Fruit sugar profile and antioxidants of peach and nectarine cultivars on almond x peach hybrid rootstocks

C Font i Forcada, Y Gogorcena and MA Moreno*

Dpto. de Pomología. Estación Experimental de Aula Dei (CSIC). Apdo. 13034, 50080 Zaragoza, Spain

***Corresponding author. Tel: +34 976 71 61 36. Fax: +34 976 71 61 45; E-mail address: mmoreno@eead.csic.es

Abstract

Individual and total sugars in fruit flesh, as well as total phenolics, flavonoids, anthocyanins, vitamin C and antioxidant capacity contents were studied on peach (*Prunus persica* cv. 'Tebana') and nectarine (*P. persica* cv. 'Queen Giant') cultivars budded on five almond x peach hybrid rootstocks (Adafuel, Adarcias, Felinem, Garnem and GF 677) and one *P. davidiana* x peach hybrid (Cadaman) grown under field conditions. Individual and total sugars were, in general, the highest on Adarcias rootstock in both peach and nectarine cultivars. The highest content of phenolics, flavonoids, anthocyanins, vitamin C and RAC was found on Adarcias, Garnem and GF 677 rootstock for 'Queen Giant' cultivar and on Cadaman (phenolics, flavonoids and RAC) and GF 677 (anthocyanins) rootstocks for 'Tebana' cultivar. Significant correlations were found among individual sugar contents, as well as between tree vigour and fruit sucrose, glucose and fructose, and between some phytochemical parameters, such as flavonoid and phenolic content. Thus, the less vigorous rootstocks, Adarcias and Cadaman, seem to induce the highest fruit quality, showing higher contents of individual and total sugars. Significant differences were found between cultivars and rootstocks for the different fruit quality traits evaluated. Selecting the right combination of the rootstock and cultivar is important for optimizing chemical characteristics of fruit, especially sugar profile, important quality parameter of peach and nectarine.

Keywords *Prunus persica*, sugars, vitamin C, antioxidant capacity, HPLC

1. Introduction

Peach [*Prunus persica* (L.) Batsch] is the second most important temperate fruit tree grown in the European Union, after apple. Peach production comes mainly from China, Mediterranean area (Italy and Spain) and United States (FAOSTAT, 2012). Breeding programs are active in the release of new rootstocks and scions, improving peach adaptability to soil (Felipe, 2009; Moreno et al., 1994) and fruit quality (Byrne et al., 1991; Monet and Bassi, 2008). Peach fruit quality is mainly determined by the genotype, although other factors such as rootstock, position of the fruit in the canopy, pruning and thinning practices, and yearly climate are known to influence fruit quality. According to previously published studies (Albás et al., 2004; Giorgi et al., 2005; Massai and Loretti, 2004; Orazem et al., 2011; Remorini et al., 2008), the rootstock influences tree vigour, yield and fruit quality parameters like soluble solids content, specific sugars, acidity, organic acids, and several antioxidant compounds although this effect is better known for agronomic and basic parameters.

In recent years, strong attention to fruit quality has been reported because it presents multiple health benefits (Prior et al., 1998). Sugars are very important parameters for market quality of peaches because they are appreciated by consumers (Subedi and

Walsh, 2009). The most abundant sugar in ripe peaches is sucrose, followed by the reducing sugars (glucose and fructose) and lower contents of sorbitol (Cantín et al., 2009a; Brooks et al., 1993). Ripe peaches are characterized by high sucrose, between 50-75% of the total sugar content, contrary to unripe peaches where sucrose content is lower (Byrne et al., 1991; Moriguchi et al., 1990). Fructose is one of the most chemically reactive of the natural sugars and sorbitol is known to play a significant role in the translocation of photosynthates (Moriguchi et al., 1990). Fructose has been shown to be sweeter than sucrose by as much as between 1.75-1.8 times (Wu et al., 2003), while glucose is reported to be less sweet than sucrose (Yamaguchi et al., 1970). Sucrose and fructose have beneficial effects on gastrointestinal health (Muir et al., 2009). In the field of human nutrition, there is also increasing interest in fruits that are rich in sorbitol, since this sugar alcohol is more beneficial than others with regard to diet control, dental health and to avoid gastrointestinal problems. Also, sorbitol can be used as a glucose substitute for diabetics and as an alternative natural sweetener to sucrose (Forni et al., 1992).

The development of some degenerative illnesses is caused by free radicals present in the human organism which cause oxidative damage to lipids or proteins. Therefore, the antioxidant compounds are capable to neutralize these free radicals, and prevent diseases such as cancer (García-Alonso et al., 2004). Eating fruits and vegetables, also reduces inflammation and blood pressure (Wang and Lin, 2000). The antioxidant activity of peach fruit is dependent on genotype, rootstock or climatic conditions such as temperature (Cantín et al., 2009b). Total antioxidant capacity (Prior and Cao, 2000) and phenolic compounds play an important role in food quality, and they can also contribute to aroma or flavour. Anthocyanins give specific coloration to fruits and vegetables and also have health benefits in the prevention of chronic diseases, such as cardiovascular disease and certain types of cancer (Ruíz et al., 2005).

In summary, a key to the commercial expansion of peach production is the promotion and maintenance of the highest possible fruit quality standards and to understand the role of rootstocks on the sugar profile and other phytochemicals substances. Until recently, this effect has been more studied in cultivars than in rootstocks and little is known about the effect of rootstock on sugar profile and phytochemical composition.

The present work aimed to evaluate the effect of different peach-almond hybrid rootstocks on sugar content and phytochemical fruit quality of peaches and nectarines when grown under typical Mediterranean conditions.

2. Materials and methods

2.1. Plant material

Five almond x peach hybrids [*Prunus amygdalus* Batsch x *P. persica* (L.) Batsch] and one *P. davidiana* x peach hybrid [*Prunus davidiana* (Carrière) Franch x *P. persica* (L.) Batsch] rootstocks were evaluated in this study. The six rootstocks were budded with 'Tebana' peach and 'Queen Giant' nectarine cultivars during the summer of 1997, and trees were established in a trial during the winter of 1998-1999. Rootstocks chosen for this study were Adafuel (Cambra, 1990) and Adarcias (Moreno et al., 1994; Moreno and Cambra, 1994) as selections from the Experimental Station of Aula Dei (CSIC); Garnem and Felinem (Felipe, 2009) as selections from the Centre of Research and Agro-food Technology of Aragón (CITA); Cadaman (Edin and Garcin, 1994) as a French-Hungarian release; and GF 677 (Bernhard and Grasselly, 1981) rootstock used as the standard, since it is the most widespread rootstock in the Mediterranean peach-growing area. The effect of almond x peach rootstocks on fruit sugar content and phytochemical constituents of peach cultivars was studied for three years (2008, 2009 and 2010) to estimate the seasonal effect on fruit quality.

