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Determining Total Phenolics and Antioxidant Activity of Selected *Fragaria* Genotypes

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Strawberries are known to have high antioxidant properties. In present study, we determined total phenolics and antioxidant activity of a group of *Fragaria* genotypes representing the Turkish diploid strawberry genotypes, dominating cultivars and some selections and hybrids. The average total phenolic compounds and antioxidant capacities were highest in wild material 7914 µg GAE/gfw and 70.2 µmol TE/gfw, respectively. The average of total phenolic compounds for hybrids was the highest (2467 µg GAE/gfw) and followed by selections (2395 µg GAE/gfw) and varieties (2318 µg GAE/gfw). The values increased during the season progress. A similar trend was observed on antioxidant activity of selected strawberries. The antioxidant capacity in the wild material was detected more than 3 fold higher than the *F. ×ananassa* groups (70.2 *vs.* 19.9, 21.4, 21.1 µmol TE/gfw). The present results showed that there is a great variability among the strawberry genotypes tested for total phenolic compounds and antioxidant capacities. Therefore, in addition to taste and aroma characteristics high antioxidant properties should be important for cultivar selection by consumers and breeders for healthy diet.

Key Words: Strawberry, Genetic resources, Diversity, Health, Antioxidant.

INTRODUCTION

The importance of the phenolic compounds on the human health has recently revised much attention. There is increasing evidence that rich diet in fruits and vegetables reduce the risk of common cancers, cardiovascular diseases and chronic degenerative diseases of aging[1,2]. A major benefit from such a diet may be increased consumption of various phytochemicals which act as antioxidants in these foods[3]. Among fruits and vegetables, small fruits are known to have strong antioxidant capacity mainly due to their

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high levels of phenolic compounds[4,5]. The phenolic compounds and the antioxidant activities are reported to be closely associated with several factors including genotypes, growing conditions, stage of maturity, fruit characteristics, size, colour, postharvest durations and treatments[6-8].

There are more than 20 *Fragaria* species described. The cultivated strawberry, *F.* ×*ananassa*, is the hybrid of two octoploid species, *F. chiloensis* and *F. virginiana*[9]. Since the cultivated strawberry has a narrow genetic base while the wild species has tremendous variation and most of the wild species are crossable with *F.* ×*ananassa*, the studies on *Fragaria* genetic resources have had an increasing trend recently. The Turkish strawberry genetics resources consist of both diploid species, *Fragaria vesca* and *F. viridis* and octoploids local varieties, *F.* ×*ananassa*. The diploid species are usually found in the woodlands of the northern part of Turkey. Fruits of these strawberry plants are collected and consumed locally while small amount of wild strawberries are frozen as well. It is difficult to harvest these berries because of their small size. However, their unique aromas make the effort worthwhile. Especially, 'Ottoman' is an old native variety grown locally in Turkey. In a previous study, we sampled more than 50 populations of *Fragaria* species from various parts of Turkey with the elevation from 6 to 2007 m. The genotypes are currently available at research station of Mustafa Kemal University.

The objective of this study was to evaluate the variation on a diverse group of *Fragaria* accessions for their total phenolic compounds and antioxidant activities. The genotypes were sampled at different times to determine changes within on the growing seasons. There is a thought among the strawberry breeders if these traits should be included among their breeding objectives. The variability is a prerequisite for such an approach although the breeders need to determine other factors on the expression of these traits as well.

EXPERIMENTAL

The genotypes studied, their groups and characteristics are listed in Table-1. Camarosa and Sweet Charlie are leading strawberry varieties currently grown in Turkey. Ottoman is an old variety whose origin is not known. Ottoman is an unusual variety having extremely strong and unique aroma, relatively small and rounded berries with very light skin and flesh colour. The plants of Ottoman look similar to pure *F. chiloensis* genotypes morphologically. The foreign selections are provided from an Italian breeding program. More information regarding these genotypes is avaliable[10]. The hybrids are from the University of Çukurova Strawberry Program. Most of these hybrids have Ottoman in their pedigrees. The diploid *Fragaria vesca* genotypes are sampled from Tokat, Samsun and Ordu in Turkey.

