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Classification of rosehip (Rosa canina L.) genotypes according to different usage purposes and further breeding objectives

Melekber Sulusoglu Durul
Kocaeli University: Kocaeli Universitesi
Kerem Mertoglu
Usak University: Usak Universitesi
Nazan Korkmaz
Mugla Sitki Kocman University: Mugla Sitki Kocman Universitesi
Tuba Esatbeyoglu (esatbeyoglu@lw.uni-hannover.de)
Leibniz University Hannover: Leibniz Universitat Hannover https://orcid.org/0000-0003-2413-6925
Ibrahim Bulduk
Afyon Kocatepe University: Afyon Kocatepe Universitesi

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Abstract

During the pandemic period, the interest in not well-known, colourful wild small fruits has increased due to their positive health effects. Within the context of the current study, eleven genotypes of the rosehip, one of these fruits, grown in nature from seeds were characterized in terms of their horticultural characteristics. As a result; fruit width, fruit length and fruit weight were found to vary between 12.96–17.25 mm; 20.74–25.49 mm and 1.39–2.72 g, respectively. While citric acid was determined as the main organic acid in all the genotypes investigated, the mean values of the examined organic acids obtained from all the genotypes were found to be as follows; citric acid (7177 mg L^{-1})>, malic acid (3669 mg L^{-1}) > tartaric acid (1834 mg L^{-1}) > oxalic acid (1258 mg L^{-1}) > carboxilic acid (631.9 mg L^{-1}) > shikimic acid (157.8 mg L^{-1}) > ascorbic acid (155 mg L^{-1}) > acetic acid (20.9 mg L^{-1}). Ellagic acid is the dominant phenolic, measured at high levels (90.1–96.24 mg L^{-1}) in all the genotypes. Genotypes are categorized according to different purposes such as suitable for wine, vinegar making etc. While the pomological characteristics were determined in a high level of positive correlation among themselves, they were generally found to be negatively correlated with the phytochemical characteristics. It is thought that genotypes categorized according to different usage purposes can contribute to rosehip agriculture, industry and breeding.

Introduction

In societies with increasing awareness, the proportion of functional foods in the daily diet is increasing due to their positive effects on health (Nguyen 2020). In this context, in places where contamination is low, species such as rosehip that grow naturally in wild, have diverse and rich biochemical content gain importance (Oszmianski et al. 2015; Li et al. 2016; Eroğul and Oğuz 2018; Çolak et al. 2022a). Rosehip fruits are rich in vitamins, polyphenols and organic acids, which have antioxidant effect (Soare et al. 2015; Fascella et al. 2019; Yaman, 2022). It has been proven that these antioxidants have positive effects in the treatment of many cardiovascular, respiratory, chronic diseases and infections etc. (Kirkeskov et al. 2011; Tumbas et al. 2012; Pekacar et al. 2021). Based on all of these it is integrated into the industry in different forms such as marmalade, jam, vinegar or food additives etc. (Johnson et al. 2022; Sahingil and Hayaloglu 2022).

Fruit seeds contain high levels of unsaturated fatty acids (Kulaitiene et al. 2020). Due to its high regeneration ability and balanced composition, rosehip can be an alternative to roughage in animal grazing (Özçelik and Doğan 2018). Also it is emphasized that if used as a landscape plant, it has a phytoremediation potential in preventing air pollution (Kalinovic et al. 2019). Rosehip, which is an important source of income for people living in rural areas, is also very important in terms of using female and child labor for agricultural enterprises (Makhorole et al. 2022). So, it is pointed out that the cultivation of rosehip, which has a high demand for all these reasons, should become widespread (Roman et al. 2013; Fascella et al. 2019; Bozhuyuk et al. 2021). However, its cultivation is still quite limited throughout the world.

Wild genotypes show higher morpho-biochemical diversity compared to cultivars (Yıldız et al 2014). These genotypes have a higher frequency of genes that trigger resistance and phytochemical accumulation, due to their tolerance to natural enemies and stress factors in their environment (Hodgkin and Hajjar 2007). The selection of these genotypes is very important in species such as rosehip, where the variety richness is low (Cheikh-Affene et al. 2013). In the short term, production with these genotypes is required, and in the long term, new superior genotypes should be developed by using these genotypes. Regarding this, cultivation of the genotypes that were selected and registered as cultivar has started in different parts of the world. Also, most of them are used as parents in order to develop new superior genotypes and studies are continuing intensively (Zlesak 2006; Güneș and Dölek 2010; Bosnjakovic et al. 2012).

