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Phytochemical characteristics of grafted watermelon on different bottle gourds (*Lagenaria siceraria*) collected from the Mediterranean region of Turkey

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Abstract: The objective of this study is to determine the sugar, organic acid, and carotenoid content of Crimson Tide watermelons grafted onto bottle gourd genotypes collected from the Mediterranean region of Turkey. The experiment was carried out during the 2008 and 2009 growing season. Grafting on the local bottle gourd rootstocks improved the total soluble solid (TSS), titratable acidity (TA), TSS/TA ratio, sugar, organic acid, and carotenoid (β-carotene and lycopene) contents of Crimson Tide fruits. Grafting also altered the ratios among the sugars of Crimson Tide watermelon fruits. Among the Crimson Tide/rootstock (CT/rootstock) combinations, the CT/09-01 and CT/07-06 graft combinations with higher sucrose, lycopene, and total carotenoid content were found promising to increase the quality of Crimson Tide fruits. Commercial rootstocks (Macis and Argentario) reduced carotenoid content compared to the control.

Key words: Bottle gourd, carotenoid, organic acid, rootstock, sugar

1. Introduction

Grafting is widely used in watermelon production to control soil-borne diseases such as *Fusarium* wilt (Yetisir and Sari 2003; Davis et al. 2008), to increase yield (Lee 1994; Oda 1995; Lee and Oda 2003), and to promote the mineral nutrition uptake (Pulgar et al. 2000), but the objectives of grafting have increased significantly over the years. For instance, grafting has been used to improve resistance against low (Bulder et al. 1990) and high (Rivero et al. 2003) temperatures, to improve resistance against iron chlorosis in calcareous soils (Romero et al. 1997), to improve the salinity tolerance of plants (Yetisir and Uygur 2010), to enhance nutrient absorption (Ruiz et al. 1997), and to improve water use (Cohen and Naor 2002). Bottle gourds have also been used routinely as a source of rootstock for watermelons and other cucurbit crops in some Asian and European countries (Lee and Oda 2003). *Lagenaria siceraria* is one of the gourd species used as rootstock for watermelon and it shows a high compatibility rate with watermelon (Lee 1994; Oda 1995; Yetisir and Sari 2003). Turkey has a rich genetic diversity of *L. siceraria*. In a previous study, 210 *L. siceraria* genotypes from ecologically different regions of Turkey were collected and evaluated for rootstock potential, and it was found that Turkish *L. siceraria* germplasm has powerful rootstock potential for watermelon against *Fusarium* wilt and is a good resource for rootstock breeding programs (Yetişir et al. 2007). We previously demonstrated that grafted watermelon plants on bottle gourd rootstocks had an increase in total yield and plant growth parameters as compared to the ungrafted control plants (Karaca et al. 2012). These rootstocks with vigorous root systems also showed superior tolerance to serious soil-borne diseases such as those caused by *Fusarium* (Yetışır et al. 2003). The rootstocks tested in this study also produced larger fruit with a thicker rind and darker fruit flesh color than the control plants (Karaca et al. 2012).

Watermelon quality is greatly affected by grafting (Yetisir and Sari 2003; Yetışır et al. 2003; Davis and Perkins-Veazie 2005). There are many conflicting reports on changes in fruit quality due to grafting. The differences in reported results may be due in part to different production environments, type of rootstock, interactions between specific rootstocks and scions, and harvest date (Davis et al. 2008). In some studies, grafted watermelon fruits had lower (Yao et al. 2003; Davis and Perkins-Veazie 2005) or similar (Miguel et al. 2004; Proietti et al. 2008; Bruton et al. 2009; Bekhradi et al. 2011) total soluble solid (TSS) and sugar content compared to ungrafted controls. However, others reported positive effects of grafting

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watermelon, including an increase in TSS (Salam et al. 2002), reducing sugar (Yetışır et al. 2003; Balazs et al. 2011), and lycopene content (Liu et al. 2003; Proietti et al. 2008). Previous studies demonstrated that quality traits can be improved by selecting rootstock–scion combinations that complement each other (Davis and Perkins-Veazie 2005). We previously reported that some of the local bottle gourd rootstocks tested in this study were found to be promising with regard to some fruit quality parameters as well as yield (Karaca et al. 2012). In this study, therefore, detailed investigation was carried out to determine some phytochemical properties such as sugar, organic acids, and carotenoid content of Crimson Tide watermelons grafted onto the selected bottle gourd genotypes collected from the Mediterranean region of Turkey.

2. Materials and methods

2.1. Plant material

Twenty-one bottle gourd genotypes were chosen as representatives of Turkish *L. siceraria* germplasm as rootstock based on the work of Yetişir et al. (2008). The sampling locations are shown in Figure 1. The genotypes were labeled with the traffic plate number of the province (01 for Adana, 07 for Antalya, 09 for Aydın, 20 for Denizli, 31 for Hatay, 33 for Mersin, 35 for İzmir, 46 for Kahramanmaraş, 48 for Muğla, and 47 for Gaziantep) and were numbered according to the order of collection. Commercial *Lagenaria* hybrid rootstocks Argentario and Macis were also used for comparison (Table 1). Crimson Tide watermelon cultivar was used as a scion.

2.2. Methods

Seeds were sown in a peat and perlite mixture (1:1 v/v) in plug trays (cell volume of 50 mL) in an unheated greenhouse (36°19′30.75″N, 36°11′40.82″E) in the middle of January in both years. Seeds (150 per genotype) were sown in a peat and perlite mixture (2:1 v/v) in plug trays (cell volume of 50 mL) for grafting. The hole-insertion grafting technique was used and plants were grafted following the procedure described by Lee (1994) and Lee and Oda (2003). Seedlings were grown in an unheated greenhouse under plastic tunnels. The grafted seedlings with 2–3 true leaves and the control plants were transplanted under low plastic tunnels in Adana (37°01′50.57″N, 35°22′00.15″E) in the middle of March in both years. The tunnels were removed when the air temperature was suitable (minimum of 22 °C and maximum of 25 °C) for watermelon. Fertilizer was applied at the rate of 180 kg N ha^{-1}, 200 kg P_2O_5 ha^{-1}, and 180 kg K_2O ha^{-1} based on soil test results. The soil was a clay-loam with pH of 8.4. Any necessary micronutrients were applied (Yetisir and Sari 2003). All of the P was applied before planting. Nitrogen and potassium were divided into 3 equal portions and applied before planting, 20 days after planting, and 40 days after planting. Seedlings were transplanted in single rows spaced 3.0 m apart with 0.6 m between plants. Water was applied by drip irrigation. Ripe fruits with completely dried stipule and tendril on the same node with fruit were harvested from 6 to 12 June 2008 and from 20 to 30 June 2009. Immediately after harvest, 5 fruits from each replicate were randomly chosen and samples were taken from the heart portion of the watermelon. Samples (40–50 g) in 50-mL centrifuge tubes were then frozen and kept at –20 °C until analysis.