TABLE-1
THE GENOTYPES, THEIR SPECIES, GROUPS AND CHARACTERISTICS FOR THE STUDY WHERE TOTAL PHENOLICS ANTIOXIDANT ACTIVITIES WERE DETERMINED ON SEVERAL DIFFERENT TIMES

Group	Species	Genotype	Characteristics
Variety	*Fragaria ×ananassa*	Camarosa	American variety from Univ. of California breeding program.
	Fragaria ×ananassa	Gaviota	American variety from Univ. of California breeding program.
	Fragaria ×ananassa	Sweet Charlie	American variety from Univ. of Florida breeding program.
	Fragaria ×ananassa	Ottoman	A local variety from Turkey.
Selection	*Fragaria ×ananassa*	Selection 1	Advance selection from Italian breeding program.
	Fragaria ×ananassa	Selection 2	Advance selection from Italian breeding program.
	Fragaria ×ananassa	Selection 3	Advance selection from Italian breeding program.
	Fragaria ×ananassa	Selection 4	Advance selection from Italian breeding program.
	Fragaria ×ananassa	Selection 5	Advance selection from Italian breeding program.
	Fragaria ×ananassa	Selection 6	Advance selection from Italian breeding program.
Hybrid	*Fragaria ×ananassa*	Hybrid 3	Selection from Cukurova University Strawberry Research Program.
	Fragaria ×ananassa	Hybrid 5	Selection from Cukurova University Strawberry Research Program.
	Fragaria ×ananassa	Hybrid 6	Selection from Cukurova University Strawberry Research Program.
	Fragaria ×ananassa	Hybrid 12	Selection from Cukurova University Strawberry Research Program.
	Fragaria ×ananassa	Hybrid 13	Selection from Cukurova University Strawberry Research Program.
	Fragaria ×ananassa	Hybrid 17	Selection from Cukurova University Strawberry Research Program.
Wild	*Fragaria vesca*	TF 22	Germplasm collected from Turkey (40º 81 N; 36º 59 E; 1172 m.)
	Fragaria vesca	TF 30	Germplasm collected from Turkey (41º 33 N; 36º 13 E; 803 m.)
	Fragaria vesca	TF 31	Germplasm collected from Turkey (40º 72 N; 37º 94 E; 1601 m.)

The *F.* ×*ananassa* genotypes (varieties, selections and hybrids) were grown in a common unheated greenhouse on a raised-bed growing system. They were planted in August 2005 as containerized fresh plants. The optimum growing conditions were applied to them. Standard fertilization and pest management was performed all plants tested.

The *F.* ×*ananassa* genotypes were sampled 3 times on 13 February, 27 April and 10 June while *F. vesca* genotypes were sampled once on 24 July. All fruit samples were harvested and immediately frozen in -20ºC until the analysis of total phenolic and antioxidant capacities.

Sample extraction and total phenolics determination: The content of total phenolic was measured according to previous work[11] with slight modifications. Briefly, 100 g of berry samples were homogenized in a blender. Aliquots were then transferred to polypropylene tubes and extracted with buffer containing acetone, water and acetic acid (70:29.5:0.5 v/v) for 1 h. Then, extract, Folin-Ciocalteu's phenol reagent and water incubated for 8 min followed by adding sodium carbonate solution. After 2 h, absorbance was measured at 750 nm. Gallic acid was used as standard. The results are expressed as µg gallic acid equivalent in g fresh weight basis (GAE/gfw).

Trolox equivalent antioxidant capacity (TEAC): For the modified TEAC assay, 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) was dissolved in acetate buffer and prepared with potassium persulfate as described in literature[12,13]. This mixture was diluted in acidic medium of 20 mM sodium acetate buffer (pH 4.5) to an absorbance of 0.700 ± 0.01 at 734 nm for longer stability[13]. For the spectrophotometric assay, 3 mL of the $ABTS^+$ solution and 20 µL of fruit extract were mixed and the absorbance was determined at 734 nm at 10 min after mixing.

