Sugars and organic acids profile and antioxidant compounds of nectarine fruits influenced by different rootstocks

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Abstract

Thirteen *Prunus* rootstocks with different genetic background [five peach-almond hybrids: Adafuel, Adarcias, GF 677, PADAC 9902-01 and Rootpac 70; one *P. persica* x *P. davidiana* hybrid: Cadaman; three hexaploid *P. insititia* plums: Adesoto 101, PM 105 AD, and PM 150 AD; three plum x peach-almond hybrids (PADAC 04-01, PADAC 04-03 and PADAC 99-05), and a plum-almond hybrid (Rootpac R)] were budded with the ‘Big Top’ nectarine and evaluated in typical Mediterranean growing conditions. Biochemical fruit compounds, such as sugars and organic acids profile, and antioxidant compounds were assessed to enhance fruit quality and beneficial effects of ‘Big Top’. For three consecutive years (2014-2016), sugars and organic acids were analysed by high-performance liquid chromatography and phenolic compounds by spectrophotometry. Statistical analyses showed that rootstocks affected strongly fruit phytochemical composition, although it was also influenced by year. Compared with peach-based rootstocks, the hexaploid ‘Pollizo’ plums Adesoto 101 and PM 150 AD, could increase the sweetness of the low-acid ‘Big Top’ nectarine, inducing higher contents of sugars and organic acids. Similarly, beneficial antioxidant effects can be enhanced on diploid plum based hybrids PADAC 04-01, PADAC

99-05 and Rootpac R, inducing higher contents on sugars and bioactive compounds (total phenols, flavonoids and vitamin C) and antioxidant capacities. High significant correlations were found between all studied compounds. New plum based rootstocks seem to induce higher fruit quality based on sugars and organic acids profile, and antioxidants compounds.

Keywords: *Prunus persica*, peach-almond hybrids, plums, fruit quality, total phenolic content, HPLC.

Introduction

Modern consumers are increasingly interested in their personal health, and they expect food not only to be tasty and attractive but also safe and healthy. Therefore, worldwide peach breeding programs are paying strong attention to improve fruit quality and biochemical composition (Byrne et al., 2012). Fruits, and especially peach and nectarine fruits, are excellent functional foods as they are high in antioxidant and nutritional compounds (Cantín et al., 2009a, 2009b). Peach fruit taste depends on its total sugar and acid contents, which has a significant impact on the perception of sweetness and sourness (Colaric et al., 2005), and its phenolic compounds, which confer astringency or bitterness in the fruit (Predieri et al., 2006). The most important fruit biochemical compounds, such as vitamin C, phenolic compounds, and sugar content, play also an important role to minimize the effect of oxidative stress, causing cancer or cardio-vascular diseases, and also helping in the gastrointestinal health (García-Alonso et al., 2004).

‘Big Top’ is considered, nowadays, the mid-season reference nectarine cultivar in Europe for fresh consumption and one of the most appreciated nectarine cultivars grown in Spain (Reig et al., 2016). It has an appreciable keeping quality, because it resembles, at ripening, the stony hard peach in firmness and crispness but melts at a slow speed at full ripening

(Bassi et al., 2008). In particular, ‘Big Top’ fruits retain flesh firmness on the tree for a longer time than typical melting nectarines, allowing full development of red colour and organoleptic qualities because of high sugar levels, juiciness and flavour (Iglesias et al., 2009).

It is well known that peach fruit quality and its biochemical composition mainly depend on the scion genotype (Cantín et al., 2010). However, as reported in several studies, they can also be influenced by rootstock (Orazem et al., 2011; Font i Forcada et al., 2014a; Reig et al., 2016). Rootstocks are an essential component in modern fruit production because of their capability of adapting a particular scion to diverse environmental conditions and cultural practices (Gainza et al., 2015). In fact, the rootstock choice for a specific scion cultivar represents one of the most important considerations for a productive and profitable peach orchard (Jiménez et al., 2011; Orazem et al., 2011).

Currently, Spain is the first peach exporter in the world, and the largest producer in the European Union (Reig et al., 2017), followed by Italy, Greece and France. In Spain, the main peach producing area is the Ebro Valley under typical Mediterranean conditions. In this area, Aragon and Catalonia regions account more than 60% of the total Spanish peach production (MAPAMA, 2018). The compact and calcareous soil growing conditions and the continuously use of the same land, lead to peach producers to deal and suffer some limiting production conditions such as root asphyxia or waterlogging, iron-chlorosis deficiency and replant conditions in Mediterranean countries (Jiménez et al., 2011; Font i Forcada et al., 2014a; Mestre et al., 2015; Ben Yahmed et al., 2016). In order to solve those limitations, which can affect tremendously peach production and fruit quality, breeding programs are very active in releasing new rootstocks to improve peach adaptability to the different types of soil and climatic conditions, with especial focus on improving fruit

quality. The Experimental Station of Aula Dei (CSIC) *Prunus* rootstocks breeding program has released new rootstocks adapted to heavy and calcareous soils typical of the Mediterranean area (Moreno, 2004). Moreover, new *Prunus* rootstocks could be released for peach production with best agronomic and fruit quality performance and tolerance/resistance to different abiotic and biotic stresses (Pinochet et al., 1999; Font i Forcada et al., 2014a; Mestre et al., 2017; Reig et al., 2016).

Very scarce studies with the ‘Big Top’ nectarine grown on different rootstocks have been reported in Europe (Mestre et al., 2015; Reig et al., 2016). In particular, and to the best of our knowledge, none study has reported the sugars and organic acids profile of ‘Big Top’, together with its antioxidant properties, budded on *Prunus* rootstocks with different genetic background. Deeping the knowledge of the main factors to increase beneficial fruit related traits will meet consumer preferences and perceptions.

Therefore, the present work aimed to assess, for the first time, the biochemical compounds (sugars, organic acids, and antioxidant compounds) affected by new released and promising rootstocks, and apply multivariate analysis to identify the best of them to enhance fruit quality attributes of ‘Big Top’.

Materials and methods

Plant material and field trial

The ‘Big Top’ nectarine cultivar was budded on five almond x peach hybrids (*P. amygdalus* x *P. persica*: Adafuel, Adarcias, GF 677, PADAC 9902-01 and Rootpac 70), one *P. persica* x *P. davidiana* hybrid: Cadaman, three hexaploid plums (*P. insititia*: Adesoto 101, PM 105 AD, PM 150 AD), three plum x peach-almond hybrids [*P. cerasifera* x (*P. amygdalus* x *P. persica*): PADAC 04-01, PADAC 04-03 and PADAC 99-05], and one plum x almond hybrid (*P. cerasifera* x *P. amygdalus*: Rootpac R) (Table 1) in the summer of 2007. Among

the thirteen rootstocks used in this study, the interspecific hybrids PADAC 04-01, PADAC 04-03, PADAC 9902-01 and PADAC 99-05, as well as the ‘Pollizo de Murcia’ plum rootstocks PM 105 AD and PM 150 AD, are under process of selection by the Aula Dei Experimental Station.

Trees were established in the field during the winter of 2008-2009. The trial was carried out at the Aula Dei Experimental Station (NE Spain; 41° 43' 42.7" N, 0° 48' 44.1" W) in the Ebro Valley area, on a heavy and calcareous soil and under a cold-semiarid Mediterranean climate (Bsk in the Köppen-Geiger climate classification system). In this area, the average annual precipitation amounts to 345 mm, the diurnal temperature variation amounts to 13.3°C, and the reference evapotranspiration ranges from 997 mm to 1140 mm (Salvador et al., 2011). The experiment was established in a randomized block design with five replicates for each rootstock. Trees were trained to a low-density open-vase system (5 m x 4 m), manual thinned and drip line irrigated. Standard pest and weed control, and fertilization practices were applied. Guard rows were used to preclude edge effects.

The effect of rootstocks on fruit sugars and organic acids content and phytochemical traits was studied for three consecutive years (2014, 2015, and 2016) to estimate the seasonal effect. Meteorological data were recorded by an automated station located very close (≈ 150 m) to the field trial. The average daily temperature (T_m), the average minimum daily temperature (T_{min}), and the average maximum daily temperature (T_{max}) were then calculated for each fruit growing period until harvest date (first fortnight of July).

