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Air layering in *Caryocar brasiliense*: effect of substrate

Alporquia em Pequizeiro: efeito do substrato

Acodo aéreo en *Caryocar brasiliense*: efecto del sustrato

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ABSTRACT
The commercial propagation of *Caryocar brasiliense* Cambess is hindered by several factors, including seed dormancy and low cutting survival rates. Air layering studies are needed to overcome these limitations. Previous studies have reported the influence of substrates on the rooting of air layers for different species. Therefore, the objective of this study was to evaluate the influence of substrates on air layering of *Caryocar Brasiliense*. An experiment was conducted with eight stock plants located at Embrapa Cerrados, Distrito Federal, Brazil, using different substrates, including Bioplant®, coconut fiber, a mixture of Bioplant® and coconut fiber in 3:1, 1:1, 1:3 proportions, rice husk biochar, vermiculite, sawdust, soil, and sand. Each treatment was replicated four times. After five months, the survival rate, callus formation, rooting percentage, root length, fresh and dry root mass, and rooting vigor were evaluated. The survival and callus formation rates in all substrates were above 95%. Bioplant® substrate provided the highest rooting percentage (77%), fresh and dry root mass (17.08g and 3.16g, respectively), while soil provided the lowest rooting percentage (17%) and fresh and dry root mass (0.1g and 0.02g, respectively). The average root length was 6.63 cm in the 1:1 mixture. Based on the evaluated characteristics, Bioplant® is the most suitable substrate for the propagation of *C. brasiliense* stock plants by air layering. The results suggest that air layering is a viable alternative to the vegetative propagation of *Caryocar brasiliense* stock plants.

Keywords: rooting, callus formation, plant propagation, Pearson’s correlation, rooting vigor.

RESUMO
A propagação comercial do pequizeiro é limitada pela longevidade e dormência das sementes e pela baixa taxa de enraizamento de estacas. A fim de superar essas limitações, a propagação vegetativa por alporquia se apresenta como uma alternativa viável. Nesse sentido, este estudo objetivou avaliar a influência de diferentes substratos na alporquia de pequizeiro. Para tal, foi conduzido um experimento em blocos casualizados com oito matrizes localizadas na Embrapa Cerrados, utilizando-se os seguintes substratos: Bioplant®, fibra de coco, misturas de Bioplant® e fibra de coco nas proporções 3:1, 1:1 e 1:3, casca de arroz carbonizada, vermiculita, serragem, subsolo e areia. Cada tratamento foi avaliado em quatro repetições de 10 alporques cada. Após cinco meses, foram realizadas as seguintes avaliações: porcentagens de sobrevivência, de calejamento e de enraizamento, comprimento da maior raiz, massa fresca e seca de raízes e vigor de enraizamento. A sobrevivência e o calejamento em todos os substratos avaliados foram superiores a 95%. O substrato Bioplant® promoveu o maior enraizamento (77%), massa fresca e seca de raízes (17,08g e 3,16g, respectivamente), enquanto o subsolo apresentou o menor

enraizamento (17%) e massa fresca e seca de raízes (0,1g e 0,02g, respectivamente). O comprimento da maior raiz foi de 6,63cm na mistura de Bioplant® e fibra de coco (1:1). Considerando as características avaliadas, o substrato Bioplant puro se mostrou o mais adequado para a propagação de pequizeiro por alporquia. Os resultados indicam que a alporquia com substrato adequado consiste em alternativa viável para a propagação vegetativa do pequizeiro.

Palavras-chave: cerrado, *Caryocar brasiliense* Cambess, clonagem, enraizamento, calejamento.