Figure 1. Map of the collection sites for bottle gourd (*L. siceraria*) genotypes in the Mediterranean region in Turkey.

Table 1. Total soluble solid (TSS) and sugar content of Crimson Tide (CT) watermelon fruits grafted onto different bottle gourd rootstocks. The values are means of 2 years ± standard error.

CT/rootstocks	TSS (%)	Fructose (%)	Glucose (%)	Sucrose(%)	Total sugar (%)
CT	11.13 ± 0.10efg	3.45 ± 0.05bcd	2.45 ± 0.02ef	2.83 ± 0.01i	8.74 ± 0.04jk
CT/01-16	10.78 ± 0.36ghi	3.51 ± 0.04b	2.52 ± 0.07de	3.00 ± 0.09h	9.03 ± 0.17efg
CT/01-17	10.83 ± 0.10ghi	2.69 ± 0.02o	1.99 ± 0.02k	2.70 ± 0.03j	7.38 ± 0.01q
CT/07-04	10.13 ± 0.30j	2.71 ± 0.05o	1.97 ± 0.03k	3.24 ± 0.04g	7.92 ± 0.05p
CT/07-06	11.25 ± 0.24def	2.74 ± 0.03o	1.95 ± 0.03k	4.61 ± 0.05a	9.30 ± 0.08bc
CT/07-42	11.40 ± 0.30cde	3.10 ± 0.05m	2.16 ± 0.03j	3.53 ± 0.06de	8.80 ± 0.05ij
CT/07-45	11.29 ± 0.24def	3.46 ± 0.08bcd	2.46 ± 0.07ef	2.89 ± 0.10i	8.81 ± 0.18hij
CT/09-01	11.68 ± 0.18bc	3.20 ± 0.05ijk	2.32 ± 0.04gh	3.70 ± 0.05bc	9.22 ± 0.04bcd
CT/20-02	10.57 ± 0.16i	3.79 ± 0.03a	3.14 ± 0.05a	1.58 ± 0.08m	8.51 ± 0.03lm
CT/20-06	10.83 ± 0.24ghi	3.38 ± 0.08def	2.88 ± 0.05b	2.40 ± 0.02k	8.66 ± 0.06jkl
CT/31-08	11.49 ± 0.23b–e	3.18 ± 0.05jkl	2.35 ± 0.07g	3.57 ± 0.11d	9.10 ± 0.09def
CT/31-09	11.80 ± 0.35b	3.43 ± 0.03cde	2.55 ± 0.03cd	3.53 ± 0.09de	9.51 ± 0.08a
CT/31-15	11.75 ± 0.08bc	3.35 ± 0.04efg	2.52 ± 0.01de	2.23 ± 0.02l	8.10 ± 0.03o
CT/31-43	11.59 ± 0.24bcd	3.34 ± 0.03fg	2.36 ± 0.04g	2.66 ± 0.05j	8.35 ± 0.12mn
CT/33-02	10.97 ± 0.32fgh	3.48 ± 0.02bc	2.59 ± 0.04cd	3.08 ± 0.01h	9.15 ± 0.07cde
CT/33-35	11.79 ± 0.28b	3.28 ± 0.04ghi	2.32 ± 0.05gh	3.76 ± 0.04b	9.35 ± 0.08b
CT/33-41	11.23 ± 0.26def	3.21 ± 0.05h–k	2.27 ± 0.09hi	3.44 ± 0.11ef	8.92 ± 0.20ghi
CT/33-45	12.57 ± 0.34a	3.35 ± 0.08efg	2.53 ± 0.04de	3.72 ± 0.01bc	9.59 ± 0.12a
CT/35-01	11.56 ± 0.29bcd	3.12 ± 0.03lm	2.25 ± 0.04hi	3.44 ± 0.08ef	8.81 ± 0.05hij
CT/46-03	10.83 ± 0.18ghi	3.28 ± 0.05gh	2.63 ± 0.05c	3.63 ± 0.05cd	9.54 ± 0.05a
CT/48-07	10.68 ± 0.13hi	3.14 ± 0.07klm	2.24 ± 0.03ij	3.58 ± 0.05d	8.96 ± 0.14fgh
CT/47745	10.93 ± 0.04f-i	3.02 ± 0.04n	2.24 ± 0.08ij	3.35 ± 0.05f	8.60 ± 0.09kl
CT/Macis	10.76 ± 0.11ghi	3.44 ± 0.01bcd	2.39 ± 0.04fg	3.47 ± 0.09e	9.30 ± 0.06bc
CT/Argentario	11.42 ± 0.12b–e	3.23 ± 0.05hij	2.35 ± 0.07g	2.63 ± 0.02j	8.22 ± 0.10no

*Mean separation was performed by Fisher's LSD test. Means (n = 3) followed by the same letter within a column are not significantly different at P < 0.05.

2.3. Total soluble solids content, juice pH, and titratable acidity

Total soluble solids (TSS) content was determined with a refractometer (Atago, Model ATC-1E), juice pH with an Orion 5-Star model digital pH meter (Thermo Fisher Scientific Inc.), and titratable acidity (TA) by titration of 10 mL of juice with 0.1 N NaOH to pH 8.1 expressed as g malic acid 100 mL^{-1} juice.

2.4. Extraction and HPLC analysis of sugars and organic acids

Sugars and organic acids were extracted following a modified version of the method described by Chisholm and Picha (1986). Briefly, frozen samples were homogenized using an Ultra-Turrax T25 model homogenizer (IKA-Labortechnik) at low speed with a 10-mm shaft. The resulting slurry was filtered through Whatman No. 4 paper with a Buchner funnel under vacuum. Exactly 1 mL of sample was diluted with deionized distilled water to a total volume of 10 mL. After vortexing for 1 min, 20 µL of sample was injected directly into the HPLC equipment after filtration through a Millex-HV 0.45 µm filter (Millipore). HPLC analysis of sugars and organic acids was performed on LC-10A equipment consisting of LC-10AD pumps, an in-line degasser, a CTO-10A column oven, an SCL-10A system controller, an SPD 10Avp, a photo diode array (PDA) detector, and a refractive index detector (RID), all operated by LC solution software (Shimadzu). Sugars were separated on an EC NUCLEOSIL Carbohydrate 250 mm × 4 mm i.d. column (Macherey-Nagel) at 25 °C. The mobile phase was acetonitrile and water (80:20, v/v) at a flow rate of 2 mL min^{-1}. Organic acids were separated on a Transgenomic™ ICSep ION300 300 mm × 7.8 mm i.d. column (Transgenomic) at 65 °C. The mobile phase was 0.0085 N H_2SO_4 at a flow rate of 0.4 mL min^{-1}. Sugars and organic acids were detected using the RID and PDA detectors at 210 nm, respectively. The quantification was performed according to external standard solution calibrations. The results were expressed as g 100 g^{-1} fresh weight.

