Australian Journal of Crop Science AJCS

Aust J Crop Sci. 19(03):236-245 (2025) | https://doi.org/10.21475/ajcs.25.19.03.p210 ISSN:1835-2707

Assessment of fruit quality of sweet orange trees grafted on different rootstocks through Mixed Models (REML/BLUP)

Lusiane de Sousa Ferreira[1*], Moises Zucoloto[2], Vinicius de Souza Oliveira[3], Renan Garcia Malikouski[4], Leonardo Lopes Bhering[5], Dimmy Herllen Silveira Gomes Barbosa[6]

[1]Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agronômicas, Botucatu, SP, Brazil, 18610-034 (ORCID 0000-0001-6625-3965)
[2]Universidade Federal do Espírito Santo, Alegre - ES, Brazil, 29.500-000 (ORCID 0000-0003-0539-4750)
[3]Universidade Federal do Espírito Santo, Alegre - ES, Brazil, 29.500-000 (ORCID 0000-0003-4068-1587)
[4]Universidade Federal de Viçosa, Viçosa, MG, Brazil, 36570-900 (ORCID 0000-0003-0957-4871)
[5]Universidade Federal de Viçosa, Viçosa, MG, Brazil, 36570-900 (ORCID 0000-0002-6072-0996)
[6]Embrapa Mandioca e Fruticultura, Cruz das Almas - BA, Brazil, 44380-000 (ORCID 0000-0002-4132-4226)

***Corresponding author: Lusiane de Sousa Ferreira** ✉

Received:* *01/09/2024

Revised:* *16/10/2024

Accepted:* *28/01/2025

Abstract: The objective was to relate, based on quality characteristics, the potential of fifty-nine (59) sweet orange cultivars grafted onto the 'Sunki Tropical' tangerine rootstock and the 'Trifoliata' citrandarin hybrids 'San Diego', 'Riverside', and 'Indio', using 236 combinations with mixed models. The orchard was established in 2015. The following parameters were evaluated at stage III (fruit maturation): fruit weight (g), juice yield (JY, %), total soluble solids content (TSS, °Brix), titratable acidity (TA, g of citric acid per L^{-1}), and the Ratio (TSS/TA). The mixed model methodology (REML/BLUP) was used to estimate/predict the fruit maturation parameters for different scion and rootstock combinations. The scion and rootstock combinations differed statistically for total soluble solids, titratable acidity, ratio, and juice yield. The rootstocks 'San Diego', 'Riverside', and 'Indio', and the 'Sunki Tropical' mandarin induced juice yields (55.05% to 60.05%), soluble solids (10.70°Brix to 12.38°Brix), and titratable acidity (0.50% to 0.66% citric acid) in sweet orange trees that were within quality standards. In the ranking, the combinations, in order, of 'Pera CNPMF D-9'/'Riverside', 'Pera Selection CNPMF C-32'/'Riverside', 'Pera Selection CNPMF D-3'/'San Diego', 'Pera Selection Olímpia'/'Sunki Tropical', 'Valência Selection CNPMF'/'Sunki Tropical', 'Diva'/'Sunki Tropical', 'Melrosa'/'Índio', 'Pera CNPMF D-6'/'Sunki Tropical', and 'Pera C-21'/'Riverside', scion and rootstock, respectively, stood out. These are combinations with greater potential for commercial exploitation because they meet quality standards.

Keywords: *Citrus* spp.; Cultivars; Commercial Potential; Maturation Parameters.
Abbreviation: CNPMF_ National Center for Research on Cassava and Fruit Crops of Embrapa; FW_ fruit weight; JW_ juice weight; JY_ juice yield; LRT_ likelihood ratio test; NaOH_ sodium hydroxide; REML/BLUP_ restricted maximum likelihood/best linear unbiased prediction; TSS_ total soluble solids; TA_ titratable acidity; TSS/TA_ total soluble solids/titratable acidity.

Introduction

Citriculture is responsible for the prominence of Brazilian fruit growing, which leads the world in orange juice production and export, in addition to its socioeconomic contributions (Alves, 2018; Soares Filho, 2019). The cultivation of sweet orange trees [*Citrus* × *sinensis* (L.) Osbeck] is concentrated in the Southeast Region, specifically in the state of São Paulo, which accounts for 82% of the national production (IBGE, 2023). The Northeast region is the second largest producer, with the states of Bahia and Sergipe accounting for 86% of the cultivated area in this region (IBGE, 2023). This prominence results from market demand, adaptability to soil and climatic conditions, availability of land, and adoption of technologies. However, approximately 90% of these citrus centers are cultivated with 'Pera' orange trees grafted onto 'Cravo' lemon trees (*Citrus* × *limonia* Osbeck), highlighting the urgent need for varietal diversification (Carvalho et al., 2020). The commercial exploitation of sweet orange trees is based on a narrow genetic base due to their horticultural characteristics (Carvalho et al., 2019). However, concentra-

Table 1. Soil analysis of the experimental area at a depth of 0-20 cm, Chão Bello Farm, Ibirapuã, BA, 2020.

pH	MO	P	K	S	Ca	Mg	Al	$H^+ + Al^+$	SB	T	V
(H_2O)	dag dm^{-1}	--- mg dm^{-3} ---			cmol$_c$ dm^{-3}						- % -
5.2	1.88	16	74	14	0.9	0.3	0.1	2.8	1.5	4.8	33.5

Extraction method for P and K: 0.05 mol/L HCl + 0.0125 mol/L H_2SO_4; S: 0.01 mol/L $Ca(H_2PO_4)^2$; Ca, Mg, and Al: 1 mol/L KCl; H^++Al^+: SMP buffer solution; pH in H_2O 1:2.5.
Source: Information provided by Chão Bello Farm regarding the soil analysis conducted in 2020.

