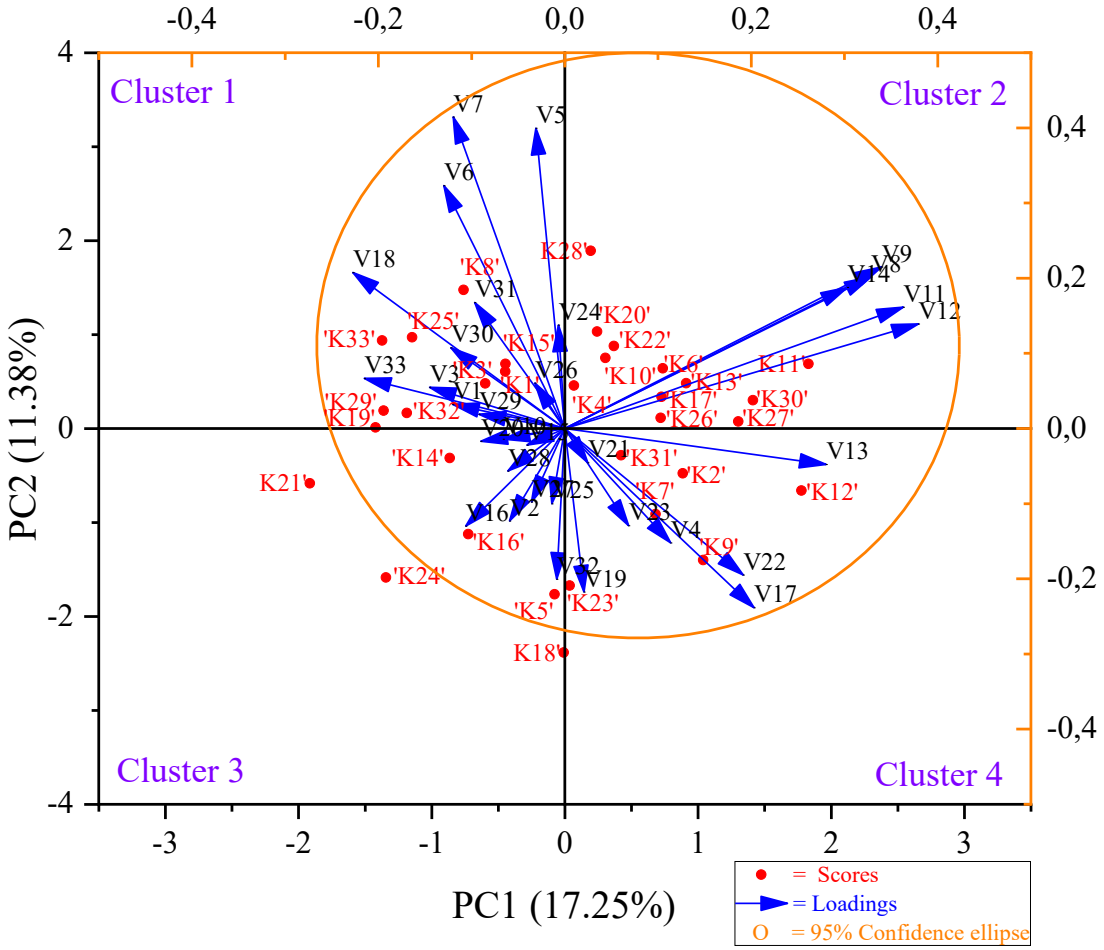

Fig. 3. The biplot displaying the PC1/PC2 distribution, which highlights the relationships between the kumquat accessions and the variables analyzed. For abbreviations, please see Table 1.

3.5. Heat Map Analysis (HMA)

Heat map analysis using Ward's hierarchical clustering method, based on Euclidean distance, is a technique for grouping similar observations in a dataset [36]. The method minimizes the variance within each cluster and maximizes the variance between clusters. The Euclidean distance metric is used to measure the similarity between data points, and the heat map visually represents these relationships. This approach helps in identifying patterns and clusters in large, complex datasets [37].

The variables were initially divided into two main groups, A and B. Subsequently, each group was further subdivided into two subgroups, A1, A2, and B1, B2. Similarly, the accessions were

first divided into two main groups, C and D, and then each group was further subdivided into two subgroups, C1, C2, and D1, D2 (Fig. 4). Accessions such as 'K32', 'K24', and 'K21' from the C1 subgroup were linked to variables from the A1 subgroup, which include fruit weight, maturity index, leaf area, petiole length, petiole index, fruit length, fruit peel a*, leaf width, fruit width, magnesium, phosphorus, zinc, total soluble solids, sulfur, and sodium. These accessions exhibit a clear relationship with the physical and mineral characteristics of the kumquat, particularly in terms of fruit size and morphology. On the other hand, the accessions 'K33', 'K29', 'K19', 'K16', and 'K14', belonging to the C2 subgroup, were found to correlate with the variables of the A2 subgroup. These variables encompass fruit peel L*, calcium, fruit peel b*, leaf length, leaf width, total flavonoids, potassium, and total phenolics. These accessions are more associated with biochemical and color-related characteristics, with an emphasis on secondary metabolites and the antioxidant potential of the fruit. The accessions 'K12', 'K26', 'K11', 'K22', 'K13', 'K17', 'K7', 'K23', 'K18', 'K9', 'K5', 'K31', 'K15', 'K28', 'K4', 'K30', 'K27', 'K10', and 'K2', from the D1 subgroup, were linked to variables from the B1 subgroup. These include fruit index, petiole thickness, titratable acidity, leaf index, total antioxidant activity, and pH. These accessions demonstrate strong relationships with chemical and metabolic properties such as acidity, antioxidant levels, and other related characteristics. Finally, the accessions 'K8', 'K6', 'K20', 'K3', 'K25', and 'K1' from the D2 subgroup were associated with variables that include aluminum, iron, copper, and manganese. These microelements are essential for plant growth and development, further highlighting their contribution to the overall health and nutritional value of the kumquat. This classification of accessions based on their associated variables offers valuable insights into the genetic diversity and complexity of kumquat germplasm, which can be used to guide breeding programs focused on improving both the quality and resilience of the kumquat. The specific relationships identified between accessions and their variables emphasize the potential for targeted selection and breeding strategies based on distinct morphological, biochemical, and nutritional characteristics.

