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Quality of rubber tree rootstock seedlings grown in protected environments and alternative substrates

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ABSTRACT. This study aimed to evaluate the quality of *Hevea brasiliensis* seedlings, a GT1 clone, produced in protected environments in a variety of substrates. The treatments consisted of 13 substrates composed of combinations of cattle manure, soil from a ravine, medium vermiculite, superfine vermiculite and washed fine sand and two environments: a greenhouse with 50% shade using aluminized screen and a plant nursery with 50% shade using a Sombrite® screen. There were no replications of the environments; therefore, each one was considered an experiment. For each environment, a completely randomized experimental design with five replications was used to evaluate the substrates. Subsequently, the average squares of the residuals of individual variance analyses of these treatments (substrates) were evaluated, and because they did not exceed an approximate ratio of 7:1, a combined analysis of experiments was performed with Sisvar software. Two hundred eighty days after sowing, the dry weight, plant height, stem diameter, diameter at five centimeters above soil and the seedling quality indices were evaluated. Results indicated that substrates with a low amount of manure and sand and a high amount of vermiculite (S9 and S13) produced rubber tree seedlings of high-quality. The environment with greater radiation availability was better for the development of high-quality rubber tree seedlings.

Keywords: *Hevea brasiliensis*; greenhouse; plant nursery; GT1 clone; cattle manure; vermiculite.

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Introduction

Among several species of the genus *Hevea*, the species *Hevea brasiliensis* [(Wild. ex. Adr. de Juss.) Muell Arg.] is the most utilized in commercial plantations due to its high yield of latex (Liu et al., 2015) and high quality for rubber manufacturing. Rubber tree cultivation is an activity that has expanded in area in the Brazilian territory, mainly to areas considered free from South American leaf blight, such as regions of São Paulo and Mato Grosso do Sul States, Brazil. This expansion has increased the requirement for phytosanitary control and high-quality seedlings. Therefore, to implant or replace plants in commercial rubber tree plantations, in addition to enhancing environmental projects that contribute to the sequestration of atmospheric carbon (Cambui, Vasconcelos, Mariano Neto, Viana, & Cardoso, 2017; Maggiotto et al., 2014), high-quality seedlings are required. To achieve this goal, multiple technologies and techniques are used, such as using selected and vigorous seeds, using suitable substrates, planting in containers and compatible protected environments, and controlling irrigation, among others.

Most rubber tree seedlings are formed from grafting, so the rootstock seedlings must be of high quality. For the production of high-quality rootstocks, the choice of the clone influences the yield potential and the uniformity of the rubber tree plantation. Therefore, care with micrometeorological factors, cultivation treatments, fertilization, and spacing, among other factors, in the formation of rootstock will influence the final quality of the seedlings (Martins, 2010). The main clones of commercial importance used are GT1, RRIM 600, PB 255, PB 235, and IAN 873, but the appropriate choice and development of these clones vary according to the location of the nurseries and rubber plantations.

According to Minami (1995), high-quality seedlings are commercially desirable, can give continuity to development after final location planting and are not susceptible to disease or damage that can jeopardize their growth and development, among other qualities. The use of protected environments and suitable substrates for high-quality seedlings has been shown to be important because it can advance the grafting stage and reduce the wait time of the productive phase (Costa, Curi, Figueiredo, Binotti, & Cardoso, 2017; Sanches, Costa, Costa, & Cardoso, 2017). Technologies that help growers improve the cultivation practices of rubber tree rootstocks are substrates, clones and protected environments, which promote a high potential for the survival of field plants and for the formation of healthy and productive rubber plantations (Vieira, Maruyama, Costa, Dias, & Pereira, 2016).

The use of protected environments is a technology that aims to identify the best conditions for plant development and growth, providing shade to avoid high, direct radiation on the plant and minimizing the entry of pests and diseases. In studies comparing different cultivation environments for the development of rubber tree rootstocks (ground nursery without protection, ground nursery with windbreak and ground nursery under greenhouse), it was verified that the greenhouse produced the best seedlings (Pezzopane, Pedro Júnior, & Ortolani, 1995). In the comparison of three environments (Sombrite®, Aluminet®, and external environment), larger root and aerial masses of rubber tree rootstocks were observed in Sombrite® and Aluminet® nurseries, as well as a greater number of leaves in the Aluminet® nursery (Vieira et al., 2016). In both studies cited above, the importance of the study of crop protection types for the formation of rubber tree rootstocks was evidenced.

