Journal of Agricultural Science; Vol. 10, No. 9; 2018
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Agronomic and Anatomical Indicators of Dwarfism and Graft Incompatibility in Citrus Plants

Waleska S. G. de Carvalho[1], Cláudia S. Marinho[1], Mayara B. de S. Arantes[1], Glaziele Campbell[2], Bruno D. Amaral[1] & Maura Da Cunha[2]

[1] Centro de Ciências e Tecnologias Agropecuárias, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ, Brazil

[2] Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ, Brazil

Correspondence: Cláudia S. Marinho, Centro de Ciências e Tecnologias Agropecuárias, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, CEP: 28013-602, Campos dos Goytacazes, RJ, Brazil. Tel: 55-(22)-2748-6213. E-mail: claudia.marinho@uenf.br

Received: May 15, 2018 Accepted: June 25, 2018 Online Published: August 15, 2018

doi:10.5539/jas.v10n9p263 URL: https://doi.org/10.5539/jas.v10n9p263

The research was financed by CNPq by scholarships.

Abstract

Poncirus trifoliata var. *monstrosa* (T. Ito) Swingle is a rootstock used in Brazilian citriculture for its potential to induce dwarfism and the viability of densified crops. Its recommendation is still restricted due to lack of research on the performance and compatibility of different types of scion under specific conditions of soil and climate. The aim of this study was to correlate plant size and productive efficiency with anatomical indicators of dwarfism or graft incompatibility between citrus scion cultivars and the 'Flying Dragon' trifoliate orange (FD) and 'Rangpur Lime' (RL) as rootstocks. The experimental design was of randomised blocks with split plots, with the experiment conducted in an orchard from the fourth to the sixth year of cultivation, under localised irrigation. The treatments consisted of two rootstocks (FD and RL) in the plots and five scion cultivars (Tahiti acid lime, and Natal, Navel, Lima Sorocaba and Pera oranges) in the sub-plots, with four replications per treatment. The agronomic performance of the plants was evaluated, together with the characteristics of samples containing the cambial zone and secondary xylem below, above and within the graft region. The FD rootstock gives the smallest size and greatest productive efficiency. The 'Navel' orange has good anatomical plasticity, adjusting itself more efficiently to the rootstocks. Visual symptoms of incompatibility between the 'Pera' orange grafted onto the FD can be seen at 64 months after planting. Vessel size and frequency, as well as the size and disposition of the cambial zone, are related to dwarfism and graft incompatibility.

Keywords: *Citrus latifolia* (Tanaka), *Citrus sinensis* (L.), *Poncirus trifoliata* var. *monstrosa* (T. Ito) Swingle, *Citrus limonia* Osbeck

1. Introduction

Citriculture is a branch of Brazilian agribusiness with a high profile on the world stage. The orange is the most produced fruit in the world, with a global production of approximately 71 million tons annually, Brazil being the largest producer and exporter of 66 Brix orange-juice concentrate (FAO, 2017).

In 2017, approximately 18.7 million tons of oranges were produced in Brazil. Citrus production in Brazil is distributed over several regions of the country. However, the State of São Paulo is responsible for most of the domestic production, with a contribution of around 14,300,000 tons of oranges and an average productivity of 37 tons ha^{-1}. In the same year, the State of Rio de Janeiro achieved a production of 51,608 tons, with an average productivity of 12 tons ha^{-1} (IBGE, 2018), demonstrating the need to carry out studies under specific growing conditions. One of the alternatives for increasing productivity and reducing the time for financial return of the investment in the orchard would be the adoption of densified plantations (Stuchi et al., 2011).

Small plants are recommended for planting densified crops. Trifoliate orange, when used as rootstock, induce the smallest growth in scions (Lima et al., 2014; Portella et al., 2016). This feature is desirable because it facilitates crop treatments and harvesting. The greater productive efficiency per plant and the increase in the number of plants per area can also result in increased productivity (Cantuarias-Avilés et al., 2012). According to Pompeu Júnior (2005), *Poncirus trifoliata* var. monstrosa 'Flying Dragon' is a citrus rootstock that emerged in Japan, capable of inducing true dwarfism in plants grafted onto it. However, recommendations of rootstocks should be made for specific conditions of soil, climate and management; not all citrus cultivars are compatible with trifoliate orange plants and their hybrids (Pompeu Junior & Blumer, 2014).

The anatomical characteristics of vascular tissue may also allow an understanding of the mechanisms involved in inducing dwarfism (Martínez-Alcántara et al., 2013) or in the early detection of problems of incompatibility between scions and rootstocks, as a hypothesis. The cambial zone is the secondary meristem responsible for the production of specific cells of the secondary xylem and phloem, from stages interrelated with cell division and differentiation (Philipson et al., 1971; Fukuda, 1996), the vascular tissue being responsible for maintaining the flow of water and the consequent performance of the scion/rootstock combinations (Appezzato-da-Glória & Carmello-Guerreiro, 2006).

The aim of this study therefore, was to correlate plant size and productive efficiency with anatomical indicators of dwarfism or graft incompatibility between citrus scion cultivars and the rootstocks 'Flying Dragon' trifoliate orange and 'Rangpur Lime', this latter being used as comparison parameter.