Within the context of the current study, the physico-chemical characteristics of eleven seed origined rosehip genotypes were determined, and the effects of these characteristics on the variation were also investigated. The genotypes were classified according to these sources of variation using multivariate analyses. In addition, by elucidating the relations among the characteristics, useful information that could be used in breeding were obtained.

Material and methods

Materials and growing conditions

The study was carried out with 11 self-grown rosehip genotypes. Genotypes were collected from Otmanlar Village of Köyceğiz district where takes place in the city of Muğla in Türkiye. Muğla is on the border of the Aegean Sea (Fig. 1A) and dominated by a subtropical climate. However, Otmanlar Village, where the samples were collected, is a mountainous area at an altitude of 1050 m (Fig. 1B) and has a continental climate.

Harvest and determination of pomological characteristics
Colouring was taken into account as a criterion in the harvesting of fruits. Fruit weight was determined by electronic balance (Sartorius - CPA 16001S, Göttingen, Germany)) sensitive to 0.001 g, and fruit width and fruit length were determined by digital caliper (VWR-6, Milan, Italy) sensitive to 0. 01 mm.

Spectrophotometric Assays

In the phytochemical analyses, all the remaining ripe fruits of the genotypes were collected, separated from the seeds and converted into fruit juice via juice extractor and used in the analyses.

Total phenolic content (TPC): TPC was evaluated using the Folin-Ciocalteu assay as reported and modified according to Lola-Luz et al. (2014). The fruit juice was mixed with Folin–Ciocalteu reagent and distilled water at a ratio of 1:1:18 (v/v/v) and left to rest for 8 min, then 7% sodium carbonate was added. After 2 h of incubation in dark, the absorbance of the bluish solution was measured at 725 nm (Varian,

Cary 100 Bio, Melbourne, Australia). Gallic acid was used as an external standard for the calibration curve and the results were expressed as gallic acid equivalents (GAE) per liter of fruit juice (mg GAE L^{-1}).
Total flavonoid content (TFC): Aluminum chloride colorimetric method was used for the determination of TFC according to Chang et al. (2002). In summary, 50 µL of juice was taken into the 10 mL tube and mixed with 950 µL of methanol and 4 mL of distilled water and then with 300 µL of sodium nitrite solution (5% in water). After the incubation, 5 mL of 300 µL of aluminium chloride solution (10% in water) was added and the mixture was allowed to stand for 6 min. Next, 2 mL of sodium hydroxyd solution (1 M, in water) was added and the final volume of the mixture was made upto 10 mL with distilled water. The mixture was left to stand for 15 min. Spectrophotometric analyzes were carried out at 510 nm. TFC was calculated from the quercetin calibration curve and the results were expressed as mg quercetin equivalent (QE) per liter.

DPPH assay: Antioxidant activity analyzes were performed using the DPPH method. Firstly, 50% inhibition concentration (IC_{50}) was calculated by drawing percent inhibition against the sample concentrations. Then, up to IC_{50} value samples were taken and the ability to remove DPPH radicals was determined according to the method specified by Mertoğlu et al. (2022). Results were expressed as a percentage (%) in which ascorbic acid used as a positive control.

Quantification of organic acids and phenolic compounds by HPLC-UV

Samples were firstly shaken for 1 h and centrifuged at 14,000 rpm for 15 min. The supernatant was filtered using a 0.45 µm membrane filter. The filtered juice was analyzed by a HPLC device using an Agilent 1260 liquid chromatographic system (Agilent Technologies, CA, USA) equipped with Chemstation software (version Rev. B.04.03), quaternary pump, autosampler and UV detector.

Determination of the organic acids were performed by using an ACE-C18 column (4 mm × 150 mm, 5 µm; Hichrom Ltd, Theale, UK) equipped with a pre-column. The mobile phase consisted of 10 mM aqueous solution of potassium phosphate (pH 2.2 with *ortho*-phosphoric acid) with a flow rate of 1 mL min^{-1}. The injection volume was 20 µL and detector was set to 245 nm for ascorbic acid and 210 nm for all other organic acids (Fu et al. 2015).

An ACE-C18 (4.6 mm × 150 mm, 5 µm; Hichrom Ltd, Theale, UK) column was used for chromatographic separation of phenolic compounds. The mobile phase flow rate was kept constant at 1.0 mL min^{-1}. Mobile phase A was ultrapure water containing 0.1% acetic acid, while mobile phase B was acetonitrile containing 0.1% acetic acid. The gradient conditions were as follows: 0–3.25 min, 8–10% B; 3.25–8 min, 10–12% B; 8–15, 12–25% B; 15–15.8 min, 25–30% B; 15.8–25 min, 30–90% B; 25–25.4 min, 90–100% B; and 25.4–30 min, 100% B. The injection volume was 10 µL and the column temperature was kept constant at 25°C. Detection wavelengths were chosen considering the wavelengths at which the phenolic compounds to be analyzed had maximum absorption. Syringic acid, protocatechuic acid and gallic acid were detected at 280 nm. Vanillic acid was detected at 225 nm. *p*-Coumaric acid was detected at 305 nm. Caffeic acid and chlorogenic acid were detected at 330 nm (Wen et al. 2005).