Table 2. Deviance analysis for soluble solids concentration, titratable acidity, juice yield, and fruit ratio of 59 sweet orange trees from different groups, using different rootstocks, in the Extreme South of Bahia State, Ibirapuã, 2021.

Deviance analysis								
Source of variation	Soluble solids		Titratable acidity		Juice yield		*Ratio*	
	Deviance	LRT	Deviance	LRT	Deviance	LRT	Deviance	LRT
Scions and Rootstocks	864.95	121.11*	539.19	44.48*	6137.02	29.32*	3782.57	53.37*
Complete model	743.84		494.71		6107.71		3729.21	

^{ns} not significant; * significant at 5% probability of error according to the Chi-square test for the variables, by deviance analysis based on the likelihood ratio test (LRT), with significance > 3.84.

ting cultivation on a limited number of scion and/or rootstock varieties exposes phytotechnical and phytosanitary problems, which can affect the longevity of the orchard. Rootstocks have intrinsic characteristics that influence scion attributes, such as plant size, ripening time, fruit quality, and tolerance to biotic and abiotic stresses, as well as graft incompatibility. These factors are essential for selecting new scion and rootstock combinations (Girardi et al., 2021). Therefore, new studies with various regional combinations adapted to different edaphoclimatic conditions are essential.

To suggest new cultivars for regions without a tradition of the crop, it is necessary to evaluate the optimal harvest timing based on ideal parameters determined at the ripening stage, such as juice yield, titratable acidity, soluble solids, and ratio. For this to occur, it is necessary to determine physicochemical parameters to define maturation periods and achieve better fruit quality, as well as to establish different production times (Beber et al., 2018). Monitoring and determining the ripening stage are essential for defining the ideal harvest point and ensuring high-quality fruits. Physical quality standards reflect external appearance aspects such as size, shape, juice yield, transverse and longitudinal diameter. For chemical parameters, they include levels of soluble solids, titratable acidity, and the ratio (Chitarra and Chitarra, 2005). These parameters should be evaluated before harvest, as they reflect the fruits' post-harvest destination, which is linked to acceptability and industrial yield.

Pera orange trees grafted onto 'San Diego' and 'Riverside' citrandarins in the municipality of Rio Real (BA) resulted in smaller-sized trees with high production efficiency and a higher rate of productive alternation (Carvalho et al., 2021). The cultivars of Pera orange trees, 'Pera CNPMF D9', 'Natal CNPMF 112', and 'BRS 002 - Sincorá', combined with the rootstocks of Santa Cruz lemon tree 'Cravo', Tropical selection of 'Sunki' tangerine tree, and Indio and Riverside citrandarins, were grown in the semi-arid region of Juazeiro, Bahia (BA). Among these, the 'Sunki' Tropical rootstock induced fruits with the best chemical characteristics (Santos et al., 2021). In the evaluation of canopy and rootstock fruits in Ibirapuã, located in the southernmost region of Bahia, statistical differences were observed for quality characteristics; however, the ripening curve was not described (Buffon et al., 2021). Found effects and interactions between canopy cultivar and rootstock, confirming that both factors influence the physicochemical quality of the fruits Oliveira et al. (2023).

The diversification of scion and rootstock combinations is a strategy that, in the long term, supports the sustainability of citrus orchards. Identifying the potential of genotypes through non-parametric data and methods emerges as an alternative to complement the methodologies (Santos et al. 2021), Carvalho et al. (2021), Buffon et al. (2021), and Oliveira et al. (2023), among which the use of mixed models via REML/BLUP stands out. In this sense, the hypothesis supporting this study is that new scion and rootstock combinations are alternative diversification options that meet the minimum quality requirements for fresh consumption and for the industrial sector. The objective was to assess, based on quality parameters, the commercial potential of fifty-nine (59) sweet orange cultivars on the 'Sunki Tropical' tangerine rootstock and the 'Trifoliata' Citrandarin hybrids 'San Diego', 'Riverside', and 'Indio' using mixed models.

Results and Discussion

Deviance analysis

Significant effects (LRT > 3.84) were observed among the different scion and rootstock combinations for JY, TSS, TA, and Ratio (Table 2). The quality of the fruits is influenced by the interaction between the scion cultivar and rootstock, as evidenced in the region where the research was conducted. The use of rootstocks, such as the 'Sunki Tropical' mandarin tree (*Citrus sunki*), and high-yielding rootstocks like the 'Indio' and 'San Diego' citrandarins, is crucial for improving productivity (Carvalho et al., 2023; Pedroso et al., 2014). The results demonstrated significant genetic variability between the combinations of scion and rootstock, resulting in distinct fruit characteristics and influencing quality parameters, where detailed analysis of these combinations is essential to identify the best options for different regions. In the southern region of Bahia, the ideal edaphoclimatic conditions and the absence of diseases such as Greening (Oliveira et al., 2024) offer potential for orange production. In the long term, the appropriate choice of scion and rootstock combinations can contribute to the sustainability of citrus farming by affecting quality indicators and allowing the determination of fruit ripening at different times. To study the changes in orange quality during maturation is crucial to identify the ideal harvest time, thus meeting consumer preferences (Shahrestani et al., 2024). The grouping of sweet orange trees and rootstocks based on permanent phenotypic values and gains after selection (Table 3) considered the ranking of scion and rootstock combinations, classified in ascending order according to JY, TSS, TA, and Ratio values.