2. Method

2.1 Description of the Area

The experiment was carried out in an orchard planted in November of 2011 in the district of Campos dos Goytacazes, in the north of the State of Rio de Janeiro (21°45′15″ S and 41°20′28″ W, at an altitude of 10 m). The climate in the region is classified as tropical humid, with a dry winter and rainy summer. The average annual temperature varies around 24 °C and the average annual rainfall is around 1,020 mm.

The orchard was planted at a spacing of 7 × 5 m, with a localised irrigation system using two drippers per plant at an average flow rate of 8 L h^{-1}. The soil in the area is characterised as a Yellow Argisol, as decribed in Jacomine (2009). Cover fertilisation and the correction calculations were carried out as per the fertilisation recommendations proposed by Ribeiro et al. (1999), based on the results of the chemical analysis of the soil sample at a depth of 0-20 cm (pH: 5.8, OM: 27.4 g dm^{-3}, P: 17 mg dm^{-3}, K^+: 141 mg dm^{-3}, Ca^{2+}: 3.4 $cmol_c$ dm^{-3}, Mg^{2+}: 1.4 $cmol_c$ dm^{-3}).

The experiment was conducted in a split-plot design, in an orchard from the fourth to the sixth year of cultivation. The plots consisted of two rootstocks, with five scion cultivars evaluated in the sub-plots, and four replications per treatment. The rootstocks used were the 'Flying Dragon' trifoliate orange [*Poncirus trifoliata* var. Monstrosa (T. Ito) Swingle] and the 'Rangpur Lime' (*Citrus limonia* Osbeck cv. Cravo). The scions under evaluation were the 'Tahiti-IAC-5' acid lime (*Citrus latifolia* Tanaka) and the sweet orange [*Citrus sinensis* (L.) Osbeck] cultivars Natal CNPMF 112, Navel, Lima Sorocaba and Pera IAC 2000.

2.2 Biometric Analysis

Biometric evaluations were made during the first half of three consecutive years (2015 to 2017), and consisted of determining the height (H) and diameter of the canopies. Canopy diameter was measured parallel to the crop row (DR) and between rows (DB). The canopy volume (VC) was estimated with the formula proposed by Zekri (2000) (Equation 1):

$$VC = (\pi/6) \times H \times DR \times DB \tag{1}$$

where, H is the height of the plants (m), DR is the diameter of the canopy parallel to the row (m) and DB the diameter of the canopy between rows (m).

The production data and calculation of productive efficiency refer to 2015 and 2016. To obtain the production per plant, the total number of fruit produced was counted, and the average weight of two samples of eight fruit per plant (16 fruit), harvested when mature, was taken. For the 'Tahiti' acid lime, which produces throughout the year, the production per plant was estimated for the first six months of each year. Productive efficiency was evaluated from the ratio between fruit production and canopy volume.

Between the fourth and sixth year of cultivation, 'windows' were opened once a year in the region of the scion-rootstock union, allowing the exchange to be exposed to evaluate the presence of symptoms typical of graft incompatibility (dots, gum lines and necroses).

For statistical analysis, biometric and production data were submitted to analysis of variance at $p < 0.05$ and comparison of the mean values was by F-test (rootstock) or Duncan's test (scions) at $p < 0.05$. The data were analyzed using the computational resources of the Sanest program (Zonta & Machado, 1984).

2.3 Anatomical Analysis

For the anatomical evaluations, samples containing the cambial zone and newly formed secondary xylem were collected from three regions (below, above and within the graft region) by a non-destructive method using a Pressler Increment Borer (SUUNTO, USA), for combinations of the 'Navel' and 'Pera' oranges and the 'Tahiti' acid lime grafted onto both rootstocks, the 'Flying Dragon' trifoliate orange or the 'Rangpur Lime'. Three individual of each combinations were colleted. The samples were fixed while still in the field with a solution consisting of 2.5% glutaraldehyde, 4% formaldehyde and 0.05 M sodium cacodylate buffer pH 7.3 (Klein et al., 2004). They then underwent stages of dehydration in an increasing ethylic series (70% to 95% ethanol), were embedded in Historesina® resin and sectioned in a rotary microtome (RM 2235, LEICA, Germany) at a mean thickness of 4 μm along the transverse plane. The sections were stained with Toluidine Blue O to prepare permanent slides (Feder & O'Brien, 1968). The permanent slides were examined under an optical microscope (Axioplan, ZEISS, USA) with a coupled camera (Moticam Pro 282A, MOTIC, Asia) for capturing the images and observation of characteristics anatomy. The best images were used to make the figures.

3. Results

3.1 Biometric Analysis

The combinations of scions grafted onto the 'Flying Dragon' trifoliate orange (FD) had the lowest values for canopy diameter parallel to the planting row when compared to those grafted onto the 'Rangpour Lime (RL). Among the scions, the 'Tahiti' acid lime had the highest values for diameter parallel to the row on both rootstocks in all the years under evaluation (Table 1), demonstrating the greater vigour of the scion in relation to the oranges trees. Among the oranges, in the last evaluation made in 2017, 'Navel' and 'Lima Sorocaba' had a larger canopy diameter parallel to the row compared to the 'Natal', when grafted onto the RL. When the FD rootstock was used, the 'Pera' had a larger diameter parallel to the row than the 'Natal'. Canopy diameter parallel to the row was similar between the other orange trees grafted onto FD and evaluated at the same time. This demonstrates the interaction between scion and rootstock in defining the final size.