Statistical analysis

The research was designed according to the randomized plot experimental design. In the pomological characteristics, measurements were made on 25 fruits collected from each of the four sides of the plants (100 fruits for each genotype). It was determined whether the examined characteristics showed statistically significant differences between the genotypes by using the one-way ANOVA (Minitab Version 17 Minitab Inc., State College, Pennsylvania, USA) program package. Tukey (HSD) multiple comparison test was used to reveal the differences between the genotypes. In the determination of the relations between the characteristics, correlation analysis was used. Principal component analysis (PCA) was used to examine the interrelations among the observed set of variables to identify the similarities and differences of the characteristics. In addition, scatter plot based on the first two principal components (PC1 and PC2) was generated (Zar 2013).

Results and discussion

The findings obtained for the physico-chemical characteristics examined in the rosehip genotypes are given in Table 1. Fruit width, fruit length and fruit weight characteristics were found to vary between 12.96–17.25 mm; 20.74–25.49 mm and 1.39–2.72 g, respectively and significant differences were observed among the genotypes (Table 1). The coefficient of variation calculated for fruit weight (18.90%) was found to be higher than fruit width (9.45%) and fruit length (7.52%). This is thought to be caused by the elliptical shape of rosehip fruits. In studies carried out in regions with different ecological characteristics in the world, fruit width, fruit length and the fruit weight of rosehip genotypes varies within the limits of 10.44 mm − 18.40 mm; 15.10 mm − 33.83 mm and 0.90 g − 4.95 g, respectively (Cheikh-Affene et al. 2013; Bozhuyuk et al. 202; Demir et al. 2021). Parallel to our study, it has been stated that these characteristics show middle-high variation in scope of selection studies conducted on minor fruit species (Eroğul and Oğuz 2018).

Different parts such as fruits, leaves and seeds of the rosehip plants contain high levels of biochemicals which shown antioxidant effects (Kulaitiene et al. 2020). So, these parts are widely preferred for product improvement in the food industry due to their rich and diverse biochemicals (Nicorescu et al. 2018; Sahingil and Hayaloglu 2022). The findings of the current study also showed that rosehip genotypes contain high levels of phenolic compounds and have antioxidant activity. Lowest coefficients of variation were obtained from TPC (3.78%) and antioxidant activity (0.67%), indicate all genotypes are similar regarding these characteristics and average values obtained from all genotypes were 3726 mg GAE 100 mL^{-1} and 93.59%, respectively. Significant differences were detected between the genotypes in terms of TFC, and the values ranged from 457.2 to 625.0 mg QE L^{-1} (Table 1). While similar amounts of TPC (3543–4807 mg GAE 100mL^{-1}) and TFC (206–672 mg QE L^{-1}) in rosehip fruits harvested at an altitude close to the current study were reported by Soare et al. (2015), higher levels of TPC (3851–7908 mg GAE 100mL^{-1}) and TFC (287–1686 mg QE L^{-1}) were reported at fruits harvested from higher altitudes (Demir et al. 2021). As known, phenyl propanoid enzymes get activated at higher levels in plants that adapt to

the typical climatic characteristics of high altitudes and trigger phytochemical accumulation (Mertoğlu et al. 2020).

