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Substrate mixing formulations for Citrus nursery management

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ABSTRACT: The citrus seedling development is determined by several factors, including the physical and chemical qualities of the substrate, which affect the quality of field seedlings. The purpose of this study was to evaluate how the 'Swingle' citrumelo rootstock developed with different substrate formulations. The experiment was carried out in a seedling nursery from seeding to grafting, and six treatments were carried out: 60% peat moss, 30% fine grade horticultural vermiculite, 10% rice hulls; 60% peat moss, 30% super fine grade horticultural vermiculite, 20% rice hulls; 50% peat moss, 30% super fine grade horticultural vermiculite, 20% rice hulls; 50% peat moss, 30% super fine grade horticultural vermiculite, 20% rice hulls; 50% peat moss, 20% fine grade horticultural vermiculite, 30% rice hulls ; 50% peat moss, 20% super fine grade horticultural vermiculite, 30% rice hulls ; 50% peat moss, 20% super fine grade horticultural vermiculite, 30% rice hulls ; 50% peat moss, 20% super fine grade horticultural vermiculite, 30% rice hulls ; 50% peat moss, 20% super fine grade horticultural vermiculite, 30% rice hulls ; 50% peat moss, 20% super fine grade horticultural vermiculite, 30% rice hulls ; 50% peat moss, 20% super fine grade horticultural vermiculite, 30% rice hulls ; 50% peat moss, 20% super fine grade horticultural vermiculite, 30% rice hulls ; 50% peat moss, 20% super fine grade horticultural vermiculite, 30% rice hulls ; 50% peat moss, 20% super fine grade horticultural vermiculite, 30% rice hulls ; 50% peat moss, 20% super fine grade horticultural vermiculite, 30% rice hulls ; 50% peat moss, 20% super fine grade horticultural vermiculite, 30% rice hulls. When container transplanting was performed, the emergence percentage of seedlings was determined. Stem height, stem diameter, leaf number, area meter, root dry matter, leaf and stem dry matter, and quality index were measured on seedlings. All substrate formulations improved seedling development until grafting, except for the formulations with 30% r

Keywords: [*Citrus paradisi* Macfad. Duncan grapefruit × *Poncirus trifoliata* (L.) Raf.]; peat moss; rice hulls; vermiculite.

Manejo de viveiro de citros com mistura de formulações de substrato

RESUMO: Muitos fatores influenciam na formação das mudas cítricas, sendo que as propriedades físicas e químicas dos substratos afetam a qualidade da muda levada a campo. O trabalho teve como objetivo avaliar o desenvolvimento do porta-enxerto citrumeleiro 'Swingle' em diferentes formulações de mistura de substratos. O experimento foi realizado em viveiro sendo as plantas conduzidas da semeadura até o ponto de enxertia. Os tratamentos utilizados foram: Substrato 60% Turfa Sphagnum, 30% vermiculita fina, 10% casca de arroz; Substrato 60% Turfa Sphagnum, 30% vermiculita super fina, 10% casca de arroz; Substrato 50% Turfa Sphagnum, 30% vermiculita super fina, 20% casca de arroz; Substrato 50% Turfa Sphagnum, 20% vermiculita fina, 30% casca de arroz; Substrato 50% Turfa Sphagnum, 20% vermiculita super fina, 30% casca de arroz; Substrato 50% Turfa Sphagnum, 20% vermiculita super fina, 30% casca de arroz; Substrato 50% Turfa Sphagnum, 20% vermiculita super fina, 30% casca de arroz; Substrato 50% Turfa Sphagnum, 20% vermiculita fina, 30% casca de arroz; Substrato 50% Turfa Sphagnum, 20% vermiculita super fina, 30% casca de arroz; Substrato 50% Turfa Sphagnum, 20% vermiculita super fina, 30% casca de arroz; Substrato 50% Turfa Sphagnum, 20% vermiculita super fina, 30% casca de arroz; Substrato 50% Turfa Sphagnum, 20% vermiculita super fina, 30% casca de arroz; Substrato 50% Turfa Sphagnum, 20% vermiculita super fina, 30% casca de arroz; Substrato 50% Turfa Sphagnum, 20% vermiculita super fina, 30% casca de arroz; Substrato 50% Turfa Sphagnum, 20% vermiculita super fina, 30% casca de arroz; Substrato de caule, número de folhas, área foliar, massa seca das raízes e parte aérea e índice de qualidade. Todas as formulações de mistura de substratos proporcionam desenvolvimento semelhante das mudas até a enxertia, exceto as formulações com 30% de casca de arroz que não promoveram o desenvolvimento adequado das plântulas de citrumeleiro 'Swingle'.