2.5. Extraction and HPLC analysis of carotenoids

Carotenoids were extracted following a modified version of the method described by Perkins-Veazie and Collins (2006). Briefly, frozen samples were homogenized using the Ultra-Turrax homogenizer at low speed with a 10-mm shaft. Three grams of puree were weighed into the centrifuge tube and extracted with HPLC-grade solvents of 10 mL of hexane, 5 mL of ethanol, and 5 mL of acetone containing 0.05% butylated hydroxytoluene (Merck KGaA). Samples were tightly sealed and placed on an orbital shaker (Heidolph Unimax 2010, Heidolph Instruments GmbH Co. KG) for 15 min at 320 rpm, and then 3 mL of deionized distilled water was added and samples were shaken again for 10 min. Afterwards, samples were put in a rack to allow solvent phase separation. The upper hexane layer was also filtered using a Millex-HV 0.45-µm filter (Millipore) and 20 µL of sample was injected directly into Shimadzu HPLC equipment (as previously described). Carotenoids were separated on a YMC carotenoid column, C30 250 mm × 4.6 mm id, 5 µm particle size (YMC Europe GMBH), operating at 30 °C with a flow rate of 1.5 mL min^{-1}. The mobile phase was solvent A (methanol, methyl tertiary butyl ether, and deionized distilled water, 81:15:41) and solvent B (methanol and methyl tertiary butyl ether, 10:90) with elution with a linear gradient of 0–16 min with 100% A and 16–60 min with 100% B (Liu et al., 2009). Detection was carried out at 503 nm for lycopene and 452 nm for β-carotene using the PDA detector. Components were identified by comparison of their retention times to those of authentic standards under analysis conditions and were quantified by external standard method and expressed as µg g^{-1} fresh weight.

2.6. Statistical analysis

The data were analyzed as a factorial experiment in a completely randomized block design by ANOVA using SAS software (SAS 1999). Each treatment was replicated 3 times, with 5 fruit in each replication. Mean separation was performed by Fisher's least significant difference (LSD) test at $P < 0.05$ using the GLM procedure of SAS. The relationships between the fruit's chemical attributes were evaluated by Pearson's correlation coefficients obtained from the CORR procedure of SAS. The means obtained for each variable were subjected to principal component analysis (PCA) to evaluate relationships among genotypes and variables using the PRINCOMP procedure of the SAS. A visual representation of these results was carried out through 3-dimensional biplot of the first 3 principal components (PCs) constructed from the output data sets of PROC PRINCOMP using SAS macros in PROC IML provided by Friendly (2007).

3. Results

3.1. TSS and sugar content

The effects of Crimson Tide/rootstock (CT/rootstock) combinations on TSS and sugar content were significant (Table 1). TSS content was highest in the CT/33-45 while it was lowest in the CT/07-04. The CT/20-02 resulted in higher fructose and glucose content and lower sucrose content. The CT/07-06 had higher sucrose and lower fructose and glucose contents. In addition to the CT/07-06, the CT/01-17 and CT/07-04 also contained lower fructose and glucose content compared to the control and other graft combinations. The higher total sugar content was determined in the CT/33-45, CT/46-03, and CT/31-09 combinations, whereas lower values were seen in CT/01-17 and CT/07-04. Fructose (3.45 ± 0.05) was the predominant soluble sugar, followed by sucrose (2.83 ± 0.01) and glucose (2.45 ± 0.02) in ungrafted Crimson Tide fruits (Table 1). A similar trend was observed with Crimson Tide fruits from the CT/01-16, CT/07-45 CT/31-43, CT/33-02, and CT/Argentario graft combinations. In the CT/20-02, CT/20-06, and CT/31-15 graft combinations, fructose and glucose were present in higher concentration than sucrose. Sucrose was the predominant soluble sugar in 13 graft combinations (CT/07-04, CT/07-06, CT/07-42, CT/09-01, CT/31-08, CT/31-09, CT/33-35, CT/33-41, CT/33-45, CT/35-01, CT/46-03, CT/48-07, and CT/47745). Crimson Tide fruits grafted onto local bottle gourd rootstocks had fructose, sucrose, and glucose contents ranging from 2.7% to 3.8%, from 1.6% to 4.6%, and from 1.9% to 3.1%, respectively. With the commercial rootstocks Macis and Argentario, fructose, sucrose, and glucose contents varied from 3.2% to 3.4%, from 2.6% to 3.5%, and from 2.3% to 2.4%, respectively.

3.2. TA, TSS/TA ratio, juice pH, and organic acid content

The TA, TSS/TA ratio, juice pH, and organic acid content were affected by grafting (Table 2). Compared to the control and other graft combinations, CT/20-02 had the highest TA and malic acid content, as well as the lowest juice pH and TSS/TA ratio. CT/07-06 showed an opposite pattern. Higher citric acid content was obtained from CT/31-15 and lower from CT/31-43 and CT/33-02. In ungrafted Crimson Tide fruits, the malic and citric acid

Table 2. Titratable acidity (TA), TSS/TA ratio, juice pH, and organic acid content of Crimson Tide (CT) watermelon fruits grafted onto different bottle gourd rootstocks. The values are means of 2 years ± standard error.