Comparative studies of leaf anatomy of GT1 and RRIM 600 clones found that GT1 presents anatomical structures that give it characteristics more resistant to environmental stress. GT1 clones have a higher number of stomata and sclerenchyma, suggesting greater resistance to water stress and wilting. GT1 clones also have a thicker palisade parenchyma, and it is possible to infer that they have a greater photosynthetic efficiency (Martins & Zieri, 2003).

The substratum is another factor that interferes with the quality of seedlings because it is where the seedlings develop their root systems and receive nutrition and irrigation; therefore, it must have adequate physical and chemical qualities, in addition to being free of pathogenic organisms. Larger plants, a greater number of leaves, larger masses and a higher quality index of seedlings of rubber tree rootstocks were obtained with the use of commercial substrate or substrate composed of soil + bovine manure in a 7:1 proportion (Vieira et al., 2016). Some studies have demonstrated that the proportion of organic material in the substrate interferes with plant growth and development according to the species or cultivar (Prado, Costa, Cardoso, & Binotti, 2016).

Based on this information, this study aims to contribute management data for the production of high-quality rubber tree seedlings for use as rootstock. The object of this study was to evaluate different substrates composed of proportions of inert materials and cattle manure, in addition to comparing two growing environments in the production of *H. brasiliensis* seedlings.

Material and methods

The experiments with the formation of rubber tree rootstocks, clone GT1, were developed at the State University of Mato Grosso do Sul (UEMS), Cassilândia, Mato Grosso do Sul State, Brazil, latitude: -19,1225º (= 19º07'21" S), longitude: -51.7208º (= 51º43'15" W) and 516 m a.s.l. According to the climatic classification of Köppen, the region has a tropical climate, with rainy summers and dry winters.

Rubber tree seeds of the GT1 clone were collected at the Instituto Agronômico (IAC), in Votuporanga, São Paulo State, Brazil. Two protected environments were used: 1) Greenhouse; arch model; galvanized steel structure; 8.00 m wide by 18.00 m long; height under the 4.00 m channel; covered with a 150 μm light diffusing polyethylene film; a zenith opening along the ridge and lateral and frontal closures with monofilament screen; thermoreflective mesh, mobile 50% aluminized shading at 3.30 m from the ground. 2) Plant nursery; galvanized steel structure; 8.00 m wide by 18.00 m long and 3.50 m high; closing at 45º inclination, with monofilament screen in all directions; mesh with 50% shading (Sombrite®).

Within each protected environment, the seedlings were produced in polyethylene bags (15.0 x 25.0 cm), with a capacity of 1.8 liters, filled with a combination (%) of substrates from cattle manure (C), soil from ravine (S), medium vermiculite (M), superfine vermiculite (V), and washed fine sand (W), on suspended benches, as recommended in Normative Instruction #29 (Brasil, 2009). Thirteen substrates were made, as shown in Table 1.

Table 1. Substrate composition (from S1 to S13) from proportions of cattle manure (C), soil from ravine (S), medium vermiculite (M), superfine vermiculite (V), and washed fine sand (W). Cassilândia, Mato Grosso do Sul State, Brazil, 2016.

S1 = 50%C +30%S + 10%M + 10%V + 0%W	S7 = 30%C + 30%S + 10%M + 20%V + 10%W
S2 = 40%C + 30%S + 10%M + 10%V + 10%W	S8 = 20%C + 30%S + 10%M + 30%V + 10%W
S3 = 30%C + 30%S + 10%M + 10%V + 20%W	S9 = 10%C + 30%S + 10%M + 40%V + 10%W
S4 = 20%C + 30%S + 10%M + 10%V + 30%W	S10 = 50%C + 30%S + 0%M + 10%V + 10%W
S5 = 10%C + 30%S + 10%M + 10%V + 40%W	S11 = 30%C + 30%S + 20%M + 10%V + 10%W
S6 = 50%C + 30%S + 10%M + 0%V + 10%W	S12 = 20%C + 30%S + 30%M + 10%V + 10%W
	S13 = 10%C + 30%S + 40%M + 10%V + 10%W

Cattle manure, obtained from a local slaughterhouse, contained rumen material. Cattle manure was composted for 45 days, beginning on July 4th and ending on August 19th, 2015, in a sheltered location, being rolled and moistened every two days. The soil from ravine was collected at UEMS/Cassilândia, Mato Grosso do Sul State, Brazil. Vermiculite and washed fine sand were obtained from commercial companies. The watering of the seedlings was carried out using a watering can, aiming not to soak the substrates but to maintain adequate moisture for root development. Cattle manure and soil from ravine were chemically characterized (Tables 2 and 3).

