



Morphoanatomical aspects of adventitious rooting and the establishment of an efficient vegetative propagation protocol for *Mimosa ophthalmocentra*

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Received: 15 November 2025 / Accepted: 9 March 2026 / Published online: 20 April 2026
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Abstract

Mimosa ophthalmocentra is a native species of the Caatinga with high ecological and economic potential, particularly valued for its timber uses and suitability for the restoration of degraded areas. Although seed propagation is efficient, vegetative propagation offers advantages for multiplying superior genotypes and standardizing desirable traits. This study evaluated the effectiveness of the mini-cutting technique as a vegetative propagation method for the species, considering the effect of indole-3-butyric acid (IBA) and the position of the mini-cuttings (apical and basal) on rooting, as well as investigating the temporal dynamics and anatomical features of the rhizogenic process. The experiment was conducted in a completely randomized design in a 2×4 factorial scheme, using four IBA concentrations (0, 2000, 4000, and 6000 mg L⁻¹). Rooting dynamics were monitored for 25 days, with morphological assessments every five days, and morphoanatomical analyses performed on histological sections stained with Alcian Blue and Safranin. Basal mini-cuttings exhibited higher rooting percentage and vigor, whereas apical mini-cuttings showed greater survival, particularly under intermediate IBA concentrations. Rooting occurred via direct rhizogenesis, originating from the vascular cambium, without prior callus formation. The results demonstrate the high rhizogenic competence of the species, even in the absence of exogenous regulators, and highlight the potential of the mini-cutting technique for large-scale vegetative propagation of *M. ophthalmocentra*, contributing to its domestication and commercial production.

Keywords Caatinga · Jurema · De · Embira · Direct rhizogenesis · Indole · 3 · Butyric acid · Vegetative propagation · Mini · Cuttings

Introduction

The growing demand for seedlings of forest species for reforestation and sustainable management is a current reality in Brazil (Silva et al. 2017; Dutra-Silva et al. 2024). This expansion is driven by legislation regulating the preservation of native vegetation and environmental compliance, as well as by advances in silviculture in response to the increasing demand for forest products (Queiroz et al. 2024; Jiang et al. 2025). In 2023, more than 10.2 million hectares of planted forests were established in the country (IBÁ, 2024). However, despite this expansion, the silviculture of native species remains limited, hindering the development of large-scale production systems capable of supporting sectoral goals.

Seed propagation continues to be the most common method for producing seedlings of native forest species because of its simplicity, efficiency, and low cost. Nevertheless, in the Brazilian semiarid region, seedling production can be influenced by factors such as genetic variability among mother trees, environmental conditions during fruiting, collection timing, and the physiological quality of seed lots (Azevedo et al. 2018; Félix et al. 2021). This variability also makes it difficult to obtain phenotypically homogeneous plants, which are essential for breeding programs and commercial production programs (Leão et al. 2018). In addition, physical dormancy, which is widespread among native legume species, together with logistical constraints related to seed collection and storage, can further limit large-scale seedling production (Galíndez et al. 2016; Nativel et al. 2015).

In this context, vegetative propagation becomes a strategic alternative for ensuring the multiplication of selected genotypes and the establishment of uniform planting material for restoration and commercial purposes. However, according to Rolim et al. (2020), vegetative propagation of native species still represents a major challenge due to insufficient technical information, which restricts the establishment of efficient large-scale asexual propagation systems.

Mimosa ophthalmocentra Mart. ex Benth (Fabaceae), a species native to the Caatinga, exhibits notable hardiness, tolerance to water stress, and multiple timber and energetic uses (Leal et al. 2015). A distinctive characteristic of the species is the natural occurrence of thorned and thornless phenotypes. Thornless individuals offer clear silvicultural and forage advantages, making them highly valuable for domestication and selection programs (Freire et al. 2025). However, sexual propagation results in genetic segregation, meaning that thornlessness is not consistently expressed in seed-derived progenies (Menegatti et al. 2018). This underscores the importance of developing vegetative propagation techniques to reliably multiply selected genotypes.