2.2. Field trial

The experimental plot was located in the Ebro Valley at the Experimental Station of Aula Dei (CSIC-Zaragoza, Northeast, Spain), on a heavy and calcareous soil, with 27% total calcium carbonate, 8% active lime, water pH 8.3, and a clay-loam texture. Trees were trained to a low density open-vase system (6 × 5 m). Cultural management practices, such as fertilization, winter pruning, and spring thinning, were conducted as in a commercial orchard. Open vase trees were pruned to strengthen existing scaffold branches and eliminate vigorous shoots, inside and outside the vase, that would compete with selected scaffolds or shade fruiting wood. Moderate-sized fruiting wood (0.3-0.6 m long) was selected. Trees were hand-thinned at 45-50 days after full bloom (DAFB) leaving approximately 20 cm between fruits. The plot was level-basin irrigated every 12 days during the summer. Guard rows were used to preclude edge effects. The experiment was established in a randomized block design (five blocks) with one single-tree replication for each scion-stock combination and block.

2.3. Growth and yield determinations

Trunk girths were measured during the dormant season 20 cm above the graft union, and the trunk cross-sectional area (TCSA) was calculated. At harvest, all fruits from each tree were counted and weighted to determine total yield per tree (kg/tree) and mean fruit weight. Cumulative yield per tree and yield efficiency (cumulative yield in kilograms per tree/TCSA) of each scion-stock combination were computed from the harvest data.

2.4. Sampling

During harvest, 20 mature fruits per year of each tree were randomly selected for three consecutive years. Fruits were peeled, and a portion of the mesocarp was removed from the middle of each opposite side and cut into small pieces. A composite sample for each rootstock of 5 g was built by mixing all pieces from all the selected fruits. It was frozen in liquid nitrogen and kept at -20ºC until analysed. Samples for vitamin C determination were kept at -20ºC in metaphosphoric solution (5% HPO_3) until analysis for preservation of oxidation. For analysis of sugar content, samples were homogenized with 10 mL of extraction solution consisting of 800 mL/L ethanol/Milli-Q water. The mixture was centrifuged at 20,000 g for 20 min at 4ºC. For analysis of antioxidant compounds, samples were homogenized using an Ultra-Turrax homogenizer (IKA Works, Inc., Wilmington) with 10 mL of extraction solution consisting of 0.5 N HCl in methanol/Mili-Q water (80% v/v). Extracts were centrifuged at 20,000 g for 20 min at 4ºC, and the supernatant was collected and stored at -20ºC. Finally, to determine vitamin C, samples were homogenized with 5% HPO_3 and then centrifuged at 20,000 g for 15 min at 4ºC, and the supernatant stored at -20ºC.

2.5. Evaluation of basic fruit quality traits

Fruit weight was calculated from the total number of fruits and the total yield per tree, as previously reported (Font i Forcada et al., 2012). Soluble solids content (SSC) of fruit juice was measured with a digital refractometer (Atago PR-101, Tokyo, Japan). The titratable acidity (TA) of samples was determined using an automatic titrator

(Metrohm Ion analysis, 807 Dosing Unit, Switzerland) and a ripening index was calculated based on the SSC/TA. Flesh firmness measurements were performed by a hand penetrometer with an 8 mm flat probe. Values of L* (brightness or lightness), a* (-a* = greenness, +a* = redness), b* (-b* = blueness, +b* = yellowness), C* (chroma) and H (lightness's angle) were measured using a colorimeter (Chroma Meter, CR-400 Konica Minolta, Japan).

2.6 Determination of sugars

For the analysis, 250 µL of the homogenized extract was incubated at 80 °C for 20 min in 200 µL of 800 mL/L ethanol/water, with 5 g/L manitol added as an internal standard. Samples were purified using ion exchange resins (Bio-Rad Barcelona, Spain) as reported by Moing et al. (1992). Samples were then vacuum concentrated and then resuspended to 1 mL of Milli-Q water, before HPLC analysis. The most important sugars found in fruit flesh were sucrose, glucose, fructose and sorbitol. They were analyzed by High Performance Liquid Chromatography (Bio-Rad, Barcelona, Spain), using a 300x7.8 mm column (Aminex® HPX-87C, CA, USA) and a refractive index detector (Waters 2410). This method consisted of a pump and manual injection (20 µl injection volume) interfaced to a PC Millenium 3.2 software (Waters) as described by Cantín et al. (2009a). A distilled deionized water solution was used as mobile phase with a flow rate of 0.6 mL/min at 85°C. HPLC peaks were identified using commercial standards of analytical grade (Panreac Quimica SA, Barcelona, Spain). Sugar concentrations were expressed as g per kg of fresh weight (FW).

2.7. Antioxidant compounds analysis

The antioxidant compounds were analysed using a spectrophotometer photodiode array detector DU 800 (Beckman Coulter, Inc., Fullerton, CA) as described by Cantín et al. (2009b). Standard calibration curves were daily prepared. The Folin-Ciocalteau reagent at 0.25 N was used to determine the total phenolic content. Absorbance was measured at 725 nm and the results were expressed as mg of gallic acid (3,4,5-trihydroxy-benzoic acid) equivalents (GAE) per 100 g FW. The flavonoid content absorbance was measured at 510 nm and the results were expressed as mg of catechin equivalents per 100 g of FW. For determining anthocyanin content, spectrophotometric readings at 535 nm were taken subtracting absorbance at 700 nm (due to turbidity). Anthocyanins were expressed as mg of cyanidin-3-glucoside equivalents (C3GE) per kg of FW using a molecular weight of 494 and a molar extinction absorptivity coefficient $\varepsilon = 25{,}965$/cm M. The relative antioxidant capacity (RAC) was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH). Absorbance was measured at 515 nm and the results were expressed as µg of Trolox equivalents per g of FW. For vitamin C determinations, absorbance was determined at 525 nm and the results were expressed as mg of ascorbic acid (AsA) per 100 g of FW.

2.8. Data analysis

The means from five replicates were analyzed statistically using SPSS 19.0 (SPSS, Inc, Chicago, USA). Data were analyzed to determine the significance of differences between rootstocks using two-way variance (ANOVA) analysis. When the F test was significant, means were separated by Duncan's multiple range ($P \leq 0.05$). In addition, the analyses of bilateral Pearson correlation were carried out to conclude relationships between parameters. Principal components analysis (PCA) was also used to study correlations among agronomic, fruit quality, sugar content and phytochemical constituents, to interpret relationships between rootstocks and to detect clustering formation and establish relationships between rootstocks and fruit quality traits. A 2D PCA plot was designed using combined data from the three years of the study.

3. Results

3.1. Influence of environmental factors on sugar and phytochemical compounds

Results for ‘Tebana’ include only Adafuel, Adarcias, Cadaman and GF 677 rootstocks, due to the high mortality of trees on Felinem and Garnem (Zarrouk et al., 2005). Table 1 shows factors affecting fruit quality parameters in both cultivars. ANOVA results showed that rootstock influenced all traits except sorbitol and RAC (relative antioxidant capacity) for ‘Queen Giant’, and sucrose and flavonoids for ‘Tebana’. Year exerted a significant effect on all traits.

