Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2016) 40: 542-551
© TÜBİTAK
doi:10.3906/tar-1601-24

Antioxidant potential of Turkish pepper (*Capsicum annuum* L.) genotypes at two different maturity stages

Davut KELEŞ[1,*], Şenay ÖZGEN[2], Onur SARAÇOĞLU[3], Atilla ATA[1], Mustafa ÖZGEN[2]
[1]Alata Horticultural Research Institute, Erdemli, Mersin, Turkey
[2]Department of Plant Production and Technologies, Ayhan Şahenk Faculty of Agricultural Sciences and Technologies, Niğde University, Niğde, Turkey
[3]Department of Horticulture, Faculty of Agriculture, Gaziosmanpaşa University, Tokat, Turkey

Received: 06.01.2016 • **Accepted/Published Online:** 08.03.2016 • **Final Version:** 14.06.2016

Abstract: Improved phytochemicals and antioxidant properties in crops are becoming important traits in many breeding programs. In this study, along with several other horticultural attributes, total phenolic content (TP) and antioxidant capacity (ferric reducing ability of plasma (FRAP) and Trolox equivalent antioxidant capacity (TEAC)) of 52 superior pepper genotypes from the Alata Pepper Breeding Program were examined. The fruits from these plants were harvested at immature and mature stages. The genotypes greatly varied for TP, FRAP, TEAC, soluble solids, vitamin C content, and fruit color as determined by L, a and b values. The range for TP was 319–4047 µg GAE/g fresh weight (fw), while FRAP and TEAC varied between 0.22 and 0.56 µmol of TE/g fw and 0.08 and 1.88 µmol TE/g fw. All these characteristics were considerably variable between immature and mature stages. These characteristics were also found to be significantly correlated. Principal component analyses conducted for all the characters used in the study and constructed separately for immature and mature stages demonstrated no obvious patterns for pepper types. Therefore, our overall results suggest that individual pepper cultivars having high total phenolic and antioxidant capacity can be utilized in developing new pepper cultivars with rich phytochemical content.

Key words: Ascorbic acid, breeding, germplasm, ferric reducing ability of plasma, phenolic, Trolox equivalent antioxidant capacity

1. Introduction

Pepper is an important vegetable crop in many countries and Turkey ranks fourth in world pepper production with 2.2×10^6 t (faostat.fao.org). Turkey has a large number of local and popular cultivars (Aktas et al., 2011; Bozokalfa and Eşiyok, 2011).

Pepper is considered an important vegetable crop, not only due to its economic importance, but also for the nutritional value of its fruits. They are rich in phytochemicals and a good source of vitamins C and E and provitamin A. A wide spectrum of antioxidants such as flavonoids, phenolic compounds, and carotenoids, are also present in pepper fruits (Guil-Guerrero et al., 2006; Carvalho et al., 2015). Materska and Perucka (2005) identified 10 specific phenolic compounds in pepper fruit. Hot cultivars contain capsaicinoids alkaloids with pharmacological properties giving the specific taste to pepper fruit (Jayaprakasha et al., 2012).

With regard to human health, some studies indicated that these compounds have an important protective role given their antioxidant activity. They can neutralize free radicals or their actions can modulate the activity of enzymes involved in detoxification, oxidation, and reduction processes (Edge et al., 1997). Antioxidant vitamins A and C help to prevent cell damage, cancer, and diseases related to aging, and support immune function (Howard et al., 2000). Red peppers are also a good source of the carotenoid called lycopene, which is earning a reputation for helping to prevent prostate cancer as well as cancer of the bladder, cervix, and pancreas (Rao and Rao, 2007). Several epidemiological studies reported an inverse correlation between a high intake of carotenoids and a reduced risk of colon cancer (Rao and Rao, 2007).

Many studies related to antioxidant activity and composition analysis of pepper have been performed. From the agronomic point of view, researchers found that

* Correspondence: davut.keles@gthb.gov.tr

other than genotypic variation (Lee et al., 1995; Frary et al., 2008), maturity (Hornero-Mendez et al., 2000; Howard et al., 2000; Fox et al. 2005) and color (Zhang and Hamauzu, 2003; Sun et al. 2007) can influence the antioxidant properties of peppers. The fruits can be consumed at different ripening stages from green to red-colored fully ripe stages. Green peppers are harvested earlier, before they have a chance to turn yellow, orange, and then red. Compared to green peppers, the red ones have almost 10 times more beta-carotene and 1.5 times more vitamin C (Howard et al., 2000; Matsufuji et al., 2007). Sun et al. (2007) demonstrated that antioxidant activity increased with fruit maturation. Biologically active carotenoids such as β-carotene and lycopene reach their highest levels in red fruits. Beneficial effects of maturation on the other antioxidant compounds showed the red stage to be optimal from the nutritional point of view. Bae et al. (2014) examined the impacts of cultivar, fruit maturity stage, and two growing season on the concentration of bioactive compounds in diverse pungent and nonpungent peppers. They observed significant interactions among cultivars, maturity stages, and growing seasons. Mature peppers generally had the highest content of ascorbic acid (782.0–2305.3 mg/g fresh weight (fw) in 2008 and 693.5–2817.2 mg/g fw in 2009) and capsaicinoids (115.5–338.9 mg/g fw in 2008 and 93.8–326.3 mg/g fw in 2009) compared to immature peppers.

After many scientific studies of the health benefits of plant-based antioxidants, consumers are seeking out rich antioxidant contents of fruits and vegetables to avoid the onset of cancer and other diseases (Scheerens, 2001). This has directly influenced plant breeders' goals for new cultivar development studies. Improved phytochemicals and antioxidant properties in crops have become important trait in many breeding programs. Although Turkey is not a germplasm center of pepper, due to consumer demand and traditional Turkish cuisine, wide ranges of pepper cultivars are grown in this region where rich genetic diversity exists. Collection and characterization of plant genetic resources and assessment of horticultural, genetic, and phytochemical variations play a fundamental role in plant breeding programs.

