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Performance of rainfed Persian lime cv. BRS EECB IAC Ponta Firme on 26 rootstocks under Aw climate

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ABSTRACT: It is necessary to diversify the scion/rootstock combinations for Persian lime cultivation, notably under tropical rainfed conditions. Therefore, in this work we evaluated the performance of a new cultivar, BRS EECB IAC Ponta Firme, up to six years after planting on 26 citrus rootstocks under Aw climate in northern São Paulo state, Brazil. Thirty single-tree randomized replications of each rootstock were evaluated without irrigation at 1,000 trees·ha^{-1}. Lemon-type, followed by mandarin rootstocks induced the largest tree size and higher mean yield, probably related to the higher drought tolerance. The true dwarfing rootstocks of Flying Dragon trifoliate orange and Lindcove citrandarin were highly drought-intolerant, but, in addition to Swingle citrumelo and four other citrandarins, they did not present huanglongbing-symptomatic trees during the evaluation period under strict control of the insect vector. Overall, BRS EECB IAC Ponta Firme fruit quality was minimally influenced by the rootstock cultivar and fulfilled the requirements for both domestic and export fresh fruit markets. Due to superior production efficiency associated with high yield in relation to the canopy size, the Goutoucheng sour orange, BRS Ary, BRS Bravo and BRS Matta hybrids were selected as potential rootstocks of rainfed BRS EECB IAC Ponta Firme lime under Aw climate (tropical savannah) conditions.

Key words: *Citrus × latifolia*, drought tolerance, essential oil, graft compatibility, huanglongbing, yield.

INTRODUCTION

The Persian or Tahiti acid lime [*Citrus × latifolia* (Yu. Tanaka) Tanaka] is grouped within the lemon types widely cultivated under tropical and subtropical conditions. Brazil is the fifth greatest producer, with 58.446 ha and a yield of 1.5 million tons in 2021 (FAO 2023). The São Paulo state comprises 78% of the Brazilian production of Persian lime, which is concentrated in areas under the Aw climate (Fundecitrus 2022b, Rolim et al. 2007).

Persian lime trees produce continuously in São Paulo state conditions, but two harvest periods are well defined over the year: the main one, between December and May, which supplies fresh fruit for export, and the off-season from July to November, when growers take advantage of higher prices in the domestic market (Figueiredo et al. 2002). In 2021, Brazil exported 126.4 thousand tons of limes, primarily to the European Union, given US$ 108.1 million, making the Persian lime the third most exported Brazilian fresh fruit (Secex 2022). Its essential oil is mainly exported to the United States and used by several industries (Di Giacomo 2002, Embrapa 2021).

In São Paulo state, Persian lime is still widely cultivated without irrigation (Fundecitrus 2022b). The cultivar BRS EECB IAC Ponta Firme was highlighted due to its earlier bearing and higher production than other clones in northern São Paulo state with and without irrigation (Bremer Neto et al., 2013). Most rainfed orchards use Rangpur lime (RL) (*Citrus* × *limonia* Osbeck) rootstock (Figueiredo et al. 2002), despite its high susceptibility to exocortis and citrus gummosis (*Phytophthora citrophthora* and *Phytophthora nicotianae* var. *parasitica*), causing high-tree mortality. Other important rootstocks for the Persian lime are the Swingle citrumelo (CS) [*Citrus* × *paradisi* Macf. × *Poncirus trifoliata* (L.) Raf.] and the Flying Dragon (FD) trifoliate orange (*P. trifoliata* var. *monstrosa*) (Girardi et al. 2021). FD is less vigorous and more intolerant to drought than the RL and CS (Espinoza-Núñez et al. 2011).

The diversification of scion/rootstock combinations is a crucial strategy to decrease the impact of adverse climatic conditions and diseases. For instance, acid limes were considered among less susceptible citrus types to the huanglongbing (HLB) disease (Folimonova et al. 2009, Ramadugu et al. 2016, Deng et al. 2019), and Bettini et al. (2019) reported less HLB incidence in Persian lime trees grafted onto FD trifoliate orange. This is particularly important because HLB is widespread in São Paulo state (Fundecitrus 2022a).

Considering the socio-economic importance and the limited rootstock varietal usage in Persian lime orchards, herein we report the performance of BRS EECB IAC Ponta Firme grafted onto 26 citrus rootstocks in Aw climate, including new hybrid genotypes adapted to rainfed cultivation.

MATERIAL AND METHODS

Plant material and experimental design

The Persian lime (*C.* × *latifolia* var. *latifolia*) cv. BRS EECB IAC Ponta Firme (PF) was grafted onto 26 citrus rootstocks, including traditional varieties and new genotypes previously evaluated with sweet orange scion and grouped according to their tree size (Costa et al. 2021) (Table 1). The seeds were provided by Embrapa Cassava & Fruits, except for the Limeira Rangpur lime (RLL) and Flying Drago trifoliate orange (FDT), obtained from a commercial nursery.

Table 1. Variety name, species or parents, and acronym of 26 citrus rootstock genotypes grafted with BRS EECB IAC Ponta Firme Persian lime in Bebedouro, northern São Paulo state, Brazil.

Rootstock variety name	Species/Parents	Acronym
CNPMF-03 Rangpur lime	*Citrus* × *limonia* Osbeck	RL03
BRS Santa Cruz Rangpur lime	*C.* × *limonia*	RLSC
Limeira Rangpur lime	*C.* × *limonia*	RLL
Florida rough lemon	*Citrus* × *jambhiri* Lush.	FRL
FM rough lemon	*C.* × *jambhiri.*	FMRL
Volkamer lemon cv. Lagoa Grande	*Citrus* × *volkameriana* (Risso) V. Ten. & Pasq.	VKL
Cleopatra mandarin	*Citrus reshni* hort. ex Tanaka	CLM
BRS Tropical Sunki mandarin	*Citrus sunki* (Hayata) hort. ex Tanaka	STM
Sunki mandarin	*C. sunki*	SM
Swingle citrumelo	*Citrus* ×*paradisi* Macfad. × *Poncirus trifoliata* (L.) Raf. - 4475	SC
Goutoucheng sour orange	*Citrus* ×*aurantium* L.	GTSO
Indio citrandarin	*C. sunki* × *P. trifoliata* cv. English-256	IC
BRS H Montenegro citrimonia	*C.* ×*limonia* × *P. trifoliata* - 001	HMC
BRS N Gimenes Fernandes citrimoniambhiri	*C.* ×*jambhiri* × (*C.* ×*limonia* × *P. trifoliata*) - 005	NGF
BRS Ary lemon	*C.* ×*volkameriana* × *C.* × *limonia* - 038	AL
Lindcove citrandarin	*C. reshni* × *P. trifoliata* cv. Barnes - 245	LC
BRS Ríos Castaño citrandarin	*C. reshni* × *P. trifoliata* cv. Rubidoux	RCC

continue...

