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# Agronomic Performance of Sweet Orange Genotypes under the Brazilian Humid Subtropical Climate

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**Abstract:** The diversification of *Citrus* spp. orchards, for both scion and rootstock genotypes, is essential to prevent outbreaks of insects and diseases, improve yield and fruit quality, and extend harvesting and industrial juice processing. Furthermore, this enables growers to obtain higher off-season profits. Citrus plantings were prohibited in most regions of the state of Paraná in the past due to the spread of citrus canker disease. Therefore, this study aimed to evaluate the agronomic performance of distinct early- and mid-season sweet orange cultivars (*C. sinensis* (L.) Osbeck) regarding vegetative growth, fruit quality, and yield under the Brazilian humid subtropical climate in order to select new alternatives of sweet orange for the industrial and fresh fruit markets. The experimental orchard was planted in 2012 with 15 sweet orange cultivars (early-maturing: Bahia Cabula, Diva, Cadenera, Marrs, Midsweet, Paulista, Rubi, and Westin; mid-season maturing: Berna Peret, Jaffa, Khalily White, Fukuhara, Seleta do Rio, Seleta Tardia, and Shamouti) grafted on Rangpur lime (*C. limonia* (L.) Osbeck). The experimental design was randomized blocks with three replicates and five trees per plot, analyzed between each maturation group. Data were submitted to analysis of variance followed by Tukey's test ($p \leq 0.05$). Regarding the early-season cultivars, Diva had the tallest trees with largest canopy diameter and volume, differing from Marrs, which had the smallest trees. Shamouti and Khalily White trees were greatly different from all other mid-season cultivars and produced low fruit load over the evaluated period. The early-season Midsweet scored the highest yield and technological index, similar to the mid-season Berna Peret, producing fruits of high juice quality. These genotypes are more effective under the current situation faced by the citrus industry, as the economic life of orchards has been reduced due huanglongbing (HLB). Altogether, Midsweet and Berna Peret genotypes, previously reported as being less susceptible to citrus canker under the same soil–climate condition, are precocious and exhibit higher agronomic potential to be planted in humid subtropical climates, including Brazil and other similar areas around the world.

**Keywords:** *Citrus sinensis*; early-season cultivar; mid-season cultivar; tree size; yield performance; fruit quality

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## 1. Introduction

*Citrus* spp. fruits are consumed worldwide, and four major citrus groups are commercially planted, including sweet oranges, mandarins, lemons, and grapefruits [1–3]. Sweet orange (*C. sinensis* (L.) Osbeck) is widely cultivated in more than 100 countries and is among the most produced and consumed fruit [1]. Brazil is the world's largest producer of sweet orange, accounting for more than one quarter of the world production [1,4]. Moreover, the citrus industry in Brazil accounts for three quarters of the global orange juice exports, mainly as frozen concentrated orange juice (FCOJ) [3]. The main citrus-growing area in Brazil is in the state of São Paulo, with 77.5% of the national production, followed by Minas Gerais with 5.6% and Paraná with 4.6% [5].

Advanced cultural practices applied in citrus orchards have been essential for Brazil to lead world orange production [6,7]. These technologies include irrigation, fertilization, and pest and disease management, in addition to healthy nursery trees and cultivars that are more productive and adapted to the Brazilian environmental conditions [7–9]. Even under high disease pressure, such as citrus tristeza virus (CTV), citrus canker, citrus variegated chlorosis (CVC), and huanglongbing (HLB), the Brazilian citrus industry has adapted to the new scenario, relying on efficient preventive control systems and migration to new production areas [4,8].

Several scion and rootstock accessions have been introduced and selected by different breeding programs in Brazil. The Instituto de Desenvolvimento Rural do Paraná (IDR-Paraná) maintains a program that constantly evaluates potential citrus selections, aiming at orchard diversification and production of high-quality cultivars for fresh and industrial markets [10,11]. Historically, citrus planting was banned in most regions of Paraná until the 1980s due to the presence of citrus canker, caused by the bacterium *Xanthomonas citri* subsp. *citri* (*Xcc*), a detrimental disease to the Brazilian citrus industry [11,12]. Several alternative strategies have been implemented to prevent and control the disease in the state [13,14]. A state-wide integrated management approach was developed, including some control measures such as resistant citrus cultivars, windbreak, and sprays with copper-based bactericides [13,15], in addition to leafminer (*Phyllocnistis citrella* Stainton) control [16].

Previous investigations have assessed and selected sweet orange cultivars with certain resistance to citrus canker in Paraná, which are currently authorized for planting, including the early-season IAPAR 73, Navelina, and Cadenera; the mid-season Pera, Salustiana, Shamouti, and Jaffa; and the late-season Valencia, Folha Murcha and Natal [11,17,18]. However, the orchards in the state are mostly composed of IAPAR 73, Pera, Valencia and Folha Murcha [17]. This limits the productive potential and increases the risk of pests and disease outbreaks due to the narrow genetic background [8,19]. In this context, it is essential to increase the genetic diversification of the citrus orchards with different scion and rootstock genotypes. Furthermore, the harvest season may be extended by planting cultivars with distinct maturing periods, which would enable the industry to expand the fruit-processing period. In addition, citrus growers may obtain higher profits when the fruit supply is low and enhance fruit yield and quality with better scion–rootstock combinations [8,19,20].

Accordingly, this study aimed to evaluate the performance of 15 early- and mid-season sweet orange cultivars grown under the humid subtropical climate of the northwest region of the state of Paraná, Brazil, in order to select new alternatives of sweet orange for the industrial and fresh fruit markets. Several horticultural characteristics were evaluated, such as tree vegetative growth, yield performance, and fruit quality over different cropping seasons, as well as estimate studies for new orchard planning.

## 2. Materials and Methods

### 2.1. Field Location

The experiment was carried out in the Unidade de Difusão de Tecnologia (UDT/Cocamar Cooperativa Agroindustrial) in Guairaçá, state of Paraná, southern Brazil, located at a latitude of 22°56′04″ S, longitude of 52°41′08″ W, and altitude of 518 m. The climate of the area is humid subtropical (Cfa), according to the Köppen–Geiger climate classification, with annual maximum and minimum temperatures of 28.4 and 17.8 °C, respectively; annual average rainfall of 1527 mm (Figure 1); and relative humidity of 69% [21]. The soil is Typic Haplustox with 85–90% sand, 1% clay [22], a base saturation of 20%, and a pH of 3.9 in the 0–40 cm layer.