In this study, Turkish and popular pepper genotypes have been evaluated for their antioxidant properties. The aim of this study was to determine antioxidant and phytochemical content of the germplasm for future breeding targets.

2. Materials and methods

2.1. Plant material and pomological analysis

From the Alata Pepper Breeding Program, 52 superior genotypes were selected for determination antioxidant and phenolic diversity of peppers. These superior genotypes were selected as they were identified as a "core collection" for the Alata Pepper Breeding Program based on their phenotypic variation. All genotypes and cultivars in this breeding program were grown in controlled greenhouse conditions in the Alata Horticultural Research Institute, Erdemli, Mersin, Turkey. The characteristics of these peppers are presented in Tables 1 and 2. Peppers at immature and mature stages were hand-harvested and transferred to the laboratory for physical and phytochemical analysis.

The color was measured at the time of harvest using a Minolta portable chromameter (Minolta, Model CR-400), which provided CIE L*, a* and b* values. Peppers were cut in half and seeds were removed. Three replicates of 10 peppers were then frozen immediately and stored at –80 °C until analyzed. For extraction, samples were thawed at room temperature and homogenized in a standard food blender. Obtained slurries were used to determine total soluble solid (TSS) contents by refractometer (Pal-1, Atago). Remaining slurries were used for antioxidant and phenolic assays.

2.2. Analytical procedures

2.2.1. Determination of total phenolic (TP) content

TP content was measured according to Singleton and Rossi (1965). Briefly, slurries were extracted with buffer containing acetone, water, and acetic acid (70:29.5:0.5 v/v) for 2 h in darkness. Samples were replicated three times and then extract, Folin–Ciocalteu phenol reagent, and water were incubated for 8 min followed by adding 7% sodium carbonate. After 2 h, the absorbance was measured with an automated UV-Vis spectrophotometer (Model T60U, PG Instruments) at 750 nm. Gallic acid was used as a standard. The results were expressed as µg gallic acid equivalent per g fresh weight (GAE/g fw).

2.2.2. Total antioxidant activity (TAC)

TAC was estimated using two standard procedures, ferric reducing ability of plasma (FRAP) and Trolox equivalent antioxidant capacity (TEAC) assays, as suggested by Özgen et al. (2006). The results were expressed as µmol Trolox equivalent per g fresh weight (TE/g fw).

2.2.2.1. FRAP

FRAP was determined according to the method of Benzie and Strain (1996). The assay was conducted using three aqueous stock solutions containing 0.1 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ [2,4,6-tris(2-pyridyl)-1,3,5-triazine] acidified with concentrated hydrochloric acid, and 20 mmol/L ferric chloride. These solutions were prepared and stored in the dark under refrigeration. Stock solutions were combined (10:1:1 v/v/v) to form the FRAP reagent just prior to analysis. For each assay a laboratory duplicate from each replicate, 2.97 mL of FRAP reagent, and 30 µL of sample extract were mixed. After 30 min, the absorbance of the reaction mixture was determined at 593 nm on a spectrophotometer.

2.2.2.2. TEAC

For the standard TEAC assay, ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) was dissolved in acetate buffer and prepared with potassium persulfate as described by Özgen et al. (2006). The mixture was diluted in an 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 (Özgen et al., 2006). For the spectrophotometric assay, 2.97 mL of the $ABTS^{·+}$ solution and 30 µL of fruit extract were mixed and incubated for 10 min and the absorbance was determined at 734 nm.

2.2.3. Chromatographic conditions and ascorbic acid determination

Pepper slurries (5 g) were diluted with metaphosphoric acid (2.5%) solution for individual organic acid analysis. The homogenate was centrifuged at 6000 rpm for 5 min. Supernatants were filtered through a 0.45-µm membrane filter (Iwaki Glass) before HPLC analysis, and the mobile phase solvents were degassed before use. The HPLC analyses were carried out using a PerkinElmer HPLC system with Totalchrom Navigator 6.2.1 software, a pump, and a UV detector (PerkinElmer, Series-200) (Waltham, MA, USA). Separation and determination of organic acids was modified from Özgen et al. (2009). The separation was carried out on an SGE Wakosil C18RS 5-µm column (250 × 4.6 mm I.D.). Detection was performed at 215 nm. Optimum efficiency of separation was obtained using sulfuric acid solution of pH 2.5 (solvent A) and methanol (solvent B). Other parameters adopted were as follows: injection volume, 20 µL; column temperature, 30 °C; and detection wavelength, 215 nm.

2.3. Statistical analysis

Data were analyzed using SAS procedures and software (SAS Institute, 2006). Means and standard deviations were obtained using TABULATE. Principal component analysis (PCA) was performed using the PRINCOMP procedure and the accessions were plotted on the first three principal components (PCs) using the G3G procedure.

3. Results and discussion

The TP and TAC and several horticultural attributes of 52 superior genotypes from a diverse genetic background were determined. The plant material contained genotypes from long green pepper, bell pepper, Kahramanmaraş, capia, Hungarian, chinense, Charleston, ornamental, and tomato-pepper types. Fruit diameter, fruit weight, and thickness of fruit wall greatly varied among these genotypes at both immature and mature stages (Tables 1 and 2). Flesh color and immature and mature fruit colors of these genotypes are presented in Table 3. The averages of TP, FRAP, TEAC, soluble solids, and vitamin C contents are also presented for two different maturation stages in Table 3. The stages had a compound effect on these variables. For instance, the averages of TP for immature and mature stages were 1349 vs. 2025 µg GAE/g fw, while the averages for FRAP and TEAC were 0.43 vs. 1.40 and 0.64 vs. 1.01 µmol TE/g fw. These represent 50%, 164%, and 195% increases for TP, FRAP, and TEAC respectively.

