



PROPAGULE AGE AND ADVENTITIOUS ROOTING IN CUTTINGS OF *Cordia oncocalyx* (Allemão), A TROPICAL DRY FOREST TREE

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ABSTRACT

The success of the cutting technique is primarily due to the rooting capacity linked to the ontogenetic aging of cell tissues, meaning that mature propagules generally root less effectively than younger ones. This raises a key question: for how long can a propagule maintain rooting potential in tree species from tropical dry forests such as *Cordia oncocalyx*? To address this, we examined how propagule maturation and indole-3-butyric acid (IBA) concentrations affect the rooting of *Cordia oncocalyx* cuttings. Cuttings were collected at three maturation stages (30, 90, and 150 days) and tested with IBA concentrations of 0, 2000, and 8000 mg.L⁻¹. For root evaluation through digital image analysis, the images were processed and analyzed using ImageJ software version 1.46. Thirty-day-old cuttings, collected at the beginning of the rainy season and treated with 2000 mg.L⁻¹ of IBA, showed the highest survival (100%) and rooting (100%) rates, producing seedlings of superior quality. Cuttings from 90- and 150-day-old propagules exhibited low rooting and survival rates, even at higher IBA levels. *Cordia oncocalyx* can rapidly lose its rooting potential; therefore, it is recommended to collect cuttings from younger propagules shortly after sprout emergence and treat them with 2000 mg.L⁻¹ of IBA. Digital image analysis effectively assessed root count and length, proving to be accurate. This study contributes to the vegetative propagation of *Cordia oncocalyx*, and successful propagation strongly depends on using young propagules combined with appropriate auxin treatment. Additionally, digital image analysis proved to be a reliable method.

Keywords: Cutting; Collection time; AIB

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IDADE DO PROPÁGULO E ENRAIZAMENTO ADVENTÍCIO EM ESTACAS DE *Cordia oncocalyx* (Allemão) Baill, ÁRVORE DE FLORESTA SECA TROPICAL

RESUMO O sucesso da técnica de corte deve-se principalmente à capacidade de enraizamento ligada ao envelhecimento ontogenético dos tecidos celulares, o que significa que os propágulos maduros geralmente enraizam de forma menos eficaz do que os mais jovens. Isto levanta uma questão fundamental: por quanto tempo um propágulo pode manter o potencial de enraizamento em espécies arbóreas de florestas tropicais secas como *Cordia oncocalyx*? Para isso, examinamos como a maturação dos propágulos e as concentrações de ácido indol-3-butírico (IBA) impactam o enraizamento de estacas de *Cordia oncocalyx*. As estacas foram coletadas em três estádios de maturação (30, 90 e 150 dias) e testadas com concentrações de AIB de 0, 2.000 e 8.000 mg.L⁻¹. Para a avaliação das raízes por análise de imagens digitais, as imagens foram tratadas e processadas com o auxílio do software Image J versão 1.46. As estacas com 30 dias, coletadas no início da estação chuvosa e tratadas com 2.000 mg.L⁻¹ de AIB tiveram as maiores taxas de sobrevivência (100%) e enraizamento (100%), produzindo mudas de melhor qualidade. Estacas provenientes de propágulos com 90 e 150 dias de idade apresentaram baixas taxas de enraizamento e sobrevivência, mesmo com níveis mais elevados de AIB. *Cordia oncocalyx* pode perder rapidamente o potencial de enraizamento; portanto, é aconselhável colher mudas de propágulos mais jovens logo após à emergência dos brotos e tratar com 2.000 mg.L⁻¹ de AIB. A análise de imagens digitais avaliou com eficácia a contagem e o comprimento das raízes, provando ser precisa. Este estudo contribui para a propagação vegetativa de *Cordia oncocalyx*, e o sucesso da propagação depende fortemente do uso de propágulos jovens combinados com tratamento adequado

com auxina. Além disso, a análise de imagens digitais provou ser um método confiável.

Palavras-Chave: Estaquia; Época de coleta; AIB

1. INTRODUCTION

Semi-arid areas are present at least in forty-eight countries. In Brazil, this comprises ten states with a territorial extension corresponding to 1.03 million km², equivalent to 12% of the country's territorial area (Sullivan et al., 2020). This region is characterized by having a semi-arid climate, irregular water distribution, saline soils, unpredictable rainfall levels, and mostly young soils (Maia et al., 2020). Regarding vegetation, the semi-arid region is represented by a seasonally dry tropical forest rich in endemic species (Mendes et al., 2020), such as *Cordia oncocalyx* (Allemão) Baill.

Cordia oncocalyx (Allemão), belongs to the Boraginaceae family, and is popularly known as pau-branco, has a good wood quality and energy potential, being used in producing firewood and charcoal, woodworking, and agricultural instruments. It is also used for recovering degraded areas and as folk medicine due to its healing action (Carvalho, 2008; Maia, 2012). These multiple uses without forest management reduced the number of *C. oncocalyx* individuals in natural populations, considered a rare species with a high probability of extinction (Brasil, 2022). Its propagation by seeds is hard due to the mechanical dormancy of diaspores (Araújo et al., 2022) and the seeds are predated by *Pachymerus nucleorum* Fabricius (Brito & Araújo, 2009). Vegetative propagation techniques, including cutting, can be used to overcome this obstacle. However, information about clones produced from adult trees is so far unknown for the species.

The cutting technique potentially allows the selection of superior genotypes with any characteristic of interest for ecological and/or silvicultural performance (Costa et al., 2024). Also, clonal plants could improve the formation of homogeneous plantations for forestry purposes and supply the wood market demand reducing illegal logging

deforestation in natural areas (Deus Silva et al., 2021). Furthermore, the propagation of adult trees from seeds can allow the production of seedlings with greater genetic diversity, aiming to obtain and conserve a broad genetic collection of species of ecological interest.

Therefore, cutting success depends on adequate development of a radicular system, which is limited by variables such as seasons, climatic conditions, matrix plant physiological and nutritional status, propagule's size, type, age, position, storage, and use of growth regulators (Hartmann et al., 1997; Fachinello et al., 2005). The season in which the propagules are collected is one of the main factors that affect the adventitious rooting due to differences in climatic conditions, which influence the ontogenetic and physiological state of the matrix plant, specifically, in the variation of the endogenous hormonal balance and sugars (Fachinello et al., 1996). It is essential to show that higher temperatures and drought can cause a decrease in sprouting activity, flowering, and high growth rates (Lima et al., 2021) increasing the rate of sprout maturation. In that sense, a question arises: How long can a propagule maintain rooting potential in a dry forest species?

The application of plant regulators prior to the rooting phase is another important factor, since auxins are the main substances that induce adventitious rooting, especially when the species has difficulty rooting (Henry et al., 1992; Gomes & Scortecci, 2021). However, the standard concentrations of plant regulators need to be adjusted according to environmental conditions and the maturation status of the propagules (Ross et al., 2021).

