



Citrus crop performance and fruit quality in response to different scion-rootstock combinations

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Abstract

Aim of study: To address diversification of citrus cultivars to increase the variety and profitability of orchards to adapt them to environmental changes.

Area of study: State of São Paulo, a subtropical region of southeastern Brazil.

Material and methods: The study evaluated the phenological intervals, thermal sum, vegetative and productive performance, and fruit quality of the sweet orange cultivars 'Rubi' (R), 'Lue Gim Gong' (LGG) and 'Valencia Delta Seedless' (VDS) grafted onto 'Rangpur' lime (RL) and 'Swingle' citrumelo (SC). The field experiment was conducted over consecutive growing seasons 2018-2021.

Main results: The duration of the phenological intervals was little influenced by the rootstocks. The harvest time was approximately 245 days after anthesis (DAA) for R, 402 DAA for LGG, and 407 DAA for VDS, regardless of rootstock. Scion cultivars grafted onto RL showed larger canopy volumes and greater weight, length, and diameter of fruits than those of SC trees, and the combinations with SC were more productively efficient than RL due to lower canopy volume. The R scion cultivar presented superior yield performance and fruit colouration than LGG and VDS. The physicochemical quality of the fruits showed improved results with the combinations of LGG/RL, LGG/SC, VDS/RL, and VDS/SC.

Research highlights: These results may be useful when planning the diversification of scion/rootstock combinations for new orchards, identifying dual-market orange cultivars for industrial processing and natural consumption, and determining the combinations that are better adapted to undesirable climatic conditions.

Additional key words: orange; degree-days; drought tolerance; phenology; ascorbic acid.

Abbreviation used: AA (ascorbic acid); DAA (days after anthesis); DD (degree-days); JY (juice yield); LGG ('Lue Gim Gong'); PCA (principal component analysis); R ('Rubi'); RL ('Rangpur' lime); SC ('Swingle' citrumelo); SS (soluble solids); TA (titratable acidity); TI (technological index); VDS ('Valência Delta Seedless').

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Introduction

Citrus fruits are rich sources of bioactive compounds with exceptional health benefits (Saini et al., 2022). Citrus cultivation areas are widespread globally, and Brazil is the world's largest orange producer, predominantly manufacturing orange juice (Domingues et al., 2021). Genetic diversity methodologies are a main challenge in expanding citrus orchards (Tietel et al., 2020), especially in relation to climate change adaptation and mitigation (Cataldo et al., 2021). There is global concern about the sustainability of citrus crops, particularly considering climate scenarios (Carvalho et al., 2021).

Brazilian citriculture is characterised by low genotypic diversification, centred on the use of a few combinations of scion-rootstock cultivars, thereby placing the culture in a position of vulnerability in relation to phytosanitary problems and economic viability (Carvalho et al., 2019).

The cultivar 'Rubi' (R) is originated in the active germplasm bank, located in the Araras municipality, São Paulo State, Brazil. The cultivar exhibits early fruit ripening, average size of 172 g, annual production of up to 40 t/ha, and juice yield of 49 % (Pio et al., 2005). 'Lue Gin Gong' (LGG) is a cold-tolerant Valencia-type cultivar which is tolerant to citrus canker (*Xanthomonas axonopodis* Starr & Garces emend. Vauterin et al. pv. *citri* (Hasse) Dye) and shows late maturation of the fruit, which under refrigeration conditions after harvest, can be preserved for longer than one month. The main limitations of this cultivar are its propensity to produce small fruits and its alternate bearing (Oliveira & Scivitaro, 2008). The 'Valencia Delta Seedless' (VDS) cultivar originated from spontaneous bud mutation of the 'Valencia' cultivar or by nucellar seedlings, with the 'Valencia' cultivar as genitor. It has tolerance to citrus canker, late ripening, and seedless fruits, but is also alternate bearing (Oliveira et al., 2008).

Grafting performed with the use of proper rootstocks can provide important improvements to the scion (Balfagón et al., 2022). Rootstocks interfere with the agronomic characteristics of scion cultivars, such as vegetative vigour; production precocity; fruit ripening time; water and nutrient absorption capacity; and drought, pest, and disease resistance (Bowman & Joubert, 2020). The rootstock can manipulate a grafted citrus tree by affecting ripening development, skin and juice colour, soluble solids, titratable acidity, and other fruit and juice qualities (Domingues et al., 2021).

The 'Rangpur' lime (RL; *Citrus limonia* Osbeck) tree is a natural hybrid of *Citrus medica* L. and mandarin (*Citrus reticulata* Blanco) and is suggested to be native to India (Curk et al., 2016). In Brazil, the rootstock of RL has been used in citrus orchards because of its compatibility with all scions, as well as its vigour, drought tolerance, high yield, precocity, and early fruit maturation (Oliveira et al., 2017). Although it is tolerant to the Citrus tristeza virus (CTV), it is susceptible to the Citrus exocortis viroid (CEVd) and

Citrus sudden death-associated virus (SCDaV) (Fadel et al., 2018).

'Swingle' [*Poncirus trifoliata* (L.) Raf × *Citrus paradisi* Macf.] (SC) is the most cultivated citrumelo in Brazil and worldwide. It is among the main rootstocks for diversifying orange groves, providing scions with high-quality fruits and low vigour. This cultivar is ideal for semi-dense planting in cooler locations although it is not compatible with all scions (Domingues et al., 2021). It is resistant to *Citrus* sudden death-associated virus and decline (Ribeiro et al., 2014).

The duration of phenological cycles, thermal requirements, vegetative and productive performance, and fruit quality attributes are among the most important variables for the evaluation of citrus scion-rootstock combinations. These variables are important for crop rationalisation and optimisation, as many cultural practices, such as pruning, fertilisation, phytosanitary management, and harvest time, depend on knowledge of the phenological cycles (Rivadeneira, 2012). The thermal sum is used in production scheduling, harvest planning, and climate zoning (Şekerli & Tuzco, 2020).

This study aimed to characterise the phenological intervals, thermal sum, vegetative and productive performance, and quality attributes of the fruits of three cultivars of sweet orange trees grafted on RL and SC rootstocks in the subtropical region of southeastern Brazil.

