



Research article

Performance of the Brasiliano 92 orange cultivar with six trifoliolate rootstocks

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Abstract: The rootstock plays a crucial role in the fruit tree. For several decades, sour orange (*Citrus aurantium* L.) has been the most widely used rootstock in citrus cultivation. However, the spread of the *Tristeza* virus (CTV) has prevented the use of sour orange rootstock in new plantings in many areas because it is sensitive to CTV.

The objective of this experiment was to study the vegetative, productive, and fruit qualitative performance of six graft combinations obtained from grafting Brasiliano N.L. 92 onto *Poncirus trifoliata* (L.) Raf. (PT); Flying dragon [*Poncirus trifoliata* (L.) Raf. cv. Monstrosa] (FD); Citrange Carrizo [*Citrus sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf.] (CC); Citrange C35 [*Citrus sinensis* (L.) Osbeck, cv. Ruby's *poncirus trifoliata* (L.) Raf., cv. Webber Fawcett] (C35); Citrange Troyer [*Citrus sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf.] (CT); and Swingle Citrumelo [*Citrus paradisi* Macf. *Poncirus trifoliata* (L.) Raf.] (SC). Sampled fruits were assessed for biometric measures, maturation index, and nutraceutical parameters. At 225 days after full bloom (DAFB), the oranges were harvested and the number and weight of fruit per tree were determined.

The differences were recorded, and statistical analysis showed that each of the six combinations had specific characteristics. Particular attention must be given to the combination B92/FD; despite the fruit being smaller, it showed better organoleptic and nutraceutical results. For the lower yield per tree higher density of planting is required compared to the other rootstocks. The cost of planting a higher number of trees using the B92/FD graft combination at planting time can be justified for an orange cultivar that guarantees high income, such as the Brasiliano 92 cultivars.

Keywords: citrus; fruit quality; maturation index; nutraceutical parameter

1. Introduction

Rootstock plays a crucial role in the fruit tree, influencing characteristics such as nutritional status and fruit quality [1], vegetative tree performance [2], yield [3], and resistance to biotic and abiotic stress [4]. Although there are many studies of scion development induced by rootstock changes [5,6], there are also some studies on the influence of scion genotype on root development. Each grafting combination gives origin to a new tree that differs by parent, and is the result of a cyclic exchange interaction between scion and root.

Citrus fruits are widely grown in more than 100 countries with tropical, subtropical, and Mediterranean climates [7]. For several decades, sour orange (*Citrus aurantium* L.) has been the most widely used rootstock in citrus cultivation. This is due to its positive effect on productivity in different soil and climate conditions, its tolerance to many fungi (*Phytophthora* spp.) and viroids (CEVd, CCaDV), and its positive influence on the quality of production [8]. However, the spread of the *Tristeza* virus (CTV) has prevented the use of sour orange rootstock in new plantings [9] because it is sensitive to CTV.

Many rootstocks can be used instead of sour orange. Among these is *Poncirus trifoliata* (L.) Raf., its mutations, and its hybrids. *Poncirus trifoliata* (L.) Raf. is a rootstock resistant to low temperatures, *Phytophthora* spp., CTV, and nematodes, but it is susceptible to CEVd [10] because it is vulnerable in calcareous soils to ferric chlorosis, preferring acidic soils. Flying dragon [*Poncirus trifoliata* (L.) Raf. var. *Monstruosa*] is a dwarfing mutation of *Poncirus trifoliata* (L.) Raf. [11]. It is planted in high density conditions to reduce the size of the tree [11].

The Citrumelo Swingle (CPB-4475) was obtained by hybridization of *Citrus paradisi* Macf “Duncan” × *Poncirus trifoliata* (L.) Raf. It is a rootstock tolerant to CTV, *Phytophthora* spp., and salt stress [12]. In Mediterranean environments, the most commonly used rootstocks are the Troyer citrange and Carrizo citrange, obtained from hybridization between the Washington navel of *Citrus sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf. Today, Carrizo is preferred over Troyer because of its greater tolerance to nematodes. A citrange of the subsequent constitution is the C35 [*Citrus sinensis* (L.) Osbeck, cv. Ruby’s *poncirus trifoliata* (L.) Raf., cv. Webber Fawcett]. All citrange rootstocks have good resistance to *Phytophthora* spp., CTV, and nematodes [13].

