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VEGETATIVE PROPAGATION FROM SHOOT CUTTINGS OF MATURE ROSEWOOD TREES (*ANIBA ROSAEODORA* DUCKE)

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Resumo

Propagação vegetativa por estaquia de cepas de árvores adultas de pau-rosa (Aniba rosaeodora Ducke*).* Uma alternativa para conciliar exploração e conservação do pau-rosa (*Aniba rosaeodora* Ducke) é a produção de mudas por estaquia. Este trabalho teve como objetivo avaliar o enraizamento de estacas das brotações epicórmicas de cepas de pau-rosa adulto, testando substratos, concentrações de auxina e tipos de estaca, assim como descrever o processo anatômico da formação das raízes adventícias. Dois ensaios foram realizados: E1 - teste de substrato (areia [A], vermiculita [V] e A+V) e concentrações de ácido indol butírico (IBA) via talco (0,0; 1,5; 3,0; 6,0 g kg^{-1}); E2 – avaliação do tipo de estaca: apical, intermediária, basal e rebroto; além de análises anatômicas em amostras de estacas enraizadas. Nos ensaios foram avaliadas as variáveis sobrevivência (%), enraizamento (%), número de raízes e comprimento da maior raiz (cm). Em E2 acrescenta-se brotações (%), número de brotos, comprimento do maior broto (cm) e calosidade (%). O enraizamento em 3,0 g kg^{-1} IBA foi melhor que o controle e a vermiculita como substrato foi superior à areia. Mesmo sem interação, a auxina tendeu a um melhor enraizamento quando utilizado com um dos substratos com vermiculita. Dentre os tipos de estaca, o rebroto foi superior para a maioria das variáveis, diferindo das demais quanto à brotação e calosidade, enquanto o basal foi inferior na concentração de carboidratos totais e amido. A origem da raiz adventícia foi evidenciada no parênquima acima do xilema, mas existe um anel de esclerênquima após o floema que pode constituir uma barreira anatômica à indução radicial.
Palavras-chave: Substratos, ácido indol butírico, tipos de estaca, rizogênese, carboidratos.

Abstract

The production of seedlings by cuttings is an alternative to reconcile exploration and conservation of Rosewood (*Aniba rosaeodora* Ducke). In this study, our objective was to evaluate the rooting of cuttings from epicormic shoots of adult rosewood strains, through substrate tests, auxin concentrations, and cutting types, as well as to describe the anatomical process of the formation of adventitious roots. We carried out two experiments: E1 - substrate test (sand [A], vermiculite [V], and A+V) and concentrations of indole butyric acid (IBA) via talc (0.0; 1.5; 3.0; 6 .0 g kg^{-1}); E2 – evaluation of the type of cutting: apical, intermediate, basal and regrowth; in addition to anatomical analyzes on samples of rooted cuttings. In the experiments, the variables survival (%), rooting (%), number of roots, and length of the largest root (cm) were evaluated. In E2, shoots (%), number of shoots, length of the largest shoot (cm), and callosity (%) were added. The rooting at 3.0 g kg^{-1} IBA was better than the control and vermiculite as a substrate was superior to sand. Even without interaction, auxin showed a tendency for better rooting when used with one of the vermiculite substrates. Among the types of cuttings, the regrowth was superior for most variables, differing from the others in terms of sprouting and callosity, while the basal was inferior in the concentration of total carbohydrates and starch.
Keywords: Plant growing media; indolebutyric acid; types of cutting; rhyzogenesis; carbohydrates.

INTRODUCTION

Rosewood (*Aniba rosaeodora* Ducke, Lauraceae), native to the Amazon Rainforest, has been explored since the beginning of the 20th century for use in the perfumery industry (LARA *et al.*, 2021). Predatory extraction, due to its high commercial value, has led the species to the danger of extinction, with populations still in decline today (IUCN, 2021). The decrease in timber resources and the increase in the price of oil stimulate the production chain, including the trade of seeds and seedlings, however, obtaining and germinating seeds are challenges that make the production of seminal seedlings difficult and expensive (LARA *et al.*, 2021), consequently, the implementation of commercial plantations.

Currently, sustainability in production is sought by replacing shallow cutting with aerial part management, which favors multiple shoots (KRAINOVIC *et al.*, 2017). This makes it possible to produce seedlings through cuttings, since the induction of shoots can promote tissue rejuvenation and enhance the rooting rate (Wendling *et al.*, 2014). Vegetative propagation by cuttings is a cloning method that aims to maximize the quality and uniformity of seedlings. This method is related to the vigor of cells to form roots, influenced by the increasing juvenile gradient towards the basal region of the plant, which can determine the best position for

collecting propagules (HARTMANN *et al*. 2011). Sampaio *et al.* (1989) emphasized that the utilization of rejuvenated Rosewood material is intricately linked to the physiological aspects of the plant and the environmental conditions provided to the cuttings during rhizogenesis. The same authors, supported by Menezes *et al.* (2018), reiterated that preserving juvenile propagules for cuttings in the species entails the cutting of adult trees to induce juvenile shoots, along with ongoing pruning and thinning. They further noted the positive effect of auxin on root quality, despite no significant increase in rooting rate, whereas Menezes *et al.* (2018) achieved promising outcomes by employing more porous substrates with enhanced water retention.

Significant advancements are yet required in the application of this technique for Rosewood seedling production, spanning both experimental and commercial levels. In this context, aiming to contribute with subsidies for the definition of rosewood cutting protocols, our objective was to evaluate the rooting of cuttings from epicormic shoots in different substrates, auxin concentrations, and types of cuttings, as well as to understand the anatomical process of formation of adventitious roots.

MATERIALS AND METHODS

Matrix and cutting management

We carried out two cutting experiments (E1 and E2) with propagules collected from an 11-year-old commercial rosewood plantation, consisting of 334 trees on 0.4 ha, from the company Magaldi Agrocomercial e Industrial Ltda, municipality of Maués (AM), Brazil. We employed epicormic shoots that emerged following selective thinning in March 2014 and February 2015, coupled with fertilization using a potassium chloride and ammonium sulfate mixture in a 1:2 ratio (30 g per strain), administered three months after the final intervention. We collected two to four shoots per strain, from which cuttings were made with an average length of 11±1 cm and a pair of leaves reduced to 25% of the leaf area. The cuttings underwent disinfection in commercial sodium hypochlorite (2.5% active chlorine) at a concentration of 0.5% for 10 minutes, followed by rinsing in running water. Subsequently, they were subjected to the treatments outlined for each test described below.