Vegetative propagation through stem cuttings and mini-cuttings is a promising alternative for overcoming limitations associated with seed propagation and for enabling the multiplication of superior genotypes of native forest species, thus contributing to the conservation of forest genetic resources (Dias et al. 2012a, 2012b). Traditional cuttings support the fixation of desirable phenotypes, such as thornless, more productive, or more tolerant individuals, which can be directed toward specific productive systems. Mini-cuttings, in turn, can contribute to the maintenance of genetic diversity when shoots are collected from multiple genotypes, since each mini-cutting represents a clone of the donor plant (Dias et al. 2012a). This characteristic is particularly relevant for restoration programs, in which genetic heterogeneity enhances population resilience and adaptive potential (Whitlock 2014). Such

flexibility strengthens the potential of the mini-cutting technique for tree production in semiarid environments.

The optimization of vegetative propagation via mini-cuttings depends on an integrated understanding of shoot production, adventitious rooting, and early growth (Hartmann et al. 2018; Xavier et al. 2013). Shoot dynamics in the mini-stump directly determine the availability and quality of propagules and are closely linked to the physiological vigor of the mini-stumps, which is influenced by rejuvenation processes induced by successive pruning, carbohydrate reserves, nutritional status, and water availability (Wendling et al. 2014a, 2014b; Brondani et al. 2014). In addition, the endogenous hormonal balance, particularly the auxin – cytokinin ratio, plays a central role in regulating sprouting capacity and adventitious root formation (Wendling et al. 2015). These physiological mechanisms are critical for the efficiency of mini-cutting systems, especially in native woody species from semi-arid environments, where water stress can restrict sprouting and rooting (Felice et al. 2024; Freire et al. 2025; Araújo et al. 2025). In Fabaceae species, such as *Anadenanthera colubrina*, additional challenges are frequently reported, including reduced rooting competence associated with tissue lignification and phenolic compound accumulation, as well as species-specific sensitivity to auxin (Dias et al. 2012a, b).

The formation of adventitious roots is essential for vegetative propagation and is regulated by the ability of stem tissues to respond to physiological and environmental signals (Wendling et al. 2014b; Díaz-Sala 2020). This process results from the integration of hormonal, anatomical, and metabolic mechanisms that culminate in the formation of root primordia (Rickli et al. 2015). Cutting position influences rooting responses, with apical segments typically associated with higher juvenility and auxin levels, while basal segments have greater reserve accumulation (Wendling et al. 2014a; Stuepp et al. 2018). The application of indole-3-butyric acid (IBA) can enhance root initiation and growth (Hunt et al. 2011; Sekhukhune & Maila 2025), although its effectiveness depends on dose, cutting position, and tissue maturation. Moreover, rooting success also depends on the ability of competent cells to reprogram, mobilize reserves, and coordinate the induction, initiation, and differentiation phases of rhizogenesis (Xavier et al. 2013; Díaz-Sala 2020). The duration under controlled rooting conditions is also critical for ensuring that rooting phases occur in a coordinated manner, favoring morphophysiological development, biomass accumulation, and the final quality of the propagated material (Díaz-Sala 2020). Therefore, a deeper understanding of how physiological maturation, auxin sensitivity, and tissue position interact is fundamental for optimizing propagation protocols, especially in native woody species.

Despite the potential of the mini-cutting technique, no specific propagation protocols have been developed for *M. ophthalmocentra*, and studies evaluating the effects of IBA concentration, mini-cutting position, and rooting period on rhizogenic processes are lacking. In this context, this study aimed to optimize the production system of *M. ophthalmocentra* through vegetative propagation via mini-cuttings by evaluating shoot dynamics in the seminal mini-stump garden and assessing the effects of different IBA concentrations, mini-cutting positions (apical and basal), and rooting duration on morphophysiological development and biomass accumulation.

Materials and Methods

Seed Collection Sites and Procedures

Seeds were collected in a Certified Natural Seed Collection Area (ACS-NM) established specifically for the *M. ophthalmocentra* population, comprising 11 georeferenced mother trees located in the municipalities of Upanema, Açú, Apodi, and Olho d'Água dos Borges, in the state of Rio Grande do Norte, Brazil (Fig. 1).

These municipalities exhibit a BSh climate according to the Köppen classification, characterized as a hot semiarid climate (Alvares et al. 2013). The region has an average temperature of 27.4 °C, a mean relative humidity of 68.9%, and irregular annual precipitation averaging 673.9 mm, with a dry period lasting 6 to 8 months (Melo et al. 2020).

Certification was based on the selection of phenotypically superior and healthy trees, considering traits such as plant height (up to 10 m), stem diameter (up to 12 cm), phytosanitary condition, vigor, and seed production capacity. The absence of thorns was also considered a desirable phenotypic trait during mother tree selection; however, within the certified area, only four individuals did not present thorns. As a result, among the 11 selected mother trees, four were thornless and seven were thorned individuals.

