Erwerbs-Obstbau (2022) 64:559–567
https://doi.org/10.1007/s10341-022-00734-2

ORIGINAL ARTICLE / ORIGINALBEITRAG

Comparison of Polyphenol Compounds of Strawberry Cultivars with Their Progeny

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Received: 11 December 2020 / Accepted: 30 July 2022 / Published online: 6 September 2022
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Abstract
In this study, an attempt was made to combine the domestic strawberry cultivars 'Sevgi,' 'Ebru,' and 'Kaşka' with the foreign cultivars 'Fortuna,' 'Sabrosa,' and 'Albion' by making use of cross-breeding methods. Superior strawberry variety candidates with the desired attributes were selected during two growing seasons. According to the results of weighted ranked testing, 4066 progenies were gradually reduced to 229 (first year) and eventually to 13 (second year). The main objective of the study was to determine the effect of cultivars and their progeny (13 hybrid lines) on polyphenol content, which is important for human health and the color quality of fruit. As a result, it was observed that genotype had a strong effect on all parameters. However, one genotype did not perform well enough to meet the expectations in terms of all parameters. As observed in previous studies, research on genetics and breeding is important to more fully understand the polyphenol content of strawberry fruits.

Keywords Antioxidants · Breeding lines · Health-related compounds · Quality

Introduction

Research on health-related compounds has been increasing in recent years. In this context, strawberry is one of the most important fruits, containing vitamin C, mineral substances, folic acid, and phenolic compounds that provide high amounts of natural antioxidants to consumers. Obesity is one of the most critical health problems in recent years. According to the results of detailed analysis, by consuming 100 g of strawberry, one obtains carotenoids and fat-soluble vitamins, such as A and E, along with just 32 kcal of energy. This can be the most important reason for strawberry to be a nutrient option against obesity. In addition, as a healthy food with a low number of calories, strawberry fruit is an excellent option for processed products due to its sweet aroma. Moreover, some strawberry varieties contain higher levels of vitamin C than citrus fruits. It has been determined that berry fruits contain antioxidant and phenolic compounds in amounts four, ten, and 40 times greater than in fruits, vegetables, and cereals, respectively (Halvorsen et al. 2002), and it is reported that strawberry fruits have two to seven times more antioxidant capacity than apples, peaches, grapes, tomatoes, oranges, and kiwifruits (Wang et al. 1996; Scalzo et al. 2005). The antioxidant content of strawberries is highly affected by the selected species and cultivar (Giampieri et al. 2013), so it is essential to choose strawberry genotypes that are high in antioxidants. Nowadays, breeding research is aimed at combining health-related compounds and quality parameters, such as yield, size, and color, at the desired level. Today's consumers have become interested not only in the taste of products but also in their health-related compounds. Functional foods, including strawberry, reduce the risk of cancer, obesity, inflammation, and cardiovascular diseases because of their high nutrient content (Kaur and Das 2011; Schulz et al. 2016).

Food flavonoids are mainly composed of flavonols such as kaemferol, quercetin, and myricetin. Flavonoids have different biological functions such as having antiallergenic, antimicrobial, antithrombotic, antioxidant, and anti-inflammatory properties; preventing constriction of the

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intravenous walls; and protecting the heart and expanding blood vessels (Manach et al. 2005). Epidemiological studies have highlighted an inverse relationship between cancer and kaempferol intake. Kaempferol significantly inhibits the formation and development of cancer cells and has also been shown to stimulate the death of cancerous cells. Kaempferol has also been found to maintain normal cell viability and, in some cases, increase their protective effects (Chen and Chen 2013).

The objective of this study was to determine the effect of cultivars on their progeny in terms of polyphenol, which is beneficial for human health. Polyphenol content profiles of the cultivars are shown under Mediterranean climate conditions.

Material and Method

Material

This experiment was conducted in Turkey at the Department of Horticulture, Faculty of Agriculture, of Çukurova University and at Alata Horticultural Research Station during the 2016–2017 growing season. Phenolic compounds were analyzed in the Food Engineering Department of Çukurova University. Three domestic strawberry varieties, namely 'Kaşka,' 'Sevgi,' and 'Ebru,' which were registered in 2009 as a result of many years of breeding research at the Department of Horticulture of Çukurova University, were used as pollinizers. Strawberry varieties of European and U.S. origin, 'Fortuna,' 'Sabrosa,' and 'Albion,' were used as female parents because of their important characteristics such as high yield and fruit size. These foreign strawberry varieties are widely cultivated in most parts of Turkey, especially around the Mediterranean coastal region. Achenes obtained from hybridizations were sown at the Alata Horticultural Research Institute (Table 1).