CT/rootstocks	TA (%)	TSS/TA	Juice pH	Citric **(%)**	Malic **(%)**
CT	0.12 ± 0.01b	95.51 ± 4.14hij	5.42 ± 0.01k	0.082 ± 0.003hi	0.400 ± 0.005bc
CT/01-16	0.11 ± 0.01bc	95.15 ± 2.01ij	5.50 ± 0.02hij	0.083 ± 0.003ghi	0.402 ± 0.003bc
CT/01-17	0.11 ± 0.01bc	95.74 ± 5.50g–j	5.74 ± 0.01b	0.082 ± 0.003hi	0.330 ± 0.001k
CT/07-04	0.11 ± 0.01bcd	92.12 ± 2.73j	5.50 ± 0.02ij	0.098 ± 0.006b–e	0.380 ± 0.009efg
CT/07-06	0.08 ± 0.01f	140.6 7 ± 2.95a	5.80 ± 0.01a	0.098 ± 0.013b–e	0.327 ± 0.003k
CT/07-42	0.12 ± 0.01b	97.88 ± 5.58f–j	5.57 ± 0.01d–g	0.107 ± 0.003b	0.372 ± 0.003f–j
CT/07-45	0.11 ± 0.01bcd	102.61 ± 2.16d–i	5.48 ± 0.03j	0.098 ± 0.008b–e	0.410 ± 0.009b
CT/09-01	0.11 ± 0.01bc	103.19 ± 5.13d–i	5.56 ± 0.03efg	0.100 ± 0.005bcd	0.382 ± 0.008ef
CT/20-02	0.13 ± 0.01a	81.33 ± 1.19k	5.30 ± 0.04l	0.088 ± 0.003e–i	0.457 ± 0.003a
CT/20-06	0.11 ± 0.01bc	95.68 ± 4.16hij	5.40 ± 0.02k	0.098 ± 0.003b–e	0.410 ± 0.001b
CT/31-08	0.11 ± 0.01cde	107.96 ± 6.18cde	5.59 ± 0.02cde	0.100 ± 0.009bcd	0.405 ± 0.001bc
CT/31-09	0.11 ± 0.01bc	104.20 ± 3.01d–g	5.55 ± 0.01efg	0.103 ± 0.003bc	0.395 ± 0.010cd
CT/31-15	0.11 ± 0.01bc	103.85 ± 5.19d–h	5.55 ± 0.02efg	0.127 ± 0.003a	0.382 ± 0.012ef
CT/31-43	0.11 ± 0.01bcd	105.88 ± 8.58def	5.54 ± 0.03f–i	0.043 ± 0.003j	0.385 ± 0.005de
CT/33-02	0.10 ± 0.01e	109.70 ± 3.24bcd	5.52 ± 0.07g–j	0.042 ± 0.006j	0.377 ± 0.003e–h
CT/33-35	0.10 ± 0.01de	114.41 ± 8.58bc	5.59 ± 0.04cde	0.095 ± 0.009c–f	0.370 ± 0.005g–j
CT/33-41	0.10 ± 0.01de	108.77 ± 3.43cde	5.62 ± 0.04c	0.093 ± 0.006c–g	0.362 ± 0.010j
CT/33-45	0.11 ± 0.01cde	118.05 ± 7.29b	5.50 ± 0.02ij	0.048 ± 0.003j	0.368 ± 0.008hij
CT/35-01	0.11 ± 0.01bc	102.59 ± 8.83d–i	5.58 ± 0.04c–f	0.085 ± 0.005f–i	0.363 ± 0.008ij
CT/46-03	0.11 ± 0.01bcd	98.42 ± 1.68f–j	5.60 ± 0.02cde	0.092 ± 0.013d–h	0.378 ± 0.008e–h
CT/48-07	0.10 ± 0.01de	103.56 ± 5.75d–i	5.61 ± 0.04cd	0.095 ± 0.009c–f	0.375 ± 0.001e–h
CT/47745	0.10 ± 0.01de	106.03 ± 6.01c–f	5.55 ± 0.04e–h	0.080 ± 0.001i	0.373 ± 0.008f–i
CT/Macis	0.11 ± 0.01cde	101.08 ± 4.72e–i	5.53 ± 0.03ghi	0.095 ± 0.001c–f	0.397 ± 0.006c
CT/Argentario	0.11 ± 0.01cde	107.21 ± 5.04cde	5.53 ± 0.04ghi	0.083 ± 0.008ghi	0.375 ± 0.005e–h

*Mean separation was performed by Fisher's LSD test. Means (n = 3) followed by same letter within a column are not significantly different at P < 0.05.

contents were 0.40% and 0.08%, respectively. Crimson Tide fruits grafted onto local bottle gourd rootstocks had malic and citric acid contents ranging from 0.33% to 0.46% and from 0.04% to 0.13%, respectively. When grafted with commercial Macis and Argentario rootstocks, the malic acid content varied from 0.38% to 0.40% and the citric acid content varied from 0.08% to 0.10%.

3.3. Carotenoid content

The effects of CT/rootstock combinations on carotenoid content were significant (Table 3). The β-carotene content was higher in CT/01-17, while the *cis*-lycopene, all-*trans*-lycopene, total lycopene, and total carotenoid contents were higher in the CT/09-01 and CT/07-06 combinations when compared to the control and other graft combinations. Among the cultivar/rootstock combinations, CT/Macis and CT/Argentario contained lower β-carotene, total lycopene, and total carotenoid content. In addition to commercial rootstocks, CT/20-02 and CT/20-06 also resulted in lower all-*trans*-lycopene, total lycopene, and total carotenoid content. Ungrafted Crimson Tide fruits contained 0.55 ± 0.01 μg g^{-1} of β-carotene, 30.85 ± 0.85 μg g^{-1} of total lycopene, and 31.40 ± 0.86 μg g^{-1} of total

Table 3. Carotenoid content (µg g^{-1}) of Crimson Tide (CT) watermelon fruits grafted onto different bottle gourd rootstocks. The values are means of 2 years ± standard error.