Juice yield (JY)

The noticeable heterogeneity in the ranking of the scion and rootstock combinations, as presented in Table 3. This variation influenced the estimated values for JY, except for the combinations belonging to the group 'Other Oranges'. The average juice yields ranged from 58.53% for the combination 'Pera C-21' / 'Riverside' to 60.79% for 'Valência Midknight' / 'Riverside'. The combination 'Valência Midknight' / 'Riverside' stood out, showing values above the minimums stipulated by CEAGESP (2011), as shown in Table 3. Among the sweet orange canopies of the Pera, Valência, and Natal groups, and the citrandarins used as rootstocks, the best performances in JY were observed. It is important to emphasize that all the combinations presented in Table 3 achieved yields higher than 58.53%, thus meeting the standards required by the industry and recommended for consumption *in natura.*

Therefore, all these combinations are suitable to significantly meet the established requirements. Additionally, characteristics such as color, flavor, presence of seeds, shape, and skin color influence consumer perception and, consequently, the economic value of fruits (Shahrestani et al., 2024). The accumulation of juice in oranges during ripening, (Almeida et al., 2021), is associated with biochemical and physiological processes such as cell division and differentiation, accompanied by an increase in water content, transformation of carbohydrates into simple sugars, and the development of cellular vacuoles, which are compartments that store water and other substances.

This results in a greater capacity for water retention in the cells, leading to an increase in the volume of available juice. The accumulation of juice is the result of an interaction between water absorption, carbohydrate transformation, cellular expansion, and the degradation of cell walls composed of pectins and cellulose, which occur during the ripening process of the orange (Crasque et al., 2020; Amaral et al., 2021).

The fruits of fruit-bearing species (sweet orange trees) require a quality standard equivalent to the physical and chemical aspects that reflect palatability and market acceptability. Therefore, the permanent phenotypic values were used to identify and select superior individuals, as they reflect the production capacity, through gain from selection and a new long-term mean. These results support demonstrating the potential of the region for the implementation of these new combinations of scion and rootstock, considering the possibility of diversifying the orchards. In this regard, Coelho et al. (2019) states that the Pera orange tree stands out for providing high yield and juice quality, averaging 52%, corroborating Coelho et al. (2019). The higher the JY, the more attractive it is for fresh consumption and desired by the citrus industry, as represented by the combinations of canopy and rootstock that have potential for implementation in commercial crops, primarily due to the differences in JY, TSS, and TA values.

Total Soluble Solids (TSS)

The estimated average values for TSS, considering the minimum and maximum, ranged from 11.63 to 12.35 °Brix, within the minimum acceptable limits for commercial exploitation, according to pre-established standards (Table 3). The combination 'Melrosa' / 'Indio' achieved 12.35°Brix, while 'Diva' / 'Sunki Tropical' reached 11.63°Brix, an increase of 6.19%. The sweet orange trees that make up the Pera, Valência, and others group once again stood out in contributing to the concentration of TSS, as well as for JY, associated with the 'San Diego' citrandarin, which achieved the highest results.

The combination 'Melrosa' / 'Indio' was observed to be in first place among those that induced the highest concentration of TSS. However, it is worth noting the participation of the citrandarin rootstock 'San Diego', with 50% of occurrences among those selected, followed by the citrandarin 'Indio' and the 'Sunki Tropical' tangerine. Citrus fruits, being non-climacteric, have a low respiration rate and ethylene production (Rashidi; Amiri; Shirzad, 2023). Oranges are rich sources of phytochemicals, vitamins, and minerals, varying with the variety and growing conditions (Vicente et al., 2022). The analysis of the chemical profile of oranges is complex and influenced by various factors, including the stage of ripeness, genetic variations, type of rootstock, cultivation methods, and climatic conditions (Shahrestani et al., 2024).

This makes the main indicators of flavor and quality of oranges the TSS and TA. The increase in sugar concentration contributes to the reduction of acidity, as the Ratio between sugars and acids is one of the main indicators of ripeness in citrus fruits. According to CEAGESP (2011), the minimum quality requirements for TSS is 10° Brix; in this case, the combinations of scions and rootstocks remained within the required standards. According to Silva et al. (2017), the concentration of soluble solids (TSS) is one of the attributes that describes the quality and indicates the degree of maturity of fruits, related to the amount of naturally soluble sugars in water. It is a widely used criterion for differentiating and selecting new cultivars (Beber et al., 2018). Therefore, the characterization of the combinations of scions and rootstocks for juice yield, as well as for the soluble solids content under the management conditions adopted in this study, serves as indicators to be incorporated into the planning of citrus orchard diversification.