Table 1. continuation...

Rootstock variety name	Species/Parents	Acronym
San Francisco citrandarin	*C. reshni* × *P. trifoliata* cv. Swingle - 287	SFC
BRS Matta citrandarin	*C. sunki* × *P. trifoliata* var. monstrosa - 006	MC
BRS Bravo citrimoniandarin	*C. sunki* × (*C.* × *limonia* × *P. trifoliata*) - 059	BRA
TSKC × (LCR × TR)-073	*C. sunki* × (*C.* × *limonia* × *P. trifoliata*) - 073	073
TSKC × CTSW-025	*C. sunki* × (*C.*× *paradisi* × *P. trifoliata*) - 025	025
TSKC × CTSW-033	*C. sunki* × (*C.* × *paradisi* × *P. trifoliata*) - 033	033
BRS Cunha Sobrinho citrumelandarin	*C. sunki* × (*C.* × *paradisi* × *P. trifoliata*) - 041	041
BRS Santana citrangor	*Citrus* ×*sinensis* (L.) Osbeck × (*C.* ×*sinensis* × *P. trifoliata*) - 069	SAC
Flying Dragon trifoliate orange	*P. trifoliata* var. *monstrosa*	FDT

The experimental design was entirely randomized, with 26 treatments (rootstocks), 30 replications, and a single tree in the unit.

Local conditions and plant maintenance

Fifteen-month-old nursery trees were transplanted in February 2016 at the experimental area in the municipality of Bebedouro, São Paulo (20°53'16"S, 48°28'11"W, 601 m a. s. l.). The local climate is classified as Aw according to the Köppen-Geiger classification, that is, tropical savannah with dry winter (Rolim et al. 2007). From planting to August 2021, the mean annual rainfall was 1,070 mm, and average maximum, mean, and minimum air temperatures were 30.2, 22.9, and 16.8 °C, respectively (Fig. 1). The water deficit (mm) and real evapotranspiration (mm) were calculated for the evaluation period according to Thornthwaite and Mather (1955), considering a water holding capacity of 100 mm.

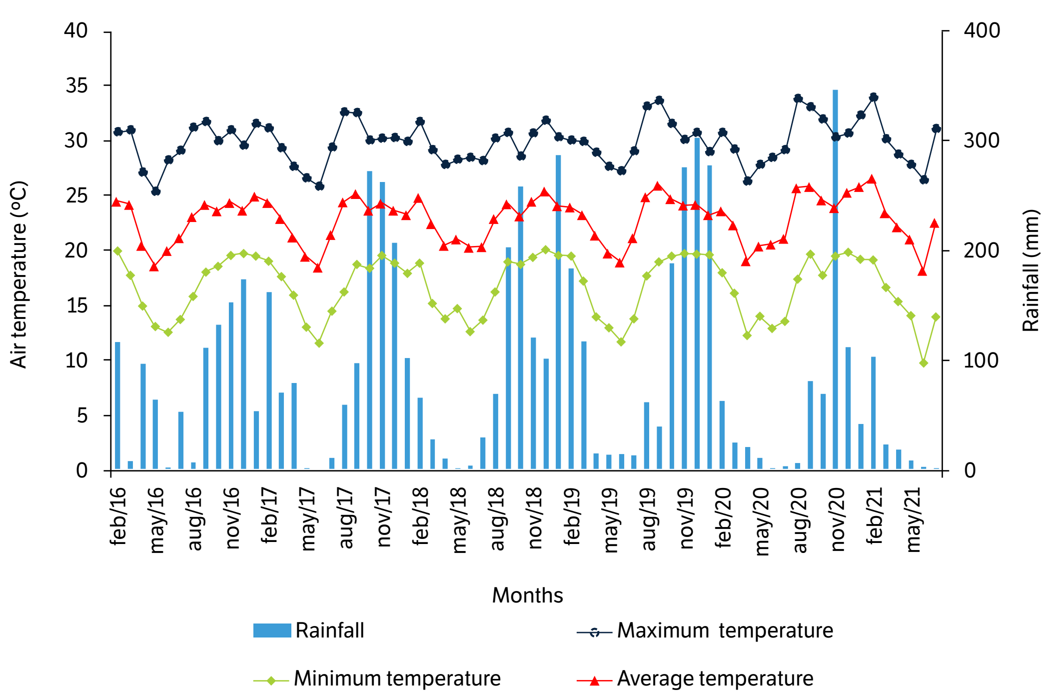

Figure 1. Monthly averages of air temperature (maximum, minimum and mean) and rainfall at the experimental area from February 2016 to September 2021 in Bebedouro, northern São Paulo state, Brazil. Campbell CR-10 meteorological station.

Tree spacing was 5 × 2 m (between and in rows, respectively), resulting in a high-tree density orchard (1,000 trees·ha^{-1}), and cultivation was rainfed. The soil at the experimental area was classified as Red oxisol, dystrophic, hypoferric, and with a medium texture (Embrapa 2006), with the following chemical attributes at 0–20 cm at the end of the evaluation [pH ($CaCl_2$) = 5.60 $mmol_c.dm^{-3}$; CEC = 114.9 $mmol_c.dm^{-3}$; Ca = 60 $mmol_c.dm^{-3}$; Mg = 20 $mmol_c.dm^{-3}$; and K = 3.90 $mmol_c.dm^{-3}$; V% = 73; P = 54 mg·dm^{-3}; O.M. = 29 g·dm^{-3}]. The annual fertilization average consisted of 160.5, 48, and 160.5 kg·ha^{-1} of N, P_2O_5, and K_2O, respectively, from 2016 to 2021, and 2.5 t·ha^{-1} of limestone from 2016 to 2017. Hedging and topping were performed twice, during the winter in 2020 and 2021, obeying an inclination of 15 and 20º at 3.5-m tree height, respectively, in relation to the tree vertical axis to maintain space between rows and to allow more sunlight interception (Azevedo et al. 2013). A hedge trimmer was used mounted on a tractor (Kortflex 400, Kamaq, Araras, São Paulo, Brazil).