The phenolic content in 2008 for both cultivars was, in general, higher than in the following two years (2009 and 2010), probably because ripening index was higher in 2008. A positive and significant correlation between ripening index and phenolic content was found in 2008 (r = 0.58; $P \leq 0.05$). This positive correlation was also reported by Remorini et al. (2008). In contrast, the contents of anthocyanins, vitamin C and RAC were generally higher in 2009 than in 2008 and 2010, for both cultivars. No significant interaction between rootstock and year was found, except for the content of flavonoids, vitamin C and RAC of ‘Queen Giant’.

3.2. Content on sugar profile

Regarding sucrose levels for the ‘Queen Giant’ cultivar, in average, the highest and lowest values were induced by Adarcias and Garnem respectively, while no significant differences were found with Adafuel, Cadaman, Felinem, and GF 677 (Table 2). Results obtained for 'Tebana' followed the same tendency as for 'Queen Giant'. In detail, we can observe that, although no significant differences were found in the sucrose content for 'Tebana', Adarcias and Cadaman induced the highest values in 2008, as well as Adarcias in 2009 and Cadaman in 2010, and GF 677 the lowest in all years. Among rootstocks, values of sucrose ranged from 36.0 (Garnem, in 2009) to 59.9 (Adarcias, in 2008) g kg^{-1} FW for 'Queen Giant' and from 60.8 (GF 677, in 2009) to 79.1 (Adarcias and Cadaman, in 2008) g kg^{-1} FW for 'Tebana' cultivar.

For glucose content in 'Queen Giant', Adarcias and Cadaman showed the highest values and Garnem and GF 677 induced the lowest, while no significant differences were found with Adafuel and Felinem rootstocks. In 'Tebana', no significant differences were found among rootstocks. Among them, values for glucose ranged from 6.98 (Garnem, in 2009) to 14.6 (Adarcias, in 2008) g kg^{-1} FW for 'Queen Giant' and from 5.9 (Adafuel, in 2009) to 13.6 (Adarcias, in 2008) g kg^{-1} FW for 'Tebana' cultivar.

For fructose content in 'Queen Giant', Adarcias again had the highest average value, although no significant differences were found with Cadaman and Felinem, while GF 677 induced the lowest value with the latter not differing from Adafuel and Garnem. In 'Tebana', Adarcias had the highest content in 2008, 2009 and for the average values although no significant differences were found with Cadaman and GF 677. Among rootstocks, values of fructose ranged from 9.6 (Garnem, in 2009) to 13.4 (Adarcias, in 2008) g kg^{-1} FW for 'Queen Giant' and from 7.9 (Adafuel, in 2010) to 13.9 (Adarcias, in 2008) g kg^{-1} FW for 'Tebana' cultivar.

For sorbitol content in 'Queen Giant', no significant differences were found among rootstocks, in spite of the tendency of Adarcias to show a higher value. In 'Tebana', Adarcias and Cadaman induced higher values than Adafuel and GF 677, both in 2008 and in the average values for the three years. Among rootstocks, values of sorbitol ranged from 1.6 (GF 677, in 2008) to 3.2 (Cadaman, in 2010) g kg^{-1} FW for 'Tebana' cultivar.

Finally, for total sugars and for 'Queen Giant', Adarcias induced the highest average value while Garnem showed the lowest one, although no significant differences were found with Adafuel, Cadaman, Felinem and GF 677. In 'Tebana', Adarcias and Cadaman also induced higher average values than Adafuel and GF 677. Among rootstocks, values of total sugars ranged from 53.9 (Garnem, in 2009) to 90.0 (Adarcias, in 2008) g kg^{-1} FW for 'Queen Giant' and from 78.6 (GF 677, in 2010) to 109.6 (Adarcias, in 2008) g kg^{-1} FW for 'Tebana' cultivar.

Summarizing, Adarcias rootstock induced the highest values of individual and total sugars for both cultivars, while Garnem showed the lowest ones for 'Queen Giant' and GF 677 for 'Tebana'. Thus, for 'Queen Giant', Adarcias induced a 21%, 28% and 8% increase in total sugars compared to Garnem, in 2008, 2009 and 2010, respectively. Regarding 'Tebana', Adarcias rootstock showed a 15%, 12% and 3% increase in total sugars compared to GF 677 in 2008, 2009 and 2010, respectively.

3.3. Content on phytochemical constituents

For 'Queen Giant', Adarcias and Garnem rootstocks showed higher values of phenolic content than Cadaman, Felinem and GF 677, while no significant differences were found among them and Adafuel (Table 3). In the case of 'Tebana', Cadaman induced the largest amount of phenolics compared to Adafuel, Adarcias and GF 677. Among rootstocks, values of phenolics ranged from 18.0 (Cadaman, in 2010) to 36.6 (Garnem, in 2008) mg GAE/100 g FW for 'Queen Giant', and from 26.9 (GF 677, in 2008) to 38.2 (Cadaman, in 2008) mg GAE/100 g FW for 'Tebana'.

Regarding flavonoid content for ‘Queen Giant’, the average of the three years showed that Adarcias induced the highest content, although no significant differences were found with Adafuel, Felinem and Garnem. The lowest value was induced by GF 677 rootstock but no significant differences were observed with Cadaman. In contrast, for ‘Tebana’, Cadaman induced the highest value for two years as well as for the average of the three years (Table 3). Among rootstocks, values of flavonoids ranged from 2.7 (GF 677, in 2009) to 13.2 (Garnem, in 2008) mg CE/100g FW for ‘Queen Giant’ and from 2.6 (Adafuel, in 2010) to 7.6 (Cadaman, in average) mg CE/100g FW for ‘Tebana’.

Fruit anthocyanin content for ‘Queen Giant’ was higher on Adarcias, but no significant differences were found with Adafuel, Garnem and GF 677 rootstocks. The lowest value was induced by Felinem but it did not differ significantly from Cadaman. For ‘Tebana’, GF 677 and Adafuel induced the highest and lowest values respectively, although they did not differ from Adarcias and Cadaman. Among rootstocks, values of anthocyanins ranged from 0.10 (Cadaman, in 2008) to 0.35 (Adarcias, in 2010) mg C3GE/kg FW for ‘Queen Giant’, and from 0.13 (Adarcias, in 2008) to 0.66 (GF 677, 2009) mg C3GE/kg FW for ‘Tebana’.

Significant differences were also found among rootstocks in the fruit vitamin C content, although it was more evident in the case of ‘Queen Giant’. For this cultivar, GF 677 induced higher value than the rest of rootstocks except for Felinem. For ‘Tebana’, the highest vitamin C content was shown on Adarcias for two years, but no significant differences were found among rootstocks when averaged for the three years. Among rootstocks, values of vitamin C ranged from 2.5 (Adafuel and Cadaman, in 2008) to 6.0 (GF 677, in 2009) mg AsA/100 g FW for ‘Queen Giant’ and from 2.5 (Cadaman, in 2008) to 12.1 (Adarcias, in 2009) mg AsA/100 g FW for ‘Tebana’.