Fruit sampling

Each year, twenty mature fruits of each tree were randomly selected at commercial harvest. After peeling the fruits, a composite sample of 5 g was built by mixing all pieces from all the selected fruits. The sample was frozen in liquid nitrogen and kept at -20°C until further

analysis. Fruit samples were analysed for the content of individual sugars (glucose, fructose, sucrose and sorbitol) and organic acids (citric, malic, quinic, succinic and shikimic). The samples kept at -20°C were homogenized with 10 mL in methanol/Milli-Q water (80% v/v) using an Ultra-Turrax homogenizer (T25D Ultra-Turrax, IKA Works Inc., Wilmington, NC, USA), then centrifuged at 20,000 g for 30 min at 4°C, and the supernatant was recovered. This hydroalcoholic extract was used for sugars, organic acids, total phenolics, flavonoids, and relative antioxidant capacity assays. In the case of vitamin C samples, 5 mL of metaphosphoric acid solution (5%) were initially added before frozen in liquid nitrogen. Previously to be analyzed, samples were homogenized with 5 mL more of the metaphosphoric solution. Then, these vitamin C extracts were centrifuged at 20,000 g for 30 min at 4°C, and the supernatant was collected.

Sugar and organic acids analysis

For the analysis of sugars and organic acids, 200 µL of the homogenized extract was vacuum concentrated and resuspended to 800 µL of Milli-Q water. The obtained solutions were passed through a 0.22 µm filter to eliminate large particles. Sugars found in fruit flesh were analysed by HPLC (Aminex HPX-87C column, 300 mm x 7.8 mm, Biorad) with a refractive index detector at 35°C (Waters 2410, Waters Corporation, Milford, USA) as previously described (Font i Forcada et al., 2013). Filtered and degassed Milli-Q water was used as mobile phase with a flow rate of 0.6 mL/min at 85°C. Organic acids were also analysed by HPLC (RezexTM ROA-Organic Acid H⁺ (8%) column, 300 mm x 7.8 mm, Phenomenex) and a photodiode array detector (Waters 2489, Milford, USA) at 210 nm. A sulfuric acid solution (0.005 N) was used as mobile phase with a flow rate of 0.5 mL/min at room temperature. The individual sugars and organic acids in nectarine extracts were identified by their retention time characteristics using the adequate standards mentioned in

the chemicals paragraph. Sugars and organic acids concentrations were expressed as g per kg of fresh weight (FW).

Antioxidant compounds analysis

The antioxidant compounds were analyzed using a 96-well microplate spectrophotometer photodiode array detector (Asys UVM 340 microplate reader; Biochrom, Cambridge, UK) as described by Reig et al. (2016). Standard calibration curves were prepared on each microplate using eight different concentrations. For the analysis of the total phenolics content (TPC), the Folin-Ciocalteu reagent (Sigma-Aldrich, Germany) at 0.25 N was used. The method consisted of mixing 10 μ L of the extract diluted in water (1:8) with 80 μ L of Folin-Ciocalteu's reagent. After 3 minutes of reaction, 30 μ L of 1 N sodium carbonate (Na_2CO_3) was added and kept 1 hour in the dark. Absorbance was measured at 725 nm and the results were expressed in mg of gallic acid equivalents (GAE) per 100 g FW. Total flavonoids content was determined using a colorimetric assay based on the method of Zhishen et al. (1999) with some modifications. Fifty μ L of the methanolic extract was diluted with water (1:2), and 15 μ L of 5% NaNO_2 was added. After 5 minutes, 15 μ L of 10% AlCl_3 were added. After 1 minute, 100 μ L of 1 M NaOH were added. Absorbance was measured at 510 nm and the results were expressed as mg of catechin equivalents (CE) per 100 g FW. The relative antioxidant capacity (RAC) was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method adapted from Brand-Williams et al. (1995) with some modifications. Briefly, 10 μ L of the methanolic extract was added to 290 μ L of cold DPPH radical solution (100 μ M in methanol) and mixed in the dark at room temperature. After 10 minutes, absorbance was measured at 515 nm and the results were expressed as mg trolox equivalents per 100 g FW. Finally, vitamin C was determined using the method for the spectrophotometric determination of ascorbic acid as described by Zaharieva and Abadía

(2003) with some modifications. Seventy-five μL of the vitamin C extract was added to 60 μL of H_3PO_4 (1:1), 60 μL of 4% bipyridil in ethanol:water (70:30) and 30 μL of 3% $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. After 60 minutes of incubation at 37°C , absorbance was measured at 525 nm. Vitamin C was expressed as milligrams of ascorbic acid (AsA) per 100 g of FW.

Chemicals

All chemicals were of analytical grade. All the sugars standards (sucrose, glucose, fructose, and sorbitol), aluminium chloride (AlCl_3), 3,4,5 trihydroxybenzoic acid (gallic acid), sodium carbonate (Na_2CO_3) and sodium hydroxide (NaOH) were purchased from PanReac Quimica SA (Barcelona, Spain). All the organic acids standards (citric, malic, quinic, succinic and shikimic), bipyridyl, catequin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), folin-ciocalteau's reagent, phosphoric acid (H_3PO_4), iron (III) chloride (FeCl_3), metaphosphoric acid (HPO_3), sodium nitrite (NaNO_2) and 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (Saint Louis, MO, USA).

Data analysis

Means from five replicates were obtained for each scion-rootstock combination and analysed statistically using IBM SPSS 23.0 (USA). Data were analysed to determine the significance of differences between rootstocks and years (seasons) using two-way variance (ANOVA) analysis. When the F test was significant, means were separated by Duncan's multiple range ($P \leq 0.05$). Pearson correlation was carried out to study the different correlations among sugars, organic acids contents and other phytochemical traits. A principal component analysis (PCA) was performed to simultaneously analyze all the biochemical fruit compounds of 'Big Top' cultivar and to understand how these traits contribute to variability, using the program Unscrambler X 10.3 (CamoASA, 2001). Rootstock genotype means were used in multivariate analysis to generate two-way

similarity cluster diagrams based on genotype similarity and variable similarity, using the program JMP statistical software package (Version 12; SAS Institute Inc., Cary, North Carolina).

Results and Discussion

Rootstock and year effect

Statistical analyses highlighted rootstock and year as main factors affecting metabolite profiles and fruit characteristics. The ANOVA statistical analysis showed the significant effect of the rootstock on all traits evaluated, except for fructose (Table 2). Thus, the levels of sugars, organic acids and biochemical compounds in ‘Big Top’ fruits differed among rootstocks as demonstrated in other peach studies (Orazem et al., 2011; Font i Forcada et al., 2013; Reig et al., 2016). The analysis also showed the significant effect of the year for all traits studied. This year effect could be explained by the annual temperatures and crop load differences observed during the three years of study, as previously reported (Serrano et al., 2005; Font i Forcada et al., 2013; Reig et al., 2016). Finally, statistically significant rootstock x year interaction was only observed for sucrose, sorbitol, citric acid and total phenolics content. This could indicate that rootstock and year had consistent effects on these traits.

The average daily temperature (T_m), the average minimum daily temperature (T_{min}), and the average maximum daily temperature (T_{max}) as well as the solar radiation (S_r) were higher, in general, in the fruit growing season of 2015 (T_m 17°C, T_{min} 10°C, T_{max} 25°C, and S_r 272 W m⁻²), compared to 2014 (T_m 17°C, T_{min} 9°C, T_{max} 24°C, and S_r 268 W m⁻²) and 2016 (T_m 15°C, T_{min} 9°C, T_{max} 22°C, and S_r 257 W m⁻²). Significant ($P \leq 0.01$) and positive correlations (data not shown) between sucrose, sorbitol, total sugars, and the ratio of total sugars/total acids (TS/TA) with T_m , T_{min} , and T_{max} average temperatures were

found. Also, citric and succinic + shikimic acids and total acids were significantly ($P \leq 0.01$) but negatively correlated (data not shown) with those environmental temperature factors. The complex quantitative nature of sugars, organic acids, and antioxidant traits can be affected by environmental conditions and agronomical management (Cirilli et al., 2016; Predieri et al., 2006). Indeed, environmental variables such as temperature, precipitation and/or solar radiation, influence the tree-growing environment providing wide variations in bioactive compounds accumulation (Cirilli et al., 2016; Abdelghafar et al., 2018).