Titratable Acidity (TA)

It was considered the combinations that resulted in lower averages, due to the fact that with the advancement of maturation, the levels of TSS, JY, and Ratio tend to increase, while a decrease in TA is expected (Beber et al., 2018). During maturation, there is an increase in the sugar content (TSS) and a decrease in acidity (TA), resulting in a higher TSS/TA Ratio. This reduction in acidity, associated with the increase in sugars, is attributed to lower enzymatic activity in the fruits, which contributes to the decrease in organic acids and the increase in sugars (Zhang et al., 2022a; Rodríguez-Concepción et al., 2018). Acidity is an intrinsic characteristic of sweet orange fruits, and its correct determination, along with juice yield and soluble solids content, defines the final quality of the oranges and

Table 3. Scions and rootstock combinations from the Pera, Valencia, Natal, and Other Orange groups for juice yield, soluble solids, titratable acidity, and Ratio via individual BLUP, in the Extreme South of Bahia, Ibirapuã, 2021.

Scions and Rootstock Combinations, respectively	Parameters			
Juice yield (%)	**fp**	**u + fp**	**Gain**	**New Mean (%)**
'Valência Midknight' / 'Riverside'	6.12	60.79	6.12	60.79
'Pera CNPMF D-9'/ 'Riverside'	5.58	60.25	5.85	60.52
'Melrosa' / 'Indio'	4.86	59.53	5.52	60.19
'Pera Seleção CNPMF C- 32' / 'Riverside'	4.61	59.28	5.29	59.96
'Pera Seleção CNPMF D-3' / 'San Diego'	3.40	58.07	4.91	59.59
'Valência CNPMF 27' / 'Indio'	3.32	57.99	4.65	59.32
'Valência Seleção L.Shaffey' / 'Indio'	3.23	57.90	4.44	59.12
'Pera Seleção CNPMF C- 32' / 'Indio'	3.19	57.87	4.29	58.96
'Valência Seleção CNPMF 21' / 'San Diego'	3.17	57.84	4.16	58.84
'Pera Seleção Olímpia' / 'Sunki Tropical'	2.96	57.63	4.04	58.72
'Pera CNPMF D-6' / 'Sunki Tropical'	2.95	57.62	3.94	58.62
'Pera C-21' / 'Riverside'	2.91	57.59	3.86	58.53
Soluble solids (°Brix)				**(°Brix)**
'Melrosa' / 'Indio'	1.55	12.35	1.55	12.35
'Valência Seleção CNPMF' / 'Sunki Tropical'	1.07	11.87	1.31	12.11
'Seleção Crescent' / 'Sunki Tropical'	0.95	11.75	1.19	11.99
'Seleção Crescent' / 'San Diego'	0.89	11.68	1.12	11.91
'Pera Seleção Vacinada' / 'San Diego'	0.78	11.57	1.05	11.84
'Seleção Crescent' / 'Indio'	0.77	11.57	1.00	11.80
'Pera Lamb's Summer' / 'Riverside'	0.73	11.53	0.96	11.76
'Pera Seleção CNPMF A-15' / 'San Diego'	0.69	11.49	0.93	11.72
'Pera C-21' / 'San Diego'	0.69	11.48	0.90	11.70
'Melrosa' / 'San Diego'	0.66	11.45	0.88	11.67
'Pera D-12' / 'San Diego'	0.65	11.44	0.86	11.65
'Diva' / 'Sunki Tropical'	0.64	11.43	0.84	11.63
Titratable acidity (% Citric acid)				**(% Citric acid)**
'Pera Seleção Ibotirama' / 'San Diego'	0.51	0.50	0.57	0.50
'Seleção Flor de Brumadinho' / 'Sunki Tropical'	0.50	0.51	0.52	0.50
'Jaffa' / 'Indio'	0.48	0.52	0.59	0.53
'Salustiana' / 'San Diego'	0.43	0.53	0.58	0.54
'Westin' / 'San Diego'	0.42	0.53	0.57	0.54
'Seleção Rubi CN-01' / 'Riverside'	0.41	0.53	0.57	0.54
'Jaffa' / 'Sunki Tropical'	0.40	0.54	0.58	0.57
'Seleta Itaborai' / 'San Diego'	0.39	0.55	0.59	0.59
'Seleção Early Oblong' / 'San Diego'	0.39	0.56	0.60	0.59
'Seleta Itaborai' / 'Sunki Tropical'	0.39	0.56	0.60	0.60
'Pera Seleção Olímpia' / 'Riverside'	0.38	0.57	0.62	0.62
'Jaffa' / 'Sunki Tropical'	0.40	0.58	0.64	0.66
Ratio				
'Seleção Flor de Brumadinho' / 'Sunki Tropical'	3.95	10.75	3.95	10.75
'Seleção Flor de Brumadinho' / 'San Diego'	3.17	9.98	3.56	10.37
'Pera Seleção Ibotirama' / 'San Diego'	2.15	8.96	3.09	9.90
'Pera Seleção Olímpia' / 'Sunki Tropical'	2.12	8.93	2.85	9.65
'Pera CNPMF E-6' / 'Riverside'	1.85	8.65	2.65	9.45
'Westin' / 'San Diego'	1.70	8.51	2.49	9.29
'Pera CNPMF D-9' / 'Riverside'	1.62	8.43	2.36	9.17
'Pera Lamb's Summer' / 'San Diego'	1.50	8.31	2.26	9.06
'Pera Seleção CNPMF D-3' / 'San Diego'	1.50	8.30	2.17	8.98
'Pera Seleção CNPMF C- 32' / 'Riverside'	1.46	8.27	2.10	8.91
'Melrosa' / 'Indio'	1.44	8.25	2.04	8.85
'Natal Seleção Ipeal' / 'Indio'	1.43	8.24	1.99	8.80

fp: Permanent phenotypic effect refers to the lasting effect of factors that influence the phenotype (observable characteristics) of an organism. u + fp: Permanent phenotypic value combines genetic value (u) and the permanent phenotypic effect (fp). Gain: Average genetic gain with selection refers to the average increase in desired traits expected to be achieved through the genetic selection process over generations. New mean: New mean and/or improved means for each combination after selection, indicating the new average of the phenotypic or genetic traits resulting from the selective process.