Material and methods

Experimental area characterisation

The experiment was conducted at the São Manuel Experimental Farm, School of Agriculture, São Paulo State University (FCA UNESP), Brazil (22°44'28" S, 48°34'37" W) located at an altitude of 740 m a.s.l. According to the Köppen-Geiger climate classification, the climate of the area is *Cwa*, or warm temperate (mesothermal) and humid, and the average temperature of the warmest month is approximately 22 °C (Cunha & Martins, 2009). The soil is classified as a sandy-textured Latossolo Vermelho distroférrico according to the Brazilian system of soil classification (Santos et al., 2013), that is, a dystrophic Typic Hapludox (Soil Survey Staff, 1999).

Plant material and crop management

A replicated trial was performed in three consecutive crop seasons (2018-2021) in a non-irrigated orchard of trees of three, four, and five years of age, respectively. Sweet orange trees were grafted onto RL and SC trees and planted with 6 m spacing between rows and 4 m spacing between trees (i.e. 416 trees/ha). The experimental area

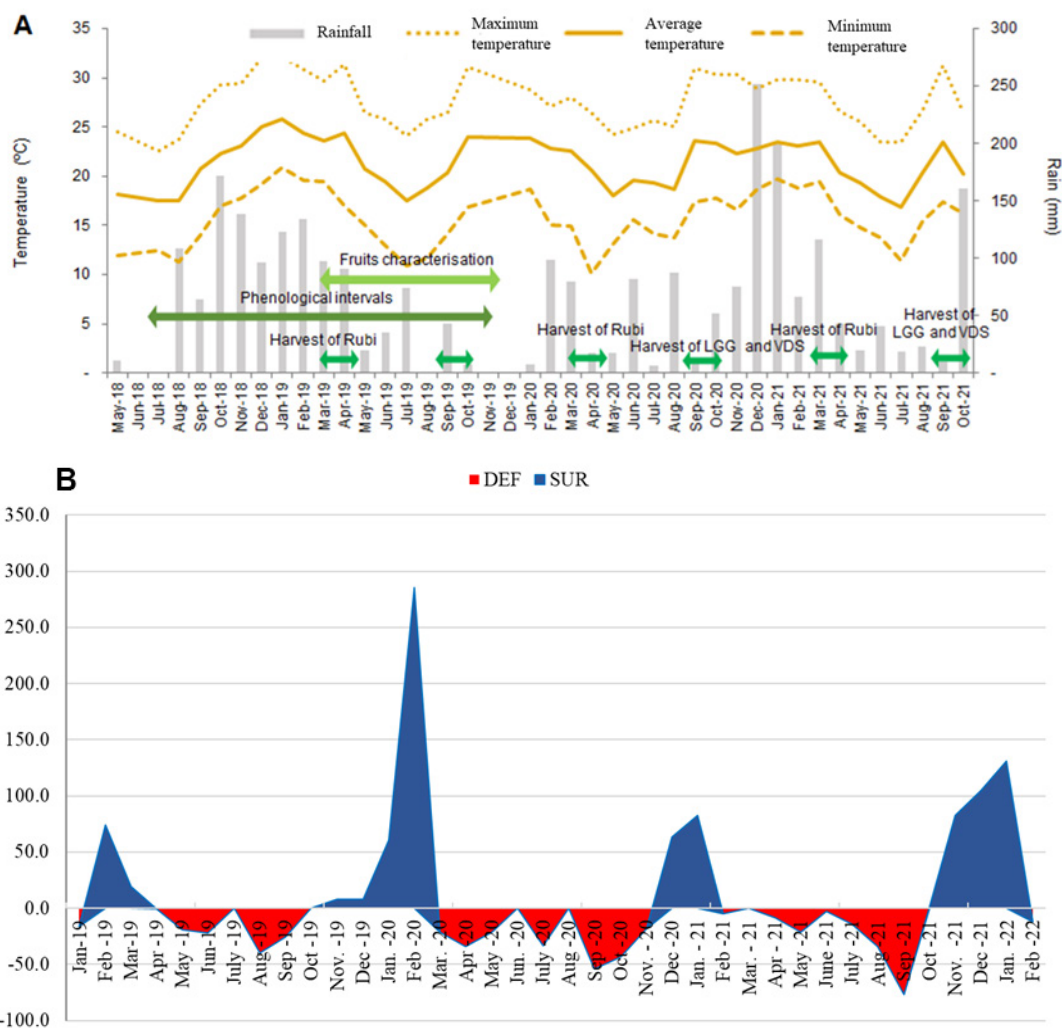


Figure 1. Precipitation, average, maximum and minimum monthly temperatures during the study period (A). Climatic water balance (B). DEF = deficit. SUR = surplus. LGG = ‘Lue Gim Gong’. VDS = ‘Valência Delta Seedless’.

was prepared based on soil analysis and orange crop recommendations, using ploughing, sorting, and liming. The trees received the standard management practices recommended for citrus orchards. Daily precipitation (mm) was obtained from a weather station located 100 m from the experimental area, along with the maximum, minimum, and average temperatures (°C) throughout the experimental period (Fig. 1A). Water balance was determined by adopting an average root system depth of 80 cm and an available water capacity of 70 mm (Thornthwaite & Mather, 1955) (Fig. 1B).

Treatments and experimental design

The treatments consisted of six scion/rootstock combinations: ‘Rubi’/‘Rangpur’ lime (R/RL), ‘Rubi’/‘Swingle’ citrumelo (R/SC), ‘Lue Gim Gong’/‘Rangpur’ lime (LGG/RL), ‘Lue Gim Gong’/‘Swingle’ citrumelo (LGG/SC), ‘Valência Delta Seedless’/‘Rangpur’ lime (VDS/RL),

and ‘Valência Delta Seedless’/‘Swingle’ citrumelo (VDS/SC). The experimental design was a randomised complete block in a 3×2 factorial scheme, with the first factor corresponding to the scion cultivars and the second factor represented by the rootstocks using five replicates. Each replicate consisted of three trees per experimental plot, with border trees external to the trial.

Phenological intervals

Six branches per tree were randomly chosen and marked in the midsection of the graft around the circumference. The phenological study began by evaluating the following stages: (3) floral opening (anthesis); (4) dried petals and stylet; (5) without petals or stylet; (6) fruits 3 cm in diameter; (7) fruits 4.5 cm in diameter; (8) unripe fruits approaching the final size; (9) fruits changing colour from green to yellow; and (10) ripeness index (RI) or ratio (soluble solid/titratable acidity) between 8.5 and 10. These

stages were based on the adapted BBCH scale (Agusti et al., 1995; Barbasso et al., 2005).

