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CROP PRODUCTION AND MANAGEMENT - Article

Compatibility and horticultural performance of Pera sweet orange clones grafted to Swingle citrumelo rootstock

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ABSTRACT: Despite its interesting agricultural traits, Swingle citrumelo (*Citrus paradisi* Macfad. 'Duncan' × *Poncirus trifoliata* L. Raf.) rootstock exhibits severe incompatibility when grafted with Pera sweet orange [*Citrus sinensis* (L.) Osb.] scions, and this fact limits its use for propagation purposes. Nonetheless, the existence of certain Pera sweet orange clones that are compatible with Swingle citrumelo represents a substantial gain for the entire citrus industry. This study investigated the compatibility between seven Pera sweet orange clones and Swingle citrumelo rootstock, regarding the presence of a necrotic line at the graft union junction, the difference in diameter between rootstock and scion, the influence of clonal cleaning by micrografting, and the horticultural performance of the selected combinations. No trees died because of grafting incompatibility, even after twenty years (trial I), except for clone CV1, or after six years and nine months (trial II) from planting. The rootstock/scion trunk diameter ratio was not related to the presence of a necrotic line in the graft-union region. Additionally, these variables did not influence fruit yield and quality, and they were not affected by clonal cleaning or pre-immunisation of the old Pera sweet orange clones.

Key words: *Citrus sinensis*, *Citrus paradisi* × *Poncirus trifoliata*, rootstock, bud-union crease.

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INTRODUCTION

Citrus has the highest production worldwide of fruits, with more than 135 million tons per year, of which Brazil provides 13.6% (FAO 2017). The Cravo Rangpur lime (*Citrus limonia* Osbeck) is one of the main rootstocks utilised in commercial citrus production in Brazil, because of its appropriate characteristics and its tolerance to citrus tristeza virus (CTV). Nonetheless, its high susceptibility to citrus sudden death demonstrates the need for rootstock diversification (Bové and Ayres 2007).

Currently, Pera sweet orange represents 35% of all the sweet orange varieties cultivated in the states of São Paulo and Minas Gerais, considering both productive and nonproductive groves (Fundecitrus 2018). In 2017, more than 50% of all the sweet orange nursery trees commercialised in São Paulo state were grafted onto Cravo Rangpur lime.

Pera sweet orange stands out because of its good fruit yield and quality, off-season blossoms (Salibe et al. 2002) and tolerance to CTV (Müller et al. 1999). Cravo Rangpur lime rootstock induces early fruit bearing to the scion and is tolerant to water stress and CTV (Yamamoto et al. 2011), although it shows variable susceptibility to gummosis (Pompeu Júnior 2005).

Swingle citrumelo (*Citrus paradisi* Macfad. 'Duncan' × *Poncirus trifoliata* L. Raf.) is characterised by its tolerance to CTV (Castle and Stover 2000), nematodes, *Phytophthora* spp. (Pompeu Júnior 2005), citrus sudden death (Bové and Ayres 2007), and the superior fruit quality it confers on the scion, when compared to Cravo Rangpur lime rootstock. However, it is incompatible with Pera Pré-Imunizada, Pera IAC and Bianchi sweet orange clones (Pompeu Júnior 2005), a fact that limits its utilisation as a rootstock. Bud union incompatibility is known to occur between selections of this sweet orange and trifoliate orange and its hybrids.

Pera sweet orange selections grafted onto Swingle citrumelo showed bud union incompatibility at the age of eight years or earlier, including scions Pera Sweet, Pera 15, Pera 7, Bianchi 25, Bianchi 26, Indiantown, Olympia, Pré-Imunizada, Rio and Vacinada. By contrast, Perão and Acidless did not present any bud union creases (Castle and Baldwin 2011). Incompatibility was reported for Pera Sweet orange IAC-1743, pre-immunised against CTV selection when budded in two citromonia rootstocks, limão Cravo × citrange Carrizo (717) and (1581), citrandarin Cleópatra × Swingle trifoliate orange (1654) and Swingle citrumelo rootstock (Pompeu Júnior and Blumer 2014).