We initiated E1 in June 2015 with epicormic shoots collected four months after the second thinning. Following collection, conducted in the early morning hours, we transported the shoots in water-filled drums to the company's nursery facilities, situated near the plantations. There, we prepared 480 cuttings, with an average diameter of 5.0 ± 1.5 mm. The cuttings were treated with indole butyric acid (IBA) in talc at concentrations of 0.0; 1.5; 3.0 and 6.0 g kg^{-1} and planted in 180 cm^3 polypropylene tubes, buried up to 1/3 of the length in three different substrates: washed sand (A), medium-grained vermiculite (V) and A +V (1:1, v:v). The experiment was designed in randomized blocks in a 3x4 factorial scheme (substrates and IBA concentrations), four replications, 12 treatments, and 10 cuttings per plot.

In November 2015, nine months after the second thinning, we initiated E2 by collecting epicormic shoots based on their location on the stump, categorized as follows: type 1, comprising younger shoots with wider spacing between leaves and some leaf loss; and type 2, consisting of branches from older, more woody shoots, with closely packed intact leaves. The shoots were gathered during the early morning hours, carefully packed in Styrofoam boxes lined with damp newspaper, and transported by river to the Experimental Field of Embrapa Amazônia Ocidental in Manaus (AM). Subsequently, they were stored in water within the greenhouse equipped with misting systems. After 24 hours, the cuttings were prepared, with the treatments defined according to positions in the type 2 shoot branch – apical, intermediate and basal (Fig. 1A) – resulting in cuttings with an average diameter of 5.0 ± 1.7 cm, and from type 1 shoots, "regrowth" treatment, formed by cuttings with an average diameter of 5.3 ± 1.9 cm without differentiating the position in the shoot (Fig. 1B). After disinfestation, the cuttings were planted in 180 cm^3 polypropylene tubes containing sand and vermiculite of medium particle size (1:1, v:v).

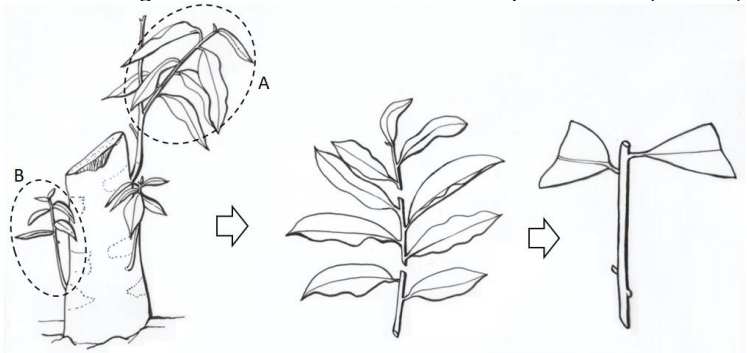

Figure 1. Propagules of epicormic shoots of rosewood (E2) with treatments according to the type of cutting: A) cuttings from the apical, intermediate, and basal positions obtained from the branching of the shoot; B) cuttings obtained directly from the sprout (regrowth type). Illustration by Josiene Rossini.

Figura 1. Propágulos das brotações epicórmicas do pau-rosa (E2) com os tratamentos em função do tipo de estaca: A) estacas das posições apical, intermediária e basal obtidas da ramificação do broto; B) estacas confeccionadas direto das brotações epicórmicas (rebroto). Ilustração por Josiene Rossini.

Our experimental design was completely randomized, with four treatments (apical, intermediate, basal, and regrowth) and four replications, with each replication or plot consisting of 12 cuttings in the regrowth treatment and 20 cuttings in the other treatments.

We used intermediate segments of branches from 15-month-old epicormic shoots for the anatomical evaluation of rooted cuttings. In the company's nursery, the cuttings were disinfested and treated by immersing the base in a hydroalcoholic solution (50% v/v), containing 3,000 mg L^{-1} of IBA or just distilled water and ethanol (50% v:v), totaling 35 cuttings per treatment. Then, we planted them in plastic trays (60 x 41 x 18 cm) with 16 drainage holes, spaced 6.0 cm apart, containing sand and medium-grained vermiculite (1:1 v/v) as substrate.

The nursery had 70% shade on the top and sides, a thermohygrometer to monitor temperature, humidity, and automatic misting irrigation (carried out from 6 am to 6 pm and activated every 12 minutes for 2 minutes). Every 30 days, the cuttings were treated with Priori® systemic fungicide (1 ml L^{-1}) and Maxiplant® foliar fertilizer (0.75 g L^{-1}). The greenhouse, E2's environment, consisted of automated intermittent nebulization, activated every 11 minutes for 60 seconds, with a temperature of 30°C ± 2°. The cuttings were treated weekly with Cercobin® fungicide (0.70 mg L^{-1}) and Forth Enraizador® foliar fertilizer (10 ml L^{-1}).

At the 120-day mark (E2) and 150-day mark (E1), we conducted assessments on roots and shoots, focusing on emissions greater than 3 mm, across the following variables: survival rate (%), rooting rate (%), number of adventitious roots per cutting, and length of the longest root per cutting (cm). Additionally, in E2, we examined sprouting rate (%), number of shoots per cutting, length of the longest shoot per cutting (cm), and callosity rate (%). However, in E1, these variables were not assessed due to the absence of shoots and limited callosity formation.