Similar to our findings, Sun et al. (2007) demonstrated that antioxidant activity increased with fruit maturation. Biologically active carotenoids such as β-carotene and lycopene reach their highest levels in red fruits. Bhandari et al. (2013) observed continuous increases in vitamin C, total phenol, vitamin E, total free sugar, beta-carotene, linolenic acid content, and antioxidant activity during ripening of pepper fruits at three different ripening stages of green mature, intermediate breaker, and red ripe stages. Bae et al. (2014) confirmed these findings with eight cultivars and three different ripening stages of peppers.

ANOVA was conducted for TP, FRAP, TEAC, soluble solids, vitamin C content, and color measurements (L, a, and b) by using both immature and mature measurements (Table 4). The range for TP was 319–4047 µg GAE /g fw, while FRAP and TEAC varied between 0.22 and 0.56 µmol TE/g fw and 0.08 and 1.88 µmol TE/g fw. Soluble solids varied between 3.8% and 12.8%. A greater deal of variation was found for Vitamin C (5.3–77.8 mg/100 g fw). The L, a, and b values were greatly varied (32.7 to 64.7, –94 to 18.7, and 16.0 to 48.3 for L, a, and b, respectively) at both maturation stages.

All these horticulturally important attributes were found to be significantly correlated with each other (Table 5). TP, FRAP, TEAC, soluble solids, vitamin C content, and "a" were positively correlated while "L" and "b" were negatively correlated with these characteristics. Bhandari et al. (2013) observed similar patterns throughout the ripening processes whereby positive correlations with antioxidant activity were observed in vitamin E (r = 0.814), beta-carotene (r = 0.772), vitamin C (r = 0.610), and total phenol (r = 0.595) contents while capsaicinoids, total flavonoid, and phytosterols exhibited no or slightly negative correlations. Bae et al. (2014) indicated a positive

Table 1. Several horticultural characteristics of 52 superior pepper genotypes from the Alata Pepper Breeding Program.

Genotype	Immature (1)						Mature (2)						
	Fruit color	Type	Thickness of fruit wall (skin)	Fruit length	Fruit diameter	Fruit weight	Fruit color	Type	Thickness of fruit wall (skin)	Fruit length	Fruit diameter	Thickness of fruit wall (skin)	Fruit weight
15	1	2	1	3	4	6	1	2	1	3	4	4	6
31	1	1	1	3	3	4	7	1	3	3	3	2	4
32	1	8	1	3	5	6	7	8	4	3	5	4	6
35	1	6	1	2	3	3	8	6	3	2	3	1	3
47	2	6	2	2	2	2	9	6	3	2	2	2	2
74	1	5	2	2	7	7	9	5	3	2	7	4	7
81	1	1	1	4	2	3	7	1	3	4	2	2	3
93	3	1	1	4	3	4	7	1	3	4	3	2	4
107	1	3	1	2	3	2	7	3	3	2	3	1	2
173	8	1	1	4	3	4	9	1	3	4	3	2	4
200	4	1	1	3	3	4	9	1	4	3	3	2	4
202	1	6	1	2	4	3	7	6	3	2	4	1	3
215	4	1	1	3	2	2	9	1	3	3	2	1	2
226	1	1	1	4	3	4	8	1	4	4	3	2	4
261	1	1	1	3	3	3	7	1	3	3	3	1	3
269	1	1	1	3	2	3	9	1	3	3	2	2	3
276	3	2	2	3	3	5	8	2	4	3	3	3	5
302	1	4	1	2	4	4	7	4	3	2	4	2	4
304	4	3	2	2	4	4	7	3	4	2	4	2	4
388	1	6	1	1	3	2	7	6	3	1	3	1	2
390	6	6	1	1	2	1	7	6	3	1	2	1	1
1029	1	9	1	1	4	3	7	9	3	1	4	4	3
1676	1	5	1	2	9	12	7	5	4	2	9	5	12
1719	2	7	2	3	5	10	9	7	4	3	5	4	10
1721	1	5	2	2	7	11	9	5	4	2	7	3	11
1738	1	7	2	4	6	10	7	7	3	4	6	4	10
1763	1	7	1	4	5	9	7	7	4	4	5	3	9
1779	1	12	1	3	6	11	9	12	4	3	6	6	11
1780	1	3	1	2	4	3	9	3	3	2	4	1	3
1788	1	3	2	3	6	9	7	3	4	3	6	6	9
1839	3	11	1	1	3	3	8	11	3	1	3	1	3
1842	1	10	1	1	2	1	7	10	3	1	2	2	1
1866	5	6	1	2	1	1	7	6	3	2	1	1	1
1882	1	5	2	2	7	10	7	5	4	2	7	4	10
1883	3	5	2	1	8	11	7	5	4	1	8	5	11
1885	6	5	2	2	8	11	5	5	4	2	8	6	11
1886	4	8	2	2	5	6	8	8	4	2	5	4	6
1888	1	5	2	2	9	13	7	5	4	2	9	5	13
161	1	6	1	1	3	2	7	6	3	1	3	2	2
19a	1	1	1	3	5	6	7	1	4	3	5	4	6
242-b	1	2	1	3	3	4	7	2	3	3	3	2	4
283a	1	2	1	4	3	4	7	2	4	4	3	2	4
3a	1	6	1	2	3	3	7	6	3	2	3	2	3
405a	1	5	2	2	6	7	7	5	4	2	6	3	7
764-2-1b	4	8	2	2	6	7	5	8	4	2	6	5	7
765-4-2b	4	8	2	2	7	10	8	8	4	2	7	5	10
765-4-3b	4	8	2	3	6	9	5	8	4	3	6	6	9
769-5	1	9	2	1	7	8	7	9	4	1	7	6	8
769-5-1b	3	9	2	1	7	8	8	9	4	1	7	4	8
771-8	2	1	1	3	1	1	9	1	3	3	1	1	1
774-3	2	1	1	3	2	2	9	1	3	3	2	1	2
774-4-2b	1	1	1	3	2	2	7	1	3	3	2	1	2

Table 2. Several characteristics and their classes used to evaluate of 52 superior pepper genotypes from the Alata Pepper Breeding Program.