Sugars profile

The sugar profile plays an important role in peach and nectarine flavour quality (Esti et al., 1997). In fact, the sugar content is one of the most important quality traits perceived by consumers (Cirilli et al., 2016). In terms of individual sugars, the sucrose values of ‘Big Top’ ranged from 51.5 g kg⁻¹ FW (Adafuel, in 2016) to 86.7 g kg⁻¹ FW (PADAC 99-05, in 2016) (Table 3). Among rootstocks, the plum x peach-almond hybrid PADAC 04-01 induced the highest sucrose values for the average of the three years, although not significant differences were found with other plum based rootstock (PADAC 99-05, PM 150 AD, and Rootpac R). For glucose content, the values ranged from 6.1 g kg⁻¹ FW (Adafuel, in 2016) to 11.2 g kg⁻¹ FW (PM 105 AD, in 2015) (Table 3). The ‘Pollizo’ plum PM 105 AD and the plum-almond hybrid Rootpac R induced the highest glucose values for the average of the three years, whereas the peach-almond hybrid Adafuel induced the lowest values, although they did not differ from the rest of the rootstocks. Regarding fructose content, values ranged from 7.2 (PADAC 99-05, in 2016) to 13.5 (Adesoto 101, in 2015) g kg⁻¹ FW. However, no significant rootstock effect on the three years average fructose content was observed in ‘Big Top’ fruits. For sorbitol content, the values ranged from 1.5 (PM 105 AD, in 2014) to 7.9 (Adesoto 101, in 2016) g kg⁻¹ FW. The plum x

peach-almond hybrids, PADAC 04-01 and PADAC 99-05, as well as the plum-almond hybrid Rootpac R had the highest sorbitol values, and Adafuel the lowest, although they did not differ significantly from the rest of the rootstocks. Finally, the values for total sugars ranged from 68.3 (Adafuel, in 2016) to 109.3 (PM 150 AD, in 2016) g kg⁻¹ FW. It is interesting to note that the three way interspecific hybrid PADAC 04-01 induced the highest values, although not significant differences were found with the hexaploid ‘Pollizo’ plums Adesoto 101 and PM 150 AD, as well as with the plum-almond hybrid Rootpac R. Sugars values were included in the range reported in the literature for peach and nectarine cultivars (Tomás-Barberán et al., 2001; Cantín et al., 2009a; Reig et al., 2013; Font i Forcada et al., 2014a, 2014b). As expected, sucrose showed the highest values followed by fructose, glucose and sorbitol, as previously reported (Reig et al., 2013; Font i Forcada et al., 2014b). In spite of the lower content of sorbitol, Colaric et al. (2005) reported this sugar as the attribute most related to peach aroma and taste. In addition, sorbitol content is more beneficial than other sugars with regard to diet control, and to avoid gastrointestinal problems (Forni et al., 1992). Consequently, the diploid plum based hybrids PADAC 04-01, PADAC 99-05, and Rootpac R could have an additional value making ‘Big Top’ nectarine more attractive for consumers.

Organic acids profile

In peaches and nectarines, taste also depends on organic acids (Cirilli et al., 2016). In the present work, malic, citric, quinic, and succinic + shikimic acids were quantified by HPLC (Table 4). The malic acid values ranged among rootstocks and years, ranging from 2.13 (PADAC 04-03, in 2014) to 4.79 (PM 105 AD, in 2015) g kg⁻¹ FW. The plum PM 105 AD rootstock induced the highest value of malic acid, although not significant differences were found with the other two hexaploid plums Adesoto 101 and PM 150 AD, and the peach x

265 *P. davidiana* Cadaman. The lowest contents of malic acid were induced by Adafuel and
 266 PADAC 04-03, although not significant differences were found with PADAC 04-01. With
 267 regards to citric acid, the values ranged from 0.82 (PADAC 04-01 and PADAC 9902-01,
 268 in 2015) to 3.08 (PM 105 AD, in 2014) g kg⁻¹ FW. Comparing rootstocks, the same
 269 tendency as malic acid was found for the citric acid. Again, the highest value was induced
 270 by the hexaploid plum PM 105 AD, although not significant differences were found with
 271 the peach-almond hybrid GF 677. Regarding quinic acid, the values ranged from 0.81
 272 (Adafuel, in 2016) to 1.78 (PM 150 AD, in 2016) g kg⁻¹ FW. The ‘Pollizo’ plums Adesoto
 273 101 and PM 150 AD induced the highest values, although they did not differ significantly
 274 of PADAC 04-01 and PM 105 AD. For the sum of succinic and shikimic, the values ranged
 275 from 0.49 (PADAC 99-05, in 2016) to 0.98 (PM 105 AD, in 2014) g kg⁻¹ FW. The
 276 maximum values were induced by the plum PM 105 AD, although not significant
 277 differences were found with the plums Adesoto 101 and PM 150 AD, as well as with the
 278 peach-almond hybrids GF 677 and Rootpac 70. Finally, the sum of the total organic acids
 279 values ranged from 5.34 (Adafuel, in 2014) to 8.32 (PM 105 AD, in 2014) g kg⁻¹ FW.
 280 Again, the maximum average value was induced by the ‘Pollizo’ plum PM 105 AD,
 281 although not significant differences were found with the other two ‘Pollizo’ plums (Adesoto
 282 101 and PM 150 AD). Values of the individual organic acids seem to be within the range
 283 reported by other authors (Wu et al., 2005; Reig et al., 2013). The predominant organic acid
 284 was malic acid, in agreement with different studies (Wang et al., 1993; Serrano et al., 2005;
 285 Reig et al., 2013), followed by citric and quinic (Byrne et al., 1991; Wang et al., 1993), and
 286 traces of succinic and shikimic (Wu et al., 2005). However, Reig et al. (2013) reported acid
 287 quinic was the second and citric the third most abundant organic acid in peaches and
 288 nectarines.

289 *Antioxidant compounds*

290 The total phenolic content varied greatly among rootstocks and years ranging from 8.7
291 (Adafuel, in 2015) to 22.3 (PM 150 AD, in 2016) mg GAE/100 g FW. In the average for
292 the three years of study, the highest values were induced by the three way interspecific
293 hybrid PADAC 04-01, although not significant differences were found with the three
294 ‘Pollizo’ plums, the peach-almond hybrid Adarcias, and the plum based hybrids. For
295 flavonoids content, the values ranged from 2.9 (Adafuel, in 2015) to 20.2 (PM 150 AD, in
296 2016) mg CE/100 g FW. Concerning the average for the three years, the ‘Pollizo’ plum PM
297 150 AD seems to induce the highest content, although not significant differences were
298 found with the ‘Pollizo’ plum Adesoto 101 and the three plum based hybrids (PADAC 04-
299 01, PADAC 04-03, and PADAC 99-05). Regarding vitamin C, values ranged from 5.3
300 (Adafuel, in 2016) to 12.5 (PADAC 04-01, in 2016) mg AsA/100 g FW. For the average
301 of the three years, Adarcias and PADAC 04-01 induced the highest vitamin C contents,
302 while the peach-almond hybrid PADAC 9902-01 induced the lowest content, although
303 differences were not significant with the rest of the rootstocks. Finally, RAC values ranged
304 greatly from 37.1 (Adafuel, in 2015) to 226.0 (PADAC 99-05, in 2016) µg trolox/g FW.
305 The highest average value was induced by the ‘Pollizo’ plum PM 150 AD, although it did
306 not differ significantly from Adarcias, Adesoto 101, Cadaman, and the plum based
307 interspecific hybrids. Values for each biochemical trait above mentioned are within the
308 range reported for peaches and nectarines in the literature (Remorini et al., 2008; Orazem
309 et al., 2011; Reig et al., 2013; Font i Forcada et al., 2014b). Nevertheless, ‘Big Top’ cultivar
310 has been characterized by low antioxidant capacity (Font i Forcada et al., 2014b; Drogoudi
311 et al., 2016).

The sugar profile, organic acids and antioxidant traits of ‘Big Top’ cultivar have been significantly affected by rootstocks according to their predominant genetic background. Thus, different rootstocks can affect fruit quality, indicating that vigor and yield are not the only parameters influenced by rootstocks (Giorgi et al., 2005; Orazem et al., 2011). ‘Big Top’ is a low acid cultivar with a medium to high sugar content. Consequently, the higher content of organic acids, as well as sugars, induced by the ‘Pollizo’ plums (Adesoto 101, PM 105 AD, and PM 150 AD) will increase greatly the sweetness sensation of the non-acid ‘Big Top’ nectarine fruit. Several agronomical studies with different peach cultivars (Font i Forcada et al., 2014a; Reig et al., 2016; Mestre et al., 2017), showed also that the ‘Pollizo’ plums Adesoto 101 and PM 105 AD and the plum-almond Rootpac R were well adapted rootstocks to heavy-calcareous soil conditions, and now conferring a good fruit quality based on sugar profile and other biochemical traits. On the other hand, it is interesting to note that the plum x peach-almond hybrids, and especially the PADAC 04-01 rootstock seem to induce higher contents on sucrose, total sugars, total phenolics, and vitamin C. Similarly, PADAC 04-01 also induced the best fruit quality to the flat peach cultivar ‘Subirana’ compared to the rest of the evaluated rootstocks (Ben Yahmed et al., 2016), confirming its suitability for the Mediterranean area.