In the first year (2015–2016 growing season), 229 genotypes were selected from 4066 crossbred lines and were evaluated by their flower and fruit structures. Pomological analyses and phenological observations were performed with these selected 229 genotypes and their parents. Thirteen genotypes came into prominence after application of a weighted ranked test of the obtained data. Eventually the selected 13 genotypes and their parents were grown under a Spanish-type high tunnel using the same cultivation practices. The experiments were designed in three replicates, each including 20 plants. These plug plants were planted on 20–21 September in trapezoidal raised beds measuring 0.70 m from the base and 0.50 m at the top, with a height of 0.30 m. The distance between the beds was 0.4 m, and the beds were covered by 0.02-mm-thick black plastic mulch. Fertilizer was applied uniformly via drip irrigation and foliar spraying. Plant protection, as needed, was carried out by spraying of agricultural pesticides. This production system was previously described by Kapur et al. (2018). Fruit samples were taken at the beginning of May, which is one of the most active harvest times under Mediterranean climate conditions.

Method

Determination of Polyphenol Compounds

The polyphenol content of the genotypes was determined using the method of Agcam and Akyıldız (2015), with small modifications. Five grams of fruit pulp was put into Teflon tubes, and 80% methanol was added to reach a volume of 50 ml. The tubes were then centrifuged at 6000 rpm for 10 min at 4 °C (Heraeus Biofuge, Primo R, Germany). After this process, the collected supernatant was passed through a 0.45-µm Teflon membrane filter (PTFE) to fill brown vials.

Finally, the supernatant in brown vials was injected into the high-performance liquid chromatography (HPLC) instrument (Fig. 1).

The quantification of phenolic contents was carried out at 280, 320, 360, and 520 nm using external standard methods. The amount of compounds was expressed as milligrams per kilogram of fresh fruit (mg kg^{-1}). The calibration curves were obtained using commercial standards, and their regression coefficients (R^2) ranged between 0.996 and 0.999 for all cases.

The mobile phase gradient program for the analysis of the phenolic compounds is given in Table 2.

Chromatography conditions:

- Column: Waters XTerra RP 18, 5 µM, 4.6×250 mm, Ireland
- Column temperature: 30 °C
- Detector: photodiode array detector (PDA)
- Wavelength: 280-320-360-520 nm
- Mobile phase flow rate: 1 ml min^{-1}
- Injection volume: 20 µl

Table 1 Hybridization combinations of foreign and domestic cultivars

Albion × Ebru (A × E)	Fortuna × Ebru (F × E)	Sabrosa × Ebru (S × E)
Albion × Kaşka (A × K)	Fortuna × Kaşka (F × K)	Sabrosa × Kaşka (S × K)
Albion × Sevgi (A × S)	Fortuna × Sevgi (F × S)	Sabrosa × Sevgi (S × S)

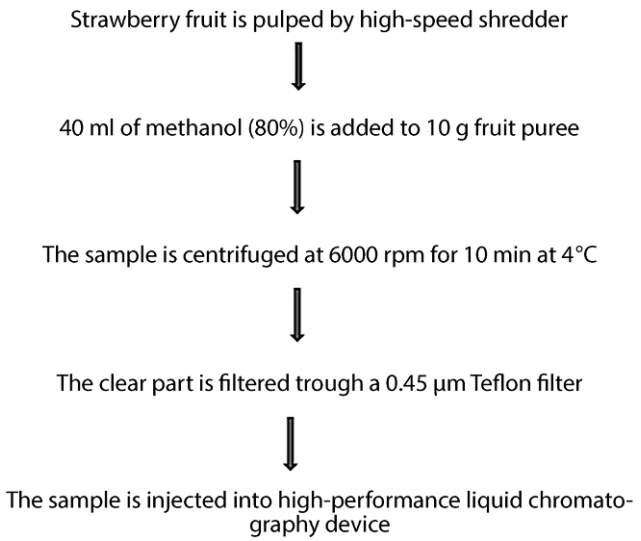

Fig. 1 Extraction method of individual phenolic compounds

Table 2 Mobile phase gradient program for the analysis of phenolic compounds

Time (min)	(A) Formic acid (5%)	(B) Acetonitrile/A (80/20) (%)
0.01	100	0
10	95	5
25	90	10
55	80	20
70	55	45
90	0	100
95	100	0
100	100	0

- Mobile phase solution: formic acid (5%) (A), acetonitrile/A (80/20) (B), gradient flow

Certified and high-purity standard materials were used during the identification of phenolic compounds of strawberry fruit. Phenolic peaks were defined by taking into account the detection times and spectra of standard substances. The quantification of peaks was conducted with calibration curves obtained by certified standards ($R^2 > 0.995$).