RESUMEN

La propagación comercial del árboles de *Caryocar brasiliense* Cambess está limitada por la longevidad y la dormancia de las semillas, así como por la baja tasa de enraizamiento de estacas. Con el fin de superar estas limitaciones, la propagación vegetativa por acodo aéreo se presenta como una alternativa viable. En este sentido, este estudio tuvo como objetivo evaluar la influencia de diferentes sustratos en la alporquia de *C. brasiliense*. Para ello, se llevó a cabo un experimento en bloques al azar con ocho matrices ubicadas en la Embrapa Cerrados, Distrito Federal, Brasil, utilizando los siguientes sustratos: Bioplant®, fibra de coco, mezclas de Bioplant® y fibra de coco en proporciones de 3:1, 1:1 y 1:3, cáscara de arroz carbonizada, vermiculita, aserrín, suelo y arena. Cada tratamiento fue evaluado en cuatro repeticiones de 10 acodos aéreos. Después de cinco meses, se realizaron las siguientes evaluaciones: porcentajes de supervivencia, callosidad y enraizamiento, longitud de la raíz, masa fresca y seca de raíces y vigor de enraizamiento. La supervivencia y el callosidad en todos los sustratos evaluados fueron superiores al 95%. El sustrato Bioplant® promovió el mayor enraizamiento (77%), masa fresca y seca de raíces (17,08g y 3,16g, respectivamente), mientras que el suelo presentó el menor enraizamiento (17%) y masa fresca y seca de raíces (0,1g y 0,02g, respectivamente). La longitud de la raíz fue de 6,63cm en la mezcla de Bioplant® y fibra de coco (1:1). Considerando las características evaluadas, el sustrato Bioplant puro se mostró el más adecuado para la propagación de *Caryocar brasiliense* Cambess por acodos aéreos. Los resultados indican que la alporquia con sustrato adecuado es una alternativa viable para la propagación vegetativa del *C. brasiliense* Cambess.

Palabras clave: cerrado, *Caryocar brasiliense* Cambess, clonación, enraizamiento, callo, acodos aéreos.

1 INTRODUCTION

Seeds are the main propagation method used for *Caryocar brasiliense* Cambess., commonly known as "pequizeiro," despite their limitations, due to dormancy, which results in low and slow germination (Nasory & Cunha 2012) and low longevity. Furthermore, plants obtained from seeds are genotypic and phenotypic different. Therefore, other propagation methods are necessary in this species to enable the commercial production of seedlings (Dutra *et al*. 2012).

The advantages of vegetative propagation include the maintenance of the genetic identity of the parental plant (clonal propagation), the uniformity in seedling development and the faster plant development (Stuepp *et al*. 2018). Therefore, cloning methods have been studied in *C. brasiliense*, especially through cutting and grafting, but with still low success rates (Leite *et al*. 2007; Santos *et al*. 2013). The technique of air layering, although more labor-intensive, can be often successful in propagating plant species with difficult rooting via cuttings (Hartmann & Kester 2014). Air layering is an efficient method for obtaining seedlings of some perennial species, such as peach (Castro & Silveira 2003), lychee (Lins *et al*. 2015) and jabuticaba (Cassol *et al*. 2015).

Various factors can affect the success rate of air layering, including stem diameter (Carmona *et al*., 2022), growth regulators and substrate. The substrate that surrounds the ringed region is particularly important because it provides the initial environment for root development and should have appropriate physical and chemical characteristics for rooting. Several studies have observed significant differences in the survival, callus formation and rooting of air layers of various species using different substrates (Dutra *et al*. 2012; Daneluz *et al*. 2009).

Dutra *et al*. (2012) evaluated the effect of three substrates (Bioplant ®, vermiculite, and sugarcane bagasse) and concluded that vermiculite provided the greatest number of roots of umbuzeiro. Similarly, Daneluz *et al*. (2009) observed significant differences in the rooting rate and number of roots using different substrates in air layering of fig tree.

Yakubu *et al*. (2019) observed a greater stem diameter, length, and number of leaves in *Dennettia tripetala* seedlings produced with air layering soil substrate compared to sawdust. Mishra (2014) reported an increase in rooting rate from 57% to 80% in air layered *Citrus aurantifolia* using soil and a sawdust:soil mix (1:1) as substrate, respectively. This author attributed the higher effectiveness of the sawdust:soil substrate to its higher aeration and water retention capacity.

Eganathan *et al*. (2000) achieved 45% rooting in air layering of *Heritiera fomes* using sphagnum substrate. Aliyu (2007) compared the effect of soil, sand and sawdust substrates mixed in different ratios on air layering of cashew trees and concluded that the mixture of soil with sand or sawdust provided the best results, considering the period for root initiation, the average number of roots and the length of the longest root. Substrate mixtures provided the best conditions for air layering rooting of this species.