Concerning RAC, for ‘Queen Giant’, Garnem induced the highest value in 2008, although no significant differences were found with Adafuel, Adarcias and Felinem. In 2009, GF 677 induced the highest value but it did no differ from Cadaman. No significant differences were found in the average values for the three years. Regarding ‘Tebana’, Cadaman induced the highest content. Among rootstocks, values of RAC ranged from 306.4 (Felinem, in 2008) to 466.5 (GF 677, in 2009) µg Trolox/g FW for ‘Queen Giant’ and from 299.7 (GF 677, in 2008) to 405.7 (Cadaman, in 2009) µg Trolox/g FW for ‘Tebana’.

3.4. Phenotypic correlations between sugar content, phytochemical components and other important quality traits

Total sugar content was positively and highly correlated with all individual sugars and SSC for both cultivars. Similarly, correlation values between total sugars and glucose or fructose were higher than between total sugars and sorbitol. Also, significant correlation values among sucrose, glucose and fructose were higher than values between these sugars and sorbitol. Also, the correlations found between SSC and individual and total sugars; and between SSC and fruit weight (FW), were significant for both cultivars. Glucose and fructose sugars were also slightly correlated with FW in the case of ‘Tebana’. In contrast, a significant negative correlation between sugar components and yield was found, especially in the case of ‘Queen Giant’, and between SSC and tree vigour (TCSA) for this cultivar.

Also, significant and negative correlations were observed between TCSA of ‘Queen Giant’ and fruit content in sucrose (r = -0.58; $P \leq 0.01$), glucose (r = -0.45; $P \leq 0.01$), fructose (r = -0.51; $P \leq 0.01$) and total sugars (r = -0.44; $P \leq 0.01$) in 2008. Similarly, for 'Tebana', a significant and negative correlation was also found between TCSA and fructose (*r* = -0.62; $P \leq 0.05$) in 2008.

On the other hand, sugar components were significantly correlated with TA and RI (SSC/TA). In the case of 'Queen Giant', significant positive correlations were found between glucose or fructose with TA, and for 'Tebana' between glucose and TA. On the contrary, significant negative correlations were found between all individual sugars and fruit firmness, with the exception of sorbitol. However, no significant correlations were found between sugar content and biochemical compounds.

Significant relationships were observed between RAC and total phenolics, flavonoids and vitamin C for both cultivars (Table 4). However, no significant correlation was found between RAC and anthocyanins. Also, positive correlations between FW and vitamin C, between SSC and phenolic content or flavonoids for 'Queen Giant', and between vitamin C and TA, SSC and phenolic content, flavonoids and RAC in the case of 'Tebana' were found.

3.5. Principal components analysis

A four component model accounted for more than 75% of total variance, with the first three components, PC1, PC2 and PC3, explaining 39.1%, 25.3% and 11% of total variance, respectively (Table 5). The distribution of individuals based on the PC1, PC2 and PC3 shows their phenotypic variation and how widely dispersed they are along axes (Fig. 1 and 2).

The PC1 mainly contributes to fruit weight, SSC, TA, RI, sucrose, total sugars, phenolics, flavonoids, anthocyanins and vitamin C. The PC2 explains TCSA, yield, cumulative yield, yield efficiency, glucose and fructose.

An examination of PC1 loadings suggested that rootstock/scion combinations in the negative side had, in general, higher values for glucose and fructose and other fruit quality traits, such as TA, firmness and fruit weight than rootstock/scion combinations on the positive side. Rootstock/scion combinations on the negative side of the PC2 loadings suggested higher values for sorbitol, SSC and total sugars than cultivars on the positive side.

The rootstocks for ‘Queen Giant’ are on the left-hand side of the PCA and the rootstocks for ‘Tebana’ are on the right-hand side of the (Fig. 2). This is probably due to the average values for glucose and fructose (left-hand side of the PCA, Fig. 1), higher on ‘Queen Giant’, and the average values for sucrose, sorbitol, total sugars, phenolics, flavonoids, anthocyanins and vitamin C (right-hand side of the PCA, according to figure 1), higher on ‘Tebana’. These results confirmed the higher contents for individual trees on the negative side of PC1 such as 6, 7, 8, 9 and 10 (Adarcias rootstock); 11, 12 and 13 (Cadaman rootstock); or 22, 26 and 27 (GF 677 rootstock) that were all budded with ‘Queen Giant’ and had higher values for glucose and fructose and other fruit quality traits (TA, firmness and fruit weight). Individuals on the positive side of PC1 such as 32, 33, 34, 35 and 36 (Adarcias rootstock) were budded with ‘Tebana’ and had higher values for sorbitol, SSC and total sugars. Also, the individuals 28, 38, 39 and 40 (Cadaman rootstock); and 42, 43 and 45 (GF 677 rootstock), all with ‘Tebana’ as the scion cultivar, had higher values on anthocyanins, phenolic content and RAC.

3.6. Discussion

Twelve years after budding, growing conditions generated varying levels of tree mortality, the highest with Felinem and Garnem rootstocks (Font i Forcada et al., 2012). In these growing conditions, tree mortality could be attributed to the sensitivity of almond × peach hybrid rootstocks to root asphyxia because these rootstocks are better adapted to well drained soils (Felipe, 2009) or susceptibility to various root rot pathogens such as *Phytophtora spp*. (Zarrouk et al., 2005). For Felinem and Garnem rootstocks, 100% of trees died for ‘Tebana’ cultivar (Font i Forcada et al., 2012). The ‘Tebana’ plot situation was established closer to the irrigation canal than the plot with ‘Queen Giant’, and it was likely more prone to flooding.

The statistical analysis showed the significant effect of the rootstocks on all quality traits, except for sorbitol and RAC content for ‘Queen Giant’ cultivar and for sucrose and flavonoids content for ‘Tebana’ cultivar. It has been shown that levels of individual sugars in peach fruit differ among rootstocks (Albás et al., 2004; Orazem et al., 2011), which agree with our results. Also, the analysis showed the significant effect of the year for all quality traits, except for phenolics in ‘Tebana’ cultivar. The year-to-year variation in fruit quality parameters may be explained by the differences in annual temperatures and crop load over the 3 years of study. However, no interaction was found between rootstock and year, except for flavonoids, vitamin C and RAC content of ‘Queen Giant’. Thus, some biochemical traits could be more influenced by the environmental conditions over the growing season than other traits, in agreement with different studies (Brooks et al., 1993; Bureau et al., 2009). Serrano et al. (2005) reported that the location, year or climate had a significant effect on the anthocyanin and flavonoid content in sweet cherry. Similarly, Tomás-Barberán et al. (2001) showed that temperature had a marked effect on anthocyanin production in apples or plums. Therefore, the chemical composition of sugar and other biochemical compounds of peach and nectarines are significantly affected by rootstocks as well as by other factors, such as climate, harvest conditions and scion genotype. All these parameters may have significant roles in determining fruit quality such as nutraceutical composition and bioactivity of the organic compounds involved.

Our study has shown that sucrose is the sugar present at the highest concentration, followed by glucose, fructose and sorbitol, as previously reported for different peach and nectarine cultivars in other studies (Abidi et al., 2011; Cantín et al., 2009a; Robertson et al., 1990). Also, rootstock influenced the levels of sucrose, glucose, fructose, sorbitol and total sugars in fruit for both cultivars. It has been shown that Adarcias and Cadaman rootstocks produce higher values of individual and total sugars. As previously reported, levels of individual and total sugar content in peach fruit differed among rootstocks (Albás et al., 2004; Orazem et al., 2011) and peach cultivars (Abidi et al., 2011; Cantín et al., 2009a).