The 13 genotypes and their parents were evaluated using a completely randomized design. The data were analyzed using the statistical program JMP, version 5.0.1 (SAS Institute, Cary, NC, USA). Analysis of variance (ANOVA) was carried out to determine the differences between the strawberry genotypes in terms of polyphenol compounds. A least significant difference test (LSD) was conducted to examine the differences among the genotypes. The comparisons that yielded $p \leq 0.05$ were considered to be statistically significant.

Results and Discussion

Polyphenol Compounds of Promising Hybrids and Their Parents

Polyphenols are a group of compounds of particular importance in plants. They are known to be responsible for the brightness, red color (anthocyanins), and sour taste (tannin) in strawberry fruits. Phenolic compounds are effective against ultraviolet rays and disease-causing agents. They are also useful in attracting pollinating insects and animals that distribute seeds (Crozier et al. 2006); this is due to the wavelengths at which they are seen under ultraviolet light. Flavanols are measured at 280 nm, hydroxycinnamic acid derivatives at 300–320 nm, and anthocyanins at 500–520 nm (Kajdžanoska et al. 2010). Phenolic compounds are divided into three basic groups: 1) flavonoids, anthocyanins, flavonols, and flavanols; 2) soluble tannins, including phenolic acids such as ellagitannins and gallotannins; and 3) hydroxybenzoic and hydroxycinnamic acids (Aaby et al. 2005; Giampieri et al. 2012). Catechin derivatives of the selected promising hybrids and their parents were investigated in this study; (–) catechin, protocatechuic acid ethyl ester (PAEE), (+) catechin, (–) epigallocatechin gallate, (–) epicatechin, (–) epicatechin gallate, and ellagic acid contents are given in Table 3. In the current study, the chromatogram peaks of these compounds obtained at 280 nm are shown in Fig. 2. Catechin derivatives of strawberry genotypes were ordered by their amounts; (–) epicatechin gallate ranged from 6.36 mg kg^{-1} ('Albion') to 249.8 mg kg^{-1} ('59'), followed by (–) catechin, which ranged from 16.3 mg kg^{-1} ('Fortuna') to 71.0 mg kg^{-1} ('33') depending on genotype. Among the hybrid lines, genotype '33' was found to be at the top in terms of (–) catechin and (–) epigallocatechin gallate, while genotype '59' was found to be superior in terms of PAEE, (–) epicatechin, and (–) epicatechin gallate. On the other hand, (+) catechin content, which is another important catechin derivative, of the '61' hybrid was found to be statistically significant and superior to the others with a value of 68.4 mg kg^{-1}.

As a result of the crossbreeding study, significant development of catechin derivatives was generally observed. It was noted that some of the hybrids reached higher values than those of their parents. Ellagic acid, known to be an important contributor to human health, is formed by the combination and oxidation of two gallic acid monomers. Ellagic acid is also known to form at different levels in strawberries (Badjakov et al. 2008). In this study, it was determined that the values of ellagic acid ranged from 1.82 mg kg^{-1} ('103') to 14.51 mg kg^{-1} ('33'). Regarding the parents, the lowest value (1.84 mg kg^{-1}) was observed in 'Kaşka' cultivar, and the highest value, 10.11 mg kg^{-1}, was found in 'E-bru' cultivar. Kim et al. (2015) reported a 50% increase of

Table 3 Catechin derivatives and ellagic acid values in hybrid genotypes and their parents (mg kg^{-1})

	Genotype	(–) Catechin	PAEE	(+) Catechin	(–) Epigallocatechin gallate	(–) Epicatechin	(–) Epicatechin gallate	Ellagic acid
Parents	Fortuna	16.3 h	2.96 efg	34.5 fgh	2.17 d–g	0.47 h	103.9 ef	6.69 def
	Sabrosa	35.6 b–f	2.97 efg	39.9 d–g	2.19 d–g	0.57 gh	84.3 fg	7.89 cd
	Albion	36.2 b–e	3.66 d	48.9 bc	3.19 c	1.55 def	6.4 j	5.12 gh
	Sevgi	27.4 c–h	2.38 gh	36.9 efg	1.64 g	1.44 d–g	94.3 efg	9.09 bc
	Kaşka	65.2 a	2.21 h	32.9 gh	1.59 g	1.65 de	101.8 ef	1.84 k
	Ebru	21.6 fgh	3.29 def	43.3 b–e	2.45 de	1.16 d–h	56.8 hi	10.11 b
Hybrids	33 (F × K)	71.0 a	5.52 b	14.2 j	4.57 a	3.05 b	56.7 hi	14.51 a
	36 (F × K)	60.9 a	2.39 gh	9.3 jk	1.77 fg	0.78 e–h	153.5 d	4.89 gh
	50 (F × S)	41.9 b	3.48 de	3.9 k	2.56 d	2.75 bc	222.3 b	6.49 ef
	59 (F × S)	44.6 b	14.10 a	28.1 hi	3.84 b	14.60 a	249.8 a	7.31 de
	60 (F × S)	69.0 a	4.58 c	9.0 jk	3.41 bc	1.88 cd	119.5 e	6.70 def
	61 (F × S)	39.4 bcd	4.63 c	68.4 a	3.34 bc	0.76 e–h	70.7 gh	7.86 cd
	87 (F × S)	19.3 gh	2.62 fgh	34.3 fgh	1.93 efg	0.97 d–h	153.5 d	4.20 hi
	103 (F × S)	19.9 gh	3.05 d–g	32.5 gh	2.26 def	0.85 e–h	147.9 d	1.82 k
	112 (F × S)	25.8 d–h	2.77 fgh	24.9 i	2.05 d–g	1.15 d–h	35.9 i	6.01 fg
	114 (F × S)	32.4 b–g	2.97 efg	41.5 c–f	2.20 d–g	2.75 bc	97.8 ef	5.98 fg
	121 (F × S)	23.2 e–h	3.12 def	41.3 def	2.55 d	1.08 d–h	112.3 e	9.46 b
	169 (S × E)	18.2 h	3.10 def	45.2 bcd	2.30 def	0.49 gh	194.0 c	2.35 jk
	171 (S × E)	40.7 bc	3.10 def	49.1 b	2.29 def	0.62 fgh	67.5 gh	3.12 ij
–	LSD	**14.2****	**0.68****	**7.59****	**0.62****	**0.97****	**26.97****	**1.25****