Table 2. Cattle manure chemical traits. Cassilândia, Mato Grosso do Sul State, Brazil, 2016.

N	P_2O_5	K_2O	Ca	Mg	S	H-65°C	C
% (natural conditions)							
0.9	0.3	0.1	0.3	0.1	0.2	2.0	11.0
Na	Cu	Fe	Mn	Zn	C/N	pH	OM
mg kg^{-1} (natural conditions)						$CaCl_2$	% (natural conditions)
324	18	12103	204	53	12/1	5.3	20.0

H = Humidity; OM = organic matter; C/N = Carbon:Nitrogen ratio.

A commercial micronutrient solution of Conmicros® Standard was applied to the substrate during April, May, June, August and October 2016 at a concentration of 0.025 g per 10 liters of water (20 mL per plant). Conmicros® Standard contains zinc (0.73%), manganese (1.82%), boron (1.82%), molybdenum (0.36%), and nickel (0.36%).

Table 3. Soil chemical traits. Cassilândia, Mato Grosso do Sul State, Brazil, 2016.

P resin	K	Ca	Mg	BS	CEC	V%
mg dm^{-3}	mmol dm^{-3}					
9	1.0	8	3	12	67	18
pH	OM	B	Cu	Fe	Mn	Zn
Water	g dm^{-3}	mg dm^{-3}				
4.4	5	0.19	0.40	30	8.8	0.3

OM = Organic Matter.

Relative humidity (RH, %), temperature (T, °C), and global solar radiation (GR, W m^{-2}) were monitored from June to November (Table 4). In the greenhouse, at the end of August and in September, October and November, the screen under the film remained closed, providing more shade and lower direct radiation to the plants (Table 4).

Table 4. Monthly mean relative humidity (RH, %), temperature (T, °C), and global solar radiation (RG, W m^{-2}) in the protected (plant nursery and greenhouse) and external environments. Cassilândia, Mato Grosso do Sul State, Brazil, 2016.

	Environments								
	External			Plant nursery			Greenhouse		
2016	RH	T	GR	RH	T	GR	RH	T	GR
June	68.9	21.0	427.9	69.9	20.8	111.0	67.7	19.8	368.0
July	66.9	21.8	521.7	67.7	21.6	173.7	56.0	22.7	398.8
August	73.4	25.0	505.0	73.1	24.5	166.2	53.2	23.2	347.0
September	65.2	25.5	591.9	63.8	25.3	209.7	50.7	24.8	198.3
October	67.7	25.9	681.9	66.4	25.9	214.7	65.2	24.9	236.8
November	73.1	25.6	599.6	72.2	25.5	191.1	75.1	25.3	308.9

Seed sowing was performed on February 27, 2016, with two seeds per container. Emergence began on March 10, 2016, at 12 days after sowing (DAS). At the beginning of seedling formation, the emergence speed index (ESI) was measured until stabilization according to Maguire (1962). At 280 DAS, the height of the seedlings (HS), stem diameter (SD), stem diameter at 5 cm from the substrate (SD5), shoot dry mass (SDM), root system dry mass (RSDM), and total dry mass (TDM) were collected. After collection, the height:stem diameter ratio (HSD), height:shoot dry mass ratio (HSR), shoot dry mass:root system dry mass ratio (SRS), and Dickson Quality Index (DQI) were estimated (Dickson et al., 1960).

$$\mathrm{DQI} = \frac{\mathrm{TDM\ (g)}}{\frac{\mathrm{HS\ (cm)}}{\mathrm{SD\ (cm)}} + \frac{\mathrm{SDM(g)}}{\mathrm{RSDM\ (g)}}}$$

Because there were no replications of the protected environment, each one was considered an experiment. For each environment, a completely randomized experimental design was used to evaluate the substrates, with five replications of four seedlings each. Subsequently, the mean squares of the residuals of the analyses of individual variances of these treatments (substrates) were evaluated, and because they did not surpass the ratio of 7:1, a joint analysis of experiments was performed with the Sisvar software to identify the best-protected environment. IVE data were transformed into $\sqrt{(x + 0.5)}$.