The branches were reviewed every 20 days, and grades of 0 to 10 were issued. These scores were calculated when 51 % of the branches were at the appropriate phenological stage. The intervals between stages were analysed for phenological characterisation of the scion/rootstock combinations. Each phenological interval was measured in days and calculated as the total degree-days (DDs), according to the models of Ometto (1981).

Yield

The total number of fruits harvested from each tree was counted. The average fruit weight (g) was obtained by weighing the fruits on a semi-analytical balance, and length and diameter (mm) were determined by measuring the longitudinal and equatorial diameters of the fruits using a digital caliper. The product of the total number of fruits and fresh weight of the fruits harvested/tree was considered the production/tree and was expressed in kg/tree. The yield (t/ha) was obtained using a stand of 416 trees/ha.

The canopy volume (m³) of each tree was estimated using Eq. 1 (Zekri, 2000).

$$\text{Canopy volume} = \frac{2}{3} \pi r^2 h, \quad (1)$$

where r = canopy radius and h = tree height.

Production efficiency (PE, kg m⁻³) was determined using Eq. 2:

$$\text{PE} = \text{PT}/\text{CAV} \quad (2)$$

where PT = production per tree (kg) and CAV = canopy volume (m³).

Fruit quality

Fruit quality evaluations began when the fruit had a ratio between 8.5 and 10. During this period, 10 fruits/tree were harvested and analysed. A colorimeter was used to assess skin and pulp colour. A texturometer with an SMS P/2 compression probe was used to measure the firmness by compressing the fruit 10 mm from the point of contact at a speed of 1.0 mm/s (N). Titratable acidity (TA) was determined by titration and expressed as a percentage of citric acid. Soluble solids (SS) were measured with a digital refractometer using three drops of juice and expressed in °Brix. The ripeness index (RI) or *ratio* was calculated as the relationship between soluble solid content and TA (Ribeiro et al., 2020).

The sugar content in the juice was estimated using the method described by Anjum et al. (2020). Ascorbic acid (AA) content was determined according to method 967.21

of the Association of Official Analytical Chemists (AOAC, 2019).

Juice yield (JY) was calculated as a percentage of the juice:fruit weight ratio and the results were expressed as percentages. The technological index (TI) was calculated using the equation proposed by Di Giorgi et al. (1990) and is expressed in kg SS/box (Eq. 3):

$$\text{Technological index} = (\text{JY} \times \text{SS} \times 40.8) / 10000, \quad (3)$$

where JY = juice yield (%); SS = soluble solids (°Brix); and 40.8 = standard weight of the box used to harvest oranges (kg).

Statistical analysis

Three-year data of the production and fruit quality variables were analysed using repeated measures, and the reported data are the mean of the two years of evaluation. Phenological intervals were evaluated in the growing season, corresponding to the period from August 2018 to November 2019. Data were subjected to analysis of variance (ANOVA) at 1 % and 5 % probability, and when the F test established significance, means were compared using Tukey's test. ANOVA was performed using AgroEstat® software. Principal component analysis (PCA) was performed (Rencher & Christensen, 2012) using XLSTAT version 2019.4.1 (Addinsoft, NY, USA), with the ellipse package for figure presentation.

To determine the correlation between variables, Pearson's correlation analysis was conducted with $\alpha = 5\%$. A heatmap was used for illustration, which was available in the corrplot, latticeExtra, and RColorBrewer packages of the R team software.

Results

Citrus orchard microclimate

The climate was atypical in the subtropical region, especially in the 2018 and 2019 crop seasons. Differences in rainfall patterns were observed during the three years of assessment (Fig. 1A). The years 2018 and 2019 were characterised by an uneven distribution of rainfall, mainly during the spring, with a dry period occurring at the time of the new flushing (Fig. 1B). The accumulated water deficit (611 mm) mainly affected the sprouting and production of the late cultivars, LGG and VDS, in the two evaluated rootstocks. The years 2020 and 2021 showed typical rainfall, and from January to March, the water balance showed a cumulative surplus, allowing the resumption of vegetative growth and subsequent flowering and fruit setting in all evaluated scion/rootstock combinations. Temperatures varied during the

Table 1. Analysis of variance and duration in days (D) and degree-days (DDs) of phenological intervals from sweet orange scion cultivars grafted onto two rootstocks.

	3-6		6-7		7-8		8-9		9-10	
	D	DDs	D	DDs	D	DDs	D	DDs	D	DDs
F_S	4.10*	5.19**	1.12 ^{ns}	0.27 ^{ns}	15.48**	14.58**	13.64**	2.38 ^{ns}	172.76**	195.55**
F_R	0.21 ^{ns}	0.04 ^{ns}	0.24 ^{ns}	0.18 ^{ns}	0.00 ^{ns}	0.00 ^{ns}	0.23 ^{ns}	0.17 ^{ns}	0.14 ^{ns}	0.00 ^{ns}
$F_{S \times R}$	1.43 ^{ns}	0.95 ^{ns}	0.40 ^{ns}	0.48 ^{ns}	0.59 ^{ns}	0.85 ^{ns}	0.32 ^{ns}	0.37 ^{ns}	0.14 ^{ns}	0.37 ^{ns}
Mean	51.66	545.14	72.20	941.52	102.08	1417.06	64.91	629.17	60.29	552.51
CV (%)	20.36	18.92	9.72	10.34	16.86	14.80	28.48	31.02	19.01	14.98
Scion										
R	43.12 ^b	450.61 ^b	74.75 ^a	953.91 ^a	83.12 ^b	1198.91 ^b	38.61 ^b	512.66 ^b	5.00 ^c	100.74 ^c
LGG	54.50 ^{ab}	577.84 ^{ab}	72.37 ^a	949.80 ^a	94.12 ^b	1315.15 ^b	70.00 ^a	621.95 ^a	111.25 ^b	897.88 ^a
VDS	57.38 ^a	606.97 ^a	69.50 ^a	920.86 ^a	129.00 ^a	1737.12 ^a	86.12 ^a	752.92 ^a	64.62 ^a	658.90 ^b