Palavras-chave: [*Citrus paradisi* Macfad. Duncan grapefruit × *Poncirus trifoliata* (L.) Raf.]; casca de arroz vermiculita; turfa de *Sphagnum*.

1. INTRODUCTION

Most seedlings are housed in containers with substrate in greenhouses, but new technologies are allowing for greater plant development. Therefore, new requirements are based on a variety of studies, such as substrate, irrigation, containers, and fertilizers (SCHÄFER et al., 2008; FERRAREZI et al., 2019). Furthermore, several citrus development plants have failed because of the type of seedlings that were picked. Although some citrus is regarded to be healthy, it is susceptible to viral infections and citrus greening. Citrus seedlings require several years to come into production, thus both growth (from seedlings to adulthood) and disease development are gradual. (DORJI; LAKEY, 2015). 'Swingle' citrumelo is a hybrid [*Citrus paradisi* Macfad. Duncan grapefruit × *Poncirus trifoliata* (L.) Raf.]. This hybrid is also one of the most significant rootstocks for being resistant to a variety of diseases that impact citriculture around the world (LIBERATO et al., 2013), besides having moderate drought tolerance, a long lifespan, and can be used in place of Rangpur lime rootstock (*Citrus limonia* L. Osbeck) (OLIVEIRA; SCIVITARO, 2007; PRADO et al., 2008). Seedling production is crucial when it comes to growing an orchard. All citrus nurseries (i.e. large or small) have obstacles during the production stage in a protected environment (MERLIN et al., 2012). Thus, selecting the appropriate substrate can be a difficult task that requires consideration of both physical and chemical features (ARCE; RIVERA, 2018). There are various substrate possibilities on the market, all of which are made from diverse material combinations; these substrates also have formulations and characteristics that are either known or unknown to the producers (FRANCO et al., 2007; ALMEIDA et al., 2018).

The physicochemical features of substrates will be a determining influence on the quality of seedlings as the root system develops, affecting plant growth and yield (MAGGIONI et al., 2014).

Pine bark, rice hulls, peat moss, fine vermiculite, perlite, pulverized coal, and coconut fiber are some of the raw materials commonly utilized in citrus seedling combinations in Brazil. Material, combinations, and formulations are also primarily determined by the cost and availability of raw materials (COSTA et al., 2005). Nonetheless, mixing more than three components in the same combination is impractical, because it results in higher expenses and poorer profit margins, according to Guerrini; Trigueiro (2004). Similar information was reported by ARCE; RIVERA, (2018) and FERRAREZI (2019).

Peat is a type of plant material that is used to create an anaerobic environment. It is widely utilized in temperate climates. These adaptable plants have a low density and an acidic pH. They are appropriate for use in the production of substrate materials because of their capacity to hold water. *Sphagnum* peat is a form of dry peat with a density of around 110 g/L⁻¹ and a water retention capacity of 15 to 30 times its weight. It usually originates from Canada, Ireland, and Germany (BRITO et al., 2012).

Aluminium, magnesium, and iron are all found in vermiculite, which is a hydrated silicate. Moreover, this mineral absorbs up to five times its volume in water; its presence in substrate composition boosts water retention capacity (REZENDE et al., 2010; ARCE; RIVERA, 2018). It also has a high capacity for cation exchange, making it a great candidate (REZENDE et al., 2010).

After going through a roasting process, rice hulls are a residue produced by the rice industry and has been used as a component of substrates. Also, rice hulls have a low density, they allow the substrate to have a higher overall porosity, which aids in the drainage and aeration process of the seedlings' root systems. Rice hulls have high macro porosity, they work best when paired with microporous materials like vermiculite (KRATZ et al., 2013).

