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Rootstock-related improved performance of ‘Pera’ sweet orange under rainfed conditions of Northeast Brazil

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ABSTRACT

Citrus orchards in the coastal tablelands of Northeast Brazil are almost exclusively composed of ‘Pera CNPMF D-6’ sweet orange, henceforth ‘Pera’, grafted on ‘Rangpur’ lime. Therefore, assessing new rootstocks for ‘Pera’ in this region is pivotal for diversification purposes, potentially leading to increased competitiveness, yield and fruit quality. In this study, vegetative growth, productive performance, pest attack and fruit quality of ‘Pera’, grafted on five rootstocks, were assessed over 10 years in Northeast Brazil. The trifoliate hybrid HTR-051 conferred the highest yield efficiency to ‘Pera’, while ‘Red Rough’ lemon and LVK x LCR - 010 induced precocity, with yields exceeding 9400 kg ha^{-1} in the first harvest, as well as the highest cumulative yields. ‘Red Rough’ lemon, LVK x LCR - 010 and HTR-051 were notable for imparting high weight and total soluble solids to ‘Pera’ fruit. Regarding pests, the highest densities of citrus rust mite *Phyllocoptruta oleivora* were found on the fruit of ‘Pera’ grafted on LVK x LCR - 010, despite low densities of this arthropod over the sampling period. Altogether, our results highlight ‘Red Rough’ lemon and LVK x LCR −010 as superior rootstocks for excelling in productive performance and fruit quality. In addition, HTR-051 manifests as an excellent option for high-density orchards because it induces dwarfism and high production efficiency.

1. Introduction

Sweet oranges [*Citrus sinensis* (L.) Osbeck] are among the most popular fruits and account for more than two-thirds of the citrus fruit produced worldwide (UNCTAD, 2004; FAO, 2017). Brazil ranks as the world’s top producer of sweet orange, mostly concentrated in the southeast (418.955 ha) and northeast (101.757 ha) regions of the country (IBGE, 2019). This country is also the leading exporter of orange juice. In Northeast Brazil, over 90 % of the citrus orchards lie within an area alongside the coastline in the neighbouring states of Bahia and Sergipe. The yields in these two states are around half and one-third, respectively, the yield of the south-eastern state of São Paulo (32.18 kg·ha^{-1}), the main producer nationwide (IBGE, 2019). Such low yields in Northeast Brazil are related to soil fertility constraints, low and irregular rainfall regime, deficiency in pest and disease management, low adoption of technologies and the presence of hard-setting subsoil layers that hamper root growth (Martins et al., 2016; Silva et al., 2017; Carvalho et al., 2019).

Apart from the yields below the potential yields, orchards in this region are composed almost exclusively of ‘Pera CNPMF D-6’ sweet orange, hereafter ‘Pera’ (Martins et al., 2016). This cultivar is characterised by medium-sized plants, mid-season maturation starting in July, and fruit suited for both *in natura* consumption and the juice industry (Bastos et al., 2014). In addition, Rangpur’ lime (*C. limonia* Osbeck) is the rootstock of choice for the majority of growers throughout the region, mainly because of its drought-tolerance and graft-compatibility to ‘Pera’ (Carvalho et al., 2016a). This lack of genetic diversification can not only compromise the ability of the regional citriculture to face a potential emergence of new pests (Bové and Ayres, 2007) but also the economic competitiveness of growers. As the rootstock influences several characteristics of the scion (Medina et al., 2005), assessing the influence of rootstocks, other than the prevailing ‘Rangpur’ lime, is pivotal for diversification, increasing competitiveness, yield and fruit quality (Carvalho et al., 2019).

As varieties present distinct genetic characteristics, the effect of rootstocks on the susceptibility to the citrus rust mite *Phyllocoptruta*

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oleivora (Acari: Eriophyidae), a major pest of citrus orchards in the region and worldwide, was also assessed (Teodoro et al., 2014; Martins et al., 2016; Silva et al., 2017; Tropea Garzia and de Lillo, 2018). This mite attacks developing fruit, leading to reduced fruit yield and aesthetic damage resembling rust (Teodoro et al., 2014). Accordingly, this study aimed to identify alternative rootstocks for 'Pera' under rainfed conditions of Northeast Brazil by assessing vegetative, productive and qualitative variables as well as susceptibility to the citrus rust mite.