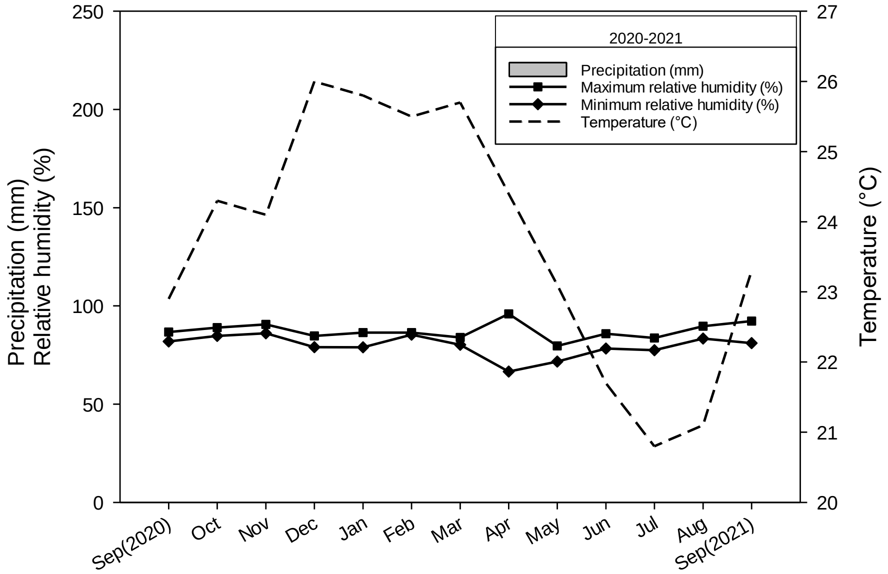

Figure 1. Precipitation, temperature, maximum and minimum humidity collected at the São Mateus (ES) automatic station during the execution of the experiment, agricultural year 2020 - 2021.
Source: INMET (2022).

indicates the ideal harvesting point. In the present study, the average values of titratable acidity ranged from 0.50 to 0.66% of citric acid (Table 3), falling within the range considered ideal by CEAGESP (2011), which is between 0.5 and 1.0%.

This range is corroborated by Pereira et al. (2006) and Bastos et al. (2014), who indicate that in ripe fruits, the acidity should be between 0.5 and 1.0% citric acid. The values found in the present work are in line with those of Stuchi et al. (2018), who reported an average value of 0.94% citric acid for ripe 'Pera' orange fruits. During the ripening process of citrus fruits, the decrease in titratable acidity results from physiological and biochemical processes such as respiration and transpiration, consequently leading to organoleptic changes that reduce the levels of sugars and organic acids, which are used as respiratory substrates (Deshi et al., 2021; Habibi et al., 2021). Moreover, changes in acidic and phenolic compounds during ripening significantly affect the flavor and nutritional value of fruits (Zhang et al., 2022b; Salvatore et al., 2022). Therefore, monitoring acidity is essential to ensure the quality of oranges and optimize their market acceptance.

At the beginning of fruit development, there is an accumulation of organic acids, such as citric acid and malic acid, in the cellular vacuoles, synthesized in the tricarboxylic acid cycle within the mitochondria of the cells (Singh et al., 2024). The reduction in the concentration of organic acids such as citric acid in the vacuoles decreases during the fruit ripening process, mainly occurring due to the increase in fruit volume (dilution), as well as cellular respiration and the conversion of these acids into other compounds (Huang et al., 2023), as well as enzymatic decarboxylation. The sweet orange varieties 'Pera Seleção Ibotirama' / 'San Diego' and 'Jaffa' / 'Sunki Tropical' stood out in this ranking. However, it is important to note that all twelve combinations of scions and rootstocks met the pre-established standards. In this context, there was a higher frequency of occurrence of citrandarins, with 'San Diego' being the most prominent rootstock. It was present in 41.66% of the listed combinations, followed by the tangerine 'Sunki Tropical' and the citrandarins 'Riverside' and 'Indio.'

Regarding the scions, those classified in the Other Oranges and Pera groups had estimated average values that likely justify the predominance of mostly early maturing sweet oranges. This may be due to the fact that this group contains genotypes that primarily exhibit early maturation. It is worth noting the citrandarins in this selection and their influence on titratable acidity under the study conditions. The evaluated fruits exhibited palatability and would be suitable for collection and consumption in their natural state. It is important to consider that the values for this characteristic represent an average of different harvest dates; however, since they were collected from April to August 2021 (the 2021 harvest), they theoretically encompass data obtained from early to late harvest.

Ratio: TSS/TA

The combinations 'Flor de Brumadinho Selection' / 'Sunki Tropical', 'Flor de Brumadinho Selection' / 'San Diego', 'Pera Selection Ibotirama' / 'San Diego', and 'Pera Selection Olímpia' / 'Sunki Tropical' exhibited the highest values for the ratio: 10.75, 10.37, 9.90, and 9.65, respectively (Table 3). These values fit within the parameters considered ideal for the quality of sweet orange fruits, with a ratio ≥ 9.5 according to CEAGESP (2011), and belong to the groups Other Oranges and Pera. However, the 'Natal Selection Ipeal' / 'Indio' oranges had an average ratio lower than 8.80, which is below the ideal value.

