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Phytochemical Variation of Native Apple Germplasm Resources from the Eastern Black Sea Region, Turkey

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Abstract

In order to contribute more to human health, revealing the nutritional potential of fruit germplasm sources that have rich phytochemical compounds has gained importance today and has become one of the breeding objectives of various fruit species. Many nutritional components of wild apples have been considered as useful sources for apple breeding efforts, but studies on the phytochemical compounds of native apple genetic resources grown in local areas are limited. This study was carried out to investigate the phytochemical compounds of 19 native apple genotypes grown in Giresun in the eastern Black Sea region of Turkey and to compare them with those of three commercial varieties ('Granny Smith', 'Fuji', and 'Royal Gala'). The total phenolic contents of the genotypes ranged from 141.7 mg per 100 g (Yesilsut) to 1036.8 mg per 100 g (Cipir). Antioxidant activity was determined to be between 505.6 µmol per 100 g (Ahmet) and 5041.8 µmol per 100 g (Cipir). The total flavonoids ranged between 11.2 mg per 100 g (Beyaz) and 95.3 mg per 100 g (Maden). Uzun had the highest content of malic acid (15.33 g l^{-1}) and tartaric acid (1.008 g l^{-1}). The highest values for succinic acid and oxalic acid were detected in Cipir (1.192 g l^{-1} and 0.484 g l^{-1}, respectively). Most of the native apple genotypes had higher levels of phytochemical compounds than those of standard apple cultivars. Principal component analysis showed that the phytochemical components could effectively explain the variability among the native apple genotypes, which exhibited wide variation in terms of phytochemical compounds. Most genotypes contained higher levels of phytochemical compounds than standard apple cultivars. The data imply that the native apple genotypes are an important source of phytochemical compounds and that native apple genotypes with higher contents of bioactive compounds can be used as genetic material for apple breeding programs. They might contribute to the development of new apple cultivars with enhanced health benefits.

Keywords Apple · Breeding · Phenolics · Antioxidant · Organic acids

Phytochemische Variation einheimischer Apfelkeimplasma-Ressourcen aus der östlichen Schwarzmeerregion, Türkei

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Introduction

Apple (*Malus* x *domestica* Borkh.) is the main fruit crop of temperate zones all over the world. Turkey, one of the leading apple producers, is among the origin centers and spreading areas of apple. Apple cultivation in Turkey is not limited to only temperate regions but extends to higher subtropical areas along the Mediterranean and Aegean Sea coasts. Today, there are many districts, neighborhoods, and villages in Turkey that have taken their name from apples. Most regions of the country have valuable apple genetic resources, but they are increasingly diminishing for various reasons and even face the danger of disappearance. A large

number of wild apple populations, especially in the Black Sea and northeastern regions of the country, are critical for conserving diversity in the gene pool (Ercisli 2004). Numerous local apple genotypes have been successfully grown without using pesticides despite the high rainfall and humidity in the coastal line of the eastern Black Sea region of northeastern Anatolia (Dumanoglu et al. 2018).

Although apple shows high genetic diversity (Cornille et al. 2014), its cultivation all over the world is carried out with a small number of varieties. This situation causes a limited number of commercial varieties to gradually replace the well-adapted old and local varieties, narrowing the genetic base and loss of genetic diversity (Urrestarazu et al. 2012; Lassois et al. 2016). In addition, traditional apple cultivars exhibit higher genetic (Gasi et al. 2010), morphological (Gasi et al. 2011), and phytochemical (Ma et al. 2015) diversity than modern international cultivars. In Turkey, studies on native apple germplasm resources, which mainly focused on investigating the breeding qualities, showed the existence of numerous local and old varieties distributed to different regions (Pirlak et al. 2003; Karlidag and Esitken 2006; Bostan and Acar 2009; Islam et al. 2009; Yarilgac et al. 2009; Karadeniz et al. 2013; Kirkaya et al. 2014; Kaya at al. 2015; Senyurt et al. 2015; Nas et al. 2019).

Indeed, apple is a useful and popular fruit for human health. Containing many sugars, acids, phenolic compounds, and antioxidants, the apple fruit constitutes an important part of the human diet (Wu et al. 2007). It is a significant source of dietary polyphenols (Lata and Tomala 2007). Flavonoids make up the majority of apple phenols (Panzella et al. 2013). Many studies (Planchon et al. 2004; Khanizadeh et al. 2008; Wojdylo et al. 2008; Vieira et al. 2011; Celik et al. 2018) have emphasized the beneficial influences of apple on human health. These influences are mainly attributed to the antioxidants it contains (Boyer and Liu 2004). Apple fruit plays an important role in prevention of and protection against various diseases in humans, such as cancer, asthma, diabetes, and cardiovascular disorders (Wardlaw et al. 2004). The amounts of bioactive compounds such as antioxidants and phenolics in apple depend on the variety, genotype, ecological conditions, fruit maturity, and harvest season (Lata 2007; Drogoudi et al. 2008). In addition, local varieties evoke ancient flavors and attract consumers today (Panzella et al. 2013).