After this adjustment, another important factor is the traditional assessment of cutting rooting, based on visual observation of the roots at the base of the tubes, which can lead to an overestimation of the time required in the propagation environment, resulting in increased costs and the risk of seedling loss. Accurately determining rooting speed allows for optimized use of facilities and reduces operational errors (Ferreita et al., 2004; Melo

et al., 2011). In this context, digital image analysis emerges as an efficient tool for measuring root characteristics such as area, perimeter, and number of roots, contributing to more assertive decisions in forest nurseries (Medeiro et al., 2018; Narisetti et al., 2019).

Therefore, studies using exogenous hormones in the rooting process of *C. oncocalyx* cuttings and analysis of root development are necessary and important to understand how to produce and improve propagation using cuttings with propagules of different ages and exogenous plant growth regulators.

In this sense, we aimed to answer the following questions: how long a sprout maintains rooting potential in *Cordia oncocalyx* in the dry forest? Is it necessary exogenous regulator to induce adventitious rooting in sprouts from *Cordia oncocalyx* collected at different ages? Is it possible to propagate *Cordia oncocalyx* sprouts from adult trees allowing us to conserve the species, produce siblings and select phenotypes? Does the use of digital image analysis have the potential to analyze the root development of the species?

2. MATERIAL AND METHODS

2.1 Study site and plant material

The vegetative material was collected from one clone at 11 years of *Cordia oncocalyx*, located at the Center for Multiplication of Wild Animals (CEMWA) (5° 03' 37" S, 37° 23' 50" W) at the Federal Rural University of Semi-Arid in the municipality of Mossoró, State of Rio Grande do Norte, northeast Brazil. The local altitude is approximately 16 m.

The region's climate, according to Thornthwaite is semi-arid and according to Köppen's classification, is BShw-dry and very hot, featuring two distinct seasons: a dry season typically from June to January, and a rainy season from February to May (Beck et al., 2018). The average climate data during the experimental period are presented in Figure 1 (EMA, 2020).

The research was conducted in three contrasting periods, considering the climatic seasonality of the region: beginning of the rainy season (January–February 2020), end

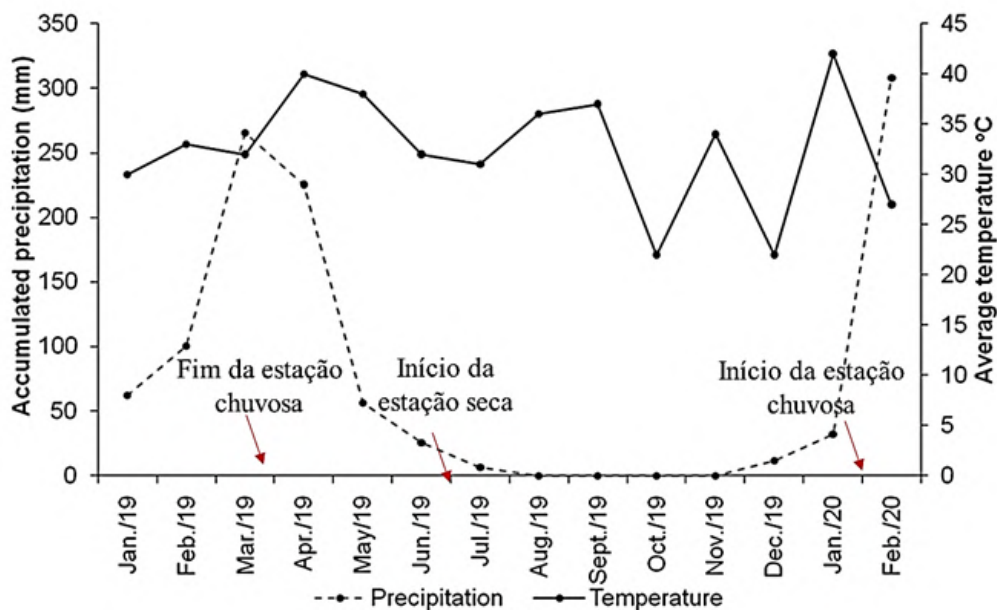


Figure 1. Monthly average of temperature (°C) and pluviosity (mm) from January 2019 to February 2020 in Mossoró, Rio Grande do Norte, Brazil. Source: Ema (2020)

Figura 1. Média mensal de temperatura (°C) e pluviosidade (mm) de janeiro de 2019 a fevereiro de 2020 em Mossoró, Rio Grande do Norte, Brasil. Fonte: Ema (2020)

of the rainy season (April–May 2019) and beginning of the dry season (June–July 2019). The cuttings collected with 90-day-old (end of the rainy season) and 150-day-old (beginning of the dry season) were hardwood, while the cuttings collected with 30 days old (beginning of the rainy season) showed to be more juveniles (softwood).

2.2 Shoot collection, cutting preparation and rooting environmental

No silvicultural practice was carried out to induce sprouts since the trees had natural sprouting at the base (Figure 2). The shoots were collected from the stem branches at the base of the trees, at a height of 40 cm from the soil and later conditioned in styrofoam containing water (to maintain the hydration of the tissues and cell turgor).

The cuttings were prepared by making a transverse cut in the basal region, keeping the apical part, with one to two pairs of expanded leaves, and a length of 12 ± 2 cm. The leaf area of propagules were reduced by 25% to avoid leaf water loss and avoid the “umbrella” effect, which prevents the substrate from being moistened.

After being prepared, the cuttings were immersed by the bases (2 cm) in a solution of

indol-3-butyric acid (IBA) or water (control) for 15 seconds before being staked in polypropylene tubes of 55 cm³ capacity, filled with commercial substrate (BioPlant®) based on vermiculite, Sphagnum peat, coconut fiber, pine bark and carbonized rice husk with the following physicochemical characteristics: pH = 6.2 (± 1.0); electrical conductivity = 0.7 (± 0.5) mS cm⁻¹; density = 150 kg m⁻³ and water retention capacity (WHC, m/m) = 100%. The basic mineral nutrition used in the substrate was composed of 8 kg.m⁻³ of NPK (10-05-30).

In sequency, the cuttings were placed in a greenhouse for rooting. The propagation environment had an opening system at the base, a 50% thermoreflective shading screen, and was equipped with an automated nebulization irrigation system activated at defined intervals (10 seconds every 30 minutes), with relative humidity controlled by a humidistat, maintaining values between 68% and 85%, and temperatures ranging from 30 °C to 34 °C.”

2.3 Experiment I: Cuttings with different ages of propagules

We set up one experiment analysing three propagule ages and three doses of

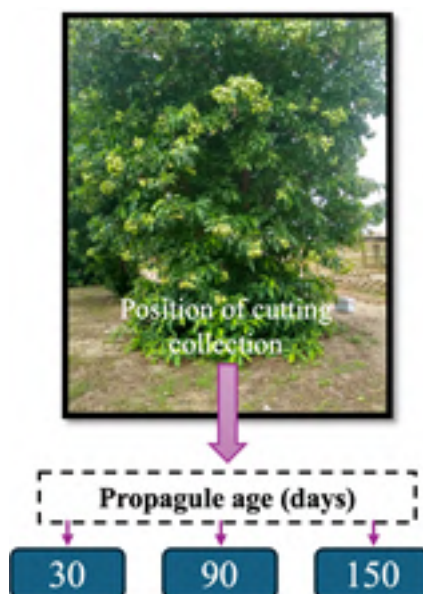


Figure 2. Propagule collection for vegetative propagation of *Cordia oncocalyx* (Allemão) by cutting technique

Figura 2. Coleta de propágulos para propagação vegetativa de *Cordia oncocalyx* (Allemão) pela técnica de estaquia

Indol-3-butyric acid (IBA-Sigma Co.), to answer the questions: how long a sprout maintains rooting potential in *Cordia oncocalyx*? Is it necessary exogenous regulator to induce adventitious rooting in sprouts from *Cordia oncocalyx* collected at different ages?