Phenotypic correlations, cluster and principal component analysis (PCA)

Significant correlations were found between sugars, organic acids and antioxidant compounds (Table 6). As expected, sucrose was positively correlated with sorbitol and total sugars (TS) content; glucose was also positively correlated with fructose, as well as sorbitol with TS content. Previous studies, in peaches and nectarines, reported similar positive correlations (Font i Forcada et al., 2013, 2014a). Regarding organic acids, a significant negative correlation was found between citric acid and malic acid in agreement with Reig

et al. (2013). Citric and malic acid were also significantly and positively correlated with quinic acid as previously reported (Esti et al., 1997; Wu et al., 2003). As expected, phenolic content was positively correlated with flavonoids and RAC, and flavonoids content was also positively correlated with RAC values. Flavonoids are an important group of phenolic compounds in peaches and nectarines (Gil et al., 2002) and both contribute significantly to the antioxidant capacity of fruits (Gil et al., 2002; Cevallos-Casals et al., 2006). Furthermore, significant and medium positive correlations were also observed between sugars and other biochemical traits. Sucrose, sorbitol, and TS contents were positively correlated with total phenolics, flavonoids, vitamin C, RAC, and the ratio TS/TA as previously reported in peaches and nectarines, in berries (Pirie et al., 1977), and in apples (Wu et al., 2007), probably due to the role of sugars in the regulation of phenolics biosynthesis (Colaric et al., 2005).

A principal component analysis (PCA) was performed to understand how biochemical traits contribute to variability among the different rootstocks budded with ‘Big Top’ nectarine cultivar (Figure 1). The first two PCs (PC1 and PC2) accounted for 48% and 28% respectively of the total variability. The PC1 mainly contributes to glucose, fructose, malic, quinic, and the sum of succinic and shikimic acids, as well as total phenolics content, flavonoids, and relative antioxidant capacity. The PC2 explains sucrose, sorbitol, total sugars, citric acid, total organic acids, and vitamin C. Rootstocks on the positive side of PC2 corresponding to hexaploid plums and diploid plum based rootstocks induced, in general, higher values of sugars (sucrose and sorbitol) and other biochemical compounds (phenols content, RAC, and vitamin C). In contrast, trees on the negative side of PC2, corresponding to peach-almond hybrids (Adafuel, GF 677, PADAC 9902-01 and Rootpac

70) and peach x *P. davidiana* (Cadaman), showed, in general, lower values for several biochemical compounds.

In addition, a hierarchical agglomerative cluster analysis was used to divide the rootstocks into four groups of increasing dissimilarity (Figure 2). This division showed that the rootstocks grouped according to their genetic origin and based on the content of the biochemical traits analyzed.

Results obtained with the PCA and hierarchical agglomerative cluster confirm that the ‘Pollizo’ plum rootstocks Adesoto 101 and PM 150 AD and the plum based hybrids PADAC 04-01, PADAC 99-05 and Rootpac R, induced higher values on sugar profile (individual and total sugars) and antioxidant compounds of ‘Big Top’ nectarine cultivar, based on their genetic background. This information will be used to develop a better selection protocol for new peach rootstocks in future breeding programs.

Conclusions

This study demonstrates that rootstocks affect strongly fruit composition based on fruit quality and phytochemical composition. Average higher contents of organic acids and sugars induced by hexaploid ‘Pollizo’ plums (Adesoto 101 and PM 150 AD) could increase the sweetness perception of the low-acid ‘Big Top’ nectarine. Some antioxidant compounds could be also increased by these plum rootstocks. Similarly, other interspecific plum based rootstocks as PADAC 04-01, PADAC 99-05 and Rootpac R greatly influences some important biochemical fruit traits, such as sugars and antioxidants content, probably because they have a greater expected plum genetic background (up to 50%). Consequently, this study is also demonstrating the availability of new rootstocks with a good performance on biochemical fruit quality compounds.

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References

Abdelghafar, A., Burrell, R., Reighard, G., Gasic, K., 2018. Antioxidant capacity and bioactive compounds accumulation in peach breeding germplasm. J. Am. Pomol. Soc. 72 (1), 40-69. <https://doi.org/10.17660/ActaHortic.2017.1172.25>.

Bassi, D., Monet, R., 2008. Botany and Taxonomy. In: Layne, D.R. and Bassi, D., Eds., The Peach: Botany, Production and Uses, CAB International, Wallingford, pp. 1-36.

Ben Yahmed, J., Ghrab, M., Moreno, M.A., Pinochet, J., Ben Mimoun, M., 2016. Performance of ‘Subirana’ flat peach cultivar budded on different *Prunus* rootstocks in a warm production area in North Africa. Sci. Hortic. 206, 24-32. <https://doi.org/10.1016/j.scienta.2016.04.031>.

Bernhard, R., Grasselly, C., 1981. Les pêchers × amandiers. L’Arboriculture Fruitière. 328, 37-42.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft und-Technologie (LWT) 28 (1), 25-30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5).

Byrne, D.H., Nikolic, A.N., Burns, E.E., 1991. Variability in sugars, acids, firmness, and color characteristics of 12 peach genotypes. J. Am. Soc. Hortic. Sci. 116 (6), 1004-1006.

CamoASA., 2001. *The Unscrambler 9.6 User Manual*; Camo: Oslo, Norway.

405 Byrne, D.H., Raseira, M.B., Bassi, D., Piagnani, M.C., Gasic, K., Reighard, G.L., Moreno,
 406 M.A., Pérez, S., 2012. The peach. In: Badenes, M.L., Byrne, D.H. (Eds.), Fruit Breeding,
 407 Handbook of Plant Breeding. Springer Science + Business Media, pp. 505-569.
 408 Cantín, C.M., Gogorcena, Y., Moreno, M.A., 2009a. Analysis of phenotypic variation of
 409 sugar profile in different peach and nectarine [*Prunus persica* (L.) Batsch] breeding
 410 progenies. J. Sci. Food Agric. 89 (11), 1909-1917. <https://doi.org/10.1002/jsfa.3672>.
 411 Cantín, C., Moreno, M.A., Gogorcena, Y., 2009b. Evaluation of the antioxidant capacity
 412 phenolic compounds, and vitamin C content of different peach and nectarine [*Prunus*
 413 *persica* (L.) Batsch] breeding progenies. J. Agric. Food Chem. 57 (11), 4586-4592.
 414 <https://doi.org/10.1021/jf900385a>.
 415 Cantín, C.M., Gogorcena, Y., Moreno, M.A. 2010. Phenotypic diversity and relationships
 416 of fruit quality traits in peach and nectarine [*Prunus persica* (L.) Batsch] breeding
 417 progenies. Euphytica 171 (2), 211-226. <https://doi.org/10.1007/s10681-009-0023-4>.
 418 Cevallos-Casals, B.A., Byrne, D., Okie, W.R., Cisneros-Zevallos, L., 2006. Selecting new
 419 peach and plum genotypes rich in phenolic compounds and enhanced functional properties.
 420 Food Chem. 96 (2), 273-280. <https://doi.org/10.1016/j.foodchem.2005.02.032>.
 421 Cirilli, M., Bassi, D., Ciacciulli, A., 2016. Sugars in peach fruit: a breeding perspective.
 422 Hortic. Research. 20 (3), 15067. <https://doi.org/10.1038/hortres.2015.67>.
 423 Colaric, M., Veberic, R., Stampar, F., Hudina, M., 2005. Evaluation of peach and nectarine
 424 fruit quality and correlations between sensory and chemical attributes. J. Sci. Food Agric.
 425 85 (15), 2611-2616. <https://doi.org/10.1002/jsfa.2316>.
 426 Drogoudi, P., Pantelidis, G.E., Goulas, V., Manganaris, G.A., Ziogas, V., Manganaris, A.,
 427 2016. The appraisal of qualitative parameters and antioxidant contents during postharvest

428 peach fruit ripening underlines the genotype significance. *Postharvest Biol. Technol.* 115,
 429 142-150. <https://doi.org/10.1016/j.postharvbio.2015.12.002>.

430 Edin, M., Garcin, A., 1994. Un nouveau porte greffe du pêcher Cadaman-Avimag.
 431 *L'Arboriculture Fruitière* 475, 20-23.

432 Esti, M., Messina, M.C., Sinesio, F., Nicotra, A., Conte, L., La Notte, E., Palleschi G., 1997.
 433 Quality evaluation of peaches and nectarines by electrochemical and multivariate analyses:
 434 Relationships between analytical measurements and sensory attributes. *Food Chem.* 60 (4),
 435 659-666. [https://doi.org/10.1016/S0308-8146\(96\)00306-8](https://doi.org/10.1016/S0308-8146(96)00306-8).