However, the bioactive compounds of native apple germplasm resources have not been extensively studied (Ma et al. 2015). It has been reported that fruits of local apple varieties contain higher amounts of bioactive compounds than those of the 'Granny Smith', 'Red Delicious', 'Fuji', and 'Royal Gala' cultivars, and varieties richer in bioactive compounds should be utilized as genetic resources in future breeding programs (Vrhovsek et al. 2004; Ma et al. 2015). Iacopini et al. (2010) determined that fruits of old local apple varieties had higher levels of phenolic compounds and antioxidant activity than 'Golden Delicious' and 'Stark Delicious.' The local apple varieties exhibited a much higher phenol content than widely consumed varieties (Panzella et al. 2013).

The trend of consumers to seek healthy lifestyles has laid the groundwork for various studies to improve the nutritional properties of fruits through breeding (Bliss 1999). Development of fruit cultivars with enhanced health benefits is among the major goals of fruit breeding. In order to contribute more to human health, revealing the nutritional potential of fruit germplasm sources that have rich phytochemical compounds has gained importance today and has become one of the breeding objectives for various fruit species. Many nutritional components of wild *Malus* species have been considered as useful sources for apple breeding efforts (Ma et al. 2015; Fang et al. 2017).

Apple genetic resources should be conserved for future breeding efforts (Urrestarazu et al. 2012; Gasi et al. 2013) and future generations. In addition, preserving biodiversity requires the selection of apple genotypes with high bioactive compound potential (Jakobek and Barron 2016). Apple germplasm resources should be evaluated not only for fruit and plant characteristics but also for phytochemical components. Evaluating the phytochemical characteristics of local and old apple genetic resources is a prerequisite for proper utilization of these genetic resources in breeding efforts (Ma et al. 2015). The main objective of this study was to determine the phytochemical components—including total phenolics, antioxidant activity, total flavonoids, and organic acids—for future breeding efforts in 19 native apple genotypes grown in the Giresun province located in the eastern Black Sea region of Turkey.

Materials and Methods

Plant Materials

The study material consisted of 19 old apple genotypes grown since ancient times in the district of Yağlidere in the Giresun province in the eastern Black Sea region of northern Anatolia. The local names of the old apple genotypes are Demir, Pasli, Uzun, Katir 1, Katir 2, Ahmet, Tatli, Cipir, Tarak, Sut, Kavni, Yesil Sut, Cingirak, Mum, Beyaz, Cal, Mis, Maden, and Sinap. They were investigated in comparison with 'Granny Smith', 'Fuji', and 'Royal Gala' apple cultivars. The research area, which takes its name from the Yaglıdere River and is located where the river flows into the Black Sea, is located between latitude 40° 51′ 53″ N and longitude 38° 37 38 24″ E. The length of the river

is about 65 km. In the research area, the altitude is 50 m, and the average annual temperature is 14 °C. The coldest month is February (down to –3 °C), and the warmest month is August (average temperature 27 °C). The annual rainfall average is 1300 mm, and relative humidity is 70% (TSMS 2020).

Reagents and Standards

Purified water was obtained using the Water Story Mini Pure II system (MDM, South Korea). Acetonitrile, methanol (MeOH), and ethanol (EtOH) were obtained from Merck Millipore (Germany). Trolox, gallic acid, quercetin, and 1,1-diphenyl-2-picryl-hydrazil (DPPH·) for bioactive compounds and standards of malic acid, succinic acid, oxalic acid, citric acid, and tartaric acid were purchased from Sigma-Aldrich (Germany).

Some Fruit Characteristics of Apple Genotypes

To represent the whole tree for each genotype and cultivar investigated, a total of 20 fruit samples were randomly hand-harvested from four different locations on the tree. The fruits were weighed using a digital balance (±0.01 g) (AS 220/C/2, Radwag, Poland), and their diameters were measured using a digital caliper (±0.01 mm) (CD-15CP, Mitutoyo, Japan). Total soluble solids content was determined with a digital refractometer (PAL-1, Atago, Japan) in fruit juice. For titratable acidity, 10 ml of extract was taken from each sample, 10 ml of distilled water was added, and sodium hydroxide (NaOH) was titrated to increase the pH of the samples to 8.1. Fruit flesh firmness was measured using the 11.1-mm penetrating tip of an Effegi penetrometer (FT-327, McCormick Fruit Tech, Yakima, WA, USA), and the measurements were expressed in newtons. Fruit shape was determined according to the scale of Watkins and Smith (1982). Fruit skin color was determined to be either green, light green, light yellow, red, or light red. After an ascorbic acid test strip was dipped into the fruit juice for 2 s for ascorbic acid analysis, the reflectometer set (RQflex 10 Plus, Merck, Germany) was started. The test strip was shaken to remove excess liquid, and after an 8-s waiting period, the reading was done until the end of the 15th second. The resulting value was expressed as milligrams per 100 g.

Bioactive Compounds

For bioactive compounds, 10 apples from each tree examined in the study were randomly hand-harvested at harvest date. A sample slice was cut with a stainless steel knife from each of the 10 fruits of each apple genotype, and the bioactive compounds in the flesh were determined. Total phenolic, total antioxidant (ferric reducing antioxidant power [FRAP] assay), total flavonoid, and organic acid (malic acid, succinic acid, oxalic acid, citric acid, and tartaric acid) contents were measured. For biochemical analyses, the fruits were placed into tubes and kept at –20 °C until the analysis. Samples were thawed at room temperature (21 °C). The samples were then homogenized, and a 2.0-g sample was extracted in 10 ml methanol in a Falcon tube for 2 d. The resulting solution was centrifuged at 6000 rpm at 4 °C for 10 min.