Determination of carbohydrate content

Seven 5 cm samples of cuttings from E2 were collected to evaluate carbohydrate and starch content at the Plant Physiology Laboratory at Embrapa Amazônia Ocidental and were kept at -18°C until biochemical analyses. According to Passos' methodology (1996), the cuttings were thawed and dried in an oven (60°C) until constant weight, crushed in a ball mill (fine granulation) to obtain samples of 200 mg of dry mass (DM) in triplicate. The alcoholic extraction of sugars was carried out with two cycles of maceration in ethanol (95% followed by 80%), with centrifugation (6,000 rpm for 15 min). 3 mL of chloroform and 5 mL of water were mixed with the supernatant to remove chlorophyll and lipids from the extracted solution and an aliquot was diluted 10x. Starch extraction was performed on the resulting precipitate, which was subsequently dried and combined with 35% (w/v) perchloric acid. The mixture was then subjected to centrifugation at 1,000 g for 15 minutes, followed by dilution of an aliquot by a factor of 100. The determinations of soluble carbohydrates and starch were made with the anthrone reagent in diluted aliquots and reading the absorbances on the spectrophotometer at 625nm, using a glucose standard curve. The results were expressed as milligrams of glucose or starch per gram of dry mass (mg g^{-1} MS).

Statistical analysis

We conducted data analysis from the experiments employing analysis of variance (ANOVA) to compare means, applying the F-test at a 5% significance level for variables exhibiting a normal distribution. For variables lacking normal distribution (such as number of shoots per cutting, rooting percentage, number of adventitious roots per cutting, and length of the longest root per cutting), we utilized the Kruskal-Wallis test. We conducted mean comparisons using the Tukey test at a significance level of 5%. Additionally, we employed the Pearson correlation test to assess the relationship between sprouting percentage, callus percentage, and rooting percentage in the cuttings. Furthermore, we investigated the impact of soluble carbohydrates (mg g^{-1}) and starch concentrations (mg g^{-1}) on survival percentage, sprouting percentage, and rooting percentage. Before analysis, percentage data were transformed using the $arc\ sen\ \sqrt{\frac{x}{100}}$ method, while count data underwent square root transformation.

Anatomical analysis

For qualitative anatomical analysis, samples were collected from the bases of the piles at intervals (t) of 7, 15, 30, 45, 60, 90, and 120 days post-piling, with four samples per interval/treatment, totaling eight samples per interval. These samples were fixed in FAA70% for 96 hours following the method by Johansen (1940), then stored in 70% (v/v) ethyl alcohol until embedding. Permanent slides were prepared using materials embedded in historesin according to the technique outlined by Feder and O'Brien (1968). Sections, 6 µm thick, were obtained using an automatic rotating microtome, stained with 1% toluidine blue as described by Feder and O'Brien (1968), and observed under an optical microscope. Starch detection was facilitated by staining with 0.5% Lugol, while phenolic compounds were identified using ferric chloride, following the protocol by Johansen (1940).

RESULTS

In E1 we did not observe the effect of the interaction between the substrate and the auxin concentration for the variables evaluated. Alone, the substrates had significant differences in the percentage of survival (P = 0.0052) and rooting (P = 0.0088) of cuttings, while IBA concentrations resulted in a significant difference in rooting (P = 0.0292) (Table 1).

Table 1. Average values of percentage survival (PES), percentage rooting (PER), number of roots per cutting (NR) and length of longest root per cutting (LLR) based on substrate types and IBA concentrations.
Tabela 1. Valores médios de porcentagem de sobrevivência (PES) e enraizamento (PER), número de raízes/estaca (NR) e comprimento da maior raiz/estaca (LLR) com base no substrato e concentrações de IBA.

Factors	PES (%)	PER (%)	NR	LLR (cm)
Substrate				
Sand (A)	69,4[b]	5,6[b]	0,8	1,0
Vermiculite (V)	83,8[a]	16,9[a]	1,5	2,5
A+V (1:1)	73,8[b]	12,5[ab]	1,0	1,8
IBA concentration (g kg^{-1})				
0,0	75,8	5,0[b]	0,7	1,3
1,5	75,0	10,8[ab]	0,8	1,5
3,0	72,5	16,7[a]	1,5	2,5
6,0	79,2	14,2[ab]	1,6	1,8
Overall average	75,6	11,7	1,1	1,8
CV (%)	17,3	67,6	73,8	108,6

Means with the same letters in the column are not significantly different from each other according to the Tukey test (P < 0.05).

Vermiculite was the most efficient substrate, with 83.8% survival and 16.9% rooting. Despite having the highest value for LLR, the concentration of 3.0 g kg^{-1} of IBA only differed from the control treatment in PER (16.7%). As for NR, the highest averages were obtained at the two highest concentrations of IBA (Table 2). Although there was no interaction between the factors (Figure 2), we observed that the increase in auxin concentration resulted in greater rooting for substrates V and A+V.

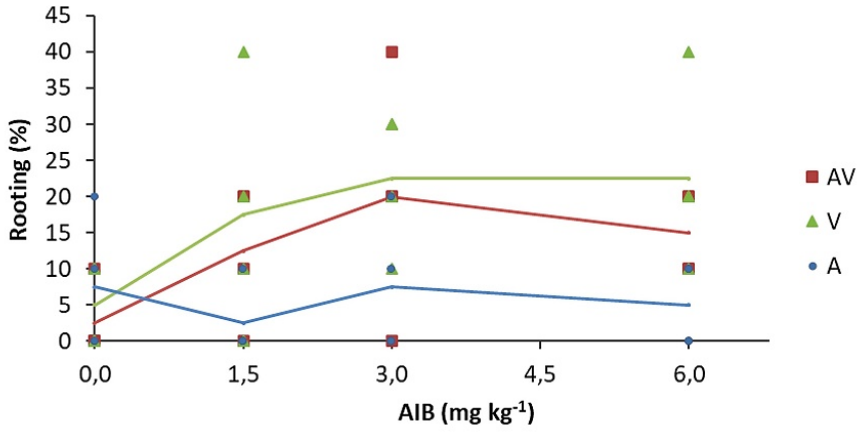

Figure 2. The average percentage of rooting in the sand (A), vermiculite (V), and A+V as a function of IBA concentrations, with points representing the average values for each substrate.
Figura 2. Porcentagem média de enraizamento em areia (A), vermiculita (V) e A+V em função das concentrações de IBA, com pontos representando os valores médios de cada substrato.

In E2, there was a significant difference observed in the percentage of sprouting and callus. The regrowth treatment exhibited higher average percentages for all variables, except survival (62.5%). Despite this, the survival rate is deemed satisfactory as it hovered around 80% for most types of piles. However, the rooting variable values were notably low, averaging only 7.6% (Figure 3).