A minimum distance of 100 m between mother trees was adopted, which is considered adequate for semiarid regions (Sena & Gariglio 2008). Seeds were collected at physiological maturity, processed, and stored in a cold chamber at 5 °C.

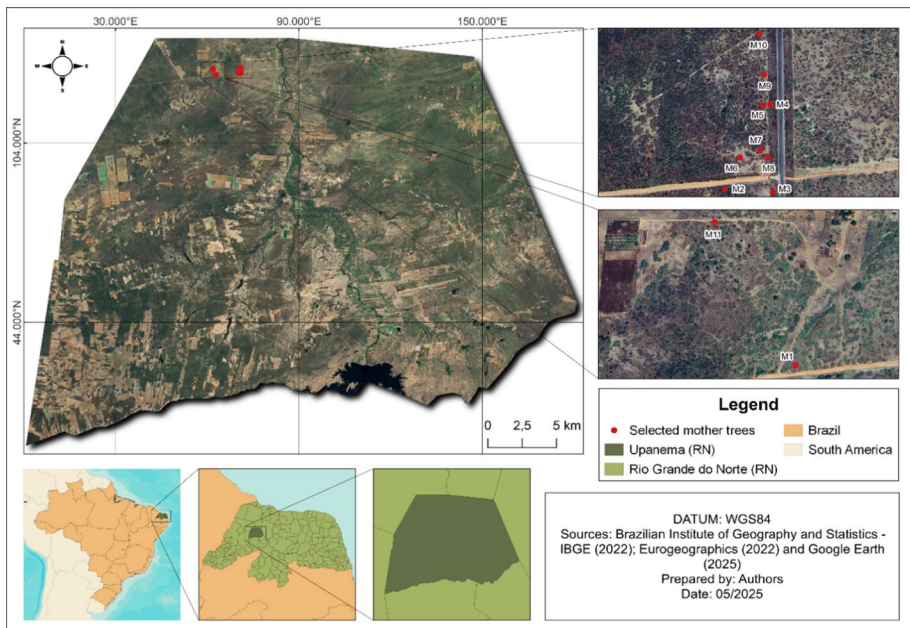


Fig. 1 Map of the state of Rio Grande do Norte showing the location of the municipalities where *Mimosa ophthalmocentra* seeds were collected for establishing the seminal mini-stump garden. Markers M1 to M11 correspond to the georeferenced mother trees. Source: Authors

Establishment of the Seminal Mini-Stump Garden

Seeds collected from the 11 selected mother trees were bulked to form a composite seed lot. From this mixed lot, a subsample of 500 seeds was taken and used for seedling production aimed at establishing the seminal mini-stump garden. Therefore, seedlings were not tracked by individual mother tree, and the experimental approach was designed to represent population-level variability rather than intra-family variation.

Seeds were manually clipped at the end opposite the hilum as a method to break physical dormancy (Nogueira et al. 2019; Freitas et al. 2018). They were then sown in polyethylene bags measuring 30 × 40 cm (width × height) with an approximate volume of 11.5 L, filled with a substrate composed of subsoil mixed with well-decomposed cattle manure in a 3:1 ratio. Sowing was carried out during the rainy season, from February to April, and seedlings were monitored under nursery conditions until reaching approximately 15 cm in height. Mini-cuttings were obtained from seminal mini-stumps established from seedlings at an early developmental stage, ensuring high juvenility.

Mini-stumps were formed 60 days after sowing, when plants were pruned at 10 cm above the collar to stimulate shoot production. These plants, which served as propagule sources (mini-stumps), were maintained under 50% shade and irrigated every two days with 500 mL of water per plant.

Plants were fertilized weekly with a nutrient solution prepared from a mixed mineral fertilizer for fertigation, containing macro- and micronutrients: nitrogen (N) at 2.4%, potassium oxide (K₂O) at 9%, iron (Fe) at 3.192%, manganese (Mn) at 1.040%, boron (B) at 1.04%, copper (Cu) at 0.66%, zinc (Zn) at 0.41%, nickel (Ni) at 0.13%, molybdenum (Mo) at 0.20%, and cobalt (Co) at 0.05%.