Table 1
Descriptive statistics of pomological and chemical characteristics

Abbreviation		Unit	Minimum	Maximum	Mean± StDev	CV (%)
General Physco-chemical characteristics						
Fruit Width	FrWi	mm	12.96	17.25	$15.09^{\pm 0.14}$*	9.45
Fruit Length	FrL	mm	20.74	25.49	$23.21^{\pm 0.17}$*	7.52
Fruit Weight	FrWe	g	1.39	2.72	$2.01^{\pm 0.38}$*	18.90
Total phenol content	TPC	mg GAE $100mL^{-1}$	3501	39567	3726± 140.80ns	3.78
Total flavonoid content	TPFlvC	mg Quercetin L^{-1}	457.2	625.0	526.2± 44.88*	8.51
Antioxidant activity	AntAc	µg Trolox m^{-}	92.54	94.78	93.59± 0.63ns	0.67
Organic acids						
Citric acid	CitA	$mg\ L^{-1}$	6352	8766	7177± 679.44*	9.46
Malic acid	MalA	$mg\ L^{-1}$	2842	4583	3669± 637.35*	17.35
Tartaric Acid	TarA	$mg\ L^{-1}$	1420	2294	1835± 318.32*	17.35
Oxalic acid	OxaA	$mg\ L^{-1}$	1010	1461	1258± 131.58*	10.45
Carboxilic Acid	CarA	$mg\ L^{-1}$	489	739.5	631.9± 82.02*	12.97
Shicimik Acid	ShiA	$mg\ L^{-1}$	135.1	182.2	157.8± 15.70*	9.95
Ascorbic acid	AscA	$mg\ L^{-1}$	100.1	211.1	154.6± 30.78*	19.92
Acetic Acid	AceA	$mg\ L^{-1}$	9.98	30.04	$20.91^{\pm 7.01}$*	33.51
Phenolic compounds						

Abbreviation		Unit	Minimum	Maximum	Mean± StDev	CV (%)
General Physco-chemical characteristics						
Ellagic Acid	EllA	mg L^{-1}	90.08	96.24	$92.55^{\pm 0.98ns}$	1.95
Sinapic Acid	SinA	mg L^{-1}	33.14	46.56	$40.32^{\pm 4.02*}$	9.98
Gentisic Acid	GenA	mg L^{-1}	23.48	35.64	$28.71^{\pm 3.56*}$	12.42
p-coumaric acid	CoumA	mg L^{-1}	2.26	3.42	$2.78^{\pm 0.37*}$	13.24
Catechic Acid	CatA	mg L^{-1}	1.22	2.81	$1.83^{\pm 0.51*}$	27.59
Chlorogenic Acid	-	-	nd	nd	-	-
Syringic Acid	-	-	nd	nd	-	-
Caffeic Acid	-	-	nd	nd	-	-

StDev: Standart deviation; CV: Coefficient of variation; *: Means statistical difference among genotypes, ns: non-significant, nd: not detected

Citric acid was detected as the main organic acid in all the genotypes examined in agreement with Koç (2020) and Igual et al. (2022). Mean values of the examined organic acids obtained from all the genotypes are ordered as follows; citric acid (7177 mg L^{-1})>, malic acid (3669 mg L^{-1}) >, tartaric acid (1835 mg L^{-1}) >, oxalic acid (1258 mg L^{-1}) >, carboxilic acid (631.9 mg L^{-1}) >, shicimic acid (157.8 mg L^{-1}) >, ascorbic acid (154.6 mg L^{-1}) > and acetic acid (20.91 mg L^{-1}). All the examined organic acids showed statistically significant differences between the genotypes. With its high coefficient of variation, acetic acid (33.51%) came to the fore among the organic acids in terms of distinguishing the genotypes. Carboxilic acid (12.97%), malic acid (17.35%), tartaric acid (17.35%) and ascorbic acid (19.92%) showed a moderate level of variation among the genotypes, while citric acid (9.46%), shikimic acid (9.95%) and oxalic acid (10.45%) contributed less. Among 25 different species, rosehip was found in the group with very rich in organic acids and it was reported that organic acids generally showed moderate variation among the genotypes (Mikulic-Petkovsek et al. 2012).

Ellagic acid with a low coefficient of variation (1.95%) came to the fore as the dominant phenolic, measured at high levels (90.08 96.24 mg L^{-1}) in all the genotypes. The same situation was reported by Fascella et al. (2019), who studied three different wild rosehip species. Among the phenolic compounds examined, sinapic acid (33.14 46.56 mg L^{-1}) and gentisic acid (23.48 35.64 mg L^{-1}) were measured in moderate amounts in the rosehip genotypes, while *p*coumaric acid (2.26 3.42 mg L^{-1}) and catechic acid (1.22 2.81 mg L^{-1}) were found at low levels. In accordance with Fascella et al. [7], chlorogenic acid, syringic acid and caffeic acid could not be detected in the rosehip genotypes.

Findings of the current study is comparable with results of the previously reported bioactive profile of *Rosa canina* genotypes (Cheikh-Affene et al. 2013; Soare et al. 2015; Bozhuyuk et al. 2021; Yaman 2022). Although it is thought that the differences are mainly due to the genotypes examined, factors such as the differences in analysis methods, ecological differences in the place of selection area, harvest time and type, maturity period etc. have important effects on the final phytochemical composition of the fruits (Kulaitiene et al. 2020; Çolak et al. 2022b; Mertoğlu 2022).