Differences between the means are shown with different letters

F × *K* Fortuna × Kaşka, *F* × *S* Fortuna × Sevgi, *S* × *E* Sabrosa × Ebru, *LSD* least significant difference, *N.S.* not significant, *PAEE* protocatechuic acid ethyl ester

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

ellagic acid in 'Dahong,' 'Keumhyang,' 'Maehyang,' 'Seolhyang,' 'Sunhong,' 'Akimime,' and 'Red Pearl' strawberries in 2012 compared with 2011. In strawberries grown in Finland and Poland, the content of ellagic acid was found to range from 3.4 mg per 100 g to 5.9 mg per 100 g (Häkkinen et al. 2000). In another study, ellagic acid was determined to range from 15.18 mg per 100 g to 26.36 mg per 100 g among 14 types belonging to *Fragaria vesca* species and in 'Camarosa' variety belonging to *Fragaria ananassa* species (Yildiz et al. 2014). Similarly, Nour et al. (2017) reported the genotypic effect on ellagic acid, which ranged from 6.79 mg per 100 g to 9.03 mg per 100 g. Ariza et al. (2018) determined that ellagic acid content was 35.78 mg per 100 g in achenes and 2.93 mg per 100 g in all fruits. Kosar et al. (2004) found variation in some specific phenolic compounds depending on maturity stage. For example, while the ellagic acid was dominant in the green period, pelargonidin-3-glucoside (P3G) and *p*-coumaric acid were highest in the pink and red periods. In another study that compared the color effect of different mulches, it was observed that ellagic acid increased by around 7% in fruits grown under white mulch compared with those grown in brown mulch (Anttonen et al. 2006). As seen in the literature, in addition to being highly determined by genotype, ellagic acid content is also affected by the part of the fruit, maturity stage, and growing conditions. In the current study, ellagic acid was similarly mainly affected by the genotypic structure. In this context, genotypes '33' and '121' have attracted attention because of their high ellagic acid values among hybrid plants and their parents.

Some values of the phenolic acids (caffeic acid, chlorogenic acid, *p*-coumaric acid, and ferulic acid) and flavonols (quercetin and kaempferol) obtained in the study are shown in Table 4. The chromatogram image of these compounds is given in Fig. 3. Flavanols (kaemferol, quercetin, and myricetin) are included in flavonoids. Researchers have reported that these have different biological functions, including having antiallergenic, antimicrobial, antithrombotic, antioxidant, and anti-inflammatory properties; preventing the narrowing of intravascular walls; and protecting the heart and expanding blood vessels (Manach et al. 2005). Flavonoids have different antioxidant capacities depending on the distribution and number of hydroxyl groups in the structure, as well as on the ability to replace electron-withdrawing and withdrawing electrons from the ring structure (Packer and Glazer 1990). The hydroxyl radical-scavenging activity of flavonoids is increased by the number of hydroxyl groups that are replaced in the B ring (especially in the 3' position) (Packer and Glazer 1990; Tsao 1997). Quercetin has the highest antioxidant activity, followed