IBA was used as a rooting inducer at concentrations of 0 (control), 2.000 and 8.000 mg. L⁻¹ in a liquid formulation, dissolved in potassium hydroxide (KOH) at 1 mol.L⁻¹ and diluted in deionised water. The experiment was conducted over the three ages of the propagules: 30-day-old (beginning of the rainy season), 90-day-old (end of the rainy season) and 150-day-old (beginning of the dry season). The experiment followed a 3 × 3 factorial scheme in a split-plot design over time, with the main plot consisting of three propagule ages and the subplots composed of three IBA concentrations. Each treatment combination had five replicates, and each replicate consisted of eight mini-cuttings, totalling 40 mini-cuttings per treatment.

After 45 days in a greenhouse, rooting time, all cuttings were evaluated for survival and rooting. The number of surviving cuttings was counted to obtain the percentage of survival (S%), considering those that were

with green and turgid leaves as alive. To determine if the cuttings have rooted, we looked for roots on polyethylene tube bases or by the removal resistance of the cuttings from the substrate according to Vilasboa et al. (2022).

2.4 Experiment II: Cutting as a propagation technique for *Cordia oncocalyx*

To answer if is it possible to propagate *Cordia oncocalyx* sprouts from adult trees in large scale by cutting technique, we collected sprouts with 30-day-old (beginning of the rainy season) and tested three IBA concentrations (0-control, 2.000 and 8.000 mg. L⁻¹). The cuttings were kept in the greenhouse for 45 days, rooting time. After, the plants were transferred to shade house (to acclimatation) for a period of ten days with 50% light intensity and finally placed under full sunlight (to rustication) for ten days. The plants were irrigated manually with a watering can twice a day (early morning and late afternoon) in the acclimatation and rustication phases to maintain moisture in the substrate.

The experiment was conducted in a randomized block design with four replicates, each consisting of 16 cuttings per treatment,

totaling 64 cuttings per treatment. To perform morphological analyses of the quality of established cuttings, evaluations of survival, rooting, height of the aerial part (H), stem diameter (SD), number of roots emitted per cutting (NR), length of the largest root (LLR), number of sprouts (NS), sprout length (SL), fresh and dry mass of the aerial part (FMAP and DMAP), and fresh and dry mass of rooted cuttings (FMR and DMR) were evaluated on the 65th day of the experiment (after the rustication period).

Cuttings with root (at least 0.1 cm) and sprout were measured. The total number of cuttings planted was considered when calculating the rooting percentage. Height was measured using a ruler from stem ground level to apical bud. The stem diameter was determined at the substrate level with digital caliper (precision of 0.01 mm). The plants were then sectioned into aerial and root sections. The roots were washed, and the NR was visually quantified by counting the roots that grow at the base of the cuttings, followed by LLR measurement using a millimeter ruler. Subsequently, the aerial part and roots were weighed in a precision analytical balance (0.001g) to obtain shoot and root fresh mass. In sequence, the aerial part and roots were packed in paper packages and placed to dry in an oven with forced air circulation at 65°C for 72 hours and weighed on an analytical balance (0.001 g) to obtain shoot and root dry mass.

2.5 Experiment III: Rooting events in hardwood propagules

To better understand the events associated with the poor rooting rate of hardwood shoots of *Cordia oncocalyx* cuttings, we set up an experiment to analyze the rooting events over 40 days with propagules at 150-day-old. After preparation, the cuttings had their bases (2 cm) dipped in a solution of IBA at a concentration of 8.000 mg.L⁻¹.

The experiment was carried out in a randomized block design, with three replications of 40 cuttings per plot, with 10 propagules being analyzed per replication in each evaluation. The sample collection was performed at 0 (following excision), 10-, 20-,

30- and 40-days post-excision. During each evaluation, the cuttings were removed from the substrate and analyzed visually the following characters: rooting, presence of callus, oxidation, size of the longest root and number of roots growing from the cutting base. Cuttings with a visible induction of root primordia at least 1 mm in length at the base of the propagule were considered rooted. The calluses consisted of undifferentiated cells at the base and the oxidations was characterized as tissue necrosis. The total number of cuttings per repetition was considered when calculating the rooting, callus, and oxidation percentages. The evaluation of the roots was carried out through visual and digital image analysis. At the visual, the size of the longest root was measured using a millimeter ruler and number of roots growing from the cutting base was visually quantified.

For the evaluation of the roots by digital image analysis, the bases of the cuttings were detached and distributed on white paper with a ruler graduated in millimeters as a reference metric and photographed with a digital camera (12 MP lens) attached to a height of 30 cm. The images were treated and processed with the help of the Image J version 1.46 software (Félix et al., 2020). The characters analyzed were area (mm²), perimeter (mm), number and length of the root.

2.6 Statistical analysis

The assumptions of data normality and homogeneity of variances were tested using the Shapiro–Wilk and Bartlett tests, respectively. When necessary, Box-Cox transformations were applied to meet these assumptions.

For Experiment I, data were analyzed using two-way ANOVA, considering the factorial arrangement of three propagule ages and three IBA concentrations. For Experiment II, one-way ANOVA was used to evaluate the effect of three IBA concentrations (0, 2000, and 8000 mg.L⁻¹) on rooting-related variables. When the F-test indicated significant effects ($p < 0.05$), means were compared using Tukey's test at the 5% significance level.

In Experiment III, rooting (%), callus formation (%), and oxidation (%) were subjected to analysis of variance at a 5%

significance level, and regression analysis was performed to assess the effect of time (days), selecting the best-fitting function for each variable based on the distribution of the data. Additionally, root length (cm.cutting⁻¹), number of roots (unit.cutting⁻¹), and root area (mm².cutting⁻¹) were analyzed using one-way ANOVA. All analyses were performed using R statistical software (R Core Team, 2024).

3. RESULTS

3.1 Influence of seasonal conditions and IBA on cutting survival and root development

Our initial objective was to understand the impact of propagule age (30, 90, and 150 days) on the survival and rooting success of cuttings. A significant interaction was observed between propagule age and IBA concentration (0, 2000, and 8000 mg.L⁻¹) for both survival (%) and rooting (%) ($p < 0.05$; Table 1).