The objectives of this study were: a) to evaluate the effect of substrates on the survival, callus formation, rooting percentage, vigor (visual scale), length of the longest root and fresh and dry root mass of air layers of *C. Brasiliense*; b) to validate a visual rooting vigor scale for *C. Brasiliense* air layers.

2 MATHERIAL AND METHODS

This study was carried out on eight adult *C. brasiliense* stock plants located at Embrapa Cerrados, in the Distrito Federal, Brazil (15°35'33.7"S 47°44'00.5"W). The region's climate is classified as Aw, according to the Köppen classification (Cardoso *et al*., 2014), with an average annual precipitation of 1,500 mm.

The stock plants were approximately twenty years old and showed similar sizes. Air layers were assembled on healthy, lignified stems with diameter between 20 and 30 mm (Carmona *et al*., 2022; Oliveira Júnior *et al*., 2024), on October 19th and 20th, 2020. The substrates (treatments) were placed in plastic containers with dimensions of 10x20 cm, with approximately 300 mL of substrate capacity.

The experiment was carried out in a randomized block design, with 10 substrates (treatments): Cerrado soil, medium-textured sand, commercial substrate Bioplant® Gold Class F, coconut fiber, a mixture of Bioplant® and coconut fiber at the ratios of 3:1, 1:1, and 1:3, carbonized rice husk, vermiculite, and medium sawdust, which was a blend of fine and coarse sawdust in a 1:1 proportion with four replications. Each plot comprised 10 air layers, totaling 400 air layers in the experiment. In order to minimize damage to the stock plants, five air layers of each treatment were assembled on each plant, totaling 50 air layers per plant.

Air layering was undertaken on the lower portion of the matrices and at the distal part of the stems. The stems were ringed with a 3 cm width plier and a lengthwise cut plastic bag containing substrate was wrapped around the ringed region and attached with transparent adhesive tape, to fix it and to prevent desiccation (Carmona *et al*., 2022; Oliveira Júnior *et al*., 2024). Air layers were labeled according to the treatment and flowers and fruits were removed from the layered stems, to avoid migration of photoassimilates to these structures.

After five months, evaluations took place. A air layer was considered "calloused" when at least one callus was formed in the girdling region and "rooted" with at least one root longer than 1 cm. The length of the longest root was measured using a ruler. To determine the fresh roots mass, they were first removed from the air layer and immediately weighed. Dry root mass was also determined, after root desiccation at 80°C for 3 days followed by a two hours period in desiccator (Maurya *et al*., 2013).

Visual rooting vigor was also estimated, using a rating scale comprising the following categories: 0 - Presence of calluses, but no root formation; 1 – Less than 20% of the calluses emitted roots; 2 – Between 21 and 40% of the calluses emitted roots; 3 – Between 41 and 60% of the calluses emitted roots; 4 – Between 61 and 80% of the calluses emitted roots; 5 – More than 80% of the calluses emitted roots (Carmona *et al*., 2022).

Before performing any statistical analysis, the data were checked for normality using the Shapiro-Wilk test, and for homogeneity of variances using the Levene test. If these assumptions were not met, the data were transformed using the formula ($\sqrt{x+1}$). The data was subjected to analysis of variance and in case of significance, to the Scott-Knott grouping test ($p \leq 0.05$). The rooting vigor were evaluated using regression analysis. The strength of the linear correlation coefficients was classified as very strong (r from ± 0.91 to ± 1.00), strong (r from ± 0.71 to ± 0.90), medium (r from ± 0.51 to ± 0.70), or weak (r from ± 0.31 to ± 0.50), according to the Carvalho *et al*. (2004) classification. The statistical analyses were performed using the "R" software (R Core Team, 2022).

3 RESULTS AND DISCUSSION

Significant differences were observed between the substrates concerning rooting, longest root length, rooting vigor, root fresh mass and root dry mass (Table 1). After five months of the air layering process, the mean percentage of survival, callus formation and rooting were 98%, 99% and 45%, respectively, with an average root length of 4.49 cm (Table 1). Inferior results were observed using sphagnum as substrate for air layering in this species by Leite *et al*. (2007), who reported 62% of survival, 79% of callus formation and 8% of rooting and an average main root length of 0.28 cm, after three months. The differences in these studies may have resulted from plant genotype, environmental conditions, substrate and the duration of the evaluation period. No significant differences, however, were observed between substrates regarding survival and callousing (Table 1).