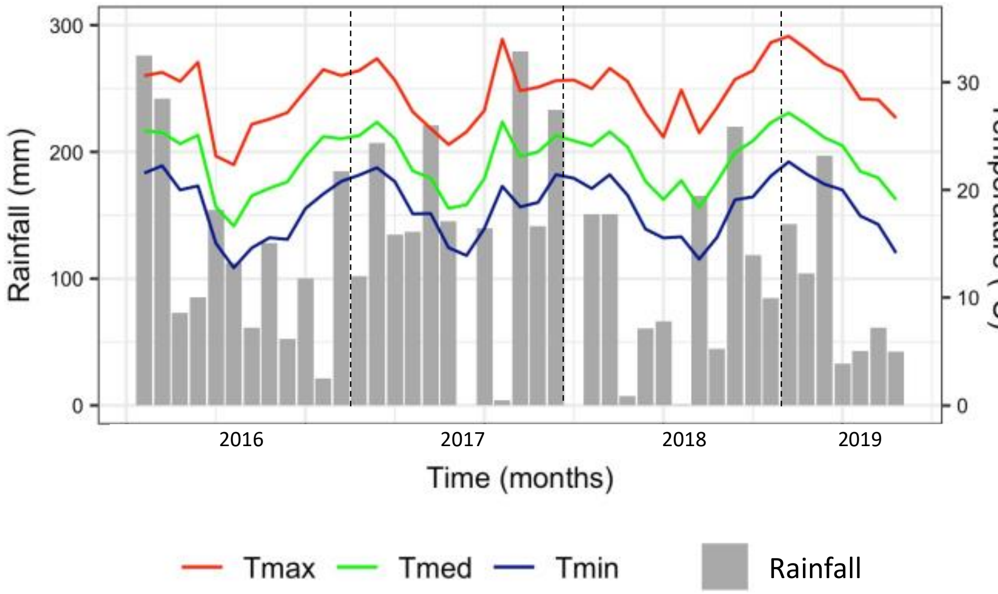


**Figure 1.** Monthly cumulative rainfall (mm) and maximum (Tmax), mean (Tmed), and minimum (Tmin) temperatures (°C) from January 2016 through July 2019 for Guairaçá, Paraná, Brazil. [21].

### 2.2. Plant Materials

The experimental orchard was planted in December 2012 at a tree spacing of 6.5 × 4.5 m with a total of 341 trees ha$^{-1}$. The nursery trees of eight early-season and seven mid-season cultivars of sweet oranges were provided by the Citrus Active Germoplasm Bank of the Instituto Agronômico de Campinas (IAC)/Centro de Citricultura "Sylvio Moreira" in Cordeirópolis, state of São Paulo, Brazil, and the Instituto de Desenvolvimento Rural do Paraná—IAPAR/EMATER (IDR-Paraná) in Londrina, state of Paraná (Table 1). All trees were grafted on Rangpur lime (*C. limonia* (L.) Osbeck), as this rootstock is compatible with most scions and adapted to a wide range of soil and climate conditions. The experimental design was a randomized block with eight (early-maturing) and seven (mid-maturing) treatments of sweet orange cultivars, and three replicates of five trees per plot. The three innermost trees were used as the experimental units and the two outermost trees were considered borders.

**Table 1.** Origin of the 15 sweet orange (*C. sinensis* (L.) Osbeck) cultivars included in the study.

| Genotypes | Sweet Oranges | Origin of Selection | Material Source | Name in the Source Institution |
|---|---|---|---|---|
| Early-season | | | | |
| Bahia Cabula | Navel | Brazil | CCSM [1] | Bahia Cabula IAC 25 |
| Diva | Common | Brazil | CCSM | Diva IAC 58 |
| Cadenera | Common | Spain | IDR [2] | IPR Cadenera |
| Marrs | Navel | USA | CCSM | Marrs IAC 1735 |
| Midsweet | Common | USA | CCSM | Midsweet IAC 1437 |
| Paulista | Common | Brazil | CCSM | Paulista IAC 567 |
| Rubi | Common | Brazil | CCSM | Rubi IAC 52 |
| Westin | Common | Brazil | CCSM | Westin IAC 115 |
| Mid-season | | | | |
| Berna Peret | Common | Spain | CCSM | Berna Peret IAC 2011 |
| Jaffa | Common | USA | IDR | IPR Jaffa |
| Khalily White | Common | Egypt | CCSM | Khalily White IAC 1345 |
| Fukuhara | Common | Japan | CCSM | Fukuhara IAC 2010 |
| Seleta do Rio | Common | Portugal | CCSM | Seleta do Rio IAC 420 |
| Seleta Tardia | Common | Portugal | CCSM | Seleta Tardia IAC 1329 |
| Shamouti | Common | Israel | CCSM | Shamouti IAC 1532 |

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### 2.3. Orchard Management

Citrus tree management was based on the recommendation for the state of Paraná, Brazil [11,23]. Fertilizations were performed for each cropping season using 150 kg N ha$^{-1}$, 140 kg $P_2O_5$ ha$^{-1}$, and 180 kg $K_2O$ ha$^{-1}$. Weeds were managed with periodic mowing using an ecological rotary mower and herbicide sprays between and within rows, respectively. Trees were irrigated by a localized drip irrigation system. The amount of water supplied to the trees was determined according to the crop evapotranspiration (*ETc*), which is determined by the crop coefficient procedure whereby the effect of the various weather conditions is incorporated into the reference crop evapotranspiration (*ETo*), and the crop characteristics into the single crop coefficient (*Kc*) [24]:

$$ETc = ETo \times Kc \quad (1)$$

where *ETc* = crop evapotranspiration, *ETo* = reference crop evapotranspiration, and *Kc* = single crop coefficient.

Disease and insect pest management activities included monthly preventative copper sprays to control citrus canker and weekly insecticide sprays (Pyriproxyfen at 0.625 g ai 100 L$^{-1}$ of water) to control the Asian citrus psyllid (*Diaphorina citri* Kuwayama), which is the vector of '*Candidatus* Liberibacter asiaticus' ('CLas'), the causal agent of huanglongbing (HLB). Top and edge pruning were not performed in order to evaluate the natural tree vegetative growth.

### 2.4. Vegetative Growth Measurements

The vegetative growth of the trees was assessed in early spring of 2019, when trees were seven years old. Tree height and canopy diameter were used to calculate the canopy volume as proposed by Mendel [25]:

$$CV = \frac{2}{3} \times \pi \times CR^2 \times TH \quad (2)$$

where *CV* = canopy volume (m$^3$), *CR* = canopy radius (m), and *TH* = tree height (m).

Trunk circumference was measured 10 cm above and 10 cm below the graft union using a cloth tape measure and converted to diameter. Trunk indices were calculated based on the relationship between trunk diameter above and below the graft union.

### *2.5. Fruit Yield*

Fruit yield was assessed annually from 2016 to 2019 in May for the early-maturing cultivars and in July for the mid-season. Cumulative yields were determined after the annual harvests. The yield efficiency was calculated based on the relationship between fruit yield (kg tree$^{-1}$) and canopy volume (m$^3$ tree$^{-1}$) determined in 2019. The results were expressed in kg m$^{-3}$. The alternate bearing index was determined according to Pearce and Doberšek-Urbanc [26]:

$$ABI = \frac{1}{n-1} \times \left\{ \frac{|a_2 - a_1|}{a_2 + a_1} + \frac{|a_3 - a_2|}{a_3 + a_2} + \ldots + \frac{|a_n - a_{n-1}|}{a_n + a_{n-1}} \right\} \quad (3)$$

where $ABI$ = alternate bearing index, $n$ = number of years, and $a_1, a_2, \ldots, a_{(n-1)}, a_{(n)}$ = yields of the corresponding years.