2. Materials and methods

The study was conducted from 2008 to 2018 at the Experimental Station of Embrapa Tabuleiros Costeiros located in Umbaúba (11°22′37″ S; 37°40′26″ W; 109 m.a.s.l.), in the north-eastern state of Sergipe. According to Köppen-Geiger classification, the climate is *As′* type (rainy tropical with dry summers) with annual means for temperature, relative humidity and precipitation of 24.6 °C, 83 % and 1309.4 mm, respectively, during the experiment.

The orchard was established in an Haplic Acrisol in 2008 and consisted of 'Pera' sweet orange grafted on five rootstocks in a completely randomised block design, with four replicates and two plants per plot. Trees were planted at a density of 416 plants ha^{-1} (6.0 m × 4.0 m) and managed under rainfed conditions (Martins et al., 2016). The rootstocks were 'Red Rough' lemon (*C. jambhiri* Lush.), 'Orlando' tangelo (*C. paradisi* Macfad. x *C. tangerina* Tanaka), and the hybrids HTR-051 (*C. limonia* x *Poncirus trifoliata* L. Raf.), LVK x LCR - 010 (*C. volkameriana* V. Ten. & Pasq. x *C. limonia*) and TSKFL x CTTR - 017 {'Sunki of Florida' mandarin [*C. sunki* (Hayata) hort. ex Tanaka] x 'Troyer' citrange (*C. sinensis* × *P. trifoliata*)}, which were obtained or introduced by the Citrus Breeding Program of Embrapa Mandioca e Fruticultura.

Vegetative growth was assessed by measuring plant height (PH) and calculating canopy volume (CV) in 2015. PH was measured with a ruler from the base of the trunk close to the soil up to the top of the plant. The CV (m^3) was calculated by the formula proposed by Zekri (2000): CV = (π/6) × PH × RD × PD, where RD and PD are the canopy diameters (m) along (RD) and perpendicular (PD) to the row. In addition, survival rate (SR; %) was obtained by counting living plants in 2018. Yields and cumulative yields (CY; kg ha^{-1}) were recorded over 8 years (2011–2018), while yield efficiency (YE; kg m^{-3}) was estimated in the 2015 harvest by calculating the quotient between per plant fruit production and CV. The alternate bearing index (ABI) was calculated using data from 2011 to 2018 by the following formula proposed by Monselise and Goldschmidt (1982):

$$ABI = \frac{\sum_{i=1}^{n} \frac{|Y_{i+1} - Y_i|}{Y_{i+1} + Y_i}}{n - 1},$$

where n is the number of years and Y_i is the yield in year i (Smith et al., 2004). Yield precocity (YP) was calculated as a percentage of the ratio between the yield in the first two harvest seasons (2011 and 2012) and total CY, allowing early bearing estimates for each rootstock.

Fruit quality was assessed during the harvests 2015–2016 by sampling nine fruit per tree, as per Pregnolatto and Pregnolatto (1985). Briefly, the height and the diameter of fruit were measured with a digital calliper. The percentage of juice was derived by dividing the juice weight by the sample weight and multiplying by 100. Juice mass was obtained by the difference between sample weight and bagasse weight (i.e., seeds, peels, and fragments sieved). Total soluble solids (TSS; ºBrix) were determined using a digital refractometer (Pallete PR-32α, Atago, Tokyo, Japan), with values corrected to 20 °C. Total titratable acidity (TTA) was measured using a semi-automatic burette, with 0.1 mol L^{-1} NaOH (titrant) and phenolphthalein indicator. The vitamin C content (mg/100 mL of juice) was determined using the oxidation–reduction volumetric technique, with 0.002 mol L^{-1} potassium iodate (KIO_3) and 1 % starch indicator solution.