Maturity period	Fruit color	Type	Thickness of fruit wall (skin)	Fruit length	Fruit diameter
1: Green maturity	1: Dark green	1: Long green pepper	1: Thin skin (unripe)	1: Very short	1: Extremely narrow
2: Red maturity	2: Green	2: Charleston	2: Thick skin (unripe)	2: Short	2: Very narrow
	3: Light green	3: Kahramanmaraş	3: Thin skin (ripe)	3: Medium	3: Narrow
	4: Yellow	4: Şanlıurfa	4: Thick skin (ripe)	4: Long	4: Medium narrow
	5: Orange	5: Bell			5: Medium
	6: Purple	6: Ornamental pepper			6: Medium wide
	7: Red	7: Hatay			7: Wide
	8: Light red	8: Hungarian			8: Very wide
	9: Dark red	9: Tomato Pepper			9: Extremely wide
		10: *Frutenses*			
		11: *Chinense*			
		12: Capia			

correlation between total phenolics and DPPH radical scavenging activity in their study that included 8 cultivars and three different ripening stages.

PCA was conducted using all 17 characteristics recorded in the study and for two maturation stages separately. The first three PCs explained 65% and 63% of the total variation for immature and mature stages (Table 6). For the immature data set, PC1 was highly correlated with fruit weight, diameter, and thickness of the fruit wall. Fruit length, color, and fruit measurements (L, a, and b) were important for PC2 while the most important characters for PC3 were fruit color and a. The importance of the characters forming the PCs had some differences for the mature stage. Although similar characters were highly correlated with PC1 for the mature stage as well, the highest correlations between PC2 and characteristics were found with FRAP and TEAC. For PC3, the most important characteristics were maturity period, fruit color, and thickness of fruit wall. The genotypes and their first three PCs for immature and mature stages are presented in Figures 1 and 2. As revealed by the figures, there are no apparent group formations for either of the maturation stages.

In conclusion, these results revealed a great deal of variation for TP and TAC for the pepper genotypes coming from diverse genetic backgrounds. TP and TAC and both TEAC and FRAP increased with fruit maturation. The results also showed that several horticultural attributes are highly correlated with these characteristics. The fact that PCA conducted for all the characteristics used in the study and constructed separately for immature and mature stages demonstrated no obvious patterns suggests that individual pepper cultivars having high total phenolic compounds and antioxidant activities can be developed in pepper types. The genetic variability in antioxidant capacity found in this study constitutes a useful genetic base for improving the phytonutrient quality of peppers. Our results also shed light on the selection of parental genotypes to develop new cultivars with high phytochemical content.

Acknowledgments
We would like to thank the Alata Horticultural Research Institute, the General Directorate of Agricultural Research and Policies, and the Republic of Turkey Ministry of Food, Agriculture, and Livestock for supporting this research.

Table 3. Several characteristics of 52 superior pepper genotypes from the Alata Pepper Breeding Program at two different maturity stages.