### *2.6. Fruit Quality Evaluations*

Fruit quality was determined based on 10-fruit samples collected from the three innermost trees of each block. Fruits were randomly collected at 1–2 m tree height in May and July of the early-maturing and mid-maturing cultivars, respectively, before the annual harvest in 2019. Fruit height and diameter were measured with a digital Vernier caliper (Mitutoyo, ABS, Kawasaki, Japan), weighed, and classified according to the fresh citrus standards [27,28]. Then, the fruit shape index was calculated based on the relationship between fruit height and diameter. The fruit samples were juiced using an industrial extractor (Croydon, Duque de Caxias, Brazil). Juice content was determined based on the relationship between juice and fruit weights and expressed in percentage:

$$JC = \frac{JW}{FW} \times 100 \quad (4)$$

where $JC$ = juice content (%), $JW$ = juice weight (g), and $FW$ = fruit weight (g).

Total soluble solids (TSS) were measured with a digital refractometer (Atago Co., Ltd., PAL-3, Tokyo, Japan) in 0.3 mL of undiluted juice. The values were corrected to 20 °C and the results were expressed in °Brix units. Titratable acidity (TA) was determined in 25 mL juice using 0.1 N NaOH in a TitroLine easy titrator (Schott Instruments GmbH, TitroLine easy, Mainz, Germany) and expressed in percentage of citric acid [29]. The TSS TA$^{-1}$ ratio was calculated to determine the fruit maturity of each cultivar. The technological index, which indicates the amount of TSS per standard citrus box (total capacity of 40.8 kg), was calculated according to the equation proposed by Di Giorgi et al. [30]:

$$TI = \frac{TSS \times JC \times 40.8}{10000} \quad (5)$$

where $TI$ = technological index (kg TSS box$^{-1}$), $TSS$ = total soluble solids (°Brix), and $JC$ = juice content (%).

### *2.7. Estimates for Planting Density and Yield*

The number of trees per hectare was estimated for all sweet orange cultivars included in the study, assuming a row spacing of 2.5 m and 25% tree overlap [31]. Fruit yield was estimated according to the theoretical number of trees per hectare and the average fruit yield per tree determined for the 2017–2019 seasons, when all trees were bearing. The technological index was determined according to the estimated yield and expressed in tons of TSS per hectare (t TSS ha$^{-1}$):

$$TI = \frac{TSS \times JC \times YE}{10000} \quad (6)$$

where $TI$ = technological index (t TSS ha$^{-1}$), $TSS$ = total soluble solids (°Brix), $JC$ = juice content (%), and $YE$ = yield estimation. Adapted from Di Giorgi et al. [30].

*2.8. Data Analyses*

The sweet orange cultivars were evaluated according to their respective maturation period for the early- and mid-maturing groups. All data were tested for normal distribution and homogeneity at $p \leq 0.05$, and then submitted to analysis of variance (ANOVA) followed by the comparison of means using Tukey's post-hoc test at $p \leq 0.05$. All significant variables were taken together and submitted to multivariate analyses using a mean value for each variable and treatment. A principal component analysis (PCA) was plotted. All data were processed using *R* v. 4.0.2 (The R Foundation for Statistical Computing, Vienna, Austria).

## 3. Results

*3.1. Tree Vegetative Growth*

Among the early-season sweet orange cultivars, Diva had the tallest trees, whereas the Marrs trees were the shortest ones, when trees were seven years old (Table 2). The trees of the eight early-season cultivars did not show any significant differences ($p \leq 0.05$) in canopy diameter, which ranged from 3.69 m for Marrs to 4.14 m for Midsweet trees. On the other hand, significant differences were observed for canopy volume. Midsweet and Diva trees had the highest canopy volume, whereas Marrs trees had the lowest volume (Table 2). Regarding trunk diameter, Diva and Midsweet trees had the largest rootstock and scion trunk diameters, differing significantly from the Marrs trees. The ratio between trunk diameter below (rootstock) and above (scion) the graft union, which is an indication of the scion–rootstock compatibility, ranged from 0.82 for Paulista sweet orange trees to 0.94 for Cadenera, but did not show any significant difference across this range (Table 2).

**Table 2.** Tree size of 15 early-season and mid-season sweet orange cultivars evaluated in 2019 in Guairaçá, Paraná, Brazil.

| Cultivar | Tree Height (m) | Canopy Diameter (m) | Canopy Volume (m$^3$) | Rootstock Trunk Diameter [1] (cm) | Scion Trunk Diameter [1] (cm) | Trunk Diameter Index [2] |
|---|---|---|---|---|---|---|
| Early-season | | | | | | |
| Bahia Cabula | 3.83 b [3] | 3.98 | 32.0 ab | 14.9 cd | 13.8 bc | 0.93 |
| Diva | 4.51 a | 3.92 | 36.6 a | 18.0 a | 16.7 a | 0.93 |
| Cadenera | 4.21 ab | 3.79 | 31.9 ab | 15.2 b–d | 14.3 b | 0.94 |
| Marrs | 3.69 b | 3.69 | 26.7 b | 14.1 d | 11.8 c | 0.83 |
| Midsweet | 4.23 ab | 4.14 | 38.2 a | 17.5 ab | 14.6 ab | 0.83 |
| Paulista | 4.08 ab | 3.81 | 31.1 ab | 16.4 a–d | 13.5 bc | 0.82 |
| Rubi | 4.17 ab | 3.84 | 32.4 ab | 14.6 cd | 13.1 bc | 0.90 |
| Westin | 4.24 ab | 3.97 | 35.2 ab | 16.9 a–c | 14.9 ab | 0.89 |
| CV (%) | 4.90 | 3.72 | 9.90 | 5.68 | 5.97 | 5.39 |
| *F* value | 4.86 ** | 2.65 ns | 3.64 * | 7.72 *** | 8.95 *** | 3.20 ns |
| Mid-season | | | | | | |
| Berna Peret | 4.41 a–c | 4.17 bc | 40.3 ab | 17.4 b | 16.2 bc | 0.93 ab |
| Jaffa | 4.28 b–d | 3.64 d | 29.8 c | 16.4 b | 13.2 d | 0.81 b |
| Khalily White | 4.53 ab | 4.58 a | 49.7 a | 21.7 a | 18.3 a | 0.85 b |
| Fukuhara | 3.91 d | 3.90 cd | 31.2 bc | 16.3 b | 16.8 ab | 1.04 a |
| Seleta do Rio | 4.13 b–d | 4.04 bc | 35.3 bc | 15.7 b | 14.7 cd | 0.94 ab |
| Seleta Tardia | 4.09 cd | 4.03 bc | 35.2 bc | 16.6 b | 14.1 d | 0.85 b |
| Shamouti | 4.81 a | 4.36 ab | 47.9 a | 20.9 a | 17.9 a | 0.86 b |
| CV (%) | 3.38 | 3.14 | 8.87 | 5.87 | 3.42 | 6.76 |
| *F* value | 12.78 *** | 16.95 *** | 15.75 *** | 15.98 *** | 38.40 *** | 5.13 ** |