Table 1. Comparison of the mean values for diameter (m) in scion cultivars grafted onto rootstocks for the fourth to the sixth year after planting. Different letters indicate significant differences. On the lines, mean values followed by the same uppercase letters do not differ by F-test (0.05) and in the columns, mean values followed by the same lowercase letters do not differ by Duncan's test (0.05)

Year of Cultivation/Cultivar	DR		
	RL	FD	Mean
2015			
Tahiti	4.20	2.49	3.35 a
Natal	3.09	1.68	2.38 b
Navel	3.41	1.62	2.51 b
Lima Sorocaba	3.38	1.71	2.55 b
Pera	3.15	1.89	2.52 b
Mean	3.45 A	1.88 B	
CV_A (%)	3.19		
CV_B (%)	8.52		
2016			
Tahiti	5.11 Aa	2.90 Ba	4.01
Natal	3.45 Ac	1.84 Bb	2.64
Navel	3.92 Ab	1.97 Bb	2.94
Lima Sorocaba	3.73 Abc	1.88 Bb	2.81
Pera	3.54 Abc	2.27 Bb	2.91
Mean	3.95	2.17	
CV_A (%)	4.20		
CV_B (%)	9.62		
2017			
Tahiti	5.99 Aa	3.26 Ba	4.63
Natal	3.73 Ac	1.97 Bc	2.85
Navel	4.42 Ab	2.22 Bbc	3.32
Lima Sorocaba	4.12 Ab	2.10 Bbc	3.11
Pera	4.04 Abc	2.49 Bb	3.26
Mean	4.46	2.41	
CV_A (%)	1.57		
CV_B (%)	7.46		

Note. DR = canopy diameter parallel to the row; RL = Rangpur Lime; FD = 'Flying Dragon' trifoliate orange; CV = Coefficient of variation.

The greatest values for height were seen in the Tahiti acid lime and Lima Sorocaba cultivars when grafted onto the RL for the first two years under evaluation (Table 2). In the last year of the evaluation, the Tahiti acid lime reached a greater height than the other cultivars. When grafted onto the FD, the Tahiti and Pera cultivars had largest sizes in all the years under evaluation.

During the first year of harvest, it can be seen that the height of the plants grafted onto FD was 1.34, 1.98 and 1.98 m for the Natal, Tahiti and Pêra cultivars respectively, following the pattern of dwarf plants.

Table 2. Comparison of the mean values for plant height (m) in scion cultivars grafted onto lime rootstocks for the fourth to sixth year after planting. Different letters indicate significant differences. On the lines, mean values followed by the same uppercase letters do not differ by F-test (0.05) and in the columns, mean values followed by the same lowercase letters do not differ by Duncan's test (0.05)

Year of Cultivation/Cultivar	Height	
	RL	FD
2015		
Tahiti	3.20 Aa	1.98 Ba
Natal	2.45 Ac	1.34 Bb
Navel	2.76 Abc	1.40 Bb
Lima Sorocaba	3.25 Aa	1.53 Bb
Pera	2.82 Ab	1.98 Ba
CV_A (%)	5.16	
CV_B (%)	10.18	
2016		
Tahiti	3.74 Aa	2.40 Ba
Natal	3.25 Ab	1.50 Bb
Navel	3.27 Ab	1.78 Bb
Lima Sorocaba	3.63 Aa	1.82 Bb
Pera	3.21 Ab	2.26 Ba
CV_A (%)	2.49	
CV_B (%)	9.16	
2017		
Tahiti	3.95 Aa	2.39 Ba
Natal	3.04 Ad	1.55 Bb
Navel	3.41 Abc	1.72 Bb
Lima Sorocaba	3.57 Ab	1.77 Bb
Pera	3.24 Acd	2.28 Ba
CV_A (%)	5.94	
CV_B (%)	6.25	

Note. RL = 'Rangpur Lime'; FD = 'Flying Dragon' trifoliate orange; CV = Coefficient of variation.

The Tahiti acid lime achieved the greatest values for canopy volume when grafted onto the RL, irrespective of the year (Table 3). Among the oranges, the Natal and Pera cultivars had the lowest values when grafted onto that rootstock. For plants grafted onto the FD rootstock, it was found that the 'Tahiti' acid lime and the 'Navel' and 'Pera' oranges had similar volumes in 2016. During the last year under evaluation (2017), the Tahiti and Pera cultivars had similar canopy volumes, demonstrating that the greater growth rate of this orange tree could already be an indication of the differential accumulation of reserves in the aboveground part, a consequence of graft incompatibility between the Pera orange and the FD.

The FD induced plants with a lower canopy volume than those found for the RL. Among the scions grafted onto FD, the Tahiti acid lime had the highest canopy volume in the last year of evaluation, 13.41 m^3, a lower value than found for the same scion grafted onto the RL, which was 72.2 m^3.