CT/rootstocks	β-Carotene	*cis*-Lycopene	All-*trans* -lycopene	Total lycopene	Total carotenoid
CT	0.55 ± 0.01c	1.27 ± 0.04h	29.58 ± 0.83g	30.85 ± 0.85hi	31.40 ± 0.86hi
CT/01-16	0.45 ± 0.01n	1.35 ± 0.04ef	28.67 ± 0.21h	30.03 ± 0.23jk	30.47 ± 0.23jk
CT/01-17	0.61 ± 0.01a	1.40 ± 0.01de	33.73 ± 0.35b	35.13 ± 0.35c	35.74 ± 0.35c
CT/07-04	0.53 ± 0.01fg	1.17 ± 0.01kl	29.75 ± 0.05g	30.91 ± 0.04hi	31.44 ± 0.04hi
CT/07-06	0.48 ± 0.01l	1.54 ± 0.01b	36.67 ± 0.31a	38.21 ± 0.31b	38.70 ± 0.32b
CT/07-42	0.46 ± 0.01m	1.18 ± 0.02jkl	30.67 ± 0.40ef	31.84 ± 0.39g	32.30 ± 0.39g
CT/07-45	0.47 ± 0.01m	1.35 ± 0.05fg	28.49 ± 0.16h	29.84 ± 0.13k	30.30 ± 0.14k
CT/09-01	0.54 ± 0.01de	2.01 ± 0.05a	37.07 ± 0.64a	39.08 ± 0.62a	39.62 ± 0.61a
CT/20-02	0.46 ± 0.01m	1.18 ± 0.04jkl	23.26 ± 0.41k	24.44 ± 0.43n	24.90 ± 0.43n
CT/20-06	0.49 ± 0.01kl	1.21 ± 0.05jk	23.23 ± 0.37k	24.44 ± 0.34n	24.92 ± 0.34n
CT/31-08	0.52 ± 0.01hi	1.47 ± 0.02c	30.03 ± 0.32fg	31.50 ± 0.31gh	32.01 ± 0.31gh
CT/31-09	0.50 ± 0.01j	1.48 ± 0.01c	32.73 ± 0.28c	34.21 ± 0.27d	34.71 ± 0.27d
CT/31-15	0.51 ± 0.01i	1.44 ± 0.03cd	31.38 ± 0.48d	32.81 ± 0.45ef	33.32 ± 0.46ef
CT/31-43	0.52 ± 0.01gh	1.14 ± 0.02l	33.70 ± 0.41b	34.84 ± 0.41cd	35.36 ± 0.41cd
CT/33-02	0.53 ± 0.01fg	1.26 ± 0.04hi	29.66 ± 0.31g	30.92 ± 0.33hi	31.44 ± 0.33hi
CT/33-35	0.54 ± 0.01cd	1.30 ± 0.02gh	31.26 ± 0.33de	32.56 ± 0.34f	33.10 ± 0.34f
CT/33-41	0.57 ± 0.01b	1.08 ± 0.01m	27.25 ± 0.28i	28.33 ± 0.28l	28.90 ± 0.28l
CT/33-45	0.49 ± 0.01kl	1.17 ± 0.03jkl	28.20 ± 0.84h	29.38 ± 0.85k	29.86 ± 0.85k
CT/35-01	0.49 ± 0.01l	1.22 ± 0.04ij	29.36 ± 0.38g	30.58 ± 0.39ij	31.07 ± 0.39ij
CT/46-03	0.50 ± 0.01jk	1.29 ± 0.02h	28.58 ± 0.64h	29.86 ± 0.64k	30.36 ± 0.63k
CT/48-07	0.49 ± 0.01l	1.21 ± 0.05jk	30.55 ± 0.16f	31.76 ± 0.19g	32.25 ± 0.18g
CT/47745	0.53 ± 0.01ef	1.22 ± 0.02ij	32.16 ± 0.02c	33.38± 0.03e	33.91 ± 0.03e
CT/Macis	0.43 ± 0.01o	0.96 ± 0.01n	24.96 ± 0.36j	25.92 ± 0.36m	26.34 ± 0.36m
CT/Argentario	0.42 ± 0.01o	0.92 ± 0.02n	25.49 ± 0.31j	26.40 ± 0.30m	26.82 ± 0.30m

*Mean separation was performed by Fisher's LSD test. Means (n = 3) followed by same letter within a column are not significantly different at $P < 0.05$.

carotenoids. Crimson Tide fruits grafted onto local bottle gourd rootstocks had β-carotene, total lycopene, and total carotenoid contents in the range of 0.45–0.61 µg g^{-1}, 25–39 µg g^{-1}, and 25–40 µg g^{-1}, respectively. With Macis and Argentario rootstocks, β-carotene, total lycopene, and total carotenoid contents varied from 0.42 to 0.43 µg g^{-1}, from 25 to 26 µg g^{-1}, and from 26 to 27 µg g^{-1}, respectively. The *cis*-lycopene was 3%–5% of the total lycopene and β-carotene was 1%–2% of total carotenoids.

3.4. Correlations among quality parameters

Significant correlations were found among the fruit quality parameters (Table 4). Fructose and glucose content was positively correlated with acid content (TA and malic acid) but negatively correlated with TSS/TA ratio, juice pH, and carotenoid content (β-carotene, all-*trans*-lycopene, total lycopene, and total carotenoids). In contrast, sucrose was negatively correlated with acid content (TA and malic acid) but positively correlated with TSS/TA ratio,

Table 4. Pearson's correlation coefficients between some quality parameters.

	TSS	Fructose	Glucose	Sucrose	Total sugar	TA	TSS/TA	Juice pH	Citric acid	Malic acid	β-Carotene	*cis*-Lycopene	All-*trans*-lycopene	Total lycopene
Fructose	0.14													
Glucose	–0.01	0.86**												
Sucrose	0.25*	–0.47**	–0.58**											
Total sugar	0.35**	0.36**	0.24*	0.62**										
TA	–0.04	0.41**	0.47**	–0.61**	–0.27*									
TSS/TA	0.46**	–0.30**	–0.40**	0.64**	0.39**	–0.89**								
Juice pH	0.14	–0.70**	–0.73**	0.65**	0.04	–0.58**	0.58**							
Citric	–0.12	–0.15	–0.09	0.05	–0.07	0.08	–0.11	0.11						
Malic	–0.20	0.76**	0.77**	–0.58**	0.08	0.54**	–0.56**	–0.83**	0.08					
β-Carotene	0.03	–0.40**	–0.31**	0.05	–0.28*	–0.06	0.04	0.31**	–0.12	–0.39**				
cis-Lycopene	0.23	–0.15	–0.10	0.28*	0.19	–0.04	0.16	0.27*	0.28*	–0.10	0.36**			
All-*trans*-lycopene	0.28*	–0.54**	–0.61**	0.50**	0.02	–0.34**	0.44**	0.64**	0.03	–0.58**	0.49**	0.69**		
Total lycopene	0.28*	–0.52**	–0.59**	0.50**	0.03	–0.33**	0.43**	0.63**	0.04	–0.56**	0.49**	0.72**	0.99**	
Total carotenoid	0.28*	–0.52**	–0.59**	0.50**	0.02	–0.33**	0.43**	0.63**	0.04	–0.56**	0.50**	0.72**	0.99**	0.99**

*, **Correlation is significant at $P < 0.05$ and $P < 0.01$, respectively.

juice pH, and carotenoid content (all-*trans*-lycopene, total lycopene, and total carotenoids). TSS and pH were significantly correlated with all of the carotenoids. Although significant relationships were observed between total sugar and sucrose, fructose, and glucose content, the correlation coefficients were quite low. There was no relationship between TSS content and TA, fructose, or glucose content. Malic acid was negatively correlated with all-*trans*-lycopene, total lycopene, and total carotenoids while citric acid was only correlated positively with *cis*-lycopene content.