[1] Trunk diameters were based on trunk circumference measurements, 10 cm above and 10 cm below the graft union. [2] Expressed as the ratio between scion and rootstock trunk diameters. [3] Means followed by the same letter in the column for each maturing group did not differ significantly according to Tukey's test ($p \leq 0.05$). Significance level: ns, non-significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Regarding the mid-season cultivars, Shamouti sweet orange trees were the tallest, whereas Fukuhara were the smallest ones (Table 2). In addition, Shamouti trees showed the highest canopy diameter, as did Khalily White. In contrast, Fukuhara trees had the lowest diameter among the mid-season cultivars (Table 2). Khalily White trees also had the largest canopy volume, whereas Fukuhara showed the smallest ones (Table 2). The rootstock and scion trunk diameters were larger for Khalily White and Shamouti sweet orange trees, with an average diameter of 21.7 and 18.3 cm for Khalily White and 20.9 and 17.9 cm for Shamouti, respectively. The ratio between scion and rootstock trunk diameter ranged from 0.81 for Jaffa to 1.05 for Fukuhara trees (Table 2).

### *3.2. Yield Performance*

The Midsweet sweet oranges were the most productive among the early-season cultivars (Table 3). On the other hand, the navel orange cultivars, Marrs and Bahia Cabula, showed the lowest performance for fruit yield (Table 3). Midsweet were also the most productive among the early-season cultivars regarding cumulative yield and yield efficiency (Table 3), contrasting with Bahia Cabula, which had the lowest values for these yield dimensions (Table 3). The alternate bearing indices fluctuated from 0.10 for Midsweet to 0.61 for Marrs, showing significant differences ($p \leq 0.05$) among the early-season cultivars.

**Table 3.** Annual yield, cumulative yield, yield efficiency, and alternate bearing index of 15 early-season and mid-season sweet orange cultivars from 2016 to 2019 in Guairaçá, Paraná, Brazil.

| Cultivar | Yield (kg Tree$^{-1}$) | | | | Cumulative Yield (kg) | Yield Efficiency (kg m$^{-3}$) [1] | Alternate Bearing Index |
|---|---|---|---|---|---|---|---|
| | 2016 | 2017 | 2018 | 2019 | | | |
| Early-season | | | | | | | |
| Bahia Cabula | 8.1 c [2] | 25.7 b | 7.5 c | 48.8 c | 90.1 c | 1.52 b | 0.55 a |
| Diva | 29.6 bc | 73.1 a | 32.0 bc | 97.8 ab | 232.5 ab | 2.70 a | 0.44 a |
| Cadenera | 18.3 bc | 50.7 ab | 51.6 ab | 78.3 a–c | 198.9 bc | 2.49 ab | 0.25 ab |
| Marrs | 2.1 c | 35.0 b | 14.2 bc | 45.3 c | 96.7 c | 1.74 ab | 0.61 a |
| Midsweet | 74.4 a | 78.0 a | 72.5 a | 103.1 a | 327.9 a | 2.69 a | 0.10 b |
| Paulista | 45.9 ab | 52.8 ab | 15.7 bc | 86.4 a–c | 200.8 a–c | 2.75 a | 0.48 a |
| Rubi | 14.3 bc | 55.1 ab | 23.8 bc | 64.4 a–c | 157.6 bc | 1.99 ab | 0.51 a |
| Westin | 21.8 bc | 50.1 ab | 30.5 bc | 51.9 bc | 154.3 bc | 1.54 b | 0.37 ab |
| CV (%) | 44.54 | 22.93 | 43.13 | 23.46 | 24.31 | 13.17 | 17.18 |
| *F* value | 11.57 *** | 6.23 ** | 7.83 *** | 5.39 ** | 9.06 *** | 2.90 * | 5.93 ** |
| Mid-season | | | | | | | |
| Berna Peret | 28.2 b | 105.0 a | 79.7 ab | 106.6 a | 319.5 a | 2.68 a | 0.30 b |
| Jaffa | 55.7 a | 86.9 ab | 94.2 a | 24.6 c | 261.4 a | 0.83 c | 0.30 b |
| Khalily White | — [3] | 18.8 c | 30.7 c | 49.5 b | 99.1 b | 1.00 bc | 0.44 ab |
| Fukuhara | — | 14.5 c | 3.9 c | 54.2 b | 72.6 b | 1.72 ab | 0.76 a |
| Seleta do Rio | 9.31 b | 50.3 bc | 33.3 bc | 52.0 b | 144.9 b | 1.48 bc | 0.33 b |
| Seleta Tardia | — | 14.6 c | 21.2 c | 90.0 a | 125.8 b | 2.63 a | 0.63 ab |
| Shamouti | — | 13.5 c | 26.0 c | 51.9 b | 91.4 b | 1.09 bc | 0.56 a |
| CV (%) | 26.46 | 42.96 | 41.46 | 13.90 | 23.73 | 9.71 | 29.91 |
| *F* value | 24.16 ** | 12.79 *** | 11.06 *** | 31.64 *** | 18.61 *** | 17.35 *** | 2.39 * |

[1] Yield efficiency was based on fruit yield and canopy volume determined for 2019. [2] Means followed by the same letter in the column for each maturing group did not significantly differ according to Tukey's test ($p \leq 0.05$). Significance levels: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$. [3] No data for the respective year.

Based on yield performance of the mid-season cultivars, Berna Peret and Jaffa were the most productive compared to all other tested cultivars. In contrast, Fukuhara, Shamouti, Khalily White, Seleta Tardia and Seleta do Rio showed the lowest yield across the evaluated period (Table 3). Most of these cultivars did not show any fruit yield in the first season, whereas Berna Peret, Jaffa and Seleta Tardia were precocious, bearing fruit in this season. Despite imparting high fruit yield, Jaffa scored low for yield efficiency, whereas Berna Peret and Seleta Tardia exhibited higher values for this parameter. Differences were also observed for the alternate bearing indices among the mid-season cultivars, in which Berna Peret, Jaffa and Seleta do Rio had the lowest indices (0.30–0.33), differing from those recorded for Fukuhara and Shamouti, with 0.56 and 0.76, respectively.

