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Comparison between diploid and tetraploid citrus rootstocks: morphological characterization and growth evaluation

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Abstract

Tetraploid citrus rootstocks may present different morphological characteristics and growth when compared to diploid ones. This work aimed at comparing morphological characteristics and height growth of diploid and tetraploid plants from the rootstocks 'Swingle' citrumelo [*C. paradise* Macf. x *Poncirus trifoliata* (L.) Raf], citrange 'Troyer' (*C. sinensis* (L.) Osb. x *P. trifoliata*) and citranges 'Fepagro C 13' and 'Fepagro C 37' [*C. sinensis* cv. Pêra x *P. trifoliata*] during twelve months. Diploid (2n=18) and tetraploid (2n=36) plants originated from the same seed were identified, cultivated and evaluated every 45 days regarding color, height, petiole length, leaf length and central leaflet width. Significant differences were observed for the evaluated characteristics: the average of petiole length was 1.78 cm in the diploid and 0.99 cm in the tetraploid plants; the average of leaf length was 2.32 cm in the diploid and 2.95 cm in the tetraploid plants; the average of central leaflet width was 1.33 cm in the diploid and 1.69 cm in the tetraploid plants. Moreover, tetraploid plants had darker and thicker leaves than the diploid ones. Variation regarding height was observed and the diploid plants presented higher growth than the tetraploid ones. As tetraploid plants are smaller, have a slow height growth and wider and longer leaves.

Key words: *Citrus*, morphology, leaf color, height, dwarfing.

Comparação entre porta-enxertos cítricos diploides e tetraploides: Caracterização morfológica e avaliação do crescimento

Resumo

Porta-enxertos cítricos tetraploides podem apresentar características morfológicas e desenvolvimento diferenciados comparados a plantas diploides. Este trabalho teve como objetivo avaliar características morfológicas e o crescimento em altura de plantas diploides e tetraploides dos porta-enxertos citrumelo Swingle [*C. paradisi* Macf. x *Poncirus trifoliata* (L.) Raf], citrange Troyer [*C. sinensis* (L.) Osb. x *P. trifoliata*] e dos citranges Fepagro C 13 e Fepagro C 37 (*C. sinensis* cv. Pêra x *P. trifoliata*) por um período de 12 meses. Plantas diploides (2n=18) e tetraploides (2n=36) oriundas da mesma semente foram identificadas, estabelecidas e avaliadas a cada 45 dias quanto a cor, altura, comprimento do pecíolo, da folha e largura do limbo do folíolo central. Diferenças significativas foram observadas nos caracteres avaliados: a média do comprimento do pecíolo nas plantas diploides foi de 1,78 cm e, nas tetraploides, de 0,99 cm; a média do comprimento das folhas nas diploides foi de 2,32cm e nas tetraploides de 2,95 cm; a média de largura dos folíolos centrais das folhas das plantas diploides foi de 1,33 cm e, nas tetraploides, de 1,69 cm; além disso, as plantas tetraploides apresentaram folhas com coloração mais escura e mais espessas do que as das diploides. Variações foram observadas na altura, com as plantas diploides apresentando maior crescimento que as tetraploides. Portanto, plantas tetraploides apresentam menor tamanho, crescimento em altura mais lento e folhas mais largas e compridas.

Palavras-chave: *Citrus*, morfologia, cor da folha, altura, ananizante.

1. INTRODUCTION

The rootstock is important in establishing a good quality orchard, since it influences several characteristics of the plant and fruit (Davies and Albrigo, 1994; Pompeu Junior, 2005; Schäfer and Dornelles, 2000), besides increasing tolerance to phytosanitary problems (Chagas et al., 2007).

In all citrus regions of the world, there is a strong tendency to use a small variety of rootstocks. In Brazil, for several decades, few varieties were used when creating orchards, jeopardizing production due to the numerous phytosanitary problems that affect the culture, exposing the productive sector to the outbreak of new diseases (Guerra et al., 2012; Moreira et al., 2010; Passos et al., 2006). To maintain the citrus production in Brazil and reduce the risk of orchards being decimated, it is essential the rational utilization of varieties, the use of rootstocks tolerant/resistance to plagues and diseases and well adapted to the distinct soil and environmental conditions (Moreira et al., 2010; Passos et al., 2006).