Total Phenolic Contents

A portion of 600 µl from each sample was diluted with 4.0 ml distilled water, and 100 µl Folin–Ciocalteu reagent was added. After an interval of 3 min, 2% sodium carbonate (Na_2CO_3) was added to 300-µl portions, and the mixture was vortexed and incubated for 30 min. After the absorbance values were detected using an ultraviolet-visible (UV-Vis) spectrophotometer (Shimadzu, Kyoto, Japan) at 760 nm, they were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of fresh weight (mg GAE per 100 g fw) (Beyhan et al. 2010).

Total Flavonoid Contents

A portion of 500 µl from each sample was diluted with 3.8 ml methanol, and 100 µl ammonium acetate ($C_2H_7NO_2$) was added, followed by the addition of 100 µl ammonium nitrate (NH_4NO_3). The mixture was vortexed and incubated for 40 min, and then the absorbance of the mixture was measured at 415 nm. The results were expressed as milligrams of quercetin equivalents (QE) per 100 g of fresh weight (mg QE per 100 g fw) (Chang et al. 2002).

FRAP Assay

The antioxidant activity of fresh fruit was determined according to the method of Benzie and Strain (1996). A portion of 250 µl from each sample was diluted with 1.0 ml monosodiumphosphate (NaH_2PO_4), and 1.25 ml potassium ferric cyanide (K3[Fe(CN)6]) was added. After incubation for 30 min, 1.25 ml trichloroacetic acid ($C_2HCl_3O_2$) and 0.25 ml iron trichloride ($FeCl_3$) were added. Then the absorbance of the mixture was measured at 700 nm. The results were expressed as micromoles of Trolox equivalents (TE) per 100 g of fresh weight (µmol TE per 100 g fw).

Organic Acids

The organic acid content of native apple genotypes was determined by modifying the method of Ma et al. (2015). Fresh apple samples were homogenized, and a 0.5-g sample was extracted in 5 ml methanol in a test tube for 8 h. Pre-

pared samples were filtered using a 0.45-mm syringe filter. After filtration, the samples were injected into a high-performance liquid chromatography system (CTO-20A, Shimadzu, Kyoto, Japan) for analysis. Organic acids were detected by using an Agelent Hi-Plex H (C18, 7-µm particle size, 300 mm × 7.7 mm, kept at 55 °C) at 210 nm wavelength via UV-Vis detector (SPD M20A). The mobile phase was 0.02 M KH_2PO_4 solution with pH of 2.4. The mobile phase flow rate was set at 0.6 ml min^{-1}. Malic acid, succinic acid, oxalic acid, citric acid, and tartaric acid as organic acids were determined. The obtained results were expressed as grams per liter.

Statistical Analysis

The data were analyzed by analysis of variance using JMP 10 software. Differences among means were determined using the least significant difference multiple-comparison test at $P < 0.05$. Principal component analysis and component plot analysis based on PC1, PC2, and PC3 and cluster analysis were performed using IBM SPSS 23.0 software. Cluster analysis of bioactive compounds was performed using a hierarchical clustering method based on Euclidean distances and Ward's method.

Results and Discussion

Some Fruit Characteristics

Fruit size is an important trait that influences consumer preferences. Fruit weight and dimensional attributes are parameters affecting the fruit size. Dimensional attributes can be used in cultivar descriptions, classification of fruit, and description of fruit shape (Beyer et al. 2002). In the apple genotypes investigated, fruit weight was determined to be between 48.9 g (Mum) and 172.5 g (Sinap). Fruit diameter ranged from 49.6 mm (Mum) to 76.1 mm (Sinap) (Table 1). Regarding fruit weight and fruit diameter, the genotypes Sinap, Pasli, Maden, and Beyaz had the closest values to

Table 1 Fruit characteristics of native apple genotypes and commercial apple cultivars