** = differ statistically at 1%; * = differ statistically at 5%; ns = did not differ statistically by F test at <0.05. F_S = F value of scion, F_R = F value of rootstock; $F_{S \times R}$ = F value from interaction scion \times rootstock. Means followed by the same letter, in the row (scion) did not differ statistically, by the Tukey test at 5% probability level. R = 'Rubi'; LGG = 'Lue Gin Gong'; VDS = 'Valencia Delta Seedless'; S = scion; R = rootstock. Phenological intervals: 3-6 = anthesis to fruit \sim 3 cm; 6-7 = fruit \sim 3 cm to fruit \sim 4.5 cm; 7-8 = fruit at \sim 4.5 cm to \sim normal fruit size; 8-9 = fruits with normal size until the fruits change colour; 9-10 = fruit with colour change until ripening index 12.

study period, as expected for the region, except in 2019, when more days and hours of minimum temperatures occurred during winter, and the temperature range was greater. The flowering and fruiting periods showed greater variation in spring 2019 due to environmental conditions and scion/rootstock combinations, with intense fruit drop, especially for the LGG and VDS cultivars.

Phenological intervals and thermal sum

The rootstocks did not influence the duration between the phenological intervals of the R, LGG, and VDS orange trees (Table 1). The cultivar R, on average, regardless of the rootstock combination, had a total cycle length of 245 days, with a thermal sum of 3216.85 DDs, classified as an early maturing orange, with fruit ripening between the months of March and April in the three seasons evaluated. Regardless of rootstock, the cultivar LGG had on average, a total cycle length of 402 days and 4493.61 DDs, whereas VDS averaged a 407-day cycle length and 4545.81 DDs, which are both classified as late-maturing oranges, with fruit ripening between the months of September and October in all seasons evaluated.

Vegetative and productive combination performance

The interaction between scion/rootstock combinations had no significant effect on the number of fruits/tree; weight, length, and diameter of fruits; yield; canopy volume; and productive efficiency (Table 2). Differences were observed between scions for number of fruits/tree, fruit diameter, yield,

canopy volume, and production efficiency. The weight and length of the fruits differed only between the rootstocks. The RL rootstock induced the production of fruits with greater weight, length, and diameter than those of the SC rootstock, regardless of the scion. The scion cultivars showed differences in the number of fruits/tree and yield, with the highest values obtained for cultivar R, followed by LGG and VDS, respectively. The largest fruit diameters were obtained with the R and VDS cultivars.

Scion cultivars grafted onto RL showed larger canopy volumes than those of the SC trees. The combinations with SC showed greater productive efficiencies than those with RL. The R scion cultivar presented better yield performance than LGG and VDS, suggesting that the higher efficiency obtained by the genotypes was associated with lower canopy volume.

Fruit quality

The scion/rootstock combinations had an effect on the TA, ripeness index, total sugars, reducing sugars, non-reducing sugars (Table 3), firmness, and juice yield (Table 4). The fruits of the LGG/RL, VDS/RL, LGG/SC, and VDS/SC combinations showed higher acidity than those of the R/RL and R/SC combinations. The rootstocks on each scion influenced the total and reducing sugar contents for the LGG and VDS scions, with fruits on the SC rootstock showing higher concentrations. On the RL rootstock, the orange cultivar VDS had the highest total and non-reducing sugar content, while on the SC rootstock, the R cultivar had the lowest total, reducing, and non-reducing sugar contents.

The rootstocks of each scion influenced the contents of total and reducing sugars for LGG and VDS, with fruits on

Table 2. Analysis of variance, vegetative, and productive variables of the sweet orange scion cultivars grafted onto two rootstocks.

	No. of fruits/tree	Weight of fruits (g)	Length of fruits (cm)	Diameter of fruits (cm)	Yield (kg/tree)	Canopy volume (m ³)	Productive efficiency (kg/m ³)
F_S	36.21**	1.00 ^{ns}	1.89 ^{ns}	4.02*	23.75**	26.87**	98.60**
F_R	0.01 ^{ns}	11.33**	15.49**	15.38**	0.77 ^{ns}	13.88**	4.35*
$F_{S \times R}$	0.33 ^{ns}	1.77 ^{ns}	0.41 ^{ns}	0.08 ^{ns}	0.51 ^{ns}	1.40 ^{ns}	1.43 ^{ns}
Mean	84.21	167.97	65.18	65.74	11.45	4.57	4.51
CV (%)	37.08	14.41	3.81	4.63	26.45	23.81	24.22

	Scion			Rootstock	
	R	LGG	VDS	RL	SC
Number of fruits/tree	189.00 ^a	51.00 ^b	13.00 ^c	-	-
Weight of fruits	-	-	-	184.60 ^a	151.34 ^b
Length of fruits	-	-	-	67.17 ^a	63.18 ^b
Diameter of fruits	67.69 ^a	63.42 ^b	66.09 ^a	68.17 ^a	63.30 ^b
Yield	20.21 ^a	10.05 ^b	4.00 ^c	-	-
Canopy volume	3.27 ^b	3.58 ^b	6.87 ^a	5.40 ^a	3.75 ^b
Productive efficiency	10.67 ^a	2.50 ^b	0.30 ^c	3.86 ^b	5.17 ^a

** = differ statistically at 1%; * = differ statistically at 5%; ns = did not differ statistically by F test at <0.05. F_S = F value of scion, F_R = F value of rootstock; $F_{S \times R}$ = F value from interaction scion \times rootstock. Means followed by the same letter, in the row (scion and rootstock) did not differ statistically, by the Tukey test at 5% probability level. R= 'Rubi'; LGG= 'Lue Gin Gong'; VDS = 'Valencia Delta Seedless'; RL= 'Rangpur' line; SC= 'Swingle' citrumelo.

the SC rootstock showing higher amounts. In the RL rootstock, the cultivar VDS had the highest total and non-reducing sugar content, and in the SC rootstock, cultivar R had the lowest total, reducing, and non-reducing sugar content.

The LGG scion differed between rootstocks in terms of the ripeness index, with a higher index in SC, and a lower index in the RL rootstock. There was no difference between the SC rootstocks. Fruits with lower firmness were observed in the R/RL, VDS/RL, and VDS/SC combinations. A lower juice yield was obtained with the VDS/SC combination.

