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The effects of genotype and altitude of the growing location on physical, chemical, and phytochemical properties of strawberry

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Abstract: The effects of genotype and altitude of the growing location on physical, chemical, and phytochemical properties of strawberry fruits were investigated. Eight strawberry genotypes obtained from diverse breeding programs were selected. The genotypes were grown at three altitudes: in Antakya (117 m), Urumu (443 m), and Saksak (755 m). The results indicated that genotype and growing location had a significant impact on both physico-chemical and phytochemical characteristics. Genotypes explained 36%–51% of total variance for fruit weight (FW), total soluble solids (TSS), total acidity (TA), color incidence chroma (C), hue (*h*°), citric acid, and total monomeric anthocyanin (TMA). Altitudes explained 23%–50% of total variance for color L, total phenolic content (TPC), glucose, fructose, and total sugar. The genotype effect was larger than that of the different altitude conditions for most of the physico-chemical and phytochemical component variables in the experiment, showing that breeding for fruit quality properties may be successful.

Key words: Altitude, anthocyanins, antioxidant capacity, quality, strawberry

1. Introduction

Berries have important nutritional components thanks to their high phytochemical contents (Tosun et al., 2009; Zorenc et al., 2016; Cuce and Sokmen, 2017). Among berries, strawberries are favored fruits, because of their high nutritional value leading to putative health benefits and unique color, flavor, and taste (Gündüz, 2016). In recent years, there has been increasing interest in determining phytochemical properties of colorful, in particular red, fruits (Moyer et al., 2002; Caliskan et al., 2017). Epidemiological studies suggest that consumption of red fruit juices such as grape, some berry juices and pomegranate correlate with reduced risk of coronary heart disease, stroke, certain types of cancers, and aging (Malik and Mukhtar, 2006). Recent studies have focused on the nutrient and phytochemical contents of the strawberry and on factors affecting the composition of this fruit. These studies have included genotype; harvest time; degree of maturity; climatic factors; postharvest storage; plant materials such as berries, fruits, vegetables, herbs, cereals, tree material, plant sprouts, and seeds; geographic origin; cultural system; pest management; and organic farming and growing conditions (Capocasa et al., 2008; Crespo et al., 2010; Jin et al., 2011; Tulipani et al., 2011; Aaby et al., 2012; Diamanti et al., 2012; Fernandes et al., 2012; Pincemail et al., 2012; Gündüz and Özdemir, 2014). Altitude of the growing location is a factor affecting fruit quality and phytochemical composition. There are several climatic factors generally associated with altitude, such as reduction in atmospheric temperature, a higher fraction of UVB radiation, and lowering total atmospheric pressure (Körner, 2007). It has been theorized that the combined action of these variables could play a role in determining the final phenolic profile of plants. However, there is limited knowledge on the effects of these climatic changes along the altitudinal range on quality characteristics of fruits such as strawberry. Only a few studies have tested this specific hypothesis. It was argued that the anthocyanin content of the strawberry was mostly managed by genetically traits rather than environmentally characteristics in an experiment conducted at two different altitudes (480 m and 1060 m) (Crespo et al., 2010). Doumet et al. (2011) compared the nutritional and nutraceutical components in *Fragaria vesca* grown under the same cultural practice and environmental conditions but at different altitudes. No significant differences were detected in polyphenols, ascorbic acid and radical scavenging activity, but sugars and organic acids were greater at higher altitudes. Guerrero-Chavez et al. (2015) found levels of anthocyanin and antioxidant declined when the altitude increased from

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900 m and 1500 m in the "Elsanta" strawberry cultivar, but altitude did not affect total phenolic, TSS, or titratable acidity.

In the present study, the objective was to compare the performances of several strawberry genotypes across different altitudes in a Mediterranean climate, to determine the relative importance of genotype and environmental variation on their physical, chemical, and phytochemical properties.