Genotype	Fruit weight (g)		Fruit diameter (mm)		Total soluble solids (%)		Titratable acidity (%)		Ascorbic acid (mg per 100 ml)	
Ahmet	56.0	jkl[a]	53.7	klm	10.2	j	0.35	g	21.6	de
Beyaz	126.8	d	70.2	cd	10.8	i	0.53	ef	17.2	f
Cal	53.1	kl	51.5	lm	12.5	fg	0.70	c	21.7	de
Cingirak	76.9	hi	58.5	g-j	15.1	a	0.26	h	25.5	a
Çıpır	65.1	ijk	57.0	h-k	11.9	h	0.21	hij	22.9	cd
Demir	107.3	ef	61.6	fg	13.2	de	0.95	a	25.1	ab
Katir-1	66.7	ij	53.0	klm	13.9	bc	0.58	de	23.5	bcd
Katir-2	75.9	hi	53.2	klm	13.7	c	0.56	ef	17.5	f
Kavni	66.9	ij	54.6	jkl	13.6	cd	0.21	hij	23.1	cd
Maden	137.4	d	71.2	cd	12.9	ef	0.62	d	14.6	g
Mis	113.7	e	60.3	fgh	14.2	b	0.78	b	22.9	cd
Mum	48.9	l	49.6	m	13.9	bc	0.69	c	25.3	ab
Pasli	152.3	c	76.0	ab	13.9	bc	0.69	c	20.8	e
Sinap	172.5	b	76.1	a	13.8	bc	0.17	j	20.1	e
Sut	93.3	g	67.0	de	13.2	de	0.21	hi	17.9	f
Tarak	94.0	g	61.7	fg	11.8	h	0.52	f	24.3	abc
Tatli	88.9	gh	60.0	f-i	13.7	c	0.16	j	18.0	f
Uzun	68.4	ij	55.9	ijk	13.3	de	0.98	a	22.7	cd
Yesilsüt	97.5	fg	63.6	ef	13.9	bc	0.24	hi	14.7	g
Cultivar										
'Granny Smith'	187.2	a	77.1	a	10.3	j	0.79	b	11.7	h
'Fuji'	170.1	b	71.8	bc	12.3	g	0.20	ij	8.6	i
'Royal Gala'	162.4	bc	71.3	c	11.7	h	0.21	hij	8.1	i
Significance	***	–	***	–	***	–	***	–	***	–
LSD (0.05)	13.23	–	4.23	–	0.43	–	0.05	–	1.92	–

[a]Differences between mean values shown on the same line with the same letter are not significant ($P < 0.05$)
LSD least significant difference

Table 2 Firmness, fruit shape, fruit skin color, harvest time, and harvest season of native apple genotypes and commercial apple cultivars

Genotype	Firmness (N)	Fruit shape	Fruit skin color	Harvest time	Harvest season
Ahmet	94	Obloid	Light red	17 October	Late
Beyaz	84	Obloid-globose	Green	15 October	Late
Cal	100	Ellipsoid	Light red	15 October	Late
Cingirak	85	Conical	Light yellow	27 September	Late
Cipir	105	Obloid	Light red	20 October	Late
Demir	114	Ellipsoid	Light red	17 October	Late
Katir-1	101	Ellipsoid	Red	20 October	Late
Katir-2	109	Conical	Light red	15 October	Late
Kavni	84	Obloid-globose	Light red	29 September	Late
Maden	65	Obloid-globose	Light red	29 September	Late
Mis	114	Conical	Light red	17 October	Late
Mum	96	Obloid	Green	20 October	Late
Pasli	96	Obloid	Green	13 October	Late
Sinap	62	Globose-conical	Light red	29 September	Late
Süt	77	Obloid-globose	Light red	5 October	Late
Tarak	92	Globose-conical	Light red	15 October	Late
Tatli	87	Globose-conical	Light red	10 September	Medium
Uzun	103	Globose-conical	Green	17 October	Late
Yesilsüt	79	Obloid-globose	Light red	15 October	Late
Cultivar					
'Granny Smith'	87	Obloid-globose	Green	15 October	Late
'Fuji'	79	Globose	Light red	15 October	Late
'Royal Gala'	70	Obloid-globose	Light red	10 September	Medium

N newtons

those of commercial apple cultivars. Soluble solids content and titratable acidity are important characteristics affecting fruit taste (Kumar et al. 2018). Consumers have different preferences for sweet or sour apples. Therefore, these characteristics may be important during the evaluation of fruit quality for consumers. In the present study, the total soluble solids content was detected to be between 10.2% (Ahmet) and 15.1% (Cingirak). Titratable acidity was between 0.17% (Sinap) and 0.98% (Uzun) (Table 1).

Ascorbic acid, an important antioxidant source promoting human health, is an important quality parameter for apple fruit (Lata and Tomala 2007). In the present study, Cingirak (25.5 mg per 100 ml), Mum (25.3 mg per 100 ml), Demir (23.5 mg per 100 ml), and Tarak (24.3 mg per 100 ml) genotypes had the highest values of ascorbic acid. The lowest value was found in Maden (14.6 mg per 100 ml). Ascorbic acid content was ranged from 8.1 ('Royal Gala') to 11.7 ('Granny Smith') mg per 100 ml in standard apple cultivars (Table 1). Ascorbic acid contents have been reported to be between 11.6 and 35.3 mg per 100 ml in different apple cultivars (Kevers et al. 2011), between 11.4 and 14.2 mg per 100 ml in apple cultivars grown in Pakistan (Maqsood et al. 2013), between 22.2 and 43.5 mg per 100 ml in standard apple cultivars in Turkey (Okatan et al. 2018), and between 50.4 and 134.4 mg per 100 ml in old and new apple cultivars grown in Germany (Kschonsek et al. 2018). Finding great variation in ascorbic acid content among 457 apple accessions, Fang et al. (2017) reported that wild apples contained significantly higher levels of ascorbic acid than cultivated apples. Planchon et al. (2004) found that old apple varieties had higher ascorbic acid content than commercial apple cultivars and also reported that genotypes with high ascorbic acid content are important for apple breeding programs. In the present study, all apple genotypes had higher ascorbic acid content than the standard apple cultivars. Therefore, they might contribute to breeding efforts as sources of ascorbic acid.