The scions presented different colour characteristics, with VDS showing lower lightness (L^*), a^* positive (red), b^* positive (yellow), colour saturation (chroma), and an angle hue closer to 90 ° (yellow) than the other scions. The cultivar R had brighter fruits, also showed a yellow hue, and did not differ from the cultivar LGG for this variable.

There was an isolated effect of scions on soluble solids and AA contents. The highest soluble solid contents were obtained in the VDS and LGG cultivars, and the highest concentrations of AA were obtained with the VDS and R cultivars. The technological index presented an isolated effect between rootstocks with higher values related to SC, in contrast to RL.

tances of the characteristics evaluated according to the regions (groups) (Fig. 2). The scion/rootstock combinations VDS/CS and VDS/LC presented clustering patterns which were distinct from the R/CS, R/LC, LGG/CS, and LGG/LC combinations. This result indicates that there was a similarity between cultivars R and LGG. The groupings between the combinations occurred mainly as a function of the scion cultivars, as there was no grouping of combinations as a function of the rootstocks, with an isolated effect of the rootstock only for some variables.

The combinations VDS/SC, VDS/RL, LGG/SC, and LGG/RL showed positive correlations with the variables days and DDs, because they are late ripening cultivars. The R/SC and R/RL combinations showed negative correlations, indicating the precocious ripening of this cultivar. The variables related to productive performance suggested that the VDS/LC and VDS/CS combinations were negatively correlated with the lowest yields. However, these combinations showed positive correlations with the concentrations of total, reducing, and non-reducing sugars, in addition to hue angle.

PCA

The total variability of the data was explained by the first three principal components. Cumulatively, the first two PCs, PC_i and PC_e, represented 53.61 % of the total variation (Fig. 3A). The PCA identified the scores of the

Correlation analysis and colourmap

Data from the correlation analysis, which was illustrated through a heatmap, allowed to detail the relative dis-

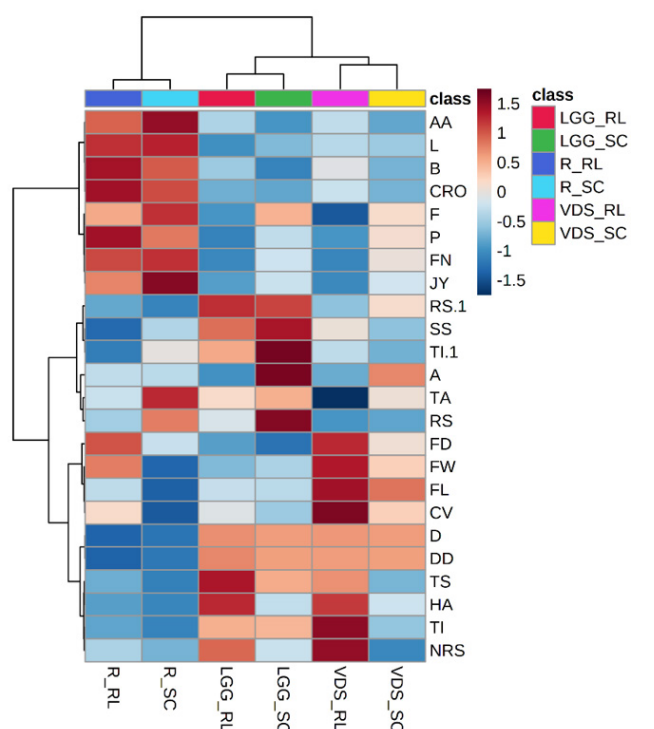


Figure 2. Heatmap for variables correlations of the sweet orange scion cultivars grafted onto two rootstocks. Variables in light blue are not significant ($p \leq 0.05$). AA= ascorbic acid; L= luminousness; B= b coordinate; CRO= chroma; F= Firmness; PE= productive efficiency; FN= fruit number; JY= juice yield; RS= reducing sugar; SS= Soluble solids; TI= technological index. A= a coordinate; TA= titratable acidity; FD= fruit diameter; FW= fruit weight; FL= fruit length; CV= canopy volume; D= days; DD= degree days; TS= total sugar; HA= Hue angle; Y= yield (kg/tree); RI= ripeness index (ratio); NRS= non-reducing sugar; Combinations: R/RL = ‘Rubi’/‘Rangpur’ lime; R/SC = ‘Rubi’/‘Swingle’ citrumelo; LGG/RL = ‘Lue Gim Gong’/‘Rangpur’ lime; LGG/SC = ‘Lue Gim Gong’/‘Single’ citrumelo; VDS = ‘Valência Delta Seedless’/‘Rangpur’ lime; VDS/SC = ‘Valência Delta Seedless’/‘Swingle’ citrumelo.

six combinations, which identified the two distinct regions: the first formed by VDS/RL, VDS/SC, and LGG/RL, and the second by LGG/SC, R/SC, and R/RL (Fig. 3B). Variability was explained by two principal components, F1 and F2, which accounted for 39.2 % and 19.4 % of the variation in the data, respectively (Fig. 3B). The PCA projection showed separation among the studied scion/rootstock combinations, that is, some combinations were grouped based on the similarity of the variables.

By positioning the scores in the PCA, it was possible to infer that the rootstocks had little influence on the distinction of clusters, which was affected mainly by the differences between the scion cultivars, as was also evidenced in the heatmap. The cultivars VDS and LGG showed high similarity. In addition to this grouping, the R cultivar had sufficient distinct characteristics to group it separately. The dissimilarity between the VDS/SC and VDS/RL group-