2. Materials and methods

2.1. Plant material and growing conditions

Eight genotypes from diverse breeding programs were selected. The genotypes included "Camarosa", "Rubygem", "Albion", "San Andreas", and "Sweet Ann" (University of California), "Florida Fortuna" (University of Florida), and "Sabrina" and "Sabrosa" (Plantas De Navarra, S.A., Spain national program, Spain). The trials were run in 2014–2015 at Antakya (117 m altitude), Urumu (443 m altitude), and Saksak (755 m altitude), designated as A, U, and S, respectively. The districts have different environmental conditions, soil pH, and soil organic matter (Table 1). Air temperature (°C) was measured at all the sites from October 2014 to June 2015, and the mean, minimum, and maximum temperature (T) per month and site were calculated from the recorded daily minimum (Tmin) and maximums (Tmax) temperature (Table 1). The genotypes were grown in three locations: A, U, and S for one growing season during 2014–2015.

The raised bed treatments consisted of beds (60 cm width and 25 cm height) with two rows of plants set at 30 × 30 cm spacing into black polyethylene mulch. The black mulch was applied to reduce weed pressure and increase soil temperature. The beds were irrigated with a drip irrigation system under the plastic mulch. There were 20 plants per plot. In addition to the initial fertilizer application, 20:20:20+Fe NPK fertilizer was applied biweekly by drip irrigation throughout the growing season.

2.2. Analytical procedures

2.2.1. Determination of physical and chemical fruit properties

Average fruit weight (FW) was determined by dividing the weight of all fruit picked in the season by the number of fruit. Total soluble solid (TSS), pH, and titratable acidity (TA) were measured in each of four replicates, using juice extracted from 100-g fruit samples blended at high

Table 1. Monthly temperature from October 2014 to June 2015, soil profile, planting time, and fruit sampling time at the three locations.

Location				A			U		
Altitude (m)				117			443		
Latitude				36°13.4′N			35°54.5′N		
Soil pH				8.1			7.9		
Soil organic matter (%)				1.07			0.91		
Planting time				22.08.2014			23.08.2014		
Sampling time				20.04.2015			5.05.2015		
Months	Temperature (°C)								
	A			U			S		
	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean
October	29.5	9.2	19.4	28.4	7.3	17.3	25.1	9.1	15.9
November	25.4	2.8	11.8	24.4	3.3	13.1	23.1	5	11.7
December	22.7	2	11.8	18.7	2	12.1	15.8	1.6	9.1
January	24.2	–2.9	7.5	19.8	–1.9	8.6	15.3	–4.5	5.4
February	22.5	–0.2	10	24.6	1.3	9.8	16.7	–1.2	6.6
March	34.3	3.3	14.4	26.2	4.4	12.7	24.7	2.9	10.7
April	41.2	6.3	17.7	32.1	4.8	15.3	33.2	5	13.5
May	48.7	12	23.8	38.9	10.6	21	31.8	9.8	18.6
June	49.5	14.7	25.9	35	13.3	22.7	28	14.5	19.5

speed in a tissue homogenizer (Ultra Turrax T25; Janke and Kunkel Co., Staufen, Germany). TSS was determined using a handheld refractometer (Westover Model RHB-32; Southwest United Industries, Tulsa, OK, USA). The results were reported in percent TSS (w/w) on a fresh weight (FW) basis. TA was determined from 10 mL of juice diluted to 100 mL with distilled water, titrated with 0.1 N sodium hydroxide (NaOH) to pH 8.2, and expressed as percentage citric acid (w/w) on a fw basis. The TSS to TA ratio (TSS/TA) was calculated as an indicator of overall sweetness. The pH measurements were made using a digital pH meter. Fruit firmness (FF) measurements were conducted using a penetrometer with a 5-mm plunger (Nippon Optical Works Co., Ltd, Japan) inserted at the equatorial region of the fruit and expressed as kg cm^{-2}. The fruit color was measured using a Minolta Chroma Meter (Minolta, Model CR-400), which provided CIE L^*, a^*, and b^* values. Chroma (C) and hue ($h°$) values were calculated from these values.

