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Evaluation of Clonal Variability of Berry Phenolics in *Vitis vinifera* L. Cv. Kalecik Karası

Nurhan Keskin[1] · Birhan Kunter[2] · Hasan Celik[3] · Ozkan Kaya[4] · Sıddık Keskin[5]

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Abstract

In clonal grapevine populations, genetic factors may have a significant effect on the amount of phenolic compounds in the grape berries. Thus, the capacity of the clones to produce distinctive chromatic profiles can be improved. This paper describes the phenolic contents and composition of grape berries as well as relationships among them for Kalecik Karası clones to reveal their wine quality potentials. Seven individual polyphenols were quantified using high-performance liquid chromatography. The clones showed a significant difference (5.01 $mg\,kg^{-1}$ protocatechuic acid and 18.80 $mg\,kg^{-1}$ gallic acid) in berry phenolic compounds. Cluster analysis and multidimensional scaling were performed, and results showed that clones were clustered into three groups regarding phenolic compounds in the berries. Based on the phenolic compounds, 18 of the 23 clones were clustered into a group. Clones 16, 13, 8, and 2 were grouped together, while clone 7 was separated from the others. Including and excluding clone 7, approximately 40% phenotypic variation and 80% similarity were observed in 'Kalecik Karası' clones, respectively. There were positive correlations between clones 2, 6, 7, 9, and 13 and *p*-coumaric, ferulic, gallic, and protocatechuic acids, as well as between clones 3, 5, 10, 14, 15, 34, 16, 19, and 20 and *q*-coumaric, vanillic, and syringic acid contents. Thus, it can be stated that multivariate methods can be used for clonal selection, and exclusive clones can be selected with high values of phenolic compounds in the future.

Keywords Grape · Clone · HPLC · Phenolic content

Introduction

The level of intravarietal diversity varies among grapevine cultivars because they are not genetically homogeneous (Keller 2015; Stajner et al. 2009). Grapevine is practically propagated by vegetative or asexual methods, in which a cutting is taken from a single parent vine (Moncada and Hinrichsen 2007). The vegetative propagated vines are also called clones as they are genetically identical to their mother vines; they were cut from and exhibit desired characteristics similar to those mother vines (Van Leeuwen et al. 2013). In the nineteenth century, the first grapevine clone selection began in Germany and then continued in other European countries, including Italy and France, in the second half of the twentieth century (Ibáñez et al., 2015). Initially, the aim of clonal selection was to achieve a healthy, virus-free population from healthy mother plants that would be resistant to different environmental conditions and capable of producing high-quality grapes (Vujović et al. 2016). In the second stage, more complex selection criteria such as yield, grape sugar concentration, skin phenolic compound concentration, nutritional values, and organoleptic characteristics have been applied (Forget et al. 2002; Artem et al. 2014). Many studies have reported that there is clonal diversity in *Vitis vinifera* varieties for a broad range of characteristics. It has indeed been determined that precocity of the phenological cycle, yield, sugar production, total acidity, seed number, disease resistance, secondary metabolites, and phenolic profiles vary among clones (Barbeau et al. 1999; Boso et al. 2004; Anderson et al. 2008; Duchene et al. 2009).

✉ Ozkan Kaya
kayaozkan25@hotmail.com

[1] Faculty of Agriculture, Department of Horticulture, Van Yüzüncü Yıl University, 65090 Van, Turkey

[2] Faculty of Agriculture, Department of Horticulture, Ankara University, 06560 Ankara, Turkey

[3] Faculty of Agriculture, Department of Horticulture, Ankara University, 06560 Ankara, Turkey

[4] Erzincan Horticultural Research Institute, 24060 Erzincan, Turkey

[5] Faculty of Medicine, Department of Biostatistics, Van Yüzüncü Yıl University, 65090 Van, Turkey