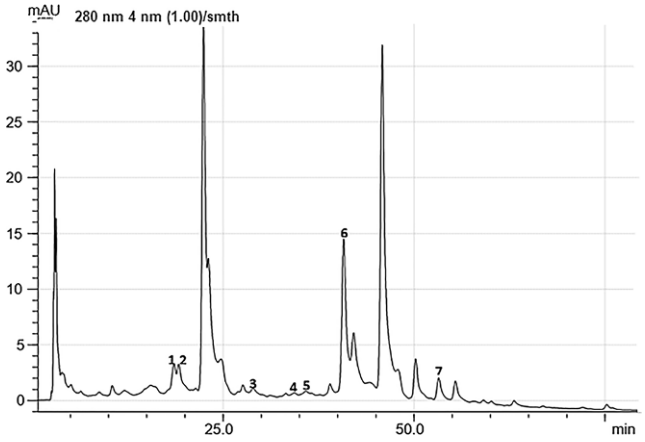

Fig. 2 Chromatogram image of selected phenolic peaks of hybrids and their parent strawberry fruits at 280 nm. *1* (–) catechin, *2* (+) catechin, *3* protocatechuic acid ethyl ester, *4* (–) epigallocatechin gallate, *5* (–) epicatechin, *6* (–) epicatechin gallate, *7* ellagic acid

by dihydroquercetin > kaempferol > quercetin chlorogenic acid = *p*-coumaric acid, respectively (Clegg and Morton 1968). An increased degree of hydroxylation in aromatic chains has been found to have a positive effect on the antioxidant activity of hydroxycinnamic acid (Fan et al. 2009). For example, caffeic acid has greater antioxidant activity than *p*-coumaric acid thanks to its higher hydroxyl group number.

Kelebek and Selli (2011) determined four phenolic acids (*p*-OH benzoic acid, *p*-coumaric acid glucoside, ferulic acid derivatives, caffeic acid) in strawberry cultivars. Similarly, in the current study, *p*-coumaric acid, ferulic acid, and caffeic acid were observed. Caffeic acid content was found to be the dominant compound among the phenolic acids of the genotypes, varying between 8.3 mg kg^{-1} ('Albion') and 79.6 mg kg^{-1} ('60'), followed by chlorogenic acid, which ranged from 11.0 ('Albion') to 67.5 ('Sabrosa'). Additionally, the *p*-coumaric acid value ranged from 1.50 mg kg^{-1} ('Sabrosa') to 4.67 mg kg^{-1} ('Kaşka'), while ferulic acid was observed to be between 0.57 mg kg^{-1} ('Albion' and '87') and 2.69 mg kg^{-1} ('171'). In terms of phenolic acids, caffeic acid, besides its high antioxidant activity, is noteworthy due to its high quantity among these genotypes. In this context, '60' and '169' were prominent among the hybrids, having values of 79.6 mg kg^{-1} and 55.9 mg kg^{-1}, respectively. Regarding chlorogenic acid, the genotype '169' had the highest value, 63.2 mg kg^{-1}, among the hybrids.

Anttonen et al. (2006), reported that quercetin and kaemferol contents, found to be main flavonols, vary depending on genotype. In the same study, it was found that the change in quercetin content was greater than that for kaemferol in different environmental conditions; however, as stated, the variation was primarily caused by genotype. Similarly, Kosińka et al. (2013) found that quercetin and kaempferol

Table 4 Polyphenols in the fruits of hybrid genotypes and their parents at 320 nm (mg kg^{-1})

	Genotype	Caffeic acid	Chlorogenic acid	*p*-Coumaric acid	Ferulic acid	Quercetin	Kaempferol
Parents	Fortuna	42.9 cde	50.9 b	3.29 de	0.95 fg	7.27 a	1.03 bcd
	Sabrosa	46.3 c	67.5 a	1.50 l	1.72 bc	2.22 fg	0.70 efg
	Albion	8.3 k	11.0 k	3.77 cd	0.57 h	6.99 a	0.50 fgh
	Sevgi	27.7 i	24.5 fgh	4.62 ab	1.23 e	1.89 fgh	0.81 cde
	Kaşka	30.9 ghi	18.6 ij	4.67 a	1.19 ef	1.31 hi	0.51 fgh
	Ebru	20.4 j	25.4 fg	4.11 bc	1.37 de	2.19 fg	1.06 bc
Hybrids	33 (F × K)	28.8 hi	20.6 g–j	3.22 def	1.34 de	1.28 hi	0.83 cde
	36 (F × K)	44.7 cd	24.9 fgh	1.71 kl	1.18 ef	1.61 gh	1.18 b
	50 (F × S)	38.2 ef	20.1 hij	2.32 hij	1.16 ef	4.69 b	0.81 cde
	59 (F × S)	18.9 j	17.9 ij	3.34 de	1.56 cd	0.78 i	0.59 e–h
	60 (F × S)	79.6 a	38.3 cd	1.86 jkl	1.25 e	0.75 i	1.19 ab
	61 (F × S)	38.5 def	15.9 j	2.67 fgh	1.18 ef	3.58 cd	0.79 cde
	87 (F × S)	17.1 j	22.0 f–i	1.99 i–l	0.57 h	3.98 c	0.44 gh
	103 (F × S)	29.1 hi	31.0 e	2.98 efg	1.89 b	2.47 ef	0.79 cde
	112 (F × S)	16.3 j	25.7 f	2.52 ghi	0.88 g	0.75 i	0.35 h
	114 (F × S)	34.8 fgh	35.0 de	2.42 ghi	1.56 cd	0.81 i	0.76 def
	121 (F × S)	30.4 ghi	42.6 c	2.58 gh	1.21 ef	2.98 de	0.70 efg
	169 (S × E)	55.9 b	63.2 a	2.25 h–k	1.17 ef	2.16 fg	1.47 a
	171 (S × E)	36.0 fg	40.7 c	3.15 ef	2.69 a	1.33 hi	0.41 h
–	LSD	**6.17****	**4.96****	**0.56****	**0.27****	**0.64****	**0.28****