Graft incompatibility is the consequence of a senescence process caused by biochemical and anatomical factors that may be enhanced by the environment (Pompeu Júnior 2005); however, it may instead be considered as just the inability of a scion and a rootstock to form a grafting union (Goldschmidt 2014). It is probably caused by anatomical abnormalities of the vascular tissue and/or infection by viruses or phytoplasmas (Hartmann et al. 2002).

The quality of the vascular connections formed in the grafting region varies according to the scion/rootstock combination, and it may affect water transport from the roots, either long term or permanently. In perennial trees, scion/rootstock incompatibility occurs around one year after grafting. However, in some cases, it may take several years to be detected (Müller et al 1996 b; Warschefsky et al. 2016).

The similarity between scion and rootstock diameters at the grafting union is proportional to the level of the compatibility between them (Ferguson et al. 1990; Aubert and Vullin 1998). Nonetheless, the existence of scion/rootstock incompatibility does not always correlate with this trait, as in some species differences in trunk diameter between both materials are commonly observed, even for compatible scion/rootstock combinations (Stuchi 2002).

This current study assessed the compatibility and horticultural performance of six Pera sweet orange clones grafted onto Swingle citrumelo rootstock, compared to the scion Pera IAC.

MATERIAL AND METHODS

The trial was carried out in Bebedouro, in the northern region of São Paulo state, Brazil (20º 53' 16" S, 48º 28' 11" W), where the mean annual rainfall was 1,550 mm and mean temperatures varied from 16.8 °C to 30.6 °C. The soil was a dystrophic, red-yellow ultisol, moderate A horizon, sandy/clay texture, with no physical or chemical restrictions, with a pH ($CaCl_2$) of 5.0 and cation exchange capacity (CEC) of 4.8 $mmolc{\cdot}dm^{-3}$ in the upper layer (0-20 cm) (Embrapa 2018). Ultisols and oxisols were regularly planted with commercial citrus orchards in São Paulo state (Corá et al. 2005).

Two trials were conducted. In trial I, four old Pera sweet orange clones selected in the 1980s from the most productive old orchards of Bebedouro county, clones Velho 1 (1CV), Velho 2 (2CV), Velho 3 (3CV), and Velho 4 (4CV), were grafted onto Swingle citrumelo trees. Planting occurred in December 1990 at the Estação Experimental de Citricultura de Bebedouro (EECB), in a 7.0 m × 3.5 m spacing (408 plants.ha^{-1}), and plants were grown without supplemental irrigation. All trees of clone Velho 1 were removed before age four; therefore, data from these plants were not considered, and this selection was not included in trial II.

In trial II, the old budlines selections of the first trial were compared with their micrografted and pre-immunised versions, with Pera IAC budded in Swingle citrumelo, and Pera IAC grafted onto Cravo Rangpur lime. In June 2003, the following eight treatments were evaluated in a grove planted at the EECB in a 6.5 m × 2.5 m spacing (615 plants·ha^{-1}): 2CV, 3CV, and 4CV; micrografted and pre-immunised clones Velho 2 (2MP), Velho 3 (3MP), and Velho 4 (4MP); and two control treatments, Pera IAC grafted to Swingle citrumelo (IAC/Sw; incompatible control) and Cravo Rangpur lime (IAC/Cr; compatible control). Both trials were conducted and statistically analysed following an unbalanced completely randomised design, with an unequal number of replications.

In 2010, the scion/rootstock incompatibility was evaluated by a 0-3 score rating scale (scores 1, 2 and 3 for a low, medium and high degree of incompatibility, respectively), adapted from Müller et al. (1996 b) (Fig. 1), using bark sections removed from the grafting line of 20 plants in trial I and 39 plants in trial II. Trunk circumference was measured 5 cm below and above the grafting line for calculating the ratio between scion and rootstock trunk diameters. Stronger scion/rootstock compatibility was indicated by ratio values closer to one.

Fruit yield (kg·tree^{-1}) was evaluated for the periods of 1995-1999 and 2003-2009 in trial I, and 2006-2009 in trial II. Yield efficiency was computed from the relationship between fruit yield and canopy volume during the period of 1995-1999 in trial I, and for the 2007 season in trial II.