Results and discussion

For all growth variables and biometric indices, the relationship between the mean squares of the residuals (RMSR) of the analyses of individual variances of the experiments did not exceed the ratio of 7:1 (Table 5), thus allowing the joint analysis of experiments (groups of experiments). Among the variables studied, the stem diameter, the HSD, HSR, and DQI showed significant interactions. For the other variables, the interactions were not significant, and the overall results for the environments and substrates were evaluated.

Table 5. Mean square of the residual of the individual variance analysis (environments) and calculated F of the variance analysis for the emergence speed index (ESI), height of the seedlings (HS), stem diameter (SD), stem diameter at 5 cm from the substrate (SD5), shoot dry mass (SDM) root system dry mass (RSDM), total dry mass (TDM), shoot:root system dry mass ratio (SRS), height:shoot dry mass ratio (HSR), height:stem diameter ratio (HSD), and Dickson quality index (DQI). Cassilândia, Mato Grosso do Sul State, Brazil, 2016.

Mean square of the residual (RMSR) of the individual variance analysis						
Environment	ESI	HS	SD	SD5	SDM	
Greenhouse	0.04	106.53	2.93	15.86	3.21	
Plant nursery	0.04	59.8	1.46	12.35	2.5	
RMSR	1.02	1.78	2.01	1.28	1.29	
Calculated F						
Environment (E)	1.02^{ns}	10.52**	55.53**	23.15**	2.72^{ns}	
Substrate (S)	10.83**	3.52**	3.51**	5.37**	4.01**	
E x S	0.57^{ns}	1.74^{ns}	2.65**	1.83^{ns}	1.22^{ns}	
CV	48.36	10.7	9.38	9.8	22.18	
Environment	RSDM	TDM	SRS	HSR	HSD	DQI
Greenhouse	4.89	11.56	2.45	10.02	2.72	0.28
Plant nursery	3.76	9.93	1.33	23.15	5.41	0.15
RMSR	1.3	1.16	1.85	2.31	1.99	1.9
Calculated F						
Environment (E)	0.51^{ns}	2.73^{ns}	0.21^{ns}	8.49**	61.48**	22.23**
Substrate (S)	10.48**	8.51**	2.97**	3.37**	3.08**	8.31**
E x S	0.85^{ns}	1.32^{ns}	0.8^{ns}	2.31*	4.33**	2.09*
CV	28.2	18.69	45.14	25.19	13.12	22.82

* = significant at 5% probability; ** = significant at 1% probability; ^{ns} = not significant.

The different environments provided similar ESIs. However, the emergence was influenced by the substrates. The substrates that had a low percentage of cattle manure and sand and a high amount of vermiculite (S9, S12, and S13) provided better conditions for a higher ESI (Table 6) because they contained larger amounts of porous material with a capacity for high water retention and aeration of the substrate. These properties directly influenced the seed germination process, allowing a higher emergence speed index.

Substrates formulated with more organic matter (cattle manure) provided a lower emergence speed index, as observed by Oliveira, Costa, Oliveira, and Jorge (2014) and Costa, Ferreira, Silva, and Nardelli

(2012). According to Artur, Cruz, Ferreira, Barretto, and Yagi (2007), a high amount of organic material in the substrate can raise the pH and interfere with emergence. However, according to Table 2, the amount of sodium (Na) is increased proportionally to the amount of cattle manure added to the substrate; therefore, salination of the substrate occurs. The emergence of the plant in this type of substrate is hampered by the excess ions that restrict the uptake of water and hinder germination processes (Marcos-Filho, 2015).

Seedlings produced in the plant nursery grew taller than those developed in the greenhouse; that is, the plants were larger in this environment. However, height measurement alone cannot characterize a seedling as being high quality, and other variables must be examined (Table 6). Plants present preference and greater adaptation to the characteristics provided by a given environment. They are expressed through different variables, such as height, diameter, mass and biometric relations, which relates to growth and differentiated morphology under a given environment and its management (Arrua, Costa, Bardiviesso, Nascimento, & Binotti, 2016, Sanches et al., 2017)

Regarding the substrates, seedlings with taller heights, by up to 40%, were observed in the substrates with cattle manure (Table 6), agreeing with the results obtained from other species, such as *Dipteryx alata* (Costa, Dias, Lopes, Binotti, & Cardoso, 2015) and *Hancornia speciosa* (Dias, Pereira, Cavacante, Raposo, & Freire, 2009).