The predominance of citrandarins 'Indio', 'Riverside', and 'San Diego' (hybrids obtained from the cross between the 'Sunki' tangerine [*Citrus sunki* (Hayata) Hort. Ex Tanaka] and trifoliata [*Poncirus trifoliata* (L.) Raf.]) is once again highlighted as a quality parameter specifically, the ratio just as was observed for JY, TSS, and TA.

The interaction and compatibility of citrandarins with the various sweet orange scions, evaluated based on quality parameters, provide scientific support for these rootstocks (citrandarins), which have been recommended since 2011 for cultivation in the edaphoclimatic conditions of the Northeast region. State that rootstocks influence fruit

Table 4. Group Pera, Natal, Valencia, and Others and their respective cultivars of sweet orange trees in the Extreme South of Bahia, Ibirapuã, 2021.

Scions	Pera Oranges	Natal Oranges	Valencia Oranges	Other Oranges
1	Selection CNPMF 01	Selection CNPMF 01	Selection CNPMF	Berna Selection
2	Selection CNPMF 02	Selection CNPMF 02	Selection CNPMF 01	'Jaffa'
3	Selection CNPMF A-15	'CNPMF 112'	Selection CNPMF 02	F- Menuda Selection
4	'B-12'	Selection Ipeal	Selection CNPMF 03	'BRS Sincorá'
5	'C-21'	'Folha Murcha'	Selection CNPMF 21	'Aquiri'
6	Selection CNPMF C-32		'CNPMF 27'	Early Oblong Selection
7	Selection CNPMF D-3		Selection CNPMF 36	Russas P.S Selection
8	'CNPMF D-6'		Selection CNPMF F-11	'Seleta de Itaboraí'
9	'CNPMF D-9'		'Midknight'	'Salustiana'
10	'D-12'		Criola Selection	'Pineapple'
11	'D-25'		Delta Selection	Rubi CN-01 Selection
12	Selection Ipeal -E3		'Late'	'Westin'
13	'CNPMF E-6'		L.Shaffey Selection	'Diva'
14	Selection Olímpia		Chapman Selection	'Hamlin 20'
15	'Bianchi'		L.White Selection	Crescent Selection
16	Selection CE-03		'CNPMF Montemorelos'	'Melrosa'
17	Vaccinated Selection		Registro Selection	Flor de Brumadinho Selection
18	Ibotirama Selection		'CNPMF Tuxpan'	
19	Lamb's Summer			

Table 5. Rootstocks mandarin 'Sunki Tropical' and the 'Trifoliata' hybrids Citrandarin 'San Diego', 'Riverside', and 'Indio'.

Rootstocks	
Citrandarins 'Indio' Citrandarins 'Riverside' Citrandarins 'San Diego'	Hybrid between 'Sunki' tangerine [*Citrus sunki* (Hayata) Hort. Ex Tanaka] and trifoliata [*Poncirus trifoliata* (L.) Raf.]
'Sunki' tangerine	Selection 'Sunki Tropical' (*C. sunki* (Hayata) Hort. Ex Tanaka).

quality through the interaction process with the scion variety, affecting the absorption of water and nutrients Silveira et al. (2022). Analyzed 37 rootstocks combined with the 'Pera' orange clone 'CNPMF-D6' in Umbaúba (SE) and identified, among others, that the citrandarin 'Indio' is a promising alternative for diversifying citrus orchards in Northeast Brazil Carvalho et al. (2022). Regarding the different groups of sweet orange scions, only those belonging to the Pera, Natal, and Other Oranges groups were identified as the most significant contributors to the ratio performance.

As is evident, this parameter is more commonly used as a ripening index. In this context, a lower ratio indicates more acidic juice due to the reduced content of soluble solids, rendering it unsuitable for harvesting and, consequently, for consumption. As noted by citrus fruits are classified as non-climacteric; therefore, the sugar and acid levels in the juice do not change after harvest, and they should be picked at the appropriate ripening stage Lemos et al. (2012). The content of soluble solids, the ratio, and juice yield in sweet orange fruits are good indicators of ripeness (Arruda et al., 2011).

These parameters should be evaluated or monitored pre-harvest to ensure that harvesting occurs at the optimal stage, with fruits at their peak quality. However, what is observed in practice in the producing region (Southern Bahia) is that fruits are harvested regardless of their ripening groups and times, as they show visible signs of ripening (such as yellowing skin). These fruits are sold directly on the farm to local markets or vendors, with a significant portion sent to CEASA in Vitória, ES. This method of harvesting may compromise the buying and selling process, as well as consumer acceptance. The combinations from the Pera, Natal, and Valencia Orange groups that stood out were: 'Pera CNPMF D-9' / 'Riverside', 'Pera Selection CNPMF C-32' / 'Riverside', 'Pera Selection CNPMF D-3' / 'San Diego', and 'Pera Selection Olímpia' / 'Sunki Tropical' in terms of juice yield and ratio, as well as rootstocks and grafting materials, respectively.