The object of the experiment was to study the vegetative, productive, and fruit qualitative performance of six graft combinations obtained grafting a sweet orange cultivar of the navel group, Brasiliano N.L. 92, onto six rootstocks of trifoliolate orange. The experiment was carried out in a cultivation area where sour orange was in crisis due to CTV, and had been replaced with other rootstocks.

2. Materials and methods

2.1. Vegetal materials, experimental site, and design of the experiment

The experiment was carried out over three years (2017–2019) at the ARSAC farm site in Lamezia Terme, (38°51'39"N; 16°16'12"E), Italy. Scion of Brasiliano 92 [*Citrus sinensis* (L.) Osbeck] was grafted onto: *Poncirus trifoliata* (L.) Raf. (PT), Flying dragon [*Poncirus trifoliata* (L.) Raf. cv. *Monstruosa*] (FD), Citrange Carrizo [*Citrus sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf.] (CC), Citrange C35 [*Citrus sinensis* (L.) Osbeck, cv. Ruby’s *poncirus trifoliata* (L.) Raf., cv.

Webber Fawcett] (C35), Citrange Troyer [*Citrus sinensis* (L.) Osbeck \times *Poncirus trifoliata* (L.) Raf.] (TC), and Swingle Citrumelo [*Citrus paradisi* Macf. *Poncirus trifoliata* (L.) Raf.] (SC). The citrus orchard has been planted in 2008. The B92/FD trees have been spaced 3.5×3.5 m apart ($816 \text{ trees ha}^{-1}$) for the dwarfing character of this rootstock. The trees have been planted 5×5 m apart ($400 \text{ plants ha}^{-1}$) for the other graft combinations. The north-south row orientation has been adopted, and the tree has been trained to shape a globe canopy. The orchard was managed using the standard integrated pest management system and stable drip irrigation and fertigation system.

The study was arranged in a randomized block design with three blocks, six graft combination per block and five trees per graft combination ($3 \text{ blocks} \times 6 \text{ treatments} \times 5 \text{ trees} = 90 \text{ trees per graft combination}$).

2.2. Canopy and yield parameters

All tree dimension parameters were calculated using the “Analysis” tool of Adobe Photoshop CS6 extended software (Adobe, San Jose, CA, USA). This image processing tool allows the definition of the variables required for the calculation of both tree height and canopy volume after setting the measurement scale [14].

The oranges were harvested at 225 days after full bloom (DAFB); the weight per tree (kg tree^{-1}) and per Hectare (q ha^{-1}) was determined.

2.3. Fruit morphometric characteristics

At 190, 210, and 225 days after flower bloom (DAFB) for each tree, six fruits for tree were randomly selected (90 fruits from each graft combination), and the following parameters were measured: fresh weight (FW), using an electronic balance (Mettler-Toledo MgbH, Grefensee, Switzerland); longitudinal diameter; transversal diameter; peel thickness using a digital calliper; and longitudinal/transversal diameter ratio.

The color of the skin was measured in terms of CIELAB space color (L^* , a^* , b^* , chroma and hue angle) using a Minolta CM-700d Spectrophotometer (Minolta, Osaka, Japan). The juice yield (JY) was obtained using a juice extractor, and the juice content was expressed as the percentage of juice volume (mL) to fruit weight (g).