Our data illustrate that, as expected, all treatments exhibited a 100% survival rate for 30-day-old propagules, with no significant differences between the control (0 mg.L⁻¹) and IBA-treated groups (Figure 3a). As propagule age increased, survival rates decreased across all treatments. In 90-day-old propagules, no significant differences in survival were observed between the control and IBA-treated groups. However, in 150-day-old propagules, both the control and 8000 mg.L⁻¹ treatments had the lowest survival rates. Overall, survival rates were not significantly improved by IBA treatment and declined notably as propagules aged, except at 150 days, which showed better survival at 2000 mg.L⁻¹.

In contrast, rooting response was significantly influenced by IBA concentration (Figure 3b). Thirty-day-old propagules treated with 2000 mg.L⁻¹ and 8000 mg.L⁻¹ IBA had significantly higher rooting percentages compared to the control ($P < 0.05$). This positive effect of IBA on rooting, however, diminished with increasing propagule age. In 90-day-old propagules, rooting percentages decreased in all treatments, with no significant differences between IBA concentrations. For 150-day-old propagules, rooting percentages were significantly lower in the control group, with 8000 mg.L⁻¹ presenting the lowest values ($P < 0.01$), with the exception of the 200 mg.L⁻¹ concentration, which promoted a small increase in rooting. These results indicate that, although IBA effectively promotes rooting in juvenile propagules (30 days), its effectiveness decreases as the propagules mature, suggesting limited benefits for older plant material.

3.2 Cuttings as a technique to multiply *Cordia oncocalyx*

One vital strategy to enhance the quality of plants propagated by cutting is the acclimatization and rustication of the cuttings after rooting. The application of IBA at varying concentrations significantly influenced several growth characters in *Cordia oncocalyx* cuttings (Table 2). However, IBA concentrations did not have a significant effect ($P = 0.26$) on the number of sprouts per cutting.

Survival and rooting rates were significantly higher at IBA concentrations of

Table 1. Two-way ANOVA to rooting (%) and survival (%) of *Cordia oncocalyx* (Allemão) cuttings subjected to propagules age (30, 90 and 150 – days) and to IBA concentrations (0 - control; 2000; and 8000 mg.L⁻¹). The numbers in table indicate the P-value

Tabela 1. ANOVA bidirecional para enraizamento (%) e sobrevivência (%) de estacas de *Cordia oncocalyx* submetidas à idade dos propágulos (30, 90 e 150 – dias) e às concentrações de AIB (0 - controle; 2000; e 8000 mg.L⁻¹). Os números na tabela indicam o valor P

Effect	P-value	
	Survival	Rooting
Propagules age	<0.0001	<0.0001
IBA concentrations	0.0025	<0.0001
Propagules age: IBA concentrations	<0.0001	<0.0001

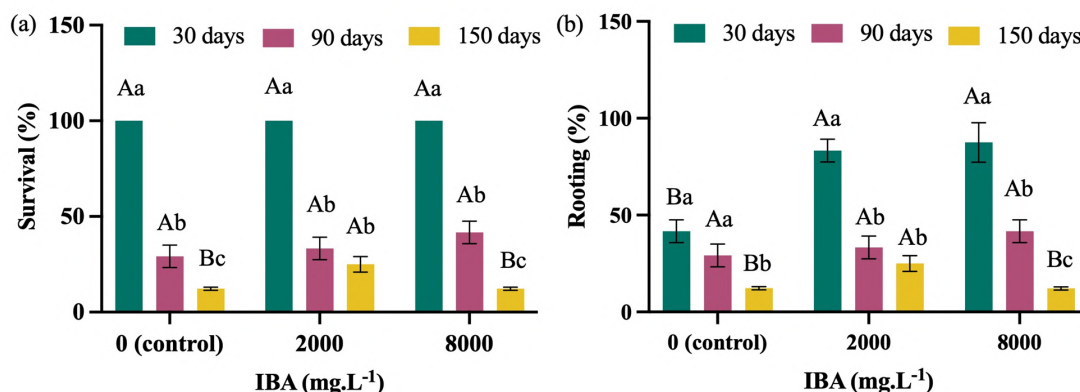


Figure 3. (a) Average of survival (%) and (b) rooting (%) in *Cordia oncocalyx* (Allemão) cuttings collected in 3 ages and treated with different IBA concentrations. Means followed by the same letters do not differ significantly, by the Tukey test at the 5% significance level. Different lowercase letters indicate differences between propagules ages (30, 90 and 150 – days) at the same IBA level, and uppercase letters indicate differences between IBA level (0-control, 2000 and 8000 mg.L⁻¹) at the same collection time. Error bars indicate standard error of the mean (SEM)

Figura 3. (a) Média de sobrevivência (%) e (b) enraizamento (%) em estacas de *Cordia oncocalyx* (Allemão) coletadas em 3 idades e tratadas com diferentes concentrações de AIB. Médias seguidas pelas mesmas letras não diferem significativamente, pelo teste de Tukey ao nível de significância de 5%. Diferentes letras minúsculas indicam diferenças entre as idades dos propágulos (30, 90 e 150 – dias) no mesmo nível de AIB, e letras maiúsculas indicam diferenças entre os níveis de AIB (0-controle, 2.000 e 8.000 mg.L⁻¹) no mesmo horário de coleta. As barras de erro indicam o erro padrão da média (SEM)

Table 2. One-way ANOVA to survival (S, %), rooting (R, %), height (H, cm.cutting⁻¹), stem diameter (SD, mm.cutting⁻¹), number of roots (NR, unit.cutting⁻¹), root length (RL, cm.cutting⁻¹), number of sprouts (NS, unit.cutting⁻¹), sprout height (SH, cm.cutting⁻¹), fresh mass of the aerial part (FMAP, mg.cutting⁻¹), fresh mass of the root (FMR, mg.cutting⁻¹), dry mass of the aerial part (DMAP, mg.cutting⁻¹) and dry mass of the root (DMR) in *Cordia oncocalyx* (Allemão) cuttings, after rustication, subjected to different IBA concentrations (0 - control; 2000; and 8000 mg.L⁻¹). The numbers in table indicate the P-value

Tabela 2. ANOVA unidirecional para sobrevivência (S, %), enraizamento (R, %), altura (H, cm.estaca⁻¹), diâmetro do caule (SD, mm.estaca⁻¹), número de raízes (NR, unidade.estaca⁻¹), comprimento da raiz (RL, cm.estaca⁻¹), número de brotos (NS, unidade.estaca⁻¹), altura dos brotos (SH, cm.estaca⁻¹), massa fresca da parte aérea parte (FMAP, mg.corte⁻¹), fresco massa da raiz (FMR, mg.estaca⁻¹), massa seca da parte aérea (DMAP, mg.estaca⁻¹) e massa seca da raiz (DMR) em estacas de *Cordia oncocalyx* (Allemão), após rusticação, submetidas a diferentes AIB concentrações (0 - controle; 2.000; e 8.000 mg.L⁻¹). Os números na tabela indicam o valor P

P-value											
S	R	H	SD	RN	RL	NS	SH	FMAP	FMR	DMAP	DMR
1 ⁻⁴	4.9 ⁻³	4.9 ⁻³	0.04	3 ⁻⁴	1 ⁻⁴	0.26	2 ⁻³	0.03	0.04	0.04	1.4 ⁻³