Statistical analyses were carried out using SAS[14]. The pair-wise comparisons, for both months and genotypes groups within each sampling months, were done by t-test assuming equal variance. The *F. vesca* genotypes and their sampling month, July, were not included in the pair-wise t-test comparisons as they are not direct counterparts for the other factors.

RESULTS AND DISCUSSION

The genotypes studied can be divided into four groups: varieties, selections, hybrids (*F.* ×*ananassa*) and wild materials (*F. vesca*). The average of total phenolic compounds and antioxidant activities were the highest on wild material (7914 µg GAE/gfw and 70.2 µmol TE/gfw, respectively) (Table-2). These averages were not compared to the averages of the others since they were harvested once when there were no berries from other groups. However, the differences between wild material and any other group were found to be differ for both of the variable studied (Table-2).

TABLE-2
TOTAL PHENOLICS (μg GAE/gfw) AND ANTIOXIDANT CAPACITIES (μmol TE/gfw) FOR SEVERAL *Fragaria* ×*ananassa* GENOTYPES GROWN ON A COMMON UNHEATED GREENHOUSE AND WILD *F. vesca* GENOTYPES COLLECTED FROM THEIR SAMPLING SITES

Group	Genotype	Total phenolics (μg GAE/gfw)					Antioxidant capacity (μmolTE /gfw)				
		February	April	June	July	Mean	February	April	June	July	Mean
Variety	Camarosa	1811	2802	2959	–	2524	19.86	17.87	25.77	–	21.2
	Gaviota	2034	2282	–	–	2158	18.76	20.89	24.95	–	21.5
	Sweet Charlie	1815	2637	2234	–	2228	18.01	20.14	19.48	–	19.2
	Ottoman	–	1989	2723	–	2356	18.35	17.42	17.73	–	17.8
	Mean	1886	2427	2639	–	2318	18.7	19.1	22.0	–	19.9
Selection	Selection 1	1687	2541	2943	–	2390	16.33	21.68	20.79	–	19.6
	Selection 2	2215	1993	3029	–	2412	17.87	19.76	29.31	–	22.3
	Selection 3	2182	2434	2547	–	2388	20.03	19.76	22.95	–	20.9
	Selection 4	1946	2581	3177	–	2568	19.21	21.89	28.89	–	23.3
	Selection 5	1558	1908	3210	–	2225	19.76	24.16	28.93	–	24.3
	Selection 6	1731	2058	3395	–	2395	16.22	19.28	28.72	–	21.4
	Mean	1887	2252	3050	–	2397	18.2	21.1	26.6	–	22.0
Hybrid	Hybrid 3	1858	1995	2654	–	2169	20.34	19.24	23.95	–	21.2
	Hybrid 5	2327	2187	2403	–	2305	21.13	20.14	20.41	–	20.6
	Hybrid 6	2622	3773	3735	–	3377	24.95	24.46	27.07	–	25.5
	Hybrid 12	2084	3275	2137	–	2499	15.91	18.80	16.60	–	17.1
	Hybrid 13	3096	3187	2815	–	3033	22.88	22.27	21.44	–	22.2
	Hybrid 17	1735	2633	2602	–	2323	17.56	20.21	18.59	–	18.8
	Mean	2287	2842	2724	–	2618	20.5	20.9	21.3	–	20.9
	Overall mean	2047	2517	2838	–	2467	19.2	20.5	23.5	–	21.1
Wild	*F. vesca*, TF 22	–	–	–	7027	7027	–	–	–	73.3	73.3
	F. vesca, TF 30	–	–	–	8695	8695	–	–	–	71.2	71.2
	F. vesca, TF31	–	–	–	8019	8019	–	–	–	66.0	66.0
	Mean	–	–	–	7914	7914	–	–	–	70.2	70.2