2.4. Fruit maturation index: Soluble solids and titratable acidity

The juice of each fruit was measured for total soluble solids (TSS), using a handheld digital refractometer (PR-1, Atago, Tokyo, Japan), and titratable acidity (TA), using an automatic titrator (Titralab AT1000 series, HACH, Colorado, USA). Twenty-five mL of orange juice was diluted (1:1) and titrated to pH 8.2 with 0.1 N NaOH (mEq. NaOH/100 g fresh fruit). Titratable acidity was expressed as a percentage of monohydrate citric acid. The TSS/TA ratio was also calculated.

2.5. Nutraceutical parameters: ascorbic acid, total antioxidant capacity, total polyphenol content

The ascorbic acid (AA) content was determined using the procedure based on the reduction of the dye 2,6-dichlorophenol-indophenol by ascorbic acid (mg ascorbic acid/100 g FW).

Total antioxidant capacity (TAC) and total polyphenol content (TPC) analyses were performed; for each block, five fruits for each graft combination were placed in polyethylene bags and frozen at $-80\text{ }^{\circ}\text{C}$ until the analysis of TAC and TPC. The extract was made after the fruit was partially defrosting: 10 g of citrus pulp was added to a solution of methanol, water, and acetic acid (80%:19%, 1% v/v/v) with a final ratio of 1:10 for weight to volume; after 24 hours, the pulp in the mixture was homogenized using an Ultra Turrax blender (20.000 rpm; T25 Basic, IKA Werke, Germany); the samples were centrifuged at 2000 g for 10min; the supernatant of each sample was recovered and then centrifuged for 15 min at 2000 g [15]. The extract obtained was used for TAC and TPC analysis. The TAC was determined using the modified TEAC assay [16–18]; results were read at 734 nm using a Lambda 35 spectrophotometer (Perkin Elmer Corporation, USA) [(blank: solution of methanol, water, and acetic acid (80%:19%, 1% v/v/v)] and expresses as ($\mu\text{mol Trolox equivalents/g}$ fresh weight (FW)). [15]. The TPC was determined using the Folin–Ciocalteu method [19]; results were read at 760 nm using a Lambda 35 spectrophotometer (Perkin Elmer Corporation, USA) [blank: 2.5 mL Folin-Ciocalteu (1:10), 450 microliters distilled water, 50 microliters Folin-Ciocalteu, 2 mL sodium carbonate]. They were expressed as milligrams of GA equivalent (GAE) per g fresh weight. Before measuring the TAC and TPC, standard curves were prepared for each test.

The data were analyzed using two (for biometric and productive parameters) and three-ways (for other parameters) ANOVA test. Mean comparisons were conducted using Tukey test and were considered significant at $p < 0.05$. Analyses were carried out using SPSS v. 22.0 (IBM Corporation, New York, SA).

3. Results and discussion

The six grafting combinations (GC) showed substantial differences in vegetative and productive performance, in agreement with observations by other authors [7,20–24]. This is because the scion grafted on different rootstocks creates individual trees with different characteristics from each other, and from their mother trees, as a result of the cyclical correlations established between rootstock and scion [25]. Therefore, each cultivar grafted on each rootstock is a unique combination.

3.1. Tree size parameters

The canopies of B92 grafted onto TC and B92 onto CC were significantly larger than other combinations, in agreement with other authors [26]. There were no significant differences in canopy size found between B92/C35 and B92/SC and B92/PT and 48% lower, respectively, than grafted combinations with TC and CC (Table 1); similar results were obtained on Satsuma, cv. Okitsu by other authors [27].

Finally, the B92/FD graft combination was 25% shorter and had a 44% smaller canopy (Table 1) than B92/C35, B92/PT and B92/CS (Table 1). The height and canopy volume of B92/FD were four times and 1.5 times lower, respectively, compared with B92/CC and B92/TC. These results highlight the dwarfing behavior of FD rootstock, which is line with other studies conducted on citrus fruits [2,28–31], and the greatest development of B92 in combinations with TC and CC rootstock was detected.