After shoot emergence, shoots were counted and measured for length. Systematic harvesting was performed every 15 days over 90 days. Shoots were collected by cutting approximately 1 cm above their base, maintaining the mini-stump training system in a bowl-shaped structure to improve aeration and promote balanced development of subsequent shoots (Fig. 2).

Experimental Design and Shoot Productivity Assessment

The experiment was conducted in a completely randomized design with three replications, each experimental unit consisting of 16 mini-stumps pruned at regular 15-day intervals to stimulate shoot production. At each evaluation time, the temporal dynamics of mini-stump performance were assessed by recording survival, shoot length, and shoot productivity. Shoot productivity was expressed as the number of shoots per square meter, calculated based on the planting density derived from the area occupied by each polyethylene bag. The area corresponding to each mini-stump was defined according to the surface area occupied by the container, allowing standardization of shoot density estimates (shoots m⁻²) across evaluation periods.

Propagule collection and measurements were standardized over time by maintaining consistent pruning intensity, evaluation intervals, and sampling procedures at each date. Regression analyses were performed to evaluate the relationships between evaluation time (days after pruning) and the measured variables (shoot length and number of shoots per



Fig. 2 Management of the seminal mini-stump garden of *Mimosa ophthalmocentra*. **a** Mini-stump trained in a bowl-shaped structure after shoot collection; **b** Mini-stumps showing vigorous shoots at the appropriate stage for a new harvest. Source: Authors

square meter). In addition, Spearman's correlation analysis was used to assess relationships among evaluation time, shoot number, and shoot length.

Effect of IBA on Adventitious Rooting

Shoots were collected from the seminal mini-stump garden following the methodology proposed by Dias et al. (2012a, b) and adapted by Souza et al. (2022). Shoots were standardized to 13 cm in length, each retaining a pair of fully expanded leaves. Mini-cuttings were classified into two types according to their position on the shoot: apical, corresponding to the upper portion containing the apical bud, and basal, originating from the lower half of the shoot. In addition to the mini-cutting position, the effect of different concentrations of the growth regulator indole-3-butyric acid (IBA) on adventitious rooting was evaluated.

The experiment was conducted in a completely randomized design (CRD) with a 2×4 factorial arrangement, consisting of two mini-cutting positions (apical and basal) and four IBA concentrations (0, 2000, 4000, and 6000 mg L⁻¹). Three replicates were used, each containing 10 mini-cuttings, totaling 30 propagules per treatment.

IBA was first dissolved in a 1% potassium hydroxide (KOH) solution and subsequently diluted in distilled water. The bases of the mini-cuttings were immersed in the respective solutions for 10 s. For the control treatment, the bases were immersed only in distilled water for the same duration. Mini-cuttings were then placed in 55 cm³ polyethylene tubes filled with a commercial substrate consisting of 70% sphagnum peat and 30% vermiculite, supplemented with limestone, with a pH of 5.5, an electrical conductivity of 0.7 mS cm⁻¹, density of 130 kg m⁻³, water holding capacity of 300%, and a maximum moisture of 60%.

Mini-cuttings were maintained for 30 days in a mini-greenhouse equipped with an automated irrigation system using micro-sprinklers delivering 10 L h^{-1} , programmed to operate every 30 min for 30 s over a 12-h daily cycle. Environmental conditions inside the structure were monitored, maintaining a temperature between 26°C and 30°C and a relative humidity of around 80%. Afterward, mini-cuttings were transferred to a 50% shade house for 10 days for acclimatization.

After 40 days, the following variables were evaluated: shoot height (cm), root length (cm), number of roots ($\text{unit.mini-cutting}^{-1}$), fresh and dry mass of shoots and roots (g), and rooting percentage (%).

Adventitious Rooting Dynamics

To assess the dynamics of adventitious rooting, shoots were collected from the mini-stump garden and sectioned into apical and basal mini-cuttings, each standardized to 13 cm in length and retaining a pair of fully expanded leaves. The experiment followed a completely randomized design (CRD) with two treatments corresponding to mini-cutting positions (apical and basal) and three replicates. All evaluations were based on destructive sampling to avoid repeated measurements on the same propagules.