### 3.3. Fruit Quality Evaluation

Significant differences were observed among the sweet orange cultivars for all physicochemical parameters in both early- and mid-season maturing groups (Tables 4 and 5). The largest size and highest weight among the early-maturing cultivars were observed in Bahia Cabula fruit, whereas Diva, Cadenera and Rubi produced the smallest fruit (Table 4). Further, Cadenera, Rubi and Westin fruit had the lowest weight, differing significantly from the Bahia Cabula and Marrs navel oranges. Based on the fruit shape index, Rubi and Westin had a moderate oblate fruit shape (Table 4). Midsweet fruit had the highest number of seeds (14 seeds per fruit) among the early-season maturing sweet oranges (Table 4).

Regarding the mid-season maturing cultivars, Berna Peret produced the smallest fruit (Table 4). On the other hand, Seleta do Rio, Khalily White, Seleta Tardia and Fukuhara produced the heaviest fruit (Table 4). The fruit shape indices were higher for Khalily White, Fukuhara and Shamouti, i.e., an oblong shape, and moderate oblate for Seleta do Rio, which had the lowest index. Moreover, Seleta do Rio fruit had a higher number of seeds (14 seeds per fruit) than all other mid-season cultivars (Table 4).

**Table 4.** Fruit quality of 15 early-season and mid-season sweet orange cultivars produced in Guairaçá, Paraná, Brazil, in the 2019 cropping season.

| Cultivar | Fruit Height FH (mm) | Fruit Diameter FD (mm) | Fruit Shape FS (FH FD$^{-1}$) | Fruit Weight FW (g) | Number of Seeds NS |
|---|---|---|---|---|---|
| Early-season | | | | | |
| Bahia Cabula | 82.8 a [1] | 88.5 a | 1.01 ab | 297.3 a | 0 d |
| Diva | 69.4 d | 70.3 c | 0.98 a–c | 161.3 bc | 1 cd |
| Cadenera | 70.4 cd | 71.7 bc | 0.98 a–c | 135.8 c | 0 d |
| Marrs | 82.8 ab | 79.7 b | 1.03 a | 215.1 b | 0 d |
| Midsweet | 75.1 b–d | 75.4 bc | 0.99 a–c | 191.8 bc | 14 a |
| Paulista | 80.9 a–c | 78.0 bc | 1.03 a | 187.3 bc | 4 c |
| Rubi | 67.6 d | 72.8 bc | 0.92 c | 141.8 c | 7 b |
| Westin | 73.7 b–d | 77.8 bc | 0.94 bc | 148.8 c | 1 cd |
| CV (%) | 5.13 | 3.93 | 2.86 | 10.98 | 27.38 |
| *F* value | 11.68 *** | 11.02 *** | 5.83 ** | 20.52 *** | 79.53 *** |
| Mid-season | | | | | |
| Berna Peret | 77.1 c | 73.2 d | 1.05 ab | 159.5 b | 1 b |
| Jaffa | 77.2 c | 79.5 bc | 0.97 c | 201.3 ab | 4 b |
| Khalily White | 83.0 b | 75.1 cd | 1.10 a | 227.8 a | 2 b |
| Fukuhara | 88.4 a | 81.6 ab | 1.08 a | 218.8 a | 0 b |
| Seleta do Rio | 78.3 bc | 86.7 a | 0.90 d | 236.5 a | 14 a |
| Seleta Tardia | 82.1 bc | 82.5 ab | 0.99 bc | 220.0 a | 3 b |
| Shamouti | 78.3 bc | 73.1 d | 1.07 a | 194.5 ab | 0 b |
| CV (%) | 2.37 | 2.75 | 2.04 | 8.28 | 41.66 |
| *F* value | 14.17 *** | 17.30 *** | 35.81 *** | 6.79 ** | 32.48 *** |

[1] Means followed by the same letter in the column for each maturing group did not differ significantly according to Tukey's test ($p \leq 0.05$). Significance level: ns, non-significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

**Table 5.** Fruit quality of 15 early-season and mid-season sweet orange cultivars produced in Guairaçá, Paraná, Brazil, in the 2019 cropping season.

| Cultivar | Juice Content JC (%) | Total Soluble Solids TSS (°Brix) | Titratable Acidity TA (g 100 mL$^{-1}$) | Ratio (TSS TA$^{-1}$) | Technological Index TI (kg TSS box$^{-1}$) |
|---|---|---|---|---|---|
| Early-season | | | | | |
| Bahia Cabula | 32.8 c [1] | 9.2 bc | 0.53 de | 17.4 ab | 1.22 b |
| Diva | 48.4 a | 11.0 ab | 0.92 b | 11.9 cd | 2.18 a |
| Cadenera | 47.6 a | 10.3 ab | 1.17 a | 8.8 d | 2.00 a |
| Marrs | 38.8 bc | 10.4 ab | 0.63 cd | 16.8 ab | 1.65 ab |
| Midsweet | 45.1 ab | 11.2 a | 0.79 bc | 14.2 bc | 2.06 a |
| Paulista | 34.6 c | 7.8 c | 0.40 e | 19.7 a | 1.10 b |
| Rubi | 44.4 ab | 10.7 ab | 0.92 b | 11.6 cd | 1.94 a |
| Westin | 48.3 a | 9.5 a–c | 0.87 b | 11.0 cd | 1.88 a |
| CV (%) | 5.66 | 6.52 | 9.02 | 8.56 | 11.46 |
| *F* value | 20.46 *** | 9.08 *** | 37.61 *** | 29.60 *** | 11.75 *** |
| Mid-season | | | | | |
| Berna Peret | 53.9 a | 10.3 ab | 0.88 ab | 11.6 bc | 2.25 a |
| Jaffa | 51.6 a | 10.3 ab | 0.98 a | 10.5 c | 2.17 a |
| Khalily White | 36.3 c | 10.9 a | 0.81 b | 13.3 ab | 1.61 bc |
| Fukuhara | 37.8 bc | 8.4 c | 0.61 c | 13.9 a | 1.29 c |
| Seleta do Rio | 43.8 b | 10.4 ab | 0.93 ab | 11.2 c | 1.87 ab |
| Seleta Tardia | 43.1 b | 9.1 bc | 0.81 b | 11.2 c | 1.60 bc |
| Shamouti | 38.7 bc | 11.2 a | 0.84 ab | 13.3 ab | 1.78 a–c |
| CV (%) | 5.29 | 5.69 | 5.99 | 5.23 | 10.42 |
| *F* value | 26.41 *** | 8.98 *** | 16.84 *** | 13.13 *** | 9.60 *** |

[1] Means followed by the same letter in the column for each maturing group did not significantly differ according to Tukey's test ($p \leq 0.05$). Significance levels: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Diva, Cadenera and Westin exhibited fruit with the highest juice content among the early-season cultivars, with mean values above 47%, whereas Bahia Cabula had the lowest content, below 33% (Table 5). Significant differences were also observed for TSS. For instance, Midsweet fruit had higher TSS content than the other cultivars in the group (Table 5). In contrast, the lowest TSS content in the juice was found for Paulista fruit, which barely scored 7.8 °Brix. The acidity ranged from 0.40% for Paulista sweet orange juice to 1.17% for Cadenera (Table 5). The Brix–acidity ratio (TSS TA$^{-1}$) ranged from 8.8 for Cadenera to 19.7 for Paulista. Furthermore, the technological indices, i.e., the TSS content per 40.8 kg industrial citrus box [30], were above 2.0 for Diva, Midsweet and Cadenera (Table 5), which showed the highest industrial potential.