The mean surface area of the trunk above the grafting point was 38.32 cm^2 (± 2.49), but significant differences between graft combinations were found. In particular, the trees grafted on FD

showed the lowest surface area of the trunk (about -40%), while no statistically significant differences were found between other graft combinations (Table 1). For all the above-mentioned parameters, the differences observed between graft combinations were confirmed in the three observation years (Y). The Y effect was recorded, but no $Y \times GC$ interaction was statistically detected (Table 1).

3.2. Fruit carpometric parameters

The fresh weight (FW) of B92/C35 did not show a significant difference compared to B92/SC only; the other hand, this last grafted combination did not show a difference compared to the remaining grafted combinations (Table 2).

The FW increased significantly from 210 to 220 DFAB, and it was 11% higher in the last sampling compared to the first. This trend was confirmed between the observation years (data not shown). The year effect was observed, where as graft x year interactions were not recorded.

The relative longitudinal diameter of fruit was not statistically different between the six grafting combinations. This indicates that the interrelationship between scion and rootstock did not modify the shape of the fruit in the graft combinations. However, the fruit of B92/CC and B92/TC were slightly longer than the other four combinations (Table 2).

The thickness of the peel (PTK) is an important parameter because it can affect the quality of the fruit in the pre-harvest and post-harvest phases [7,21]. The value recorded in the fruit of tree B92/FD was similar to B92/PT and higher than the other combinations. There was no skin thickness difference between B92/PT and B92/C35, and the lowest values were B92/TC, SC, and CC (Table 2).

The PTK increased when the sampling was delayed. The differences changed between years, but no $GC \times Y$, $GC \times \text{sampling Time (ST)}$, and $GC \times ST \times Y$ interaction were recorded.

The JY is an important parameter for the orange industry [7,32]. It ranged from 40 to 48%; however, statistical differences were found among graft combinations. The JY was similar in the fruit of B92/C35, B92/SC. They showed higher values compared to the combinations with PT, TC and FD, whereas JY of fruit by B92/CC did not show significant differences with all other combinations (Table 2). The JY was also increased by about 14% between the first and last sampling dates. There was an observed difference between the three years for this parameter, but no $GC \times Y$, $GC \times ST$, and $GC \times ST \times Y$ interaction were observed.

The lightness (L^*) was higher in the flavedo of fruit from the B92/FD tree. The darkest flavedo was observed in the fruit of B92/PT and B92/SC graft combination. In the flavedo of the other graft combinations, L^* value was intermediate compared to the above-mentioned graft combinations. The chromatic component a^* was statistically higher in the flavedo of B92/FD and B92/TC graft combinations compared to the other graft combinations. The a^* was also significantly higher (more reddish) in B92 grafted with C35, SC, and PT rootstocks compared to CC. In this last graft combination, the lowest value (less reddish) for this parameter was recorded (Table 3).

The chromatic component b^* did not show significant differences in the grafting combinations in the comparison (Table 3). All three parameters increased from the first to last sampling date.

Table 1. Tree biometric and productive parameters of Brazilian 92 in six grafted combinations (a = average of the three years of observation; b cumulative yield).