The control of the Asian citrus psyllid, *Diaphorina citri* Kuwayama, which is the vector of *Candidatus Liberibacter* spp. associated with HLB, was based on bimonthly drench application of thiamethoxam or imidacloprid in the rainy season from 2016 to 2018, in addition to the fortnightly spraying of contact insecticides in rotation (thiamethoxam, dimethoate, imidacloprid, acetamiprid, thiamethoxam + lambda-cyhalothrin, lambda-cyhalothrin + chlorantraniliprole, etofenprox, cypermethrin, carbamazepine, deltamethrin, azadirachtin, formetanate hydrochloride, and bifenthrin) since planting. Other cultural practices followed the standard practices for acid lime cultivation in Brazil (Mattos Junior et al. 2014).

Tree size

The tree size was measured every year just after summer harvesting with a topographic ruler. The tree height (A, m) was measured from the collar end to the apex, and the mean canopy diameter (D, m) was calculated by the average between the perpendicular and parallel diameters to the row at half tree height. The canopy volume (V, m^3) was calculated by Eq. 1:

$$V = (2/3)\ \pi\ (D^2/4)\ A \tag{1}$$

Moreover, tree size was classified as adapted from Castle and Phillips (1977): super standard (> 110%), standard (80 to 109%), substandard (60 to 80%), semi-dwarfing (40 to 60%), and dwarfing (< 40%) to the standard commercial rootstock, the Limeira RL.

Yield, production efficiency, and drought tolerance

Fruit production was weighed from 2018 to 2021 with a digital scale (Líder, PR30, São Paulo, Brazil). Every year, the fruit of each tree was harvested whenever the fruits equatorial diameter reached 50 mm, and the weight of all harvests was summed. Harvests in the second semester of 2021 were too low due to severe drought. Therefore, they were not considered. The mean fruit yield per tree and mean production efficiency per unit of canopy volume in the evaluation period were calculated by dividing the total yield by the number of evaluations and by the mean ratio between the fruit yield and the canopy volume for each year, respectively. Moreover, the drought tolerance of rootstocks was evaluated during the most severe water deficit period (August to September) from 2017 to 2021. Four evaluators attributed visual scores tree by tree based on the leaf wilting and drop, as described by Cantuarias-Avilés et al. (2012).

Incidence and severity of huanglongbing

HLB incidence and severity were evaluated under natural infection by *D. citri* over the evaluation period, that is, trees were not inoculated under controlled conditions. Every three months, from March 2016 to August 2021, trained personnel scouted the experimental orchard for HLB-symptomatic trees, and infection was confirmed by quantitative polymerase chain reaction (qPCR). The cumulative HLB incidence - CI (%) was calculated by the relation between the number of infected trees and the total number of trees per rootstock. The severity of HLB was evaluated on the tree canopy of all infected trees in November 2021 based on a visual score index used by Bassanezi et al. (2011).

Graft compatibility and tree survival

In November 2021, a 3 × 1 cm bark strip was removed with a penknife at the graft union of all trees per rootstock to evaluate graft compatibility by visual scores (Costa et al. 2020, 2021). Tree survival was calculated in 2021, just after drought periods, by the ratio between the cumulative number of dead trees and the total initial number of trees per rootstock.

Fruit quality and essential oil evaluations

The physical traits of the fruit were evaluated during the main harvest season (January to March) from 2019 to 2021. Fruits were sampled based on a minimal 50-mm equatorial diameter and dark green rind color. Samples of 21 fruits per tree were randomly collected from five replications per rootstock at half tree height on the outer surface of all four quadrants of the canopy. From each sample, six fruits were sorted and evaluated for fruit weight (g) on a digital scale (Filizola, MF-6, São Paulo, Brazil), and equatorial and longitudinal lengths (cm) using a gutter-type ruler. The peel thickness (mm) was measured with a digital caliper (Mitutoyo, São Paulo, Brazil), being calculated by the average of two random measurements of the mesocarp at the equatorial diameter on both fruit halves. For fruit rind color evaluation, three measurements were carried out at different points on the equatorial circumference of the fruit with a colorimeter (CR-300, Konica Minolta, Tokyo, Japan). CIELAB space was registered as L* (luminosity), a*, and b* (CIE 1986), and the rind color index (RI) was calculated by Eq. 2 (Jimenez-Cuesta et al. 1983):

$$RI = 1{,}000 \cdot a/(L \cdot b) \tag{2}$$

The other 15 fruits from the sample were processed in a point-of-sale extractor (Otto 1800, OIC, Limeira, São Paulo, Brazil), and the juice was weighed on a digital scale to calculate the juice content. Data presented are the average values for 2019–2021.

To evaluate the yield of the essential oil in the Persian limes fruit peel, nine rootstock varieties were selected for their commercial importance or good performance in the experiment till 2020 (RLL, swingle citrumelo – SC, FDT, Volkamer lemon cv. Lagoa Grande – VKL, STM, BRS Tropical Sunki mandarin – BRA, Indio citrandarin – IC, San Francisco citrandarin – SFC, and BRS Matta citrandarin – MC). Three rootstock varieties were sampled in successive weeks during March 2021. Ten trees grafted on each rootstock variety were randomly sorted, and three pooled samples of 20 fruits were randomly collected as aforementioned. Fruits presenting an equatorial diameter of 50 to 60 mm and no deformation, lesion, or spots on the peel, were collected at noon and stored at room temperature. The next morning, they were analyzed in a commercial processing plant in Itajobi, São Paulo state, following the methodology described in Redd et al. (1987)[1].

Data analysis

Data were tested for homoscedasticity (Levene 1960) and normality (Shapiro and Wilk 1965), except for the HLB incidence and severity. Data were analyzed by analysis of variance (ANOVA), and the means were grouped by the Scott-Knotts' test ($p \leq 0.05$), using the Sisvar v. 5.6 software (Ferreira 2011). The scores of drought tolerance and graft compatibility were analyzed by the non-parametric Kruskal-Wallis' test (Kruskal and Wallis 1952), and the means were grouped by the Scott-Knotts' test ($p < 0.05$), using the R v. 3.6.1 software. The data were standardized for hierarchical clustering of rootstocks and multivariate principal component analysis (PCA). Six groups of rootstocks were arbitrarily selected according to the clustering estimates proposed by the dendrogram, avoiding the clustering of only one individual. Euclidean distance was used to measure similarity, and the average method was employed to assess linkage (Hair et al. 2006). Cluster analysis was calculated with the "NbClust" package of R 3.6.1 (Charrad et al. 2014). PCA was run using the FactoMineR package in R 3.6.1 (Lê et al. 2008). Only components with Eigenvalues equal to or greater than one was considered (Kaiser 1958).