Table 3. Comparison of the mean values for canopy volume (m^3) in scion cultivars grafted onto rootstocks for the fourth to sixth year after planting. Different letters indicate significant differences. On the lines, mean values followed by the same uppercase letters do not differ by F-test (0.05) and in the columns, mean values followed by the same lowercase letters do not differ by Duncan's test (0.05)

Year of Cultivation/Cultivar	VC	
	RL	FD
2015		
Tahiti	29.79 Aa	6.37 Ba
Natal	12.78 Ac	1.85 Ba
Navel	16.90 Abc	2.04 Ba
Lima Sorocaba	20.58 Ab	2.30 Ba
Pera	14.30 Ac	4.42 Ba
CV_A (%)	5.96	
CV_B (%)	26.64	
2016		
Tahiti	51.31 Aa	11.25 Ba
Natal	21.39 Ac	2.39 Bb
Navel	28.14 Abc	4.34 Bab
Lima Sorocaba	28.88 Ab	3.51 Bb
Pera	21.46 Ac	7.07 Bab
CV_A (%)	7.34	
CV_B (%)	25.29	
2017		
Tahiti	72.17 Aa	13.41 Ba
Natal	23.03 Ad	2.83 Bb
Navel	35.55 Ab	4.25 Bb
Lima Sorocaba	32.00 Abc	4.21 Bb
Pera	27.14 Acd	8.06 Bab
CV_A (%)	11.30	
CV_B (%)	17.94	

Note. V = canopy volume; RL = 'Rangpur Lime'; FD = 'Flying Dragon' trifoliate orange; CV = Coefficient of variation.

Table 4. Mean values for production (kg plant^{-1}) and productive efficiency (kg m^{-3}) in scion cultivars grafted onto lime rootstocks for the fifth and sixth year after planting. Different letters indicate significant differences. On the lines, mean values followed by the same uppercase letters do not differ by F-test (0.05) and in the columns, mean values followed by the same lowercase letters do not differ by Duncan's test (0.05)

Year of Cultivation/Cultivar	Production			Productive efficiency		
	RL	FD	Mean	RL	FD	Mean
2015						
Tahiti	62.00	37.89	49.95 a	2.17	6.39	4.28 ab
Natal	42.87	14.07	28.47 b	3.72	7.57	5.65 a
Navel	45.17	12.42	28.79 b	2.82	5.88	4.35 ab
Lima S.	38.47	10.42	24.45 b	1.91	4.47	3.19 b
Pera	42.05	20.19	31.12 b	2.92	4.60	3.76 b
Mean	46.11 A	19.00 B		2.71 B	5.78 A	
CV_A (%)	20.10			15.36		
CV_B (%)	24.99			32.98		
2016						
Tahiti	48.35	28.20	38.27 a	0.96	2.55	1.76 b
Natal	48.07	11.02	29.55 abc	2.42	4.68	3.55 a
Navel	42.37	12.76	27.57 bc	1.53	3.08	2.30 b
Lima S.	38.67	8.79	23.73 c	1.38	2.52	1.95 b
Pera	50.44	22.20	36.32 ab	2.43	3.23	2.83 ab
Mean	45.58 A	16.59 B		1.75 B	3.21 A	
CV_A (%)	22.65			14.71		
CV_B (%)	27.98			43.76		

Note. RL = Rangpur Lime; FD = 'Flying Dragon' trifoliate orange; CV = Coefficient of variation.

For the scion/rootstock compatibility index, the cultivars proved to be similar (Table 5). Generally, the cultivars grafted onto the RL rootstock had values greater than one. This shows that the diameter of the scion was slightly greater than the diameter of the rootstock. The closer this index is to one, the greater the compatibility between the scion/rootstock combination. In the combinations grafted onto FD, these values were less than one, which is considered normal when this rootstock is used, although it demonstrates less graft compatibility between these combinations.

In the present work, no gum rings were seen in the combinations under evaluation. However, a rupture line was found at the graft region for the Pera/FD combination only; this is a sign of incompatibility.

Table 5. Mean values for the scion-trunk diameter to rootstock-trunk diameter ratio (STD/RTD) in scion cultivars grafted onto rootstock under irrigated cultivation. Different letters indicate significant differences. On the lines, mean values followed by the same uppercase letters do not differ by F-test (0.05) and in the columns, mean values followed by the same lowercase letters do not differ by Duncan's test (0.05)

Cultivar	STD/RTD		
	RL	FD	Mean
Tahiti	1.08	0.79	0.93 a
Natal	1.01	0.77	0.89 a
Navel	1.05	0.79	0.92 a
Lima Sorocaba	1.10	0.74	0.92 a
Pera	1.16	0.74	0.95 a
Mean	1.08 A	0.76 B	
CV_A (%)	3.26		
CV_B (%)	11.71		

Note. RL = Rangpur Lime; FD = 'Flying Dragon' trifoliate orange; CV = Coefficient of variation.

3.2 Anatomical Analysis

Analysis of the exchange anatomy and the newly formed secondary xylem showed differences, mainly related to vascular elements and development of the exchange between the five genotypes under evaluation (the 'Pera' and 'Navel' oranges, 'Tahiti' acid lime, RL and the FD) (Figures 1 and 2). The FD has vascular elements that are visually more grouped and with smaller diameters when compared to the vascular elements of the 'Pera orange' and the RL, which are more similar to each other. On the other hand, in the 'Tahiti' acid lime and 'Navel' orange a clear variation can be seen in the quantity and presence of vascular elements with different diameters. Furthermore, in the RL, it can be seen that vascular elements tend to appear more frequently than do the others.

Similarly, it could be seen that in the cambial zone region of the FD, the exchange is apparently smaller, whereas in the RL this region appears to be better developed. In addition, a possible variation in the cambial zone was found in the Navel orange for the different treatments, *i.e.* when on the FD the region tends to be smaller and when on the RL it tends to be larger (Figures 1 and 2), following the characteristics of the rootstock.