Graft combination (GC)	Plant density Trees-ha ⁻¹	Height canopy m	Canopy volume m ³	Trunk area cm ² ;	Cumulate yield per tree (9th–11th year); kg tree ⁻¹	Yield ha ⁻¹ q ha ⁻¹	Yield per tree Kg tree ⁻¹	Yield Efficiency Kg m ⁻³
B92/FD	816	1.56 ± 0.05b	2.25 ± 0.30c	26.18 ± 1.23a	62.11 ± 4.93c	309.301 ± 9.2a	37.89 ± 1.13c	16.84 ± 0.5a
B92/CC	400	2.54 ± 0.11a	8.74 ± 0.30a	43.25 ± 1.28b	127.81 ± 8.35a	307.24 ± 8.1a	76.81 ± 2.35a	8.71 ± 0.3c
B92/TC	400	2.50 ± 0.11a	8.64 ± 0.43a	40.96 ± 1.66b	121.31 ± 6.15a	287.82 ± 7.3b	71.98 ± 3.10a	8.26 ± 0.4c
B92/PT	400	2.10 ± 0.12b	4.84 ± 0.28b	38.77 ± 1.45b	104.85 ± 4.03b	247.4 ± 9.4c	61.85 ± 2.83b	12.71 ± 0.3b
B92/SC	400	2.05 ± 0.11b	4.51 ± 0.54b	40.45 ± 1.98b	95.92 ± 2.12b	224.4 ± 14.1c	56.10 ± 2.45b	12.44 ± 0.4b
B92/C35	400	2.0 ± 0.09b	4.18 ± 0.48b	40.33 ± 1.34b	89.27 ± 2.33b	203.0 ± 5.5d	50.57 ± 1.15b	12.10 ± 0.23b
Year								
2017	–	1.88 ± 0.22b	4.15 ± 0.42b	26.9 ± 4.85b	–	248 ± 14.1b	53.8 ± 5.5b	10.1 ± 1.1b
2018	–	2.1 ± 0.18ab	5.55 ± 0.21ab	30.6 ± 4.25ab	–	259 ± 15.5ab	58.8 ± 5.8ab	12.0 ± 0.8ab
2019	–	2.39 ± 0.12a	6.59 ± 0.08a	39.48 ± 6.11a	–	282 ± 16.5a	65.8 ± 5.7a	13.4 ± 1.2a
GC × Y		n.s.	n.s.	n.s.		n.s.		

Note: In the column, different letters indicate significant differences for $p \leq 0.05$. n.s. = not significant.

As for the Chroma parameter, the highest value was observed in B92/PT, whereas the lowest value was recorded in B92/CC. In the flavedo of B92 grafted onto the other rootstocks, this parameter was significantly similar but statistically different both B92/TC that B92/PT.

As for hue, the B92/FD, B92/TC showed lower values compared to B92/SC, B92/C35, and B92/PT; in the peel of B92/CC fruit, the Hue was statistically like other graft combinations. The lower value corresponds to a more red color. The a/b was higher in B92/FD and B92/TC graft combinations compared to B92 grafted onto C35, SC, PT, whereas B92/CC was like all graft combinations. The higher a/b index also corresponds to a more red colour (Table 3). All colorimetric parameters changed between the three years, but no GC × Y, GC × ST, and GC × ST × Y interaction were recorded.

Although flavedo color is influenced by many factors [33–37], our experiment confirms that rootstock contributes to flavedo color patterns, according to reports by other authors [38].

With regard to color evolution among harvest dates, the a/b ratio increased from the first to the last harvest. Finally, it reached the optimum value at 220 DAFB (Table 3). A change was observed between years, but no GC × Y, GC × ST, and GC × ST × Y interaction were observed.

Table 2. Carpological parameters (Fresh Weight, FW; Relative Length, RL; Peel Thickness; PTK) and juice yield (JY) of Brazilian 92 fruit according to the time of harvest and the rootstock (average of the three years of observation).