In contrast to E1, visible shoots emerged in the first month in E2. This occurrence did not appear to hinder rooting despite potential competition for resources. There was a notable and significant positive correlation of 58.2% (P = 0.0179) between shoot and root emissions, despite the relatively low rooting percentage. Moreover, positive correlations were observed between sprouting and callus (78.8%, P = 0.0003), as well as between callus and rooting (72.2%, P = 0.0016) (Figure 3).

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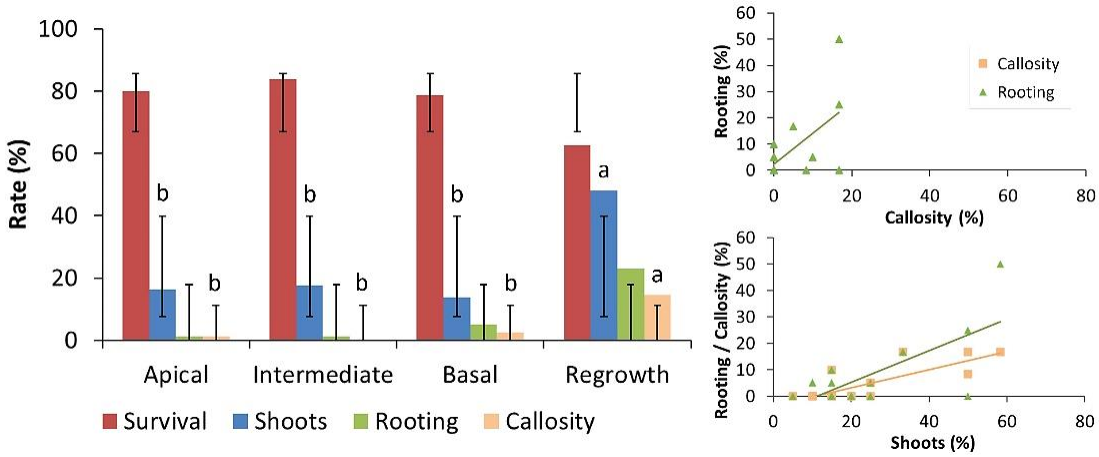

Figure 3. Percentage of survival, sprouting, rooting, and callus for each type of cutting, along with the Pearson's Correlation between callosity and rooting, and between shoots with rooting and callosity. Identical letters within the same variable indicate no significant difference by the Tukey test ($P < 0.05$).

Figura 3. Porcentagem de sobrevivência, brotação, enraizamento e calosidade por tipo de estacas e a Correlação de Pearson entre calosidade e enraizamento, e entre brotação com enraizamento e calosidade. Para letras iguais na mesma variável, não há diferença significativa no teste Tukey ($P < 0,05$).

There were no differences between the soluble carbohydrate contents in the types of cuttings studied. However, starch and total carbohydrate contents were significantly lower in basal-type cuttings, with values of 28.6 mg g^{-1} DM ($P = 0.0249$) and 85.99 mg g^{-1} DM ($P = 0.0752$) respectively (Figure 4). Soluble carbohydrate values were positively correlated with cutting survival (57.9%, $P = 0.0483$), while starch content was positively correlated ($P = 0.0229$) with sprouting (64.7%). The percentage of rooting did not correlate with any of the variables (Figure 4).

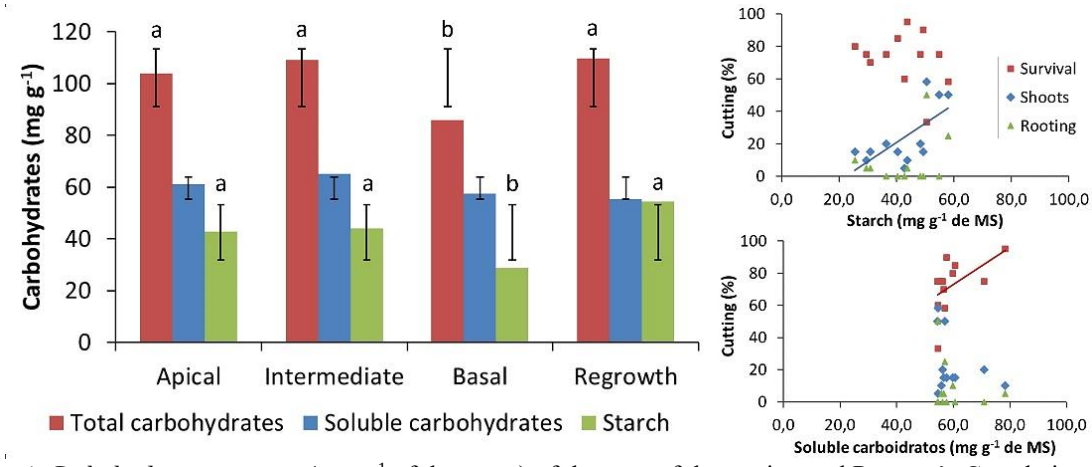

Figure 4. Carbohydrates contents (mg g^{-1} of dry mass) of the type of the cutting and Pearson's Correlation of the percentages of live cuttings, with sprouts and rooted with soluble Carbohydrates and Starch. For equal letters in the same variable, there is no significant difference between them - Tukey test ($P < 0.05$).

Figura 4. Teores de carboidratos (mg g^{-1} de massa seca) por tipo de estaca e a Correlação de Pearson das porcentagens de sobrevivência, brotação e enraizamento com os Carboidratos solúveis e Amido. Para letras iguais na mesma variável, não há diferença significativa entre si - teste Tukey ($P < 0,05$).

Histological analysis

Anatomical analyses in control cuttings (t0) revealed secondary growth structures and the presence of sclerenchymatic tissue cells in the form of a continuous ring (fibers and sclereids), between phloem and cortex, and as solitary or in groups, in the cortex and the marrow, as shown in Figure 5A. At 15 days, there was a visual change in the color of the base of the cutting, from dark brown to orange-brown. There is the formation of a disorganized parenchymatic tissue in the periderm and evidence of lenticels with filling tissue, in addition to cells derived from the cambium that begin to break through the shell (Figure 5B). After 30 days, the divisions intensified, leading to the formation of parenchyma tissue, which became visible in the xylem region due to the

activities of the vascular cambium. Additionally, some new cells were derived in the phloem from the same cambial activity (Figure 5C).