Findings related to the correlations between the fruit characteristics are given in Fig. 2. A high level of positive correlation was detected between fruit width and fruit length (0.63). In plants, after fertilization, firstly an increase in cell number is observed, followed by cell enlargement. The coexistence of transversal and longitudinal development in the cell during the cell expansion phase explains the strong relationship between these two characteristics. The increase in the volume lead to increase of the weight. In this context, a strong positive correlation was determined between fruit weight and fruit width and fruit length respectively at r: 0.75*** and r: 0.80***. The increase in fruit size causes a decrease in the amount of dry matter per unit area by increasing the intercellular space (Mertoğlu et al. 2022). For this reason, generally medium-high negative correlations were found between the pomological characteristics and chemical characteristics of the fruits [34]. As expected, the increase in the amount of individual phenolic compounds increase the TPC and TFC in general. The results of the study are consistent with studies conducted with different minor fruit species (Yaman 2022; Çolak et al. 2022a).

It has been reported that organic and phenolic acids with acidic character are in a positive relationship both within themselves and with each other (Mertoğlu et al. 2022; Çolak et al. 2022b). However, as a result of the study, generally negative relations were found between phenolic acids and organic acids, and also increase in organic acids caused a decreases in the amounts of TPC and TFC. In addition, TPC and TFC, known with their antioxidant properties, were found to be negatively correlated with antioxidant activity respectively at r: -0.75 and r: -0.18. It is thought that this situation is caused by the fact that the amount of organic acid in rosehip fruits is much higher than that of phenolic compounds. Except for carboxylic acid, all the examined organic acids were found to be positively correlated with antioxidant activity. So, main antioxidant effect is shaped by organic acids in rosehip. Although the co-location of major quantitative trait loci (QTLs) for phenolics and organic acids were reported on the same linkage groups in plum (LG1 and LG6) (Salazar et al. 2017), in case of need, these compounds can be converted into each other (Sha et al. 2011). For this reason, results can be obtained from time to time as if there is a negative relationship between them (Mertoğlu 2022).

Principal component analysis (PCA) was carried out in order to obtain a broad view to see the distribution of genotypes according to the characteristics (Fig. 3). PCA has been used for the same purpose in different minor fruits such as blackberry (Oszmianski et al. 2015), blueberry (Li et al. 2016), European cranberrybush (Çolak et al. 2022b) and rosehip (Soare et al. 2015).

In line with the results, it was determined that the rosehip genotypes have the potential to be used for different purposes. Genotypes 5 and 8 seem suitable for vinegar production with their high acetic acidity,

and genotype 1 seems suitable for wine making due to its richness in flavonoids that give astringent and bitter taste to the products (Nishiyama et al. 2015). Genotypes 3 and 6 are suitable to be processed in industry with their high organic acid content. As known, organic acids help maintain stability in products by limiting microorganism activity (Tejero-Sarinena et al. 2012). Genotypes 7 and 10 stand out with their large fruit and can be used as parents for the development of new genotypes with large fruit in breeding studies. The biggest advantage of selection studies is that genotypes for different purposes can be created.

The results of the cluster analysis of the genotypes are given in Fig. 4. It seems that the genotypes are gathered under two main groups. Genotypes 7 and 10, which form one of the branches of Group 1, differ from the others in terms of pomological characteristics, while genotypes 4, 9 and 11, which form the other branch, are rich in TPC. The genotype 1, constituting one of the branches of Group 2 on its own, was found to be distinguishing in terms of TFC. Same was seen for genotype 8 in terms of acetic and ellagic acids. Other genotypes 2, 3, 5 and 6 in this group also showed higher results than Group 1 in terms of their chemical content.

Conclusion

Within the context of the study, physico-chemical characteristics of 11 different seed-originated rosehip genotypes from Muğla province, where selection studies were not carried out before, were determined. In line with the results, high variation was observed and genotypes were identified for different purposes. In order to increase the biochemical content and quality of industrial products, the high organic acid contents of genotypes 3 and 6 may have positive effects on product stabilization. Genotypes 7 and 10 stand out with their large fruit and can be used as parents for the development of new genotypes with large fruit in breeding studies. Genotypes 5 and 8 seem suitable for vinegar production with their high acetic acidity, and genotype 1 seems suitable for wine making due to its richness in flavonoids that give astringent and bitter taste to products.

Declarations

Author contributions Conceptualization: KM, Resource for plant materials: NK, Methodology: TE, KM, MSD, NK and IB software: TE, KM and MSD, Original draft preparation: KM and MSD, Review and editing: TE, KM and MSD, Supervision: TE, KM and MSD

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Data availability The data that support the fndings of the study are available from the corresponding authors upon reasonable request.