Graft Combination (GC)	FW g	RL	JY %	PTK mm
B92/FD	293.17 ± 11.89b	0.98 ± 0.07n.s.	40.97 ± 2.93b	6.38 ± 0.22a
B92/C35	361.55 ± 9.96a	0.95 ± 0.06	47.74 ± 1.03a	5.6 ± 0.25b
B92/TC	295.58 ± 9.17b	1.18 ± 0.15	42.19 ± 3.10b	4.98 ± 0.15c
B92/PT	307.07 ± 7.56b	0.98 ± 0.08	40.03 ± 2.35b	5.85 ± 0.22b
B92/SC	331.97 ± 9.05ab	1.00 ± 0.08	45.05 ± 1.12a	5.22 ± 0.18b
B92/CC	295.03 ± 11.42b	1.17 ± 0.15	44.50 ± 2.33ab	4.72 ± 0.12c
Sampling time (ST) (DAFB)				
190	303.78 ± 5.20b	0.99 ± 0.08n.s.	39.55 ± 1.12b	4.95 ± 0.21b
210	304.31 ± 6.18b	1.07 ± 0.07	40.9 ± 1.04b	5.70 ± 0.40a
220	335.86 ± 6.15a	1.07 ± 0.07	43.80 ± 1.22a	5.95 ± 0.42a
Year (Y)				
2017	295.12 ± 9.22a	1.042 ± 0.04 n.s.	42.1 ± 1.05a	5.12 ± 0.25b
2018	314.22 ± 10.15a	1.048 ± 0.02	43.90 ± 1.33ab	5.51 ± 0.20ab
2019	335.78 ± 9.120b	1.041 ± 0.04	44.28 ± 1.02a	5.58 ± 0.18a
GC × ST	n.s.	n.s.	n.s.	n.s.
GC × Years	n.s.	n.s.	n.s.	n.s.
GC × ST × Year	n.s.	n.s.	n.s.	n.s.

Note: In the column, different letters and * indicate significant differences for $p \leq 0.05$. n.s. = not significant.

3.3. Maturation indices

The total soluble solids (TSS) increased by 12% from 190 to 210 DAFB, and it remained stable in the next sampling. The differences between years were significant. For titratable acidity (TA), the fruit of B92/FD showed a significantly lower value compared to the other grafting combinations. In contrast, a higher value was detected in the fruit of B92 grafted onto C35, TC, and CC. The TA in juice fruit of B92/SC and B92/PT fruit was intermediate compared to all grafted combinations (Table 4).

The TSS/TA ratio gives a clear indication of the sweetness of the fruit: the higher the ratio, the sweeter the fruit.

It reports that the sugar/acid ratio must be higher than 8.0 to be considered acceptably ripe in citrus fruit [39]. All graft combinations showed higher values than 8.0 at the date of the first harvest (Table 4). However, the highest ratio was obtained with the B92/FD (11.31 ± 0.93); B92/TC showed significantly lower values than B92/FD, whereas the B92/PT was similar to B92/FD and B92/TC. The significantly lowest values were recorded with B92/CC, B92/SC, and B92/C35 (Table 4) without significant differences among them.

The role of rootstock on the maturation index was also reported by another author [40]. Several authors reported that hydraulic conductivity, and therefore the anatomy of the conductive tissues of rootstocks, could modify the water potential of both leaves and fruits. Citrus fruits contain sucrose

and other osmotically active sugars, unlike pome fruits. Therefore, the result is conditioning the main ripening indices through a dilution/concentration-effect [41–43].

Therefore, the sweetness of pulp result depending on ripening indexes and dilution/concentration-effect [41–43]. For all maturation indexes, no GC × Y, GC × ST, and GC × ST × Y interaction were observed.

Table 3. Effect of grafted combination on fruit peel and pulp colour characteristics (CIE LAb and HSB colour space) in sweet orange tree, cv. Brasiliano 92. The measures reported representing the average of the observations made in the last three years.