2.2.2. Determination of phytochemical properties

2.2.2.1. Extraction

Sampling for phytochemical analysis was conducted at the peak of the harvest season in each location and sampling time as presented in Table 1. Fruits were harvested when 75% of the fruit had the typical color formation of the cultivar. Next 500 g of fruit was sampled and stored at –20 °C until the analysis. Four replicates were utilized for each analysis. Later, samples were thawed at room temperature and then homogenized in a food processor. Chemical analysis was completed within 20 days of storage.

2.2.2.2. Determination of total phenolic content (TPC)

TPC was measured according to the procedure described by Singleton and Rossi (1965). Fruit samples were extracted with buffer containing acetone, water, and acetic acid (70:29.5:0.5 v/v) for 1 h in the dark room conditions. Then extract, Folin–Ciocalteu's phenol reagent, and water were incubated for 8 min followed by the addition of 7% sodium carbonate. After 2 h, the absorbance was measured by an automated UV-VIS spectrophotometer at 750 nm. Gallic acid was used as standard. A standard curve was used to determine the concentration of total phenolics. For this purpose 0, 50, 100, 200, 400, 800, and 1600 mg/L of gallic acid concentrations were used as a quality assurance. The results were expressed as mg gallic acid equivalent in kg fresh weight (GAE/kg FW).

2.2.2.3. Total monomeric anthocyanins (TMAs)

TMAs were estimated by a pH differential method (Giusti and Wrolstad, 2005), using a UV-VIS spectrophotometer (model T60U, PG Instruments). Absorbance was measured at 533 nm and 700 nm in buffers at pH 1.0 and 4.5 using A = (A533 – A700) pH 1.0 – (A533 – A700) pH 4.5 with a molar extinction coefficient of 31,600. Results were expressed as mg Pg-3-glk/kg fresh weight (FW).

2.2.2.4. The total antioxidant capacity (TAC)

Ferric reducing ability of plasma (FRAP) was determined according to the method described by 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 (1000:3.3 v/v), 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 in duplicate, 2.97 mL of FRAP reagent and 30 µL of sample extract were mixed. After 10 min, the absorbance of the reaction mixture was determined at 593 nm in a spectrophotometer. The results were expressed as mmol TE/kg FW.

2.2.3. Extraction of organic acids and individual sugars for HPLC

Fruit slurries (5 g) were diluted with purified water or meta-phosphoric acid (2.5%) solutions for individual sugar and organic acid analysis, respectively. 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. All samples and standards were injected four times for each and mean values were calculated.

2.2.3.1. Chromatographic conditions

The HPLC analyses were carried out using a PerkinElmer HPLC system with Totalchrom navigator 6.2.1 software, a pump, and UV detector (PerkinElmer series-200) (Waltham, MA, USA). Procedures for separation and determination of organic acids were modified from Shui and Leong (2002). The separation was carried out on a 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 a pH 2.5 sulfuric acid solution (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.

Analysis of sugars was performed according to the method reported by Bartolome et al. (1995) using a refractive index (RI) detector (PerkinElmer series-200). The separation was carried out on a SGE SS Exsil amino column (250 × 4.6 mm I.D.). The elution solvent used was 80% acetonitrile and 20% deionized water. The column was operated at 30 °C with 0.9 mL/min flow rate. Sample injection volume was 20 µL. The results were expressed as mg/100 g FW.

2.3. Statistical analysis

The data were analyzed using SAS procedures and software (SAS, 2005). Means were obtained using the TABULATE

procedure. The ANOVA tables were constructed using the GLM procedure. In the construction of ANOVA tables and partitioning of the variance components of all factors (locations, genotype, and their interactions) were considered random. The significant means were separated by Tukey's test at $P < 0.05$. Variance for each variable was partitioned into location, genotype, and error using VARCOMP.