The juice content in the fruit of the mid-season cultivars was higher than that observed for the early-season sweet oranges (Table 5). Berna Peret and Jaffa had the highest values, above 50%, and Khalily White the lowest, with only 36.3% of juice (Table 5). TSS content in Shamouti and Khalily White juice was higher than in Fukuhara. Juice acidity remained below 1.0% for all mid-season sweet oranges, ranging from 0.61%, for Fukuhara to 0.98% for Jaffa (Table 5). The Brix–acidity ratio showed lower variation among the cultivars of this maturity group than among the early-season cultivars. The ratio ranged from 10.5 to 13.9 for Jaffa and Fukuhara, respectively. Berna Peret and Jaffa had the highest technological indices, whereas Fukuhara had the lowest index.

### *3.4. Estimates for Planting Density and Yield*

The early-season cultivars did not show differences in the planting estimates for row and tree spacing (Table 6). However, significant differences were observed for the yield and technological indices estimates. Midsweet had higher yield performance, with 40.8 t ha$^{-1}$, and a better index, 2.07 t TSS ha$^{-1}$, than the other early-season cultivars, particularly in comparison with the Bahia Cabula and Marrs navel oranges (Table 6). The estimates also indicate that, among the mid-season cultivars, Khalily White and Shamouti trees require the lowest planting density, i.e., the largest row and tree spacing, whereas Jaffa and Fukuhara cultivars demanded the closest spacing (Table 6). The two latter cultivars had higher tree density, with 598 and 535 trees ha$^{-1}$, respectively, compared to the other mid-season cultivars (412–511 trees ha$^{-1}$). Berna Peret scored the highest technological index and yield estimates among this maturity group, similar to Jaffa (Table 6).

**Table 6.** Estimates [1] of minimum row and tree spacing and maximum tree density, yield, and technological index for 15 early-season and mid-season sweet orange cultivars based on field performance in Guairaçá, Paraná, Brazil, from 2012 to 2019.

| Cultivar | Row Spacing (m) | Tree Spacing (m) | Tree Density (Trees ha$^{-1}$) | Fruit Yield (t ha$^{-1}$) | Technological Index (t TSS ha$^{-1}$) |
|---|---|---|---|---|---|
| Early-season | | | | | |
| Bahia Cabula | 2.98 | 6.48 | 518 | 14.1 c [2] | 0.42 c |
| Diva | 2.94 | 6.42 | 532 | 35.9 ab | 1.92 a |
| Cadenera | 2.84 | 6.29 | 560 | 33.9 ab | 1.70 ab |
| Marrs | 2.77 | 6.20 | 582 | 18.4 bc | 0.75 bc |
| Midsweet | 3.10 | 6.64 | 486 | 40.8 a | 2.07 a |
| Paulista | 2.86 | 6.31 | 554 | 28.5 a–c | 0.77 bc |
| Rubi | 2.89 | 6.35 | 546 | 26.1 a–c | 1.22 a–c |
| Westin | 2.98 | 6.47 | 523 | 23.6 a–c | 1.08 a–c |
| CV (%) | 3.71 | 2.27 | 5.89 | 24.30 | 31.13 |
| *F* value | 2.65 ns | 2.65 ns | 2.62 ns | 5.38 ** | 7.25 *** |
| Mid-season | | | | | |
| Berna Peret | 3.13 bc | 6.67 bc | 480 b–d | 46.9 a | 2.59 a |
| Jaffa | 2.73 d | 6.14 d | 598 a | 40.9 a | 2.17 a |
| Khalily White | 3.43 a | 7.08 a | 412 d | 13.6 b | 0.54 b |
| Fukuhara | 2.92 cd | 6.40 cd | 535 ab | 12.8 b | 0.41 b |
| Seleta do Rio | 3.03 bc | 6.54 bc | 506 bc | 23.0 b | 1.07 b |
| Seleta Tardia | 3.02 bc | 6.53 bc | 511 bc | 21.4 b | 0.84 b |
| Shamouti | 3.27 ab | 6.86 ab | 448 cd | 13.7 b | 0.58 b |
| CV (%) | 3.14 | 1.95 | 5.41 | 25.19 | 25.77 |
| *F* value | 16.95 *** | 19.95 *** | 15.04 *** | 15.02 *** | 24.46 *** |

[1] Estimate study was based on vegetative, yield, and fruit quality data of the evaluated cultivars; tree density and row/tree spacing projections were calculated according to De Negri and Blasco [31] and used to estimate fruit yield and technological index. [2] Means followed by the same letter in the column for each maturing group did not significantly differ according to Tukey's test ($p \leq 0.05$). Significance levels: ns, non-significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

### 3.5. Multivariate Analysis

In the multivariate analysis, the first two principal components explained 62% of the total variance in the dataset (Figure 2). Principal component 1 (Dim1) and principal component 2 (Dim2) represented 37% and 25% of the variation, respectively. The projection of the first two principal components showed a segregation among the sweet orange cultivars. Three distinct groups were formed according to their similarity.

Fukuhara, Paulista, Bahia Cabula, Seleta Tardia and Marrs formed the first group (Figure 2, Col. 1). These cultivars were characterized in terms of bearing the largest fruit, size and weight, and having the highest Brix–acidity ratios and alternate bearing indices. On the other hand, Khalily White and Shamouti were grouped together (Figure 2, Col. 3) due to their similarities in the vegetative measurements. These mid-season cultivars had trees with the largest size among the evaluated cultivars, including tree height, canopy diameter, volume, and trunk diameter. Similarities were also found among the early-season cultivars Diva, Midsweet, Westin, Cadenera and Rubi and the mid-season Berna Peret, Jaffa and Seleta do Rio for some characteristics, such as yield and technological index, and fruit quality parameters including juice content, TA, TSS, and number of seeds per fruit (Figure 2, Col. 2). All these cultivars scored high for almost all the above-mentioned parameters.