Substrates and rootstocks are the most expensive agronomic materials, accounting for 20.7 percent of total

production expenses, according to the ViveCitrus Association and Conplant Consulting Company (BATAGLIA et al., 2008). Furthermore, studies demonstrate that lowering the use of discarded rootstocks and other agronomic supplies, as well as making optimal use of substrate and fertilizers, can increase the profitability of commercial nursery plants (BREMER NETO et al., 2015).

New substrate formulations, when compared to those on the market, include low-cost and greater availability materials that can positively impact the final cost of the seedling. The seedlings' quality is directly connected to the substrate on which they are grown. The purpose of this study was to evaluate how the 'Swingle' citrumelo rootstock developed with different substrate formulations until grafting, to then develop innovative formulations for citrus nurseries that are possibly cheaper, more available and mainly, that promote better seedling development.

2. MATERIALS AND METHODS

The experiment was carried out in a nursery at the School of Agriculture, Sao Paulo State University (FCA/UNESP) in Botucatu, State of Sao Paulo, Brazil. The seeds of 'Swingle' citrumelo were collected at the Horticulture Department and sieved under running water before being dried for 48 hours without the tegument. The experiment was then divided into two stages: a seedling nursery in tubes (stage 1) and a planting phase in bags (stage 2).

This study used six mixtures of substrate formulations that were prepared and supplied by the Carolina Soil® Company, as follows: 60% peat moss, 30% fine grade horticultural vermiculite, 10% rice (Oriza sativa) hulls (1); 60% peat moss (Sphagnum spp), 30% super fine grade horticultural vermiculite, 10% rice hulls (2); 50% peat moss, 30% fine grade horticultural vermiculite, 20% rice hulls (3); 50% 30% peat moss, super fine grade horticultural vermiculite, 20% rice hulls (4); 50% peat moss, 20% fine grade horticultural vermiculite, 30% rice hulls (5); peat 50%20% super fine grade moss, horticultural vermiculite, 30% rice hulls (6).

Table 1 summarizes the physical parameters of each moisture-sensitive substrate (Kämpf, 2001): moisture substrate (Comité Europeén de Normalisation - CEN, 1999); volumetric density, water retention capacity, porosity and granulometry (Ministério da Agricultura, Pecuária e Abastecimento - MAPA, 2008).

Table 1. Total porosity (TP), macroporosity (MA), microporosity (MI), water retention capacity (WRC), volumetric density (VD) and moisture (M) of various substrate formulations.

Tabela 1. Po	rosidade	e total (ГР),	macroporosidade	(MA),	microporosidade	(MI),	capacidade	de	retenção	de	água	(WRC),	densidade
volumétrica (VD) e u	midade (MO) das diferentes for	nulaçõ	ões de substrato.								

			3			
Substrates ¹	TP	MA	MI	WRC	VD	MO
	(%)	(%)	(%)	(55 mL cm ⁻³)	(Kg m ⁻³)	(%)
1	85.4	21.6	63.7	33.1	433.3	68.6
2	72.5	20.1	52.4	27.2	283.3	52.9
3	77.5	33.1	44.3	23.0	253.3	47.9
4	79.9	29.2	50.6	26.3	293.3	51.9
5	80.7	40.1	40.6	21.2	243.3	49.5
6	78.2	35.5	42.7	22.3	240.0	43.5

1: 60% peat moss, 30% vermiculite fine, 10% rice hulls; 2: 60% peat moss, 30% vermiculite super fine, 10% rice hulls; 3: 50% peat moss, 30% vermiculite fine, 20% rice hulls; 4: 50% peat moss, 30% vermiculite super fine, 20% rice hulls; 5: 50% peat moss, 20% vermiculite fine, 30% rice hulls; 6: 50% peat moss, 20% vermiculite super fine, 30% rice hulls; 6: 50% peat moss, 30% vermiculite super fine, 30% rice hulls; 6: 50% peat moss, 30% vermiculite super fine, 30% rice hulls; 6: 50% peat moss, 30% vermiculite super fine, 30% rice hulls; 6: 50% peat moss,

2.1. Stage 1

Stage 1 began with the filling of 50 cm³ conical plastic tubes with substrates, followed by planting one seed per tube. These were placed in polypropylene trays (176 cells) 1 m above the ground, on concrete benches in a greenhouse with automated irrigation (i.e. a timer set for 10 minutes twice a day and a medium irrigation plate of 6 mm). The shading screen (sombrite) used was 0.87×0.30 mm, respectively on the covering and on the sides.