Fruit quality was evaluated only in trial II during the period of 2006-2009. For trial II, 15 fruits per replication were collected. Analyses were carried out at the Bebedouro Experimental Station, São Paulo state, Brazil. Mean fruit weight (g), fruit length and diameter (cm) were measured, followed by juice extraction in a mechanised extractor (Otto 1800 juice extractor, OIC, Limeira, São Paulo, Brazil). The total juice mass of each replicate was determined, and the juice content (JC) was expressed as percentage of juice (%).

The total soluble solids (TSS) were determined in a digital refractometer (Palette PR-101, ATAGO, Tokyo, Japan), with a sample of 5 mL of juice per plot being collected after extraction. The values were obtained by direct reading on the refractometer, expressed in Brix. Total acidity was determined by titration with 0.1 N sodium hydroxide, according to Stenzel et al (2003). The technological index (TI), expressed in kg of TSS per box of 40.8 kg (kg TSS·box^{-1}), was determined by the equation: TI = (JC × TSS × 40.8) ÷ 10,000.

Data of both trials were submitted to analysis of variance, and the means were compared by Tukey's test ($p < 0.05$). Kruskal-Wallis and Wilcoxon tests were used to analyse data of scion/rootstock incompatibility showing no homogeneity of variance and normality, as determined by these signed rank nonparametric tests ($p < 0.05$). A regression model was calculated to analyse the relationship between scion/rootstock incompatibility (independent variable) and scion/rootstock trunk diameter ratios (dependent variable) (Pimentel-Gomes (2000).

Figure 1. Illustration of the 0-3 score rating scale utilized for assessing incompatibility symptoms in the grafting region in Pera sweet orange plants grafted on Swingle citrumelo. Score 1: absence of a necrotic line or stem pitting; Score 2: presence of a light necrotic line, continuous or not, with or without stem pitting; Score 3: presence of a stressed necrotic line, with or without the presence of resin.

RESULTS AND DISCUSSION

In trial I, Pera IAC sweet orange trees grafted to clone 4CV had higher yields in the 1995-1999 period (Fig. 2a), but showed similar yield efficiency when compared to the other scion/rootstock combinations (Fig. 2c). By contrast, in the 2003-2009 period (13-19 years after planting), this combination showed the lowest yield (Fig. 2b). The poor performance of clone 4CV during this period could be associated with rootstock incompatibility developing only after ten years from planting (Pompeu Júnior 2005; Oliveira et al. 2008). In contrast, no significant differences in intensity of incompatibility (score), with mean values ranging from 2.5 to 2.83, or in scion/rootstock trunk diameter, with mean values ranging from 0.64 to 0.68, were observed in trees grafted either with clones 2CV, 3CV or 4CV (Table 1).

A combined analysis of the results obtained in both trials indicated that the combinations with different clones in trial I showed higher mean incompatibility intensity than those in trial II (Fig. 3). This incompatibility, expressed as a necrotic line at the grafting union, might have increased in intensity over the lifespan of the tree without limiting plant growth or fruit yield during the first twenty years after planting, as reported by Müller et al. (1996 b).

In trial II, the scion/rootstock diameter ratio varied among the different combinations (Table 1). The Pera IAC sweet orange scion showed the strongest compatibility when grafted on Cravo Rangpur lime, and it differed significantly from clones 2CV, 3CV and 4MP grafted on Swingle citrumelo, which showed the lowest compatibility.

The absence of significant differences between the combinations, including the MP and CV clones (Table 1), indicated that there was no influence of clonal cleaning and cross-protection on grafting compatibility. Therefore, the incompatibility was not caused by any element translocating from the scion to the rootstock, as reported by Roistacher (1991), who associated the presence of a graft-transmissible agent (viroid or virus) with the occurrence of a bud-union crease in citrus trees. Galipienso et al. (2000) also observed the same symptoms in Nagami SRA-153 kumquat (*Fortunella margarita* Lour.) and Troyer citrange (*C. sinensis* L. Osb. × *P. trifoliata* L. Raf.), and related them with a graft-transmissible pathogen. Müller et al. (1996 a) described a physiological origin for the incompatibility of virus-free budwood of Pera sweet orange budded in Swingle citrumelo, similar to the results observed in the current study, except for CTV involvement.