Genetic factors may also have a significant effect on the phenolic content in crop populations, with most observed changes being quantitative rather than qualitative (Parr and Bolwell 2000). Therefore, clonal selection may be used as a common technique to improve grape phenolic content (Revilla et al. 2009). Some clones of grapevine have the capacity to produce wines with higher phenolic content, chromatic profile, and a distinctive color. For example, differences in chromatic properties were found in wines made from different clones of Monastrell grapes. Similarly, differences were determined in the chromatic properties of wines made from different clones of Cabernet Sauvignon (two different clones) and Monastrell grapes (Gómez-Plaza et al. 2000; Burin et al. 2011). In general, determination of the phenolic profile of red wine plays a key role for various reasons, such as astringency, bitterness, and color stability, and the profile affects the organoleptic properties of the wine (Đorđević et al. 2017). Moreover, these compounds can act as neutralizing free radicals against oxidative stress–related diseases, contributing in such a way to the maintenance of human body homeostasis (Youdim et al. 2002). These compounds also protect essential macromolecules, such as nucleic acids, enzymes, structural proteins, membrane lipids, and lipoproteins, from oxidation by free radicals (Schroeter et al. 2000). Consumption of red wine (together with olive oil) was found to be one of the key explanations for the "French paradox"—a low incidence of cardiovascular disease, even if fatty foods are consumed (Renaud and de Lorgeril 1992). Many studies have also reported that moderate daily consumption of wine protects against many chronic diseases, such as cardiovascular diseases, dementia, and certain cancers (Garaguso and Nardini 2015).

The grape phenolic compounds are affected by different parameters, including species, variety, ripening stage, climatic conditions, clone, soil characteristics, canopy management, environmental stress, vine health status, vineyard management, and viticulture practices such as irrigation, nutrition, and soil management (Rodríguez-Montealegre et al. 2006; Rusjan et al. 2012; Di Lecce et al. 2014). Clarifying the effect of individual factors on the synthesis and profile of phenolic compounds is difficult due to the interactions among all these complex factors. Therefore, the study of the phenolic profile of grapes, as well as the factors affecting it, has attracted great interest among both viticulturists and scientific researchers. Considering all the above, the clonal selection of grapevine seems like a never-ending story, and it is a common technique for upgrading the quality of both grape and wine. In Turkey at the beginning in the 1970s, clone selection studies were conducted by the Faculty of Agriculture in Ankara, Tekirdag Viticulture Research Institute, Yalova Atatürk Horticultural Central Research Institute, and Manisa Viticultural Research Institute (Ağaoğlu 1999; Köse 2002). A pioneer clonal selection study was performed by the Ankara University Faculty of Agriculture on Kalecik Karası, which is one of Turkey's most prominent red wine grape varieties. Almost 40 years later, a detailed and unique reelevation was carried out on 23 clones of Kalecik Karası, focused on both agronomic characteristics and wine performance (Çelik et al. 2019). Additionally, our previous study on these 23 clones of Kalecik Karası revealed significant differences in trans-resveratrol (t-RSV; 3, 5, 40-trihydroxy-trans-stilbene) and organic acid content (Keskin et al. 2020, 2021). However, our knowledge of the phenolic compounds of these clones is still limited. Thus, the aim of this study was to describe the phenolic contents and composition of grape berries as well as relationships among them for Kalecik Karası clones to reveal their wine quality potentials throughout the consecutive vintages in 2016 and 2017.

Materials and Methods

Chemicals

All chemicals used in this study were analytical reagent grade, and all solvents were high-performance liquid chromatography (HPLC) grade. These solvents were degaussed and filtered before use. Analytical standards and chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Plant Materials and Growth Conditions

This experiment was carried out in the experimental vineyard in the Viticulture Research Station (University of Ankara, Faculty of Agriculture) in Kalecik, which was the homeland of Kalecik Karası in 2016–2017 for the selected 23 clones of *Vitis vinifera* L. cv Kalecik Karasıred wine variety. Kalecik Karasıclones were grown on the rootstock 41 B clone 172, with 3 m × 2 m row spacing in 1999. Bilateral Guyot training and 75-cm trunk height were applied to the vines. The vineyard soil was clay loam with almost natural pH (7.65), total lime 14.6%, salinity 0.30 mmhos/cm, organic matter 2.18%, and boron content 1.01 ppm.

Samples of Grape

For berry sampling, four zones were defined on each grape cluster to be sampled: the tail and middle sections and the left and right shoulders. In the vintage of 2016 and 2017, clusters of the clones were harvested at around 23% °Bx in berries. The study followed a defined protocol for berry collection to avoid bias in sampling. The samples were selected alternately from each of these cluster zones for berry selection, moving from cluster to cluster throughout the

vine. In addition, while berry samples were collected, the samples were alternated from the rear to the front of the cluster. For example, if there was one cluster, the first four berries would be selected from the outer-facing or front side of the cluster (one berry from each of the four zones), and the second four berries would come from the rear-facing side (in sequence with the four cluster zones). In total, 16 clusters from 16 vines were taken for each clone, and eight berries were sampled from each cluster (128 berries in total for each clone). After harvest, one subsample of 128 berries for each harvested clone was directly stored at –80 °C.