					TP[a]		FRAP[b]		TEAC[c]		Soluble solids[d]		Vitamin C[e]	
Genotype	Type	Flesh	Immature color	Mature color	IM	M	IM	M	IM	M	IM	M	IM	M
15	Long green pepper	Thin	Green	Light red	1517	1998	0.32	1.12	0.23	0.29	4.2	8.6	18.9	52.4
31	Long green pepper	Thin	Green	Red	1373	2156	0.67	1.41	0.49	0.92	6.5	7.2	26.3	58.8
32	Bell	Thin	Green	Red	1309	1625	0.31	0.88	0.21	0.65	5.2	6.4	21.8	44.3
35	Ornamental pepper	Thin	Green	Light red	1516	2209	0.45	1.52	0.29	0.79	4.9	10.1	41.0	51.3
47	Ornamental pepper	Thin	Dark green	Dark red	2091	2521	0.46	0.74	0.41	0.66	7.0	8.8	20.2	44.2
74	Bell	Thick	Green	Dark red	1222	3141	0.34	1.49	0.20	1.28	4.4	8.3	23.0	84.1
81	Long green pepper	Thin	Green	Green	1567	2421	0.90	1.67	0.72	1.18	6.4	9.1	23.1	53.5
93	Long green pepper	Thin	Light green	Red	1181	1870	0.40	1.10	0.30	0.89	5.4	7.9	24.6	40.6
107	Long green pepper	Thin	Green	Red	1422	2088	1.04	1.40	0.37	1.05	4.3	11.0	5.1	10.9
173	Long green pepper	Thin	Light red	Dark red	1130	1587	0.67	1.48	0.47	1.14	5.6	8.7	10.6	76.3
200	Long green pepper	Thick	Green	Dark red	1597	1768	0.42	1.46	0.31	1.21	5.6	6.9	25.1	58.0
202	Bell	Thin	Green	Red	1279	1862	0.37	1.03	0.26	0.86	4.8	8.7	43.4	74.7
215	Long green pepper	Thin	Green	Dark red	1310	4047	0.46	2.31	0.38	1.58	5.6	20.0	31.7	51.0
226	Long green pepper	Thick	Green	Light red	784	1869	0.15	1.28	0.13	1.06	1.8	6.6	17.4	57.9
261	Long green pepper	Thin	Green	Red	2442	2763	0.89	1.67	0.56	1.42	6.1	9.5	40.8	42.0
269	Long green pepper	Thin	Green	Dark red	782	2367	0.37	1.71	0.27	0.79	2.5	8.3	17.6	76.5
276	Long green pepper	Thick	Light green	Light red	1215	1643	0.45	1.22	0.30	0.87	4.9	7.7	17.7	37.4
302	Long green pepper	Thin	Green	Red	777	1044	0.29	0.86	0.19	0.85	3.2	4.4	18.8	43.4
304	Kahramanmaraş	Thick	Green	Red	948	2008	0.34	1.41	0.31	1.07	4.9	8.2	29.6	61.3
388	Ornamental pepper	Thin	Green	Red	1389	3184	0.80	1.51	0.56	1.36	6.0	10.9	44.8	99.3
390	Ornamental pepper	Thin	Purple	Red	3092	2247	1.31	1.30	1.11	1.08	7.1	16.2	4.5	16.6
1029	Tomato pepper	Thin	Green	Red	2166	3003	0.57	1.91	0.37	1.65	6.6	16.7	36.6	76.6
1676	Bell	Thin	Green	Red	2204	2337	1.18	0.98	0.45	0.64	6.1	10.2	28.7	63.1
1719	Bell	Thick	Dark green	Dark red	1209	1470	0.36	1.06	0.23	0.82	5.1	8.5	29.9	62.9
1721	Bell	Thick	Green	Dark red	1187	3185	0.24	2.21	0.16	1.86	5.0	14.5	29.0	84.6
1738	Bell	Thick	Green	Green	319	3657	0.10	2.53	0.08	1.78	1.5	16.7	26.3	56.7
1763	Hatay	Thick	Green	Red	955	1722	0.28	1.16	0.22	0.89	5.1	8.0	27.7	67.6
1779	Capia	Thick	Green	Dark red	707	1466	0.25	0.71	0.18	0.52	3.2	7.1	23.0	22.4
1780	Kahramanmaraş	Thin	Green	Dark red	1816	2368	0.85	1.14	0.34	0.81	5.3	12.4	47.3	59.3
1788	Kahramanmaraş	Thick	Green	Red	834	1460	0.34	0.84	0.23	0.59	5.1	7.2	31.5	37.7
1839	*Chinense*	Thin	Light green	Light red	1605	2219	0.67	1.82	0.50	1.25	5.1	11.6	13.1	68.8
1842	Ornamental pepper	Thin	Green	Red	1771	2305	0.80	1.12	0.44	0.92	8.3	11.5	6.3	10.1
1866	Long green pepper	Thin	Yellow-orange	Red	1329	2072	0.55	1.56	0.42	1.34	6.1	9.3	44.7	41.4
1882	Bell	Thick	Green	Green	749	1026	0.25	2.27	0.55	1.25	4.7	3.7	89.3	89.9
1883	Bell	Thick	Light green	Light red	888	1144	0.40	3.76	0.24	0.74	5.0	7.1	56.2	74.8
1885	Bell	Thick	Purple	Orange	879	1241	0.46	0.93	0.34	0.75	4.9	6.4	48.9	70.2
1886	Hungarian	Thick	Yellow	Light red	1006	1241	0.63	1.24	0.31	0.86	4.7	6.4	17.4	80.7
1888	Bell	Thick	Green	Red	1183	1471	0.24	1.05	0.19	0.86	4.0	6.2	29.2	72.0
16-1	Ornamental pepper	Thin	Green	Red	938	1601	0.20	0.60	0.16	0.63	4.0	6.6	29.8	40.9
19a	Long green pepper	Thick	Green	Red	1603	1253	0.42	0.65	0.32	0.50	5.3	8.0	21.6	45.8
242-b	Long green pepper	Thin	Green	Red	1065	2163	0.41	0.70	0.23	0.58	4.4	8.6	19.2	37.9
283a	Charleston	Thick	Green	Red	1115	1121	0.20	0.91	0.14	0.62	2.3	7.0	10.1	25.2
3a	Ornamental pepper	Thin	Green	Red	813	1789	0.55	1.46	0.30	0.94	6.3	7.5	16.6	68.3
405a	Bell	Thick	Green	Red	931	1661	0.23	1.27	0.19	1.04	4.2	7.1	20.9	59.8
764-2-1b	Hungarian	Thick	Yellow	Orange	1683	2488	0.89	2.03	0.26	1.34	4.7	5.3	61.6	94.0
765-4-2b	Bell	Thick	Yellow	Light red	1202	2216	0.57	1.95	0.41	1.88	4.3	8.7	33.2	100.5
765-4-3b	Bell	Thick	Yellow	Orange	1258	1467	0.58	1.00	0.39	0.88	4.3	5.2	41.4	61.2
769-5	Tomato pepper	Thick	Green	Red	1096	1320	0.38	0.72	0.23	0.54	4.0	5.3	21.4	33.9
769-5-1b	Hungarian	Thick	Light green	Light red	1218	1653	0.61	1.67	0.44	1.07	5.0	8.3	35.9	72.5
771-8	Long green pepper	Thin	Dark green	Dark red	2431	2313	0.69	1.78	0.49	1.47	7.2	14.0	47.4	55.1
774-3	Long green pepper	Thin	Dark green	Dark red	1702	2004	1.06	1.54	0.36	1.27	6.4	11.6	48.8	54.4
774-4-2b	Long green pepper	Thin	Green	Red	2358	2461	1.26	1.70	0.61	1.44	6.0	10.8	9.2	46.7
Mean					1349	2025	0.53	1.40	0.34	1.01	5.0	9.1	29.0	57.1
% increase						50		164		195		81		97

[a]Total phenolic contents were estimated by the Folin–Ciocalteu assay of Singleton and Rossi (1965). Values are expressed as µg GAE/g fw; [b]FRAP values were determined by the method of Benzie and Strain (1996). Values are expressed as µmol TE/g fw; [c]TEAC values were determined by the method of Özgen et al. (2006). Values are expressed as µmol TE/g fw; [d]TSS values were determined by digital refractometer and values are expressed as % basis. [e]Vitamin C values were determined by the method of Özgen et al. (2008). Values are expressed as mg/100 g fw.

Table 4. Variation of pomological properties of 52 superior pepper genotypes from the Alata Pepper Breeding Program.