Firmness is one of the quality parameters of apple, affecting the preferences of apple consumers. Consumers prefer firmer apples, and in the apple genotypes investigated, firmness varied from 62 N (Sinap) to 114 N (Demir and Mis) (Table 2). Fruit shape and color are also characteristics affecting consumer decisions (Beyer et al. 2002). The apple genotypes showed wide variation in fruit shape and color (Table 2).

Total Phenolic and Total Flavonoid Contents

Phenolic compounds, which make important contributions to antioxidants, constitute part of the secondary metabolites

in the plant. For plants, phenolics and flavonoids are necessary components for withstanding biotic and abiotic stress (Kschonsek et al. 2018). They also play important roles in prevention of and protection against various diseases in humans, including cancer, asthma, arteriosclerosis, and heart disease (Wardlaw et al. 2004). The quantities of phenolic compounds and their antioxidant capacities are significant in terms of evaluation of apple varieties. Although there have been many studies on the total phenolics content and antioxidant activity of the commercial apple cultivars, data are still limited on old varieties grown in local regions (Jakobek et al. 2013). While commercial apple varieties are grown for appearance, size, and flavor, local apple varieties are characterized by higher amounts of phenolic compounds (Wojdylo et al. 2008; Jakobek et al. 2013).

The total phenolic content (TPC) of native apple genotypes and standard apple cultivars showed significant variations ($P < 0.05$) (Table 3). In the genotypes, the highest TPC value was obtained from Cipir (1036.8 mg per 100 g), followed by Mis, Cal, and Maden genotypes (1029.1, 960.7, and 892.4 mg per 100 g), respectively. The lowest TPC value was found in Yesilsüt (141.7 mg per 100 g). The TPC of standard apple cultivars was determined to be between 318.5 mg per 100 g ('Royal Gala') and 567.8 mg per 100 g ('Granny Smith'). Most of the native apple genotypes had clearly higher TPC content than the standard apple cultivars (Table 3). The TPC values in many apple varieties and genotypes have been reported as 194.0–479.0 mg per 100 g by Khanizadeh et al. (2008), 105.5–2269.8 mg per 100 g by Vieira et al. (2009), 164.1–472.7 mg per 100 g by Rop et al. (2011), 265.1–686.0 mg per 100 g by Jakobek et al. (2013), and 164.8–472.7 mg per 100 g by Wang et al. (2015). In this study, the TPC of apple genotypes was higher than in some apple varieties and genotypes reported by many researchers. With higher TPC, the genotypes Cipir, Mis, Cal, and Maden were remarkable. They can be used as a good source of total phenolics and genetic material for apple breeding programs. Many studies have noted that the TPC of the fruit can be influenced by genetic factors as well as by the growing ecology (McGhie et al. 2005; Vieira et al. 2011).

There were significant differences related to total flavonoid content (TFC) between native apple genotypes and standard apple cultivars ($P < 0.05$) (Table 3). In ad-

Table 3 Total phenolics, total flavonoids, and antioxidant activity (FRAP) of native apple genotypes compared with commercial cultivars

Genotype	Total phenolics (mg per 100 g)		Total flavonoids (mg per 100 g)		FRAP (μmol per 100 g)	
Ahmet	164.6	n[a]	13.9	jk	505.6	r
Beyaz	209.2	m	11.2	l	598.3	q
Cal	960.7	b	40.1	d	4589.5	b
Cingirak	634.1	h	41.2	d	1857.5	ı
Cipir	1036.8	a	65.4	b	5041.8	a
Demir	837.9	d	27.2	g	2978.3	f
Katir-1	153.2	no	14.9	j	522.5	r
Katir-2	621.8	h	23.9	h	1852.8	ı
Kavni	224.0	m	18.7	ı	770.5	o
Maden	892.4	c	95.3	a	4282.0	c
Mis	1029.1	a	32.0	f	4035.1	d
Mum	697.9	g	44.4	c	2707.6	h
Pasli	879.7	c	40.5	d	3752.9	e
Sinap	321.8	l	24.5	h	1070.2	m
Süt	422.9	k	31.6	f	1277.3	k
Tarak	755.6	f	35.3	e	2808.6	g
Tatli	416.7	k	23.2	h	1181.0	l
Uzun	791.3	e	37.1	e	2834.9	g
Yesilsüt	141.7	o	14.4	jk	512.9	r
Cultivar						
'Granny Smith'	567.8	ı	14.9	j	1607.2	j
'Fuji'	454.8	l	13.9	kl	992.0	p
'Royal Gala'	318.5	j	12.6	jk	663.3	n
Significance	***	–	***	–	***	–
LSD (0.05)	17.77	–	1.21	–	33.23	–

[a]Differences between mean values shown on the same line with the same letter are not significant ($P < 0.05$)
FRAP ferric reducing antioxidant power assay, *LSD* least significant difference

Table 4 Organic acid contents of native apple genotypes and commercial apple cultivars