3. Results

3.1. The influence of genotype and altitude on physical and chemical fruit properties

The effects of genotype and altitude on fruit quality properties were tested by comparing the FW, TSS content, TA, TSS/acidity, pH, FF, and fruit color parameters of eight genotypes grown at the A, S, and U locations. ANOVA of the tested characteristics is presented in Table 2. Locations had significant effects on the traits except for FF. Genotype had significant effect on all characteristics studied (Table 2). The highest value for FW was at the U location; TSS and TA were highest at the A and S locations. The lowest values for FW and TSS were observed at S, whilst the lowest value for TA was at U (Table 3). The highest *L*, *C*, and *h*° values were obtained from A (*L* = 35.6, *C* = 43.9, and *h*° = 27.0). The highest value for FW was in "Rubygem" (15.1 g/fruit), while the lowest value was in "Sabrosa". "Sabrina" (7.40%) showed the highest TSS, followed by the "Rubygem", "Sabrosa", and "Camarosa" (respectively 7.23%, 7.18%, and 7.05%) cultivars (Table 3). "Camarosa" and "Albion" (0.70%) had the highest value in terms of TA, while the lowest values were in "Rubygem" and "Fortuna" (0.54% and 0.58%). The highest FF was in "Sabrina" (0.87 kg cm^{-2}), followed by "Fortuna", "San Andreas", and "Camarosa". The lowest FF was observed in "Rubygem" (0.75 kg cm^{-2}).

Table 2. Variance analysis of physical and chemical fruit properties.

Sources	df	FW	TSS	TA	TSS/TA	pH	FF	*L*	*C*	*h*°
Location (L)	2	11.2*	1.4*	0.06*	2.9	0.27*	0.01	146.5*	368.3*	296.5*
Rep (R)	8	4.7	0.5	0.01	1.1	0.01	0.00	3.9	11.2	6.0
Genotype (G)	7	26.6*	2.8*	0.03*	24.2*	0.04*	0.02*	31.2*	60.7*	49.8*
L × G	14	4.8*	0.4	0.00*	4.4*	0.02*	0.01*	8.4*	13.8*	9.7*
Error	51	2.5	0.3	0.00	1.1	0.01	0.00	2.6	5.5	3.9

* represent significance at $P \leq 0.05$. TSS: Total soluble solids; TA: Titratable acidity

Table 3. Effect of genotype and locations on physical and chemical fruit properties of strawberries.

Source	Pomological properties						Fruit color		
Growing location	FW (g)	TSS (%)	TA (%)	TSS/TA (%)	pH	FF (kg cm^{-2})	*L*	*C*	*h*°
Antakya (117 m)	12.4 ab	7.01 a	0.69 a	10.0	3.38 c	0.84	35.6 a	43.9 a	27.0 a
Urumu (443 m)	13.2 a	6.92 ab	0.58 b	10.3	3.56 a	0.80	33.9 b	38.7 b	20.1 c
Saksak (755 m)	11.3 b	6.53 b	0.65 a	9.7	3.49 b	0.82	31.1 c	36.5 c	22.5 b
Genotype									
Camarosa	11.1 de	7.05 ab	0.70 a	10.0 b	3.44 cd	0.84 ab	32.0 cd	37.0 c	20.7 d
Rubygem	15.1 a	7.23 ab	0.54 d	13.4 a	3.58 a	0.75 c	33.5 b	37.5 c	22.6 bc
Albion	13.9 b	6.80 b	0.70 a	9.7 bc	3.50 abc	0.82 b	31.5 d	38.7 bc	22.4 cd
San Andreas	12.0 cd	6.21 c	0.66 ab	9.4 bc	3.45 bcd	0.85 ab	33.3 bc	43.1 a	24.6 b
Sweet Ann	12.8 bc	6.28 c	0.61 c	9.8 ab	3.43 bcd	0.76 c	37.7 a	44.2 a	28.7 a
Fortuna	12.2 cd	6.04 c	0.58 d	9.23 bc	3.42 bcd	0.85 ab	33.9 b	39.4 bc	23.1 bc
Sabrina	10.7 de	7.40 a	0.64 bc	8.7 c	3.52 ab	0.87 a	33.1 bcd	38.7 bc	21.8 cd
Sabrosa	10.6 e	7.18 ab	0.67 ab	9.1 bc	3.41 d	0.82 b	34.7 b	40.4 b	23.0 bc

Values in the same column that are followed by different letters are significantly different ($P \leq 0.05$) using Tukey's comparison test.