436 Font i Forcada, C., Gogorcena, Y., Moreno, M.A., 2013. Fruit sugar profile and
 437 antioxidants of peach and nectarine cultivars on almond $\times$ peach hybrid rootstocks. *Sci.*
 438 *Hortic.* 164, 563-572. <https://doi.org/10.1016/j.scienta.2013.10.020>.

439 Font i Forcada, C., Gogorcena, Y., Moreno, M.A., 2014a. Agronomical parameters, sugar
 440 profile and antioxidant compounds of 'Catherine' peach cultivar influenced by different
 441 plum rootstocks. *Int. J. Mol. Sci.* 15 (2), 2237-2254. <https://doi.org/10.3390/ijms15022237>.

442 Font i Forcada, C., Gradziel, T.M., Gogorcena, Y., Moreno, M.A., 2014b. Phenotypic
 443 diversity among local Spanish and foreign peach and nectarine [*Prunus persica* (L.) Batsch]
 444 accessions. *Euphytica* 197 (2), 261-277. <https://doi.org/10.1007/s10681-014-1065-9>.

445 Forni, E., Erba, M.L., Maestrelli, A., Polesello, A., 1992. Sorbitol and free sugar contents
 446 in plums. *Food Chem.* 44 (4), 269-275. [https://doi.org/10.1016/0308-8146\(92\)90049-8](https://doi.org/10.1016/0308-8146(92)90049-8).

447 Gainza, F., Opazo, I., Guajardo, V., Meza, P., Ortiz, M., Pinochet, J., Muñoz C., 2015.
 448 Rootstock breeding in *Prunus* species: Ongoing efforts and new challenges. *Chilean J.*
 449 *Agric. Res.* 75, 6-16. <http://dx.doi.org/10.4067/S0718-58392015000300002>.

450 García-Alonso, M., Pascual-Teresa, S., Santos-Buelga, C., Rivas-Gonzalo, J.C., 2004.
 451 Evaluation of the antioxidant properties of fruits. *Food Chem.* 84 (1), 13-18.
 452 [https://doi.org/10.1016/S0308-8146\(03\)00160-2](https://doi.org/10.1016/S0308-8146(03)00160-2).
 453 Gil, M., Tomás-Barberán, F.A., Hess-Pierce, B., Kader, A.A., 2002. Antioxidant capacities,
 454 phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum
 455 cultivars from California. *J. Agric. Food Chem.* 50 (17), 497-4982.
 456 <https://doi.org/10.1021/jf020136b>.
 457 Giorgi, M., Capocasa, F., Scalzo, J., Murri, G., Battino, M., Mezzetti, B. 2005. The
 458 rootstock effects on plant adaptability, production, fruit quality, and nutrition in the peach
 459 (cv. "Suncrest"). *Sci. Hortic.* 107 (1), 36-42. <https://doi.org/10.1016/j.scienta.2005.06.003>.
 460 Iglesias, I., Echeverría, G., 2009. Differential effect of cultivar and harvest date on nectarine
 461 color: quality and consumer acceptance. *Sci. Hortic.* 120 (1), 41-50.
 462 <https://doi.org/10.1016/j.scienta.2008.09.011>.
 463 Jiménez, S., Pinochet, J., Romero, J., Gogorcena, Y., Moreno, M.A., Espada, J.L., 2011.
 464 Performance of peach and plum based rootstocks of different vigor on a late peach cultivar
 465 in replant and calcareous conditions. *Sci. Hortic.* 129 (1), 58-63.
 466 <https://doi.org/10.1016/j.scienta.2011.03.006>.
 467 MAPAMA, 2018. (Accessed 01 September 2018) <https://www.mapama.gob.es/es/>
 468 Mestre, L., Reig, G., Betrán, J.A., Pinochet, J., Moreno, M.A., 2015. Influence of peach-
 469 almond hybrids and plum-based rootstocks on mineral nutrition and yield characteristics of
 470 'Big Top' nectarine in replant and heavy-calcareous soil conditions. *Sci. Hortic.* 192, 475-
 471 481. <https://doi.org/10.1016/j.scienta.2015.05.020>.
 472 Mestre, L., Reig, G., Betrán, J.A., Moreno, M.A., 2017. Influence of plum rootstocks on
 473 agronomic performance, leaf mineral nutrition and fruit quality of 'Catherina' peach

474 cultivar in heavy-calcareous soil conditions. Span. J. Agric. Res. 15 (1), e0901.
 475 <https://doi.org/10.5424/sjar/2017151-9950>.
 476 Moreno, M.A., Tabuenca, M.C., Cambra, R., 1994. Performance of Adafuel and Adarcias
 477 as peach rootstocks. HortScience 29 (11), 1271-1273.
 478 Moreno, M.A., Tabuenca, M.C., Cambra, R., 1995. Adesoto 101, a plum rootstock for
 479 peaches and other stone fruit. HortScience 30 (6), 1314-1315.
 480 Moreno, M.A., 2004. Breeding and selection of *Prunus* rootstocks at the Aula Dei
 481 Experimental Station, Zaragoza, Spain. Acta Hort. 658, 519-528.
 482 <https://doi.org/10.17660/ActaHortic.2004.658.79>.
 483 Orazem, P., Stampar, F., Hudina, M., 2011. Quality analysis of ‘Redhaven’ peach fruit
 484 grafted on 11 rootstocks of different genetic origin in a replant soil. Food Chem. 124 (4),
 485 1691-1698. <https://doi.org/10.1016/j.foodchem.2010.07.078>.
 486 Pinochet, J., Calvet, C., Hernández-Dorrego, A., Bonet, A., Felipe, A., Moreno, M.A.,
 487 1999. Resistance of peach and plum rootstocks from Spain, France, and Italy to root-knot
 488 nematode *Meloidogyne javanica*. HortScience 34 (7), 1259-1262.
 489 Pinochet, J., 2010. ‘Replantpac’ (Rootpac® R), a plum-almond hybrid rootstock for replant
 490 situations. HortScience 45 (2), 299-301.
 491 Pirie, A., Mullins, M.G., 1977. Interrelationships of sugars, anthocyanins, total phenols and
 492 dry weight in the skin of grape berries during ripening. Am. J. Enol. Vitic. 28 (4), 204-209.
 493 Predieri, S., Ragazzini, P., Rondelli, R., 2006. Sensory evaluation and peach fruit quality.
 494 Acta Hort. 713, 429-434. <https://doi.org/10.17660/ActaHortic.2006.713.63>.
 495 Reig, G., Iglesias, I., Gatus, F., Alegre, S., 2013. Antioxidant capacity, quality, and
 496 anthocyanin and nutrient contents of several peach cultivars [*Prunus persica* (L.) Batsch]

497 grown in Spain. *J. Agric. Food Chem.* 61 (26), 6344-6357.
 498 <https://doi.org/10.1021/jf401183d>.
 499 Reig, G., Mestre, L., Betrán, J.A., Pinochet, J., Moreno, M.A., 2016. Agronomic and
 500 physicochemical fruit properties of ‘Big Top’ nectarine budded on peach and plum based
 501 rootstocks in Mediterranean conditions. *Sci. Hortic.* 210, 85-92.
 502 <https://doi.org/10.1016/j.scienta.2016.06.037>.
 503 Reig, G., Alegre, S., Cantín, C.M., Gatiús, F., Puy, J., Iglesias, I., 2017. Tree ripening and
 504 postharvest firmness loss of eleven commercial nectarine cultivars under Mediterranean
 505 conditions. *Sci. Hortic.* 219, 335-343. <https://doi.org/10.1016/j.scienta.2017.03.001>.
 506 Remorini, D., Tavarini, S., Degl’Innocenti, E., Loreti, F., Massai, R., Guidi, L., 2008. Effect
 507 of rootstock and harvesting time on the nutritional quality of peel and flesh of peach fruits.
 508 *Food Chem.* 110 (2), 361-367. <https://doi.org/10.1016/j.foodchem.2008.02.011>.
 509 Salvador, R., Martínez-Cob, A., Cavero, J., Playán, E., 2011. Seasonal on-farm irrigation
 510 performance in the Ebro basin (Spain): crops and irrigation systems. *Agric. Water Manag.*
 511 98 (4), 577-587. <https://doi.org/10.1016/j.agwat.2010.10.003>.
 512 Serrano, M., Guillén, F., Martínez-Romero, D., Castillo, S., Valero, D., 2005. Chemical
 513 constituents and antioxidant activity of sweet cherry at different ripening stages. *J Agric*
 514 *Food Chem.* 53 (7), 2741-2745. <https://doi.org/10.1021/jf0479160>.
 515 Tomás-Barberán, F.A., Gil, M.I., Cremin, P., Waterhouse, A.L., Hess-Pierce, B., Kader,
 516 A.A., 2001. HPLC-DAD-ESIMS analysis of phenolic compounds in nectarines, peaches,
 517 and plums. *J. Agric. Food Chem.* 49 (10), 4748-4760. <https://doi.org/10.1021/jf0104681>.
 518 Wang, T., Gonzalez, A.R., Gbur, E.E., Aselage, J.M., 1993. Organic acid changes during
 519 ripening of processing peaches. *J. Food Sci.* 58 (3), 631-632.
 520 <https://doi.org/10.1111/j.1365-2621.1993.tb04343.x>.