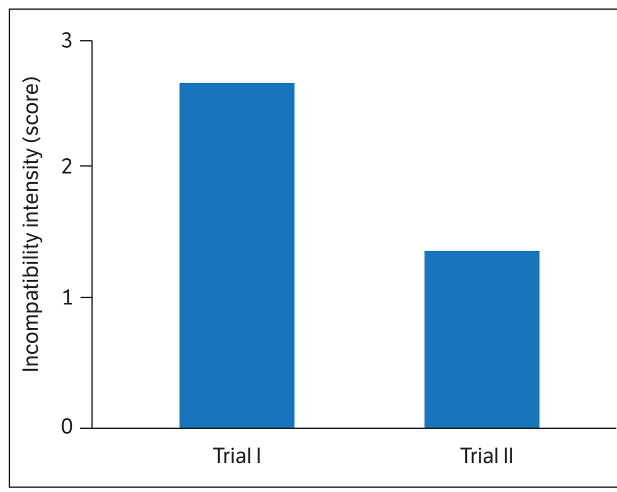

Figure 3. Mean incompatibility intensity score at the grafting region in Pera sweet orange old clones and on micrografted and pre-immunised clones grafted on Swingle citrumelo (trials I and II). Significant by the Wilcoxon's test at 5% significance level.

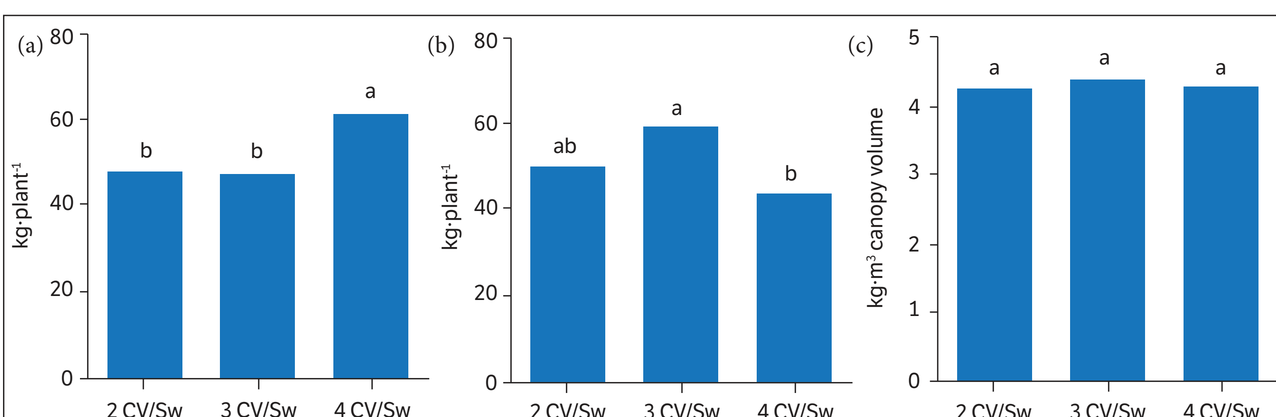

Figure 2. Mean fruit yield of Pera sweet orange old clones (CV) grafted on Swingle citrumelo in the 1995-1999 period (a) and the 2003-2009 period (b), and yield efficiency (kg of fruit per m^3 canopy volume) in the 1995-1999 period (c), EECB, Bebedouro, SP, Brazil. Means followed by the same letters in columns are not significantly different by the Tukey's test ($p < 0.05$).

Table 1. Incompatibility intensity at the grafting union and scion/rootstock trunk diameter ratio in Pera sweet orange old clones (CV) and micrografted and pre-immunised clones (MP) grafted on Swingle citrumelo (Sw) and on Cravo Rangpur lime (Cr) after 6 years and 9 months from planting. EECB, Bebedouro, SP, Brazil, 2010.