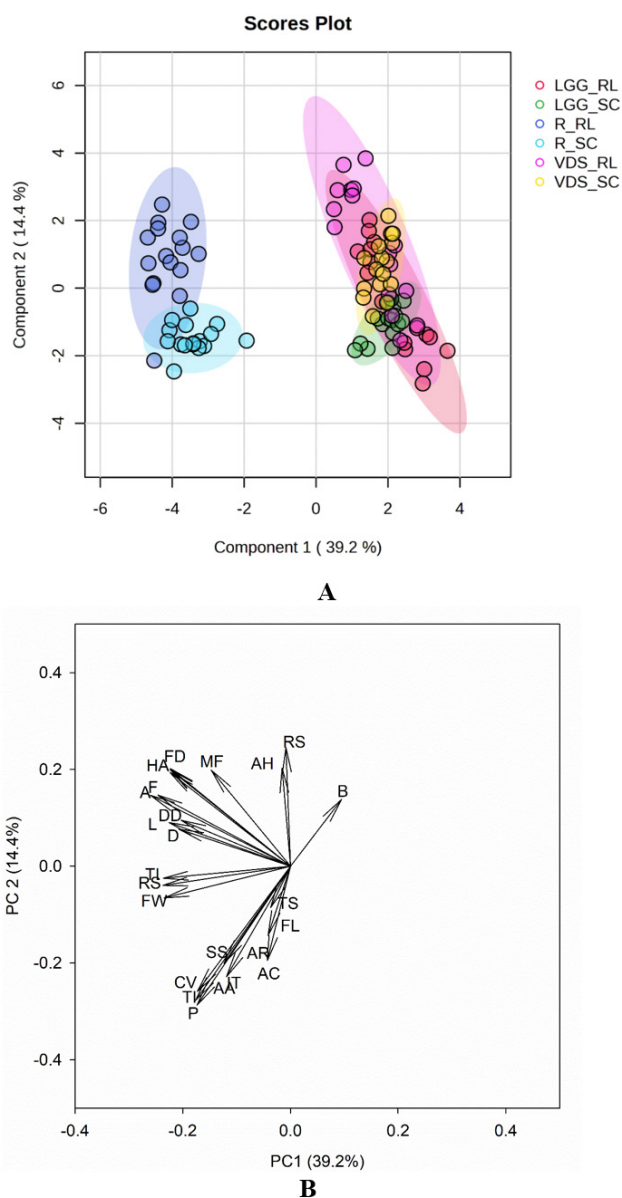


Figure 3. Principal component analysis (PCA) of the of the sweet orange scion cultivars grafted onto two rootstocks: Biplot of the scion/rootstock combinations (A); PCA of the F1 and F2 components (B).

ings can be explained by the physicochemical variables of VDS.

The R/RL and R/SC combinations were positively correlated with the fruit number, canopy volume, fruit firmness, chromaticity, and AA concentration. The LGG/SC and R/SC combinations were positively correlated with the yield.

The LGG/RL and VDS/RL combinations were negatively correlated with fruit diameter, length and weight; canopy volume; and production efficiency. The TA and RI were positively correlated with the R/SC and LGG/RL combinations. VDS/SC and LGG/SC were negatively correlated with reducing, non-reducing, and total sugar content; soluble solids; ripeness index; and hue angle.

Table 3. Analysis of variance and physicochemical variables of the sweet orange scion cultivars grafted onto two rootstocks.

	Titrateable acidity (% citric acid)	Soluble solids (°Brix)	Ripeness index (ratio)	Total sugars (%)	Reducing sugars (%)	Non-reducing sugars (%)	Ascorbic acid (mg/100g)
F_S	15.74*	34.32*	0.41 ^{ns}	61.90**	17.09**	62.67**	12.08**
F_R	7.83*	20.80*	0.61 ^{ns}	7.51**	11.02*	0.68 ^{ns}	0.00 ^{ns}
$F_{S \times R}$	3.43*	3.19 ^{ns}	3.65*	8.75**	6.20*	6.34**	0.41 ^{ns}
Mean	1.34	10.41	7.96	7.10	3.56	3.36	68.85
CV (%)	10.41	7.72	12.90	10.23	13.08	16.58	9.31
	R		LGG		VDS		
Soluble solids	8.52 ^b		11.06 ^a		11.66 ^a		
Ascorbic acid	72.81 ^a		59.78 ^b		73.96 ^a		
	R/RL	R/SC	LGG/RL	LGG/SC	VDS/RL	VDS/SC	
Titrateable acidity	1.02 ^{Ab}	1.20 ^{Ab}	1.48 ^{Aa}	1.44 ^{Aa}	1.28 ^{Ba}	1.61 ^{Aa}	
Ripeness index	8.20 ^{Aa}	7.45 ^{Aa}	6.87 ^{Ba}	8.76 ^{Aa}	8.30 ^{Aa}	8.15 ^{Aa}	
Total sugars	5.35 ^{Ab}	4.54 ^{Ab}	6.31 ^{Bb}	8.49 ^{Aa}	8.40 ^{Ba}	9.52 ^{Aa}	
Reducing sugars	2.98 ^{Aa}	2.72 ^{Ab}	3.81 ^{Ba}	4.60 ^{Aa}	2.94 ^{Ba}	4.31 ^{Aa}	
Non-reducing sugars	2.26 ^{Ab}	1.73 ^{Ac}	2.37 ^{Bb}	3.70 ^{Ab}	5.18 ^{Aa}	4.95 ^{Aa}	

** = differ statistically at 1%; * = differ statistically at 5%; ns = did not differ statistically by F test at <0.05. F_S = F value of scion, F_R = F value of rootstock; $F_{S \times R}$ = F value from interaction scion × rootstock. Means followed by the same letter, upper case in the column (scion) and lower case in the row (rootstock) did not differ statistically, by the Tukey test at 5% probability level. RL= ‘Rangpur’ lime; SC= ‘Swingle’ citrumelo; R= ‘Rubi’; LGG= ‘Lue Gin Gong’; VDS= ‘Valência Delta Seedless’.

Discussion

The ability of citrus trees to adapt to different environmental fluctuations allows for a wide geographical distribution, which has resulted in distinct vegetative and productive performances (Nascimento et al., 2018). The subtropical wetlands of southeast Brazil have highly variable climatic conditions caused by conflicting tropical and subtropical air masses, which result in insufficient water balances in certain years or locations and enhance the risk of citrus flushes with reduced intensities (Carvalho et al., 2021). The vegetative growth of citrus trees occurs during seasonal cycles or flushes throughout the year. Flushes are normally more intense in spring, less intense in early autumn, and absent in winter (Marks, 2022). New shoots emerge in late spring and early summer, coinciding with an increase in temperature and water availability (Kistner et al., 2016).