At 45 days, a pronounced phase of cell proliferation occurred, resulting in the development of a tissue abundant in mucilages and pectins within the cambium region (refer to Figure 5D). This tissue intertwines with the newly formed xylem tissue, merges with the phloem, and extends beneath the sclerenchymatic ring. Consequently, there is a notable expansion in the diameter of the pile base, often leading to the detachment of the bark. Within this tissue, meristematic nuclei are generated, typically with xylem parenchyma predominantly located at the core, and sclerenchymatic nuclei surrounding the sclerenchyma bundles.

By the 60th day (Figure 5E), a notable increase in meristematic nuclei was evident, alongside the presence of vascular nuclei and the initiation of root primordia. By day 90, the initial roots were discernible, originating within the parenchymatic tissue of the xylem, a result of cambial activity, as illustrated in Figure 5F.

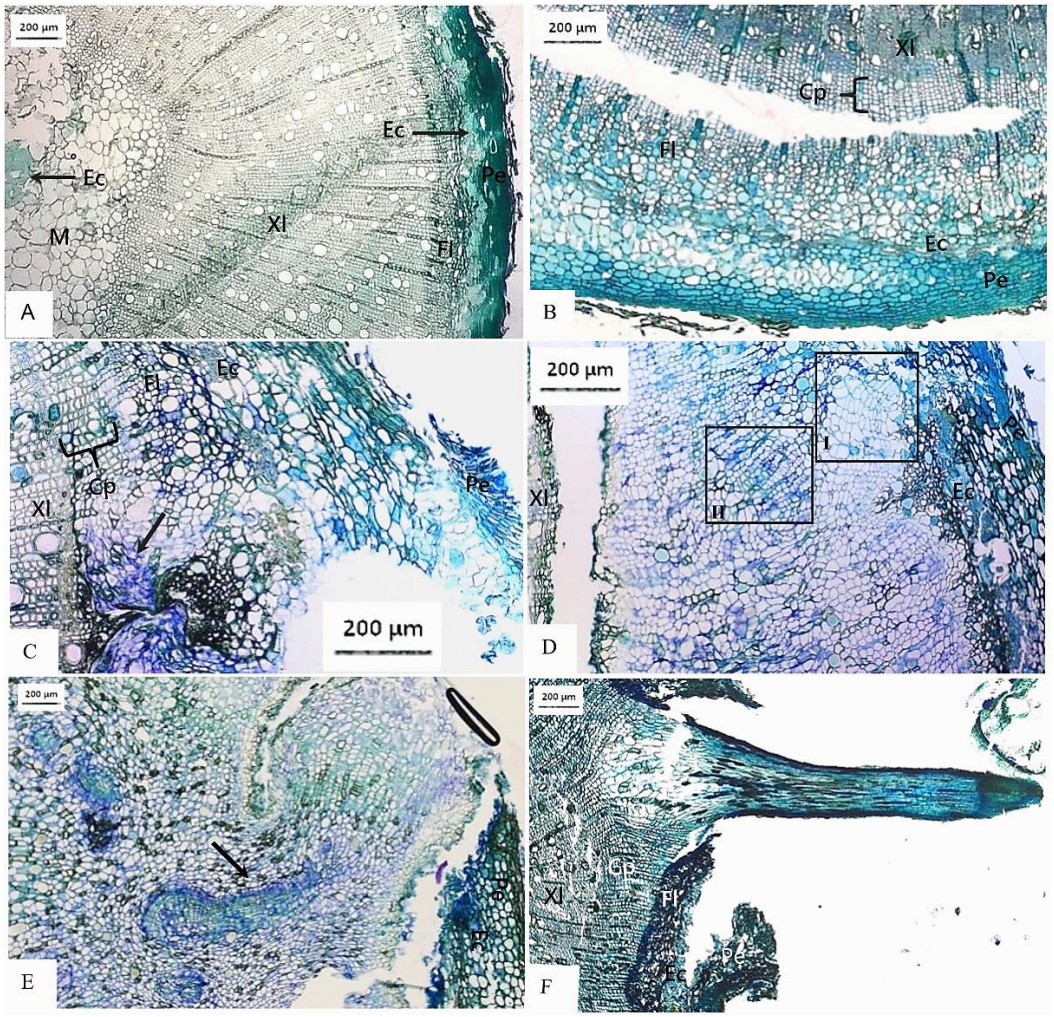

Figure 5. Transverse sections of the base of rosewood cuttings at 0, 15, 30, 45, 60 and 90 days (t) after test installation, where: A (t0) - Evidence of secondary growth in the cuttings and presence of sclerenchyma in the form of a continuous ring and grouped (arrows); B (t15) – Section new cells derived from the vascular cambium (bracket) and formation of disorganized of parenchyma tissue in the periderme; C (t30) – Formation of xylem parenchyma, derived by cambium (backet), and start the formation of tissue rich in mucilages and pectins (arrows); D (t45) – Increase of the mucilages and pectins tissue and the detail of the group of initial cells (I) and meristematic nucleus (II); E (t60) – Development of roots primordium (arrow) with surrounding vascular nuclei; F (t90) – Development of the root from the parenchymal tissue of the xylem. M = Medulla, Xl = secondary xylem, Fl = phloem, Cp = parenchyma cells Ec = sclerenchyma (bundles and/or sclereids), Pe = periderm.

Figura 5. Secções transversais da base da estaca do pau-rosa aos 0, 15, 30, 45, 60 e 90 dias (t) após a instalação do ensaio, onde: A (t0) - Evidência do crescimento secundário e presença de esclerênquima em forma de anel contínuo e agrupado (setas); B (t15) – Novas células derivadas do câmbio (colchete) e a formação de tecido parenquimático desorganizado na periderme; C (t30) – Formação do parênquima xilemático

derivado do câmbio (colchete) e início da formação do tecido rico em mucilagens e pectinas (seta); D (t45) – Crescimento do tecido com mucilagens e pectinas e detalhes do grupo de células iniciais (I) e do núcleo meristemático (II); E (t60) – Desenvolvimento de primórdio radicular (seta) com núcleos vasculares ao redor; F (t90) – Desenvolvimento da raiz a partir do tecido parenquimático do xilema. M = Medula, Xl = xilema secundário, Fl = floema, Cp = células parenquimáticas, Ec = esclerênquimas (feixes e/ou esclereídes), Pe = periderme.