Pure Bioplant® substrate and its mixtures with coconut fiber resulted in the highest rooting rates. Rooting percentage of 77 was achieved with pure Bioplant®, which was considerably higher than the rooting rate observed in other studies using different substrates with this species (16%) (Leite *et al.*, 2007), with umbuzeiro (20%) (Dutra *et al.*, 2012) and with tamarindo (30%) (Da Silva *et al.*, 2017).

Table 1: Summary of analysis of variance (ANOVA) for the substrates effect on survival (Su), callousing (Ca), rooting (Ro), longest root length (Lr), rooting vigor (Vi), root fresh mass (FM) and root dry mass (DM) of *C. brasiliense* air layering. Brasília-DF, 2020-2021. **= significant ($p \leq 0.01$); ns= not significant.[1] transformed data ($\sqrt{x+1}$).

	df	Su	Ca	Ro	Lr	Vi	FM[1]	DM[1]
Substrates	9	16,94^{ns}	4,44^{ns}	1553,89**	0,44**	2,78**	7,210**	1,197**
Blocs	3	49,17^{ns}	3,33^{ns}	870,83^{ns}	0,45^{ns}	1,34^{ns}	0,094^{ns}	0,025^{ns}
Residue	27	17,69	5,19	168,52	0,28	0,40	0,470	0,101
Mean		98,25	99,50	45,25	4,49	2,40	6,17	0,99
CV (%)		4,28	2,29	28,69	13,76	26,26	22,31	15,36

Source: The authors

The substrates soil, sand, coconut fiber, carbonized rice husks, vermiculite and sawdust entailed lower rooting percentages, ranging from 17% to 37% (Table 2). A possible explanation for this phenomenon could be the lower nutrient availability in these substrates compared to Bioplant® (Carlos *et al.*, 2014). However, sawdust has been reported to provide relatively high rooting percentages in air layering of some species, such as cashew (60%) (Aliyu, 2007) and citrus (57%) (Mishra, 2014).

Sphagnum is still a widely used substrate for air layering process, providing high rooting percentages in some species, such as tamarind (60%) (Da Silva *et al.*, 2017) and lychee (95%) (Lins *et al.*, 2015). Coconut fiber provided (40%) and (75%) rooting in tamarind and lychee, respectively. The superior rooting performance of sphagnum may be due to its higher capacity for water retention and aeration compared to coconut fiber (Lins *et al.*, 2015). However, sphagnum is not easily available and it is relatively more expensive compared to the other substrates evaluated in this study.

Table 2: Effect of substrates on survival (Su), callousing (Ca), rooting (Ro), length of the longest root (Lr), rooting vigor (Vi), fresh root mass (Fm) and dry root mass (Dm) of *C. brasiliense* air layering. Brasília - DF, 2020-2021. Means followed by the same letter in each column do not differ according to the Scott-Knott test ($p \leq 0.05$).

Substrate	Su (%)	Ca (%)	Ro (%)	Vig	Lr (cm)	Fm (cm)	Dm (g)
Soil	95,0a	100a	17,5b	1,06b	2,31b	0,10c	0,020c
Sand	97,5a	100a	27,5b	1,52b	3,58b	0,80c	0,119c
Fiber (F)	97,5ª	97,5a	37,5b	1,84b	3,11b	3,08c	0,362c
Bioplant® (B)	100a	100a	77,5a	3,49a	6,35a	17,08a	3,163a
B:F (3:1)	100a	100a	66,5a	3,10a	5,82a	13,54a	2,127b
B:F (1:1)	100a	100a	62,5a	3,58a	6,63a	13,22a	1,939b
B:F (1:3)	95,0a	100a	60,0a	2,78a	5,40a	7,30b	0,847c
Rice husk	100a	100a	32,5b	2,38b	4,39b	1,64c	0,276c
Vermiculite	100a	100a	37,5b	2,23b	3,60b	3,84c	0,875c
Sawdust	97,5a	97,5a	33,5b	2,02b	3,75b	1,12c	0,186c
Average	98,5	99,50	45,5	2,40	4,49	6,17	0,993