Water scarcity and high temperatures are among the most common adverse conditions worldwide (Balfagón et al., 2022). Under favourable temperatures, citrus can be forced to grow at almost any time by manipulating the water supply (Teodoro et al., 2020). The results obtained in this study confirm these reports and reinforce the high drought tolerance of the RL rootstock, resulting in high flushing and subsequently, flowering and fruit setting, regardless of the scion. The SC rootstock had medium tolerance to water shortages, which rendered it more suscep-

tible to unfavourable conditions, mainly in the 2018 and 2019 seasons, which affected the performance within the scion combinations (Ribeiro et al., 2014). Drought induces stomatal closure, reduces CO₂ intake and photosynthetic activity, increases oxidative stress, and causes membrane damage (Vincent et al., 2020). The differences between the rootstocks evaluated can be attributed to the root distribution, water absorption and transport efficiency, and the opening and closing of leaf stomata (Castle, 2010). França et al. (2016) reported that the production of trees grafted onto RL was greater than that of trees grafted onto SC in a dry land system using the ‘Valencia Tuxpan’ orange. These observations suggest that, depending on the region and the rootstock used, irrigation may be beneficial, as with the SC rootstock, as water deficit can have a major impact on fruit setting.

The results showed that rootstocks had either no effect or a minor effect on the duration of the phenological intervals in all crop seasons evaluated. To determine the proper harvest time for optimal fruit quality, it is important to identify growth, flowering, and fruit development intervals (Nascimento et al., 2018). According to Rivadeneira (2012), the thermal sum of phenological intervals is unique to each cultivar and may influence the growing region, which reinforces the importance of local studies. Neves et al. (2018) observed similar results to this study, concluding that some mandarin cultivars did not present variations in the interval between flowering and harvest.

Table 4. Analysis of variance, colour parameters, firmness, juice yield and technological index of the sweet orange scion cultivars grafted onto two rootstocks.

	L*	a*	b*	Chroma	Hue angle	Firmness (N)	Juice yield (%)	Technological index
F_S	37.57**	21.99**	48.83**	98.69**	16.67**	77.59**	1.28 ^{ns}	17.52*
F_R	0.15 ^{ns}	2.15 ^{ns}	2.56 ^{ns}	0.67 ^{ns}	3.05 ^{ns}	5.51*	1.50 ^{ns}	15.17*
$F_{S \times R}$	0.25 ^{ns}	1.41 ^{ns}	0.29 ^{ns}	1.56 ^{ns}	0.88 ^{ns}	3.45*	3.60*	1.10 ^{ns}
Mean	59.49	12.39	45.94	6.99	76.47	108.56	46.96	1.99
CV (%)	3.64	18.10	10.65	7.37	9.50	12.96	4.80	9.70

	Scion			Rootstock	
	R	LGG	VDS	RL	SC
L*	63.49 ^a	60.66 ^b	54.32 ^c	-	-
a*	16.05 ^a	19.92 ^a	11.20 ^b	-	-
b*	56.76 ^a	48.19 ^b	32.88 ^c	-	-
Chroma	59.71 ^a	53.01 ^b	33.00 ^c	-	-
Hue angle	74.37 ^b	67.19 ^b	87.85 ^a	-	-
Technological index				1.83 ^b	2.14 ^a

	R/RL	R/SC	LGG/RL	LGG/SC	VDS/RL	VDS/SC
Firmness (N)	101.55 ^{Bb}	136.32 ^{Aa}	143.97 ^{Aa}	148.60 ^{Aa}	59.93 ^{Ac}	61.01 ^{Ab}
Juice yield (%)	46.42 ^{Aa}	48.67 ^{Aa}	45.71 ^{Aa}	49.13 ^{Aa}	47.07 ^{Aa}	44.77 ^{Ab}

** = differ statistically at 1%; * = differ statistically at 5%; ns = did not differ statistically by F test at <0.05. F_S = F value of scion, F_R = F value of rootstock; $F_{S \times R}$ = F value from interaction scion \times rootstock. Means followed by the same letter, upper case in the column (scion) and lower case in the row (rootstock) did not differ statistically, by the Tukey test at 5% probability level. R= 'Rubi'; LGG= 'Lue Gin Gong'; VDS = 'Valencia Delta Seedless'; RL= 'Rangpur' line; SC= 'Swingle' citrumelo.

Selection of an appropriate scion-rootstock combination is necessary for successful citrus production, and can result in important improvements for the scion, such as reduction of the juvenile period, promotion of homogeneous tree architecture, increase in yield, improvement of fruit quality, protection against pests and diseases, and suitable tolerance to abiotic stress factors (Rasool et al., 2020).

Canopy volume is among the factors that determine the most appropriate spacing for a given scion/rootstock combination and consequently allows for better management of trees (Pompeu Júnior & Blumer, 2011). Forner-Giner et al. (2014) and Bacar et al. (2017) stated that rootstocks that provide low vigour and high yield are more convenient than those with high vigour and yield, as these characteristics can compensate for the yield by an increased density of plants. The results showed that the scion cultivars grafted onto RL had greater canopy volume and lower productive efficiency than those grafted onto SC. Similar results were reported by Rodrigues et al. (2019), who evaluated the performance of the orange tree 'Pêra' on RL and SC rootstocks. Knowledge of the impact of rootstock on the vegetative growth of citrus tree canopies can be used to programme the planting density of new orchards and subsequent cultural practices, such as pruning, fertilising, and harvesting (Carvalho et al., 2021).

The greater fruit weight, length, and diameter produced in combination with the RL rootstock may be attributed to the robustness of this rootstock, as it has a deep root system

that allows greater absorption of water and nutrients (Prieto et al., 2012). Petry et al. (2015) found that the average mass of 'Monte Parnaso' orange fruit grown on RL rootstock was greater than that grown on SC rootstock. Cruz et al. (2019) reported the opposite when analysing the quality of 'Navelina' orange fruit grown on these rootstocks, indicating that this is a genotype-dependent variable.

There are few reports on the vegetative and productive performance of VDS, although it is important to evaluate this orange cultivar because most consumers prefer to consume seedless oranges. The VDS cultivar had trees with larger canopies than R and LGG. The data obtained in the present study (4 kg/tree) showed that this cultivar had a smaller yield than the others evaluated, but it was within the expected range for this cultivar, since Arenas-Arenas et al. (2016), who evaluated the production of VDS in a young three-year-old commercial orchard, obtained an average yield of 2.5 kg fruit/tree.

The effect of rootstock on fruit quality can be attributed to several factors such as nutrient absorption and transport, compatibility, hormonal signalling, and gene expression (Tietel et al., 2020). The rootstock plays an important role in fruit ripening because it can accelerate or delay citrus tree development (Domingues et al., 2021).