Most citrus species are diploid ($2n=2x=18$), however polyploid plants, like triploid ($2n=3x=27$) and tetraploid ($2n=4x=36$), can be found in diploid populations (Aleza et al., 2011; Cameron and Frost, 1968; Hussain et al., 2012; Lee, 1988), being originated sexually through the fusion of non-reduced gametes or somatically, by chromosome duplication in nuclear cells and the non-division of these cells in two during mitoses (Cameron and Frost, 1968; Lee, 1988).

In citrus, tetraploid plants can be used as an alternative for the diversification of rootstocks, since they present a greater genetic variability due to the new possibilities of recombination, besides being able to be used as dwarf rootstocks (Cameron and Frost, 1968; Lee, 1988), since the new selections of rootstocks should confer productivity and quality to the fruit of the scion, be tolerant to the main plagues and diseases of the culture, and also induce plant size reduction (Gonzatto et al., 2011; Pompeu Junior and Blumer, 2009).

This study aimed at identifying polyploid plants obtained from diploid citrus rootstocks and describe the morphological characterization and growth analysis of height in diploid and tetraploid citrus plants.

2. MATERIAL AND METHODS

Fruits of the citrus rootstocks citrumelo 'Swingle' [*C. paradise* Macf. x *Poncirus trifoliata* (L.) Raf] and citranges 'Troyer' (*C. sinensis* (L.) Osb. x *P. trifoliata*), 'Fepagro C 13' and 'Fepagro C 37' [*C. sinensis* cv. Pêra x *P. trifoliata*] were collected in a green-house in the year of 2009 in order to conduct the experiment.

In a laboratory, the fruits collected were washed and disinfected by being immersed in alcohol 70% for 10 minutes, and afterwards being immersed in a solution of sodium hypochlorite (2% of active ingredient) for five minutes and a triple wash with autoclaved deionized water. Afterwards, the fruits were opened and the seeds collected and disinfected following the methodology described above. In a sterile laminar flow chamber, the outer (testa) and inner (tegmen) teguments were removed and the seeds put to germinate in test tubes containing 30 mL of MS medium (Murashige and Skoog, 1962), which were kept in a controlled environment, with a photoperiod of 16 hours and a temperature of 25 ± 2 °C until the plants reached 10 cm in height. In several species of citrus, polyembryony, is a common phenomenon. In the present study, when more than one seedling per seed occurred, these were identified with colored cotton swabs, with one color for each seed and transplanted to vases containing Rendmax® citrus substrate and acclimatized in a green-house. The plants were evaluated according to ploidy level using the technique of flow cytometry with the help of a Partec "Cy Flox Ploidy Analyser" cytometer, following the methodology described by Aleza et al. (2009).

Tetraploid and diploid plants were found in the same seed in the rootstocks of citrumelo 'Swingle', citrange 'Troyer' and citranges 'Fepagro C 13' and 'Fepagro C 37'. The ploidy level of these plants was confirmed by chromosome count in somatic cells of root tips according to the methodology described by Dahmer et al. (2013). After confirming the ploidy level, the plants were transplanted to bigger vases, with capacity of three liters, filled with the same citrus substrate and kept side by side in the green house. The irrigation was manual and the fertilization based on NPK fertigation.

Diploid and tetraploid plants originated from the same seed were transplanted and evaluated regarding their height one day after transplantation (DAT), on 08/03/2010, and afterwards every 45 days, on 09/17/2010, 11/01/2010, 12/16/2010, 01/31/2011, 03/17/2011, 05/02/2011, 06/16/2011 and 08/01/2011. In total, each material was evaluated eight times. The morphological evaluation of the leaves consisted on measuring three leaves completely expanded from each plant and their respective leaflets, by measuring the length of the petiole, width (at the widest point of the leaf blade) and leaf length with the help of a pachymeter. The analyses of these trifoliated rootstocks were conducted in the central leaflet, i.e., the largest one. The leaves were evaluated and classified according to the classes in table 1, according to type, color, petiole length compared to the blade, winged petiole, petiole and blade shape and shape of the edges according to the descriptors proposed by the International Board for Plant Genetic Resources (IBPGR, 1988). The plant growth in height was measured with the help of a graduated rule, beginning at the substrate surface until the highest growth point, on the dates mentioned above.