'Pera' sweet orange clone/rootstock	Incompatibility intensity (0-3)[1]	Scion/rootstock trunk diameter ratio
2CV/Sw	1.00 a	0.70 b
3CV/Sw	1.00 a	0.68 b
4CV/Sw	1.50 a	0.77 ab
2MP/Sw	1.25 a	0.77 ab
3MP/Sw	1.14 a	0.72 ab
4MP/Sw	1.25 a	0.68 b
IAC/Sw	2.60 b	0.72 ab
IAC/Cr	1.00 a	0.89 a
CV (%)	36.78	10.88

[1]Incompatibility intensity means followed by same letters in columns are not significantly different by the Kruskal-Wallis's test ($p < 0.05$). Scion/rootstock trunk diameter ratio means followed by same letters in columns are not significantly different by the Tukey's test ($p < 0.05$).

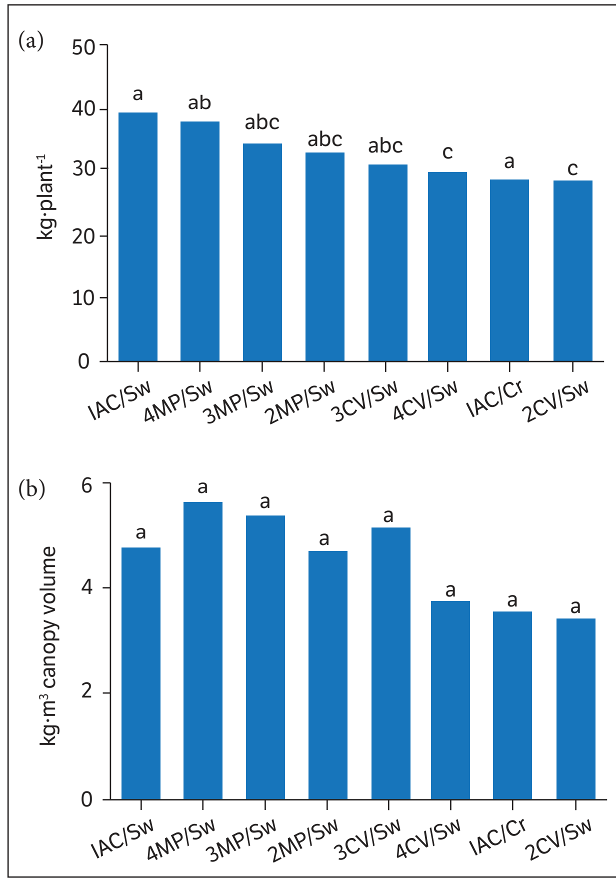

Figure 4. Mean fruit yield in the 2006-2009 period (a) and yield efficiency in 2007 (b) of Pera sweet orange old clones (CV) and micro-grafted and pre-immunised clones (MP) grafted on Swingle citrumelo (Sw) and on Cravo Rangpur lime (Cr) after six years and nine months from planting. EECB, Bebedouro, SP, Brazil, 2010. Means followed by the same letters in columns are not significantly different by the Tukey's test ($p < 0.05$).

The IAC/Sw combination showed the greatest incompatibility intensity and significantly differed from the other clones, although the combinations including old clones or micrografted clones did not differ from the compatible control (IAC/Cr). These results also demonstrated the necessity for more detailed studies with the 2CV and 3CV selections to assess their potential use as scions on Swingle citrumelo, since these combinations did not show any incompatibility symptoms, such as plant size or fruit yield reductions. According to Oliveira et al. (2012), it is possible that different combinations of sweet orange on Swingle citrumelo will not show these effects, even if typical incompatibility symptoms are observed in the bud-union region.

In trial II, the incompatible control combination (IAC/Sw) was more productive in fruit yield per plant compared to the compatible control combination (IAC/Cr) (Fig. 4). In 2007, all the treatments showed similar yield efficiencies (kg·m^{-3} canopy volume) (Fig. 4), even though the incompatible control combination developed a more pronounced necrotic line in the graft union (Table 1).

These results showed partial consistency with those reported by Barbasso et al. (2005), who observed compatibility of different mandarin selections grafted onto Swingle citrumelo, even though all the evaluated combinations showed differences between scion and rootstock trunk diameters. In general, trifoliate and its hybrid rootstocks show larger trunk diameter than several scions grafted on them, and nevertheless, these combinations are found to be compatible (Carlos et al. 1997; Stuchi 2002).