Genotype	Organic acid (g l^{-1})									
	Malic acid		Succinic acid		Oxalic acid		Citric acid		Tartaric acid	
Ahmet	8.27	j[a]	0.306	gh	0.062	o	0.248	h	Nd	–
Beyaz	7.15	m	0.176	k	0.081	n	0.215	h	0.218	g
Cal	13.66	d	0.716	c	0.006	p	Nd	–	0.923	b
Cingirak	7.16	m	0.464	f	0.216	d	0.705	b	0.316	f
Cipir	7.28	l	1.192	a	0.484	a	Nd	–	Nd	–
Demir	15.06	b	0.551	d	Nd	–	Nd	–	0.948	b
Katir-1	8.92	h	0.512	def	0.177	e	0.498	d	0.287	f
Katir-2	7.92	k	0.260	hij	0.227	c	Nd	–	0.440	e
Kavni	4.54	q	0.350	g	0.138	ghi	0.430	ef	0.204	g
Maden	10.09	f	0.487	ef	0.119	k	0.347	g	0.048	i
Mis	11.28	e	0.229	ijk	0.234	c	Nd	–	Nd	–
Mum	14.27	c	1.040	b	0.331	b	1.736	a	Nd	–
Pasli	9.48	g	0.239	ij	0.061	o	0.312	g	Nd	–
Sinap	3.75	r	0.212	jk	0.130	hij	0.232	h	0.130	h
Süt	3.82	r	0.257	hij	0.093	m	0.323	g	Nd	–
Tarak	9.00	h	0.562	d	0.006	p	0.393	f	0.607	c
Tatli	5.08	no	0.275	hi	0.142	g	0.311	g	0.060	i
Uzun	15.33	a	0.748	c	0.140	gh	0.468	de	1.008	a
Yesilsüt	5.00	op	0.300	gh	0.129	ij	0.541	c	Nd	–
Cultivar										
'Granny Smith'	8.58	i	0.512	def	0.155	f	0.693	b	0.403	e
'Fuji'	5.16	n	0.560	d	0.125	jk	0.481	d	0.522	d
'Royal Gala'	4.97	p	0.534	de	0.107	l	0.464	de	0.430	e
Significance	***	–	***	–	***	–	***	–	***	–
LSD (0.05)	0.094	–	0.054	–	0.009	–	0.040	–	0.043	–

[a]Differences between mean values shown on the same line with the same letter are not significant ($P < 0.05$)
LSD least significant difference, *Nd* not detected

dition, great variability in TFC was observed among the native apple genotypes. The TFC was highest (95.3 mg per 100 g) in Maden, followed by the Cipir and Mum genotypes (65.4 and 44.4 mg per 100 g), respectively. The lowest TFC was found in Beyaz (11.2 mg per 100 g). The TFC in standard apple cultivars ranged between 12.6 mg per 100 g ('Royal Gala') and 14.9 mg per 100 g ('Granny Smith'). Standard apple cultivars had lower values of TFC than most of the native apple genotypes (Table 4). In previous studies, the TFC was detected to be 16.0 mg per 100 g in 'Limoncella' apple variety (D'Abrosca et al. 2007), 2.1–22.3 mg per 100 g in apple genotypes and 0–9 mg per 100 g in apple cultivars (Khanizadeh et al., 2008), 7.9–28.3 mg per 100 g in various apple varieties from Brazil (Vieira et al. 2011), 8.5–145.5 mg per 100 g in old local apple varieties (Jakobek et al. 2013), and 121.5–304.5 mg per 100 g in many varieties (Wang et al. 2015). The results showed that Cipir and Maden are also a good source of total flavonoids and can be used as genetic material in nutritional improvement studies of apple. In addition, the TFC values in the present study agree with those of previous studies, except for the study done by Wang et al. (2015). Vieira et al. (2011) reported that the composition and concentration of TFC in apple are influenced by ecological conditions and genetic structure. In addition, Wojdyło et al. (2008) reported that the sunshine period and overnight temperature during maturity might also affect the TFC.

Antioxidant Activity

The antioxidant activity of foods is a significant attribute that consumers focus on (Leguchi et al. 2015), and fruit species that have high antioxidant activity increase the interest of consumers. Antioxidants decrease the risk of diseases induced by oxidative stress related to the formation of an excessive number of reactive oxygen species (Valko et al. 2007). In this study, according to the FRAP assay, significant differences were found between native apple genotypes and standard apple cultivars in terms of antioxidant activity ($P < 0.05$; Table 3). Cipir (2529.4 µmol per 100 g) accumulated higher levels of antioxidant than the other apple genotypes investigated, followed by Cal, Maden, and Mis

genotypes (4589.5, 4282.0, and 4035.1 µmol per 100 g, respectively). The lowest antioxidant activity was found in Ahmet (506.6 µmol per 100 g). The antioxidant activities of standard apple cultivars ranged from 663.3 µmol per 100 g ('Royal Gala') to 1607.2 µmol per 100 g ('Granny Smith'). The obtained results showed that most apple genetic resources had clearly greater antioxidant activity than the standard apple cultivars (Table 3). In relevant studies conducted in various countries, the antioxidant activities, according to the FRAP assay, of various apple genotypes and varieties have been reported to be within ranges of 323–1246.0 µmol per 100 g (Khanizadeh et al. 2008), 335.2–739.9 µmol per 100 g (Vieira et al. 2009), 13.5–127.9 µmol per 100 g (Wojdylo et al. 2008), and 140.2–261.7 µmol per 100 g (Vieira et al. 2011). The antioxidant activities of some native apple genotypes in the present study were markedly higher than those reported by other researchers. In particular, the genotypes Cipir, Cal, and Maden, because of their high antioxidant activities, might be good sources of natural antioxidants. As is known, the breeding of varieties with high antioxidant activity is one of the most important objectives of breeding programs. Leguchi et al. (2015) reported that fruit genetic resources with high antioxidant activity can be used as health foods and breeding materials in fruit breeding. The findings indicate that the genotypes Cipir, Cal, and Maden could be utilized as genetic material for apple breeding programs. In addition, it has been reported that the antioxidant activity of apple genotypes can be influenced by various factors such as genetic structure (Vieira et al. 2011), growing season, and ecological conditions (McGhie et al. 2005).