The results obtained were analyzed statistically with the Tukey at the 1% significance test ($p < 0.01$), using the statistical program SAS (SAS, 2000).

3. RESULTS AND DISCUSSION

In the flow cytometry analysis 406 plants from 83 seeds were evaluated, whereas four seeds, one of each rootstock of citrumelo 'Swingle', citrange 'Troyer' and citranges 'Fepagro C 13' and 'Fepagro C 37' produced a tetraploid and a diploid plant simultaneously in the same seed. The ploidy level of these plants was confirmed as tetraploid (Figure 1) by analyzing somatic cells from root ends. The presence of diploid and tetraploid plants in the same seed appears to be common in citrus and has already been described by Aleza et al. (2011).

In the morphological evaluations of the diploid and tetraploid plants according to the morphological descriptor of the IBPGR (1988) (Table 1) all the plants presented type 2 leaves, that is, trifoliolate (Table 2 and Figure 2).

Trifoliolate leaves have already been described in the morphological characterization of the citrumelo 'Swingle' and citrange 'Troyer' rootstocks, while the characterization

of this study for the citranges 'Fepagro C 13' and 'Fepagro C 37' confirms the results already expected, since the plants evaluated were obtained from the breeding with the *P. trifoliolate* rootstock, that presents leaves of this type, being this characteristic governed by a dominant gene (Cameron and Frost, 1968; Saunt, 1990).

In terms of color, the diploid plants presented leaves with green coloring, while the tetraploids presented a more intense green, considered a darker green, besides presented more leathery leaves than the diploids (Table 2). In this study, the differences observed in color and consistency of the leaves of the tetraploid plants corroborate the description made by Latado et al. (2007) that the leaves of the polyploid plants of citrus present a more intense coloring than the diploids, and are consistent with the results obtained by Allario et al. (2011) that observed that the leaves of tetraploid 'Cravo' lime tree plants (*C. limonia* Osb.) presented a more intense coloring than the diploids. The difference observed in the leaf coloring of the diploid and tetraploid plants (Table 2) agrees with Machado et al. (2005) and Ollitrault and Jacquemond (1994) that report a difference regarding consistency and coloring of citric plant leaves with distinct ploidy levels. According to Leechet et al. (1985), in several species ploidy is associated to the increase of photosynthetic enzymes and cell pigments, therefore intensifying leaf coloring. Therefore, this may be the factor responsible for a more intense coloring observed in the tetraploid plant leaves in this study (Table 2).

Regarding the characteristic of length of the petiole compared to the blade, all plants were short-petiolated, with a narrow and obovate shaped petiole. Regarding the blade shape, the leaves were classified as ellipsoids, with the exception of the tetraploid plant of the 'Fepagro C 13' rootstock, which was classified as orbiculate. As for the format of the margin, all plants presented leaves with serrated edge (Table 2).

When comparing diploid and tetraploid plants, there were variations regarding petiole length and length and

Table 1. Morphological descriptors of the leaves and their classes used in morphological characterization of the diploid and polyploid plants according to the IBPGR (1988)

Characteristic	Classes
Type	Simple, Trifoliolate
Color	Light Green, Green, Dark Green
Petiole Length X Blade	Sessile, Short-petiolate, Longipetiolate
Petiole Winged	Absent, Narrow, Large
Petiole Shape	Cordate, Deltoid, Obovate
Blade Shape	Ellipsoids, Ovate, Obovate, Lanceolate, Orbiculate
Shape of the edges	Crenade, Serrated, Full, Rolling