Considering each parameter, 'Valencia Selection CNPMF' / 'Sunki Tropical' and 'Diva' / 'Sunki Tropical' excelled in TSS and TA. For JY, TSS, and Ratio, the combination 'Melrosa' / 'Indio' also stood out. 'Pera CNPMF D-6' / 'Sunki Tropical' and 'C-21' / 'Riverside' significantly contributed to the average JY values. The combinations listed can serve as alternatives for planting in new areas, particularly to meet industry needs, as the suggested combinations exhibited JY greater than 58%.

The citrandarins 'San Diego', 'Riverside', and 'Indio' excelled in terms of JY, TSS, TA, and Ratio, with the tangerine 'Sunki Tropical' following closely. The effective contribution of citrandarins indicates their potential use as a diversification strategy, enhancing performance in various combinations. The recommended sweet orange scions include 'Pera Selection CNPMF C-32', 'Pera Selection CNPMF D-3', 'Pera CNPMF D-9', 'Melrosa', 'Pera Selection Olímpia', 'C-21', 'Valencia Selection CNPMF', 'Crescent Selection', 'Pera Lamb's Summer', 'Diva', 'Valencia Selection Chapman', 'Natal Selection CNPMF 02', and 'Flor de Brumadinho Selection'.

The Citrandarins are hybrids of trifoliate orange with mid-season ripening, and they have potential for cultivation when grafted onto sweet orange, tangerine, acid lime, and grapefruit (Bastos et al., 2014). Simonetti (2015) and Marcolini et al. (2015) identified the potential of Citrandarins as rootstocks for 'Valencia' oranges, highlighting that these rootstocks enhance the fruit quality of the scions by inducing better commercial characteristics, such as higher production of soluble solids and fruits. Carvalho et al. (2019), in their investigation of new scion and rootstock combinations for diversifying sweet orange orchards in tropical soils, concluded that 'Sincorá' oranges and 'Valencia Tuxpan' grafted onto 'Sunki Tropical' tangerine are suitable for the genetic diversification of orchards when planted at conventional densities.

The combinations of scion and rootstock have the potential for large-scale commercial use as a diversification strategy for citrus orchards. Therefore, the interaction process between plants, along with the management practices adopted, genetic potential, and edaphoclimatic conditions of the region, influences the characteristics of the fruit. The cultivation of citrus in the Northeast plays a crucial role in job creation, income generation, and the promotion of regional development. This significance underscores the necessity for studies aimed at evaluating the feasibility of new varieties suited for the region (Silva et al., 2014). This is supported by the results from combinations that JY percentages ranging from 55.05% to 60.05%, TSS between 10.70 and 12.38 °Brix, and citric acid levels from 0.50% to 0.66%. These findings align with the proposals from CEAGESP (2011) and present excellent alternatives for the Extreme South region of Bahia, with values exceeding those required for the states of São Paulo and Minas Gerais.

The cultivation of sweet oranges with varying ripening times contributes to the sustainability of citrus orchards, taking into account the management practices applied and the specific region where the orchard is located. Therefore, it is important to evaluate ripening indices individually and determine the optimal harvest point to ensure that higher-quality fruits can be available throughout the year. New research should be conducted focusing on monitoring and tracking the ripening of sweet orange fruits in different regions, aiming to achieve specific results for the key indices that determine the harvest stage. This will be crucial for supporting and guiding the harvest process and expanding the variety of fruit offerings. Additionally, it will facilitate production scheduling, allowing for marketing during off-seasons and enabling the industry to extend the processing period for juice production, thus balancing the need for labor throughout the year.

Materials and Methods

Description of the experimental area

The experiment was conducted in the experimental area of Chão Bello Farm, located in the municipality of Ibirapuã, in the extreme south of the state of Bahia, Brazil, at the geographical coordinates: 18° 03' 09.4" S latitude and 39° 52' 26.2" W longitude, at an altitude of 95 m.

The soil is classified as Dystrophic Yellow Latosol, and the region's climate is tropical Am, according to the Köppen classification, with an average annual temperature of 23.6 °C (Alvares et al., 2014). Temperature and relative humidity data were collected at the automatic weather station in São Mateus - ES, code A616, latitude: -18.6761111 and longitude: -39.86416666, located 126 km from the farm headquarters (Figure 1) (INMET, 2023).

Soil chemical analysis

The planting took place on April 21, 2015, with a spacing of 6 meters between rows and 3 meters between plants. Irrigation was carried out using a localized micro-sprinkler system with a flow rate of 72 L/h, employing a KSB Meganorm 50-200 centrifugal pump with a power of 40 hp and a maximum operating pressure of 10 bar. The irrigation was performed in two fixed shifts, applying 6 mm/day (Oliveira et al., 2023).

Fertilizations were carried out through fertigation based on the results of the soil chemical analysis in the 0-20 cm depth layer (Table 1). Cultural practices were conducted according to the management guidelines established by the company Bello Brazilian Exotic Fruit, as proposed by Siqueira and Salomão (2017), including monitoring and control of pests and diseases.

Treatments and experimental design

The experimental area (2.36 hectares) consists of fifty-nine (59) combinations of sweet orange trees on four rootstocks, resulting in a total of 236 combinations. Each combination included three plants that were not randomly located in the experimental field.

Plant materials: Scions and rootstock combinations

The canopies were categorized into groups named Pera Oranges, Valencia Oranges, Natal Oranges, and Other Oranges (Table 4).