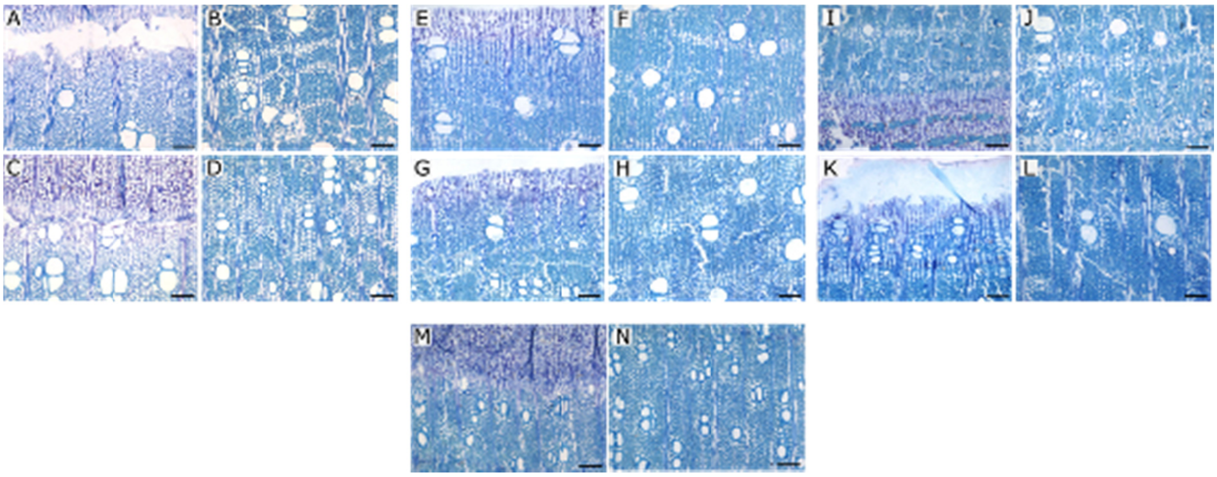

Figure 1. Anatomy of the cambial zone and the secondary xylem for combinations of the Tahiti acid lime scion (A-D), the Pera orange scion (E-H) and the Navel orange scion (I-L) on the 'Flying Dragon' trifoliate orange as rootstock (M-N). The combinations represent the regions above the grafting point (A-B; E-F; I-J) and at the grafting point (C-D; G-H; K-L). Bars: 100 µm

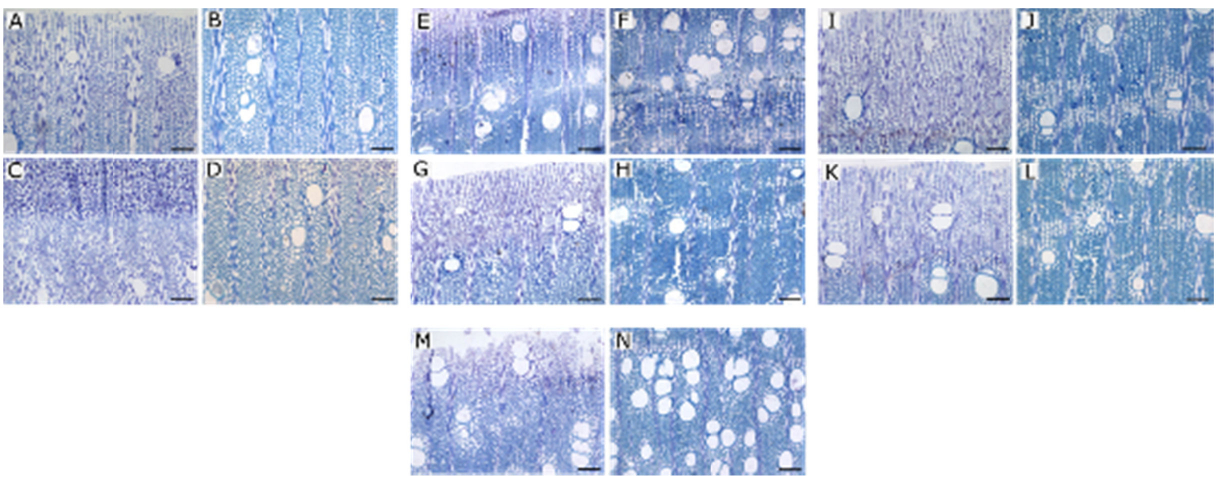

Figure 2. Anatomy of the cambial zone and the secondary xylem of combinations of the Tahiti acid lime scion (A-D), the Pera orange scion (E-H) and the Navel orange scion (I-L) on the Rangpur Lime rootstock (M-N). The combinations represent the regions above the grafting point (A-B; E-F; I-J) and at the grafting point (C-D; G-H; K-L). Bars: 100 µm

4. Discussion

It could be seen that height in the plants grafted onto the FD was significantly less than in those grafted onto the 'Rangpur Lime', with more pronounced effects when the 'Lima Sorocaba', 'Natal' and 'Navel' scions were used. The Tahiti and Pera cultivars had the largest size among the plants with the FD rootstock, for each year of the evaluation. However, the height of these plants was no greater than 2.5 m, maintaining the pattern of dwarf plants until that age. Nevertheless, it can be seen that the height of the plants had not yet stabilised, and might reach heights greater than 2.5 m, which would confirm the semi-dwarfing effect of FD on the Tahiti acid lime.

Mademba-Sy et al. (2012) found that FD was not considered as having stunted the Tahiti acid lime, the Lisboa lemon or the St. John Satsuma, when their height was evaluated 13 years after planting, as they reached heights of 3.0, 3.0 and 2.5 m respectively.