The experiment was conducted in a completely randomized design with six treatments consisting of substrate mixtures, four replicates, with 51 seedlings per replicate, totalizing 204 seedlings per treatment, utilizing a split plot layout. The plots consisted of substrate mixtures, with the subplots representing the evaluation time.

The number of seeds that emerged once a week until 102 days after sowing (DAS) was counted to determine the emergence percentage of each treatment (MAGUIRE, 1962).

When the plants were about 20 cm tall, (BATAGLIA et al., 2008), eight plants were randomly selected from each treatment to assess the following: stem height (cm) that was measured from the base to the apices of the stem; stem diameter (mm) that was measured at a height of around 1 cm above the substrate (mm); leaf number, root and aerial dry matter were calculated using a traditional drying method (LACERDA et al., 2009).

2.2. Stage 2

Two months after sowing, the most robust seedlings from stage 1 were transplanted into polyethylene bags (4 L) containing the same mixtures of substrates used in the previous stage. The rootstocks were grown in benches at a height of about 1 m above ground in a greenhouse and the fertilizations started with a solution of calcium nitrate (0.8 g L^{-1} equivalent to 0.11 g L^{-1} N + 0,13 g L^{-1} Ca) + magnesium sulphate (0.4 g L^{-1} equivalent to 0,036 g L^{-1} Mg + 0,044 g L^{-1} S) + mono ammonium phosphate (0.4 g L^{-1} equivalent to $0,24 \text{ g L}^{-1} \text{ P}_2\text{O}_5 + 0,05 \text{ g L}^{-1} \text{ N}$), potassium chloride (0.4 g L⁻¹ ¹ equivalent to 0.24 g L^{-1} K) + urea (0.3 g L^{-1} equivalent to $0.14 \text{ g L}^{-1} \text{ N}$) + micronutrients solution was applied (1 g L⁻¹), according with the recommendations of Bataglia et al., (2008) and Bremmer Neto et al., (2015) adapted. Once a week, 200 ml of the solution was administered to each bag, for all the treatments evaluated and according to the recommendations of Bremmer Neto et al., (2015) adapted. Drainage of substrate mixtures was minimal.

A solution portion was tested for electrical conductivity and pH value by using a DIGIMED brand conductivity and pH meter. Values ranged from CE: 0.52 to 0.63 dS m⁻¹and pH: 6.02 to 6.71.

Stage 2 was undertaken in a completely randomized design with four replicates of twenty plants per parcel, using a split plot design. The plots contained six substrate mixtures, whereas the subplots represented the evaluation period. Three plants per replication of each treatment were randomly gathered every 28 days to obtain a growth curve and examine the effect of varied mixing amounts on 'Swingle' citrumelo development. Thus, measuring stem height and diameter, number of leaves, root, and aerial dry matter, as well as leaf area meter - derived using the LI-Cor model LI-3100C in cm²; and Dickson Quality Index (DQI) (DICKSON et al., 1960).

Data were subjected to analysis of variance, the mean between substrates was compared using the Tukey test (p<0.05) and regression analysis was used to determine the mean emergence and seedling growth over time. The model was chosen according to the determination coefficient (R²). The emergence percentage data was converted according to equation 1.

$$\arcsin \sqrt{x/100}$$
 (01)

3. RESULTS

3.1. Stage 1

The interaction between substrate mixtures and days after sowing for seedling emergence was not significant throughout the emerging stage and early seedling growth, although there was an isolated influence of the variables. Substrate 1 stimulated higher seedling emergence than substrate 2, but not substantially different from 3, 4, 5, and 6 (Figure 1A). At 28 DAS, the seedlings started to emerge. The seedling emergence rate had a quadratic growth from this point on, reaching 105 DAS (Figure 1B).



The error bars represent the Tukey test least significant difference (LSD) at a probability of 5%.

Figure 1. Seedling emergence (%) of 'Swingle' citrumelo rootstock as a function of substrates (A) and days after sowing (DAS) (B). FCA/UNESP. Botucatu, 2017.

Figura 1: Porcentagem de emergência do porta-enxerto citrumeleiro 'Swingle' em função das formulações dos substratos (A) e dias depois da semeadura (DAS) (B). FCA/UNESP. Botucatu, 2017.