Regarding phytochemical components, the same tendency was found as for sugar content, showing that rootstock influenced the levels of antioxidants in fruit for 'Queen Giant' and 'Tebana' cultivars. Phenolic compounds, flavonoids, vitamin C and anthocyanins contents could be increased or lowered with the selection of a certain rootstock as reported by other authors (Tomás-Barberán et al., 2001) and with different peach cultivars. In general, we note that, Adarcias rootstock induce higher values for these compounds, although some rootstocks did not differ significantly between them. Among rootstocks, values of RAC ranged from 299.7 to 466.5 µg Trolox/g FW. This content of RAC agrees with the values reported by different authors (Abidi et al., 2011; Cantín et al., 2009b; Remorini et al., 2008).

The positive correlation observed between total sugar content with all individual sugars and SSC has been previously reported (Cantín et al., 2009a; Drogoudi et al., 2008). The highest values of sugars were shown between total sugars and sucrose for both cultivars (Wu et al., 2003) probably because sucrose is the major sugar in peach flesh. Similarly, the correlations found between SSC and individual and total sugars were significant for both cultivars, as reported in peaches and nectarines (Cantín et al., 2009a; Wu et al., 2003) and in apricots (Drogoudi et al., 2008). Also, a significant and positive correlation between SSC and fruit weight (FW) is probably due because the rate of fruit growth is determined by the amount of carbohydrates (Morandi, 2008). All individual sugars showed a significant positive correlation with RI with highest values for sucrose, as previously reported by other authors (Cantín et al., 2009a; Wu et al., 2003). However, no significant correlations were observed between total sugar content and several biochemical compounds. In contrast, Abidi et al. (2011) reported a positive and significant correlation between total sugars and total phenolics, vitamin C and RAC. Pirie and Mullins (1977) also found a positive correlation between sugar content in berries and levels of phenolic contents, probably due to the role of sugars in the regulation of phenolic biosynthesis.

On the other hand, the negative correlations found between TCSA and SSC or sugar contents can be due to a stronger sink competition of vegetative development in more vigorous rootstocks compared to fruit as shown by Morandi (2008). Also, the negative correlation between vigour and SSC and the positive correlation between SSC and sugars, confirm that less vigorous rootstocks like Adarcias and Cadaman have the possibility to induce sweeter fruits. Peach fruits from the less vigorous trees also had the highest SSC and sugar contents in the study of Giorgi et al. (2005) probably because dwarfing rootstocks are generally able to send more nutrients to the fruit because there is less competition from the vegetative parts (Chalmers et al., 1981).

The significant and positive relationships observed between RAC and total phenolics, flavonoids and vitamin C for both cultivars were also found in peaches and nectarines by Gil et al. (2002) and in other fruit species such as apples (Lata et al., 2007), cherries (Serrano et al., 2005) and plums (Gil et al., 2002). Gardner et al. (2000) also showed the contribution of vitamin C to the antioxidant capacity of different fruit juices, such as orange, apple and pineapple. These results showed that phenolic acids, flavonoid compounds and vitamin C are the main source of the antioxidant capacity in fruits (Cevallos-Casals et al., 2006; Gil et al., 2002). Positive correlations between FW and vitamin C, between SSC and phenolic content or flavonoids for ‘Queen Giant’, and between SSC and phenolic content, flavonoids and RAC in the case of ‘Tebana’ were found to be in agreement with other studies in peach (Cantín et al., 2009b), apricot (Bureau et al., 2009) and sweet cherry (Serrano et al., 2005). The positive correlation between vitamin C and TA for ‘Tebana’ may be due to the contribution of ascorbic acid to the fruit acidity according to Cantín et al. (2009b). These correlations showed a tendency of bigger and sweeter fruits to have higher levels of these bioactive compounds. The relationship of fruit weight with bioactive compounds could be explained by the well-known influence of the sink size (i.e., fruit weight) on the ability to attract photosynthates from the plant sources, because a sufficient accumulation of sugars in or near the fruit is essential for phenolic compounds synthesis during fruit growth (DeJong, 1999).

In summary, Adarcias and Cadaman rootstocks seem to induce, in general, higher fruit sweetness based on individual and total sugars, for both cultivars. For the other biochemical compounds, Adarcias also induced higher values on phenolics, flavonoids and anthocyanins for ‘Queen Giant’ and on vitamin C for ‘Tebana’. Cadaman induced higher values on phenolics, flavonoids and RAC for ‘Tebana’, and GF 677 induced higher values on vitamin C and RAC for ‘Queen Giant’ and on anthocyanins for ‘Tebana’. This would have a crucial impact on the quality of peach fruit. The chemical characteristics of peach fruit could be greatly affected by selecting the right combination of the rootstock and cultivar, and it should become a more important parameter to be considered in new plantings.

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Figure captions

Fig. 1. Principal components analysis of the 19 agronomic and fruit quality traits evaluated on different rootstocks budded with 'Queen Giant' nectarine and 'Tebana' peach cultivars. Symbols: (♦) agronomical traits, (●) basic fruit quality traits, (▲) sugars and (■) phytochemical compounds.

Fig. 2. Principal components analysis of the 46 individuals evaluated on different rootstocks budded with 'Queen Giant' nectarine (left side) with the symbols: (○) Adafuel, (Δ) Adarcias, (◊) Cadaman, (◊) Felinem, (ж) Garnem and (□) GF 677 rootstocks and 'Tebana' peach (right side) with the symbols (●) Adafuel, (▲) Adarcias, (♦) Cadaman and (■) GF 677 rootstocks.

Tables

Table 1

ANOVA results for the effect of rootstock and year on fruit quality traits in ‘Queen Giant’ and ‘Tebana’ cultivars for the average of the 3 years of study

Cultivar	Source of variation[1]	Sucrose	Glucose	Fructose	Sorbitol	Total Sugars	Phenolics	Flavonoids	Anthocyanins	Vitamin C	RAC
‘Queen Giant’	Rootstock (R)	**	***	***	ns	**	*	***	**	**	ns
	Year (Y)	***	***	***	**	**	***	***	**	***	***
	RxY	ns	ns	ns	ns	ns	ns	***	ns	*	**
‘Tebana’	Rootstock (R)	ns	*	***	***	*	**	ns	***	***	**
	Year (Y)	***	***	***	*	**	ns	***	***	***	*
	RxY	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

[1]Data were evaluated by two-way ANOVA; ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; ns, not significant. Abbreviations: RAC, relative antioxidant capacity.