Organic Acids

Organic acids that impact the organoleptic quality of the fruit have a significant effect on fruit taste and aroma. They are extremely important in the protection of human health (Wu et al. 2007; Kumar et al. 2018). In recent years, the selection of genetic materials with high organic acid content has become one of the most important goals of fruit tree breeding programs (Basha et al. 2012). Malic acid is the main acid in apple fruit. It accounts for about 90% of total organic acids (Zhang et al. 2010) and also highly affects fruit flavor. Organic acid content can be influenced by many factors, such as cultivar, genotype, growing season, technical and cultural practices (Hudina and Stampar 2006), and ecological and genetic factors (McGhie et al. 2005; Vieira et al. 2011).

With respect to malic acid, succinic acid, oxalic acid, citric acid, and tartaric acid content, significant differences ($P < 0.05$) were found between apple genotypes and cultivars in the present study (Table 4). In apple genotypes, malic acid content was higher than other organic acid contents. Uzun (15.33 g l^{-1}), Demir (15.06 g l^{-1}), Mum (14.27 g l^{-1}), and Cal (13.66 g l^{-1}) genotypes had the highest malic acid contents, whereas the lowest value of malic acid was detected in Sinap (3.75 g l^{-1}). The highest value of succinic acid was determined to be 1.192 g l^{-1} and was found in Cipir, while the lowest value was 0.176 g l^{-1} and found in Beyaz. The highest values of oxalic acid, citric acid, and tartaric acid were 0.484 g l^{-1} (Cipir), 1.736 g l^{-1} (Mum), and 1.008 g l^{-1} (Uzun), respectively. The standard apple cultivars had a range of 4.97 ('Royal Gala') to 8.58 ('Granny Smith') g l^{-1} for malic acid, 0.512 ('Granny Smith') to 0.560 ('Fuji') g l^{-1} for succinic acid, 0.107 ('Royal Gala') to 0.155 ('Granny Smith') g l^{-1} for oxalic acid, 0.464 ('Royal Gala') to 0.693 ('Granny Smith') g l^{-1} for citric acid, and 0.403 ('Granny Smith') to 0.522 ('Fuji') g l^{-1} for tartaric acid (Table 4).

In previous studies, Wang et al. (2010) recorded a range of 5.60–21.70 g l^{-1} for malic acid and 0.8–9.7 g l^{-1} for succinic acid in 62 heritage apple cultivars grown in Germany. Ma et al. (2015) reported that cultivated and wild apples contained malic acid content between 0.53 and 22.74 g l^{-1}. Kumar et al. (2018) reported that different apple cultivars had a range of 4.40 to 9.5 g l^{-1} for malic acid, 0.0 to 5.1 g l^{-1} for succinic acid, and undetected to 11.0 g l^{-1} for citric acid. Having determined that cultivated and wild apples contained malic acid content between 0.17 and 29.27 g l^{-1} and citric acid content between undetected and 2.42 g l^{-1}, Ma et al. (2018) reported that the great majority of apple varieties had low malic acid content. Ma et al. (2015) and Ma et al. (2018) reported that wild apples had greater or-

Table 5 Principal component analysis of bioactive compounds of native apple genotypes

Variable	Component		
	1	2	3
Malic acid	0.896*	0.339	0.011
Succinic acid	0.852*	0.346	0.311
Tartaric acid	0.930*	–0.055	0.022
Titratable acidity	0.874*	0.217	–0.173
Total phenolics	0.436	0.849*	0.002
Total flavonoids	0.047	0.971*	0.038
FRAP	0.365	0.917*	–0.099
Oxalic acid	–0.213	–0.047	0.868*
Citric acid	0.360	0.059	0.868*
Total soluble solids	–0.217	0.099	0.915*
Ascorbic acid	0.430	–0.487	0.543*
Fruit skin color	0.345	–0.413	0.609*
Eigenvalue	4.84	3.47	2.06
Percentage of variance	40.33	28.88	17.19
Cumulative percentage	40.33	69.21	86.40