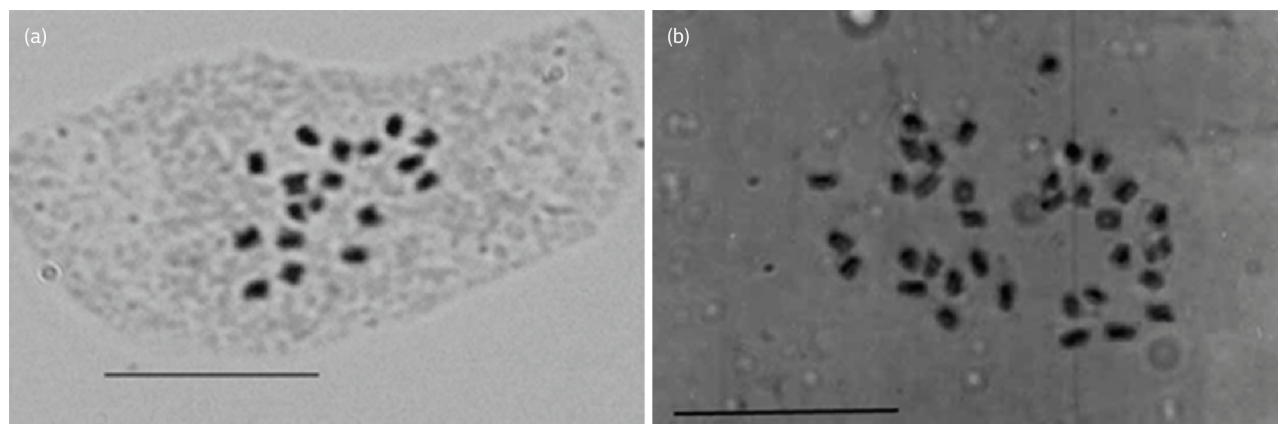


Figure 1. Determination the ploidy level by chromosome count in somatic cells of root tips of citrus rootstocks. (a) Diploid plant rootstock Citrange 'Fepagro C 13'; (b) Tetraploid plant rootstock Citrange 'Fepagro C 13'. Scale Bar = 10 μ m.

Table 2. Morphological characterization of leaves of the diploid and tetraploid plants of citrus rootstocks

Rootstock	Ploidy	Type	Color	Petiole x Blade	Winged Petiole	Petiole Shape	Blade Shape	Edges Shape
'Swingle'	2X	2	G	SP	Narrow	Obovate	Ellipsoid	Serrated
	4X	2	DG	SP	Narrow	Obovate	Ellipsoid	Serrated
'Troyer'	2X	2	G	SP	Narrow	Obovate	Ellipsoid	Serrated
	4X	2	DG	SP	Narrow	Obovate	Ellipsoid	Serrated
'Fepagro C 13'	2X	2	G	SP	Narrow	Obovate	Ellipsoid	Serrated
	4X	2	DG	SP	Narrow	Obovate	Orbiculate	Serrated
'Fepagro C 37'	2X	2	G	SP	Narrow	Obovate	Ellipsoid	Serrated
	4X	2	DG	SP	Narrow	Obovate	Ellipsoid	Serrated