There was no significant difference between the growing environments for stem diameter at 5 cm from the substrate (SD5) (Table 6). It was observed that there was a trend of higher SD5 for the seedlings in the substrate with a lower amount of manure. In rubber trees, this variable is used as an indicator for green grafting, requiring a minimum value of 8.0 mm (Hassan, 1980; Pezzopane et al., 1995) or 10.0 mm, according to Gonçalves, Bataglia, Ortolani, and Fonseca (2001). The seedlings in the present study were not fit to be grafted at 280 days.

The environments generated similar results for shoot, root and total dry masses; however, for the substrates, lower total dry mass (MST) was observed when higher amounts of cattle manure were used (Table 7). These results were similar to those reported by Trindade, Muchovej, Neves, and Barros (2001), who showed that an increase of organic matter decreased the assimilation of mass in *Eucalyptus grandis*, as verified by Costa et al. (2015) for *Dipteryx alata* and Dias et al. (2009) for seedlings of *Hancornia speciosa*.

Increasing the proportion of cattle manure results in increased nutrition and porosity of the substrate and reduced apparent density; however, the increase of this component proportionally influences the pH due to the presence of sodium. Sodium can cause disturbances in nutrient absorption and impair plant growth. Trazzi, Caldeira, Colombi, Peroni, and Godinho (2012), when using different sources of organic matter, found that the cattle manure provided a higher pH to the substrates than poultry litter and quail manure. In a comparison of physicochemical characteristics of substrates containing manure and commercial substrates, a pH ranging from 6.1 to 7.2 was found with the use of 15 to 35% manure, while the commercial substrate had a pH equal to 4.6.

Table 6. Emergence speed index (ESI), the height of the seedlings (HS), and stem diameter at 5 cm from the substrate (SD5) of rubber tree seedlings in different substrates in protected environments. Cassilândia, Mato Grosso do Sul State, Brazil, 2016.

Substrate	ESI	HS (cm)	SD5 (mm)
S1 = 50%C + 30%S + 10%M + 10%V + 0%W	0.11 c	52.55 b	4.84 b
S2 = 40%C + 30%S + 10%M + 10%V + 10%W	0.14 c	56.12 a	5.47 a
S3 = 30%C + 30%S + 10%M + 10%V + 20%W	0.06 c	55.17 a	4.95 b
S4 = 20%C + 30%S + 10%M + 10%V + 30%W	0.10 c	48.50 b	4.71 b
S5 = 10%C + 30%S + 10%M + 10%V + 40%W	0.10 c	61.98 a	5.20 b
S6 = 50%C + 30%S + 10%M + 0%V + 10%W	0.10 c	53.50 b	5.39 a
S7 = 30%C + 30%S + 10%M + 20%V + 10%W	0.17 b	56.20 a	5.22 b
S8 = 20%C + 30%S + 10%M + 30%V + 10%W	0.21 b	57.31 a	5.68 a
S9 = 10%C + 30%S + 10%M + 40%V + 10%W	0.23 a	58.14 a	5.79 a
S10 = 50%C + 30%S + 0%M + 10%V + 10%W	0.08 c	53.54 b	4.95 b
S11 = 30%C + 30%S + 20%M + 10%V + 10%W	0.18 b	57.90 a	5.24 a
S12 = 20%C + 30%S + 30%M + 10%V + 10%W	0.22 a	56.58 a	5.80 a
S13 = 10%C + 30%S + 40%M + 10%V + 10%W	0.28 a	60.96 a	5.80 a
Plant nursery	0.14 a	57.74 a	5.09 a
Greenhouse	0.15 a	54.33 b	5.53 a

Means followed by the same letter do not differ by the Scott-Knott test for the substrates and by the t-test for the environments, both at 5% probability. C = cattle manure, S = soil from ravine, M = medium vermiculite, V = superfine vermiculite, and W = washed fine sand.