FRAP ferric reducing antioxidant power assay
*Factor loadings ≥ |0.54|

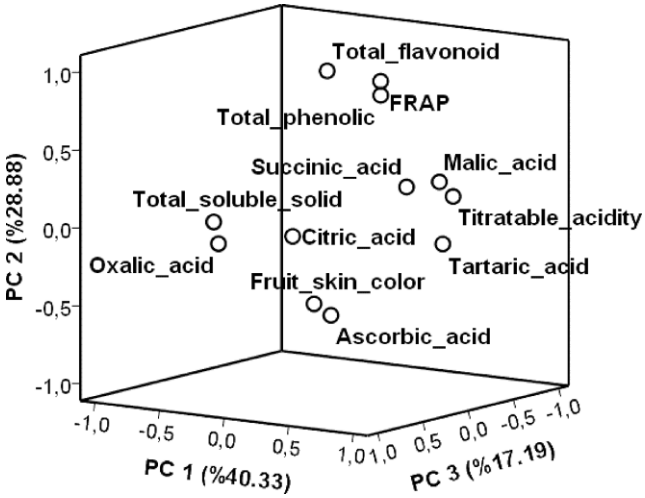

Fig. 1 Component plot of the first three principal components (PC1, PC2, and PC3) in the native apple genotypes based on phytochemical components

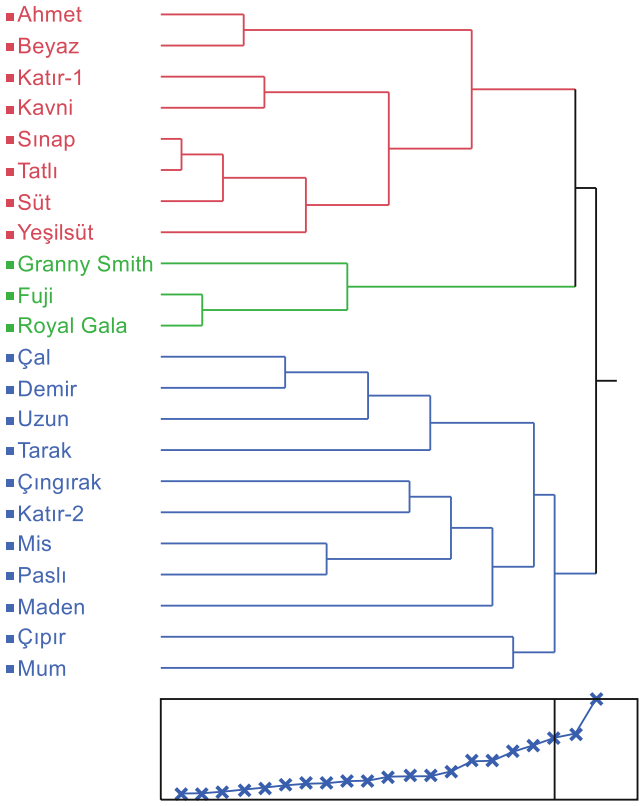

Fig. 2 Dendrogram grouping of native apple genotypes and standard apple cultivars based on phytochemical compounds

ganic acid content than that of cultivars. Similarly, in the present study, most of the apple genotypes contained higher organic acid content than the standard apple cultivars. Mum, Demir, and Uzun genotypes, with their higher organic acid contents, might be an important source of organic acids. Related findings indicate that these genotypes can be used as genetic material for apple breeding efforts.

Principal Component Analysis

Twelve traits were used for principal component (PC) analysis (Table 5; Fig. 1). Of the 12 principal components, three PCs had an eigenvalue greater than 1.0 and accounted for 86.40% of the total variation. PC1 and PC2 accounted for more than 69% of total variation. PC1 was strongly related to malic acid, succinic acid, tartaric acid, and titratable acidity, and it accounted for 40.33% of the total variation. PC2 was mainly related to total phenolics, total flavonoids, and antioxidant activity (FRAP assay) and accounted for 28.88% of the total variance. PC3 was defined by oxalic acid, citric acid, ascorbic acid, total soluble solids, and fruit skin color and accounted for 17.19% of the total variation (Table 5; Fig. 1). Thus, the phytochemical components could effectively explain the variability among the apple genotypes.

A cluster analysis of phytochemical components using three factors obtained from principal component analysis was performed (Fig. 2). Apple genotypes and cultivars were divided into two subclusters. The first group included eight apple genotypes and the three standard apple cultivars. The second group consisted of 11 apple genotypes. The means of all bioactive compounds in apple genotypes in the second group were higher than the means in the first group. The apple genotypes and cultivars investigated were divided into three subgroups. The first subgroup consisted of eight apple genotypes. The second subgroup included the standard apple cultivars ('Fuji', 'Royal Gala', and 'Granny Smith'). The third subgroup included 11 apple genotypes. The means of all bioactive compounds in the apple genotypes in the three subgroups were higher than the means of the other subgroups (Fig. 2).

Conclusion

Although many studies on the determination of phytochemical components of apple have been reported, the majority have focused on commercial apple cultivars, and studies on the phytochemical compounds of native apple genetic resources grown in local areas are limited. In the present study, phytochemical compounds of native apple genotypes in the eastern Black Sea region were compared with those of standard apple cultivars. The native apple genotypes exhibited wide variation in terms of phytochemical components. The data imply that the native apple genotypes are an important source of phytochemical compounds. Most of the native apple genotypes contained higher levels of

phytochemical compounds than the standard apple cultivars investigated in the present study as well as other apple varieties examined in different studies. This indicates that native apple genotypes are useful sources of genetic variation for nutritional breeding programs of apple and might contribute to the development of new apple cultivars with enhanced health benefits.

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Conflict of interest M.F. Balta, O. Karakaya, H. Kurt, M. Yılmaz, S. Uzun, and F. Balta declare that they have no competing interests.

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