"Sweet Ann" had the highest value in terms of fruit color property *L*, and the lowest values for these parameters were observed in "Albion" (Table 3). For *C*, "San Andreas" and "Sweet Ann" showed the highest values, the lowest *C* was observed in "Camarosa" and "Rubygem". The highest value for *h*° value was in "Sweet Ann", while the lowest value was in "Camarosa".

3.2. The influence of genotype and locations on bioactive compounds

The effect of genotype and location on fruit bioactive compounds was tested by comparing the TPC, TMA, TAC (FRAP), organic acids, and organic sugar contents of 8 genotypes, grown in A, S, and U locations. ANOVA of the tested characteristics is presented in Table 4. Location had significant effects on all traits except total acidity. Genotype had significant effects on all traits. Location A had the strongest impact on TMA and malic acid, while S had the greatest effect on glucose and total sugars (Table 5). Values were highest at S for TPC, TAC, citric acid, glucose, fructose, and total sucrose (respectively, 2762.2 mg GAE/kg FW, 8.9 mmol TE/kg FW, 0.46 g/100 g, 3.82 g/100 g, 2.39 g/100 g, and 6.20 g/100 g). Among the cultivars, TPC was highest in "Camarosa", "Sabrosa", "San Andreas", "Fortuna", "Albion", and "Rubygem" (2731.2–2422.9 mg GAE/kg FW), followed by "Sabrina". The lowest TPC was found in "Sweet Ann" (2069.7 mg GAE/kg FW). For TMA, "Fortuna", "Camarosa", and "Sabrina" showed the highest values (respectively, 123.9, 122.6, and 117.3 mg Pg-3-glk/kg FW), while the lowest TMA was observed in "Sweet Ann" (38.7 mg Pg-3-glk/kg FW). TAC (FRAP) was highest in "Camarosa" (9.4 mmol TE/kg FW), followed by "Albion" (9.0 mmol TE/kg FW). The lowest TAC was observed in the "Sweet Ann" and "Sabrina". "Camarosa" (0.67 g/100 g) and "Sabrina" (0.63 g/100 g) showed the highest TA content. "Rubygem" had the lowest TA content. "Rubygem" had the highest value for total sugar content, followed by "Sabrina"; the lowest value was observed in "Fortuna" (Table 5).

3.3. Partitioning of variability due to genotypes and locations on bioactive compounds

The proportions of variability obtained from genotypes and locations on fruit physical and chemical properties and bioactive compounds are given in Tables 6 and 7. Genotypes had significant effects on FW, TSS, TA, and color properties *C* and *h*°. Locations only affected fruit color (Table 6). Effects of locations upon bioactive compounds were determined on TPC, glucose, fructose, and total sugars (respectively, 23%, 39%, 50%, and 50%). Genotypes had significant effects on TMA (51%) and total acidity (42%) (Table 7).

4. Discussion

Interest in the nutritional quality of fruits is steadily increasing. People are becoming more and more aware of the need to consume healthy foods for a healthy life. It is necessary to establish new growing systems that protect fruit quality as well as breed new strawberry cultivars having high quality. In fact, it is important to know the effect of growing conditions on the fruit quality of each strawberry cultivar developed by a breeding program (Gündüz and Özdemir, 2014). In the present study, it was determined that growing altitudes have less effect on fruit quality than genetic factors. These results are parallel to those observed across three growing locations by Gündüz and Özdemir (2014). Genotype had strong effects on FW, TSS, TA, *C*, and *h*°, but locations had significant effects only on *L*. Altitude had little effect on FW. Hansche et al. (1968) stated a fruit quality criterion of strawberry which is strongly influenced by environment and accordingly FW had low heritability. Scott and Lawrence (1975) found that genetic structure, environmental factors, and cultural practices (irrigation, fertilizing, mulching, etc.) all had significant effects on FW. Variance in TSS was particularly high in "Sabrina", "Rubygem", "Sabrosa", and "Camarosa". Herrington et al. (2007) and Saraçoğlu (2013) found that altitudes had a weak effect on TSS. Similar results were

Table 4. Variance analysis of TMA, TPC, TAC (FRAP), organic acid, and individual sugars.