Leaves type 2= Trifoliate; G=Green coloring; DG= Darkgreen; SP= Short-petiolate

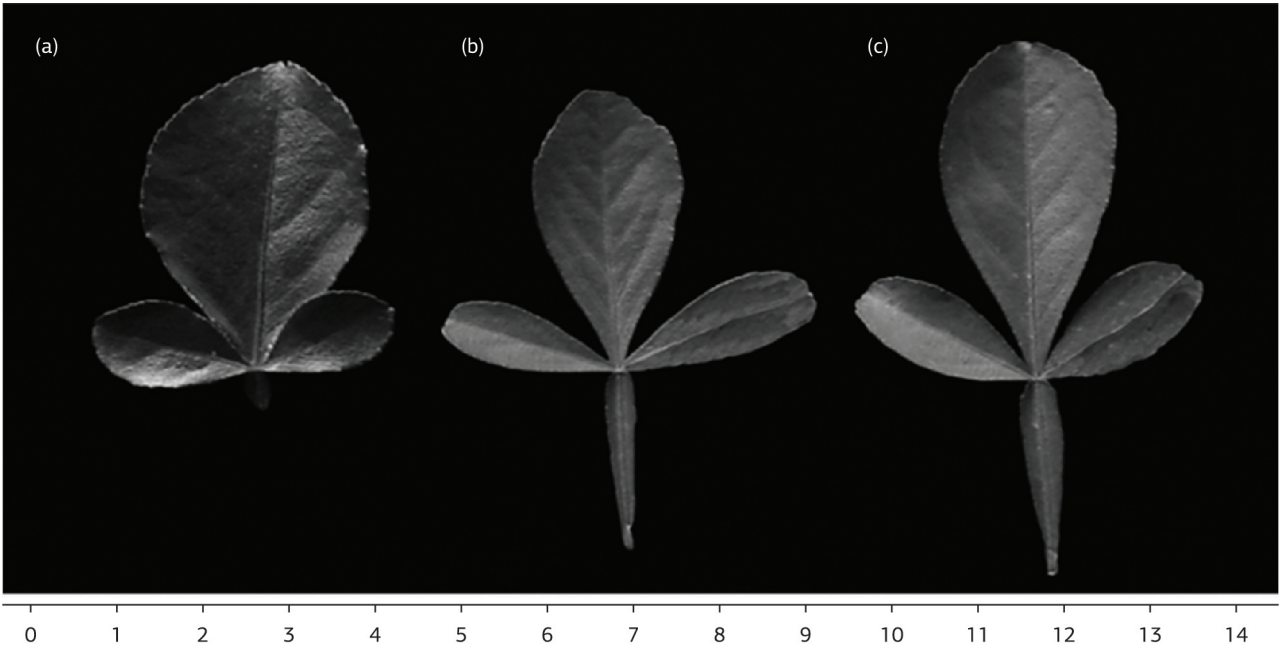


Figure 2. Morphological characterization of the leaves. (a) Leaf of the tetraploid plant rootstock Citrange 'Fepagro C13'. (b) and (c) Leaves of the diploid plant rootstock citrange 'Fepagro C13'. Scale Bar = cm.

width of the leaf (Table 3). The highest values of petiole length were found in the diploid plants (Table 3), with the longest petiole length in diploid plants being 1.45 cm in the 'Fepagro C13' rootstock, while 0.85 cm in the tetraploid. The longest petiole length in diploid plants was found in the 'Fepagro C37' with 2.37 cm, whereas it was 1.23 cm in the tetraploid rootstock (Table 3).

In the overall average of rootstocks, there were significant statistical differences and the diploid plants presented longer petioles, averaging 1.78 cm, while the tetraploids presented an average of 0.99 cm (Table 3). These results are consistent with those proposed by Frost and Soost (1968), that the diploid plants present longer petioles than the polyploids.

When analyzing the length of the leaves, in most evaluations the leaves of the diploid individuals were shorter than those of the tetraploids. In diploid plants, the lowest average length was 2.18 cm in 'Troyer', while the tetraploid presented 2.84 cm and the greatest leaf was

2.56 cm in 'Fepagro C13', while the tetraploid presented 3.18 cm (Table 3 and Figure 2).

In the overall average there were significant statistical differences between diploids and tetraploids, where the tetraploid plants had a longer leaf length with a mean of 2.95 cm and the diploids a mean of 2.32 cm (Table 3).