Genotype	TP[a]	FRAP[b]	TEAC[c]	TSS[d]	Vitamin C[e]	L	a	b
15	1758	0.72	0.26	6.4	27.1	55.6	8.9	36.3
31	1765	1.04	0.70	6.8	30.7	50.2	5.2	39.2
32	1467	0.60	0.43	5.8	23.2	50.0	4.3	37.7
35	1862	0.99	0.54	7.5	7.7	50.4	7.7	40.4
47	2306	0.60	0.54	7.9	17.2	35.1	9.8	18.8
74	2181	0.91	0.74	6.4	43.2	55.9	8.8	34.8
81	1994	1.28	0.95	7.8	27.9	53.2	6.6	44.0
93	1526	0.75	0.60	6.6	21.5	58.3	10.8	42.2
107	1755	1.22	0.71	7.7	8.0	48.2	7.4	40.0
173	1359	1.07	0.80	7.2	43.5	59.8	11.1	37.2
200	1683	0.94	0.76	6.3	30.3	48.6	6.1	38.2
202	1571	0.70	0.56	6.7	9.0	49.2	6.1	39.6
215	2678	1.38	0.98	12.8	27.1	45.1	6.0	33.1
226	1327	0.72	0.60	4.2	29.8	56.0	9.7	38.1
261	2602	1.28	0.99	7.8	23.0	48.3	4.3	38.3
269	1575	1.04	0.53	5.4	39.1	53.7	7.2	40.0
276	1429	0.83	0.58	6.3	19.6	61.5	7.8	45.8
302	910	0.57	0.52	3.8	22.6	47.8	6.0	34.5
304	1478	0.88	0.69	6.6	32.1	49.4	4.2	37.0
388	2286	1.16	0.96	8.5	7.2	49.6	6.1	37.8
390	2669	1.30	1.09	11.7	10.5	32.7	18.7	16.0
1029	2584	1.24	1.01	11.7	40.1	46.9	4.9	35.8
1676	1269	0.40	0.39	5.3	21.9	52.2	3.1	40.5
1719	1340	0.71	0.53	6.8	32.9	43.1	3.5	30.0
1721	2186	1.23	1.01	9.7	43.7	48.9	3.4	37.1
1738	1988	1.32	0.93	9.1	29.7	43.6	4.7	30.9
1763	1339	0.72	0.55	6.5	35.2	46.5	5.4	35.1
1779	1087	0.48	0.35	5.1	12.4	49.8	5.2	37.8
1780	2092	1.00	0.58	8.9	5.3	47.2	4.8	37.7
1788	1147	0.59	0.41	6.1	20.4	50.6	4.5	39.4
1839	1912	1.25	0.88	8.3	40.9	56.6	9.5	40.9
1842	2038	0.96	0.68	9.9	8.2	51.2	9.8	42.2
1866	1701	1.05	0.88	7.7	23.0	62.5	15.0	40.0
1882	887	1.26	0.90	4.2	49.4	53.7	-2.3	45.1
1883	1016	2.08	0.49	6.0	65.5	58.1	-9.4	48.3
1885	1060	0.70	0.54	5.7	37.5	37.6	14.7	23.6
1886	1124	0.93	0.59	5.6	49.1	58.6	9.9	35.2
1888	1327	0.65	0.53	5.1	37.4	42.0	3.0	28.3
16_1	2270	1.08	0.54	8.1	33.0	47.0	5.8	34.0
19a	1428	0.54	0.41	6.6	24.0	56.5	7.5	44.0
242-b	1614	0.55	0.40	6.5	19.9	58.5	14.8	43.7
283a	1118	0.55	0.38	4.6	13.1	58.5	8.8	43.1
3a	1301	1.00	0.62	6.9	42.4	41.7	5.5	28.8
405a	1296	0.75	0.62	5.7	31.0	53.8	6.6	33.2
764-2-1b	2085	1.46	0.80	5.0	77.8	64.7	9.0	38.9
765-4-2b	1709	1.26	1.15	6.5	66.8	58.6	7.8	31.7
765-4-3b	1362	0.79	0.63	4.7	32.7	61.5	7.7	32.5
769-5	1208	0.55	0.38	4.7	18.0	49.7	2.5	36.6
769-5-1b	1435	1.14	0.76	6.7	54.2	49.9	2.4	37.7
771-8	2372	1.24	0.98	10.6	29.9	42.1	9.4	30.6
774-3	1853	1.30	0.81	9.0	29.7	41.0	8.7	32.4
774-4-2b	2410	1.48	1.03	8.4	27.9	48.2	10.4	35.5
Mean	1687	0.97	0.68	7.0	29.9	50.8	6.9	36.5
St. dev.	486	0.33	0.22	2.0	15.6	7.0	4.3	6.2

[a]Total phenolic contents were estimated by the Folin–Ciocalteu assay of Singleton and Rossi (1965). Values are expressed as µg GAE/g fw.
[b]FRAP values were determined by the method of Benzie and Strain (1996). Values are expressed as µmol TE/g fw.
[c]TEAC values were determined by the method of Özgen et al. (2006). Values are expressed as µmol TE/g fw.
[d]TSS values were determined by digital refractometer and values are expressed as % basis.
[e]Vitamin C values were determined by the method of Özgen et al. (2008). Values are expressed as mg/100 g fw.

Table 5. Correlation coefficients and significance of several pomological properties used to evaluate 52 superior pepper genotypes from the Alata Pepper Breeding Program.

Variable	FRAP[a]	TEAC[b]	TSS[c]	Vit C[d]	L	a	b
TP[e]	0.64*[d]	0.74*	0.80*	0.36*	–0.52*	0.53*	–0.45*
FRAP		0.84*	0.67*	0.71*	–0.46*	0.63*	–0.25*
TEAC			0.76*	0.71*	–0.58*	0.76*	–0.42*
TSS				0.41*	–0.57*	0.66*	–0.45*
Vit C					–0.45*	0.70*	–0.37*
L						–0.69*	0.74*
a							–0.60*

[a]Ferric reducing ability of plasma (FRAP) values were determined by the method of Benzie and Strain (1996). Values are expressed as µmol TE/g fw.
[b]Trolox equivalent antioxidant capacity (TEAC) values were determined by the method of Özgen et al. (2006). Values are expressed as µmol TE/g fw.
[c]Total soluble solids (TSS) values were determined by digital refractometer and values are expressed as % basis.
[d]Vitamin C values were determined by the method of Özgen et al. (2008). Values are expressed as mg/100 g fw.
[e]Total phenolic (TP) contents were estimated by the Folin–Ciocalteu assay of Singleton and Rossi (1965). Values are expressed as µg GAE/g fw.