For the different combinations evaluated in trial II, no relationship was found between the presence and intensity of a necrotic line (as assessed by scores) and differences in trunk diameter between the scion and the rootstock, after six years and nine months from planting (Fig. 5). These results suggested that the observed differences in scion/rootstock trunk diameter were not always associated with incompatibility between the materials (Wutscher 1979; Barbasso et al. 2005). The difference between trunk diameters in scion and rootstock combinations of several species is mostly related to genetic traits of plant growth, rather than to true incompatibility between both materials (Hartmann et al. 2002). In this context, for future studies on scion/rootstock incompatibility, →

we suggest running separate evaluations of the presence of a necrotic line at the grafting union and determining a difference between scion and rootstock trunk diameters.

In Pera sweet orange, the intensity of scion/rootstock incompatibility might vary with the involved genotypes. Incompatibility occurs in both old and nucellar clones, and severe symptoms of precocious incompatibility and plant death were even observed in the nursery when grafting Pera Comprida sweet orange (Salibe et al. 2002).

The IAC/Sw combination (incompatible control) outperformed the 3CV/Sw, 3MP/Sw and 4MP/Sw combinations in mean fruit weight, as well as 2MP/Sw, 3MP/Sw and IAC/Cr (compatible control) for TI (Table 2). Nonetheless, both the IAC/Sw and IAC/Cr combinations performed similarly regarding the other evaluated fruit quality variables (Table 2). The average JC and TSS values observed in this study were lower than numbers founded for seven clones in Florida (Castle and Baldwin 2011). Similar results were reported by Wutscher and Shull (1972) for grapefruit, and more recently by Espinoza-Núñez et al. (2007), who observed higher fruit yield for Fremont mandarin grafted on Swingle citrumelo, compared to Cravo Rangpur lime rootstock.

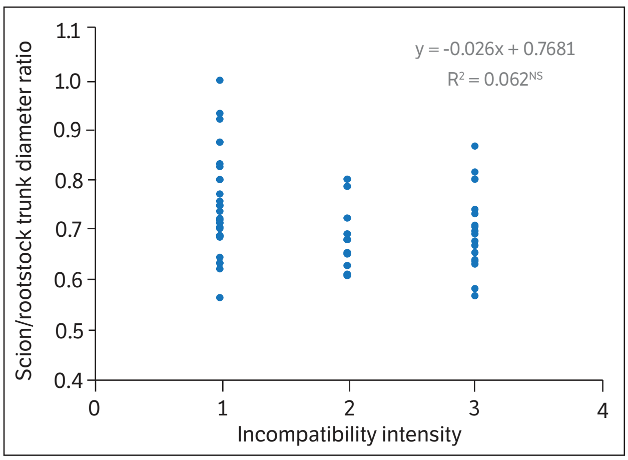

Figure 5. Linear regression between the scores of incompatibility intensity and scion/rootstock trunk diameter ratio in Pera sweet orange clones grafted on Swingle citrumelo after 20 years from planting. EECB, Bebedouro, SP, Brazil, 2010. NS: not significant ($p < 0.05$).

Grafting incompatibility occurred in Florida for seven Pera clones grafted on Swingle citrumelo, including Pera Pré-Imunizada and Pera Vacinada. Several clones declined as a result of bud union incompatibility. Others, such as Pera 7, declined later or did not exhibit symptoms of decline, including Perão and Pera Acidless (Castle and Baldwin 2011). Probably, Pera Pré-Imunizada and Pera Vacinada, which showed symptoms of incompatibility, were the same as Pera IAC in the current study, but without the protective CTV isolate, once they were studied as juvenile scions. This fact, and the severe incompatibility presented by CV1 in trial II, indicated a possible genetic component in the occurrence of the bud union crease for Pera sweet orange budded in Swingle citrumelo.