3.5. PCA

The PCA carried out in our work showed that 75.80% of the observed variability was explained by the first 3 PCs (Table 5). PC1, PC2, and PC3 accounted for 51.41%, 14.78%, and 9.61%, respectively, of the variability. Table 6 shows the correlation between the original variables and the first 3 PCs: PC1 was positively correlated with juice pH, sucrose, all-*trans*-lycopene, total lycopene, and total carotenoid content and was negatively correlated with fructose, glucose, malic acid content, and TA. PC2 explains mainly *cis*-lycopene content. PC3 was positively correlated with citric acid and negatively with TSS content. Positive values for PC1 indicate CT/rootstock combinations with higher juice pH and lycopene, total carotenoid, and sucrose contents. CT/07-06, CT/01-17, and CT/09-01 belong to this group (Figure 2). CT/rootstock combinations (CT/20-02, CT/20-06, CT/Macis, and CT/Argentario) with negative values for PC1 were rich in malic acid, fructose, and glucose and had higher TA. The positive PC2 values correspond to CT/rootstock combinations with higher *cis*-lycopene content such as CT/09-01 and CT/31-09. The negative PC3 values indicate CT/rootstock combinations (CT/07-04) with lower TSS content while the positive PC3 values correspond to CT/rootstock combinations (CT/31-15) with higher citric acid content.

3.6. Cluster analysis

The CT/rootstock combinations were divided into 4 main clusters (Figure 3). The dendrogram indicated that this grouping was not correlated with the geographical origin of the bottle gourd genotypes collected. Cluster 1 grouped CT/07-06 and CT/09-01 combinations; Cluster 2 grouped CT/20-02, CT/20-06, CT/Macis, and CT/Argentario combinations; Cluster 3 consisted of CT/01-17, CT/31-43, CT/31-09, CT/47745, CT/31-15 and CT/33-35; and Cluster 4 included the rest of the CT/rootstock combinations.

4. Discussion

Grafting on the local bottle gourd rootstocks increased TSS/TA ratio and TSS, fructose, glucose, and sucrose contents of Crimson Tide watermelon fruits compared to the control and commercial rootstock grafts. In contrast to our results, some previous reports showed no difference in TSS content between grafted and ungrafted watermelon fruits (Miguel et al. 2004; Proietti et al. 2008, Bruton et al. 2009; Balazs et al. 2011; Bekhradi et al. 2011). Proietti et al. (2008) reported a similar content of soluble sugars (glucose, fructose, and sucrose) in ungrafted and grafted miniwatermelon fruits onto the commercial hybrid rootstock. In other studies, the total sugar and TSS content of watermelons grafted onto bottle gourd rootstock was reported to be lower than in self-rooted watermelons (Yao et al. 2003; Davis and Perkins-Veazie 2005; Roberts et al. 2007). There are many conflicting reports on changes in fruit quality due to grafting. The differences in reported results may be due in part to different production

Table 5. Eigenvalues and proportion of total variability among CT/rootstock combinations as explained by the first 10 principal components.

PCs	Eigenvalue	Percent (%) of total variation	Cumulative percent (%) of total variation
1	6.6828	51.41	51.41
2	1.9214	14.78	66.19
3	1.2499	9.61	75.80
4	1.1829	9.10	84.90
5	0.6443	4.96	89.86
6	0.5137	3.95	93.81
7	0.3247	2.50	96.31
8	0.1837	1.41	97.72
9	0.1521	1.17	98.89
10	0.0811	0.62	99.51

Table 6. Component loadings for quality variables and component scores for 23 CT/rootstock combinations.

Variables/factors	Component loading			CT/rootstocks	Component scores		
	PC1	PC2	PC3		PC1	PC2	PC3
TSS	0.093	0.304	–0.540	CT	–0.515	0.844	0.079
Fructose	–0.298	0.315	–0.240	CT/01-16	–0.586	0.198	0.050
Glucose	–0.315	0.292	–0.102	CT/01-17	1.451	–0.408	0.825
Sucrose	0.270	–0.153	–0.177	CT/07-04	0.285	–1.334	1.843
Citric acid	0.027	0.001	0.698	CT/07-06	2.381	–0.836	0.006
Malic acid	–0.321	0.273	0.150	CT/07-42	0.182	–0.359	0.583
TA	–0.262	0.251	0.189	CT/07-45	–0.570	0.465	0.337
Juice pH	0.339	–0.211	–0.022	CT/09-01	1.364	2.587	0.732
β-Carotene	0.191	0.155	0.053	CT/20-02	–2.649	1.060	0.930
cis-Lycopene	0.207	0.513	0.244	CT/20-06	–1.549	–0.321	0.631
All-*trans*-lycopene	0.349	0.264	0.010	CT/31-08	0.181	0.530	0.422
Total lycopene	0.347	0.283	0.023	CT/31-09	0.256	1.435	–0.142
Total carotenoid	0.347	0.283	0.024	CT/31-15	0.042	1.136	1.055
				CT/31-43	0.215	0.805	–1.740
				CT/33-02	–0.166	0.117	–1.619
				CT/33-35	0.592	0.138	–0.492
				CT/33-41	0.147	–1.235	–0.422
				CT/33-45	–0.219	0.169	–2.848
				CT/35-01	0.214	–0.538	–0.367
				CT/46-03	–0.152	–0.301	0.166
				CT/48-07	0.362	–0.843	0.611
				CT/47745	0.599	–0.401	–0.024
				CT/Macis	–1.001	–1.491	0.162
				CT/Argentario	–0.863	–1.415	–0.779

CT: Crimson Tide.

environments, type of rootstock, interactions between specific rootstocks and scions, and harvest date (Davis et al. 2008). Bruton et al. (1999) observed a field and year effect due to soil and climatic conditions on watermelon quality parameters besides rootstock effect. In agreement with our results, others reported an increase in TSS content (Salam et al. 2002) and reducing sugars (Yetışır et al. 2003; Balazs et al. 2011) in watermelon fruits when grafted onto bottle gourd rootstocks. The fruits of the nongrafted Bonta watermelon plants had higher sucrose content than the fruits from the grafted plants on the interspecific hybrid rootstock RS 841 and the *Lagenaria* rootstock FR Strong, while the reducing sugar content (glucose and fructose) showed an opposite pattern (Balazs et al. 2011). Yetisir et al. (2003) suggested that TSS and sugar content were greatly affected by grafting, but the results were dependent on the rootstock used. Our results confirmed this finding. Grafting onto the local bottle gourd rootstocks improved the taste of Crimson Tide fruits, as evidenced by the higher TSS/TA ratio, which describes a good balance between sweetness and acidity in fruits (Proietti et al. 2008).