Conflict of Interest The authors declare that they have no conficts of interest.

Human or animal rights There are no human or animal participants in this study

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Figures

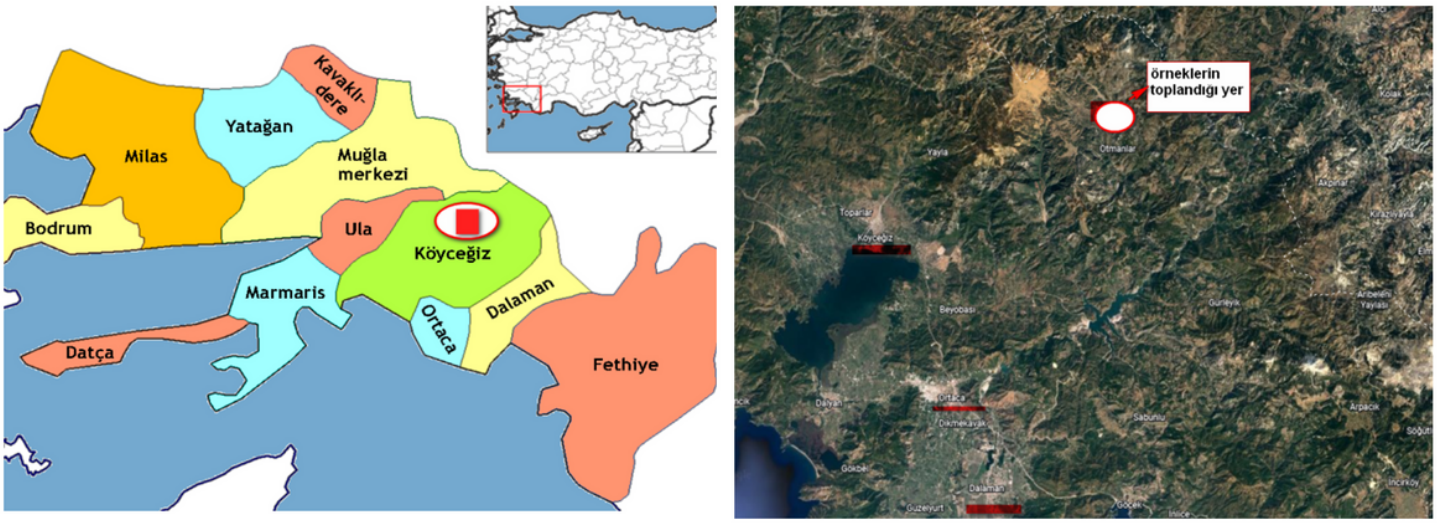

Figure 1

Districts and location of Mugla (A), collection site; Otmanlar Village (B)