521 Wu, B.H., Quilot, B., Kervella, J., Génard, M., Li, S.H., 2003. Analysis of genotypic
 522 variation in sugar and acid contents in peaches and nectarines through principle component
 523 analysis. *Euphytica* 132 (3), 375-384. <https://doi.org/10.1023/A:1025089809421>.

524 Wu, B.H., Quilot, B., Génard, M., Kervella, J., Li, S.H., 2005. Changes in sugar and organic
 525 concentrations during maturation in peaches, *P. davidiana* and hybrids as analyzed by
 526 principal component analysis. *Sci. Hortic.* 103 (4), 429-439.
 527 <https://doi.org/10.1016/j.scienta.2004.08.003>.

528 Wu, J., Gao, H., Zhao, L., Liao, X., Chen, F., Wang Z., Xiaosong, H., 2007. Chemical
 529 compositional characterization of some apple cultivars. *Food Chem.* 103 (1), 88-93.
 530 <https://doi.org/10.1016/j.foodchem.2006.07.030>.

531 Zaharieva, T.B., Abadía, J., 2003. Iron deficiency enhances the levels of ascorbate,
 532 glutathione, and related enzymes in sugar beet roots. *Protoplasma* 221 (3-4), 269-275.
 533 <https://doi.org/10.1007/s00709-002-0051-6>.

534 Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid contents
 535 in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* 64 (4), 555-
 536 559. [https://doi.org/10.1016/S0308-8146\(98\)00102-2](https://doi.org/10.1016/S0308-8146(98)00102-2).

Table 1. List of studied rootstocks, description and origin.

Rootstock	Species	Genetic background	Origin ^a	References
Adafuel	<i>P. amygdalus</i> x <i>P. persica</i>	Open-pollinated	CSIC, Spain	Moreno et al. (1994)
Adarcias	<i>P. amygdalus</i> x <i>P. persica</i>	Open-pollinated	CSIC, Spain	Moreno et al. (1994)
Adesoto 101	<i>P. insititia</i>	Open-pollinated	CSIC, Spain	Moreno et al. (1995)
Cadaman	<i>P. persica</i> x <i>P. davidiana</i>	Controlled cross	INRA (France-Hungary)	Edin and Garcin (1994)
GF 677	<i>P. amygdalus</i> x <i>P. persica</i>	Open-pollinated	INRA, France	Bernhard and Grasselly (1981)
PADAC 04-01	<i>P. cerasifera</i> x (<i>P. amygdalus</i> x <i>P. persica</i>)	Controlled cross	CSIC, Spain	Moreno (2004)
PADAC 04-03	<i>P. cerasifera</i> x (<i>P. amygdalus</i> x <i>P. persica</i>)	Controlled cross	CSIC, Spain	Moreno (2004)
PADAC 99-05	<i>P. cerasifera</i> x (<i>P. amygdalus</i> x <i>P. persica</i>)	Controlled cross	CSIC, Spain	Moreno (2004)
PADAC 9902-01	(<i>P. amygdalus</i> x <i>P. persica</i>) x (<i>P. persica</i> x <i>P. davidiana</i>)	Controlled cross	CSIC, Spain	Moreno (2004)
PM 105 AD	<i>P. insititia</i>	Open-pollinated	CSIC, Spain	Moreno (2004)
PM 150 AD	<i>P. insititia</i>	Open-pollinated	CSIC, Spain	Moreno (2004)
Rootpac 70	<i>P. persica</i> x (<i>P. amygdalus</i> x <i>P. persica</i>)	Controlled cross	AI, Spain	Jiménez et al. (2011)
Rootpac R	<i>P. cerasifera</i> x <i>P. amygdalus</i>	Controlled cross	AI, Spain	Pinochet (2010)

^a AI = Agromillora Iberia S.L. private nursery, Spain; CSIC = Consejo Superior de Investigaciones Científicas; INRA = Institut National de la Recherche Agronomique.

Table 2. ANOVA results for the effect of rootstock and year on biochemical traits of ‘Big Top’ cultivar for the average of the 3 years of study.

Source of variation ^a	Units/Description	Rootstock (R)	Year (Y)	R x Y
Sucrose	g kg ⁻¹ FW	***	**	**
Glucose	g kg ⁻¹ FW	*	***	ns
Fructose	g kg ⁻¹ FW	ns	***	ns
Sorbitol	g kg ⁻¹ FW	**	***	*
Total sugars (TS)	g kg ⁻¹ FW	***	**	**
Citric acid	g kg ⁻¹ FW	**	***	**
Malic acid	g kg ⁻¹ FW	***	***	ns
Quinic acid	g kg ⁻¹ FW	***	**	ns
Succinic + Shikimic acid	g kg ⁻¹ FW	***	***	ns
Total acids (TA)	g kg ⁻¹ FW	***	**	ns
Vitamin C	mg AsA/100 g FW	*	*	ns
Total phenolics	mg GAE/100 g FW	***	***	*
Flavonoids	mg CE/100 g FW	***	***	ns
Relative Antioxidant Capacity (RAC)	μg TE/g FW	***	***	ns

^a Data were evaluated by two-way variance (ANOVA); ns, not significant; *P≤0.05; **P≤0.01; ***P≤0.001.

Table 3. Soluble sugars of ‘Big Top’ nectarine on thirteen rootstocks, in the fifth (2014), sixth (2015) and seventh (2016) year after budding.