[1] Redd, J. B., Hendrix, C. M. and Hendrix, D. L. (1987). Quality control manual for citrus processing plants, v. 1. Safety Harbor: Intercit, Inc.

RESULTS AND DISCUSSION

Five years after planting, the evaluated rootstocks induced different tree sizes to the PF scion and were grouped into four and five classes of plant height, canopy diameter, and volume, respectively, which ranged from 1.60 to 2.89 m; 1.45 to 2.48 m; and 2.29 to 9.23 m^3 (Table 2). The vigor of PF on the traditional rootstocks, such as RLL, VKL, and SC, was like that reported for the Persian lime IAC 304 clone (Espinoza-Núñez et al. 2011, Cantuarias-Avilés et al. 2012), and most new hybrid rootstocks evaluated led to large trees in relation to previous works with Valencia sweet orange scion (Costa et al. 2020, 2021). Although Persian lime is generally a more vigorous variety than sweet oranges, in this work BRS Matta citrandarin (MC) and Lindcove citrandarin (LC) citrandarins should be highlighted as potential alternative dwarfing rootstocks to the FDT in ultra-high-density orchards (Stuchi et al. 2003).

Table 2. Tree height, canopy diameter and volume, and tree size classification of the BRS EECB IAC Ponta Firme Persian lime grafted onto 26 rootstocks six years after rainfed planting in Bebedouro, northern state of São Paulo, Brazil, 2021#.

Rootstock	Tree height (m)	Canopy diameter (m)	Canopy volume (m^3)	Tree size classification*
RL03	2.83 a	2.42 a	8.87 a	Standard
RLSC	2.75 a	2.38 a	8.19 a	Standard
RLL	2.81 a	2.34 a	8.09 a	Standard
FRL	2.82 a	2.33	8.10 a	Standard
FMRL	2.81 a	2.48 a	9.02 a	Super standard
VKL	2.89 a	2.47 a	9.23 a	Super standard
CLM	2.69 a	2.32 a	7.64 b	Standard
STM	2.87 a	2.37 a	8.57 a	Standard
SM	2.71 a	2.33 a	8.00 a	Standard
SC	2.36 b	2.06 b	5.51 c	Substandard
GTSO	2.52 b	2.11 b	6.31 c	Substandard
IC	2.70 a	2.30 a	7.67 b	Standard
HMC	2.77 a	2.14 b	6.75 b	Standard
NGF	2.54 b	2.12 b	6.20 c	Substandard
AL	2.60 d	2.27 a	7.07 b	Standard
LC	1.60 c	1.75 c	2.95 e	Dwarfing
RCC	2.26 c	1.99 c	4.89 c	Substandard
SFC	2.41 b	2.13 b	5.89 c	Substandard
MC	2.09 c	1.89 c	4.15 d	Semi-dwarfing
BRA	2.68 a	2.27 a	7.38 c	Standard
073	2.60 b	2.16 b	6.48 c	Standard
025	2.50 b	2.10 b	6.02 c	Substandard
033	2.37 b	1.95 c	5.05 c	Substandard
041	2.63 b	2.12 b	6.21 c	Substandard
SAC	2.51 b	1.94 c	5.13 c	Substandard
FDT	1.94 c	1.45 d	2.29 e	Dwarfing
Mean	2.59	2.18	6.78	-
CV (%)	13.01	11.75	27.87	-
P	< 0.0001	< 0.0001	< 0.0001	-

#Averages followed by the same letter in columns belong to the same group by Scott-Knott's test ($p \leq 0.05$); *according to Castle and Phillips (1977): super standard (> 110%), standard (80 to 109%), substandard (60 to 80%), semi-dwarfing (40 to 60%), and dwarfing (< 40%), tree size in relation to the commercial standard, the RLL; CV: coefficient of variation.

From 2018 to 2021, all evaluated selections of RL, FM rough lemon (FMRL), VKL, STM, Goutoucheng sour orange (GTSO), and BRA rootstocks induced the highest average yield to the Persian lime (Table 3). However, only RLL was grouped among the most productive rootstocks in all years. In 2021, the yield was limited by severe drought conditions (Fig. 1), which suggests that some rootstocks were more tolerant and, consequently, more productive (Table 3). Less vigorous rootstocks resulted in a five-fold decrease in the yields related to the most productive ones. As a result, in this work vigorous rootstocks were 56% more efficient than dwarfing ones and some traditional rootstocks such as SC and CLM (Table 3), even though large tree size usually relates to a lower production efficiency of the canopy volume (Costa et al. 2021). In this sense, GTSO and BRA induced yield like that on the RLL but with reduced tree size, whereas MC was more productive than the other dwarfing rootstocks (Table 3), corroborating their good performance from previous works with Valencia sweet orange scion (Costa et al. 2020, 2021). When grafted with Valencia Late orange and Clementina mandarinin the Aw climate, GTSO rootstock induced low and inconsistent fruit production, on par with CLM (Hussain et al. 2013, Benyahia et al. 2017), suggesting that GTSO rootstock is more suitable for acid limes.

Table 3. Fruit yield and production efficiency of the BRS EECB IAC Ponta Firme Persian lime grafted onto 26 rootstocks over five years after rainfed planting in Bebedouro, northern state of São Paulo, Brazil, 2018–2021*.