Table 2

Mean values of individual and total soluble sugars of ‘Queen Giant’ and ‘Tebana’ budded on different rootstocks, in the tenth (2008), eleventh (2009) and twelfth (2010) year after grafting

Cultivar	Character	Rootstock	2008	2009	2010	Average
‘Queen Giant’	Sucrose	Adafuel	53.45 ab	43.01 ab	44.72 a	47.06 ab
		Adarcias	59.91 b	46.23 b	49.43 b	51.86 b
		Cadaman	52.35 ab	40.85 ab	43.71 a	45.64 ab
		Felinem	52.94 ab	42.84 ab	45.19 a	46.99 ab
		Garnem	48.78 a	36.02 a	44.76 a	43.19 a
		GF 677	54.54 ab	46.84 b	45.12 a	48.83 ab
	Glucose	Adafuel	12.86 ab	7.84 ab	10.01 ab	10.24 ab
		Adarcias	14.58 c	9.38 c	10.87 ab	11.61 b
		Cadaman	13.48 bc	8.71 b	11.13 b	11.11 b
		Felinem	13.38 bc	7.52 ab	11.22 b	10.71 ab
		Garnem	11.67 ab	6.98 a	10.52 ab	9.72 a
		GF 677	10.68 a	8.73 b	9.80 a	9.74 a
	Fructose	Adafuel	12.50 ab	10.7 ab	10.49 a	11.23 ab
		Adarcias	13.40 b	11.98 b	11.34 a	12.24 c
		Cadaman	13.30 b	10.62 ab	11.28 a	11.73 bc
		Felinem	13.25 b	10.89 ab	11.44 a	11.86 bc
		Garnem	12.03 ab	9.59 a	10.79 a	10.80 ab
		GF 677	10.98 a	9.79 a	10.02 a	10.26 a
	Sorbitol	Adafuel	1.41 a	1.84 a	1.73 a	1.66 a
		Adarcias	2.05 a	1.76 a	2.21 a	2.01 a
		Cadaman	1.74 a	1.51 a	2.13 a	1.79 a
		Felinem	1.34 a	1.66 a	1.90 a	1.63 a
		Garnem	1.61 a	1.33 a	2.27 a	1.74 a
		GF 677	1.41 a	1.79 a	2.14 a	1.78 a
	Total Sugars	Adafuel	80.21 ab	63.41 ab	67.05 a	70.22 ab
		Adarcias	89.95 b	69.36 b	73.86 b	77.72 b
		Cadaman	80.87 ab	62.18 ab	68.25 a	70.43 ab
		Felinem	80.91 ab	62.90 ab	69.76 a	71.19 ab
		Garnem	74.09 a	53.91 a	68.34 a	65.45 a
		GF 677	77.61 a	67.15 b	67.09 a	70.62 ab
‘Tebana’	Sucrose	Adafuel	72.82 a	63.78 a	62.19 a	66.26 a
		Adarcias	79.13 b	67.01 b	63.76 a	69.97 a
		Cadaman	79.10 b	64.40 a	66.10 b	69.87 a
		GF 677	71.36 a	60.81 a	61.20 a	64.46 a
	Glucose	Adafuel	9.87 a	5.95 a	6.67 a	7.50 a
		Adarcias	13.61 a	7.64 a	6.82 a	9.36 a
		Cadaman	12.06 a	7.80 a	7.53 a	9.13 a
		GF 677	10.99 a	7.43 a	6.84 a	8.42 a
	Fructose	Adafuel	9.95 a	9.10 a	7.86 a	8.97 a
		Adarcias	13.88 b	10.84 b	8.08 a	10.93 b
		Cadaman	12.41 ab	9.73 a	8.81 a	10.32 ab
		GF 677	11.11 a	8.85 a	8.02 a	9.33 ab
	Sorbitol	Adafuel	1.72 a	1.63 a	2.31 a	1.89 a
		Adarcias	2.96 b	2.58 b	2.61 a	2.72 b
		Cadaman	2.80 b	2.32 ab	3.21 a	2.78 b
		GF 677	1.59 a	1.80 ab	2.57 a	1.99 a
	Total Sugars	Adafuel	94.36 a	80.51 a	79.05 a	84.64 a
		Adarcias	109.58 a	88.10 b	81.27 a	92.98 b
		Cadaman	105.86 a	83.87 a	85.65 a	91.79 b
		GF 677	95.05 a	78.90 a	78.56 a	84.17 a

For each year, means followed by the same letter in each column are not significantly different at $P \leq 0.05$ according to Duncan’s Multiple Range Test. All individual sugars and total sugars are expressed as g kg $^{-1}$ FW.

Table 3

Effect of rootstock on total phenolics, flavonoids, anthocyanins, vitamin C and antioxidant capacity (RAC) of ‘Queen Giant’ and ‘Tebana’ in the tenth (2008), eleventh (2009) and twelfth (2010) year after budding

Cultivar	Character	Rootstock	2008	2009	2010	Average
‘Queen Giant’	Total phenolics	Adafuel	31.4 ab	21.5 a	20.2 a	24.4 ab
		Adarcias	34.4 ab	24.9 b	21.8 a	27.0 b
		Cadaman	25.5 ab	21.8 a	18.0 a	21.8 a
		Felinem	30.3 ab	23.0 ab	18.5 a	23.9 a
		Garnem	36.6 b	23.0 ab	21.0 a	26.9 b
		GF 677	23.4 a	22.8 a	19.2 a	21.8 a
	Flavonoids	Adafuel	7.6 ab	3.4 ab	3.2 a	4.7 bc
		Adarcias	10.3 bc	4.6 b	4.6 b	6.5 c
		Cadaman	4.5 a	3.4 ab	3.8 ab	3.9 ab
		Felinem	6.5 a	2.7 a	3.3 a	4.2 bc
		Garnem	13.2 c	2.9 a	2.9 a	6.3 bc
		GF 677	5.2 a	2.7 a	3.0 a	3.4 a
	Anthocyanins	Adafuel	0.25 c	0.30 b	0.29 ab	0.28 bc
		Adarcias	0.20 bc	0.33 b	0.35 b	0.29 c
		Cadaman	0.10 a	0.26 ab	0.19 a	0.18 ab
		Felinem	0.14 ab	0.21 a	0.19 a	0.18 a
		Garnem	0.20 bc	0.25 ab	0.26 ab	0.24 bc
		GF 677	0.22 bc	0.21 a	0.19 a	0.21 bc
	Vitamin C	Adafuel	2.5 a	4.2 a	4.0 b	3.6 a
		Adarcias	3.1 a	4.7 ab	3.9 b	3.9 a
		Cadaman	2.5 a	3.8 a	4.0 b	3.4 a
		Felinem	3.4 a	4.6 ab	4.1 a	4.0 ab
		Garnem	3.7 a	3.4 a	3.3 ab	3.5 a
		GF 677	3.0 a	6.0 b	5.1 b	4.7 b
	RAC	Adafuel	370.7 ab	407.6 ab	385.6 a	388.0 a
		Adarcias	390.6 ab	394.4 ab	360.8 a	381.9 a
		Cadaman	380.4 a	427.0 bc	389.2 a	399.0 a
		Felinem	306.4 ab	360.9 a	357.0 a	341.4 a
		Garnem	399.0 b	378.0 ab	358.5 a	378.5 a
		GF 677	310.4 a	466.5 c	384.0 a	387.0 a
‘Tebana’	Total phenolics	Adafuel	31.3 a	27.7 a	23.5 a	27.5 a
		Adarcias	32.9 a	29.3 ab	27.8 ab	30.0 a
		Cadaman	38.2 a	34.8 b	32.7 b	35.2 b
		GF 677	26.9 a	29.7 ab	27.8 ab	28.1 a
	Flavonoids	Adafuel	9.1 a	3.7 a	2.6 a	5.1 a
		Adarcias	10.1 a	4.2 a	3.7 a	6.0 a
		Cadaman	9.6 a	7.6 b	5.6 b	7.6 b
		GF 677	6.1 a	4.8 a	3.9 a	4.9 a
	Anthocyanins	Adafuel	0.19 a	0.36 a	0.33 a	0.30 a
		Adarcias	0.13 a	0.56 b	0.52 ab	0.40 ab
		Cadaman	0.15 a	0.59 b	0.48 ab	0.41 ab
		GF 677	0.24 b	0.66 b	0.59 b	0.50 b
	Vitamin C	Adafuel	2.7 ab	9.9 ab	10.1 a	7.6 a
		Adarcias	3.5 b	12.1 b	11.3 a	9.0 a
		Cadaman	2.5 ab	8.7 a	10.9 a	7.4 a
		GF 677	2.8 a	10.8 ab	10.3 a	8.0 a
	RAC	Adafuel	334.1 a	389.6 ab	350.5 a	365.1 a
		Adarcias	343.7 a	374.0 a	337.4 a	351.7 a
		Cadaman	400.3 b	405.7 b	403.1 a	403.0 b
		GF 677	299.7 a	392.3 ab	399.4 a	363.8 a