Character	Rootstock	2014	2015	2016	Average
Sucrose (g kg ⁻¹ FW)	Adafuel	63.9 abcde	58.0 a	51.5 a	57.8 a
	Adarcias	62.8 abcde	61.2 a	70.0 cde	64.6 abc
	Adesoto 101	56.2 abc	62.4 a	70.7 de	63.1 abc
	Cadaman	60.8 abcd	63.0 a	70.0 bcde	64.6 abc
	GF 677	52.5 a	67.1 a	58.4 abc	59.3 ab
	PADAC 04-01	73.7 e	70.1 a	85.3 e	76.4 d
	PADAC 04-03	58.4 abc	67.5 a	65.8 abcd	63.9 abc
	PADAC 99-05	70.6 de	61.6 a	86.7 e	72.9 cd
	PADAC 9902-01	68.1 cde	66.7 a	59.4 abc	64.7 abc
	PM 105 AD	55.9 ab	70.5 a	57.4 abc	61.3 ab
	PM 150 AD	61.8 abcd	61.6 a	83.2 e	68.9 bcd
	Rootpac 70	56.9 abc	63.8 a	56.2 ab	58.9 ab
	Rootpac R	66.3 bcde	69.8 a	70.7 bcde	68.9 bcd
Glucose (g kg ⁻¹ FW)	Adafuel	6.5 a	8.0 a	6.1 a	6.9 a
	Adarcias	8.9 ab	9.0 abc	6.2 a	8.0 ab
	Adesoto 101	10.1 b	9.4 abc	6.2 a	8.6 ab
	Cadaman	9.4 ab	9.5 abc	6.3 a	8.4 ab
	GF 677	8.8 ab	8.4 ab	7.4 ab	8.2 ab
	PADAC 04-01	8.1 ab	9.6 abc	6.9 a	8.2 ab
	PADAC 04-03	8.4 ab	10.4 abc	6.5 a	8.4 ab
	PADAC 99-05	9.0 ab	10.2 abc	6.6 a	8.6 ab
	PADAC 9902-01	7.9 ab	9.5 abc	6.4 a	7.9 ab
	PM 105 AD	9.7 b	11.2 c	6.4 a	9.1 b
	PM 150 AD	8.9 ab	8.3 a	8.9 b	8.7 ab
	Rootpac 70	7.8 ab	8.9 abc	6.7 ab	7.8 ab
	Rootpac R	10.2 b	10.8 bc	6.8 ab	9.3 b
Fructose (g kg ⁻¹ FW)	Adafuel	7.7 a	9.8 a	8.8 abc	8.8 a
	Adarcias	10.8 ab	11.6 ab	7.8 ab	10.1 a
	Adesoto 101	11.8 b	13.5 b	7.6 ab	10.9 a
	Cadaman	11.3 b	11.5 ab	8.5 abc	10.4 a
	GF 677	10.9 ab	12.2 ab	7.6 ab	10.2 a
	PADAC 04-01	10.8 ab	12.2 ab	7.6 ab	10.2 a
	PADAC 04-03	9.0 ab	13.4 b	7.6 ab	10.0 a
	PADAC 99-05	9.8 ab	11.9 ab	7.2 a	9.6 a
	PADAC 9902-01	9.5 ab	12.9 b	10.1 c	10.8 a
	PM 105 AD	9.1 ab	12.3 ab	8.4 abc	9.9 a
	PM 150 AD	9.9 ab	12.5 ab	9.7 bc	10.7 a
	Rootpac 70	9.1 ab	11.9 ab	8.7 abc	9.9 a
	Rootpac R	11.4 b	13.0 b	7.2 a	10.5 a
Sorbitol (g kg ⁻¹ FW)	Adafuel	1.8 ab	2.7 a	1.9 a	2.2 a
	Adarcias	2.1 ab	4.3 a	4.7 ab	3.9 ab
	Adesoto 101	1.7 a	4.9 a	7.9 b	4.5 ab
	Cadaman	2.1 ab	4.1 a	5.5 ab	3.9 ab
	GF 677	1.5 a	5.2 a	3.7 a	3.5 ab
	PADAC 04-01	2.4 ab	5.6 a	7.7 b	5.2 b
	PADAC 04-03	1.6 ab	5.3 a	4.3 ab	3.7 ab
	PADAC 99-05	2.3 ab	3.9 a	7.8 b	4.6 b
	PADAC 9902-01	2.5 b	4.1 a	2.3 a	2.9 ab
	PM 105 AD	1.5 ab	6.5 a	2.9 a	3.7 ab
	PM 150 AD	1.7 ab	4.3 a	7.5 b	4.5 ab
	Rootpac 70	1.9 ab	4.3 a	3.2 a	3.2 ab
	Rootpac R	2.5 b	6.4 a	5.3 ab	4.7 b
Total sugars (g kg ⁻¹ FW)	Adafuel	80.1 abcd	78.5 a	68.3 a	75.6 a
	Adarcias	84.7 abcde	86.2 ab	92.7 bcd	87.8 bcd
	Adesoto 101	79.6 abcd	90.2 ab	98.3 cd	89.4 bcde
	Cadaman	83.7 abcde	88.1 ab	90.1 bcd	87.3 abcd
	GF 677	73.8 a	92.9 ab	77.1 ab	81.3 abc
	PADAC 04-01	95.1 e	97.5 ab	106.6 d	99.7 e
	PADAC 04-03	77.5 abc	96.6 ab	84.1 abc	86.1 abcd
	PADAC 99-05	91.7 de	87.7 ab	107.3 d	95.6 de
	PADAC 9902-01	88.4 bcde	93.2 ab	78.3 abc	86.6 abcd
	PM 105 AD	76.4 ab	100.6 b	75.2 ab	84.1 abcd
	PM 150 AD	82.6 abcde	86.7 ab	109.3 d	92.9 cde
	Rootpac 70	75.8 ab	89.1 ab	74.9 ab	79.9 ab
	Rootpac R	90.5 cde	100.0 b	90.0 bcd	93.5 de

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.

Table 4. Organic acids of ‘Big Top’ nectarine on thirteen rootstocks, in the fifth (2014), sixth (2015) and seventh (2016) year after budding.

Character	Rootstock	2014	2015	2016	Average
Malic acid (g kg ⁻¹ FW)	Adafuel	2.18 a	2.99 a	2.66 a	2.61 a
	Adarcias	2.89 bcd	3.53 abc	3.57 bcd	3.33 bcde
	Adesoto 101	3.04 cd	3.85 bc	3.86 cd	3.58 defg
	Cadaman	3.12 cd	4.01 c	4.08 d	3.74 efg
	GF 677	2.50 abc	3.78 bc	3.46 bcd	3.25 bcd
	PADAC 04-01	2.26 ab	3.50 abc	3.24 abc	3.00 ab
	PADAC 04-03	2.13 a	3.07 ab	2.93 ab	2.71 a
	PADAC 99-05	2.55 abc	3.80 bc	3.76 cd	3.37 bcde
	PADAC 9902-01	2.62 abc	3.68 abc	2.97 ab	3.06 bc
	PM 105 AD	3.10 cd	4.79 d	3.58 bcd	3.83 g
	PM 150 AD	2.86 bcd	4.00 c	3.90 cd	3.59 defg
	Rootpac 70	3.40 d	4.03 c	3.89 cd	3.77 fg
	Rootpac R	2.94 cd	3.81 bc	3.48 bcd	3.41 cdef
Citric acid (g kg ⁻¹ FW)	Adafuel	1.52 ab	0.94 a	2.15 c	1.54 b
	Adarcias	2.39 abc	1.05 a	1.48 ab	1.64 b
	Adesoto 101	2.13 ab	1.02 a	1.67 abc	1.61 b
	Cadaman	2.16 ab	1.20 a	1.40 ab	1.59 b
	GF 677	2.39 bc	1.14 a	1.56 abc	1.70 bc
	PADAC 04-01	1.34 a	0.82 a	1.13 a	1.10 a
	PADAC 04-03	1.77 ab	0.90 a	1.42 ab	1.36 ab
	PADAC 99-05	1.86 ab	1.13 a	1.36 ab	1.45 b
	PADAC 9902-01	2.04 ab	0.82 a	1.71 abc	1.52 b
	PM 105 AD	3.08 c	1.03 a	1.82 bc	1.98 c
	PM 150 AD	2.31 bc	1.14 a	1.23 ab	1.56 b
	Rootpac 70	2.34 bc	0.87 a	1.77 bc	1.66 b
	Rootpac R	2.60 bc	0.88 a	1.74 abc	1.74 b
Quinic acid (g kg ⁻¹ FW)	Adafuel	1.03 a	0.98 a	0.81 a	0.94 a
	Adarcias	1.38 ab	1.17 abcd	1.05 abc	1.20 bc
	Adesoto 101	1.63 b	1.43 bcde	1.41 bcd	1.49 e
	Cadaman	1.18 a	1.10 ab	1.11 abc	1.13 abc
	GF 677	1.18 a	1.14 abc	0.99 ab	1.10 abc
	PADAC 04-01	1.37 ab	1.46 cde	1.45 cd	1.43 de
	PADAC 04-03	1.26 ab	1.27 abcd	1.12 abc	1.22 bcd
	PADAC 99-05	1.30 ab	1.28 abcd	1.25 abc	1.28 cd
	PADAC 9902-01	1.10 a	1.10 ab	0.85 a	1.02 ab
	PM 105 AD	1.66 b	1.50 de	1.16 abc	1.44 de
	PM 150 AD	1.32 ab	1.70 e	1.78 d	1.60 e
	Rootpac 70	1.26 a	1.23 abcd	1.01 abc	1.17 abc
	Rootpac R	1.34 ab	1.36 bcd	1.04 abc	1.25 cd
Succinic + Shikimic acid (g kg ⁻¹ FW)	Adafuel	0.61 ab	0.55 a	0.65 ab	0.60 ab
	Adarcias	0.80 abcd	0.54 a	0.55 a	0.63 ab
	Adesoto 101	0.93 cd	0.71 ab	0.75 abc	0.80 de
	Cadaman	0.81 abcd	0.59 ab	0.59 ab	0.66 abcd
	GF 677	0.85 bcd	0.59 ab	0.74 abc	0.73 bcde
	PADAC 04-01	0.58 a	0.56 a	0.66 abc	0.60 ab
	PADAC 04-03	0.77 abcd	0.50 a	0.66 abc	0.64 abc
	PADAC 99-05	0.65 ab	0.59 ab	0.49 a	0.58 a
	PADAC 9902-01	0.70 abc	0.53 a	0.66 abc	0.63 abc
	PM 105 AD	0.98 d	0.66 ab	0.85 bc	0.83 e
	PM 150 AD	0.85 bcd	0.79 b	0.68 abc	0.77 de
	Rootpac 70	0.81 abcd	0.55 a	0.91 c	0.76 cde
	Rootpac R	0.73 abc	0.52 a	0.59 ab	0.61 ab
Total acids (g kg ⁻¹ FW)	Adafuel	5.34 a	5.45 a	6.28 ab	5.70 a
	Adarcias	7.48 bcd	6.30 abc	6.65 abc	6.77 bcde
	Adesoto 101	7.73 cd	7.01 cd	7.70 c	7.47 efg
	Cadaman	7.28 bcd	6.83 cd	7.18 abc	7.12 def
	GF 677	6.91 abc	6.67 bcd	6.77 abc	6.78 bcde
	PADAC 04-01	5.55 a	6.31 abc	6.49 abc	6.12 ab
	PADAC 04-03	5.91 ab	5.74 ab	6.13 a	5.91 a
	PADAC 99-05	6.36 abc	6.80 cd	6.86 abc	6.65 bcd
	PADAC 9902-01	6.47 abc	6.13 abc	6.19 ab	6.27 abc
	PM 105 AD	8.32 d	7.99 e	7.41 bc	8.08 g
	PM 150 AD	7.34 bcd	7.62 de	7.60 c	7.52 fg
	Rootpac 70	7.57 cd	6.68 bcd	7.59 c	7.28 def
	Rootpac R	7.27 bcd	6.56 bc	6.85 abc	6.90 cdef

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.