Source: The authors

Bioplant® and its mixtures with coconut fiber also outperformed the other substrates concerning the longest root length (Table 2). Root lengths superior to 5.0 cm were observed with the most suitable substrates in the present study. Inferior main root lengths were reported in other air layering studies with *C. Brasiliense*, of 0.8 cm (Leite *et al*., 2007); with jabuticabeira, of 0.85 cm in Plantmax® (Cassol *et al*., 2015); and with umbuzeiro, of 2.05 cm in vermiculite and 4.83 cm in Bioplant® (Dutra *et al*., 2012). Dutra *et al*. (2012) emphasized that Bioplant® has more suitable physical and chemical characteristics than vermiculite, including higher organic matter content and greater total porosity, which are crucial for substrate aeration. Furthermore, these characteristics lead to less physical resistance for root growth. Therefore, Bioplant® can be considered a superior substrate for *C. brasiliense* layering compared to vermiculite.

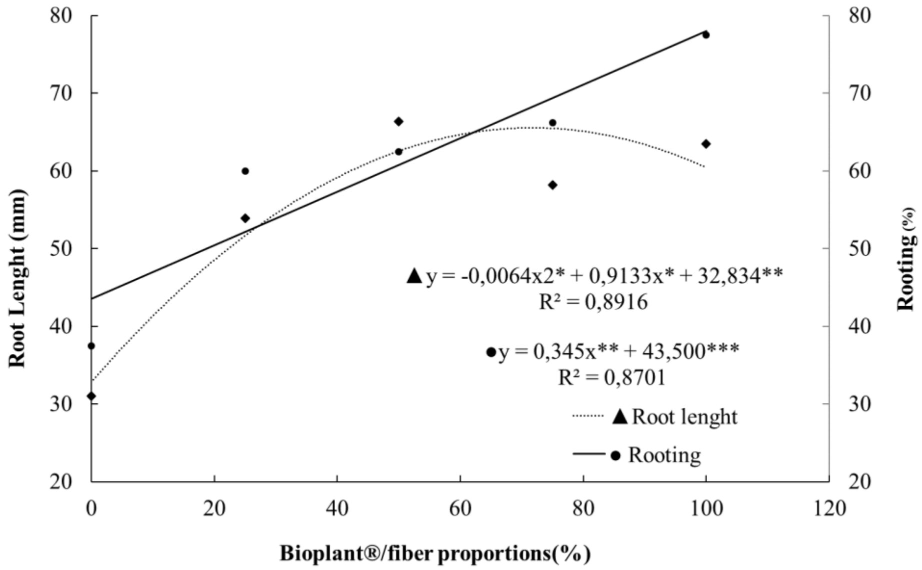
However, DaSilva *et al*. (2017) reported longer air layers roots with coconut fiber substrate (2.38 cm) compared to the commercial substrate Basaplant® (1.05 cm) for tamarind tree. These authors attributed this difference to the high moisture retention capacity of coconut fiber, despite its lower nutrient and organic matter content. On the other hand, water in excess can also be detrimental, as it can lead roots to rot. Therefore, it is important to maintain an appropriate balance of moisture in the substrate for optimal root growth.

Root fresh mass varied considerably among substrates (Table 2), from less than 1 g of roots per air layering with sand and soil, which did not differ significantly from the results obtained with coconut fiber (F), rice husk, vermiculite and sawdust, to more than 13 g with Bioplant® (B), B:F (3:1) and B:F (1:1). Concerning root dry mass, the highest value was observed using Pure Bioplant®.

Bioplant® and vermiculite did not result in significant differences in fresh and dry root mass in air layers of umbuzeiro (Dutra *et al*., 2012). Sphagnum was superior to coconut fiber in these characteristics in lychee (Lins *et al*., 2015) and Basaplant® was superior to sphagnum and to coconut fiber in tamarind tree air layering (Da Silva *et al*., 2017). A substrate composed of soil, sand and cow manure provided greater fresh and dry root mass than soil alone, probably due to the better aeration, nutrition, and moisture retention provided by the compost (Mishra, 2014).