Data were subjected to ANOVAs, and the means were grouped by the Scott–Knott test ($P < 0.05$). Multivariate analyses were then performed using XLSTAT 2014® add-in for Excel® in order to identify homogenous groups of rootstocks considering all variables that showed significant differences by univariate ANOVAs. These variables were: plant height and canopy volume for vegetative growth; cumulative yield (2011–2018), yield efficiency, ABI and yield precocity for productive performance; fruit mass and total soluble solids for fruit quality. Following the approach used by Carvalho et al. (2019), a principal component analysis (PCA) was conducted to reduce the dataset into a few synthetic and uncorrelated variables (i.e., the first principal components or PCs). Then, the rootstocks were grouped by agglomerative hierarchical clustering (AHC) applied to the PCs scores that complied with Kaiser criterion, that is, those whose eigenvalues were ≥1.0 (Kaiser, 1960). Euclidean distance was used as a measure of dissimilarity and the method for linkage to identify the clusters was the Ward's minimum-variance in the AHC. Automatic truncation option was used for cluster splitting. This approach creates homogenous groups based on the largest decrease in Shannon's entropy between a node and the next one. The resulting clusters were then interpreted from the PCA results and examined by reference to the results of the univariate ANOVAs.

We further assessed the influence of rootstocks on population levels of *P. oleivora*. The number of adults was counted monthly from June 2011 to June 2012 (months with zero mite counts for all rootstocks were excluded from analyses). Briefly, two randomly-chosen fruit taken from two plants each were evaluated, totalling four fruit per rootstock in each evaluation, and the mites were counted in a 1 cm x 1 cm area. A repeated-measures ANOVA, followed by post hoc Fisher LSD test, was carried out to assess the effect of rootstocks on population levels of the pest mite, removing variance explained by time, as evaluations were carried out monthly. Data were $\sqrt{(x)} + 1$ transformed to adjust for normal distribution.

3. Results

The rootstocks influenced vegetative parameters of 'Pera' sweet orange (Table 1), in that HTR-051 trifoliate hybrid conferred low plant height and canopy volume, which, in turn, led to the highest yield efficiency. Except for 'Orlando' tangelo (12.5 % mortality), plant mortality was 0 % that could indicate compatibility between 'Pera' and the rootstocks after 10 years of planting.

The productive performance of 'Pera' plants was also influenced by the rootstocks (Table 2). 'Pera' grafted on 'Red Rough' lemon exhibited the highest fruit yields throughout all harvests, except in 2016. 'Pera' on this rootstock, followed by LVK x LCR – 010 exhibited the highest cumulative yields (Table 2). The lowest ABI values were measured for 'Pera' grafted on LVK x LCR - 010, which, together with TSKFL x CTTR - 017 and 'Red Rough' lemon, induced precocity to 'Pera', with yields exceeding 9400 kg·ha^{-1} in the first harvest.

Most variables of 'Pera' fruit quality were not influenced by the rootstocks (Table 3). However, 'Red Rough' lemon and LVK x LCR –

Table 1
Plant height (PH), canopy volume (CV), and yield efficiency (YE) of 'Pera' sweet orange trees grafted on five rootstocks.

Rootstock	PH (m)	CV (m^3)	YE (kg m^{-3})
TSKFL x CTTR - 017	2.34 b[1]	14.67 c	6.80 c
'Orlando' Tangelo	2.84 a	20.02 a	6.06 c
HTR-051	2.11 b	6.99 d	12.14 a
LVK x LCR - 010	2.74 a	13.58 c	8.07 c
'Red Rough' Lemon	2.76 a	16.90 b	9.46 b
CV (%)	10.17	13.90	14.94
P-value	0.01	< 0.001	< 0.001

[1] Means followed by the same letter within columns do not differ from each other (Scott-Knott test, $P < 0.05$).

Table 2
Annual and cumulative yields (kg ha^{-1}), alternate bearing index (ABI) and yield precocity (YP) of 'Pera' sweet orange trees grafted on five rootstocks.

Rootstock	Annual yield								Cumulative yield[2]	ABI	YP (%)
	2011	2012	2013	2014	2015	2016	2017	2018	2011 - 2018		
TSKFL x CTTR-017	12013 b[1]	4045 d	17895 a	21091 b	41160 d	18307 c	7115 a	10800 b	132424 (16553) c	0.37 a	12.1 a
'Orlando' Tangelo	3302 e	6194 c	13528 b	13651 c	50203 b	26209 a	4781 b	10092 b	127959 (15995) c	0.38 a	7.4 b
HTR-051	5432 d	3792 d	9530 c	16509 c	34913 e	19357 c	5063 b	10103 b	104697 (13087) d	0.35 a	8.8 b
LVK x LCR - 010	9439 c	8164 b	20793 a	19312 b	45833 c	20254 c	9646 a	11633 b	145073 (18134) b	0.26 b	12.2 a
'Red Rough' Lemon	13673 a	9231 a	19928 a	31997 a	67098 a	24528 b	8573 a	15122 a	190149 (23769) a	0.34 a	12.1 a
CV (%)	13.95	11.73	13.96	15.40	7.29	5.11	20.60	13.55	5.86	10.15	11.76
P-value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.002	0.003	< 0.001	0.004	< 0.001