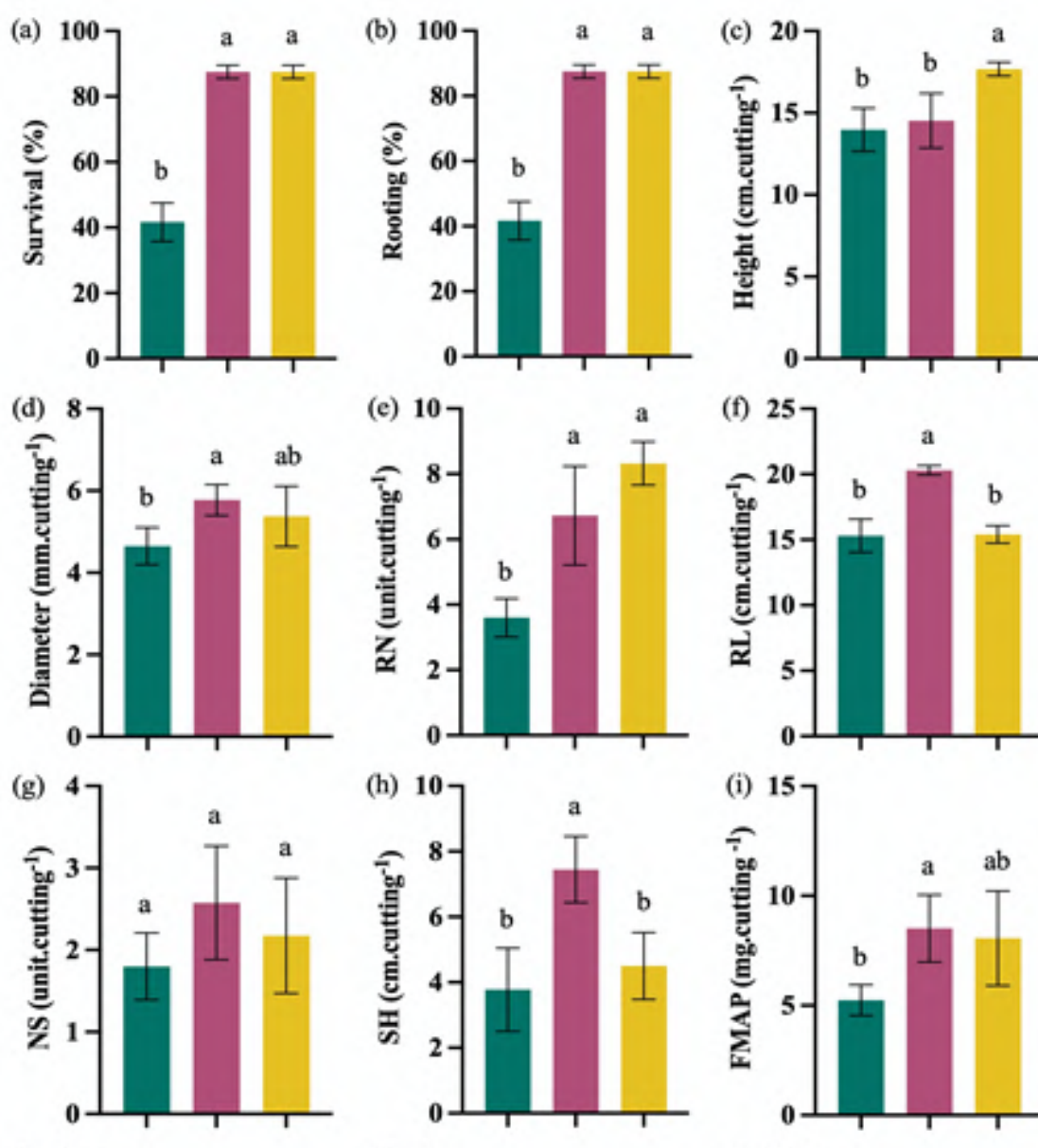
2000 mg.L⁻¹ and 8000 mg.L⁻¹, reaching nearly 90% compared to 40% in the control treatment (0 mg.L⁻¹) (Figure 4a, b). While plant height was greatest, up to 15 cm per

cutting, at 8000 mg.L⁻¹ IBA (Figure 4c), stem diameter was maximized in cuttings treated with 2000 mg.L⁻¹ IBA compared to the control (Figure 4d). Sprout height was

also significantly improved at 2000 mg.L⁻¹ (Figure 4h), suggesting that this concentration positively impacts early shoot development.

Root development showed a pronounced response to IBA application, with 2000 mg.L⁻¹ and 8000 mg.L⁻¹ concentrations producing significantly more roots (Figure 4e). Additionally, 2000 mg.L⁻¹ produced longer root systems than both the control and 8000 mg.L⁻¹ treatments (Figure 4f). Biomass accumulation followed a similar trend: the

fresh and dry mass of both roots and aerial parts was significantly greater in cuttings treated with 2000 mg.L⁻¹ IBA compared to the control (Figure 4i-l). In contrast, higher concentrations (8000 mg.L⁻¹) did not enhance biomass accumulation and, in some cases, produced results comparable to the control, indicating a potential inhibitory effect at elevated IBA levels. Notably, cuttings treated with 2000 mg.L⁻¹ IBA accumulated over 0.4 mg of root dry mass per cutting, the highest value among all treatments.



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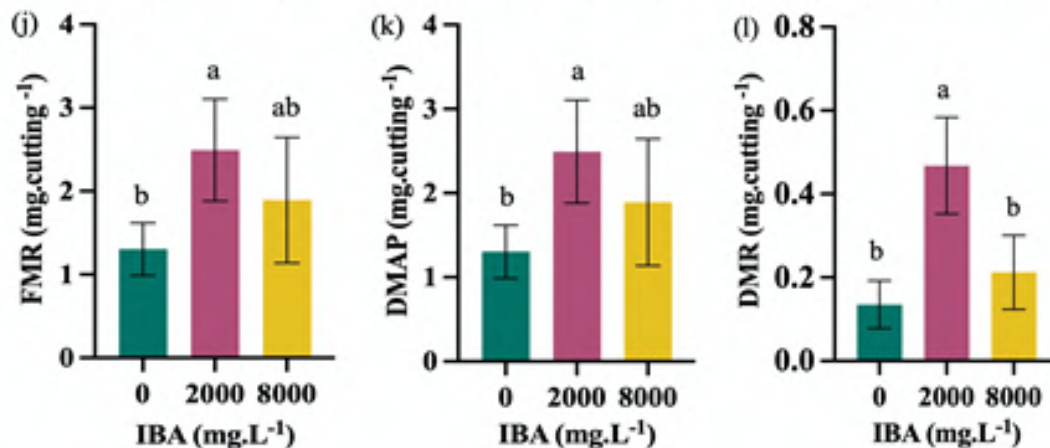