Although the rooting percentage did not differ significantly between the Bioplant® substrate and its mixtures (Table 2) in the Scott-Knott test ($p \leq 0.05$), a significant linear increasing was observed between the amount of Bioplant® in the mixture and the rooting percentage (Figure 1). The dry and fresh root mass also increased linearly with the incremental of the Bioplant® proportion in the mixture (Figure 2).

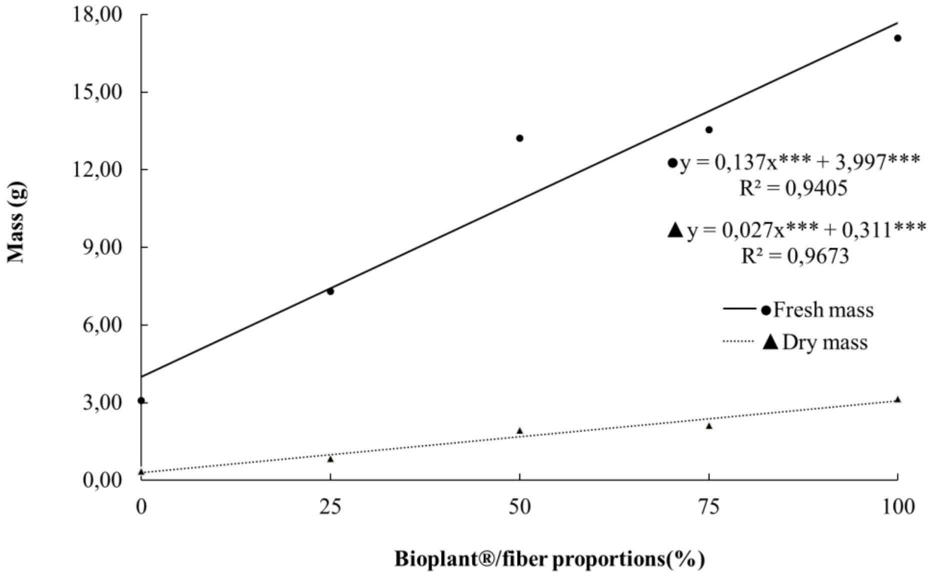
Figure 1: Rooting and root length of *C. brasiliense* air layers as affected by Bioplant®/fiber proportions. Brasília - DF, 2021. Where: ***, ** and *= significant at $p \leq 0.001$, $p \leq 0.01$ and $p \leq 0.05$, respectively. Brasília - DF, 2020-2021.



Source: The authors

A quadratic increase in the root length as the proportion of Bioplant® increased in the mixture, with the maximum at 1:1 Bioplant® and coconut fiber ratio, was observed (Figure 1). Rooting vigor, evaluated visually (Figure 3), also showed a quadratic incremental with the increase of Bioplant® in the mixture and the maximum vigor was observed at the 1:1 ratio. This parameter indicates mainly the number of roots per air layering, which affects plant survival and development.

Figure 2: Fresh and dry roots mass of *C. brasiliense* air layering as affected by Bioplant®/fiber proportions. Brasília – DF, 2021. Where: ***= significant (p ≤ 0.001). Brasília - DF, 2020-2021.



Source: The authors

Very strong correlations were observed between rooting and the other evaluated parameters, except survival and callousing (Table 3), although rooting was only observed in *C. brasiliense* air layers that initially produced callus. Consistently with these results, no significant correlation between callusing and rooting was also observed in *Persoonia virgata* cuttings (Bauer *et al*., 1999) and in small peach palm cuttings (De Deus *et al*., 2021). Negative correlations have even been reported between callousing and the emission of adventitious roots in air layering of some tropical species (Singh and Ansari, 2014). Likewise, positive and significant correlations occurred between rooting and number of roots, survival and fresh root mass in air layering of *Conocarpus erectus* L (Abdul-Hafeez, 2020). Ibrahim (2020) observed positive correlation between rooting percentage and fresh root mass in air layered *Dracaena marginata* Lam., as well as the presence of carbohydrates in the tissues, suggesting that these compounds contribute to root formation in the ringed region.