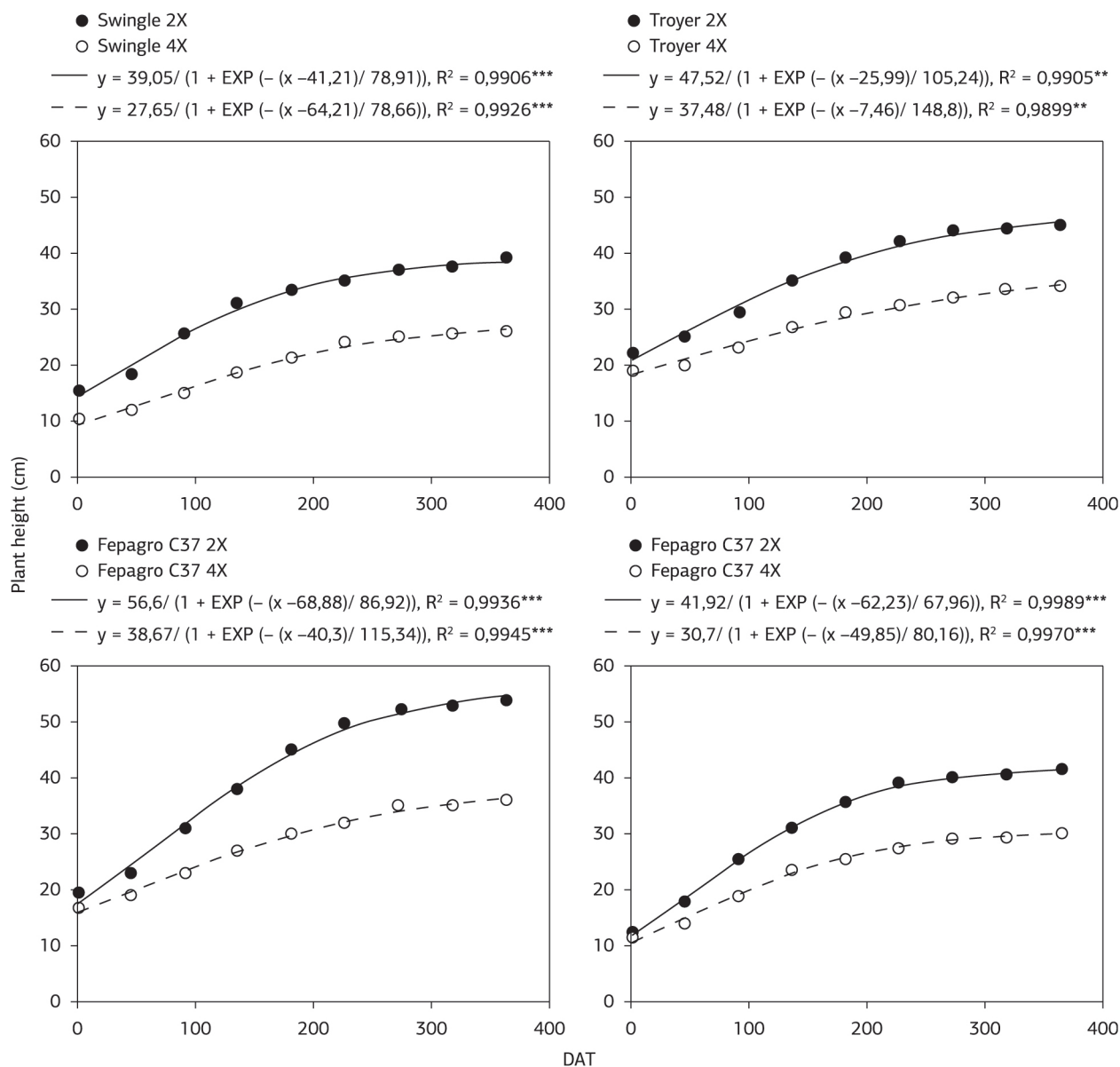
Regarding the width of the blade of the central leaflet of the leaves, diploid plants showed smaller width than the tetraploids (Table 3). In the overall average the tetraploid plants presented a central leaflet blade with a width of 1.69 cm and the diploids 1.33 cm (Figure 2), but in average there was no significant statistical difference between diploid and tetraploid plants, even if the tetraploid presented a tendency for leaves with a width greater than the diploids.