A ratio of shoot dry mass:root dry mass (SRS) varying from 1 to 2 is considered an adequate ratio, with a balanced distribution (1/1), to, at most, double (2/1). It was verified that the plants presented better mass distribution when the substrates contained less cattle manure (S5, S9, S13), with the possibility of better development in the field. Larger seedlings indicate that the shoot system is much more developed than the root system, which is undesirable for transplantation because they are susceptible to tipping and because of lower efficiency in soil fixation and exploitation (Simões, Alves, Ferreira, & Araujo Neto, 2015), as verified in substrates S1 and S10.

Table 7. Shoot dry mass (SDM), root system dry mass (RSDM), total dry mass (TDM), and shoot:root system dry mass ratio (SRS) of rubber tree seedlings on different substrates in protected environments. Cassilândia, Mato Grosso do Sul State, Brazil, 2016.

Substrate	SDM (g plant^{-1})	RSDM (g plant^{-1})	TDM (g plant^{-1})	SRS
S1 = 50%C + 30%S + 10%M + 10%V + 0%W	4.89 b	1.97 c	6.86 d	3.11 a
S2 = 40%C + 30%S + 10%M + 10%V + 10%W	5.61 a	2.56 c	8.16 c	2.29 a
S3 = 30%C + 30%S + 10%M + 10%V + 20%W	4.70 b	2.19 c	6.89 d	2.41 a
S4 = 20%C + 30%S + 10%M + 10%V + 30%W	5.18 b	2.50 c	7.68 c	2.20 a
S5 = 10%C + 30%S + 10%M + 10%V + 40%W	5.38 b	3.64 b	9.02 b	1.64 b
S6 = 50%C + 30%S + 10%M + 0%V + 10%W	3.76 c	2.43 c	6.19 d	1.66 b
S7 = 30%C + 30%S + 10%M + 20%V + 10%W	5.26 b	2.55 c	7.80 c	2.22 a
S8 = 20%C + 30%S + 10%M + 30%V + 10%W	6.14 a	3.43 b	9.57 b	1.83 b
S9 = 10%C + 30%S + 10%M + 40%V + 10%W	6.33 a	4.70 a	11.03 a	1.42 b
S10 = 50%C + 30%S + 0%M + 10%V + 10%W	5.75 a	2.18 c	7.93 c	2.93 a
S11 = 30%C + 30%S + 20%M + 10%V + 10%W	5.66 a	3.00 c	8.67 b	1.92 b
S12 = 20%C + 30%S + 30%M + 10%V + 10%W	6.46 a	2.72 c	9.18 b	2.41 a
S13 = 10%C + 30%S + 40%M + 10%V + 10%W	6.40 a	4.32 a	10.72 a	1.55 b
Plant nursery	5.33 a	2.88 a	8.21 a	2.09 a
Greenhouse	5.68 a	2.99 a	8.67 a	2.16 a

Means followed by the same letter do not differ by the Scott-Knott test for the substrates and by the t-test for the environments, both at 5% probability. C = cattle manure, S = soil from ravine, M = medium vermiculite, V = superfine vermiculite, and W = washed fine sand.

In the plant nursery, there was no difference in the stem diameter for the different substrates, and in the greenhouse, the substrates S1, S2, S4, S5, and S10 presented the lowest results (Table 8). The greenhouse seedlings produced larger stem diameters than the seedlings in the plant nursery. In the present study, at 280 DAS, the seedlings had a diameter ranging from 7.02 to 8.16, higher than that observed by Vieira et al. (2016) at 150 DAS, who observed a neck diameter of 4.88 to 4.98 mm in GT1 clones, 5.00 to 5.19 mm in PR255 clones and 5.61 to 6.11 mm in RRIM600. Barreto et al. (2016), at 300 DAS, observed results of stem diameter ranging from 6.09 to 7.07 mm in seedlings submitted to treatments by fertigation, which were lower diameters than the present study.

Table 8. Stem diameter (SD) and height:stem diameter ratio (HSD) of rubber tree seedlings on different substrates in protected environments. Cassilândia, MS, 2016.