Table 6. First three principal component (PC) scores of the variables used to evaluate immature and mature pepper genotypes of the Alata Pepper Breeding Program.

	Immature			Mature		
Variable	PC1	PC2	PC3	PC1	PC2	PC3
Maturity period	0.00	0.00	0.00	0.02	0.21	0.48
Fruit color	–0.05	0.27	0.51	–0.11	0.23	0.47
Type	0.17	0.23	–0.30	0.19	0.08	0.14
Thickness of fruit wall (skin)	0.31	0.25	0.06	0.30	0.04	0.39
Fruit length	0.01	–0.30	0.41	–0.10	–0.15	0.06
Fruit diameter	0.37	0.19	–0.16	0.38	0.06	0.11
Thickness of fruit wall (skin)	0.37	0.18	–0.02	0.37	–0.08	0.10
Fruit weight	0.39	0.14	–0.05	0.37	0.02	0.15
Maturity	0.00	0.00	0.00	0.00	0.00	0.00
TP[a]	–0.34	0.21	–0.13	–0.24	0.36	–0.04
FRAP[b]	–0.32	0.21	–0.07	0.06	0.48	–0.23
TEAC[c]	–0.33	0.27	0.02	–0.10	0.50	0.02
TSS[d]	–0.31	0.22	–0.20	–0.26	0.32	–0.01
Vitamin C	0.11	0.15	–0.18	0.17	0.32	–0.04
L	0.10	–0.26	0.27	0.27	0.11	–0.36
a	0.03	0.36	0.50	–0.36	–0.15	0.07
b	0.00	–0.45	–0.18	0.25	0.13	–0.36
Eigenvalue	4.76	3.41	1.66	5.13	2.95	2.06
Proportion	0.32	0.22	0.11	0.32	0.18	0.13

[a]TP: Total phenolic contents.
[b]FRAP: Ferric reducing ability of plasma.
[c]TEAC: Trolox equivalent antioxidant capacity.
[d]TSS: Total soluble solids.

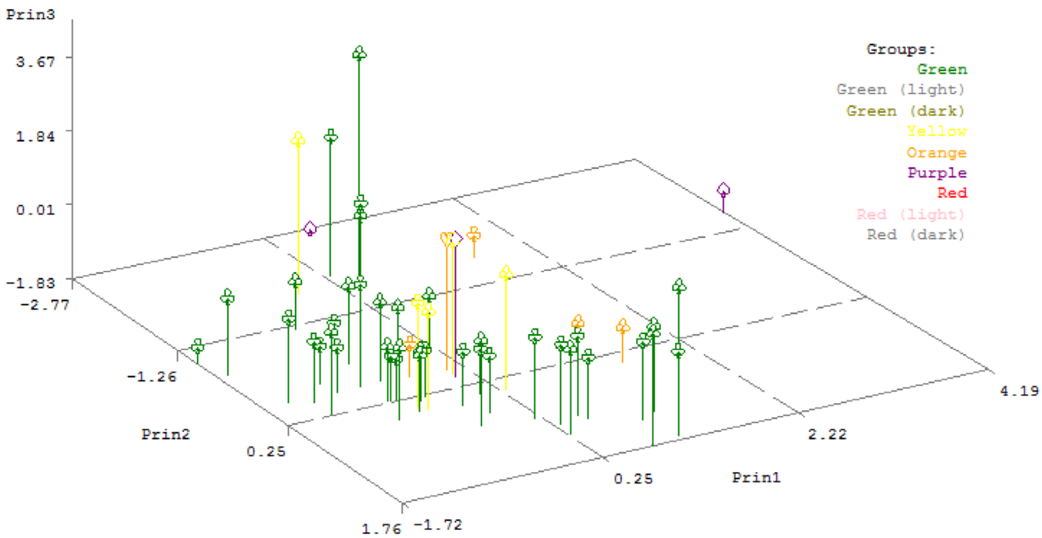

Figure 1. Plots of immature 52 superior pepper genotypes from the Alata Pepper Breeding Program on the first three principal components (PCs) that resulted from principal component analysis conducted for 17 characteristics. The genotypes were grouped based on their fruit color.