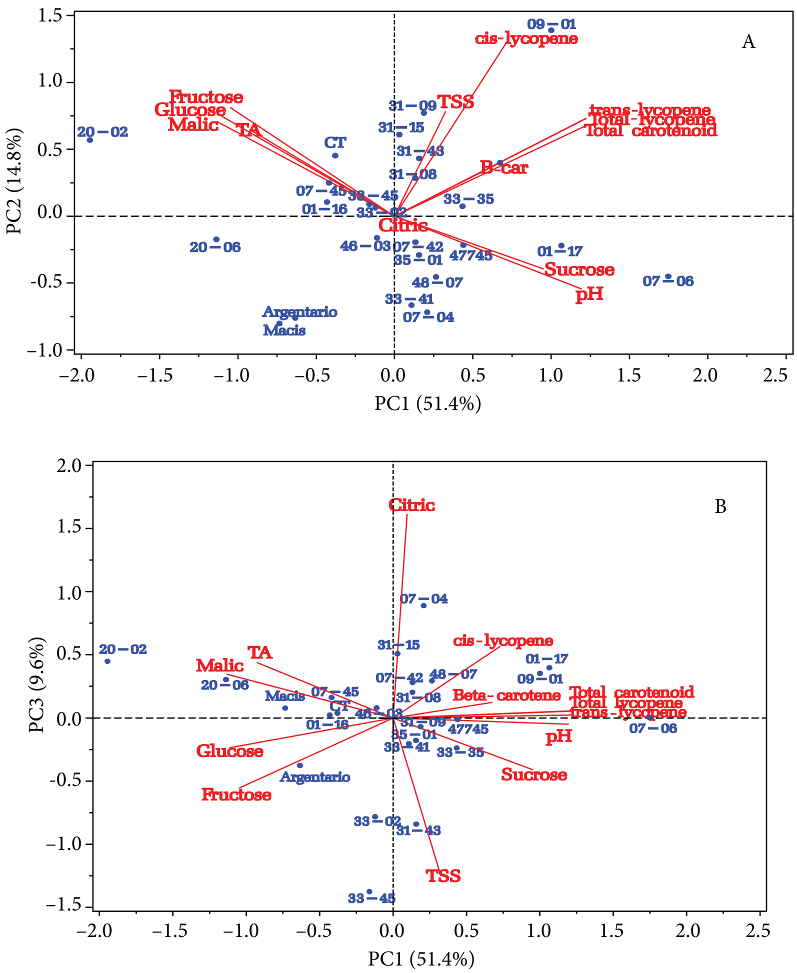

Figure 2. Two-dimensional biplots of PC2 vs. PC1 (A) and PC3 vs. PC1 (B) based on principal components analysis (PCA) of first 3 principle components (PCs) depicting relationship among Crimson Tide (CT) watermelon fruits ungrafted or grafted onto local and commercial bottle gourd rootstocks. The analysis was conducted using 13 quality parameters.

Sugar composition in watermelon includes sucrose, fructose, and glucose (Chisholm and Picha 1986; Yativ et al. 2010). In mature commercial watermelon fruits, proportions of sucrose and glucose are in the range of 20%–40% of total sugars, while the proportion of fructose is in the range of 30%–50% (Yativ et al. 2010). Fructose was reported to be the predominant sugar in some red-fleshed watermelon cultivars (Elmstrom and Davis 1981; Chisholm and Picha 1986; Proietti et al. 2008). The relative concentration of these sugars is important since they vary in perceived sweetness. According to Elmstrom and Davis (1981), cultivars or maturity that results in high fructose concentrations is a desirable feature since the relative sweetness of fructose is greater than that of sucrose. In this study, fructose was the predominant sugar, followed by sucrose and glucose in the control fruits and the fruits of the 5 graft combinations that taste sweeter than those from other graft combinations. Fructose and glucose were present in higher concentrations than sucrose in 3 graft combinations, while sucrose was the predominant soluble sugar in 13 graft combinations. This indicated that grafting altered the ratios among the sugars of watermelon fruits, confirming a previous report examining watermelon and muskmelon fruits (Gao and Liao 2006; Liu et al. 2010). Fructose, sucrose, and glucose concentrations were reported to be in the range of 2%–5%, 2%–4%, and 1.5%–4.5%, respectively, for commercial watermelon varieties (Chisholm and Picha 1986; Leskovar et al. 2004; Proietti et al. 2008; Yativ et al. 2010). Individual sugar contents of ungrafted or grafted Crimson Tide fruits were within the range of previous studies.

An increase in TA, juice pH, and malic and citric acid contents occurred in some graft combinations with local bottle gourd rootstocks. Consistent with our results, Proietti et al. (2008) reported an increase in TA by grafting. Chisholm and Picha (1986) reported that malic acid (in

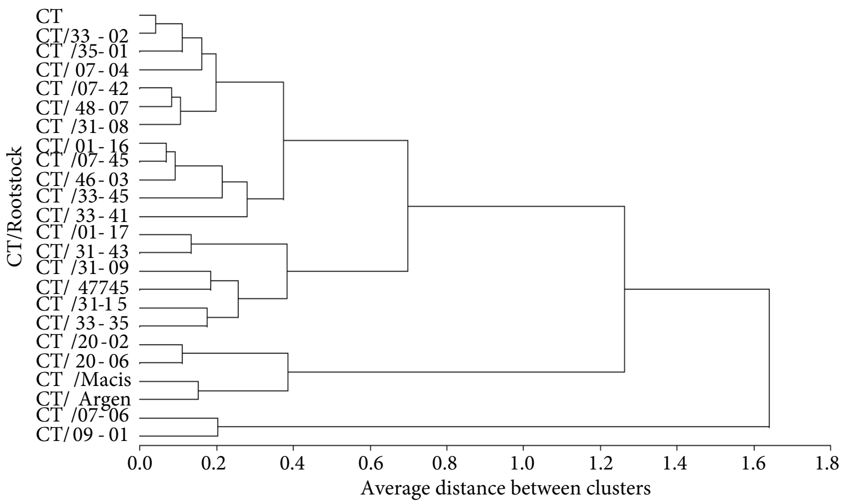

Figure 3. Dendrogram from cluster analysis of Crimson Tide (CT) watermelon fruits ungrafted or grafted onto local and commercial bottle gourd rootstocks.

the range of 0.19%–0.30%) was the primary organic acid, followed by citric acid (in the range of 0.06% to 0.09%) in red-fleshed watermelon fruits. Malic and citric acid were consistently the major organic acids in ungrafted and grafted Crimson Tide fruits, with malic acid (0.33%–0.46%) found in higher quantities than citric acid (0.04%–0.08%). We could not quantify fumaric and succinic acids because of extremely low levels of fumaric acid or the instability of succinic acid as reported by Chisholm and Picha (1986).