Rootstock	Yield (kg·planta^{-1})					Production efficiency (kg·m^{-3})				
	2018	2019	2020	2021	Mean	2018	2019	2020	2021	Mean
RL03	15.70 a	34.86 b	41.90 a	12.60 a	26.26 a	2.61 a	6.38 a	3.68 a	1.43 a	3.52 a
RLSC	11.77 a	37.57 b	25.18 b	12.67 a	21.80 a	2.00 b	6.02 a	2.71 a	1.78 a	3.13 a
RLL	14.74 a	41.17 a	38.22 a	18.06 a	28.04 a	2.30 b	6.62 a	4.27 a	2.17 a	3.84 a
FRL	5.52 c	39.98 a	33.54 a	5.38 b	21.10 b	0.93 d	6.80 a	3.72 a	0.71 b	3.04 a
FMRL	4.92 c	41.91 a	39.72 a	8.11 a	23.66 a	0.79 d	7.29 a	3.63 a	0.93 b	3.16 a
VKL	6.95 c	47.13 a	48.64 a	5.92 b	27.16 a	1.06 d	8.21 a	4.65 a	0.62 b	3.63 a
CLM	5.46 c	23.81 d	34.60 a	7.93 a	17.95 b	0.96 d	4.01 b	3.62 a	1.03 b	2.40 b
STM	10.59 b	34.83 b	37.90 a	10.37 a	23.42 a	1.62 c	6.46 a	5.38 a	1.21 b	3.67 a
SM	6.30 c	23.19 d	38.02 a	8.38 a	18.97 b	1.52 c	6.26 a	5.00 a	1.60 b	3.59 a
SC	4.05 c	25.41 d	22.36 b	0.28 b	13.02 b	0.93 d	4.81 b	3.76 a	0.07 b	2.39 b
GTSO	5.43 c	27.69 c	43.44 a	12.02 a	22.14 a	0.98 d	6.44 a	6.53 a	1.91 a	3.96 a
IC	8.35 c	23.06 d	27.14 b	7.52 a	16.51 b	1.63 c	4.50 b	3.05 a	0.09 b	2.32 b
HMC	10.02 b	20.62 d	29.48 a	6.06 b	16.54 b	2.17 b	3.97 b	4.53 a	0.93 b	2.90 b
NGF	7.79 c	32.29 c	33.70 a	4.09 b	19.47 b	1.52 c	4.31 b	4.75 a	0.68 b	2.81 b
AL	8.54 c	26.67 d	27.98 b	10.74 a	18.48 b	1.98 b	6.21 a	4.13 a	1.54 a	3.46 a
LC	2.74 c	6.13 e	9.10 c	0.43 b	4.60 c	1.52 c	1.63 c	2.35 a	0.36 b	1.46 b
RCC	4.91 c	30.09 c	31.20 a	3.13 b	17.33 b	0.99 d	8.35 a	5.86 a	0.70 b	3.97 a
SFC	2.30 b	28.86 c	31.30 a	2.10 b	16.14 b	1.83 b	6.92 a	4.34 a	0.30 b	3.35 a
MC	12.37 b	24.48 d	25.16 b	7.67 a	17.42 b	2.94 a	6.12 a	3.83 a	2.69 a	3.89 a
BRA	14.59 a	29.74 c	42.24 a	9.11 a	23.92 a	2.54 a	7.14 a	6.19 a	1.15 b	4.25 a
073	10.36 b	32.24 c	32.18 a	7.54 a	20.58 b	2.02 b	6.28 a	4.22 a	1.18 b	3.42 a
025	9.06 b	23.95 d	42.52 a	5.91 b	20.36 b	1.81 b	6.19 a	5.42 a	1.13 b	3.64 a
033	6.78 c	21.95 d	37.32 a	0.89 b	16.73 b	1.53 c	4.98 b	4.6 a	0.15 b	2.81 b
041	10.26 b	31.04 c	34.68 a	4.47 b	20.11 b	2.18 b	6.52 a	4.81 a	0.70 b	3.55 a
SAC	11.67 b	30.59 c	25.42 b	2.21 b	17.47 b	2.95 a	9.21 a	4.33 a	0.47 b	4.24 a
FDT	1.82 c	3.75 e	5.50 c	0.40 b	2.87 c	1.26 c	2.00 c	2.44 a	0.34 b	1.51 b
Mean	8.45 c	28.49 b	32.25 a	6.72 d	18.93	1.71 c	5.91 a	4.3 b	1.05 d	3.23
CV (%)	69.95	42.03	37.02	104.02	29.6	81.29	58.87	43.14	117.2	30.26
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0447	< 0.0001	0.0030

*Averages followed by the same letter in columns belong to the same group by Scott-Knott's test ($p \leq 0.05$); CV: coefficient of variation.

Yield directly correlates to drought tolerance in rainfed citriculture (Costa et al. 2020). All selections of Rangpur lime, notably RLL, were the most drought-tolerant rootstocks over the evaluation period (Table 4). AL, which is a hybrid between RL and VKL, stood out as well, but it was less productive due to the smaller tree size (Tables 2 and 3). This same hybrid induced good performance and water use efficiency for Pera sweet orange under As climate in Northeastern Brazil (Carvalho et al. 2016). Moreover, six new hybrid rootstocks were as drought tolerant as mandarin and sour orange types and superior to SC and FDT (Table 4). The tree density in the experiment was double the average tree density in the citrus belt of São Paulo state (Fundecitrus 2022b), which may have enhanced rootstock competition for water. Notwithstanding, some less vigorous rootstocks should be considered for further evaluation in irrigated conditions, such as RCC, SFC, 025, 041, SAC, MC, and LC, due to the major impact of irrigation on the production of drought-intolerant rootstocks (Espinoza-Núñez et al. 2011). Furthermore, in this work, fruit yield was negligible in the second semester of the year due to the severe drought conditions over the evaluation period (Fig. 1), which reinforces the importance of irrigation for off-season production of Persian lime irrespective of the rootstock (Bremer Neto et al. 2013).

Table 4. Drought tolerance visual scores of the BRS EECB IAC Ponta Firme Persian lime grafted onto 26 rootstocks over five years after rainfed planting in Bebedouro, northern state of São Paulo, Brazil, 2017–2021#.