At 7 days, we observed a notable abundance of starch grains within both the pith region and the xylem rays in the sections, a phenomenon absents in the other time frames. Phenolic compounds became increasingly apparent from the 45th day onward, detectable within the xylem rays and the callus and cell mass undergoing differentiation.

DISCUSSION

Of the three types of substrates, those with vermiculite were better for rooting due to their water retention capacity. In rooted cuttings, especially those with a greater number and length of roots, the substrates were more humid, especially those containing vermiculite. Menezes *et al.* (2018) corroborate this hypothesis, as when comparing the influence of substrates on rosewood cuttings from juvenile material, they observed that the vermiculite and carbonized rice husk substrate showed greater survival, rooting, and callus formation when compared to sand and Plantmax® commercial substrate. This result reflected good water retention, greater porosity, and lower density in the upper substrate with vermiculite.

According to Xavier *et al.* (2003), the development of the root system of plant species that root easily is not affected by the type of substrate, while species that are difficult to root are strongly influenced by the substrate, such as adequate aeration to oxygenate the tissues and humidity favorable to development and root growth. Hence, the low porosity and high permeability of sand lead to swift drainage with reduced water retention, coupled with increased density. Consequently, this can induce compaction owing to its weight and inadequate substrate aeration. Despite yielding suboptimal results, sand may undergo testing under alternative rooting conditions and in combination with the composition of other substrates. By amalgamating its attributes with materials conducive to rooting, sand holds the potential for enhanced efficacy (MASIERO *et al*., 2020).

IBA tended to improve rooting quality (NR and CMR) with increasing concentrations up to 3.0 g kg^{-1}, after which it decreased. At the same concentration, there was a significant difference with the control (0.0 g kg^{-1}) for rooting (Figure 2), indicating the influence of auxin on this variable. Although Menezes *et al.* (2018) did not find a relationship between the rooting of *A. rosaeodora* cuttings with the use of IBA (liquid), the authors observed a relative improvement in the quality of rooting with the presence of the regulator, as they found an increase in the number of roots per cuttings. It is important to highlight that the need to use IBA may vary depending on the cutting collection position (HERNANDEZ *et al.,* 2013), the degree of the youth of the material, the collection period, and the cultivars used (CAVUSOGLU & SULUSOGLU, 2014). Furthermore, the presence of IBA can influence rooting if it reaches the optimal hormonal balance in the tissues, or can promote phytotoxicity if it is exceeded (WENDLING *et al*., 2015).

Similar to Menezes *et al.* (2018), Sampaio *et al*. (1989) did not find it necessary to apply IBA for rooting in rosewood. The variance in the efficacy of IBA observed in this study could be attributed to the method of application; while the former authors employed the liquid route, the talc route was utilized in our study. In a comparative assessment of IBA application methods in *Annona squamosa*, the powder route exhibited 20% greater efficiency due to the prolonged and moderate contact of the plant material with auxin (SALVADOR *et al.*, 2014). The powder adheres to the cutting's base, ensuring sustained contact for an extended period, thereby facilitating gradual absorption. A similar mechanism may have contributed to the rooting process in rosewood in our study, evidenced by an 11.7% difference in rooting with increased IBA concentration compared to the control (see Table 1). Although the outcome still falls short in terms of the cost-effectiveness of auxin application, it presents an opportunity for further research to explore avenues for improved results.

While there exists a notable disparity in the average rooting values within each factor (substrates and IBA concentrations), they remain relatively low in percentage when juxtaposed with the overall average of rooted cuttings at 11.7% compared to live cuttings at 75.6%. This underperformance can be attributed to other factors, including fluctuations in external environmental conditions such as strong winds and excessive sunlight, which may have influenced E1. Furthermore, the average temperature range in the nursery was high (13.4°C) and the minimum relative humidity was low (40%) in October and November, the fourth and fifth months of the test. These extremes may have had a negative impact, as the temperature is directly linked to the metabolism of the cuttings (MARAGON & BIASIN, 2013) and the humidity of the environment has the function of maintaining the turgidity of the tissues until the formation of the roots (XAVIER *et al*., 2013). Large temperature fluctuations and low humidity values may have interfered with the synthesis of rooting promoters (beneficial endogenous cofactors)

and/or caused dehydration of the plant material. These factors, combined with the high genetic variability (35 matrices), significant differences between rooting blocks (P = 0.0467), and the length of the largest root (P = 0.0220), may have favored the high variety of data.

In E2, the regrowth treatment exhibited superior performance across nearly all variables. This notable performance can be attributed to the gradient of juvenileness, as this treatment was located closer to the base of the tree compared to the others. This gradient can be more or less accentuated, depending on the species, and positively interferes with the plant material's ability to root the closer it is to the center of origin of the first cells, as in this region the cells have a greater capacity to conserve totipotent properties (HARTMANN *et al*., 2011; WENDLING *et al*., 2014). The other treatments were obtained from shoot branches, therefore they underwent a greater succession of cell divisions and may have a lower capacity for dedifferentiation.

The best results in regrowth-type stems can also be explained by the higher average concentration of starch (54.4 mg g^{-1} of DM), but this treatment had the lowest content of soluble carbohydrates (55.4 mg g^{-1} of DM), consequently, lower survival. Soluble carbohydrates serve as rapid sources of energy, and rising temperatures accelerate respiration, increasing the energy demand (OLIVEIRA *et al*., 2012). Thus, the high temperature may have led to the rapid consumption of the cuttings reserve before the starch was broken down into smaller molecules, suitable for consumption, resulting in a lack of free energy to meet the metabolic demand and causing mortality.