For each year, means followed by the same letter in each column are not significantly different at $P \leq 0.05$ according to Duncan’s Multiple Range Test. Units: Total phenolics (mg GAE/100 g FW); flavonoids (mg CE/100g FW); anthocyanins (mg C3GE/kg FW); vitamin C (mg AsA/100 g FW); RAC, relative antioxidant capacity (µg Trolox/g FW).

Table 4

Pearson's correlations coefficients among traits observed over three years in almond x peach hybrid rootstocks budded with 'Queen Giant' and 'Tebana' cultivars

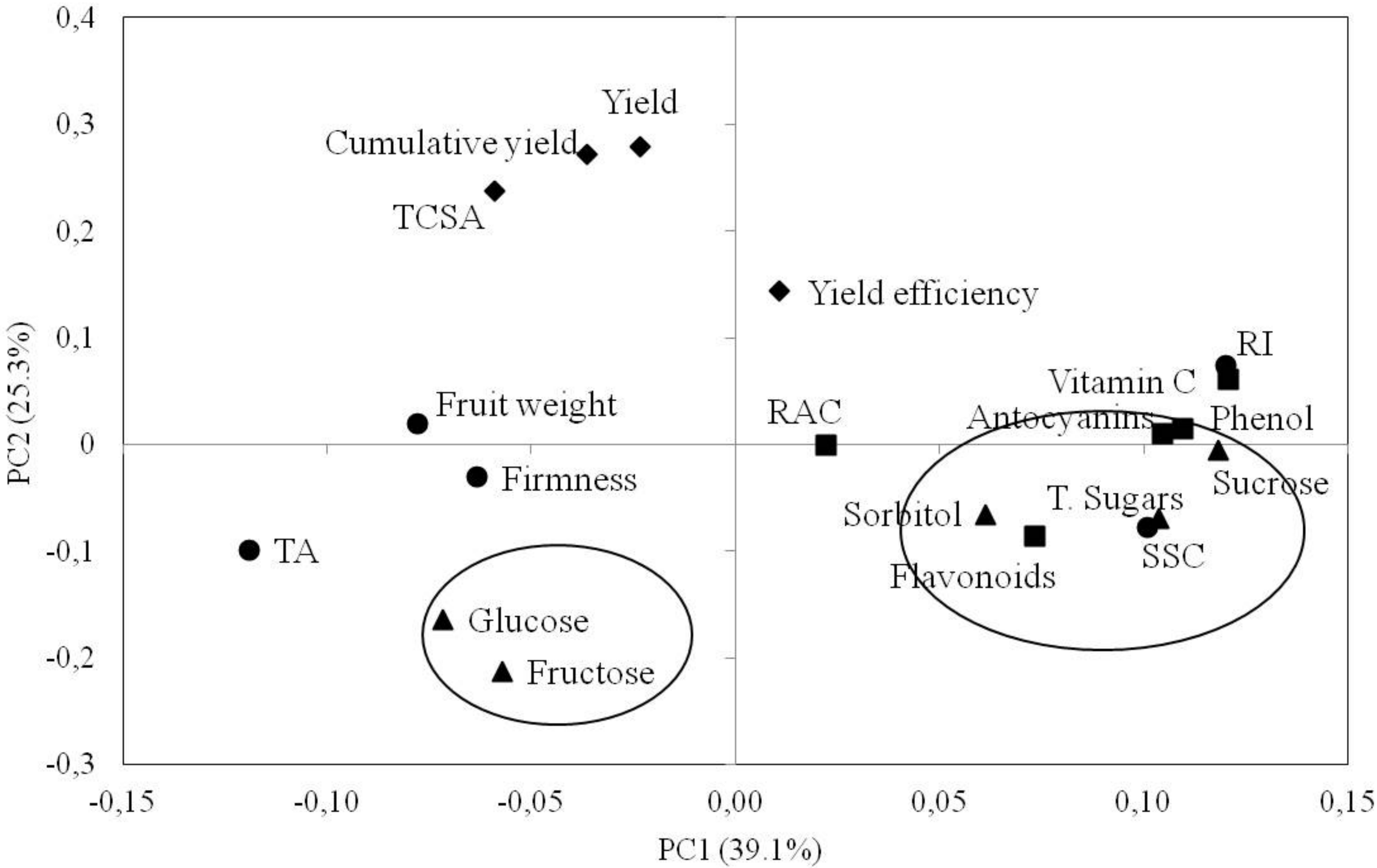
Cultivar	Trait	SSC	Sucrose	Glucose	Fructose	Sorbitol	Total sugars[a]	Phenolics	Flavonoids	Vitamin C	RAC
'Queen Giant'	Yield	-0.48**	-0.58**	-0.59**	-0.63**	ns	-0.63**	ns	ns	ns	ns
	TCSA	-0.49**	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Harvest date	0.42**	ns	ns	ns	ns	ns	0.28*	ns	ns	ns
	Fruit weight	0.39*	ns	ns	ns	ns	ns	ns	ns	0.27*	ns
	SSC		0.46**	0.48**	0.53**	ns	0.52**	0.32*	0.38**	ns	ns
	Titratable acidity		ns	0.29*	0.36**	ns	ns	ns	ns	-0.31*	ns
	Flesh firmness		-0.48**	-0.53**	-0.39**	ns	-0.51**	ns	ns	ns	ns
	Ripening index		0.42*	0.39*	0.40*	0.33*	ns	ns	ns	ns	ns
	Sucrose		-	0.73**	0.69**	0.31**	0.97**	ns	ns	ns	ns
	Glucose			-	0.81**	ns	0.86**	ns	ns	ns	ns
	Fructose				-	ns	0.82**	ns	ns	ns	ns
	Sorbitol					-	0.27*	ns	ns	ns	ns
	Phenolics							-	0.78**	ns	0.68**
	Flavonoids								-	ns	0.68**
	Vitamin C									-	0.36*
'Tebana'	Yield	ns	ns	-0.40*	ns	ns	ns	ns	ns	ns	ns
	TCSA	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Harvest date	0.39**	ns	ns	ns	ns	ns	0.37*	ns	ns	ns
	Fruit weight	0.56*	ns	0.35**	0.38*	ns	ns	ns	ns	ns	ns
	SSC	ns	0.43*	0.37*	0.35*	0.77*	0.48**	0.44*	0.34*	ns	0.47**
	Titratable acidity		ns	0.35*	ns	ns	ns	ns	ns	0.46**	ns
	Flesh firmness		-0.49**	-0.63**	-0.49**	ns	-0.59**	ns	ns	ns	ns
	Ripening index		0.54*	0.42*	0.35*	0.41*	ns	ns	ns	-0.39*	ns
	Sucrose		-	0.58**	0.63**	0.47**	0.95**	ns	ns	ns	ns
	Glucose			-	0.87**	0.44**	0.80**	ns	ns	ns	ns
	Fructose				-	0.49**	0.83**	ns	ns	ns	ns
	Sorbitol					-	0.56**	ns	ns	ns	ns
	Phenolics							-	0.73**	ns	0.67**
	Flavonoids								-	ns	0.60*
	Vitamin C									-	0.43*