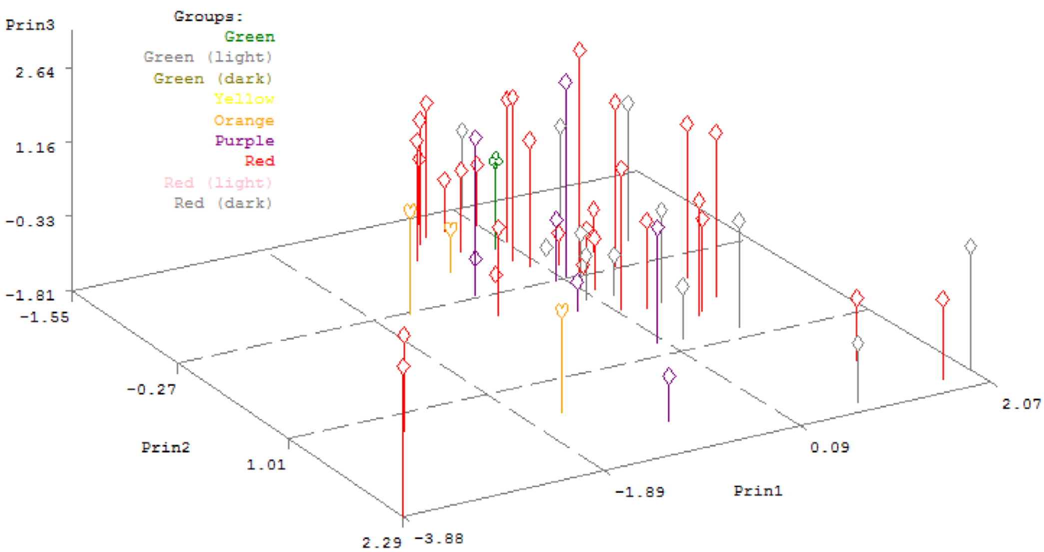

Figure 2. Plots of mature 52 superior pepper genotypes from the Alata Pepper Breeding Program on the first three principal components (PCs) that resulted from principal component analysis conducted for 17 characteristics. The genotypes were grouped based on their fruit color.

References

Aktas H, Abak K, Sensoy S (2011). Genetic diversity in some Turkish pepper (*Capsicum annuum* L.) genotypes revealed by AFLP analyses. Afr J Biotechnol 8: 4378-4386.

Bae H, Jayaprakasha GK, Crosby K, Yoo KS, Leskovar DI, Jifon J, Patil BS (2014). Ascorbic acid, capsaicinoid, and flavonoid aglycone concentrations as a function of fruit maturity stage in greenhouse-grown peppers. J Food Comp Anal 33: 195-202.

Benzie IFF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239: 70-76.

Bhandari SR, Jung BD, Baek HY, Lee YS (2013). Ripening-dependent changes in phytonutrients and antioxidant activity of red pepper (*Capsicum annuum* L.) fruits cultivated under open-field conditions. HortScience 48: 1275-1282.

Bozokalfa MK, Eşiyok D (2011). Evaluation of morphological and agronomical characterization of Turkish pepper accessions. Int J Veg Sci 17: 115-135.

Carvalho AV, Mattietto RD, Rios AD, Maciel RD, Moresco KS, Oliveria TCD (2015). Bioactive compounds and antioxidant activity of pepper (*Capsicum* sp.) genotypes. J Food Sci Tech-Mysore 52: 7457-7464.

Edge R, Mcgarvey DJ, Truscott TG (1997). The carotenoids as antioxidants – a review. J Photochem Photobiol B Biol 41:189-200.

Fox AJ, Del Pozo-Insfran D, Lee JH, Sargent SA, Talcott ST (2005). Ripening-induced chemical and antioxidant changes in bell peppers as affected by harvest maturity and postharvest ethylene exposure. HortScience 40: 732-736.

Frary A, Keçeli MA, Ökmen B, Şığva HÖ, Yemenicioğlu A, Doğanlar S (2008). Water-soluble antioxidant potential of Turkish pepper cultivars. HortScience 43: 631-636.

Guil-Guerrero JL, Martines-Guirado C, Rebolloso-Fuentes MM, Carrique-Perez A (2006). Nutrient composition and antioxidant activity of pepper (*Capsicum annuum*) varieties. Eur Food Res Tech 224: 1-9.

Hornero-Mendez D, Gomez-Ladron R, Mınguez-Mosquera MI (2000). Carotenoid biosynthesis changes in five red pepper (*Capsicum annuum* L.) cultivars during ripening. Cultivar selection for breeding. J Agric Food Chem 48: 3857-3864.

Howard LR, Talcott ST, Brenes CH, Villalon B (2000). Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum species*) as influenced by maturity. J Agric Food Chem 48: 1713-1720.

Jayaprakasha GK, Bae H, Crosby K, Jifon JL, Patil BS (2012). Bioactive compounds in peppers and their antioxidant potential, Hispanic foods: chemistry and bioactive compounds. In: Tunick MH, González de Mejía E. Hispanic Foods: Chemistry and Bioactive Compounds. Washington, DC, USA: ACS, pp. 43-56.

Lee Y, Howard LR, Villalon B (1995). Flavonoids and antioxidant activity of fresh pepper (*Capsicum annum*) cultivars. J Food Sci 60: 43-476.

Materska M, Perucka I (2005). Antioxidant activity of the main phenolic compounds isolated from hot pepper fruit (*Capsicum annuum* L.). J Agric Food Chem 53: 1750-1756.

Matsufuji H, Ishikawa K, Nunomura O, Chino M, Takeda M (2007). Anti-oxidant content of different coloured sweet peppers, white, green, yellow, orange and red (*Capsicum annuum* L.). Food Sci Tech 42: 1482-1488.

Özgen M, Reese RN, Tulio AZ, Miller AR, Scheerens JC (2006). Modified 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) method to measure antioxidant capacity of selected small fruits and comparison to ferric reducing antioxidant power (FRAP) and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) methods. J Agric Food Chem 54: 1151-1157.

Özgen M, Serce S, Kaya C (2009). Phytochemical and antioxidant properties of anthocyanin-rich *Morus nigra* and *M. rubra* fruits. Sci Hortic 119: 275-279.

Rao AV, Rao LG (2007). Carotenoids and human health. Pharmacol Res 55: 207-216.

SAS Institute (2005). SAS Online Doc, Version 8. Cary, NC, USA: SAS Institute.

Scheerens JC (2001). Phytochemicals and the consumers: factors affecting fruit and vegetable consumption and the potential for increasing small fruit in the diet. HortTechnology 11: 547-556.

Singleton VL, Rossi JL, (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Viticult 16: 144-158.

Sun T, Xu Z, Wu CT, Janes M, Prinyawiwatkul W, No HK (2007). Antioxidant activities of different colored sweet bell peppers (*Capsicum annuum* L.). J Food Sci 72: S98-S102.

Zhang D, Hamauzu Y (2003). Phenolic compounds, ascorbic acid, carotenoids, and antioxidant properties of green, red, and yellow bell peppers. J Food Agric Environ 1: 22-27.