Grafted combination (GC)	Peel L*	a*	b*	Chroma	Hue	a/b
B92/FD	65.35 ± 0.08a	+28.81 ± 1.15a	66.27 ± 0.93ns	72.26 ± 0.82b	66.49 ± 0.55b	0.44 ± 0.01a
B92/CC	67.65 ± 0.08b	+23.25 ± 0.97c	66.96 ± 0.93	70.88 ± 0.55c	67.52 ± 0.48ab	0.41 ± 0.01ab
B92/TC	66.98 ± 0.04b	+29.52 ± 1.12a	66.53 ± 1.10	72.78 ± 0.81b	66.06 ± 0.56b	0.45 ± 0.02a
B92/PT	68.67 ± 0.06c	+27.57 ± 0.81b	69.86 ± 1.03	74.39 ± 0.87a	68.89 ± 0.81a	0.39 ± 0.01b
B92/SC	69.60 ± 0.08c	+26.58 ± 1.04b	67.79 ± 0.93	72.81 ± 0.85b	68.58 ± 0.58a	0.39 ± 0.01b
B92/C35	66.61 ± 0.08b	+26.14 ± 1.20b	67.81 ± 0.93	72.67 ± 0.82b	68.91 ± 0.49a	0.38 ± 0.02b
Sampling time (ST) DAFB						
190	56.69a	18.91 ± 1.10b	64.19 ± 1.11b	66.91 ± 1.25b	68.15 ± 0.55a	0.17 ± 0.05c
210	60.88b	25.90 ± 1.22a	68.12 ± 1.82a	72.87 ± 1.15a	68.20 ± 0.81a	0.29 ± 0.08b
220	67.49c	26.96 ± 1.05a	67.55 ± 1.10a	72.73 ± 1.18a	60.15 ± 0.49b	0.40 ± 0.09a
Year (Y)						
2017	65.61 ± 0.61b	+25.11 ± 0.91b	68.11 ± 0.55a	73.21 ± 0.45a	67.48 ± 0.45b	0.40 ± 0.02ab
2018	68.64 ± 0.52a	+28.12 ± 0.80a	66.45 ± 0.48b	77.55 ± 0.82b	68.72 ± 0.39a	0.43 ± 0.02a
2019	65.55 ± 0.66b	+27.35 ± 0.88a	68.22 ± 0.45a	72.15 ± 0.82a	67.02 ± 0.41b	0.39 ± 0.01b
GC × Y	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
GC × ST	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
GC × ST × Y	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Note: In the column, different letters and * indicate significant differences for $p \leq 0.05$. n.s. = not significant.

3.4. Fruit nutraceutical parameters

As far as nutraceutical aspects are concerned, it is known that the total antioxidant capacity (TAC) of fruit takes into account the synergistic activity carried out by several biomolecules present in variable quantities, depending on the type of fruit. Among these biomolecules, polyphenols and ascorbic acid play an important role. Ascorbic acid (AA) is present in high concentrations, although there are several factors, pre and post-harvest, that can influence its content.

The fruit harvested early had a higher AA content than fruit harvested last, which supports results found by another author [44]. Furthermore, the Brasiliano 92 fruit grafted onto FD showed higher AA content compared to the other rootstocks. The lowest value of AA in B92 fruit was found in PT/C35 (Table 5).

The molecules that contribute to defining the content of total polyphenols (TPC) also have a decisive role in determining the sensory qualities of the fruit, such as color, astringency, and

flavor [24]. The TPC was significantly higher in B92/FD, B92/CC, and B92/C35 than other combinations (Table 5).

The TAC was higher in combination with FD, CC, SC, and C35 compared other grafting combinations (Table 5).

The TPC did not change from 190 to 225 DFAB, whereas TAC and AA decreased. This shows a strong correlation between TAC and ascorbic acid. For all nutraceutical parameters no GC × Y, GC × ST, and GC × ST × Y interaction were observed (Table 5).

Table 4. Maturation indices of Brazilian 92 fruit according to the time of harvest and the rootstock (average of the three years of observation).

Graft Combination (GC)	Total soluble solids (TSS) °brix	Titrateable acidity (TA) %	TSS/TA
B92/FD	11.2 ± 0.08a	0.95 ± 0.10c	11.31 ± 0.93a
B92/C35	10.08 ± 0.05b	1.14 ± 0.47a	8.78 ± 1.35c
B92/TC	10.06 ± 0.04b	1.13 ± 0.11a	10.0 ± 1.10b
B92/PT	10.24 ± 0.0b	1.01 ± 0.12b	10.16 ± 1.03ab
B92/SC	9.98 ± 0.13c	1.07 ± 0.09b	9.35 ± 1.03c
B92/CC	9.71 ± 0.11c	1.15 ± 0.06a	8.42 ± 2.33c
Sampling time (ST) (DAFB)			
190	9.6 ± 0.01c	1.13 ± 0.05a	8.45 ± 0.92c
210	10.0 ± 0.02b	1.10 ± 0.04b	9.03 ± 0.37b
220	10.8 ± 0.02a	0.96 ± 0.06c	11.21 ± 0.37a
Year			
2017	9.88 ± 0.15b	1.05 ± 0.01b	9.38 ± 0.42n.s.
2018	10.45 ± 0.20a	1.09 ± 0.02a	9.55 ± 0.38
2019	10.32 ± 0.18a	1.08 ± 0.02ab	9.45 ± 0.22
GC × ST	n.s	n.s	n.s
GC × Years	n.s	n.s	n.s
GC × ST × Years	n.s	n.s	n.s