Figure 4. (a) Survival (%), (b) rooting (%), (c) height (cm.cutting⁻¹), (d) stem diameter (mm.cutting⁻¹), (e) number of roots (NR, unit.cutting⁻¹), (f) root length (RL, cm.cutting⁻¹), (g) number of sprouts (NS, unit.cutting⁻¹), (h) sprout height (SH, cm.cutting⁻¹), (i) fresh mass of the aerial part (FMAP, mg.cutting⁻¹), (j) fresh mass of the root (FMR, mg.cutting⁻¹), (k) dry mass of the aerial part (DMAP, mg.cutting⁻¹) and (l) dry mass of the root (DMR, mg.cutting⁻¹) in *Cordia oncocalyx* (Allemão) plantlets, after rustication, subjected to different IBA concentrations (0 - control; 2000; and 8000 mg.L⁻¹). Means with the same lowercase letter do not differ by Tukey's test at the 5% significance level. Error bars indicate in standard error of the mean

Figura 4. (a) Sobrevivência (%), (b) enraizamento (%), (c) altura (cm.estaca⁻¹), (d) diâmetro do caule (mm.estaca⁻¹), (e) número de raízes (NR, unidade.estaca⁻¹), (f) comprimento da raiz (RL, cm.estaca⁻¹), (g) número de brotos (NS, unidade.estaca⁻¹), (h) altura dos brotos (SH, cm .cutting⁻¹), (i) massa fresca da parte aérea (FMAP, mg.estaca⁻¹), (j) massa fresca da raiz (FMR, mg.estaca⁻¹), (k) massa seca da parte aérea (DMAP, mg.estaca⁻¹) e (l) massa seca da raiz (MSR, mg.estaca⁻¹) em mudas de *Cordia oncocalyx* (Allemão) , após rusticação, submetidas a diferentes concentrações de AIB (0 - testemunha; 2000; e 8000 mg.L⁻¹). Médias com a mesma letra minúscula não diferem entre si pelo teste de Tukey ao nível de significância de 5%. Barras de erro indicam o erro padrão da média

3.3 Rooting events in hardwood propagules

Rooting success increased progressively over time, showing a marked rise around day 30 and peaking at 28% on day 40, with high statistical significance ($P < 0.0001$) (Figure 5a). Callus formation followed a distinct temporal pattern, beginning to increase around day 10 (20%), reaching a peak of approximately 70% on day 20, and then declining by day 30 ($P < 0.01$) (Figure 5b). Oxidation levels also showed a significant trend ($P < 0.01$), increasing steadily over time, peaking at 30% on day 30, and then slightly decreasing by day 40 (Figure 5c). Overall, these results highlight distinct timelines for rooting, callus formation, and oxidation, with callus formation peaking earliest, followed by rooting success, and oxidation reaching its highest level at day 30.

We analyzed the root development characteristics of *Cordia oncocalyx* propagules using both visual and digital methods at two time points (30 and 40 days), focusing on root length, root number, and root area. Earlier time points (0, 10, and 20 days) were not included in this analysis, as no adventitious roots were observed in any of the cuttings during these periods. Therefore, measurements for root-related traits were only recorded from 30 days onwards, when rooting effectively began. Both analysis methods showed an increase in root length from day 30 to day 40, with root length reaching a maximum observed value of 6 cm per cutting by day 40 (Figure 6a). The number of roots increased at 40 days (Figure 6b). The similarity in measurements obtained from visual and digital methods indicates a

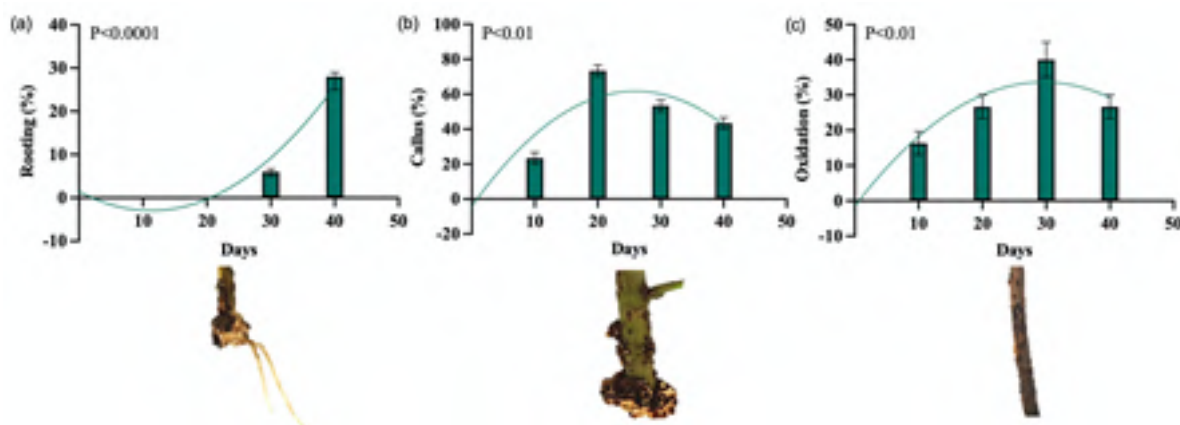


Figure 5. (a) Rooting (%), (b) callus (%), and (c) oxidation (%) in *Cordia oncocalyx* (Allemão) propagules at 150-day-old, relative to the days in greenhouse. Error bars indicate SEM

Figura 5. (a) Enraizamento (%), (b) calo (%) e (c) oxidação (%) em propágulos de *Cordia oncocalyx* (Allemão) aos 150 dias de idade, em relação aos dias em casa de vegetação. Barras de erro indicam SEM

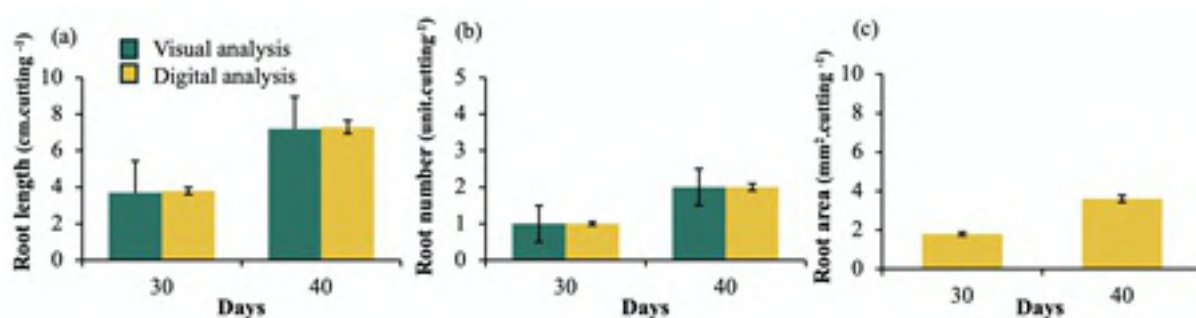


Figure 6. (a) Root length (cm.cutting⁻¹), (b) Root number (unit.cutting⁻¹), and (c) root area (mm².cutting⁻¹) in *Cordia oncocalyx* propagules at 150-day-old, relative to visual analysis and digital analysis. Error bars indicate in standard error of the mean. Evaluations at 0, 10, and 20 days after cutting were not included in the figure because no adventitious root formation was observed at those time points

Figura 6. (a) Comprimento da raiz (cm.estaca⁻¹), (b) Número da raiz (unidade.estaca⁻¹) e (c) área radicular (mm².estaca⁻¹) em propágulos de *Cordia oncocalyx* aos 150 dias -antigo, relativo à análise visual e à análise digital. Barras de erro indicam o erro padrão da média. As avaliações realizadas em 0, 10 e 20 dias após a estaquia não foram incluídas na figura, pois não foi observada formação de raízes adventícias nesses intervalos de tempo

high consistency between these approaches, with a slight bias for visual analysis to overestimate or provide higher values, as we can see at error bars (Figure 6a, b). Root area measurements, available only through digital analysis, showed an increase from day 30 to day 40 (Figure 6c). These findings suggest a significant phase of root growth occurring between these time points, especially in terms of length and area.