When plants were examined till the transplanting period (stage 1), the only variation between the substrate combinations was the aerial and root dry matter (Table 2). Substrates 4 and 5, which differed only from substrate 6, yielded the highest root dry matter. Substrate 5 had the highest aerial dry matter, while substrates 3 and 6 had lower values (Table 2).

Liberato et al.

Table 2. Height (H), diameter (D), leaf number (LN), root dry matter (RM) and aerial dry matter (AM) of 'Swingle' citrumelo rootstocks with substrates in proportions till transplanting time. FCA/UNESP. Botucatu, 2017.

Tabela 2: Altura (H), diâmetro (D), número de folhas (LN), matéria seca de raízes (RM) e matéria seca da parte aérea (AM) do porta-enxerto citrumeleiro 'Swingle' em função das formulações de substrato até o período de transplantio.

Substrates ¹	Н	D	LN	RM	AM
	(cm)	(mm)		(mg/cm^3)	(mg/cm^3)
1	21.83a	1.90a	15.00a	0.11ab	0.37ab
2	21.46a	1.91a	11.38a	0.12ab	0.36ab
3	20.61a	1.94a	10.38a	0.10ab	0.31b
4	22.08a	2.14a	12.75a	0.13a	0.39ab
5	22.36a	2.28a	15.38a	0.15a	0.47a
6	20.93a	1.96a	12.50a	0.08b	0.27b
CV (%)	5.02	9.07	20.30	20.93	22.62
DMS	2.43	0.41	5.88	0.05	0.18

Tukey test results show that means preceded by the same letter do not differ by 5% probability.



 $-\cdot \circ -\cdot$ Subst. 6 y = 0.0009x²- 0.0012x + 22.09, R² = 0.90, p<0.01, y_{min} = 0.7 The error bars represent the Tukey test least significant difference (LSD) at a probability of 5%.

Figure 2. Stem diameter (A) and seedling height (B) of 'Swingle' citrumelo in various mixes of subtracts, as a function of days after transplanting. Botucatu-SP, 2017.

Figura 2: Diâmetro de caule (A) e altura de muda (B) do portaenxerto citrumeleiro 'Swingle' em diferentes formulações de substratos em função dos dias após o transplantio. Botucatu-SP, 2017.





 $\begin{array}{lll} -\cdot \bullet -\cdot & \text{Subst. 5} & y = 0.027 x^2 \cdot 1.78 x + 94.61, R^2 = 0.80, p < 0.01, y_{\text{min}} = 33.0 \\ -\cdot \bullet -\cdot & \text{Subst. 6} & y = 0.027 x^2 \cdot 1.86 x + 101.86, R^2 = 0.88, p < 0.01, y_{\text{min}} = 34.4 \end{array}$

- Subst. 4 $y = 0.037x^2 - 2.00x + 111.85$, $R^2 = 0.89$, p < 0.01, $y_{min} = 27.0$

The error bars represent the Tukey test's least significant difference (LSD) at a probability of 5%.

Figure 3. Leaves per seedling (A) and leaf area (B) of 'Swingle' citrumelo rootstock in various mixes of subtracts, as a function of days after transplanting. Botucatu-SP, 2017.

Figura 3: Folhas por mudas (A) e área foliar (B) do porta-enxerto citrumeleiro 'Swingle' em diferentes formulações de substratos. Em função dos dias após o transplantio.

3.1. Stage 2

The relationship between substrates and days after transplanting was significant for all variables in the second stage of rootstock development. Until 112 days after transplanting (DAT), the substrates did not induce variations in stem diameter, leaf number, or root dry matter across plants (Figures 2A, 3A and 4A). Only 84 DAT had a substrate influence on height, aerial dry matter, and leaf area (Figures 2B, 3B and 4B).

Larger stem diameters were encouraged by substrates 1, 2, 3, and 4, which increased linearly up to 168 DAT (Figure 2A). Stem diameter is a critical variable since it determines the optimal time for grafting (i.e. 8 mm in diameter) and, as a result, seedling precocity. At 168 DAT, that criterion was not attained in treatments 5 and 6. At 168 DAT, the substrates with 30% rice hulls produced plants with a 26.98% lower stem diameter than the others. Therefore, the seedlings grown on these substrates (5 and 6) could not be grafted at this time. After 112 DAT, the increase in stem diameter halted on these substrates (Figure 2A).