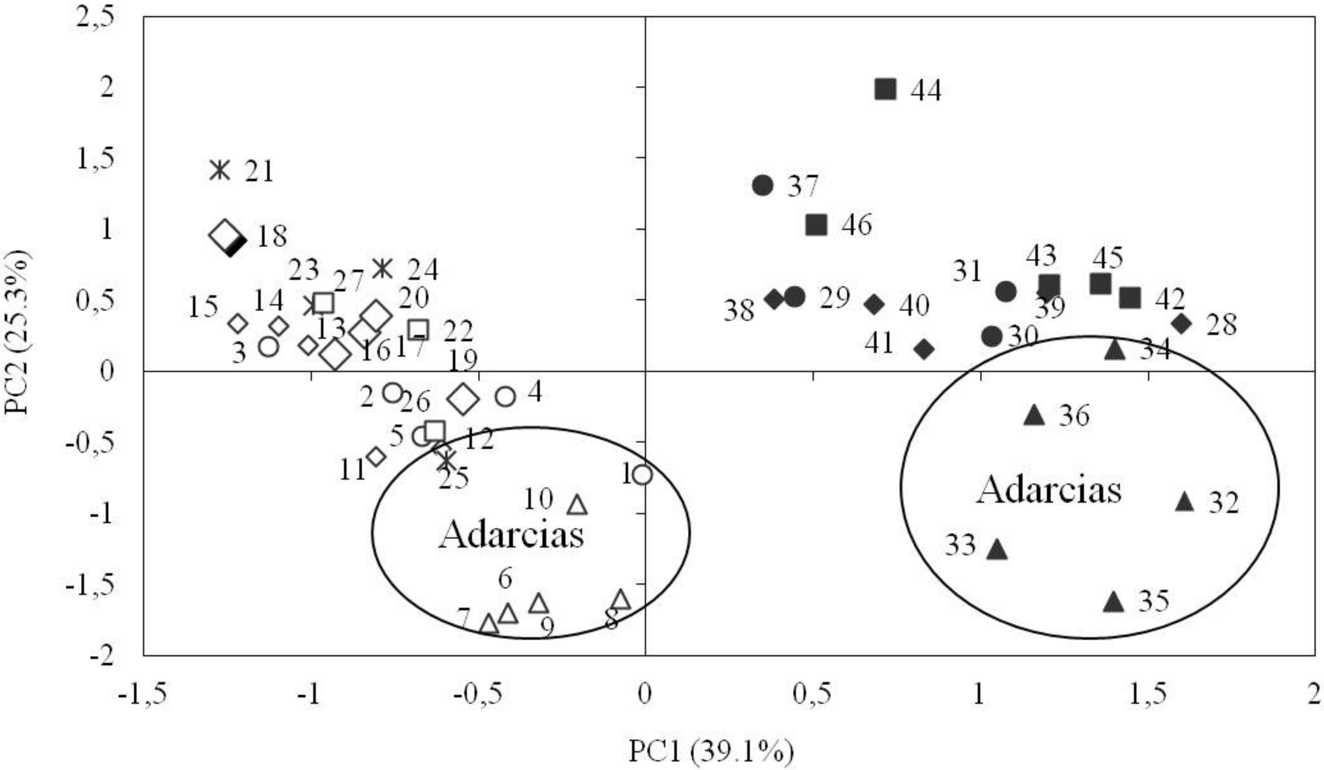
* and ** represent statistical significance at $P \leq 0.05$ and $P \leq 0.01$ respectively; ns, not significant. Abbreviations: TCSA, trunk-cross sectional area; SSC, soluble solids content; Total sugars[a] are the sum of sucrose, glucose, fructose, and sorbitol for each genotype, analyzed by HPLC; RAC, relative antioxidant capacity.

Table 5

Eigenvectors of the two principal component (PC) axes for the 19 agronomic and fruit quality traits evaluated on different rootstocks budded with ‘Tebana’ peach and ‘Queen Giant’ nectarine cultivars

	Component loading	
Traits	PC1 (39.1%)	PC2 (25.3%)
Trunk cross-sectional area	-0.438	0.689
Yield	-0.173	0.811
Cumulative yield	-0.269	0.790
Yield efficiency	0.081	0.418
Fruit weight	-0.577	0.054
Soluble solid content	0.749	-0.228
Flesh firmness	-0.469	-0.090
Titratable acidity	-0.884	-0.288
Ripening index	0.893	0.214
Sucrose	0.880	-0.016
Glucose	-0.532	-0.479
Fructose	-0.423	-0.620
Sorbitol	0.455	-0.192
Total Sugars	0.770	-0.203
Phenolic content	0.814	0.043
Flavonoids	0.546	-0.253
Antocyanins	0.778	0.027
Vitamin C	0.898	0.177
Relative antioxidant capacity	0.166	-0.003


0,4
0,3
0,2
0,1
0
-0,1
-0,2
-0,3
-0,15
-0,10
-0,05
0,00
0,05
0,10
0,15
PC2 (25.3%)
PC1 (39.1%)
Yield
Cumulative yield
TCSA
Yield efficiency
RI
Vitamin C
Fruit weight
RAC
Antocyanins
Phenol
Firmness
Sucrose
T. Sugars
Sorbitol
SSC
Flavonoids
TA
Glucose
Fructose


PC2 (25.3%)
PC1 (39.1%)
Adarcias
Adarcias
2,5
2
1,5
1
0,5
0
-0,5
-1
-1,5
-2
-1,5
-1
-0,5
0
0,5
1
1,5
2