Substrate	SD (mm)		HSD	
	Greenhouse	Plant nursery	Greenhouse	Plant nursery
S1 = 50%C + 30%S + 10%M + 10%V + 0%W	6.80 Ab	6.50 Aa	7.00 Bb	8.93 Ab
S2 = 40%C + 30%S + 10%M + 10%V + 10%W	7.02 Ab	6.18 Aa	7.87 Bb	9.38 Ab
S3 = 30%C + 30%S + 10%M + 10%V + 20%W	7.72 Aa	5.80 Ba	6.62 Bb	11.80 Aa
S4 = 20%C + 30%S + 10%M + 10%V + 30%W	6.54 Bb	6.69 Aa	8.35 Aa	7.52 Ab
S5 = 10%C + 30%S + 10%M + 10%V + 40%W	7.02 Ab	6.66 Aa	9.24 Aa	9.29 Ab
S6 = 50%C + 30%S + 10%M + 0%V + 10%W	8.74 Aa	6.28 Ba	6.54 Bb	7.96 Ab
S7 = 30%C + 30%S + 10%M + 20%V + 10%W	7.80 Aa	6.92 Ba	7.47 Ab	7.76 Ab
S8 = 20%C + 30%S + 10%M + 30%V + 10%W	7.44 Aa	6.36 Ba	7.27 Bb	9.60 Ab
S9 = 10%C + 30%S + 10%M + 40%V + 10%W	7.88 Aa	7.00 Ba	6.93 Ab	8.83 Ab
S10 = 50%C + 30%S + 0%M + 10%V + 10%W	7.08 Ab	6.55 Aa	7.09 Bb	8.70 Ab
S11 = 30%C + 30%S + 20%M + 10%V + 10%W	7.34 Aa	6.84 Aa	7.62 Ab	8.84 Ab
S12 = 20%C + 30%S + 30%M + 10%V + 10%W	7.82 Aa	6.96 Ba	7.06 Ab	8.37 Ab
S13 = 10%C + 30%S + 40%M + 10%V + 10%W	8.16 Aa	7.38 Aa	7.33 Ab	8.52 Ab

Means followed by the same uppercase letters on rows and lowercase letters on columns do not differ by the Scott-Knott for substrates and by the t-test for environments, both at 5% probability. C = cattle manure, S = soil from ravine, M = medium vermiculite, V = superfine vermiculite, and W = washed fine sand.

The HSD indicates balanced growth in height and diameter of plants (Carneiro, 1995). Lower values of HSD indicate plants with robust architecture; in contrast, higher values represent seedlings with a fragile stem to support the shoot. In this way, the greenhouse generally presented the most robust seedlings with HSDs lower than the plant nursery. The substrates S4 and S5 in the greenhouse provided less robust seedlings than the other substrates in the same environment. In the plant nursery, the highest HSD was verified for substrate S3. The substrates that presented less robust seedlings had a sand percentage greater than 10%.

The HSR presented results ranging from 7.95 to 14.04 in the greenhouse, while in the plant nursery, results ranged from 9.07 to 16.12 (Table 9). In the greenhouse, the substrates that provided the highest HSRs were S5 and S6 and in the plant nursery were S1, S3, and S6. In this case, high values indicate lower quality seedlings, which can indicate stretching of the seedlings. Through the study of this index (HSR), the greenhouse could be the most suitable environment for seedlings because it presented the better distribution of phytomass in the shoots. The lower values of this index indicate a greater capacity for survival in the field, characterized by lignified stems, with higher potential for survival in the field (Gomes, Couto, Leite, Xavier, & Garcia, 2002).

The DQI is a determinant of seedling quality, and the highest values indicate high-quality seedlings (Gomes et al., 2002; Vargas & Marques 2016; Goulart, Paiva, Leite, Xavier, & Duarte 2017). The results (Table 9) show that the interaction between the greenhouse and the substrates produced higher quality seedlings when a lower proportion of manure and a high concentration of fine vermiculite was used. The DQI values found in this study ranged from 0.45 to 1.47; however, Vieira et al. (2016) found values ranging from 0.26 to 0.68 in trials with three different clones, GT1, PR255, and RRIM600.

Table 9. Height:shoot dry mass ratio (HSR) and Dickson quality index (DQI) of rubber tree seedlings on different substrates in protected environments. Cassilândia, Mato Grosso do Sul State, Brazil, 2016.