Plants of lower height are desirable to facilitate handling; however, a very small size can lead to ergonomic problems during harvesting. For this reason, it is important to know the characteristics of the scion and rootstock to be used, since the combination of less-vigorous scions and dwarfing rootstocks can result in an excessively small size. A joint observation between the canopy diameter parallel to the row and the plant height shows that the size of the Natal orange grafted onto FD was the smallest of all the combinations. This information is important, not only for estimating plant spacing, but also for use or cover of the area during the early years of the orchard.

Machado et al. (2017), evaluating rootstocks for the Tahiti acid lime, found that the Limeira trifoliate originated plants with lower canopy sizes compared to other rootstocks such as the Rangpur Lime, and that these plants with smaller canopy volumes could be used at higher planting densities, increasing production by area.

The use of FD as rootstock allows a smaller spacing to be adopted in the row, this being stipulated according to the peculiarity of each scion cultivar. In a study by Mademba-Sy et al. (2012), in which FD was evaluated as a dwarfing rootstock for citrus plants in a 13-year-old orchard, the authors concluded that this rootstock can be used to reduce plant vigour. For this reason, the authors estimated the plant population per hectare according to each cultivar, and stated that it would be possible to cultivate from 519 to 1,111 plants ha^{-1}, allowing the adoption of denser planting.

The values for canopy volume found in this study ranged from 2.83 to 72.2 m^3 for the Natal orange grafted onto FD, and the Tahiti acid lime grafted onto the RL respectively. This variation in canopy volume demonstrates the strong impact of the scion and rootstock combination on the correct choice of spacing and on obtaining a greater financial return by efficient use of the area under cultivation.

FD is a rootstock that leads to the formation of plants with a smaller canopy volume, and for this reason the fruit load supported by these plants is less. However, when compared to combinations using more-vigorous rootstocks, FD can induce greater productivity per area if a smaller spacing is adopted.

The degree of compatibility between rootstock and scion can be determined by the uniformity of the trunk diameter near the graft line, with those displaying incompatibility reactions typically exhibiting less development of the scion trunk (Bordignon et al., 2003). However, it is emphasised that the larger diameter of the rootstock trunk in relation to that of the scion or vice versa is not always taken as a reference for assessing the degree of incompatibility. The trifoliates and their hybrids have a rootstock-trunk diameter greater than that of the scion, and yet they originate productive plants of great longevity, showing no symptoms of incompatibility such as nutritional deficiencies or the presence of necrotic lines in the graft region (Pompeu Júnior, 2005).

Emmanouilidou & Kyriacou (2017) evaluated graft combinations between two sweet oranges (the Delta and Lane Late cultivars) grafted onto six rootstocks established in a limestone soil. It was found that the Swingle citrumelo rootstock [*Citrus paradisi* Macf. × *Poncirus trifoliata* (L.) Raf.] was incompatible with both scions (four years after planting), while the GouTou Chen rootstock (*Citrus aurantium* L.) showed low compatibility with the Lane Late cultivar. The trunk compatibility index of scion cultivars on Citrumelo was 0.49 for Lane Late and 0.58 for the Delta cultivar. In this case, these indices demonstrated the abnormal development of the graft union that culminated in the decline of these combinations. On the other hand, when the Lane Late and Delta cultivars were grafted onto the *Citrus aurantium* rootstock, these indices were 0.85 and 0.91 respectively, indicating greater graft compatibility between the two scions and the rootstock.

It is known that the Pera orange is incompatible with trifoliates and their hybrids. This incompatibility is a consequence of the gradual girdling that results from the formation of a gum ring in the graft region, leading to the accumulation of photoassimilates in the scion, according to Pompeu Júnior (2005). The presence of a gum ring was not seen in any of the combinations evaluated in the present work; however, a rupture line was found in the graft region only for the Pera/Flying Dragon trifoliate orange combination, this being a sign of incompatibility. It is emphasised that the performance of this combination in the field proved not to have been compromised up to the last evaluation. Since these signs occurred between the fifth and sixth year after planting, the importance of prior knowledge of scion and rootstock compatibility should be emphasised, since the short shelf life of these plants can cause serious damage for farmers.

Early evaluation of the degree of incompatibility can be made through studies in plant anatomy. Analysis of the exchange anatomy and the newly formed secondary xylem showed differences, especially related to the vascular elements and development of the exchange between the five genotypes under evaluation (the Pera and Navel oranges, Tahiti acid lime as scions and RL and the FD as rootstocks).

It could be seen that in the region of the cambial zone of the FD the exchange is apparently smaller, while in the RL there appears to have been greater development of this region. The cambial zone regions differ from the combination of the FD and the Pera orange, which in this case has the visually largest exchange; this may confer incompatibility between the tissues of the combinations due to the different rates of radial growth provided by the exchange.

The size of the cambial zone may be a decisive factor in re-establishing the connections between the xylem and phloem of the scion and rootstock. Therefore, the rates of radial growth achieved by the activity of this meristem will be different; growth will not be proportional, *i.e.* in the plant with the apparently larger cambial zone (Pera), there will be intense cell division to form new xylem and phloem. On the other hand, in the plant with the smaller cambial zone (FD) this division will be less intense, and will delay the formation of new conducting vessels. This mismatch will make it difficult to re-establish the connection of tissues formed after grafting. The success of the graft depends on the tissues of the plants involved being soldered in such a way that the vascular connection of both is restored, and there are no problems such as the interruption of water and nutrient transport.