Error bars correspond to the least significant difference (LSD) obtained by Tukey test at 5% probability.

Figure 4. Root (A) and aerial (B) dry matter of 'Swingle' citrumelo rootstock in various mixes of subtracts, as a function of days after transplanting. Botucatu-SP, 2017.

Figura 4: Massa seca da raiz (A) e aérea (B) do porta-enxerto citrumeleiro 'Swingle' em diferentes formulações de substratos em função dos dias após o transplante. Botucatu, 2017.

Moreover, height, leaf number, leaf area, root and aerial dry matter, and Dickson Quality Index (DQI) showed a quadratic increase as a function of DAT on the substrates.

At 168 DAT, the rootstocks in substrate 4 had grown to a height of 102.68 cm. When compared to the others, substrates 1 and 2 promoted intermediate vegetative growth, with no difference between them. Substrates 5 and 6 exhibited lower height from 84 to 168 DAT (Figure 2B).

In all substrates, the number of leaves per seedling increased more between 140 and 168 DAT. Seedlings from substrate 5 had more leaves at the ending of the evaluation period, whereas those from substrate 4 had the lowest average (Figure 3A). Meanwhile, rootstocks grown in substrate 1 had a greater leaf area at 168 DAT, indicating that their leaves were larger (Figure 3B). This might be due to the substrate's increased moisture holding capability (Table 1). The leaves of the seedlings from substrates 5 and 6 are already smaller, despite their abundance, because of their reduced leaf area (Figure 3B).

Plants grown in substrates 1, 2, 3, and 4 performed better than those grown in the other treatments, accumulating more dry roots matter at 140 and 168 DAT, respectively (Figure 4A). Their physical differences can explain this finding. Higher aerial dry matter was also promoted by these substrates. When the first seedlings reached grafting stage (168 DAT), it was discovered that substrate 4 outperformed the others in terms of aerial dry matter, weighing 18.92 g, while substrates 5 and 6 weighed 9.61 g and 9.42 g, respectively (Figure 4B).

Higher DQI was possible with substrates 1, 2, 3, and 4 that showed high averages at 168 DAT (Figure 5). The DQI enables for accurate selection since it combines important parameters (such as bud and root dry matter, stem height and diameter) into one marker (DIAS et al., 2012). In the same time frame, the DQI of seedlings from substrates 5 and 6 was 28.49 percent lower (Figure 5). Figure 6 depicts the growth of seedlings derived from each of the substrates.



1 ve error vars represent toe 1 ukey test least significant difference (LSD) at a probability of 5%.

Figure 5. Dickson Quality Index (DQI) of 'Swingle' citrumelo rootstock in various mixes of subtracts, as a function of days after transplanting. Botucatu-SP, 2017.

Figura 5. Índice de Qualidade Dickson (DQI) do porta-enxerto citrumeleiro 'Swingle' em diferentes formulações de substratos em função dos dias após o transplante. Botucatu-SP, 2017.



1: 60% peat moss, 30% vermiculite fine, 10% rice hulls; 2: 60% peat moss, 30% vermiculite super fine, 10% rice hulls; 3: 50% peat moss, 30% vermiculite fine, 20% rice hulls; 4: 50% peat moss, 30% vermiculite super fine, 20% rice hulls; 5: 50% peat moss, 20% vermiculite fine, 30% rice hulls; 6: 50% peat moss, 20% vermiculite super fine, 30% rice hulls.

Figure 6: 'Swingle' citrumelo seedlings grown on various substrates. Figura 6. Mudas de citrumeleiro 'Swingle' produzidas em diferentes substratos.

4. DISCUSSION

The substrate mixtures evaluated only presented effects after 84 DAT. This can be explained by roots acclimating to new plastic bags as well as increased substrate volume exposing roots.