Differences between the means are shown with different letters

F × *K* Fortuna × Kaşka, *F* × *S* Fortuna × Sevgi, *S* × *E* Sabrosa × Ebru, *LSD* least significant difference, *N.S.* not significant

***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

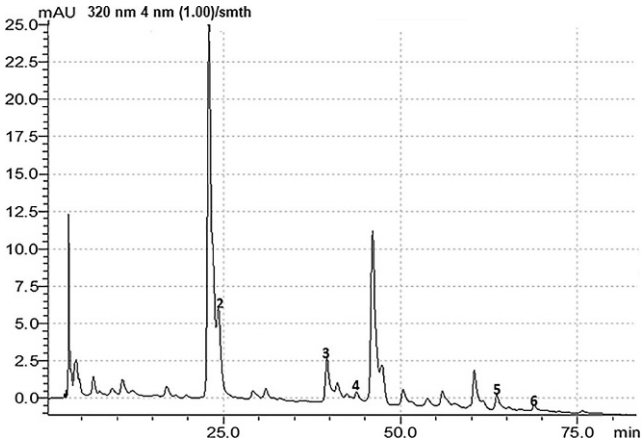

Fig. 3 Chromatogram image of selected phenolic peaks in hybrids and their parent strawberry fruits at 320 nm. *1* caffeic acid, *2* chlorogenic acid, *3 p*-coumaric acid, *4* ferulic acid, *5* quercetin, *6* kaempferol

are principal flavonols in strawberry fruits. Aaby et al. (2012) determined quercetin derivatives to be 1.18 mg per 100 g and kaemferol derivatives to be 0.84 mg per 100 g in strawberry. Similarly, in the current study, quercetin content was greater than kaempferol content except in the '60' hybrid. The quercetin content of superior genotypes and their parents ranged from 0.75 mg kg^{-1} ('60' and '112') to 6.99 mg kg^{-1} ('Albion'), and the other important flavonol, kaempferol, ranged from 0.35 mg kg^{-1} ('112') to 1.47 mg kg^{-1} ('169'). Additionally, Vinson et al. (1998) reported that flavonols, such as myricetin, quercetin, and kaemferol, are stronger than conventional vitamins. Chen and Chen (2013) showed a relationship between kaempferol consumption and lower risk of chronic diseases such as cancer. In this context, '169' and '60' contained higher levels of kaempferol than even their parents.

Individual anthocyanin values are presented in Table 5, and the chromatogram of these compounds is shown in Fig. 4. It has been reported that the change in anthocyanin content of fruits is related to the color of the fruit and that it is an important part of the appearance influencing consumer fruit preferences (Ngo et al. 2007). Anthocyanins are naturally present in six different forms (pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin), depending on the number and position of the hydroxyl groups and their degree of methylation (Jaakola 2013). The main anthocyanin in fruits is P3G, which was first identified by Robinson and Robinson (1931). Many other researchers (Yoshida et al. 2002; Da Silva et al. 2007; Kim et al. 2015), as well as the current study, have shown the main anthocyanin to be P3G, and in this study it ranged from 207.9 mg kg^{-1} ('Sabrosa') to 538.1 mg kg^{-1} ('Sevgi'). This compound was followed by unknown 2, which varied highly depending on the genotype. Pelargonidin-3-rutinoside (P3R) ranged from

Table 5 Individual anthocyanin content of hybrid genotypes and their parents (mg kg^{-1})