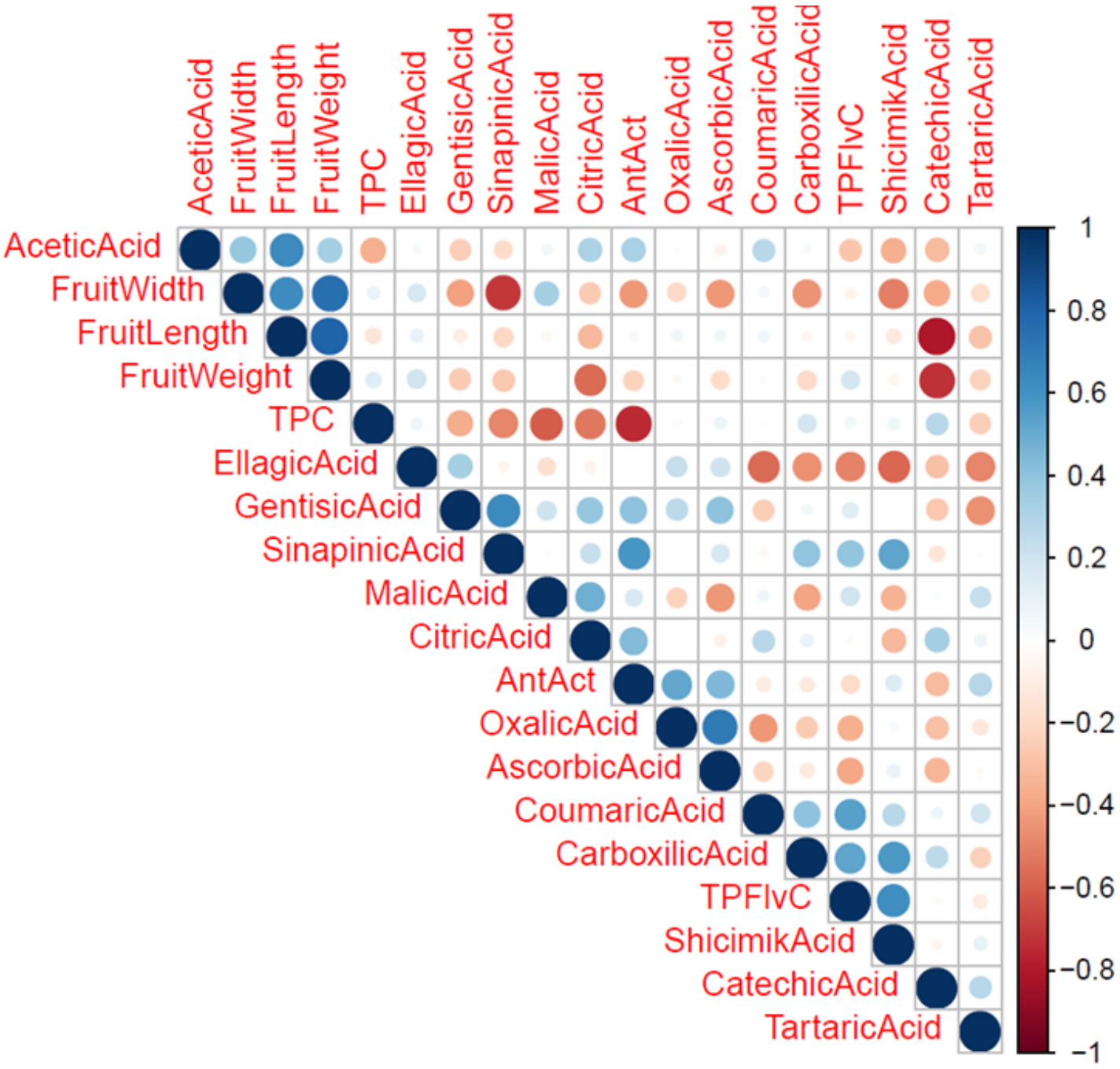

Figure 2

Correlations among investigated characteristics (Blue colour represents a positive correlation and higher darkness and larger circles means stronger positive correlation, same for red, but red represents a negative correlation).

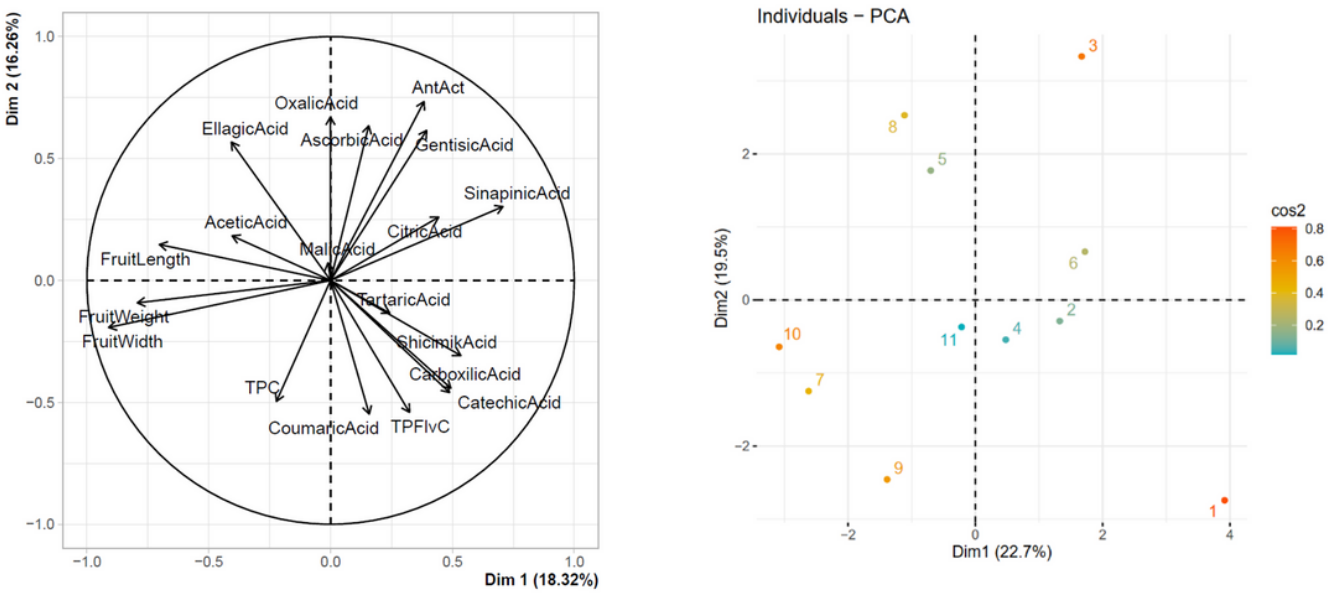

Figure 3

PCA-Biplot analysis of pomological and chemical characteristics (left) and segregation of genotypes according to characteristics (right)