The increased density, porosity, and water retention of substrate 1 (Table 1) encouraged an acceptable balance between water and O_2 , allowing for improved seedling emergence throughout the initial stage of seedling production. On the other hand, substrate 2 had the lowest overall porosity and water retention among the examined substrates. Higher micro porosity in substrates is reported to hinder seed germination and root aeration by Fernandes et al. (2006); however, this was not found in our study. Therefore, we should bear in mind that the percentage of different component groups and the combination type must be created with the adequacy of the physical characteristics, according to Kratz et al. (2013). Chemistries may be modified with fertilization and irrigation management methods.

Substrates 1, 2, 3, and 4 allowed the development of more robust seedlings in stage 2. When opposed to 5 and 6, these substrates stand out because of the reduced proportion of rice hulls. The irrigation levels were the same for all treatments and could explain the worst performance of treatments 5 and 6. This hypothesis can be attributed to lower water availability due to higher percentages of rice hulls. The results allow us to suggest that there was a possible hydric stress in treatments 5 and 6 and that if there had been a greater frequency of irrigation on these substrates mixtures, their performance could have been better. However, according to per manufacture information in substrates mixtures evaluated it was used roasted rice hull, in which the material undergoes a high temperature process followed by cooling for maintaining the structure of the particles. The method used is a differential, as it maintains the characteristics of the size of the rice hulls particles, which can influence the drainage properties in the substrate, in addition to reducing the tendency to break during the transport process.

Schäfer et al. (2008) found that lower volumetric density substrates increased macro porosity of mixtures and lowered substrate water retention capacity and the need for specialized fertilization and irrigation. The poorer performance of seedlings in substrates 5 and 6 can be explained by the fact that the rice hulls supplied more macro porosity to these substrates and consequently, the lowest humidity percentage and water retention (Table 1).

The presence of a higher percentage of rice hulls (material with higher porosity) can be advantageous for the aeration of the root development, but at the same time, worrying due to the deficiency in retaining water, since the smaller pores are responsible for the function.

The pores oversee gas exchange in the quest for a balance between substrate and surrounding atmosphere, besides determining water flow in the container (BRITO et al., 2012). The presence of a larger proportion of rice hulls (a substance with a higher porosity) might be beneficial for aeration of the root environment, but it can also be concerning owing to a lack of water retention, as the smaller pores are important for this function.

When rice hulls are used in significant numbers, they can cause water shortages in plants, especially if irrigation is done infrequently (REZENDE et al., 2010). As a result, it is recommended to combine it with high micro porosity elements, such as vermiculite.

The size and arrangement of the particles determine the physical properties of a substrate; high proportions of larger fractions enhance aeration space, while smaller particles minimize void spaces in rice hulls; thus, increasing micro porosity and, consequently, decreasing macro porosity.

Seedlings on substrates 1, 2, 3, and 4 may have more leaf area due to their better moisture retention ability, as water is a vital component in cell growth. Leaf area is an excellent indication of production, according to Taiz et al. (2017), because leaf area and photosynthetic efficiency are closely related. Plants can also influence variations in radiation interception, resulting in increased gas exchange efficiency.

The substrates that supported larger accumulation of shoot dry matter also offered root system growth, indicating that the seedlings were growing in a balanced manner (FONSECA et al., 2002). The differences in dry matter accumulation in the aerial part and in the root system observed between substrates can be explained as dry matter accumulation is the best indicator of plant growth, since it is less variable than fresh matter, which can vary throughout the day due to a variety of factors, such as temperature and the amount of water available to plants (MARTÍNEZ, 2002; MERLIN et al., 2012). Plant photosynthesis has a significant impact on dry matter development. Thus, greater accumulation indicates improved physiological performance.

After 112 days, the differences arising from the usage of different substrate mixtures were noticeable. The least interference occurs at the start of this phase due to transplanting to larger bags, when plants have most likely experienced environmental changes and need time to restore reserves for growth. The restricted capacity of container and substrate fertility impact plant development in plastic bags (MOURÃO FILHO et al., 1998). This fertility depends on substrate components, and cover fertilizers are generally required to boost fertility (DECARLOS NETO et al., 2002).

5. CONCLUSIONS

All substrates supported greater seedling emergence, apart from the substrate comprising 60% peat moss, 30% fine vermiculite, and 10% rice hulls. Most substrate formulations allowed for the generation of superior quality seedlings, except for substrates containing 30% rice hulls,

which produced seedlings with lower development and cannot be recommended for 'Swingle' citrumelo rootstock seedlings.

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