	Genotype	Cyanidin-3,5-diglucoside	Cyanidin-3-glucoside	Pelargonidin-3-glucoside	Unknown 1	Pelargonidin-3-rutinoside	Unknown 2
Parents	Fortuna	2.94 b	1.63 n	412.7 de	37.2 d	26.4 efg	48.2 f
	Sabrosa	2.24 ghi	8.89 e	207.9 k	17.0 h	18.8 jkl	1.0 h
	Albion	2.41 d–h	8.04 ef	449.2 c	36.9 d	42.9 a	5.6 h
	Sevgi	2.37 e–h	17.19 b	538.1 a	46.1 b	37.9 b	87.1 c
	Kaşka	3.18 ab	12.65 c	454.3 c	35.1 de	28.2 de	71.6 d
	Ebru	2.59 de	13.28 c	492.1 b	43.9 bc	33.1 c	88.9 c
Hybrids	33 (F × K)	2.93 bc	5.52 hi	397.9 ef	85.5 a	35.9 b	85.9 c
	36 (F × K)	2.29 f–i	5.29 hij	229.2 k	35.6 de	12.2 m	34.0 g
	50 (F × S)	2.49 d–g	2.62 k–n	325.0 h	46.6 b	21.6 hi	0.9 h
	59 (F × S)	3.38 a	10.56 d	435.9 cd	36.1 d	19.8 ijk	59.2 e
	60 (F × S)	2.19 hi	4.12 ijk	263.0 j	32.8 ef	29.0 d	37.8 g
	61 (F × S)	2.04 i	2.27 mn	312.2 hi	36.5 d	20.3 ijk	1.1 h
	87 (F × S)	2.51 d–g	3.39 klm	322.8 h	32.6 ef	17.9 kl	0.3 h
	103 (F × S)	2.61 de	3.82 jkl	367.6 g	37.7 d	22.1 hi	120.1 a
	112 (F × S)	2.55 def	3.28 klm	324.9 h	30.9	17.1 l	41.7 fg
	114 (F × S)	2.48 d–g	6.02 gh	380.9 fg	40.9 c	24.1 gh	101.1 b
	121 (F × S)	2.65 cd	2.57 lmn	296.9 hi	26.5 g	25.1 fg	0.6 h
	169 (S × E)	2.24 ghi	7.33 fg	292.6 ij	25.9 g	20.8 ij	35.9 g
	171 (S × E)	2.51 d–g	22.57 a	493.4 b	46.3 b	26.9 def	2.9 h
–	LSD	**0.28*****	**1.52*****	**29.6*****	**3.22*****	**2.52*****	**8.04*****

Differences between the means are shown with different letters. Unknown compounds were calculated in accordance with pelargonidin-3-glucoside
F × *K* Fortuna × Kaşka, *F* × *S* Fortuna × Sevgi, *S* × *E* Sabrosa × Ebru, *LSD* least significant difference, *N.S.* not significant
***$p < 0.001$, **$p < 0.01$, *$p < 0.05$

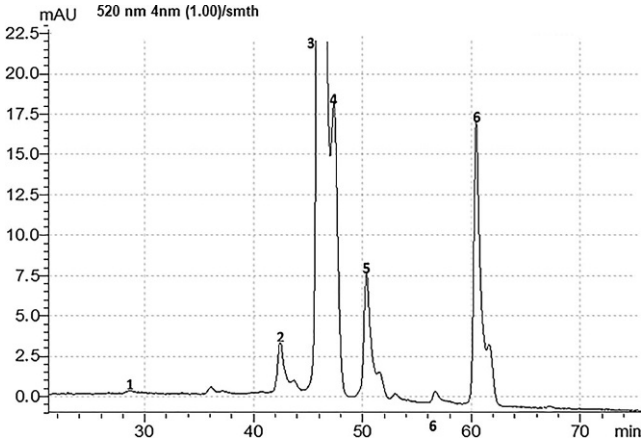

Fig. 4 Chromatogram image of colored phenol peaks of selected hybrids and their parent strawberry fruits at 520 nm. *1* cyanidin-3,5-diglucoside, *2* cyanidin-3-glucoside, *3* pelargonidin-3-glucoside, *4* unknown 1, *5* pelargonidin-3-rutinoside, *6* unknown 2

12.2 mg kg^{-1} ('36') to 42.9 mg kg^{-1} ('Albion') depending on the genotype. Cyanidin-3-glucoside (S3G) content, which is responsible for bright, vivid red color, was found to be significantly higher in the '171' hybrid, at 22.6 mg kg^{-1}, than in the others. It was followed by 'Sevgi' and 'Ebru' varieties, having values of 17.2 mg kg–1 and 13.3 mg kg–1, respectively. The lowest value (1.6 mg kg^{-1}) was seen in the 'Fortuna' variety.