Extraction and Determination of the Phenolic Compounds

The whole berry (pulp, skin, and seeds) was used in the study. The berries obtained from clusters were triturated with a conventional beater until a homogeneous berry sample was obtained for the analysis. The crushed berry samples were stored in a freezer at –20 °C until its analysis. Seven phenolic compounds (protocatechuic acid, vanillic acid, gallic acid, syringic acid, *p*-coumaric acid, ferulic acid, *q*-coumaric acid) as phenolic acids were analyzed. The phenolic compounds of the clones were identified by HPLC using the modified method of Rodriguez-Delgado et al. (2001), with three replications. The triturated berry samples were mixed with distilled water at a ratio of 1:1 and then centrifuged at 15,000 rpm for 15 min. In these samples, the upper part was filtered with 0.45-µm MF-Millipore filters and injected into the HPLC device. Chromatography assays were determined with the Agilent 1100 HPLC device by using a diode-array detector (Agilent, Santa Clara, CA, USA) and a 4-µm octadecyl-silica column, 4.6 × 250 mm (Hichrom, Reading, UK). A methanol:water:acetic acid (10:28:2) and B methanol:water:acetic acid (90:8:2) were utilized for a mobile phase. Extraction of the samples was done at 254 nm and 280 nm, 20 µl injection volume, and 1 ml min^{-1} flow rate.

Statistical Analysis

There were no statistically significant differences between years regarding the phenolic compounds of the clones, so data were pooled. Both univariate descriptive statistics and multivariate statistical techniques were used for data analy-

Table 1 Descriptive statistics for phenolic compounds of Kalecik Karası clones (mg kg^{-1})

Clones	Protocatechuic acid	Vanillic acid	Gallic acid	Syringic acid	*p*-coumaric acid	Ferulic acid	*q*-coumaric acid
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM
1	3.960 ± 0.566	0.215 ± 0.007	4.545 ± 0.305	0.812 ± 0.011	0.125 ± 0.025	0.310 ± 0.011	0.185 ± 0.015
2	5.015 ± 0.215	0.165 ± 0.005	13.24 ± 0.141	0.722 ± 0.011	0.165 ± 0.025	0.215 ± 0.025	0.340 ± 0.020
3	3.861 ± 0.060	0.185 ± 0.002	5.495 ± 0.315	1.055 ± 0.015	0.030 ± 0.201	0.145 ± 0.005	0.480 ± 0.020
4	4.035 ± 0.165	0.115 ± 0.001	1.655 ± 0.135	1.235 ± 0.165	0.135 ± 0.015	0.320 ± 0.011	0.130 ± 0.011
5	4.195 ± 0.095	0.125 ± 0.003	4.660 ± 0.121	0.975 ± 0.055	0.040 ± 0.001	0.425 ± 0.025	0.220 ± 0.021
6	3.965 ± 0.085	0.195 ± 0.005	5.580 ± 0.361	0.675 ± 0.045	0.180 ± 0.011	0.555 ± 0.015	0.235 ± 0.015
7	3.911 ± 0.610	0.210 ± 0.020	18.180 ± 0.411	0.605 ± 0.020	0.080 ± 0.101	0.365 ± 0.005	0.270 ± 0.012
8	3.841 ± 0.170	0.275 ± 0.015	12.18 ± 0.310	0.823 ± 0.030	0.045 ± 0.005	0.365 ± 0.015	0.410 ± 0.011
9	3.735 ± 0.235	0.215 ± 0.005	5.415 ± 0.175	0.635 ± 0.035	0.105 ± 0.005	0.360 ± 0.021	0.235 ± 0.005
10	3.681 ± 0.180	0.265 ± 0.025	6.200 ± 0.160	0.675 ± 0.075	0.080 ± 0.130	0.305 ± 0.005	0.330 ± 0.030
11	4.141 ± 0.130	0.140 ± 0.011	5.765 ± 0.105	0.385 ± 0.005	0.095 ± 0.005	0.335 ± 0.005	0.150 ± 0.020
12	2.841 ± 0.100	0.200 ± 0.011	7.140 ± 0.481	0.455 ± 0.005	0.070 ± 0.101	0.325 ± 0.025	0.160 ± 0.010
13	4.321 ± 0.120	0.140 ± 0.012	15.265 ± 0.605	0.385 ± 0.005	0.175 ± 0.015	0.265 ± 0.005	0.285 ± 0.015
14	3.895 ± 0.095	0.265 ± 0.025	7.385 ± 0.035	0.725 ± 0.025	0.090 ± 0.011	0.220 ± 0.011	0.415 ± 0.015
15	3.711 ± 0.190	0.215 ± 0.005	7.135 ± 0.075	0.635 ± 0.035	0.085 ± 0.015	0.095 ± 0.005	0.260 ± 0.020
16	4.115 ± 0.515	0.275 ± 0.015	13.140 ± 0.291	0.821 ± 0.030	0.130 ± 0.011	0.210 ± 0.011	0.485 ± 0.005
17	3.765 ± 0.735	0.210 ± 0.022	4.360 ± 0.603	0.603 ± 0.020	0.085 ± 0.005	0.145 ± 0.005	0.265 ± 0.015
18	4.005 ± 0.205	0.145 ± 0.005	5.280 ± 0.060	0.365 ± 0.005	0.420 ± 0.011	0.215 ± 0.025	0.200 ± 0.020
19	4.112 ± 0.180	0.180 ± 0.023	7.600 ± 0.151	0.445 ± 0.045	0.045 ± 0.005	0.210 ± 0.011	0.340 ± 0.040
20	4.035 ± 0.235	0.225 ± 0.015	5.470 ± 0.421	0.615 ± 0.005	0.050 ± 0.001	0.155 ± 0.005	0.330 ± 0.011
21	1.865 ± 0.055	0.120 ± 0.012	3.420 ± 0.303	0.380 ± 0.020	0.030 ± 0.011	0.110 ± 0.011	0.125 ± 0.025
22	1.925 ± 0.025	0.115 ± 0.005	5.505 ± 0.275	0.430 ± 0.020	0.045 ± 0.001	0.060 ± 0.011	0.050 ± 0.011
23	2.265 ± 0.105	0.140 ± 0.026	6.345 ± 0.105	0.615 ± 0.015	0.105 ± 0.001	0.135 ± 0.015	0.160 ± 0.021
Overall	3.704 ± 0.159	0.189 ± 0.01	7.694 ± 1.013	0.656 ± 0.047	0.105 ± 0.017	0.254 ± 0.025	0.264 ± 0.017
p	0.001	0.001	0.001	0.001	0.001	0.001	0.001