4. DISCUSSION

4.1 Influence of seasonal conditions and IBA on cutting survival and root development

The results indicate that while IBA promotes rooting in juvenile propagules, its effectiveness declines as the propagules mature. IBA treatment did not significantly improve survival rates, which decreased as the propagules matured, especially by shoots

collected at 150 days. The low rooting and survival rates observed in shoots collected at 90 and 150 days (during the end of the rainy season and the start of the dry season) can be explained by two main factors: increased maturity of the propagules and reduced water available, which affects sprout vigor. In contrast, shoots collected at the beginning of the rainy season (30 days old) showed a much greater tendency to root successfully.

As a deciduous plant, *C. oncocalyx* loses its leaves during the dry season but begins to regrow leaves when the rains return (Mendes et al., 2013). At the time of 30-day shoot collection, early in the rainy season, the trees had young, herbaceous, and vigorous sprouts. These juvenile traits likely contributed to the higher survival and rooting percentages observed. In many plant species, the best rooting rates occur during specific periods that align with active vegetative growth (Stuepp et al., 2015). The degree of lignification in the primary phloem of mature cuttings limits root formation, as it hinders the differentiation of root primordia. These critical tissues become incapable of dedifferentiating efficiently under auxin treatment, drastically reducing rooting capacity. This behavior has been observed in species such as *Campomanesia phaea* stems and *Prunus mume* Sieb. et Zucc. (Santoro et al., 2022; Muniandi et al., 2022; Wang et al., 2025). In contrast, the sprouts collected later in the season, at 90 and 150 days, were already lignified and mature, which likely explains the lower rooting rates for these collection times.

The development of adventitious rooting and tissue differentiation has been inversely linked to the xylogenesis programme in forest species such as *Zizyphus jujuba* (Shao et al., 2018), *Acca sellowiana* (Ross et al., 2021), *Ilex paraguariensis* (Pimentel et al., 2021) and *Eucalyptus urophylla* (Masullo et al., 2022), that is, more lignified cuttings present greater difficulties in root formation than herbaceous cuttings (Paiva & Gomes, 1995). Less lignified cuttings may have higher rooting rates due to a higher concentration of promoters, proximity to auxin synthesis sites, and less tissue differentiation (Hartmann et al., 1997). Lignified tissues lose flexibility, hindering

cell division and callus formation, which are necessary for adventitious root initiation. Furthermore, the presence of lignin reduces the absorption and transport of hormones such as IBA, compromising their action in root induction (Boerjan et al., 2003). Thus, the timing of cutting collection is closely linked to the plant's hormonal balance, maturity, and lignification level (Fachinello et al., 1995). Together, these factors they can explain the low rooting in shoots collected at 90 and 150 days.

Furthermore, the use of IBA at 2000 mg.L⁻¹ increased rooting percentages in propagules collected at 30 days, at the beginning of the rainy season. This result aligns with the established role of auxins, which are known to be effective in promoting rooting (Tate & Page, 2018). However, later in the season (end of the rainy season and start of the dry season), IBA did not significantly improve rooting in cuttings. This may be due to increased tissue maturity, which reduces the effectiveness of auxins (Fachinello et al., 1995; Masullo et al., 2022). Therefore, in older propagules (90 and 150 days), endogenous auxin levels were likely not the primary factor limiting rooting.

In addition to the collection period and the use of IBA, the temperature is another important factor that plays an essential regulatory role in plant metabolism and contributes to rooting. Temperature can influence several aspects of the rooting process, from the growth rate of the stock plants to the root development of the cuttings (Moe & Andersen, 1988). Woody species subjected to low temperatures go to a state of dormancy to protect their meristems, reducing activity in the young tissues of the secondary phloem, such change leads to an increase in the synthesis of phenolic and inhibitory compounds, reducing and ceasing its growth temporarily (Wit et al., 2014). The Brazilian semi-arid region has not shown considerable thermal variation, and the average temperatures ranged from 27.0 °C to 28.9 °C during this study (Figure 1) leading to a temperature ranged from 30 °C to 35 °C at the greenhouse, evidencing that the temperature is not a limiting factor to the vegetative propagation in this region.

In summary, these findings indicate that the maturity of propagules strongly affects



rooting success. For effective vegetative propagation in the semi-arid region of this species, shoots should be collected at the beginning of the rainy season, when propagules are younger and more vigorous. These younger propagules have significantly higher rooting rates compared to those collected later in the season. Optimal environmental conditions, such as sufficient light, stable temperature, and water availability, also enhance rooting by supporting auxin and carbohydrate metabolism (Xavier et al., 2021; Gomes et al., 2022). Overall, IBA appears to be more effective at promoting rooting in juvenile propagules rather than in ensuring long-term survival under the tested conditions.

4.2 Cuttings as a technique to multiply *Cordia oncocalyx*

Our results demonstrate that IBA at a concentration of 2000 mg/L effectively promotes rooting in cuttings, leading to better development in both the aerial parts and root systems. The need for exogenous auxin application suggests that *C. oncocalyx* cuttings produce insufficient quantities of endogenous auxins to achieve optimal rooting on their own. This aligns with findings by Masullo et al. (2022), who noted that exogenous IBA application encourages protein breakdown, mobilizes stored sugars from leaves to stems, and stimulates the synthesis of endogenous IAA. Together, these processes facilitate de novo root regeneration through multiple physiological pathways.

The IBA, as the other auxins, in adequate concentrations, promotes the extrusion of hydrogen protons out of the cell, acidifying and, consequently, loosening the cell walls. In sequence, the entrance of water into these cells causes their elongation and starts cell division (Taiz et al., 2016). However, we observed that IBA concentrations above 2000 mg/L reduced both survival and rooting. This may result from disruptions in auxin homeostasis associated with phase changes in the plant tissues, which can negatively impact adventitious rooting (Rasmussen et al., 2014). High doses of IBA have also been reported to promote lignification and

secondary growth, further inhibiting rooting in cuttings, this effect being observed in *Pterocarya fraxinifolia* (Poiret) Spach (Hartmann et al., 2011; Wendling et al., 2015; Çetin & Bas, 2025). Thus, IBA can either stimulate or inhibit tissue growth and differentiation depending on; the concentration, with an optimal level required for desired physiological responses (Shao et al., 2018). Thus, an adequate endogenous and exogenous auxin balance is needed for root stated in cuttings of *C. oncocalyx*.