The base of each mini-cutting was immersed in a 2000 mg L^{-1} IBA solution for 10 s, then placed in the substrate and maintained in a mini-greenhouse under controlled conditions (temperature, relative humidity, and shading as described in the previous section). Every 5 days over a 25-day period, 12 mini-cuttings per treatment (four per replicate) were destructively sampled at each evaluation time. Sampling was performed on days 5, 10, 15, 20, and 25 after staking, resulting in a total of 60 mini-cuttings per treatment evaluated over the course of the experiment. Mini-cuttings were washed under running water and assessed for the occurrence of swelling, characterized by visible thickening at the base; callus formation, defined as scar tissue or disorganized parenchymatous masses at the base; and rooting, identified by the presence of root primordia exceeding 2 mm in length. For rooted propagules, the number of roots and the average root length (RL) were also measured. Root length was calculated as the mean length of all roots per mini-cutting, measured from the base to the apex of each root, yielding a single average value per propagule.

After morphological characterization, morphoanatomical analyses were performed on 10 mini-cuttings per position (apical and basal), previously preserved in glass vials containing 70% ethanol (v/v). For each sample, five semipermanent histological slides were prepared from freehand transverse sections made with a scalpel blade approximately 2 mm above the base of the mini-cuttings or at the point of root emergence when present.

Histological samples were bleached in a diluted sodium hypochlorite solution (1:1 v/v with distilled water) to remove natural pigments. Sections were then rinsed twice in distilled water and subjected to sequential staining with 1% Alcian Blue (w/v) and 1% Safranin (w/v) for 30 s. After staining, samples were transferred to a Petri dish containing acidified 80% ethanol (v/v) for dye fixation, followed by immersion in absolute ethanol (100% v/v) for 60 s to remove excess stain. Sections were subsequently hydrated in distilled water and mounted on slides using 50% glycerin (v/v), sealed with clear nail polish, following the protocol adapted from Kraus and Arduin (1997).

Morphoanatomical observations were carried out using an optical microscope (Olympus CX21) equipped with a digital camera (Sony Cyber-Shot, 14.1 megapixels). The analy-

ses allowed identification of anatomical patterns associated with the rooting process, with emphasis on the origin of cell dedifferentiation and the formation of root primordia.

Statistical Analyses

The Shapiro–Wilk normality test indicated violation of normality assumptions for most variables ($p < 0.05$), justifying the use of nonparametric statistical procedures for all analyses.

To investigate shoot number and shoot length dynamics over time in the seminal mini-stump garden and the temporal dynamics of adventitious rooting of apical and basal mini-cuttings, the Friedman test for repeated measures was applied, followed by Dunn's test with Holm correction to identify significant temporal differences. Associations between quantitative variables were assessed using Spearman's correlation. Nonlinear regression models were also fitted to temporal data to describe growth trends, and coefficients of determination (R^2) were calculated to evaluate model fit.

For comparisons among independent groups, such as different IBA concentrations, the Kruskal–Wallis test was applied followed by Dunn's post hoc test to identify statistically distinct treatments. Additionally, the Mann–Whitney test was used to evaluate the effect of mini-cutting position (apical or basal) within each IBA concentration, allowing direct comparison between positions for each morphological variable. This approach enabled the identification of interactions between cutting position and growth regulator even in the absence of parametric assumptions. Nonlinear regression models were fitted to IBA concentrations, and coefficients of determination (R^2) were calculated to evaluate model fit.

All analyses were performed in R software (version 4.7.1) using a 5% significance level ($= 0.05$).

Results

Mini-Stump Garden Production

The statistical analysis evidenced significant temporal variation in shoot production and shoot length of seminal mini-stumps of *M. ophthalmocentra* over the 90-day experimental period (Fig. 3). No mortality of mini-stumps was observed during the experimental period, and all plants remained alive throughout the 90 days of evaluation. Considering the mean number of shoots per square meter, obtained by extrapolation from individual matrices, a significant effect of evaluation time on this variable was observed (Table S1; Friedman test: $\chi^2(5) = 819.84$, $p < 0.001$).

The number of shoots per m^2 differed significantly among the evaluated periods (Fig. 3a; Table S1). The highest value was recorded at 90 days (111.07 ± 1.60 shoots m^{-2}), differing significantly from all other evaluation times. At 15 days, the mean value (77.87 ± 1.03 shoots m^{-2}) was statistically higher than those observed at 30, 60, and 75 days, but lower than the value recorded at 90 days. The lowest shoot production per m^2 occurred at 75 days (45.08 ± 1.28 shoots m^{-2}), which differed significantly from all other periods. No significant difference was observed between the values recorded at 30 and 60 days (54.10 ± 1.19 shoots m^{-2}), which showed intermediate behavior.