SEM standard error of the mean

Table 2 Phenotypic similarities (%) between clones in terms of phenolic compounds of Kalecik Karası clones with cluster analysis and multidimensional scaling

Number of clusters	Similarity	Joined cluster		New cluster	Number of clones in new cluster
22	98.428	1	5	1	2
21	98.426	6	9	6	2
20	98.298	14	15	14	2
19	98.295	6	20	6	3
18	98.272	1	17	1	3
17	98.087	14	19	14	3
16	97.943	6	11	6	4
15	97.783	3	6	3	5
14	97.723	3	18	3	6
13	96.757	3	10	3	7
12	95.929	1	3	1	10
11	95.902	12	14	12	4
10	95.874	2	16	2	2
9	95.843	22	23	22	2
8	95.724	1	12	1	14
7	95.498	1	22	1	16
6	95.487	2	8	2	3
5	90.732	1	21	1	17
4	90.377	2	13	2	4
3	87.564	1	4	1	18
2	79.554	1	2	1	22
1	60.380	1	7	1	23

sis. Descriptive statistics were expressed as mean ± standard error of the mean for the obtained data. Hierarchical cluster analysis was performed to identify phenotypic similarities among the clones in terms of these characteristics. The average linkage and Euclidean distance methods were used for linkage and distance methods, respectively. In addition, multidimensional scaling was also applied to visualize the clusters on a two-dimensional map.