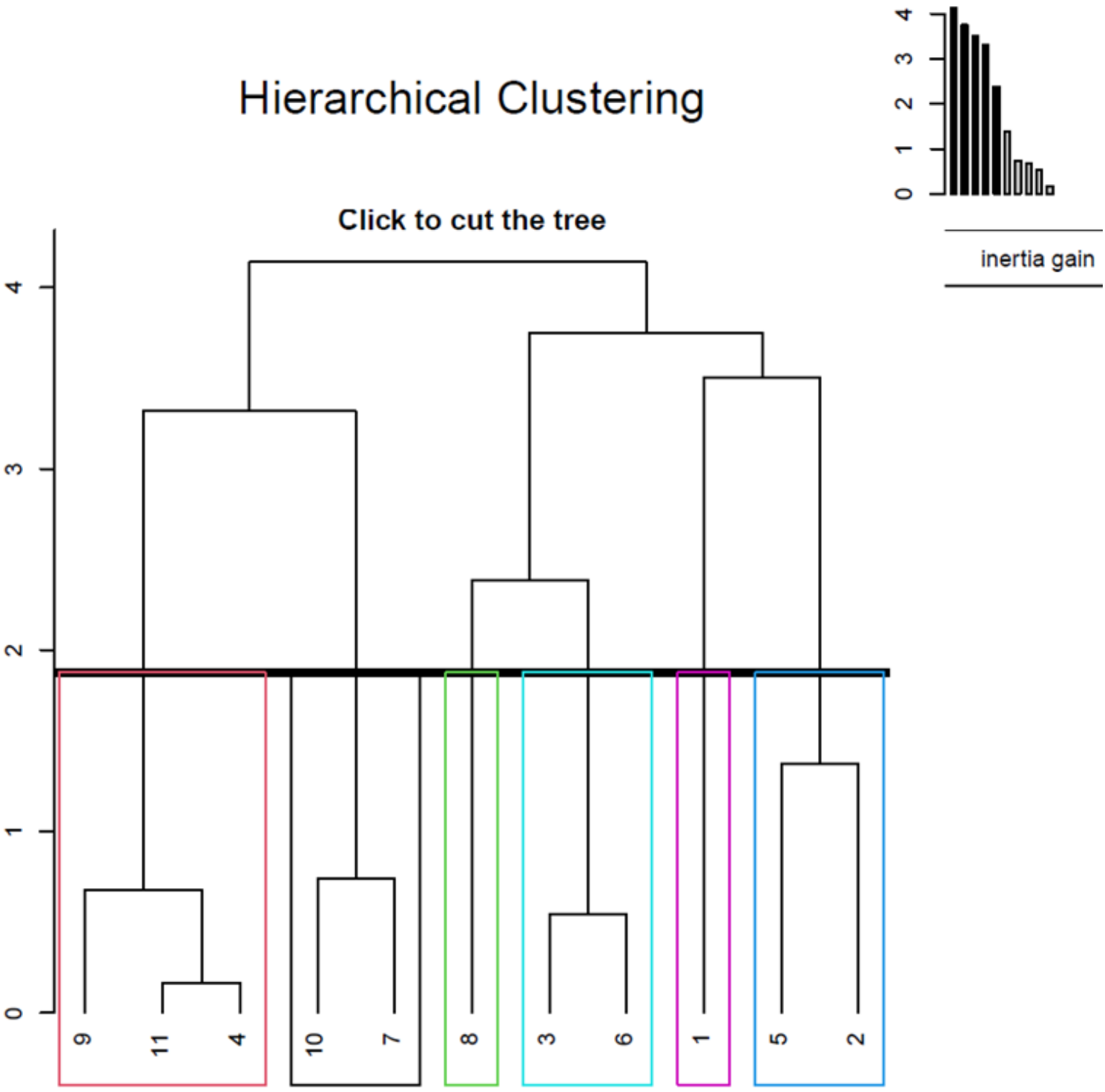

Figure 4

Hierarchical clustering of Genotypes