[1] Means followed by the same letter within columns do not differ from each other (Scott-Knott test, $P < 0.05$).
[2] Average yields throughout the harvests are given within brackets.

010, followed by HTR-051 imparted the highest weight to Pera, and HTR-051, LVK x LCR - 010 and 'Red Rough' lemon conferred the highest total soluble solids to fruit. Total titratable acidity values ranged from 0.70 % (TSKFL x CTTR – 017 and 'Orlando' tangelo) to 0.89 % (LVK x LCR – 010) and total soluble solids from 10.4 (TSKFL x CTTR - 017) to 12.2°Brix (HTR-051).

The first two axes of the PCA explained near 67 % of the total variability (Fig. 1a). The square cosine of the variables showed that those contributing to most of the variation of PC1 were plant height, canopy volume, yield efficiency and total soluble solids, whereas cumulative yield, alternate bearing index and fruit mass contributed to most of the variation along PC2.

The AHC grouped the rootstocks into three groups that showed similar results considering the set of variables (Fig. 1b). The first one included 'Orlando' tangelo and TSKFL x CTTR - 017 hybrid, and the main characteristic of this group is that the rootstocks have higher alternate bearings and lower fruit mass and total soluble solids. The second group enclosed 'Red Rough' lemon and the LVK x LCR - 010 hybrid and share similarities regarding lower alternate bearings associated with bigger fruits, high cumulative yields and yield precocity. The third one was composed only by the trifoliate hybrid HTR-051 and shows higher yield efficiency and total soluble solids but lower plant height, canopy volume and cumulative yield than the other two groups.

The population levels of the rust mite *P. oleivora* in 'Pera' plants were influenced by the evaluated rootstocks ($F_{4,14} = 3.38$; $P = 0.04$). The highest densities of *P. oleivora* were found on the fruit of 'Pera' grafted on LVK x LCR - 010 in comparison to rootstocks TSKFL x CTTR - 017, HTR-051 and 'Orlando' tangelo (data not shown).

4. Discussion

The HTR-051 trifoliate hybrid conferred lower heights and canopy volume to 'Pera' plants, which led to the highest yield efficiency. Similar patterns were found for mandarin–tangor 'Piemonte' (Carvalho et al., 2016a), and for 'Pera' and 'Valencia' sweet oranges (Ramos et al., 2015) grafted on HTR-051, corroborating that low plant height and canopy volume are intrinsic traits of this rootstock. Induction of higher yield efficiency has also been reported for other trifoliate rootstocks (Cantuárias-Avilés et al., 2011 and references therein). The results obtained here indicate that this hybrid is suitable for high-density orchards (> 1000 trees per hectare) (Pompeu, 2005; Cantuárias-Avilés et al., 2011). Pest and crop management, as well as harvest, may also be facilitated in small-sized trees (Lima et al., 2014; Pompeu and Blumer, 2014).

Independently of the rootstock, fruit yield peaked in the fifth harvest, after which yields decreased. 'Red Rough' lemon, followed by LVK x LCR - 010, conferred the highest cumulative yields to 'Pera'. Consistent with these results, high cumulative yield was previously reported for mandarin-tangor 'Piemonte' [*C. clementina* hort. ex Tanaka x (*C. sinensis* x *C. reticulata* Blanco)] when grafted on 'Red Rough' lemon cultivated in the same region of this study (Carvalho et al., 2016a). Furthermore, lemon rootstocks are known to impart higher productivity to scion cultivars (Wutscher and Bowman, 1999).