According to Moore (1984), scion/rootstock incompatibility does not depend, or is not related to cell recognition processes. However, incompatibility increases with the genetic distance between the grafted species (Flaishman et al. 2008). According to Pina et al. (2009), the lack of plasmodesmata in the grafting →

Table 2. Mean values of fruit quality attributes in Pera sweet orange old clones (CV) and micrografted and pre-immunised clones (MP) grafted on Swingle citrumelo (Sw) and Cravo Rangpur lime (Cr) in the 2006-2009 period. EECB, Bebedouro, SP, Brazil, 2010.

Pera sweet orange clone/rootstock	Fruit weight (g)	Fruit diameter (cm)	Fruit height (cm)	Total soluble solid (TSS, Brix)	Titratable acidity (TA, %)	TSS/TA ratio	Juice content (%)	Technological index kg TSS·box^{-1} (40.8 kg)
2CV/Sw	191.72 abc	6.95 ab	7.61 a	9.96 ab	0.80 bc	12.59 cd	51.85 ab	2.09 abc
3CV/Sw	183.03 bc	6.84 ab	7.50 a	9.22 c	0.71 d	14.38 a	51.52 ab	2.12 abc
4CV/Sw	187.74 abc	6.95 ab	7.56 a	10.07 a	0.72 cd	14.03 ab	52.90 ab	2.15 ab
2MP/Sw	187.32 abc	6.57 b	7.64 a	10.26 a	0.84 b	11.30 de	51.86 ab	1.98 bc
3MP/Sw	186.24 bc	6.87 ab	7.63 a	9.32 bc	0.73 cd	12.79 cd	50.07 b	1.90 c
4MP/Sw	180.36 c	6.85 ab	7.18 b	9.22 c	1.01 a	10.84 e	52.41 ab	2.14 ab
IAC/Sw	202.34 a	7.12 a	7.59 a	10.05 ab	0.69 d	15.07 a	53.89 a	2.27 a
IAC/Cr	196.18 ab	7.10 ab	7.56 a	9.87 abc	0.65 d	14.69 a	52.36 ab	2.00 bc
CV %	7.94	7.47	3.42	7.40	10.88	10.35	6.16	10.07

Means followed by same letters in columns are not significantly different by the Tukey's test ($p < 0.05$).

union region may be one of the possible reasons for this observation.

The mechanisms involved in graft incompatibility have not yet been clearly elucidated; however, some characteristics such as vascular regeneration, changes in endogenous auxin flux in the grafting region (Soumelidou et al. 1994) and the occurrence of oxidative stress (Aloni et al. 2008) are major factors in determining the success of a grafting union.

Other factors such as pathogens and environmental conditions may determine or potentialise scion/rootstock incompatibility. In addition, the possibility of genetic influences cannot be excluded, since Stegemann and Bock (2009) demonstrated the transference of genetic information via grafting in tobacco plants.

The Pera sweet orange clones compatible with Swingle citrumelo in this study, represent a considerable gain for the whole citrus production chain, mainly because it allows the growing of this combination without interstock.

CONCLUSION

Graft-incompatibility and the difference in trunk diameter between scion and rootstock were not related, and did not influence horticultural performance of Pera sweet orange clones grafted on Swingle citrumelo, six years and nine months and twenty years after grafting.

Clonal cleaning by shoot tip grafting and pre-immunisation with a CTV mild strain did not affect the intensity of graft-incompatibility between Pera sweet orange clones and Swingle citrumelo rootstock.

The 2MP, 3MP and 4MP Pera sweet orange clones were alternative scions to the IAC clone for successful growing of Pera sweet orange grafted onto Swingle citrumelo rootstock.

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AUTHORS' CONTRIBUTION

Conceptualization, Stuchi E. S., Donadio L. C. and Müller G. W.; Methodology, Fadel A. L., Stuchi E. S. and Silva R. S.; Investigation, Fadel A. L., Stuchi E. S., Silva R. S., Parolin L. G. and Oliveira C. R.; Writing – Original Draft, Fadel A. L. and Stuchi E. S.; Writing – Review and Editing, Fadel A. L., Stuchi E. S., Silva R. S. and Oliveira C. R.; Funding Acquisition, Stuchi E. S.; Resources, Stuchi E. S. and Donadio L. C,; Supervision, Stuchi E. S.

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