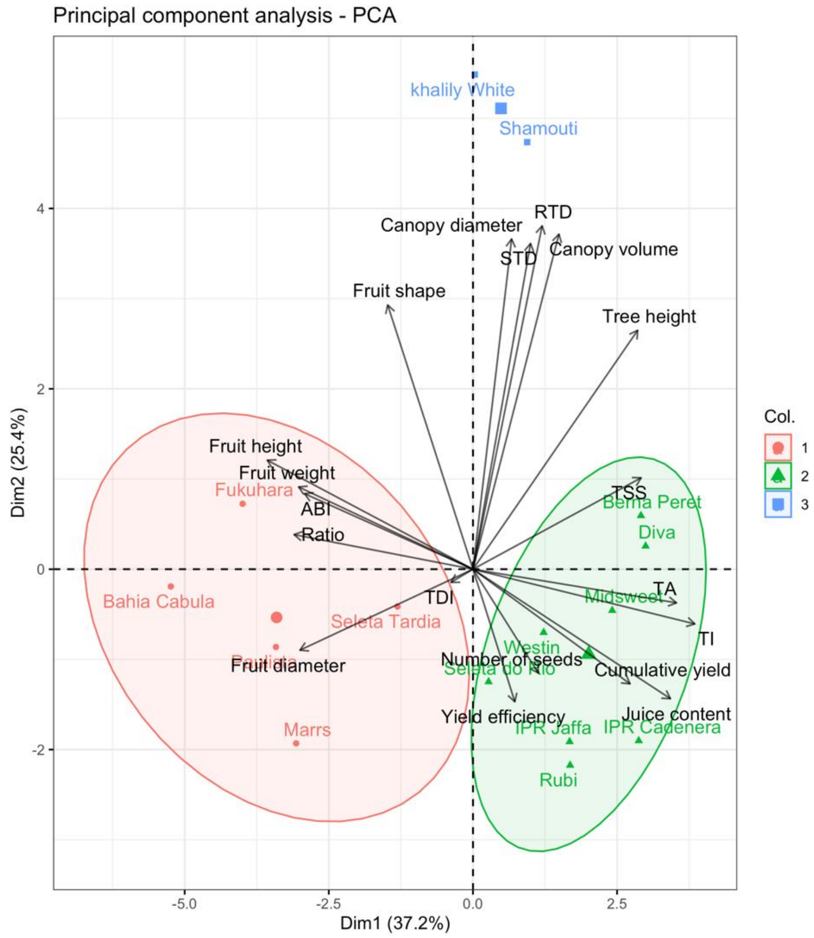


**Figure 2.** Principal component analysis (PCA) for vegetative growth, yield, and fruit quality variables of young trees of 15 early-season and mid-season sweet orange cultivars. The variables were arranged according to their principal component scores and the individuals (sweet orange cultivars) were grouped into three distinct clusters (Col.): 1, 2, and 3. Variables: tree height (m); canopy volume (m); canopy diameter (m); RTD—rootstock trunk diameter (cm); STD—scion trunk diameter (cm); TDI—trunk diameter index (STD RTD$^{-1}$); cumulative yield (kg tree$^{-1}$); yield efficiency (kg m$^{-3}$); ABI—alternate bearing index; TSS—total soluble solids (°Brix); TA—titratable acidity (g 100 mL$^{-1}$); ratio (TSS TA$^{-1}$); juice content (%); TI—technological index (kg TSS box$^{-1}$); fruit weight (g); fruit height (mm); fruit diameter (mm); fruit shape; and number of seeds per fruit.

## 4. Discussion

In our study, we evaluated the potential of early-season and mid-season sweet orange cultivars in the northwest region of the state of Paraná, Brazil. The lowest canopy volumes and tree heights were observed for the early-maturing Marrs and the mid-season Jaffa sweet oranges (Table 2). Both cultivars support high-planting density, which was demonstrated by the estimate study (Table 6). Tree height and canopy diameter are the main growth characteristics that determine adequate tree spacing for citrus orchards [32], and hence, the tree density. Lower canopy volume and tree height can provide higher planting densities and optimization of the field operations [32,33]. Based on the canopy volume, Marrs allows 582 trees ha$^{-1}$, with a tree spacing of 6.2 × 2.8 m between and within rows, respectively, whereas Jaffa can be planted at density of 598 trees ha$^{-1}$ using 6.1 × 2.7 m spacing. These densities are similar to those currently used in Brazilian citrus orchards [34]. In addition, these findings agree with previous descriptions of these two cultivars, which indicated their trees having a medium size and low vegetative vigor [35–38]. On the other hand, the mid-season cultivars Shamouti and Khalily White had the tallest trees with the largest canopy volume, which resulted in the lowest estimates of planting densities, with less than 450 trees ha$^{-1}$ (Table 6). Hodgson [35] described these two sweet orange cultivars as moderately vigorous and similar in appearance. According to Albrigo et al. [39], Shamouti trees have the most upright growth compared to all other commercially sweet orange cultivars.

Tree size is a valuable characteristic to the current citrus industry, as it has a major impact on orchard planning and management, including pruning, harvesting, and spraying for the control of insect pests and diseases. Furthermore, it is also known that small to medium-sized trees help to reduce the impact of HLB [40,41]. Under HLB pressure, tree density has been increased in citrus orchards to minimize the economic and detrimental impact caused by the removal of affected trees [7,40], where the HLB vector, the Asian citrus psyllid (*Diaphorina citri* Kuwayama), is regularly controlled [42].

In regard to the graft compatibility studies, trees of all early-season and mid-season cultivars grafted on Rangpur lime showed trunk diameter indices close to 1.0 (Table 2). These indices indicated that the rootstock and the scions were fully graft-compatible [43], at least during the first seven years of trees being in the field, as no overgrowth or tree decline was observed. These results support the findings of Bastos et al. [44], who reported a high graft affinity of Rubi, Valencia and Pera with Rangpur. In that study, the trunk diameter indices for Rubi and Rangpur lime combination were high and similar to our findings (0.87). The graft compatibility of different citrus species with Rangpur was studied by Alves et al. [45]. They showed that most species were graft-compatible with this rootstock, except for *Limonia acidissima* (L.). Similarly, 15 scions showed adequate compatibility when grafted on Rangpur [46].

Sweet orange trees start to bear fruit within two to five years after planting [20,30], but become fully productive by age 10 to 12 [47]. However, early productions are demanded by the citrus growers due HLB, which tends to reduce the economic life of the orchards [47,48].

In our study, we observed that the less vigorous sweet oranges started to bear fruit at a younger stage than the vigorous cultivars (Tables 2 and 3). Moreover, most mid-season cultivars did not produce fruit before the trees were four years old (Table 2), including Khalily White, Fukuhara, Seleta Tardia and Shamouti. According to Barry et al. [2], early-maturing trees are usually less vigorous than mid- to late-maturing cultivars, which agrees with our findings (Table 2). Vigorous vegetative growth may limit the fruit production in young trees [19]. The most vigorous trees, with the highest tree height and largest canopy volume, were Shamouti and Khalily White mid-season sweet oranges (Table 2), scoring the lowest fruit load across the four harvest seasons. In general, the early-maturing Midsweet and the mid-season Berna Peret cultivars had higher yield performance in terms of yield efficiency, as well as annual and cumulative yields. Midsweet trees grafted on Swingle citrumelo are very productive at the early stage [49], and yield from 112 kg tree$^{-1}$ in year three to 297 kg tree$^{-1}$ after 10 years. The productive stability of Midsweet and Berna Peret cultivars was also confirmed in our study, as low alternate bearing indices were observed after four cropping seasons, similar to the mid-season Jaffa and Seleta do Rio.