For the total phenolic compounds, the average of the hybrids was the highest (2467 µg GAE/gfw) and followed by selections (2395 µg GAE/gfw) and varieties (2318 µg GAE/gfw). The values increased as the season progressed. The overall averages for these three groups were 2047, 2517 and 2838 µg GAE/gfw for February, April and June, respectively (Table-2). The differences were found to be statistically significant for all pair-wise comparisons for the months except for April *vs.* June (Table-3). For the varieties, different varieties had the highest values for different dates indicating genotype × environment interaction. Similarly, different selections were found to be superior for different sampling dates. The trend was present for the hybrids as well, although some genotypes were among the high groups. For example, hybrid number 6 had the second highest values on February and the highest values for April and June (Table-2). When these three groups were compared by t-test within each sampling date, no significant difference were revealed for any of the pair-wise comparisons indicating no groups were superior for the total phenolic compounds.

The trends obtained for the antioxidant activities were found to be similar to those of total phenolic compounds. First, the antioxidant activities in the wild material was more than 3 fold higher than the *F.* ×*ananassa* groups (70.2 *vs.* 19.9, 21.4, 21.1 µmol TE /gfw) (Table-2). Second, the antioxidant activities for the three groups of *F.* ×*ananassa* genotypes increased as the season progressed (19.2, 20.5 and 23.5 µmol TE /gfw). Third, different genotypes had the highest values for different months although there were some highest numbers (hybrid 6, *e.g.*, had the highest numbers for all three sampling dates) (Table-2). Also, sampling dates were found to be statistically significant for all months except February *vs.* April comparisons (Table-3). Finally, when the groups were compared within each month they were not significantly different except for selection *vs.* hybrid in June suggestion no superior groups in terms of antioxidant capacities (Table-3). Indeed, total phenolic compounds and antioxidant capacities were found to be highly correlated when the average of each genotype in each sampling date for antioxidant capacity was plotted over total phenolic compounds (Fig. 1).

The present study revealed that there is a great variability among the strawberry genotypes tested in this study for their total phenolic compounds and antioxidant activities among strawberry genotypes from various backgrounds. Indeed, there are numerous studies indicating genotypic variability for these traits within *F.* ×*ananassa* groups[15]. Kosar *et al.*[16] also studied the phenolic composition of Ottoman and its several hybrids along with Camarosa, Dorit and Chandler using a high pressure liquid chromatography method and recovered the highest phenolic contents from an Ottoman hybrid. There is a genetic variability for the traits both within *F.* ×*ananassa*

TABLE-3

PAIR-WISE COMPARISONS OF SAMPLING DATES AND GROUPS OF *Fragaria* GENOTYPES FOR TOTAL PHENOLICS (µg GAE/gfw) AND ANTIOXIDANT CAPACITY (µmol TE /gfw) FOR SEVERAL *Fragaria* GENOTYPES GROWN ON A COMMON UNHEATED GREENHOUSE SAMPLED FROM FEBRUARY TO JUNE. THE FRUITS OF THE WILD *F. vesca* GENOTYPES WERE COLLECTED FROM THEIR SAMPLING SITES