Substrate	HSR		DQI	
	Greenhouse	Plant nursery	Greenhouse	Plant nursery
S1 = 50%C + 30%S + 10%M + 10%V + 0%W	8.76 Bb	14.73 Aa	0.73 Ac	0.57 Ab
S2 = 40%C + 30%S + 10%M + 10%V + 10%W	10.78 Ab	9.69 Ab	0.73 Ac	0.79 Aa
S3 = 30%C + 30%S + 10%M + 10%V + 20%W	10.08 Bb	16.12 Aa	0.83 Ac	0.45 Bb
S4 = 20%C + 30%S +10%M + 10%V + 30%W	10.24 Ab	9.07 Ab	0.71 Ac	0.81 Aa
S5 = 10%C + 30%S +10%M + 10%V + 40%W	13.33 Aa	11.05 Ab	0.78 Ac	0.90 Aa
S6 = 50%C + 30%S + 10%M + 0%V + 10%W	14.04 Aa	14.82 Aa	0.85 Ac	0.57 Ab
S7 = 30%C + 30%S + 10%M + 20%V + 10%W	9.98 Ab	11.68 Ab	0.91 Ac	0.71 Ab
S8 = 20%C + 30%S + 10%M + 30%V + 10%W	9.99Ab	9.51 Ab	1.05 Ac	0.85 Aa
S9 = 10%C + 30%S + 10%M + 40%V + 10%W	7.95 Ab	11.19 Ab	1.47 Aa	0.98 Ba
S10 = 50%C + 30%S + 0%M + 10%V + 10%W	9.13 Ab	11.41 Ab	0.85 Ac	0.66 Ab
S11 = 30%C + 30%S + 20%M + 10%V + 10%W	9.36 Ab	11.69 Ab	0.93 Ac	0.80 Aa
S12 = 20%C + 30%S + 30%M + 10%V + 10%W	8.11 Ab	9.79 Ab	1.03 Ac	0.80 Aa
S13 = 10%C + 30%S + 40%M + 10%V + 10%W	9.48 Ab	10.21 Ab	1.22 Ab	1.09 Aa

Means followed by the same uppercase letters on rows and lowercase letters on columns do not differ by the Scott-Knott for substrates and by the t-test for environments, both at 5% probability. C = cattle manure, S = soil from ravine, M = medium vermiculite, V = superfine vermiculite, and W = washed fine sand.

Considering the results presented, in the growth variables and the ratio of masses that did not present interaction, it is possible to observe the influence of environment and substrate on the formation of rootstocks. The environments did not differ for most of the studied variables, except for height, where larger plants were verified in the plant nursery. For the substrates, it was verified that those with a low percentage of cattle manure and sand and more vermiculite (S9 and S13) had a positive influence on the growth of the seedlings.

Biometric indices (except SRS), resulting from the interaction between the environmental factors and substrates, allowed us to note that the best seedlings were formed in the agricultural greenhouse. In such interactions, substrates with a low amount of manure and sand and a high amount of vermiculite (S9 and S13) provided the best seedlings. These results show that in addition to the growth variables, it is necessary to evaluate the biometric indices to verify the formation of a quality seedling.

Regarding the cultivation environment, the rubber tree seedlings showed greater adaptation to the conditions provided by the greenhouse, mainly due to the availability of the global radiation (Table 4) and, consequently, the greater availability of photosynthetically active radiation. Photosynthetically active radiation is approximately 50% of global radiation (Assis & Mendez, 1989). The rubber tree, a plant with heliophyte characteristics under conditions of greater luminosity, promotes the conversion of solar radiation to carbohydrates in a more efficient way; therefore, there was greater assimilation of organic matter and reserves in the plants grown in the greenhouse.

A high content of cattle manure in the substrates (above 30%), even though it provided a high amount of nutrients and organic matter, was detrimental to the seedlings due to the high amounts of sodium chloride present (Table 2). The sodium chloride hindered the diffusion of water to the plant because of the salinity of the substrate. On the other hand, a high amount of vermiculite in the substrate was beneficial to vegetal growth because, according to Monis (1975), this material has a high cation exchange capacity (CEC), can absorb of a large volume of water, and has a large retention capacity of air and nutrients transferable to the vegetable. Thus, the addition of vermiculite to the substrate benefitted the growth due to the increase of the CTC, reducing the leaching of nutrients and favoring the absorption of the nutrients by the vegetable.

Conclusion

Substrates with a low percentages of manure are more suitable to produce rubber tree seedlings, indicated by S9 (10% E + 30% S + 10% M + 40% F + 10% A) and S13 (10% E + 30% S + 40% M + 10% F + 10% A), with a low amount of manure and sand and a high amount of vermiculite. The greenhouse, an environment with higher radiation availability, was adequate for the growth of high-quality rubber tree seedlings.

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