Results

There were significant differences ($p < 0.01$) among the phenolic compounds in the extracts of the 23 clones (Table 1). The phenolic compounds with the greatest amounts in all 23 clones were gallic acid (18.180 mg kg^{-1}) and protocatechuic acid (5.015 mg kg^{-1}), while *p*-coumaric acid (0.030 mg kg^{-1}) was present in the least amounts. For protocatechuic acid, the lowest mean was obtained from clone 21, 1.865 mg kg^{-1}, while the highest was from clone 2, 5.015 mg kg^{-1}. Moreover, the smallest mean values for vanillic acid were observed in clones 4, 5, 11, 13, 18, 21, 22, and 23 (0.115, 0.125, 0.140, 0.140, 0.145, 0.120, and 0.140 mg kg^{-1}, respectively). Similar results were obtained with gallic acid; indeed, the lowest value was in clone 4 (1.655 mg kg^{-1}), followed by clones 21, 17, 1, and 5. The gallic acid contents of the clones ranged from 1.655 mg kg^{-1} (clone 4) to 24.180 mg kg^{-1} (clone 7), and the overall mean was found to be 7.694 mg kg^{-1}. Furthermore, the overall syringic acid mean of the 23 clones was found to be 0.656 mg kg^{-1}; the highest mean was obtained from clone 4, at 1.235 mg kg^{-1}, while the lowest mean was obtained from clone 18, at 0.365 mg kg^{-1}. It is evident that *p*-cumaric acid

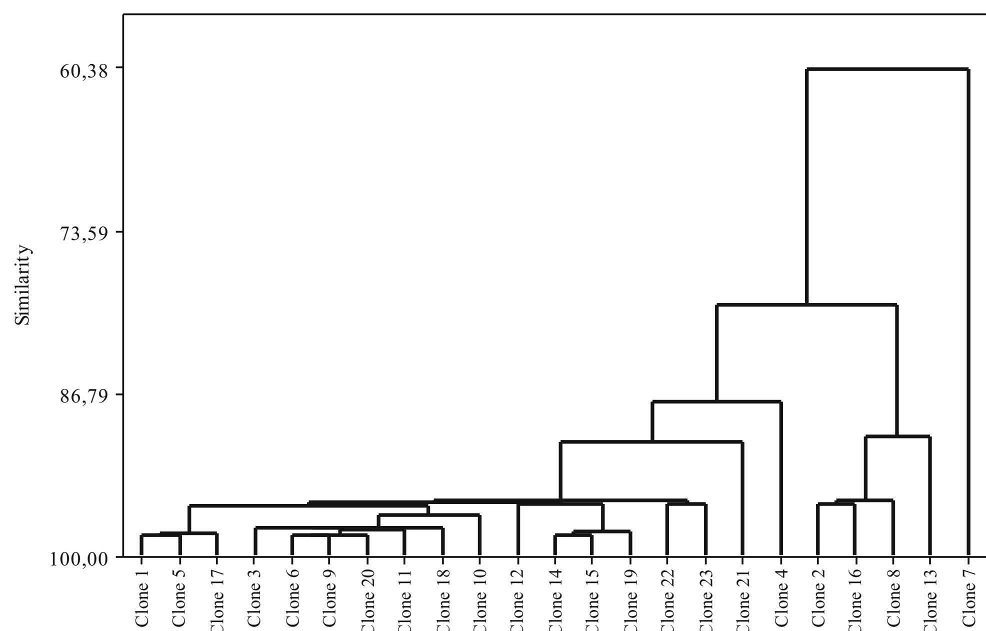

Fig. 1 Dendrogram for the phenotypic similarities among the Kalecik Karası clones