The treatments consisted of combinations of each scion cultivar with four rootstocks: 'Indio', 'Riverside', and 'San Diego' citrandarins, developed by the Citrus Breeding Program of Embrapa Cassava and Fruits (Table 5). Sequentially, the combinations are represented by the scion followed by the rootstock.

Assessments for quality analysis

Fruit samples were collected at five different harvest times, from April to August, corresponding to the 2021 harvest (April 12, May 3, May 20, June 10, July 7, July 27, and August 17, 2021). Four fruits per plant were collected, distributed at the four cardinal points around the canopy in the middle third, totaling 12 fruits per experimental plot, and 2832 fruits in total for each evaluation.

The physicochemical parameters were evaluated at the Plant Processing Laboratory of the Federal University of Espírito Santo in São Mateus (ES). At stage III (fruit maturation), the following parameters were evaluated: fruit weight (FW) and juice weight (JW), both measured in grams (g). These values were then used to calculate the juice yield (JY) as a percentage (%). The fruits were weighed, and the juice was extracted to determine the juice weight (g). Subsequently, the juice yield was calculated using the following equation:

$$\text{JY (\%)} = \left(\frac{\text{FW}}{\text{JW}}\right) \text{x } 100$$

The content of total soluble solids (TSS) in °Brix was measured using a digital refractometer from Reichert Technologies at 25 ºC, following the methodology of the Adolfo Lutz Institute (2008). The titratable acidity (TA) in % citric acid was determined using the titration method with a 0.1 mol L^{-1} NaOH (sodium hydroxide) solution and 1% phenolphthalein indicator (AOAC, 2012). The maturation index or Ratio was calculated by dividing total soluble solids in °Brix (TSS) by titratable acidity in % citric acid (TA), as proposed by Chitarra and Chitarra (1990), an adimensional Ratio. The quality parameters were classified according to the pre-established standards set by the Brazilian Program for the Modernization of Horticulture of the São Paulo General Warehouses and Warehouses Company for table citrus. The minimum requirements for fresh fruits include a juice yield of 35% to 45%, 10 °Brix, 0.5% to 1% acidity, and a ratio of ≥ 9.5 (CEAGESP, 2011).

Statistical analysis

The data were analyzed using mixed models methodology (REML/BLUP), where variance components were estimated using the restricted maximum likelihood method (REML) proposed by Patterson and Thompson (1971), and phenotypic values were predicted using best linear unbiased prediction (BLUP) (Henderson, 1973). Model 63 (a basic repeatability model without design) from the Selegen-REML/BLUP software, as developed by Resende (2007), was used. The statistical model employed was y=Xm/Wp/ey = X m / W p / ey=Xm/Wp/e, where 'y' represents the data vector, 'm' is the vector of measurement effects (assumed to be fixed) added to the overall mean, 'p' is the vector of permanent plant effects (including genotypic effects and permanent environmental effects) (assumed to be random), and 'e' is the vector of errors or residuals (random). X and W are the incidence matrices for the aforementioned effects.
The significance of the models was assessed using the likelihood ratio test (LRT), based on deviance analysis (ANADEV), which was obtained by comparing the full model with the reduced model that does not include the effect being tested. The LRT was compared with a Chi-square distribution table at the 5% significance level (Resende, 2016). Analyses were conducted on the scion and rootstock combinations to evaluate their effects on the variables studied. The selection of scion and rootstock combinations was based on the permanent phenotypic effect (fp) for each characteristic, with a selection intensity of 5% applied to the 236 combinations, resulting in 12 selected combinations. Additionally, information on the average genetic gain from selection and the new and/or improved means for each combination and variable studied were obtained.

Conclusion

The citrandarin rootstocks 'San Diego', 'Riverside', and 'Indio', and the tangerine 'Sunki Tropical', induced in the canopies of sweet orange trees 'Pera Seleção CNPMF C-32', 'Pera Seleção CNPMF D-3', 'Pera CNPMF D-9', 'Melrosa', 'Pera Seleção Olímpia', 'C-21', 'Valência Seleção CNPMF', 'Seleção Crescent', 'Pera Lamb's Summer', 'Diva', 'Valência Seleção Chapman', 'Natal seleção CNPMF 02', and 'Seleção Flor de Brumadinho' higher juice yield (55.05 to 60.05%), total soluble solids (10.70 to 12.38 °Brix), and titratable acidity (0.50 to 0.66% citric acid).
The combinations 'Pera CNPMF D-9' / 'Riverside', 'Pera Seleção CNPMF C-32' / 'Riverside', 'Pera Seleção CNPMF D-3' / 'San Diego', 'Pera Seleção Olímpia' / 'Sunki Tropical', 'Valência Seleção CNPMF' / 'Sunki Tropical', 'Diva' / 'Sunki Tropical', 'Melrosa' / 'Indio', 'Pera CNPMF D-6' / 'Sunki Tropical', and 'C-21' / 'Riverside' presented for their potential for commercial exploitation due to the good performance of fruit quality under the conditions of the Extreme South of Bahia State.

Acknowledgments

Chão Bello Farm (Bello Brazilian Exotic Fruit Company) and Embrapa Mandioca e Fruticultura (EMBRAPA). This work was supported by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Financing Code 001.
Author contributions: LSF (Conducting the experimental project, analysis, and manuscript writing); MZ and DHSGB (Planning and review); VSO (Experimental project management and manuscript review); RGM and LLB (Statistical analysis).

Conflict of interest

The authors declare no conflict of interest.

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