Da Silva et al. (2007) found that the total amount of anthocyanin varies between 200 mg kg^{-1} and 600 mg kg^{-1}. They reported that the majority of total anthocyanin content (77%–90%) was composed of P3G, followed by P3R (6%–11%) and S3G (3%–10%), respectively. Also, they found variation in anthocyanin content in the same variety and, due to ripening stage, even at the same harvest time. Similarly, in another study that compared 24 antioxidant-rich short-day strawberry varieties, 'Red Pearl' and 'Sachinola' were found to have three times more P3G than the others. The other two identified anthocyanins (S3G and P3R) were found to be highest in 'Dahong' and 'Keumhyang' varieties (Kim et al. 2015). In mature fruit it has been reported that P3G accounts for 60% to 95% of total anthocyanin, whereas pelargonidin-3-malonylglucoside is responsible for 0% to 33%, P3R for 0% to 7%, and S3G for 1% to 6% (Yoshida et al. 2002; Määttä-Riihinen et al. 2004; Buendia et al. 2010; Aaby et al. 2012). In another study, while it was found that pelargonidine-3-malonylglucoside was present in nine of 20 Japanese cultivars, the other 11 varieties did not produce this compound (Yoshida et al. 2002). Similarly, Misran et al. (2015) reported that the main anthocyanin compound is P3G, which provides about 71% to 87% of the total anthocyanin content, and that it is cultivar dependent. In quantitative assessment, P3G was found to vary in varieties: 47.1 mg per 100 g ('Mira') and 65.8 mg per 100 g ('Pierre St. Pierre'). In the same study, 'Pierre St Pierre' had the highest level of P3R, 2.9 mg per 100 g, while 'Mira' had the lowest level, 0.5 mg per 100 g. S3G, which is another important anthocyanin pigment, was not identified in the 'Jewel' and 'Mira' varieties.

As can be understood from previous studies, the genetic structure is predominant regarding the anthocyanin content of fruits, along with many other factors, such as maturity and climatic conditions. Cassidy et al. (2013) reported that the consumption of rich anthocyanin-containing strawberries prevented the development of cancerous tumors in the colon. Duarte et al. (2018) showed that P3G is an important anti-inflammatory and its mechanism of action is by preventing the activity of IkB-α and decreasing phosphorylation in the JNK-MAPK pathway. In addition, P3G, which has been found to be dominant in strawberries, significantly decreases the loss of white blood cells in pleural fluid at doses of 1 mg kg^{-1} and 10 mg kg^{-1} (up to 40.8% at 1 mg kg^{-1} and 58% at 10 mg kg^{-1}). As has been seen, anthocyanins are not only related to color but also have important beneficial health effects. These compounds, which are mainly concerned with the intensity of the color, also affect consumer preference by influencing the appearance of the fruit. Although the high anthocyanin content of strawberry has significant effects on health, fruits containing excessive anthocyanins are not preferred by customers because of their waited appearance. Considering all these factors, the '59' and '33' genotypes, which are in same statistical group as 'Albion' and 'Fortuna' in terms of P3G, come to the fore.

Conclusion

In summary, this study indicates that genotype has a strong effect on the polyphenol compounds of strawberry fruits. Unfortunately, other than positive improvements in many of the polyphenols, none of the genotypes stand out in terms of all of these polyphenols. Even so, genotypes '33' and '59' were superior in terms of catechin derivatives and ellagic acid content, while the genotype '169' was found to be superior regarding chlorogenic acid and kaempferol content. Ferulic acid and caffeic acid contents were found to be highest in the '171' and '60' hybrids, respectively.

Another important point of this study is that many hybrids had values similar to those of the foreign cultivars in terms of anthocyanin compounds. According to our outcomes, breeding studies, especially in strawberry, need to be continued to produce healthier generations by increasing the compounds related to health and taste. Therefore, our recommendation to breeders is that they should choose varieties that include high levels of health and taste-related compounds in their hybridization combinations, such as ours.

Funding Partial financial support was received from the Coordination Unit of the Scientific Research Projects of the Çukurova University (project number FDK-2016-5582) and the Council of Scientific and Technological Research of Turkey (project number TUBİTAK-TOVAG 214 O 138).

Author Contribution Mehmet Ali Saridas: conceptualization, methodology, formal analysis, writing of original draft, project administration. Erdal Agcam: investigation, resources, writing review and editing. Mustafa Bircan, Mustafa Unlu, Şenay Karabiyik, Şule Hilal Attar, Ebru Kafkas: resources, investigation. Sevgi Paydas Kargi: writing review and editing, supervision.

Declarations

Conflict of interest M.A. Sarıdaş, E. Ağçam, M. Bircan, M. Ünlü, Ş. Karabıyık, Ş.H. Attar, E. Kafkas, and S. Paydaş Kargı declare that they have no competing interests.

Ethical standards For this article no studies with human participants or animals were performed by any of the authors. All studies mentioned were in accordance with the ethical standards indicated in each case.

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