Fruit weight and size were significantly different among the sweet orange cultivars (Table 4; Figure 2). The early-maturing Bahia Cabula and Marrs navel oranges had the heaviest and largest fruit, as well as the fruit produced by the mid-season Fukuhara. Based on Brazilian fresh citrus standards [28], the navel oranges were classified as medium size, ranging from 80 to 90 mm. The Paulista and Fukuhara common sweet oranges were also large, as the fruit diameters were all above 71 mm. On the other hand, the early-maturing Diva had the smallest fruit and was classified as medium size, at 65 to 71 mm in diameter [28]. All other sweet orange cultivars produced fruit of large size [27,28].

The number of seeds per fruit is an important attribute, as seedless fruit, or fruit with a low number of seeds, is preferred by consumers and by the processing industry. According to Hodgson [35], each citrus cultivar usually has a certain number of seeds per fruit, but the number of seeds may vary depending on several factors, including environmental conditions that can affect gamete growth, pollination, fertilization, development, and survival of the zygotic and nucellar embryos. The highest number of seeds per fruit was found in Midsweet and Seleta do Rio fruits. These two cultivars were classified as moderately seedy, with nine to 15 seeds, whereas all other cultivars were commercially seedless, with fewer than eight seeds per fruit [39].

Juice content is dependent on water relations, field management [20], and soil–climate conditions [39]. Fruit produced in humid subtropical growing areas usually develops high-quality orange juice, including higher juice content and a better Brix–acidity ratio [39,50]. Sweet orange fruit must have at least 35% juice content for the fresh market [27,28,50] and a minimum of 36 to 40% for the processing industry [50,51]. Low juice contents may affect fruit appearance and firmness, reducing the saleable weight, which may limit its marketability [50]. Based on these parameters, the fruit of almost all the sweet orange cultivars surpassed these standards when trees were seven years old. The fruit of the early-maturing Bahia Cabula and Paulista did not score the minimum juice content for fresh consumption and industrial processing. This fact may be related to the tree age, which plays an important role in fruit quality [52]. Young trees usually produce lower fruit quality than mature trees, as the photoassimilates address vegetative growth expenses instead of fruit quality. In this way, qualitative attributes such as juice content, TSS, TA, and maturation index (TSS $TA^{-1}$) in sweet oranges are directly affected by the tree age [53].

Juice quality is a major concern for sweet orange fruit destined for industrial processing, particularly TSS content [20]. The levels of TSS in orange juice also affect the fruit palatability and marketability [50]. This parameter relies mainly on fruit maturation at harvest and climate conditions [54], similarly to TA content. The early-maturing Midsweet and the mid-maturing Shamouti and Khalily White had the highest levels of TSS, similar to previous reports [35,49,55]. In general, fruit produced by almost all sweet orange cultivars had appropriate and acceptable TA levels to meet the fresh citrus market and the citrus industry requirements [27,56].

Citrus fruit is considered marketable when a minimum TSS $TA^{-1}$ ratio is reached, based on consumer's preference [50]. The minimum Brix–acidity ratio acceptance varies from one country to another and is regulated by different agencies. For fresh consumption, the Organization for Economic Cooperation and Development [27] requires 6.5:1 as a threshold before commercialization. The General Warehouses Company of São Paulo [28] demands a minimum ratio of 9.5 for the domestic market. On the other hand, the citrus-processing industry requires a ratio between 12 and 18 for orange juice production [56]. In this context, almost all sweet orange cultivars assessed in this study produced fruit that meets the standards for fresh consumption, apart from Cadenera, which scored the lowest TSS $TA^{-1}$ (8.8). The low ratio for this genotype was due to the elevated acidity, as the TSS met the market standards (Table 5; Figure 2). A similar result was found for this cultivar grown in northern Paraná, Brazil [57]. Regarding the industrial standards, few cultivars evaluated there reached the established ratio ($\geq$12), including the early-maturing Midsweet and the mid-season cultivars Khalily White, Fukuhara and Shamouti, since navel oranges are not recommended for processing as frozen concentrated orange juice (FCOJ) and not-from-concentrate (NFC) juice. Navel oranges have an excessively bitter taste, resulting from the development of limonin from limonoate A-ring lactone, which limits their use for juice processing [58].

The technological index (TI) is also taken into consideration to assess fruit quality in sweet oranges for industrial processing. This index indicates the amount of TSS in a standard citrus box of 40.8 kg and is based on juice content and TSS [30]. In our study, the most productive sweet orange cultivars also had the highest TIs, due to the high juice content and amount of TSS, including Midsweet, Berna Peret and Jaffa. Similar TIs were reported for the early-maturing Midsweet, at 2.85 kg $box^{-1}$, in a previous study [49], and for the mid-season Jaffa, at 1.83–2.82 kg $box^{-1}$ [49,59].

Taken together, based on the distinct agronomic traits, our results show that trees of the early-season Midsweet and the mid-season Berna Peret excelled in our field trial in northwestern Paraná. These genotypes also demonstrated some level of resistance to citrus canker in previous field experiments conducted in the same region [60] and even under greenhouse conditions [61]. Besides the agronomic performance, a certain resistance level to citrus canker is required for citrus planting in the state of Paraná, where the disease is endemic. These results reinforce the suitability of these cultivars in the humid subtropical climate of Brazil, similar to other important citrus-growing areas, such as those in eastern and south-central China, the southeastern United States, coastal areas of Mexico, northern Argentina, and Uruguay [62], contributing to orchard diversification and horticultural practices. This is particularly important in the state of Paraná, as few sweet orange cultivars are currently legally authorized for planting.

## 5. Conclusions

The early-season Diva had the tallest trees and the largest canopy diameter and volume, whereas Marrs trees were the smallest ones. Similarly, Shamouti and Khalily White trees were the most vigorous of all other mid-season cultivars but had low fruit yield at early ages.

Remarkably, the Midsweet trees exhibited the highest yield performance and technological indices among the early-season cultivars, similar to those found for the mid-season Berna Peret. These genotypes also produced fruit of high juice quality, satisfying the requirements imposed by the fresh and industrial markets.

In general, Midsweet and Berna Peret are more effective under the current situation faced by the citrus industry, as the economic life of orchards has been reduced due to HLB, in which genotypes that have a precocious tendency are advantageous. Taken together, these genotypes, previously reported as less susceptible to citrus canker under the same soil–climate condition, are precocious and exhibit higher agronomic potential to be planted in humid subtropical climates, including Brazil and other similar areas around the world.

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