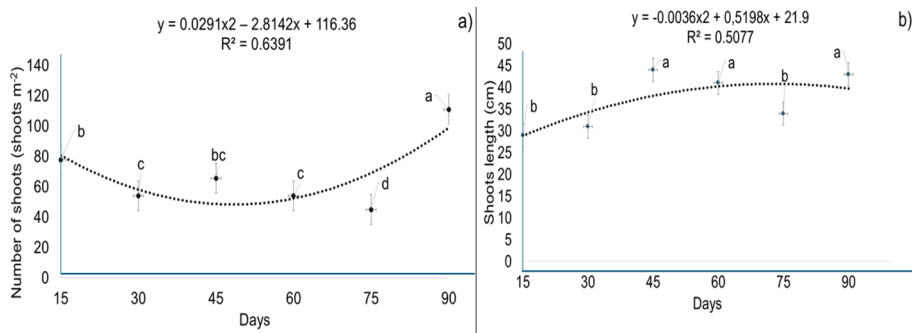


Fig. 3 Temporal variation in **a** shoot density (shoots m⁻²) and **b** shoot length (cm) of *Mimosa ophthalmocentra* mini-stumps during a 90-day evaluation period. Points represent means $\pm$ SE. Source: Authors

For shoot length, significant differences were also observed among evaluation periods (Fig. 3b; Table S1; Friedman test: $\chi^2(5) = 744.32$, $p < 0.001$). The greatest mean shoot lengths were recorded at 45, 60, and 90 days, which did not differ from each other and were higher than those observed at 15 and 30 days. The lowest mean shoot length occurred at 15 days (28.99 ± 0.14 cm), differing significantly from the periods of greater shoot development. The value observed at 75 days (34.01 ± 0.18 cm) showed intermediate behavior, not differing statistically from either the initial periods or the periods with greater shoot length.

These results indicate that although shoot production per m² fluctuates over time, the 90-day period stands out as the most favorable for maximizing shoot production, while shoot length reaches high values as early as 45 days, remaining stable until the end of the evaluated period.

Correlation analysis was performed to assess relationships among the variables Days, Number of Shoots (NS), and Shoot Length (SL). A moderate positive correlation was found between Days and SL ($r = 0.6$), indicating that longer evaluation time was associated with greater shoot length. The correlation between Days and NS was nearly zero ($r = -0.03$), suggesting no linear relationship. Between NS and SL, a weak positive correlation was observed ($r = 0.2$), indicating a slight direct association. Overall, these results suggest that time exerted a stronger influence on shoot length.

Influence of IBA on Adventitious Rooting and Shoot Development

The effect of indole-3-butyric acid (IBA) concentrations on adventitious rooting and shoot development of apical and basal mini-cuttings of *M. ophthalmocentra* is shown in Fig. 4. Rooting percentage, number of roots, mean root length, sprout length, shoot fresh mass, shoot dry mass, root fresh mass, and root dry mass varied according to mini-cutting position and IBA concentration. Mean values ($\pm$ standard error) for all evaluated variables are presented in Supplementary Table 2 (Table S2).

Statistical analysis confirmed that both mini-cutting position and IBA concentration significantly influenced the morphological performance of the mini-cuttings. Significant differences between apical and basal mini-cuttings were detected for several variables based on the Mann–Whitney test (Table 1), while the effect of IBA concentrations was evaluated using regression analyses (Fig. 4).