Our findings also indicate that treatments with IBA produced a greater number of roots and increased root fresh and dry mass, which in turn supported better development of the aerial parts. The enhancement of the root system is likely due to IBA's role as a rooting inducer, which accelerates root formation and promotes the development of a larger number of roots with greater length and volume (Costa et al., 2013; Hartmann et al., 2011; Rehana et al., 2020). A well-developed root system improves water and nutrient absorption, leading to more vigorous growth of the aerial parts, which is essential for successful establishment and growth in the field (Silva et al., 2012).

These results suggest that 2000 mg/L is an optimal concentration of IBA for promoting both root quantity and quality, which are critical for nutrient and water uptake in *C. oncocalyx* cuttings. The substantial increase in root fresh and dry mass at this concentration supports the conclusion that IBA at 2000 mg/L effectively promotes a robust root system, contributing to the overall health and development of the plant. This finding reinforces the potential of vegetative propagation via cuttings as a viable strategy for *C. oncocalyx*, a species that faces challenges in seedling production (Araújo et al., 2022).

4.3 Rooting events in hardwood propagules

The physiological responses of *Cordia oncocalyx* propagules over time in a greenhouse setting reveal significant temporal patterns in rooting, callus formation, and oxidation. These results offer insights into the optimal conditions and timeframes for successful propagation. The finding suggests that hardwood propagules of

Cordia oncocalyx requires a remarkable period of tissue induction before initiating significant root development. The observed rooting peak around day 40 implies that physiological and cellular changes conducive to rooting become fully active at this period, underscoring days up to 40 as a critical timeframe for optimal rooting. Therefore, it is recommended that the propagules remain in a greenhouse environment for at least 45 days, ensuring maximum expression of rooting potential and reducing losses due to oxidation or excessive callus.

The delay in the rooting process and the need for an extended stay in the propagation environment may be related to tissue maturation and lignin content, which is influenced by the time of collection of the propagules. Juvenile plant material has a higher rooting capacity than adult material due to physiological conditions, such as hormonal balance, that favor rooting (Hartmann et al., 2011; Stuepp et al., 2018).

The rooting time and duration of propagules in a greenhouse vary from species to species, depending on genetic control, anatomical barriers, and the degree of juvenility of the propagules (Brondani et al., 2012). Ferreira et al. (2004) concluded that the ideal rooting time for mini-cuttings of *Eucalyptus* spp. clones is between 20 to 30 days. In this context, knowing the optimal time to remove cuttings from the greenhouse optimizes the use of this structure, as well as reduces costs and the incidence of diseases, given that it decreases the exposure of propagules to humidity.

Also, callus formation often represents a preparatory stage for root differentiation, providing a mass of undifferentiated cells from which roots can develop. The peak at day 30, followed by a decline, suggests a physiological transition where the callus tissue may have provided the necessary cellular framework for subsequent root formation observed at day 40. This early peak indicates that callus formation is most active early in the propagation timeline.

The formation of adventitious roots can occur directly or indirectly. Direct formation involves root initiation near the vascular system, which is typical of species that root easily (Mhimdi & Pérez-Pérez, 2020). In indirect formation, root primordia initiate in callus tissues, connect to the vascular system,

and begin the root regeneration process (Fachinello et al., 2005; Ferriani et al., 2008). However, adventitious root formation and callus formation are independent processes, and their simultaneous occurrence may be due to cell division in both, which may depend on similar internal and environmental conditions (Hartmann et al., 2011).

Oxidation levels, measured as an indicator of tissue stress or metabolic change, increased over time. Oxidative stress often accompanies intense metabolic activity, including cellular differentiation and tissue restructuring. The peak oxidation at day 30 could be associated with the active rooting phase, suggesting that the physiological processes underpinning root development may also increase the metabolic demands or stress on the propagules.

Understanding these temporal physiological markers provides valuable insights for optimizing *Cordia oncocalyx* propagation. Monitoring callus formation around day 30 can serve as an indicator for the subsequent rooting phase, while recognizing the oxidative peak at day 30 as a potential marker for peak rooting activity. This timeline suggests that callus formation acts as a preparatory phase, creating a conducive environment for root initiation and growth. The peak in oxidation may reflect the increased metabolic activity required for rooting, potentially signaling the culmination of cellular differentiation processes.

The increase in root length over time confirms active root growth between 30 and 40 days (Figure 6a). The stable root number across both time points (30 and 40 days) suggests limited additional root initiation between days 30 and 40, implying that root formation may peak early in the propagation phase. Root initiation may have reached a plateau by day 30, with subsequent growth primarily focused on root elongation rather than the formation of additional roots.

The observed increase in root area in the digital data aligns with the root length expansion seen across both methods, suggesting an enlargement in the root system's absorptive capacity over time.

The visual and digital methods yield similar measurements, suggesting that both methods are reliable for assessing root length and root number, with a slight tendency for

visual analysis to overestimate or provide higher values. The use of digital image analysis to assess root number and length proved to be efficient and accurate, constituting a low-cost and applicable tool, minimizing operational errors and time spent analyzing root number and length of *C. oncocalyx* roots. Additionally, it provided other characters, such as area, which is essential for understanding root volume.

Digital image analysis has the potential to be used in determining seedling height with a high degree of efficiency and accuracy, minimizing the time required for testing (Medeiros et al., 2018). However, its use in root analysis of cuttings is still limited, and the present study demonstrates that this tool can be used precisely to obtain characteristics that aid decision-making in propagation nurseries.

Digital image analysis makes a significant contribution and holds a promising future in forestry, especially for nursery managers who produce seedlings on a large scale and need tools that optimize the evaluation process of cuttings as they leave the propagation environment.

5. CONCLUSION

C. oncocalyx is not a species with hard rooting, and the vegetative propagation by cutting is benefited when carried out with 30 days of propagules, collected in the rainy season using an IBA concentration of 2000 mg/L. However, the cutting technique can be an alternative to multiplied *C. oncocalyx* with quality and on a large scale, overcoming the problems found in the sexual propagation. Digital image analysis makes a significant contribution and holds a promising future to root analyze. In addition, in semi-arid areas, the definition of the period for propagules collection is an important factor for optimizing operational costs in plant propagation and obtaining plants in quantity and quality.

6. ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS

Araújo, J. K. P.: Conceptualization, Data Curation, Research, Project Administration, Visualization, Writing – Original Draft, Writing – Review & Editing; Santos, D. F.: Data Curation, Formal Analysis, Review; Araújo, J. S. O.: Data Curation, Review; Silva, F. E.: Data Curation; Freire, A. C.: Data Curation; Nicolau, J. P. B.: Data Curation; Pacheco, M. V.: Supervision, Data Curation, Review; Araújo, P. C. D.: Formal Analysis, Writing – Review & Editing.

DATA AVAILABILITY

The entire dataset supporting the findings of this study has been published within the article.

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