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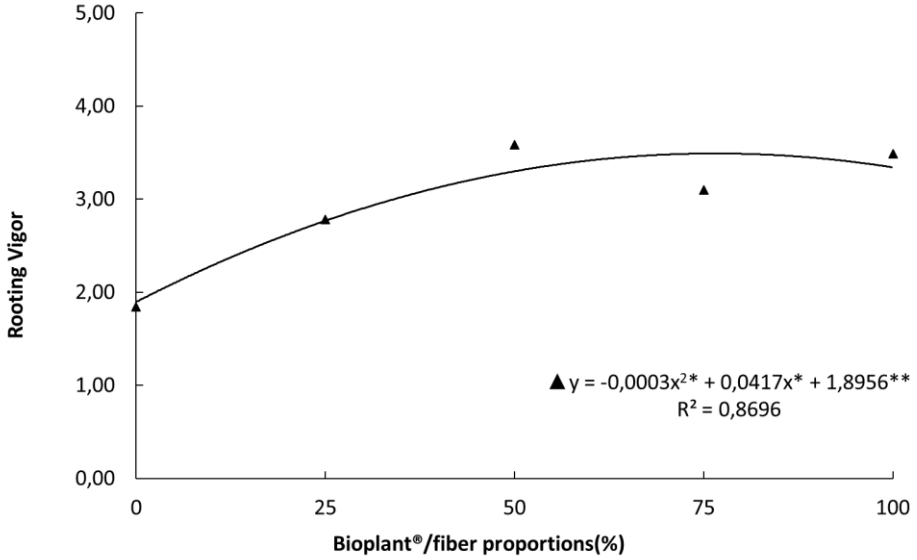
Figure 3: Rooting vigor (visual scale) of *C. brasiliense* air layering as affected by Bioplant®/fiber proportions. Brasília – DF, 2021. Where: ** and *= significant at $p \leq 0.01$ and $p \leq 0.05$, respectively. Brasília - DF, 2020-2021.



Source: The authors

Root length correlated very strongly with vigor and root fresh mass and strongly with root dry mass (Table 3). A strong positive correlation was observed between the root length and root dry mass for lychee (Lins, 2013). On the other hand, a significant but negative correlation was observed between root length, root dry mass and root fresh mass in air layers of *Conocarpus erectus* L. (Abdul-Hafeez, 2020).

Table 3: Pearson's linear correlation between the following variables evaluated in air layering of *C. brasiliense*: survival (Su), callus formation (Ca), rooting (Ro), root length (Rl), rooting vigor (Vig), fresh root mass (Fm) and dry root mass (Dm). Brasília - DF, 2020-2021. **= significant ($p \leq 0.01$). Brasília - DF, 2020-2021.

	Su	Ca	Ro	Rl	Vi	Fm	Dm
Su	1	0,1921	0,4314	0,5525	0,6043	0,5200	0,5738
Ca	-	1	0,2574	0,3346	0,2959	0,2849	0,3543
Ro	-	-	1	0,9199**	0,9382**	0,9869**	0,9446**
Rl	-	-	-	1	0,9765**	0,9183**	0,8513**
Vi	-	-	-	-	1	0,9492**	0,9184**
Fm	-	-	-	-	-	1	0,9654**
Dm	-	-	-	-	-	-	1

Source: The authors

Visual rooting vigor correlated very strongly rooting percentage, length of the longest root, root fresh and dry mass (Table 3). The evaluation of this rooting vigor parameter is easy and fast and it is non-destructive, which allows the use of the air layers for transplanting purposes.

These results indicate that this vigor scale can replace, with advantages, the other evaluated rooting parameters for *Caryocar brasiliense* air layering studies.

These results suggest the viability of the air layering process for *Caryocar brasiliense* propagation, but the efficacy of this method highly depends on the selection of an adequate substrate. They also suggest that the visual rooting vigor scale used in the present study can be recommended for future air layering rooting studies for *C. Brasiliense*, with many advantages compared to other rooting vigor evaluation methods.

4 CONCLUSIONS

The substrate used for air layering process affects rooting percentage and rooting vigor in *C. brasiliense*. Considering the substrates soil, sand, coconut fiber, Bioplant® alone and mixed in different proportions with coconut fiber, rice husk, vermiculite and sawdust, pure Bioplant® generates the highest rooting vigor and percentage in air layers of *C. brasiliense*. A visual rooting vigor scale is recommended for air layering rooting studies.

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