				TAC		Organic acids			Individual sugars		
Sources	df	TMA	TPC	FRAP	df	Malic acid	Citric acid	Total acids	Glucose	Fructose	Total sugars
Location (L)	2	996.4*	1,650,134.2*	2.8*	2	0.03*	0.03*	0.00	3.5	2.92	12.8
Rep (L)	8	528.3	190,310.4	0.7	3	0.00	0.00	0.00	0.2	0.04	0.3
Genotype (G)	7	6361.0*	435,864.9*	3.6*	7	0.01*	0.05*	0.04*	0.8*	0.51*	2.0*
L × G	14	715.5*	156,371.6	2.1*	14	0.00*	0.01*	0.01*	0.3*	0.20*	0.9*
Error	51	288.1	134,247.3	0.5	21	0.00	0.02	0.00	0.1	0.03	0.2

* represent significance at P ≤ 0.05. TMA: total anthocyanin; TPC: total phenol content; FRAP: total antioxidant capacity.

Table 5. Effect of genotype and locations on nutritional fruit quality characteristics of strawberries.

Source			TAC	Organic acids[D]			Individual sugars[E]		
Growing location	TPC[A]	TMA[B]	FRAP[C]	Malic acid	Citric acid	Total acids	Glucose	Fructose	Total sugars
Antakya (117 m)	2347.4 b	106.1 a	8.3 b	0.16 a	0.38 b	0.54	2.90 b	1.54 c	4.43 b
Urumu (443 m)	2373.8 b	87.8 b	8.6 ab	0.10 b	0.43 ab	0.52	3.50 a	2.04 b	5.54 a
Saksak (755 m)	2762.2 a	91.1 b	8.9 a	0.09 b	0.46 a	0.55	3.82 a	2.39 a	6.20 a
Genotype									
Camarosa	2731.2 a	122.6 a	9.4 a	0.06 d	0.62 a	0.67 a	3.52 abc	2.19 b	5.71 bc
Rubygem	2422.9 a	90.6 b	8.9 abc	0.14 b	0.30 c	0.45 d	3.85 a	2.43 a	6.28 a
Albion	2487.6 a	90.5 b	9.0 ab	0.09 c	0.43 b	0.52 bc	3.38 bc	2.01 bc	5.38 bcd
San Andreas	2592.0 a	89.2 b	8.3 cd	0.08 cd	0.42 b	0.50 bcd	2.95 d	1.94 c	4.89 de
Sweet Ann	2069.7 b	38.7 c	7.6 d	0.11 c	0.40 b	0.50 bcd	3.18 cd	2.01 bc	5.19 cd
Fortuna	2532.4 a	123.9 a	8.6 bc	0.10 c	0.38 b	0.48 cd	2.88 d	1.56 d	4.44 e
Sabrina	2363.0 ab	117.3 a	7.9 d	0.21 a	0.42 b	0.63 a	3.68 ab	2.16 b	5.85 ab
Sabrosa	2664.1 a	91.9 b	8.8 abc	0.14 b	0.42 b	0.56 b	3.79 ab	1.62 d	5.41 bcd

Values in the same column that are followed by different letters are significantly different ($P \leq 0.05$) using Tukey's comparison test. TMA: total anthocyanin; TPC: total phenol content; FRAP: total antioxidant content

[A]TPC contents were estimated by the Folin–Ciocalteu assay of Singleton and Rossi (1965). Values are expressed as mg gallic acid equivalents (GAE)/kg FW, by spectrophotometer.