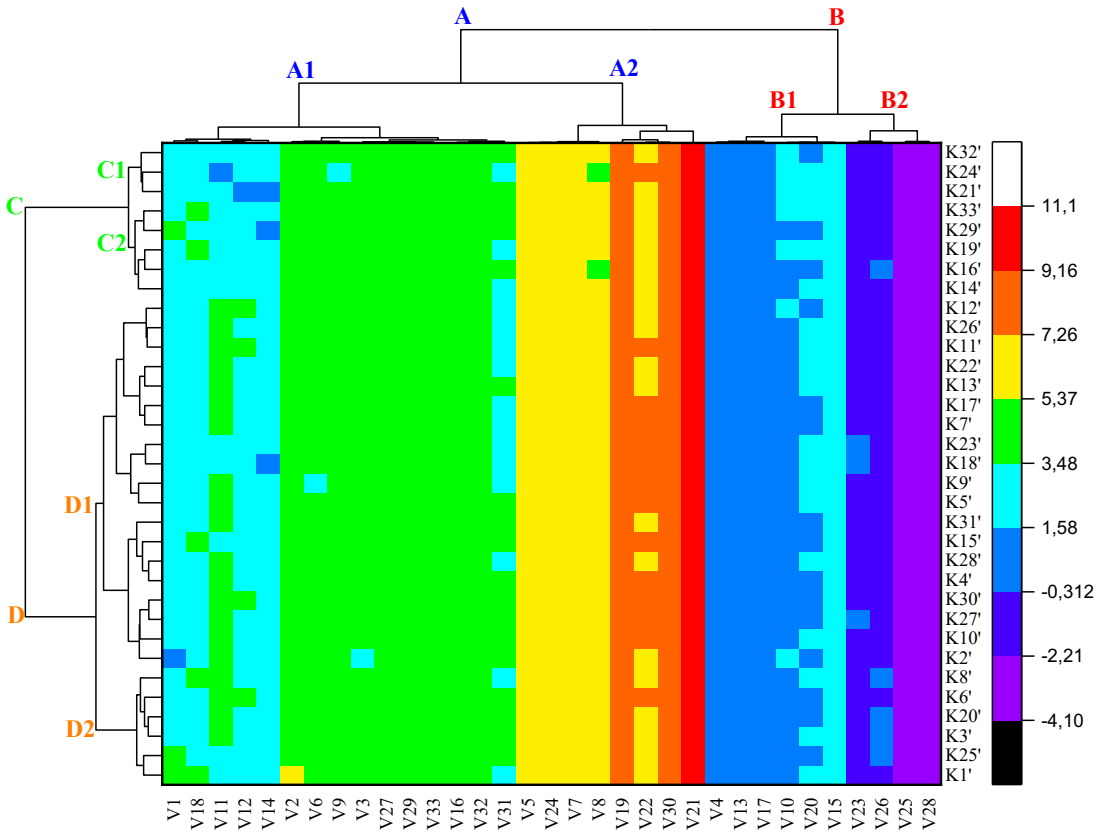

Fig. 4. Visualization of clustering patterns of kumquat accessions and variables using heat map. For abbreviations, please see Table 1.

4. Conclusion

In this study, pomological, morphological, biochemical, antioxidant, and nutritional characteristics of kumquat accessions were evaluated using multivariate analysis methods. The findings revealed significant variation among the accessions, demonstrating the potential of naturally grown kumquats in genetic diversity and selection programs.

When all datasets were collectively analyzed, the accessions with the highest values were identified as ‘K13’, ‘K12’, ‘K15’, ‘K10’, and ‘K9’. These accessions exhibited superior characteristics, making them promising candidates for further breeding and cultivation strategies. Additionally, the accessions ‘K18’, ‘K21’, and ‘K24’ were found to be outside the 95% confidence ellipse, indicating that they possess unique traits that differentiate them from the majority of the studied accessions. These accessions require further investigation to determine their potential significance in breeding programs and genetic conservation efforts.

The results of this study contribute valuable insights into the phenotypic and biochemical diversity of kumquat accessions, which may support future research focused on trait selection, breeding strategies, and conservation of genetic resources. The identification of high-performing accessions and unique genotypes provides a foundation for improving fruit quality,

stress tolerance, and overall adaptation of kumquat cultivars to different environmental conditions. Future studies should explore the underlying genetic mechanisms contributing to these variations and assess their agronomic performance under different ecological conditions.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

YT experimented, collected data, analyzed data, and wrote, and edited the manuscript. Author approved the final manuscript.

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