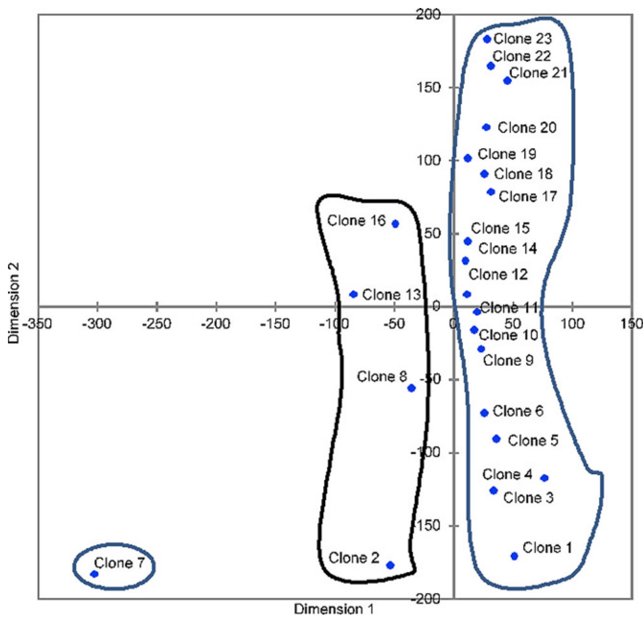

Fig. 2 Configuration of the Kalecik Karası clones on two-dimensional map

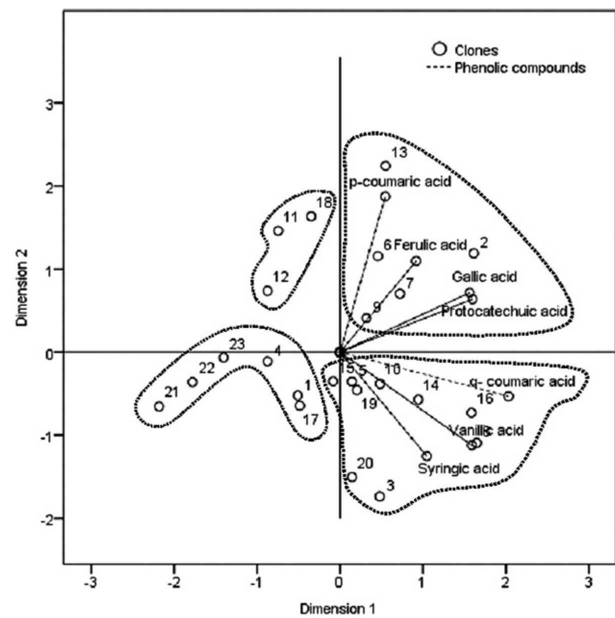

Fig. 3 Configuration of the Kalecik Karası and phenolic compounds on two-dimensional map

content was very low in all 23 clones, with the lowest mean values recorded compared to the other phenolics. For ferulic acid, clone 22 had the lowest mean, 0.06 $mg\,kg^{-1}$, while clone 6 had the highest, 0.555 $mg\,kg^{-1}$, and the overall mean of the clones was 0.254 $mg\,kg^{-1}$. Finally, the *q*-cumaric acid values of the clones changed between 0.050 $mg\,kg^{-1}$ (clone 22) and 0.485 $mg\,kg^{-1}$ (clone 16), with 0.264 $mg\,kg^{-1}$ the overall mean (Table 1).

Two sequential multivariate statistical techniques (cluster analysis and multidimensional scaling) were used to identify phenotypic similarities among the clones in terms of phenolic compounds due to no natural grouping of the Kalecik Karası clones. Based on the similarity dendrogram of the phenolic compounds, there was a high similarity rate among the 23 clones, with the similarity level among the clones ranging from 98.428% to 60.380%. The highest similarity, 98.428%, was observed between clones 1 and 5, followed by 98.298% for clones 6 and 9 and 98.298% for clones 14 and 15. Additionally, the lowest similarity value was determined between clones 1, 2, and 7 (Table 2). We conducted a cluster analysis to determine the appropriate number of clusters and to stop the agglomeration process for clones. In this context, 23 clones of Kalecik Karası were broadly classified into three major groups: 18 clones formed one group in cluster 1, while four clones (clones 2, 16, 8, and 13) were in cluster 2. However, clone 7 was in a different group (Fig. 1). Additionally, the configuration map of multidimensional scaling supported the cluster analysis results and showed that those clones were split into three different clusters, or 23 clones were grouped into three clusters on the two-dimensional configuration (Fig. 2).

The configuration of phenolic compounds and clones of the two-dimensional map formed four main groups (Fig. 3). Based on Fig. 3, *p*-coumaric, ferulic, gallic, and protocatechuic acids as well as clones 2, 6, 7, 9, and 13 were in the upper-left region, which is the positive part for both dimensions of the map. There were high positive correlations among these phenolic compounds. Similarly, clones 2, 6, 7, 9, and 13 were strongly and positively correlated with the cluster of these phenolic compounds. In the lower-right region of the configuration map, *q*-coumaric, vanillic, and syringic acids were grouped with clones 3, 5, 10, 14, 15, 16, 19, and 20. There were also high positive correlations between these phenolic compounds. Again, clones 3, 5, 10, 14, 15, 16, 19, and 20 were strongly and positively correlated with the cluster of these three phenolic compounds. According to the first dimension, there were negative correlations between the remaining clones and phenolic compounds (Fig. 3).