Pair-wise comparison	Total phenolics (µg GAE/gfw)				Antioxidant capacity (µmolTE /gfw)			
	Mean[1]	Mean[2]	T value	P value	Mean[1]	Mean[2]	T value	P value
Sampling time comparisons[3]								
February *vs.* April	2047	2517	-2.78	0.010[4]	19.2	20.50	-1.66	0.110
February *vs.* June	2047	2838	-5.15	0.000	19.2	23.47	-3.44	0.002
February *vs.* July	2047	7914	-11.84	0.007	19.2	70.16	-22.65	0.002
April *vs.* June	2517	2838	-1.83	0.078	20.5	23.47	-2.49	0.021
Group comparisons[3]								
February								
Variety *vs.* Selection	1887	1886	0.00	1.000	18.745	18.24	0.64	0.550
Variety *vs.* Hybrid	1887	2287	-1.81	0.120	18.745	20.46	-1.21	0.280
Selection *vs.* Hybrid	1886	2287	-1.70	0.130	18.240	20.46	-1.46	0.190
April								
Variety *vs.* Selection	2427	2253	0.80	0.460	19.08	21.09	-1.77	0.120
Variety *vs.* Hybrid	2427	2842	-1.24	0.260	19.08	20.85	-1.46	0.190
Selection *vs.* Hybrid	2253	2842	-1.92	0.100	21.09	20.85	0.20	0.840
June								
Variety *vs.* Selection	2639	305	-1.68	0.190	21.98	26.60	-1.84	0.120
Variety *vs.* Hybrid	2639	2724	-0.28	0.790	21.98	21.34	0.25	0.810
Selection *vs.* Hybrid	3050	2724	1.29	0.240	26.60	21.34	2.43	0.038

[1]The average of the first date or group; [2]The average of the second date or group; [3]Group comparisons exclude comparisons with wild material as the wild materials were collected in July while the others were harvested from February to June; [4]The significant values, at 5%, were bolded.

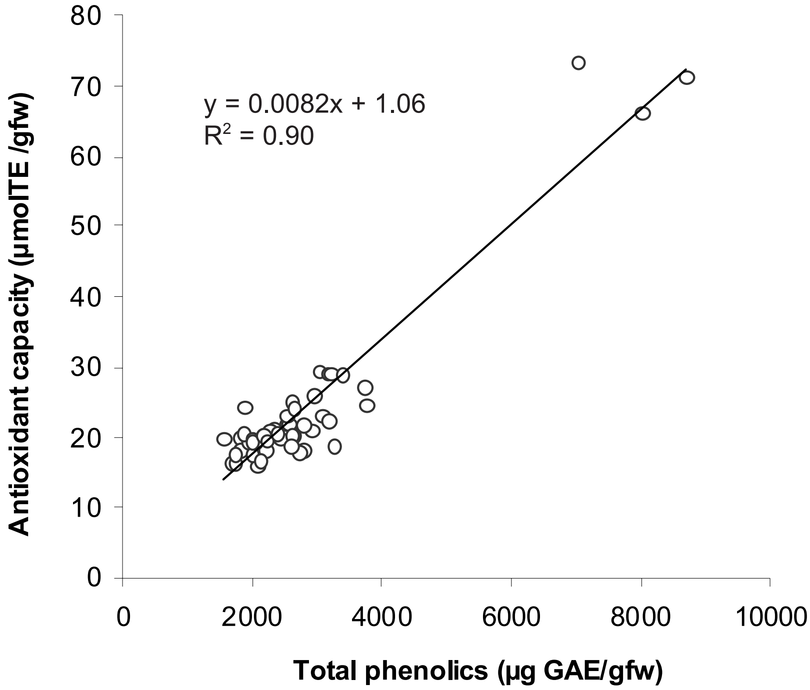

Fig. 1. Correlation between total phenolics (µg GAE/gfw) and antioxidant capacities (µmol TE/gfw) of several fragaria genotypes grown on a common unheated greenhouse and sampled from february to June 2006 or wild *Fragaria vesca* genotypes collected from their sampling sites in July 2006

and the wild materials. It looks a reasonable option to breed for genotypes having high phenolic contents. However, it is also documented that these traits are highly affected by several other factors such as harvesting date, growing conditions and storage duration and conditions[15,17]. Hence, there is a need to partition the variance components for total phenolic compounds and compare the genotypic variance to the environmental variance. Finally, most of the studies conducted in the area are based on laboratory studies. It is not known if the values obtained in the laboratory studies correlate with the activities of the phenolic compound on human body. It is suggested that more detailed studies are needed to breed strawberry cultivars that give higher total phenolic compounds and antioxidant activities within human body. Meanwhile, enjoying more of delicious strawberries seems the best option to have high antioxidant for human health.

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