The largest length and width of leaves observed in tetraploid plants in this study (Table 3 and Figure 2) agree with what has been described by Latado et al. (2007), who observed a greater width and thickness in leaf blades of polyploidy citrus plants compared with diploids, and also

Table 3. Comparison between diploid and tetraploid plants of citrus rootstocks on the petiole length and length and width of the leaf

Rootstock	Petiole Length (cm)		Leaf Length (cm)		Leaf Width (cm)	
	Plant 2X	Plant 4X	Plant 2X	Plant 4X	Plant 2X	Plant 4X
'Swingle'	2.02 a	1.39 b	2.49 b	3.08 a	1.39 b	1.89 a
'Troyer'	1.62 a	1.10 b	2.18 b	2.84 a	1.21 b	1.66 a
'Fepagro C 13'	1.45 a	0.85 b	2.56 b	3.18 a	1.53 a	1.86 a
'Fepagro C 37'	2.37 a	1.23 b	2.27 b	2.82 a	1.33 a	1.66 a
Mean	1.78 a	0.99 b	2.32 b	2.95 a	1.33 a	1.69 a

Means followed by the same letter in the row in the comparison between diploid and tetraploid rootstocks did not differ significantly from the test Tukey ($p>0.01$)

**Figure 3.** Plant height between diploid and tetraploid plants rootstocks 'Swingle', 'Troyer', 'Fepagro C13' and 'Fepagro C37' conducted side by side at greenhouse in the eight evaluation dates. DAT= Days after transplantation; ***=significant regression, $P<0.0001$.

with what was reported by Frost and Soost, (1968) and Machado et al. (2005) that the tetraploid citrus plants have larger, thicker and wider leaves than the leaves of diploid plants, characters possibly associated to fewer, but larger stomata (Syvertsen et al., 2000).

The growth evaluations found variations in plant height between diploid and tetraploid plants, and the diploids were always higher.

The greatest initial height in diploid plants was found in the 'Troyer' rootstock, with 22.2 cm in the first evaluation

and 45.0 cm in the second one, while the tetraploid plant presented 19.2 cm and 34.0 cm, respectively (Figure 3).

The lowest initial height in diploid plants was 12.4 cm in the 'Fepagro C37' rootstock in the first evaluation and 41.5 cm in the last, while in tetraploids the height was 11.2 cm and 30.0 cm, respectively (Figure 3).

In this study, the results obtained with the height evaluations of diploid and tetraploid citrus plants over a year, allowed the observation that tetraploids presented a smaller and more compact form (Figure 3), agreeing with Cameron and Frost (1968) and Lee (1988), that morphological characteristics that are different among plants with different levels of ploidy, while polyploid plants have a more compact form and reduced size when compared to diploid plants. The results of this study are also consistent with data obtained by Allario et al. (2011) who compared the height between diploid and tetraploid plants derived from the same seed, the fruit of 'Rangpur' lime (*C. limonia* Osbeck) and observed that polyploid seedlings were smaller.

Frost and Soost (1968) highlighted that, regarding cytogenetics, the only variation between citrus autotetraploid plants and their diploid siblings is the duplicated number of chromosomes. However, according to these authors, regarding morphological and growth characters, the difference between diploid and tetraploids can easily be verified, since the tetraploid plants present slower growth, compacter shape, fewer and less vigorous sprouting. According to Syvertsen et al. (2000), the lowest growth rates observed in citrus seedlings from by tetraploid rootstocks are associated to the reduced rates of transpiration due to the lower number of stomata. Therefore, the lower growth and the more compact shape of the tetraploid plants observed in this study (Figure 3) agree with the affirmation of Frost and Soost (1968) that the distinction between diploids and tetraploids can be easily verified by analyzing morphological characters.

Citrus rootstocks should confer good characteristics to cultivate canopy, such as tolerance to drought and salinity, good quality and productivity of fruit (Allario et al., 2011; Hussain et al., 2012; Saleh et al., 2008), besides inducing reduction of the plant size (Pompeu Junior and Blumer, 2009), therefore facilitating orchard management. In this study, differences in morphological characteristics between diploid and tetraploid plants were observed, such as differences in growth, whereas tetraploid plants were always smaller and more compact.

4. CONCLUSION

Tetraploid plants present petioles smaller in length, but leaves larger in length and width;

Tetraploid plants are more compact and present smaller growth than diploids.

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