In the case of the combination of the Navel orange grafted onto the FD, the cambial zone region is apparently similar between combinations, suggesting better compatibility for tissue formation. The 'Navel' orange displayed good anatomical plasticity, adjusting more efficiently to the rootstocks. One suggestion may be the compatibility of this cultivar with the FD as rootstock.

In a field experiment ten years after planting, the FA-517 hybrid rootstock [*Citrus nobilis* Lour × *Poncirus trifoliata* L. (Raf.)] and the FA-418 hybrid rootstock [Troyer citrange × *C. deliciosa* (Ten)] induced dwarfism in the 'Navel' orange, with a higher productive efficiency than plants grafted onto the Carriso citrange, producing good-quality fruit and proving to be suitable for high-density planting and alkaline soils (Forner-Giner et al., 2014). In young plants, the same authors found that the capacity to control plant size was associated with low hydraulic conductivity. In the case of the FA-418 rootstock, the low hydraulic conductivity was related to the smaller lumen area of the xylem root vessels. It was also found that hydraulic resistance in the stem decreases as plant vigour increases. Combinations grafted onto dwarfing rootstocks may also show resistance to sucrose transport, which may result in carbohydrate deficiency in the roots, reducing plant growth.

The studies cited corroborate the results for low hydraulic conductivity in the FD seen by Martinez-Alcântara et al. (2013), who stated that this characteristic may be associated with the smaller number of vessels present in the region of the xylem. This low hydraulic conductivity can be an important factor for the induction of dwarfism in plants.

In the present work, the canopy volumes of all the cultivars grafted onto FD were smaller in relation to the RL. However, among the cultivars grafted onto FD, the 'Pera' orange together with the 'Tahiti' acid lime had the largest canopy volume. In the case of the 'Pera' orange, symptoms of grafting incompatibility were noted, and in this case the accumulation of carbohydrates in the aboveground part may have contributed to the greater vigour recorded during this evaluation period. However, in the future, depletion of the root system may lead to the decline of these plants.

The macroscopic manifestation of symptoms is a result of problems during the healing stages of the grafted tissue; this is a sequence of events, beginning with the production of callus tissue (parenchyma cells). Once the two components of the graft are in contact, new cells of parenchyma tissue proliferate, forming the callus that intertwines, interconnects and fills the spaces between the components, connecting graft and rootstock. Subsequently, the new exchange cells differentiate from the newly formed callus and start to connect the original graft and rootstock exchange, in this way new vascular tissue is produced by the new exchange, allowing the passage of nutrients and water between the graft and rootstock (Hartmann, 2014). For some authors this is considered the basic requirement for a successful graft (Oliveira et al., 2002). According to Moraes et al. (2011), vascular discontinuity between scion and rootstock causes necrotic processes from the death of cells at the point of grafting and the decline and death of the plants, and can be triggered by soil and climate factors that lead to the incompatibility of combinations, even of those previously known to be compatible in other regions.

5. Conclusions

The ‘Flying Dragon’ trifoliate orange as rootstock provides smaller size and greater productive efficiency to the canopy of citrus plants. Visual symptoms of incompatibility between the ‘Pera’ orange grafted onto the ‘Flying Dragon’ can be seen 64 months after planting. The ‘Navel’ orange has good anatomical plasticity, adjusting itself more efficiently to the rootstocks. The size and frequency of vessels, as well as the disposition and size of the cambial zone, are related to dwarfism and graft incompatibility.

References

Appezzato-da-Glória, B., & Carmello-Guerreiro, S. M. (Eds.). (2006). *Anatomia Vegetal* (2nd ed.). Viçosa: Editora UFV.

Bordignon, R. I. T. A., Medina Filho, H. P., Siqueira, W. J., & Pio, R. M. (2003). Características da laranjeira ‘Valência’ sobre clones e híbridos de porta-enxertos tolerantes a tristeza. *Bragantia, 62*(3), 38-395. https://doi.org/10.1590/S0006-87052003000300005

Cantuarias-Avilés, T., Mourão Filho, F. de A. A., Stuchi, E. S., Silva, S. R. da, Espinoza-Nuñez, E., & Bremer Neto, H. (2012). Rootstocks for high fruit yield and quality of ‘Tahiti’ lime under rain-fed conditions. *Scientia Horticulturae, 142*, 105-111. https://doi.org/10.1016/j.scienta.2012.05.008

Emmanouilidou, M. G., & Kyriacou, M. C. (2017). Rootstock-modulated yield performance, fruit maturation and phytochemical quality of ‘Lane Late’and ‘Delta’sweet orange. *Scientia Horticulturae, 225*, 112-121. https://doi.org/10.1016/j.scienta.2017.06.056

FAO (Food and Agriculture Organization of the United Nations). (2017). *FAOSTAT*. Retrieved January 30, 2017, from http://www.fao.org/faostat/en/#data/QC

Feder, N., & O’Brien, T. P. (1968). Plant microtechnique: Some principles and new methods. *American Journal of Botany, 55*, 123-142. https://doi.org/10.1002/j.1537-2197.1968.tb06952.x

Forner-Giner, M. A., Rodriguez-Gamir, J., Martinez-Alcantara, B., Quinones, A., Iglesias, D. J., Primo-Millo, E., & Forner, J. (2014). Performance of ‘Navel’ Orange trees grafted onto two new dwarfing rootstocks (Forner-Alcaide 517 and Forner-Alcaide 418). *Scientia Horticulturae, 179*, 376-387. https://doi.org/10.1016/j.scienta.2014.07.032

Fukuda, H. (1996). Xylogenesis: Initiation, progression, and cell death. *Annual Review of Plant Biology, 47*(1), 299-325. https://doi.org/10.1146/annurev.arplant.47.1.299

Hartmann, H. T., Kester, D. E., Davies Jr., F. T., & Geneve, R. L. (2014). *Hartmann & Kester’s Plant Propagation: Principles and Practices* (8th ed., p. 443). Prentice-Hall Inc.