In general, starch concentrations correlated positively with the percentage of cuttings with shoots (64.7%, P = 0.0229) and soluble carbohydrate concentrations with the percentage of live cuttings (57.9%, P = 0.0229) 0483) (Figure 4). Oliveira *et al*. (2012) studied the carbohydrate content during the rooting of blueberries and observed that the period during the emission of shoots and roots is related to the greater consumption of reserves, due to their mobilization for the developing buds that act as a drain. Due to the low percentage of rooting obtained, it was not possible to establish a relationship between the use of reserves to promote rooting. However, even acting as a drain, the correlation between shoot emission was positive concerning rooting (58.2%, P = 0.0179) (Figure 5), revealing that one did not interfere with the other. Endogenous auxins and rooting cofactors are produced in buds and new parts of the plant (HARTMANN *et al*., 2011), therefore, emerging shoots may have provided such substances that collaborated with rooting, compensating for competition for carbohydrates.

The relatively low percentage of rooting observed in comparison to the sprouting of cuttings in the greenhouse (Figure 3) could be attributed to the elevated air humidity coupled with a temperature of 30°C. The environmental conditions within the enclosed space likely fostered high relative air humidity, potentially disrupting the dormancy of upper buds and stimulating shoot emission. The lower temperature of the substrate with the air generates a thermal gradient from the base to the apex of the cutting, increasing transpiration of the aerial part, resulting in greater metabolic activity and greater flow of endogenous substances in that direction, making it a preferential drain (OLIVEIRA *et al*, 2012). A hormonal balance in the auxin/cytokinin relationship may have occurred in favor of sprouting since the emission of shoots is favored by their low proportion, while a greater presence of auxin than cytokinin favors rooting (XAVIER *et al.* 2013; DA COSTA *et al*., 2013). The use of a growth regulator in this assay could rebalance the relationship in favor of rooting.

In this study, the environmental fluctuations and the responses observed in *A. rosaeodora* underscore the necessity for stricter control of humidity and temperature conditions within the greenhouse or nursery setting. It is relevant to define the minimum ideal conditions required for effectively conducting and managing the propagation environment.

Histological analysis

In most woody species that are difficult to root, the root is formed indirectly, derived from callus, while in those that are easy to root, the root normally originates directly, in the vascular cambium (HARTMANN *et al*., 2011). In the present study, the formation of a callous tissue was often observed where vascular centers of different sizes were formed, with tracheids in the center, and some elongated towards the end, characterizing the root primordium. However, unrooted callous cuttings with tracheids were found - which may suggest the need for more time to root. Also, there were rooted cuttings without evidence of callus, which suggests no dependence on callus for rooting. When analyzing the origin of root formation, the results obtained corroborate Sampaio *et al*. (1989), as they confirm that its origin is not from callus tissue, but from parenchyma cells formed in the xylem by the activity of the vascular cambium. In general, parenchymatic cells surrounded by meristematic cells are encompassed by callus tissue, after a while, vascular nuclei predominate that will derive root primordia.

Despite not being directly related to root formation, callogenesis may have favored rooting, as the rupture of the bark in the cambium region can eliminate the barrier formed by the sclerenchyma, facilitating root emission. There are disagreements regarding whether sclerenchymatic tissue limits rooting (HARTMANN *et al*. 2011). Nonetheless, a study involving woody cuttings of native forest species established a correlation between the proportion of sclerenchyma tissue present in larger diameter propagules and both the percentage and quality of

rooting - even in cases where sclerenchyma was not continuously distributed in rings (SANTOS *et al.*, 2011). This same study also reported the difficulty in making incisions in unrooted cuttings.

We hypothesize that besides sclerenchyma, various other biochemical and physiological factors could impede rooting. The presence of starch grains solely within the initial seven days of propagation likely indicates their utilization to fulfill the energy demands of emerging cell growth (OLIVEIRA *et al*., 2012). The visualization of phenolic compounds occurring solely after 45 days of planting aligns with the timing of callus mass formation rich in mucilages and pectins. This correlation suggests their potential role as cofactors during the early stages of root primordia formation. Rooting cofactors, substances that influence rhizogenesis, can function as either root inhibitors or inducers depending on their mode of action. Some inducers are essential to physiological functions and act as inducers or protectors of endogenous auxin, while some inhibitors bind to auxin, preventing its action (HARTMANN *et al*., 2011). Some of these cofactors may be interfering with rosewood rooting.

In general, there was high variability in the data, particularly in the rooting of the cuttings, resulting in a low rooting rate. This outcome is likely attributed to several factors, including genetic variability at both the species and individual levels, which influences the predisposition of cells to differentiate (HARTMANN *et al*., 2011; SANTOS *et al.*, 2011). Additionally, the production of biochemical substances that inhibit rooting (inhibitory cofactors), unsuitable environmental conditions, the presence of a sclerenchyma ring acting as a mechanical barrier to root emergence (SANTOS *et al.*, 2011), and the efficiency of rejuvenation processes (WENDLING *et al*., 2014) may have contributed to these results. Nevertheless, our study underscores the potential for producing rosewood seedlings through cutting propagation, utilizing 3.0 g kg^{-1} of IBA powder, incorporating vermiculite into the substrate, and collecting propagules from regrowth shoots directly from the stump. However, further research is necessary to adapt environmental conditions, overcome the physical barrier posed by sclerenchyma, address potential biochemical barriers, and select genetically predisposed individuals capable of rooting. These efforts are crucial for making cutting-based seedling production economically viable, while also promoting conservation actions.

CONCLUSIONS

- Vermiculite was the substrate that exhibited superior adventitious rooting of the cuttings. However, it did not differ significantly from a 1:1 mixture with sand. This suggests the possibility of exploring alternative proportions to achieve a balance between rooting performance and cost-effectiveness.
- The application of IBA auxin via talc was more effective in promoting the rooting of cuttings at a concentration of 3.0 g kg^{-1}.
- Cuttings obtained directly from sprouts rejuvenated by cutting off adult plants showed greater potential for adventitious rooting.
- Adventitious roots originate from xylem parenchyma cells, formed due to cambial activity.
- Factors that affect the rooting process: a ring of sclerenchyma after phloem, which can act as a physical barrier; the callous tissue, formed from the cambium, which indirectly helps break down the epidermis and the barrier; and the existence of endogenous inducing cofactors, such as phenolic compounds, which may also have inhibitors.