Rootstock	Drought tolerance visual score*					Mean
	2017	2018	2019	2020	2021	
RL03	2.56 a	2.80 a	2.52 b	2.64 a	2.32 a	2.57 a
RLSC	2.68 a	2.68 a	2.44 b	2.36 a	2.24 a	2.48 a
RLL	2.68 a	2.80 a	2.72 a	2.56 a	2.36 a	2.62 a
FRL	2.08 b	2.12 b	2.16 c	2.00 b	1.72 b	2.02 c
FMRL	2.20 b	2.40 b	2.32 b	2.04 b	1.88 b	2.17 b
VKL	2.28 b	2.64 a	2.36 b	2.20 b	1.84 b	2.26 b
CLM	2.20 b	2.16 b	1.80 c	1.12 e	1.20 d	1.70 c
STM	1.80 c	2.20 b	1.96 c	1.48 d	1.40 c	1.77 c
SM	2.08 b	2.12 b	1.88 c	1.48 d	1.60 c	1.83 c
SC	1.32 d	1.60 d	1.08 e	1.00 e	1.12 d	1.22 d
GTSO	2.12 b	2.20 b	2.00 c	1.64 d	1.56 c	1.90 c
IC	2.28 b	2.44 b	1.84 c	1.20 e	1.40 c	1.83 c
HMC	2.48 a	2.16 b	2.32 b	1.80 c	1.48 c	2.05 c
NGF	2.16 b	1.60 d	1.84 c	1.28 e	1.08 d	1.59 c
AL	2.76 a	2.80 a	2.76 a	2.60 a	2.32 a	2.65 a
LC	1.20 d	1.48 d	1.00 e	1.04 e	1.00 d	1.14 d
RCC	1.72 c	1.36 d	1.28 d	1.00 e	1.00 d	1.27 d
SFC	1.48 d	1.96 c	1.08 e	1.04 e	1.14 d	1.34 d
MC	2.32 b	1.76 c	1.52 d	1.20 e	1.40 c	1.64 c
BRA	2.16 b	2.40 b	1.80 c	1.32 d	1.48 c	1.83 c
073	2.28 b	2.36 b	1.92 c	1.52 d	1.60 c	1.94 c
025	1.32 d	1.44 d	1.16 e	1.12 e	1.12 d	1.23 d
033	1.96 b	1.68 d	1.32 d	1.00 e	1.04 d	1.40 d
041	1.92 b	1.56 d	1.40 d	1.12 e	1.04 d	1.41 d
SAC	1.60 c	2.01 b	1.40 d	1.16 e	1.16 d	1.47 d
FDT	1.48 d	1.92 c	1.00 e	1.00 e	1.00 d	1.28 d
CV (%)	25.59	29.06	25.30	30.93	30.76	10.43
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
PPT (mm)	70.78	30.0	37.0	8.9	12.3	-
ETP (mm)	118.74	114.31	122.34	150.70	111.24	-
DEF (mm)	43.19	73.42	61.22	110.24	73.51	-
NDT ≥ 32 °C	5	0	6	28	10	-

#Averages followed by the same letter in columns belong to the same group by Scott-Knott test ($p \leq 0.05$); *according to Cantuarias-Avilés et al. (2012); PPT: cumulative rainfall of the 90 days prior to the evaluation; ETP: cumulative evapotranspiration of the 90 days prior to the evaluation; DEF: cumulative water deficit of the 90 days prior to the evaluation; NDT ≥ 32 °C: number of days with air temperature ≥ 32 °C of the 90 days prior to the evaluation; CV: coefficient of variation.

Up to six years after planting, the cumulative incidence of HLB-infected trees of PF was the highest (~10%) on RLL, FMRL, VKL, and the hybrids AL, BRA, 025, and SAC (Table 5). No symptomatic trees were detected on SC, FDT, LC, RCC, SFC, 041, and 033. Lower incidence and inconsistent tolerance to HLB has been reported for citrus trees grafted onto *P. trifoliata* and its hybrids, especially some citrandarins (hybrids of mandarin × trifoliate orange) in field conditions in Florida and São Paulo state (Bettini et al. 2019, Tardivo et al. 2023). In this work, all HLB-symptomatic trees presented low severity indices (≤ 20% of the canopy with symptoms) regardless of the rootstock variety (Table 2). It is worth noting that the incubation period was unknown and that these results are preliminary since the experimental orchard was relatively young. In Florida (Ramadugu et al. 2016) and Porto Rico (Viteri et al. 2021), lower HLB severity was also reported for Persian lime trees. However, Lopes (2021) demonstrated a significant decrease in the number of fruits in HLB-symptomatic branches of Persian lime. Therefore, the disease progress must be evaluated in the long term for an assertive assessment of scions/rootstocks responses to HLB infection.

Table 5. Cumulative incidence (CI) and average disease severity (HLB) of huanglongbing disease, graft incompatibility score, and tree survival in 2021 of the BRS EECB IAC Ponta Firme Persian lime grafted onto 26 rootstocks over five years after rainfed planting in Bebedouro, northern state of São Paulo, Brazil, 2016–2021.

Roostsock	CI (%)	HLB*	Graft incompatibility (%)**			Tree survival (%)
			1	2	3	
RL03	6.67	0.44	100	0	0	100
RLSC	6.67	1.00	100	0	0	100
RLL	10.00	0.63	100	0	0	100
FRL	6.67	0.75	100	0	0	100
FMRL	10.00	0.42	100	0	0	100
VKL	10.00	0.46	100	0	0	100
CLM	3.33	0.13	100	0	0	100
STM	6.67	0.25	100	0	0	93.3
SM	6.67	0.75	100	0	0	96.6
SC	0.00	0.00	100	0	0	80
GTSO	6.67	0.50	100	0	0	90
IC	0.00	0.00	100	0	0	100
HMC	3.33	0.50	100	0	0	96.6
NGF	6.67	0.38	100	0	0	100
AL	10.00	0.46	100	0	0	100
LC	0.00	0.00	100	0	0	10
RCC	0.00	0.00	100	0	0	80
SFC	0.00	0.00	100	0	0	50
MC	3.33	0.63	100	0	0	86.6
BRA	10.00	0.67	100	0	0	96.6
073	3.33	0.63	100	0	0	90
025	13.33	0.31	100	0	0	96.6
033	0.00	0.00	100	0	0	96.6
041	0.00	0.00	96.7	0	3.3	100
SAC	10.00	0.54	100	0	0	100
FDT	0.00	0.00	100	0	0	53.3
Mean	5.13	0.36	-	-	-	-
CV (%)	-	-	-	-	-	-

*According to Bassanezi et al. (2011); **according to Costa et al. (2021); CV: coefficient of variation.

Graft incompatibility is critical to the commercial use of the rootstock for a given scion variety (Girardi et al. 2021). Some studies reported that the CLM rootstock induces low yield and graft uncongeniality to the Persian lime (Figueiredo et al. 2002, Stenzel and Neves 2004, Piña-Dumoulín et al. 2006). In this work, CLM induced an intermediate performance, but symptoms of graft incompatibility on the graft union, and dead plants were not observed up to five years after planting, except by the overgrowth of the Persian lime trunk, which was also noted on both selections of Sunki mandarin rootstock evaluated (Table 5). Amongst all evaluated rootstocks, only 041 presented symptoms of graft incompatibility in a few trees. In dead plants of the LC, SFC, and FDT rootstocks, it was observed that the scion tissues were dry whereas the rootstock remained alive, which suggests that tree mortality was related to the high intolerance of these rootstocks to drought. No visual symptoms typical of diseases such as gummosis and Tristeza were observed at the experimental area.