The harvest quality and optimal citrus harvest time are based on the SS content and TA and their relationship (RI). The RI represents the balance between the sugar and organic acid content in the fruit; it is associated with juice taste and

is widely used in the orange juice industry as an indicator of ripening and fruit quality (Domingues et al., 2021).

The acidity of fruits is influenced by nutritional and climatic factors, mainly temperature and water supply. During fruit ripening, there is a natural decrease in acidity due to dilution caused by the accumulation of water in the fruit; that is, the amount of water influences the concentration of acids (Fonfria, 2020). All the scion/rootstock combinations studied, especially the fruits of the cvs. LGG and VDS, presented TA concentrations higher than the 9.5 %, established by the classification standard of CEAGESP (2011) for fruits intended for natural consumption. This result may be related to climatic conditions. The harvests of October 2019 and 2020 were conducted during a dry period, and the trees were under water deficit. This may have been responsible for the loss of water from the fruits and, consequently, for the increase in acidity of the fruits.

The highest concentrations of SS and RI were obtained in the fruits of the trees grafted onto SC and the scion cultivars LGG and VDS. According to Teodoro et al. (2020), fruits kept longer on the trees finish their maturation stage and tend to present a higher content of soluble solids. Therefore, they have a higher RI compared to early- and mid-season fruits. This also favoured the increase in sugars in the late-maturing fruits of cvs LGG and VDS, which had the highest sugar concentrations. Another hypothesis for the superiority of SC relates to the length and diameter of the fruits by the concentration effect, since the fruits of the trees grafted on SC were smaller.

‘Swingle’ citrumelo is among the main rootstocks used in the diversification of orange groves because it provides scions with high-quality fruits with high juice yield and SS contents (Castle, 2010; Pompeu Júnior & Blumer, 2011). Scions on trifoliolate orange rootstocks and their hybrids, as SC, produced better-quality fruits than those on other commonly used rootstocks. This has been well documented, but the genetic factors affecting fruit quality through the interaction between the scion and rootstock remain unclear. The results obtained by Hu et al. (2022) demonstrated consistent correlations with the fruit quality of four ‘Daya’ mandarin cultivars grafted onto *P. trifoliata* rootstocks related to the differential gene expression of small RNAs.

The AA concentration varied according to the scion and growing season. This may have been influenced by weather conditions, since rainfall occurred from March onwards and temperatures remained high, resulting in an increase in concentration due to water loss in the fruits. There were periods of severe drought during the assessment period. AA biosynthesis is generated from D-glucose, with nucleotides and sugars as intermediates, and the SC rootstock presents lower tolerance to water deficit, limiting the photosynthetic capacity of the tree, which may explain these results (Loannidi et al., 2009). Photosynthesis, temperature, and light exposure can affect AA synthesis and production (Lee & Kader, 2000). Environmental factors, such as irradiation and stress, can stimulate the expression of genes involved

in AA production (Loannidi et al., 2009). Orange is a rich source of AA, which has several biological functions related to the immune system, collagen formation, iron absorption, nitrosamine inhibition, and antioxidant activity; therefore, the VDS and R scions which have higher AA contents, are relevant (Castillo-Velarde, 2019).

The colour of the fruit is the most important variable in attracting consumers. The change in colour occurs due to the ripening of the fruit and the climatic conditions of the growing region. The ripening of citrus fruits passes through different phenological stages, and the final stage is characterised by a reduced growth rate and changes in skin colour (Domingues et al., 2021). Thermal amplitude with hot days (20-25 °C) followed by cool nights (10 °C) favours fruit pigmentation because it stimulates the synthesis of carotenoids and the breakdown of chlorophyll (Balfagón et al., 2022). Temperatures above 20 °C were recorded during the fruit ripening period, benefiting skin colour, and the data obtained explained the greater pigmentation of the dominant yellow fruit in the RL/RL and RL/SC combinations. Moreover, the LGG and VDS scion cultivars, regardless of rootstock, produced fruits with a colour tending towards yellow, which is classified as a suitable colour according to the scale proposed by Pathare et al. (2013). The fruits of late ripening cultivars such as ‘Valencia’ orange also showed a yellowish-green hue near the harvest stage (Jomori et al., 2014).

Changes in fruit firmness result from advanced maturity. Lower firmness was likely associated with a more advanced stage of ripening, since the VDS/RL and VDS/SC combinations had the highest concentrations of sugars and soluble solids. The reduction in fruit firmness over the ripening period was related to the solubilisation of pectic compounds or the change from insoluble pectin to soluble pectin as fruit ripening progresses. These insoluble compounds are found in unripe fruits and are composed of carboxylic acids bound to calcium, resulting in calcium pectate (Légua et al., 2011).

Juice yield is a variable that can be used in planning new orchards to produce high-quality fruits with desirable features for the orange juice industry (Domingues et al., 2021). The SC rootstock produces thin-skinned fruits (Castle, 2010), which are positively correlated with higher juice yield (Légua et al., 2011). According to Domingues et al. (2021), when grafted onto *P. trifoliata* hybrid rootstocks, orange trees produce fruits with higher SS content and juice yield. All scion/rootstock combinations evaluated showed a JY above 35 %, according to the OECD (2010) standard. All scions grafted onto the SC rootstock presented a higher TI than that of the RL rootstock. The technological index includes both fruit weight and soluble solids, and the outcome of this variable is affected by the factors that influence the performance of these characteristics (Volpe et al., 2002). A decrease in juice yield causes a decline in the technological index (Di Giorgi et al., 1990).

In summary, the orange scion cultivars R, LGG and VDS, grafted onto the rootstocks RL and SC, completed the cycle from anthesis to harvest in 245 days with an accumulation of 3216.85 DDs; 402 days and 4493.61 DDs; and 407 days and 4545.81 DDs, respectively. Scion cultivars grafted onto RL showed larger canopy volumes and greater weight, length, and diameter of fruits, and the combinations with SC had greater productive efficiencies. The R cultivar showed better fruit yield and colouration and can be indicated for a dual-purpose market, both for fresh consumption and for processing into juice. The majority of the physicochemical variables of the fruit showed that the best results were found with the combinations LGG/RL, LGG/SC, VDS/RL, and VDS/SC, indicating the use of these combinations for the market of fruit for consumption *in natura*.

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