TSKFL x CTTR – 017, 'Red Rough' lemon and LVK x LCR - 010 induced yield precocity to 'Pera'. Similar yield precocity patterns were reported for 'Pera' and mandarin-tangor 'Piemonte' grafted, respectively, on 'Santa Cruz' Rangpur lime and 'Red Rough' lemon, in the same region of this study (Carvalho et al., 2016a, b). In addition to inducing high cumulative yield and yield precocity, LVK x LCR - 010 imparted 'Pera' trees with lower propensity for alternate bearing. From an economic perspective, this is a desirable trait as severe alternate bearing can have a negative impact on growers' income and industry.

The highest fruit mass was obtained from 'Pera' grafted on 'Red Rough' lemon and LVK x LCR – 010, followed by HTR-051. 'Pera' grafted on these rootstocks also produced sweeter fruit. Yield and juice quality are two important traits for fruit intended for the juice industry (Castle et al., 2010). Juice yield and total soluble solids measured in this study met standards for fresh fruit markets in Brazil, which should fall within 35 %–45 % and 9–10, respectively (CEAGESP, 2011). Total titratable acidity values were also within the requirements for orange fruit intended for frozen and concentrated juice (Petto Neto and Pompeu, 1991).

Although *P. oleivora* is a key pest that frequently inflicts damage to fruit in citrus orchards of Northeast Brazil, low densities of this mite

Table 3
Height (FH) and diameter (FD) of fruit, fruit mass (FM), juice content (JC), total titratable acidity (TTA), total soluble solids (TSS) and vitamin C of 'Pera' sweet orange in relation to rootstocks.

Rootstock	FH (mm)	FD (mm)	FM (g)	JC (%)	TTA (%)	TSS (°Brix)	Vitamin C (mg/100 mL juice)
TSKFL x CTTR - 017	75.5[1]	76.1	191.4 c	59.5	0.70	10.4 b	40.9
'Orlando' Tangelo	72.7	74.1	195.5 c	58.9	0.70	10.5 b	40.3
HTR-051	71.3	72.4	202.4 b	58.6	0.87	12.2 a	43.9
LVK x LCR - 010	72.8	72.5	209.0 a	57.7	0.89	11.7 a	46.5
'Red Rough' Lemon	72.1	72.6	210.6 a	57.9	0.86	11.6 a	45.5
CV (%)	2.88	2.63	2.74	1.90	17.13	7.38	7.14
P-value	0.126	0.077	< 0.001	0.194	0.181	0.046	0.056

[1] Means followed by the same letter within columns do not differ from each other (Scott-Knott test, $P < 0.05$).