Overall, the evaluated rootstocks had a limited effect on the physical attributes of fruits of the PF (Table 6). Most rootstocks were grouped with the RLL and induced larger fruit weight, in an average of 90.7 g, in relation to that of SC, IC, LC, RCC, BRA, SAC, 073, and 033 (an average of 79.6 g), though fruit on the FDT was the lightest (60.8 g). The fruit weight was directly related to the fruit dimensions; thus, similar rootstock groups were discriminated against. The juice content and rind thickness of limes were similar among the evaluated rootstocks, on average 48.5% and 2.25 mm, respectively. As a result, the fruit quality for most of the rootstocks was acceptable for export, that is, fruit diameter from 46.5 to 60 mm, fruit weight from 90 to 125 g, and rind thickness of 1.5 to 2.5 mm (Cantuarias-Avilés et al. 2012, Castricini et al. 2017), and for the domestic market that requires a minimum juice content of 40% (Ceagesp 2011).

Table 6. Physical attributes, color variables, and oil yield of fruit of the BRS EECB IAC Ponta Firme Persian lime grafted onto 26 rootstocks from three to six years after rainfed planting in Bebedouro, northern state of São Paulo, Brazil, 2019–2021#.

Rootstock	Fruit weight (g)	Equatorial diameter (cm)	Longitudinal diameter (cm)	Juice content (%)	Rind thickness (mm)	L*	a*	b*	RI[1]	Oil yield (g·box^{-1})
RL03	99.68 a	5.37 a	6.10 a	48.42	2.44	51.46 a	-19.14 a	36.74 a	-10.53	-
RLSC	87.40 a	5.19 a	5.88 a	49.98	2.37	51.23 a	-19.38 a	36.62 a	-10.16	-
RLL	92.61 a	5.29 a	6.12 a	49.53	2.21	50.44 a	-19.24 a	35.64 a	-10.92	172.77
FRL	90.72 a	5.13 a	6.02 a	49.14	2.47	49.97 b	-19.29 a	35.52 a	-11.12	-
FMRL	91.60 a	5.21 a	5.90 a	47.42	2.42	47.72 b	-18.80 b	32.91 b	-12.24	-
VKL	90.45 a	5.28 a	6.05 a	45.59	2.60	48.34 b	-18.60 b	33.50 b	-11.97	181.85
CLM	91.68 a	5.18 a	5.98 a	47.38	2.54	47.86 b	-19.25 a	33.15 b	-12.22	-
STM	90.44 a	5.32 a	6.03 a	47.56	2.33	47.37 b	-18.44 b	32.13 b	-12.46	156.01
SM	93.29 a	5.19 a	5.98 a	49.45	2.66	47.98 b	-19.09 a	33.46 b	-12.07	-
SC	70.95 b	5.00 b	5.56 b	48.88	1.49	47.87 b	-18.78 b	33.08 b	-12.01	177.69
GTSO	89.32 a	5.12 a	5.91 a	50.21	2.29	48.37 b	-18.84 b	33.39 b	-12.03	-
IC	84.17 b	5.12 a	5.88 a	48.06	2.40	47.83 b	-19.05 a	32.97 b	-12.13	179.31
HMC	90.31 a	5.14 a	6.04 a	48.03	2.45	48.62 b	-19.16 a	33.86 b	-11.69	-
NGF	85.02 a	5.20 a	5.77 a	48.01	2.28	48.60 b	-18.95 b	33.32 b	-11.84	-
AL	91.54 a	5.37 a	6.09 a	47.31	2.49	47.93 b	-18.60 b	32.85 b	-11.85	-
LC	79.68 b	5.15 a	5.81 a	46.11	1.56	48.34 b	-19.75 a	33.89 b	-12.13	-
RCC	81.89 b	5.17 a	5.90 a	48.20	1.95	50.14 a	-19.52 a	35.47 a	-11.00	-
SFC	85.61 a	5.26 a	5.95 a	47.79	2.29	49.83 a	-19.10 a	34.88 a	-10.89	145.50
MC	97.07 a	5.36 a	6.06 a	50.14	2.22	50.81 a	-19.25 a	36.07 a	-10.70	169.05
BRA	83.07 b	5.22 a	6.07 a	49.43	2.58	48.32 b	-19.33 a	33.68 b	-11.91	166.01
073	83.43 b	5.24 a	5.85 a	48.73	2.44	49.17 b	-19.39 a	34.44 a	-11.84	-
025	88.87 a	5.15 a	5.95 a	50.01	2.25	47.93 b	-18.75 b	32.54 b	-12.11	-
033	73.06 b	4.72 b	5.30 b	48.43	1.48	46.64 b	-19.08 a	31.66 b	-12.93	-
041	87.30 a	5.26 a	5.99 a	48.07	2.51	47.82 b	-18.80 b	32.69 b	-12.14	-
SAC	81.02 b	5.14 a	5.94 a	47.80	2.30	48.93 b	-19.42 a	34.59 a	-11.64	-
FDT	60.85 c	4.77 b	5.34 b	51.35	1.53	47.53 b	-17.99 c	32.09 b	-11.91	168.78
Mean	86.81	5.18	5.91	48.53	2.25	48.77	-19,03	33.92	-11.70	168.55
CV (%)	13.33	6.96	7.07	11.59	30.34	9.61	8.71	16.18	16.49	7.76
P	<0.0001	0.0003	<0.0001	0.7816	0.7296	<0.0001	<0.0001	<0.0001	0.9974	0.059

#Averages followed by the same letter in columns belong to the same group by Scott-Knott's test ($p \leq 0.05$); L* (luminosity), a* (green/red ratio), and b* (blue/yellow ratio), according to Commission Internationale de L'Eclairage (1986); 1rind color index, according to Jimenez-Cuesta et al. (1983); CV: coefficient of variation.