Local bottle gourd rootstocks resulted in higher β-carotene, *cis*-lycopene, all-*trans*-lycopene, total lycopene, and total carotenoid contents compared to the control and commercial rootstocks. Consistent with our results, previous studies have typically shown higher lycopene content in watermelon fruit from grafted plants (Davis and Perkins-Veazie 2005; Proietti et al. 2008). Liu et al. (2003) demonstrated higher carotene content in fruit of watermelon grafted onto 5 different rootstocks of *L. siceraria* or *Cucurbita ficifolia* versus nongrafted watermelon. Davis et al. (2008) reported that grafting watermelon could increase lycopene and total carotenoids by 20%. Proietti et al. (2008) noted a 40% increase in lycopene content in a miniwatermelon grafted onto a *C. moschata* × *C. maxima* rootstock. In our study, we found an 11%–27% increase in total lycopene content in some graft combinations when compared to control fruits. However, the β-carotene content of fruits from plants grafted on local bottle gourd rootstocks was lower or similar to control fruits, except the CT/01-17 graft combination, which resulted in a 12% increase in β-carotene content. Contrary to our results, Bruton et al. (2009) reported less than 5% of increase in lycopene content due to grafting and suggested that environment may provoke a greater influence on lycopene than grafting, since the lycopene content of watermelon exhibited both field and year effects.

Perkins-Veazie et al. (2006) reported that the total carotenoid content varied from 37 to 87 μg g^{-1} among red-fleshed seeded diploid watermelon cultivars. Lycopene contributed 84%–97% of total carotenoids and ranged from 35 to 76 μg g^{-1} in those cultivars. In watermelon germplasm, lycopene varies from 36 to 120 μg g^{-1} of fresh weight (Perkins-Veazie et al. 2001; Leskovar et al. 2004; Perkins-Veazie et al. 2006). Perkins-Veazie et al. (2006) reported *cis*-lycopene was 2%–18% of the total lycopene and the remainder was all-*trans*-lycopene in red-fleshed seeded diploid watermelon cultivars. β-Carotene made up 2%–11% of total carotenoids in those cultivars. In our study, *cis*-lycopene was 3%–5% of the total lycopene and β-carotene was 1%–2% of total carotenoids. Perkins-Veazie and Collins (2006) reported that β-carotene, *cis*-lycopene, all-*trans*-lycopene, total lycopene, and total carotenoid contents of some red-fleshed watermelon cultivars were in the ranges of 0.3–1.0 μg g^{-1}, 4.4–5.8 μg g^{-1}, 26.9–51.3 μg g^{-1}, 32.6–55.7 μg g^{-1}, and 33.4–58.1 μg g^{-1}, respectively. Our values obtained from CT/rootstock combinations were within the ranges of previous studies.

All carotenoids were positively correlated. In agreement with results reported by Perkins-Veazie et al. (2006), CT/rootstock combinations with more total lycopene also contain more β-carotene. TSS and pH were significantly correlated with all of the carotenoids, as reported previously by Perkins-Veazie and Collins (2006). A correlation between soluble solids and lycopene has been also found in tomato (Young et al. 1993) and papaya (Zhou and Paull 2001) fruits. In our study, juice pH and carotenoid content were positively correlated with sucrose content, but were negatively correlated with fructose and glucose concentration. Similarly, in melon genotypes, Stepansky et al. (1999) observed a relation between fruit pH and sucrose level, in which the high sucrose genotypes had high pH values and acidic genotypes had low sucrose. Burger et al. (2003) confirmed that high sucrose levels were associated with low acid levels in the mature melon fruit flesh. Perkins-Veazie (2007) suggested that increased sugar content may enhance carotenoid biosynthesis in watermelon fruits. Sucrose was previously shown to promote a color break of citrus fruit epicarp (Iglesias et al. 2001). Telef et al. (2006) showed that sucrose availability favored lycopene biosynthesis in tomato fruit pericarp disks, and its effect on carotenoid accumulation was stronger than that of hexoses (glucose and fructose).

Significant relationships were observed between content of total sugar and individual sugars, but sucrose was negatively correlated with fructose or glucose content. In high-sucrose–accumulating watermelon cultivars, the sharp increase in sucrose level was accompanied by reductions in fructose and glucose, resulting in a level of total sugars that was comparable to that in fruits that accumulate only low amounts of sucrose (Yativ et al. 2010). Stepansky et al. (1999) also reported that the high sugar concentration was attributed to the accumulation of sucrose, while the contributions of glucose and fructose to the fruit sugar content were much lower. In general, CT/rootstock combinations with higher sucrose content could indicate low acidity and high lycopene content, while the combinations with high reducing sugars and acidity could be related to a lower carotenoid content.

The PC1 component represented juice pH and lycopene, total carotenoid and sucrose, malic acid, fructose, glucose, and TA contents, while *cis*-lycopene was explained by the PC2 component and TSS and citric acid content by the PC3 component. Cluster analysis confirmed the biplot of PCA and indicated that the grouping was not correlated with the geographical origin of the bottle gourd genotypes collected, as reported by Yetişir et al. (2008), who conducted a study that included the genotypes of our study. The authors reported that although there were some groupings based on the provinces, overall, distinct and apparent geographical patterns were not detected for 182 bottle gourd genotypes. They suggested that the provinces of Turkey are artificial geographical areas and may not necessarily represent distinct ecological regions. For example, the neighboring provinces of Adana and Osmaniye are small and similar in ecological conditions. Antalya and Mersin are also neighbors with much larger land areas. The sampling of 2 genotypes from east of Antalya and west of Mersin could be closer to each another than 2 locations within Antalya or Mersin. Given that Turkey is not the center of origin, it is possible that the bottle gourds have been introduced to Turkey from multiple locations. Finally, they observed that bottle gourd genotypes have been well mixed in the Mediterranean region. Cluster 1 grouped the CT/07-06 and CT/09-01 combinations. These CT/rootstock combinations were characterized by higher juice pH and lycopene, total carotenoid, and sucrose contents. Cluster 2 grouped the CT/20-02, CT/20-06, CT/Macis, and CT/Argentario combinations, which were rich in malic acid, fructose, and glucose and had higher TA. PCA has been usefully employed for fruit characterization and germplasm evaluation of different fruit species to study relationships among cultivars/genotypes and correlations among fruit traits within each species. According to our study, PCA may help to select CT/rootstock combinations with better fruit quality performances, which might be indicated in CT/09-01 and CT/07-06. The data showed that scion/rootstock combinations have significant effects on the fruit quality parameters of grafted watermelon. Therefore, appropriate scion/rootstock combinations should be chosen for satisfying quality parameters in grafted watermelon production.

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