Table 5. Total phenolics, flavonoids, vitamin C and antioxidant capacity (RAC) of ‘Big Top’ nectarine on thirteen rootstocks, in the fifth (2014), sixth (2015) and seventh (2016) year after budding.

Character	Rootstock	2014	2015	2016	Average
Total phenolics (mg GAE/100 g FW)	Adafuel	14.7 ab	8.7 a	11.9 a	11.8 ab
	Adarcias	14.7 ab	10.7 abc	16.9 ab	14.1 abcd
	Adesoto 101	16.4 b	11.6 abc	22.1 b	16.7 cd
	Cadaman	13.2 ab	9.8 abc	16.8 ab	13.5 abc
	GF 677	13.5 ab	10.2 abc	13.4 a	12.4 ab
	PADAC 04-01	16.2 b	13.2 c	22.1 b	17.2 d
	PADAC 04-03	14.3 ab	13.0 c	20.3 b	15.9 bcd
	PADAC 99-05	17.6 b	10.3 abc	22.2 b	16.8 cd
	PADAC 9902-01	13.8 ab	8.9 ab	13.1 a	11.9 ab
	PM 105 AD	14.2 ab	12.5 bc	16.5 ab	14.4 abcd
	PM 150 AD	15.9 b	13.1 c	22.3 b	17.1 cd
	Rootpac 70	10.9 a	9.9 abc	12.5 a	11.1 a
	Rootpac R	15.0 ab	11.2 abc	14.6 a	13.6 abcd
Flavonoids (mg CE/100 g FW)	Adafuel	8.2 ab	2.9 a	4.8 a	5.3 ab
	Adarcias	8.1 ab	3.6 a	8.1 a	6.6 ab
	Adesoto 101	10.9 b	4.1 a	13.0 ab	9.3 bc
	Cadaman	7.0 ab	3.3 a	7.9 a	6.1 ab
	GF 677	7.7 ab	3.2 a	5.9 a	5.6 ab
	PADAC 04-01	10.1 ab	4.6 a	12.8 ab	9.2 abc
	PADAC 04-03	7.5 ab	4.9 a	11.6 a	8.0 abc
	PADAC 99-05	11.1 b	3.1 a	12.3 a	8.8 abc
	PADAC 9902-01	7.0 ab	3.0 a	5.0 a	5.0 a
	PM 105 AD	7.5 ab	3.9 a	7.9 a	6.4 ab
	PM 150 AD	9.5 ab	4.8 a	20.2 b	11.5 c
	Rootpac 70	5.4 a	4.7 a	4.6 a	4.9 ab
	Rootpac R	8.2 ab	3.5 a	6.2 a	5.9 ab
Vitamin C (mg AsA/100 g FW)	Adafuel	9.7 c	8.8 a	4.6 a	7.7 ab
	Adarcias	7.5 abc	10.9 a	8.9 abcd	9.1 b
	Adesoto 101	6.2 ab	8.6 a	10.1 abcd	8.3 ab
	Cadaman	6.7 ab	9.2 a	8.5 abcd	8.1 ab
	GF 677	7.4 abc	9.0 a	6.9 abcd	7.8 ab
	PADAC 04-01	8.2 bc	8.5 a	12.5 d	9.8 b
	PADAC 04-03	7.9 abc	8.3 a	10.7 cd	8.9 ab
	PADAC 99-05	7.3 abc	6.6 a	10.2 abcd	8.0 ab
	PADAC 9902-01	7.3 abc	6.2 a	4.7 ab	6.1 a
	PM 105 AD	5.3 a	8.1 a	6.7 abc	6.7 ab
	PM 150 AD	6.6 ab	7.6 a	10.4 bcd	8.2 ab
	Rootpac 70	6.3 ab	7.3 a	6.6 abc	6.7 ab
	Rootpac R	6.5 ab	7.8 a	10.2 abcd	8.2 ab
RAC (µg Trolox/ g FW)	Adafuel	129.2 abc	37.1 a	119.1 a	95.1 ab
	Adarcias	122.1 abc	76.6 ab	150.4 abc	116.4 abcd
	Adesoto 101	139.6 abc	63.0 ab	208.4 bc	137.0 bcd
	Cadaman	116.0 abc	56.2 ab	152.7 abc	108.3 abcd
	GF 677	109.2 abc	57.7 ab	124.3 ab	97.1 abc
	PADAC 04-01	150.6 bc	82.5 ab	212.1 d	148.4 cd
	PADAC 04-03	111.8 abc	69.9 ab	184.4 bcd	122.0 abcd
	PADAC 99-05	166.0 c	55.1 ab	226.0 d	149.0 cd
	PADAC 9902-01	106.8 ab	39.4 a	100.5 a	82.2 a
	PM 105 AD	118.4 abc	57.7 ab	117.4 a	97.8 abc
	PM 150 AD	145.3 abc	96.8 b	225.8 d	155.9 d
	Rootpac 70	85.9 a	58.4 ab	123.1 ab	89.1 ab
	Rootpac R	123.4 abc	65.2 ab	131.4 ab	106.7 abcd

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.

Table 6. Pearson's correlations coefficients between traits observed over three years (2014, 2015, and 2016) of 'Big Top' nectarine on thirteen different rootstocks.

Trait	Fructose	Sorbitol	TS	Citric acid	Malic acid	Quinic acid	Suc+Shi acid	TA	Phenolics	Flavonoids	Vitamin C	RAC	TS/TA
Sucrose	ns	0.76**	0.94**	ns	ns	ns	-0.19**	ns	0.53**	0.40**	0.59**	0.49**	0.65**
Glucose	0.68**	ns	0.19*	ns	ns	ns	ns	ns	-0.16*	ns	ns	-0.29**	ns
Fructose	-	ns	ns	-0.17*	ns	0.37**	ns	ns	-0.28**	-0.19*	-0.16*	-0.39**	ns
Sorbitol		-	0.81**	-0.22**	0.10**	ns	-0.25**	ns	0.37**	0.18*	0.59**	0.27**	0.46**
TS			-	ns	0.23**	0.27**	-0.11**	ns	0.44**	0.34**	0.56**	0.35**	0.67**
Citric acid				-	-0.63**	0.58**	0.24**	0.54***	ns	-0.16*	-0.23**	-0.21**	-0.69**
Malic acid					-	0.64**	ns	0.55***	ns	ns	0.20**	ns	ns
Quinic acid						-	0.23**	0.53**	0.18**	ns	0.21**	0.33**	ns
Suc+Shi acid							-	0.61***	ns	0.23**	-0.15**	ns	-0.73**
Phenolics								ns	-	0.90**	0.39**	0.91**	ns
Flavonoids										-	0.31**	0.84**	ns
Vitamin C											-	0.31**	0.415**
TA													-0.68**

* and ** represent statistical significance at $P \leq 0.05$ and $P \leq 0.01$ respectively; ns, not significant. Abbreviations: TS, Total sugars, the sum of sucrose, glucose, fructose, and sorbitol; Suc+Shi, succinic and shikimic; RAC, relative antioxidant capacity; TA, the sum of citric, malic, quinic, succinic, and shikimic acids.

Figure 1. Principal component analysis for the biochemical traits evaluated on the nectarine ‘Big Top’ budded on thirteen *Prunus* rootstocks. Analysis was performed using mean data of the three years of study (2014-2016). Symbols: (◇) sugars, (○) organic acids, (▲) antioxidants compounds and (●) assessed rootstocks.

Figure 2. Hierarchical agglomerative cluster analysis grouped for the different rootstocks based on their genetic origin and content of biochemical traits in the budded cultivar.