The color of the fruit rind is a determinant trait for the commercialization of Persian lime, with dark green fruit being preferred (Khan and Singh 2017), which in turn relates to low L*, negative a*, and low b* values in the CIELAB space (McGuire 1992). In this sense, there was a significant effect of the rootstock variety on PF rind color (Table 6). Brighter and more yellow lime fruits were observed for some rootstocks, notably RL selections and three citrandarins, whereas other traditional rootstocks and most new hybrids induced darker or greener fruit. However, the rind color index was not influenced by the rootstock varieties, which generally indicated green fruits with an average of -11.70. Our results confirm that RLL rootstock reduces the number of exportable limes due to a poor rind color compared with those fruits from trees on the FDT, which may be related to the earlier fruit maturation of the former rootstock (Cantuarias-Avilés et al. 2012).

Furthermore, other vigorous rootstocks resulted in both larger and darker fruit, such as FMRL, VKL, AL, STM, and GTSO, in addition to producing high fruit yield (Table 3). They thus could be considered better alternatives to RLL and FDT for fresh fruit exports. In addition, the essential oil yield of PFs fruits was equivalent between the selected rootstocks, which may have been a result of the scion/rootstock interaction that regulates the translocation of water, nutrients, plant hormones, and photoassimilates (Aguilar-Hernández et al. 2020). Differences in the essential oil components of the fruit peel by the rootstock variety have been reported in other citrus types, such as lemons and bergamot (Verzera et al. 2003, Reuss et al. 2020).

According to the PCA analysis, the rootstocks were arranged into six groups, considering the significant variables, except for the color indices (Fig. 2). These were not used in this analysis because they formed clusters with isolated rootstocks. The PC1 explained 69.3% of the total variance, and YL, CD, and CV were the variables that best represented this component, with r values = 0.89, 0.87, and 0.85, respectively. In contrast, the PE variable showed the highest correlation (0.52) in PC2. Group 1 was represented by the RLL and comprised vigorous, productive, and drought-tolerant rootstocks. Group 2 clustered standard-sized, highly efficient rootstocks bearing heavy fruits, as represented by SM. Group 3 comprised drought-tolerant substandard rootstocks such as GTSO. The MC represented Group 4, with substandard/semi-dwarfing yet more productive rootstocks. Finally, Group 5 and Group 6 were represented by SC (higher tree vigor) and FDT (dwarfing rootstocks), respectively, and included low-yielding, drought-intolerant rootstocks.

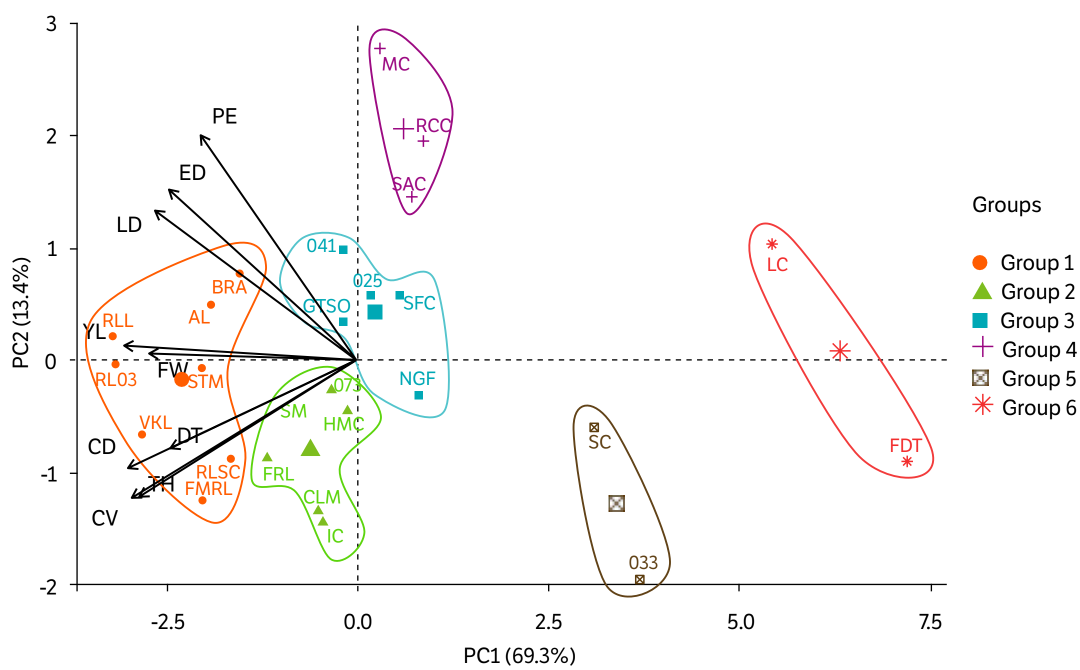

Figure 2. Principal components analysis (PC1 and PC2) shows the distribution of 26 rootstocks associated with production efficiency (PE), equatorial diameter (ED), longitudinal diameter (LD), yield (YL), fruit weight (FW), drought tolerance (DT), canopy diameter (CD), canopy volume (CV) and tree height (TH). Group 1: RL03, RLSC, RLL, FMRL, VKL, STM, AL, and BRA. Group 2: FRL, CLM, SM, IC, HMC, and 073. Group 3: GTSO, NGF, SFC, 025, and 041. Group 4: RCC, MC, and SAC. Group 5: SC, and 033. Group 6: LC, and FDT.

CONCLUSION

Lemon-type rootstocks and Sunki BRS Tropical mandarin are vigorous and productive rootstocks when grafted with the Persian lime cv. BRS EECB IAC Ponta Firme in rainfed cultivation under Aw climate. Goutoucheng sour orange, BRS Ary, and BRS Bravo hybrids induced similar yield to the vigorous rootstocks yet smaller trees without irrigation. The BRS Matta citrandarin has the potential as an alternative rootstock to the dwarfing FD trifoliate orange in irrigated high-density orchards.

AUTHORS' CONTRIBUTION

Conceptualization: Girardi, E. A. and Stuchi, E. S.; **Investigation:** Silva, L. N., Vitória, M. F. and Moreira, A. S.; **Formal Analysis:** Silva, L. N., Vitória, M. F. and Moreira, A. S.; **Methodology:** Silva, L. N.; **Funding acquisition:** Girardi, E. A. and Stuchi, E. S.; **Writing – Review and Editing:** Silva, L. N., Vitória, M. F., Moreira, A. S., Girardi, E. A. and Stuchi, E. S.; **Supervision:** Girardi, E. A. and Stuchi, E. S.; **Project Administration:** Girardi, E. A. and Stuchi, E. S.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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