Discussion

Polyphenols present in the whole berry are mainly those extracted from the grape seed (60%–70% of total soluble phenolics) and, to a lesser extent, those extracted from the grape pulp (10%) and skin (28%–35%) (Shi et al. 2003). However, total soluble phenolic in grape berries are distributed in different parts of the berry, such as pulp, seed, and skin (about 23.8, 374.6 $mg\,g^{-1}$ and 2178.8 $mg\,g^{-1}$ gallic acid equivalent, respectively; Pastrana-Bonilla et al. 2003). Additionally, the polyphenolic profile in grape berries is

affected by different factors such as soil characteristics (Cheng et al. 2015); variety; clone climatic conditions and seasonal weather variations (Di Lecce et al. 2014; Đorđević et al. 2017); canopy management; environmental stress; agronomic practices of irrigation, soil management, and nutrition (Beslic et al. 2015); vine health status (Rusjan et al. 2012); and vineyard management and vineyard environmental conditions (Rodríguez-Montealegre et al. 2006). Our findings, which are consistent with those of previous studies, show that there were significant differences ($p < 0.01$) among the phenolic compounds of the 23 clones. In our study, the gallic acid content of the 23 clones was quite high compared to other phenolic compounds. Higher contents of gallic acid was the most important factor that led to the separation of clones 7, 13, 2, 16, and 8 from the other clones (Table 1). It was reported in previous studies that concentrations of gallic acid in white grape seeds ranged from 91.13 mg kg^{-1} DW (Petra) to 54.66 mg kg^{-1} DW (Welschriesling), while in red grape seeds concentrations were between 289.13 mg kg^{-1} DW (in Prokupac) and 78.10 mg kg^{-1} DW (in Merlot) (Gođevac et al. 2010). It has also been reported that the gallic acid value in the berries of Ekşikara grape variety varies between 22.23 mg/kg DW and 19.05 mg kg^{-1} DW at high and low altitudes (Coklar 2017). Similar findings were also reported by Breksa et al. (2010), who determined the phenolic profiles of 16 cultivars and selections of *Vitis vinifera*. Results for gallic acid content in the berries obtained herein agree with those reported in a previous publication, while gallic acid values found in our berry samples were lower when compared with the literature data since the whole berry (seed, skin, and pulp together) was analyzed (Rockenbach et al. 2011).

On the other hand, high contents of protocatechuic acid influenced the distinguishing of clones. The content of protocatechuic acid of clone 2 was three times higher in comparison with clones 21 and 22. Pantelić et al. (2016) reported that the protocatechuic acid content of seeds of the Cabernet Sauvignon, Merlot, Cabernet Franc, Shiraz, Sangiovese, Pinot Noir, Prokupac, Riesling, Petra, Sauvignon Blanc, Welschriesling, Chardonnay, and Pinot Gris grape cultivars were 1.02, 2.34, 0.82, 0.98, 0.95, 0.83, 1.43, 3.80, 0.97, 3.50, 1.88, 2.22, and 0.92 mg kg^{-1}, respectively. The protocatechuic acid content of the other clones, except for clones 9, 10, 12, 15, 17, 21, 22, and 23 were quite high compared to the results for Riesling cultivar reported in the literature (Pantelić et al. 2016). Additionally, the phenolic acids, including vanillic, ferulic, syringic, *p*-coumaric, and *q*-coumaric acids, showed differences among the clones. As compared with the other clones, the most abundant phenolic acids were determined in clones 8 and 16 as vanillic acid, in clones 3 and 4 as syringic acid, in clone 18 for *p*-coumaric acid, in clone 6 as ferulic acid, and in clone 15 as *q*-coumaric acid (Table 1). It was previously determined that the ferulic acid content of the seeds of Cabernet Sauvignon, Merlot, Cabernet Franc, Shiraz, Sangiovese, Pinot Noir, Prokupac, Riesling, Petra, Sauvignon Blanc, and Pinot Gris were 1.09, 0.65, 0.41, 0.78, 0.44, 2.14, 1.75, 2.66, 0.75, 1.65, 3.24 mg kg^{-1}, respectively (Pantelić et al. 2016). When our findings are compared with the results of that study, the ferulic acid content of the clones (because the full berry was analyxed, including skin, seed, and pulp) was quite high. In a study conducted on Karaerik grape variety clones, it was reported that *p*-coumaric acid content ranged from 0.05 mg l^{-1} (clones 13, 15, 19, and 23) to 0.06 mg l^{-1} (clones 18 and 30) (Karadogan and Keskin 2017). The values obtained for *p*-coumaric acid in other clones, except for clones 3, 5, 8, 19, 21, and 22, were slightly higher than previously reported ones, while the concentrations obtained for *p*-coumaric acid in clones 7, 10, 12, 14, 15, 17, and 20 were in the common range. Additionally, Karadogan and Keskin (2017) reported that ferulic acid content ranged from 0.04 mg l^{-1} (clone 15) to 0.05 mg l^{-1} (clones 13, 18, 19, 23, and 30), and in our findings the ferulic acid content of all clones was higher than previously published values.