IBGE (Instituto Brasileiro de Geografia e Estatística). (2018). *Levantamento Sistemático da Produção Agrícola*. Retrieved April 11, 2018, from https://sidra.ibge.gov.br/home/lspa/brasil

Jacomine, P. K. T. (2009). A nova classificação brasileira de solos. *Anais da Academia Pernambucana de Ciência Agronômica, 5*, 161-179.

Klein, D. E., Gomes, V. M., Silva-Neto, S. J., & da Cunha, M. (2004). The structure of colleters in several species of *Simira* (Rubiaceae). *Annals of Botany, 94*, 733-740. https://doi.org/10.1093/aob/mch198

Lima, C. F., Marinho, C. S., Costa, E. S., & Amaral, C. O. (2014). Qualidade dos frutos e eficiência produtiva da laranjeira ‘Lima’enxertada sobre ‘Trifoliata’, em cultivo irrigado. *Revista Brasileira de Ciências Agrárias, 9*(3), 401-405. https://doi.org/10.5039/agraria.v9i3a4433

Machado, D. L., Siqueira, D. L. D., Salomão, L. C. C., Cecon, P. R., & Silva, D. F. P. D. (2017). Evaluation of rootstocks for ‘Tahiti’ acid lime in northern state of Minas Gerais. *Revista Brasileira de Fruticultura, 39*(1). https://doi.org/10.1590/0100-29452017790

Mademba-Sy, F., Lemerre-Desprezz, Z., & Lebegin, S. (2012). Use of Flying Dragon trifoliate orange as dwarfing rootstock for citrus under tropical climatic conditions. *HortScience, 47*(1), 11-17.

Martínez-Alcántara, B., Rodriguez-Gamir, J., Martínez-Cuenca, M. R., Iglesias, D. J., Primo-Millo, E., & Forner-Giner, M. A. (2013). Relationship between hydraulic conductance and citrus dwarfing by the Flying Dragon rootstock (*Poncirus trifoliata* L. Raft var. monstruosa). *Trees, 27*(3), 629-638. https://doi.org/10.1007/s00468-012-0817-1

Moraes, L. A. C., Moreira, A., Moreira, A., & Pereira, J. C. R. (2011). Incompatibility of 'Cleopatra' mandarin rootstock for grafting citrus in Central Amazon, State of Amazonas, Brazil. *Revista de Ciências Agrárias/Amazonian Journal of Agricultural and Environmental Sciences, 54*(3), 299-306. https://doi.org/10.4322/rca.2012.026

Oliveira, I. V. M., Damião Filho, C. F., & Carvalho, S. A. (2002). Enxertia em citros por substituição de ápice caulinar. *Revista Brasileira de Fruticultura, 24*(3), 744-747. https://doi.org/10.1590/S0100-29452002000300046

Philipson, W. R., Ward, J. M. & Butterfield, B. G. (1971). *The vascular cambium: its development and activity*. Chapman and Hall, London.

Pompeu Júnior, J. (2005). Porta-enxertos. In D. Mattos Júnior, J. D. De NegrI, R. M. Pio, & J. Pompeu Júnior (Eds.), *Citros* (pp. 61-104). Campinas, SP: Instituto Agronômico e Fundag.

Pompeu Junior, J., & Blumer, S. (2014) Híbridos de trifoliata como porta-enxertos para laranjeira Pêra. *Pesquisa Agropecuária Tropical, 44*(1), 9-14. https://doi.org/10.1590/S1983-40632014000100007

Portella, C. R., Marinho, C. S., Amaral, B. D., Carvalho, W. S. G., Campos, G. S., Silva, M. P. S. da, & Sousa, M. C. de. (2016). Desempenho de cultivares de citros enxertadas sobre o trifoliateiro 'Flying Dragon' e limoeiro 'Cravo' em fase de formação do pomar. *Bragantia, 75*(1), 70-75. https://doi.org/10.1590/1678-4499.267

Ribeiro, A. C., Guimarães, P. T. G., & Alvarez, V. V. H. (1999). *Recomendações para o uso de corretivos e fertilizantes em Minas Gerais-5ª Aproximação* (pp. 219-225). Viçosa: CFSEMG.

Stuchi, E. S., & Girardi, E. A. (2011). Adensamento de plantio deve ser o quarto elemento no manejo do HLB. *Citricultura Atual, 16*(81), 12-16.

Zekri, M. (2000). Citrus rootstocks affect scion nutrition, fruit quality, growth, yield and economical return. *Fruits, 55*, 231-239.

Zonta, E. P., & Machado, A. A. (1984). *Sistema de Analise Estatística* (SANEST). IFM.