Further research is crucial to establish a protocol for the commercial rooting of rosewood cuttings. This necessitates studies to ascertain the microclimate of the environment and the optimal humidity levels for the substrate. Additionally, there is a need to investigate cutting preparation methods aimed at overcoming anatomical barriers, ensuring that the rupture of the sclerenchyma ring is not solely reliant on callus production. Moreover, it is imperative to examine the influence of endogenous cofactors on both inducing and inhibiting adventitious rooting.

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REFERENCES

CAVUSOGLU, A.; SULUSOGLU, M. Effects of indole-3-butyric acid (IBA) and 1-naphthaleneacetic acid (NAA) on rooting of female and male Laurus nobilis L. cuttings. **International Journal of Biosciences (IJB)**, Bangladesh, v. 4, n. 11, p. 206-216, 2014.

DA COSTA, C. T.; DE ALMEIDA, M. R.; RUEDELL, C. M.; SCHWAMBACH, J.; MARASCHIN, F. S.; FETT-NETO, A. G. When stress and development go hand in hand: main hormonal controls of adventitious rooting in cuttings. **Frontiers in plant science**, China, v. 4, p. 133, 2013.

FEDER, N.; O'BRIEN, T. P. Plant microtechnique: some principles and new methods. **American journal of Botany**, United State of America, p. 123-142, 1968.

HARTMANN, H.T.; KESTER, D.E.; DAVIES JÚNIOR, F.T.; GENEVE, R.L. **Plant propagation: principles and practices**. New York, Englewood Clipps, 8 ed. 2011, 880 p.

RED LIST OF THREATENED SPECIES (IUCN). **The IUCN Red List of Threatened Species**. Version 2022-2. BARSTOW, M. 2021. *Aniba rosodora*. ISSN 2307-8235. Available in: <https://www.iucnredlist.org> Accessed on: 20 April 2023.

HERNANDEZ, W.; Xavier, A.; PAIVA, H. N. D.; WENDLING, I. Propagação vegetativa do jequitibá-rosa (*Cariniana estrellensis* (Raddi) Kuntze) por estaquia. **Revista Árvore**, Viçosa/MG, v. 37, p. 955-967, 2013.

JOHANSEN, D.A. **Plant Microtechnique**. New York: McGraw-Hill, 1940, 523 p.

KRAINOVIC, P. M.; ALMEIDA, D. R. A.; DESCONCI, D.; VEIGA-JÚNIOR, V. F.; SAMPAIO, P. T. B. Sequential management of commercial rosewood (*Aniba rosaeodora* Ducke) plantations in central Amazonia: Seeking sustainable models for Essential oil production. **Forests**, China, v. 8, n. 12, p. 438, 2017.

LARA, C. S.; COSTA, C. R.; SAMPAIO, P. T. B. O mercado de sementes e mudas de pau-rosa (*Aniba* spp.) no Estado do Amazonas. **Revista de Economia e Sociologia Rural**, Brasília/DF, v. 59, n.3, 2021.

MARANGON, M. A.; BIASI, L. A. Estaquia de mirtilo nas estações do ano com ácido indolbutírico e aquecimento do substrato. **Pesquisa agropecuária brasileira**, Brasília/DF, v. 48, p. 25-32, 2013.

MASIERO, M. A.; VIANA, C. M. S. S.; DE LIMA, D. M.; DA SILVA, E. C.; OTALAKOKI, J.; FELICETI, M. L.; ALMEIDA, J. S.; PINHEIRO, J. F. Substratos no enraizamento de estacas de *Mentha piperita* L. **Revista Brasileira de Agropecuária Sustentável**, Viçosa/MG, v. 10, n. 1, p. 234-240, 2020.

MENEZES, A.; SAMPAIO, P. T. B.; BLIND, A. D. Propagação de pau-rosa (*Aniba rosaeodora* Ducke) por estacas e miniestacas. **Nucleus**, Ituverava/SP, v. 15, n.1, p. 515-522, 2018.

OLIVEIRA, R. J. P. D.; BIANCHI, V. J.; AIRES, R. F.; CAMPOS, Â. D. Teores de carboidratos em estacas lenhosas de mirtileiro. **Revista Brasileira de Fruticultura**, Jaboticabal/SP, v. 34, n. 4, p. 1199-1207, 2012.

PASSOS, L. P. **Métodos analíticos e laboratoriais em fisiologia vegetal.** Coronel Pacheco/MG, Embrapa-CNPGL, 1996, 226 p.

SALVADOR, T. D. L.; SALVADOR, T. D. L.; LEMOS, E. E. P. D.; BARROS, P. G.; CAMPOS, R. D. S. Rooting cuttings of sugar apple (*Annona squamosa* L.) with indolebutyric acid. **Revista Brasileira de Fruticultura**, Jaboticabal/SP, v. 36 (SPE1), p. 310-314, 2014.

SAMPAIO, P. T. B.; NODA, H.; PARENTE, R. Enraizamento de estacas de material juvenil de pau-rosa (*Aniba rosaeodora* Ducke - Lauraceae). **Acta Amazônica**, Manaus/AM, v. 19, n. 1, p. 391-400, 1989.

SANTOS, J. D. P.D.; DAVIDE, A. C.; TEIXEIRA, L. A. F.; MELO, A. J. S.; MELO, L. A. D. Enraizamento de estacas lenhosas de espécies florestais. **Cerne**, Lavras/MG, v. 17, p. 293-301, 2011.

WENDLING, I.; BROOKS, P. R.; TRUEMAN, S. J. Topophysis in *Corymbia torelliana* × *C. citriodora* seedlings: adventitious rooting capacity, stem anatomy, and auxin and abscisic acid concentrations. **New forests**, Netherlands, v. 46, n. 1, p. 107-120, 2015.

WENDLING, I.; TRUEMAN, S. J.; XAVIER, A. Maturation and related aspects in clonal forestry – Part I: concepts, regulation and consequences of phase change. **New Forests**, Netherlands, v. 45, p. 449-471, 2014.

XAVIER, A.; WENDLING, I.; SILVA, R. L. **Silvicultura clonal**: princípios e técnicas. 2. ed., Viçosa: Ed. UFV. 2013. 279 p.