[B]TMA were determined by the pH-differential method of Giusti and Wrolstad (2005). Values are expressed as mg Pg-3-glk/kg FW, by spectrophotometer.

[C]FRAP values were determined by the method of Benzie and Strain (1996). Values are expressed as mmol TE/kg FW, by spectrophotometer.

[D]Organic acids were determined by the method of Shui and Leong (2002). Values are expresses as mg/100 g FW, determined by HPLC.

[E]Organic sugars ere determined by method of Bartolome et al. (1995). Values are expressed as mg/100 g FW, determined by HPLC

Table 6. Variance component of genotype and location conditions on physical and chemical fruit quality characteristics of strawberries.

Variance component	FW	TSS	TA	TSS/TA	pH	FF	*L*	*C*	*h*°
L	1(0)	0.06(10)	0.002(25)	0.0(0)	0.007(36)	0.000(4)	571(45)	2(0)	1.2(0)
G	215(45)	0.22(36)	0.003(37)	2.1(53)	0.002(12)	0.001(19)	254(20)	462(36)	416.0(42)
L × G	1(0)	0.02(4)	0.001(7)	0.7(17)	0.001(3)	0.001(24)	140(11)	304(24)	0.2(0)
Error	259(54)	0.29(49)	0.003(31)	1.2(31)	0.009(49)	0.002(53)	305(24)	515(40)	585.0(58)

*Numbers in parentheses show the variation percentage. L: Location; G: Genotype; TSS: Total soluble solid; TA: Titratable acidity

reported by Andreotti et al. (2014) for different altitudes in South Tyrol (Italy). Herrington et al. (2007) found the genotype effect was larger than the growing location for TA. Perkins-Veazie (1995) reported that TA content in strawberries varied more strongly due to fruit maturity, genotype, and nutrition than ecologic factors.

Fruit color is an important quality factor in strawberry production. Bright red genotypes are sought for the fresh market, but dark red pulp is favored in industry. We recorded the darkest fruits in "Camarosa" among the genotypes and at S among the locations. The high color saturation produced at S may be explained by its having high altitude and night-day temperature ratios. Similar results have been reported by Shiow and Camp (2000), Ordidge et al. (2010), and Pincemail et al. (2012).

Phenolic compounds are known to be important antioxidant compounds in strawberry (Aaby et al., 2012). In our study, the S location produced fruit that had 17.6% higher TPC than the A location. This result indicated that growing locations have a significant effect on TPC. The high levels of TPC at high altitude (S) may be explained by ecological differences (Table 1). TPC was highest in

Table 7. Variance component of genotype and location conditions on nutritional fruit quality characteristics of strawberries.

Variance component	TPC	TMA	TAC	Organic acids			Individual sugars		
			FRAP	Malic acid	Citric acid	Total acids	Glucose	Fructose	Total sugars
L	54.0(23)	7.1(6)	0.01(1)	0.001(29)	0.001(9)	0.000(0)	0.197(39)	0.17(50)	0.7(50)
G	27.0(12)	58.4(51)	0.17(15)	0.002(33)	0.006(46)	0.004(42)	0.084(17)	0.05(15)	0.2(12)
L × G	0.0(0)	11.2(10)	0.45(39)	0.001((30)	0.004(31)	0.003(30)	0.113(22)	0.09(26)	0.3(22)
Error	149.0(65)	37.9(33)	0.53(46)	0.000(9)	0.002(15)	0.003(29)	0.114(22)	0.03(9)	0.2(15)

*Numbers in parentheses show the variation percentage. L: Location; G: Genotype; TPC: total phenol content; TMA: total anthocyanin; FRAP: total antioxidant content

"Camarosa", "Sabrosa", "San Andreas", "Fortuna", "Albion", and "Rubygem", followed by "Sabrina". The lowest TPC was in the "Sweet Ann" cultivar. Phenolics, which greatly affect TPC, can vary greatly amongst cultivars (Tulipani et al., 2008; Aaby et al., 2012; Gündüz and Özdemir, 2014), environmental factors such as light exposure (Ordidge et al., 2010), cultural system, and storage temperature (Jin et al., 2011).