Note: In the column, different letters and * indicate significant differences for $p \leq 0.05$. n.s. = not significant.

3.5. Tree yield parameters

The cumulate yield per tree and yield per tree was higher in B92 onto CC and TC compared to the other combinations, followed C35, SC, and PT; the lowest value was observed onto FD (Table 1).

The production efficiency was highest for B92/FD, followed by SC, C35, and PT, with the lowest efficiency recorded with CC and TC combinations (Table 1). However, the yield.ha⁻¹ was similar for B92/FD, B92/TC, and B92/CC, and 20% lower in B92/PT and B92/SC compared with B92/FD and B92/CC. The lowest value was calculated for B92/C35.

The highest yield efficiency, the lowest canopy volume, and better quality fruit confirm the favorable role of FD for higher planting density for B92.

Table 5. Nutraceutical parameters of Brazilian 92 fruit according to the time of harvest and the rootstock (average of three years of observation).

Graft combination (GC)	Total polyphenols content (TPC) mg GAE/g FW	Total Antioxidant Capacity (TAC) µmoles Trolox/g FW	Ascorbic Acid (AA) mg ascorbic acid/100 mL
B92/FD	1.01 ± 0.08a	4.60 ± 0.10a	60.01 ± 0.93a
B92/C35	1.05 ± 0.05a	4.77 ± 0.47a	52.14 ± 1.35c
B92/TC	0.95 ± 0.04b	4.14 ± 0.11b	56.61 ± 1.10b
B92/PT	0.91 ± 0.06b	4.10 ± 0.12b	51.20 ± 1.03c
B92/SC	0.89 ± 0.13b	4.69 ± 0.09a	56.23 ± 1.12b
B92/CC	1.03 ± 0.11a	4.84 ± 0.06a	55.26 ± 2.33b
Sampling time (ST) (GDPF)			
190	1.02 ± 0.06n.s.	4.61 ± 0.05a	62.10 ± 0.92a
210	0.98 ± 0.04	4.55 ± 0.04b	56.09 ± 0.37b
225	0.91 ± 0.06	3.18 ± 0.06c	52.17 ± 0.37c
Year			
2017	0.91 ± 0.2b	4.42 ± 0.13b	53.11 ± 1.42b
2018	0.95 ± 0.03b	4.45 ± 0.10b	55.15 ± 1.07ab
2019	1.06 ± 0.02a	4.7 ± 0.12a	57.48 ± 1.357a
GC × ST	n.s.	n.s.	n.s.
GC × Years	n.s.	n.s.	n.s.
GC × ST × Years	n.s.	n.s.	n.s.

Note: In the column, different letters and * indicate significant differences for $p \leq 0.05$. n.s. = not significant

4. Conclusion

The differences recorded and statistical analysis carried out in the six grafting combinations showed that each graft combination had specific characteristics. Particular attention must be paid to the combination B92/FD. The fruit is smaller, but it is within the optimal range for B92 and showed better organoleptic and nutraceutical parameters. However, the lower yield per tree means higher density of planting is required to have competitive production results compared to the other rootstocks. The cost for the higher number of trees using B92/FD graft combinations at planting time can be justified for an orange cultivar that guarantees high income such as the Brasiliano 92 cultivars.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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