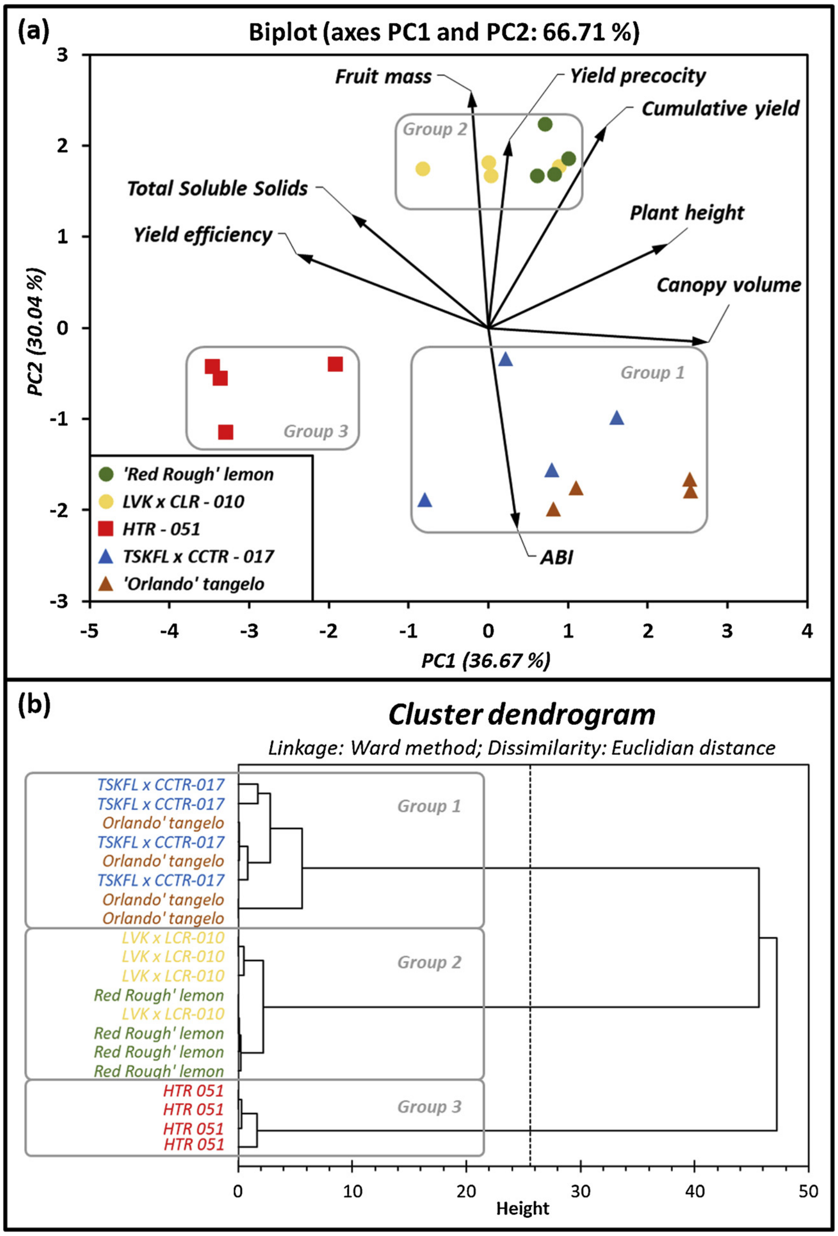

Fig. 1. Representation of rootstock groups resulting from Hierarchical Clustering on Principal Component Analysis. (**a**) PCA-Biplot showing observations and variables and (**b**) Dendrogram generated by Agglomerative Hierarchical Clustering with the three groups of rootstocks.

were found throughout the sampling period in this study, and fruit showed few visible symptoms, suggesting no yield losses due to its attack. Nevertheless, the population levels of the rust mite *P. oleivora* on 'Pera' was influenced by the rootstocks, being highest when grafted on LVK x LCR - 010 in comparison with the other rootstocks. These results are supported by Silva et al. (2017), who reported that natural infestations of *P. oleivora* and *Tetranychus mexicanus* on 'Pera' and 'Valencia Tuxpan' are affected by rootstocks. Contrarily, another study suggested that the population densities of this mite are not influenced by the rootstock in mandarin-tangor 'Piemonte' (Carvalho et al., 2016a).

The rootstock can not only influence agronomical traits, but also resistance mechanisms against arthropod pests (Bruessow et al., 2010; Agut et al., 2016). Variations in water and mineral uptake by rootstocks can modify chemical characteristics of upper parts (Parvaneh et al., 2019 and references therein), which in turn, can affect plant-arthropod interactions. In addition, there is evidence of translocation of defence-signalling molecules from roots to upper parts that confer citrus scion systemic resistance to *Tetranychus urticae* (Acari: Tetranychidae), and this systemic resistance has been proven to be rootstock-dependent (Agut et al., 2016). Therefore, possible mechanisms underlying the responses of some rootstocks to *P. oleivora* presented here require additional confirmatory studies.

5. Conclusions

Overall, the results suggest 'Red Rough' lemon, followed by hybrid LVK x LCR - 010, as superior rootstocks for imparting high cumulative yield, large fruit, high Brix and yield precocity to 'Pera' sweet orange in Northeast Brazil. In addition, HTR-051 holds potential for high-density orchards in this region, conferring lower plant height, smaller canopy volume and high yield efficiency associated with larger fruit and high total soluble solids to 'Pera'.

CRediT authorship contribution statement

Hélio Wilson Lemos de Carvalho: Methodology, Investigation, Formal analysis, Writing - original draft. **Adenir Vieira Teodoro:** Investigation, Formal analysis, Writing - original draft, Writing - review & editing. **Inácio de Barros:** Formal analysis, Writing - review & editing. **Luciana Marques de Carvalho:** Writing - review & editing. **Walter dos Santos Soares Filho:** Funding acquisition, Writing - review & editing. **Eduardo Augusto Girardi:** Writing - review & editing. **Orlando Sampaio Passos:** Writing - review & editing. **Delia M. Pinto-Zevallos:** Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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