Anthocyanins are water-soluble vacuolar pigments that may appear red, purple, or blue depending on the pH. A notable variability was found among the anthocyanin concentrations in samples of the same variety and harvest date depending on degree of maturity, climatic factors, and postharvest storage (Lopes da Silva et al., 2007; Gündüz and Özdemir, 2014). In the present study, the low altitude A location produced 14.1% higher TMA content than the high altitude S location. Similar findings were reported by Guerrero-Chavez et al. (2015). There were large differences in the TMA content among the cultivars (between 38.7 and 123.9 mg Pg-3-glk/kg FW) (Table 5). "Sweet Ann" had the lowest TMA content (38.7 mg Pg-3-glk/kg FW); "Fortuna", "Camarosa", and "Sabrina" had the highest amount of TMA.

The S location, which has the highest altitude, gave the highest TAC ingredient. There were differences in the TAC contents amongst the cultivars. "Camarosa" had the highest TAC, followed by "Albion". It has been suggested that differences in maturation stages (Koşar et al., 2004), cultural systems (covered with black polyethylene mulch and without mulch) (Wang et al., 2002), postharvest storage methods (Cordenunsi et al., 2005), cultivated vs. wild forms (Özgen et al., 2007), and genotypes (Tulipani et al., 2008; Gündüz and Özdemir, 2014) influence the concentration of antioxidants. Pincemail et al. (2012) reported that the antioxidant capacity in the open field was higher than under tunnels. Moreover, Gündüz and Özdemir (2014) reported that the antioxidant capacity in the open field and plastic tunnel was higher than that in the greenhouse. Our results indicated that different altitude conditions had little effect on antioxidant capacity in strawberries.

Organic acids are minor components of strawberry fruit, but they are important attributes of flavor and, in combination with sugars, have a major impact on sensory quality (Gündüz and Özdemir, 2014). There were distinct differences in organic acid content previously shown among the genotypes examined (Wang et al., 2002). In the present study, two major organic acids were found in the cultivars studied: malic and citric acid. Citric acid was the major acid and accounted for 77.7% of the total acid content, similar to other data found in the literature (Crespo et al., 2010; Gündüz and Özdemir, 2014). "Camarosa" and "Sabrina" showed the highest total acid content, while "Rubygem" had the lowest acid content.

Sugar content is an important taste attribute for strawberries and is highly correlated with consumer acceptance (Jouquand et al., 2008). Glucose, fructose, and sucrose are by far the most abundant soluble components in strawberries. In the present study, levels of sucrose were very low and are not presented. Wang et al. (2002) also reported that strawberry fruit contains lower sucrose concentrations compared to fructose and glucose. Glucose was highest at the S and U locations, which are at high altitudes, and lowest at the A location, at a low altitude. Similar findings were reported by Doumett et al. (2011). These observations can be explained by the variation in day and night temperatures at the A location (Table 1). When the difference between day and night temperatures is low, fruits mature faster and their sugar contents remain low (Shiow and Camp, 2000). The amounts of fructose and glucose in the fruit were quite different among genotypes. "Rubygem" had the highest total sugar. Differences among cultivars were also reported by Herrington et al. (2007).

In conclusion, we observed higher genotypic effects than environmental ones for most of the physical, chemical, and bioactive compound variables. However, a significant high altitude effect was observed on some fruit

quality characteristics (such as fruit color parameters, individual glucose, fructose, total antioxidant capacity, and total phenolic content). Low altitude significantly affected TSS, TA, and TMA. These effects can be ascribed to a combination of several climatic factors (temperature, humidity, daily average radiation, rain, etc.) acting at different altitudes. Taken together, these results indicate that as breeding programs are improving the nutritional quality of new cultivars, environmental factors must be taken into consideration.

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