Based on cluster analysis and multidimensional scaling results, there were more than 90% phenotypic similarities among clones, except for clones 1, 2, 4, and 7. In addition, phenotypic similarities were quite high (>98%) between clones 1 and 5, clones 6 and 9, clones 14 and 15, clones 6 and 20, clones 1 and 17, and clones 14 and 19 (Table 2). In our study, three groups of clones were formed in the score plot along the PC1 axis or a cluster (Fig. 1). Indeed, 18 of the 23 clones were distinguished from all clones by forming a separate group (group 1). Within this group, the clones 16, 13, 8, and 2 (group 2) were separated from the other clones; clone 7 (group 3) was further separated from the remaining 22 clones (Fig. 2). There were also four main groups according to the configuration of the phenolic compounds of the clones on the two-dimensional map. The separation of clones was strongly influenced along the PC1 axis by phenolic compounds (Fig. 3). There was a positive correlation between clones 2, 6, 7, 9, and 13 and the *p*-coumaric, ferulic, gallic, and protocatechuic acids. Similarly, there was also a positive correlation between clones 3, 5, 10, 14, 15, 16, 19, and 20 and the *q*-coumaric, vanillic, and syringic acids, while there were negative correlations between the remaining clones and phenolic compounds (Fig. 3). It may be stated that clones 3, 5, 10, 14, 15, 16, 19, and 20 are likely to be appropriate for selection as the superior clones in terms of *q*-cumaric, vanilic, and syringic acids. Similarly, clones 2, 6, 7, 9, and 13 can be considered superior for *p*-coumaric, ferulic, gallic, and protocatechuic acids. In fact, the differences and similarities between the phenolic compounds of the clones can be explained by their genetic intravarietal variability. The intravarietal variability has been reported to result from somatic mutations that oc-

cur at a very low rate in any cell division, including large deletions, point mutations, illegal recombinations, or variable numbers of repeats in microsatellite sequences (Pelsy et al. 2010). Considering that many grape varieties are vegetatively propagated, rare mutations are likely to accumulate for grapevines over the centuries, and these mutations explain the differences between the phenolic contents of the 23 clones. Additionally, although current clones are populations of very similar vines, it should be noted that they carry mutations in different chimerical states and in different regions of their DNA sequence.

Conclusions

This first report on the phenolic profile of the clones of Kalecik Karası, which is one of the leading red wine varieties of Turkey, revealed significant differences between the clones with respect to the content of protocatechuic, vanillic, gallic, syringic, *p*-coumaric, ferulic, and *q*-coumaric acids. The most abundant phenolic compounds in Kalecik Karası clones were protocatechuic acid and gallic acids, while *p*-coumaric acid was the lowest. There was a high phenotypic similarity among clones 1 and 5, clones 6 and 9, clones 14 and 15, clones 6 and 20, clones 1 and 17, and clones 14 and 19 for phenolic compounds, while this similarity was lower between the clones 1, 2, 4, and 7. In the study, the similarity level in 22 clones of Kalecik Karası was about 80% except for clone 7. By adding clone 7 into this group, the similarity level decreased to about 60%. According to the cluster analysis results, there was approximately 40% phenotypic variation among clones. There was also a positive correlation between clones 3, 5, 10, 14, 15, 16, 19, and 20 and the *q*-coumaric, vanillic, and syringic acids, as well as between clones 2, 6, 7, 9, and 13 and the *p*-coumaric, ferulic, gallic, and protocatechuic acids. Taking this all together, clones 2, 6, 7, 9, and 13 for for *p*-coumaric, ferulic, gallic, and protocatechuic acids and clones 3, 5, 10, 14, 15, 16, 19, and 20 for *q*-cumaric, vanilic, and syringic acids can be considered as superior clones. Thus, it can be stated that multivariate methods can be used for clonal selection, and exclusive clones can be selected with high values of phenolic compounds in the future. In addition, further studies regarding phenolic compounds could be greatly helpful for clonal selection.

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Conflict of interest N. Keskin, B. Kunter, H. Celik, O. Kaya, and S. Keskin declare that they have no competing interests.

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