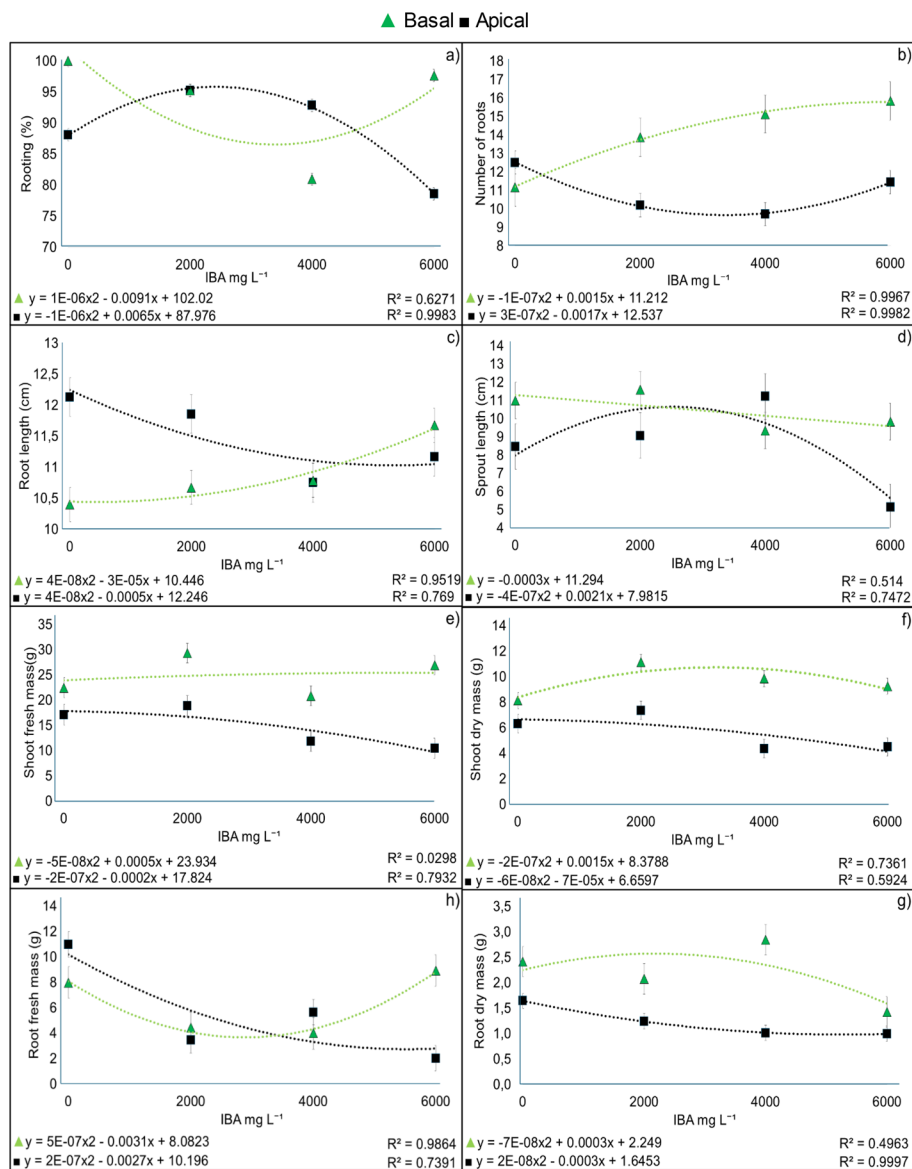


Fig. 4 Effect of indole-3-butyric acid (IBA) concentrations on the development of apical (black filled square) and basal (green filled triangle) mini-cuttings of *Mimosa ophthalmocentra*. **a** rooting percentage, **b** number of roots, **c** root length (cm), **d** sprout length (cm), **e** shoot fresh mass (g), **f** shoot dry mass (g), **g** root fresh mass (g), and **h** root dry mass. Dashed lines represent the fitted regression models, and vertical bars indicate the standard error of the mean. The second regression equation in each graph corresponds to apical mini-cuttings (black filled square), and the first to basal mini-cuttings (green filled triangle). Source: Authors

Table 1 Mean values ($\pm$ standard error) of shoot length, root length, and number of roots for apical and basal mini-cuttings of *Mimosa ophthalmocentra* under different IBA concentrations. p-values refer to comparisons between mini-cutting positions (apical vs. basal) within each IBA concentration, as assessed by the Mann–Whitney test

IBA (mg L ⁻¹)	Position	Shoot length (cm)	p (Shoot)	Root length (cm)	p (Root)	Number of roots	p (Number of roots)
0	Apical	8.59 $\pm$ 0.38 *	6.29299E-05	12.11 $\pm$ 0.44 *	0.012095	12.68 $\pm$ 0.66 ns	0.492015
0	Basal	10.99 $\pm$ 0.56 *		10.43 $\pm$ 0.27 *		11.17 $\pm$ 0.49 ns	
2000	Apical	8.65 $\pm$ 0.55 *	0.012254	11.36 $\pm$ 0.48 ns	0.155023	9.67 $\pm$ 0.78 *	0.00075
2000	Basal	11.04 $\pm$ 0.94 *		10.15 $\pm$ 0.63 ns		13.19 $\pm$ 0.95 *	
4000	Apical	7.23 $\pm$ 0.60 ns	0.135146	9.38 $\pm$ 0.63 ns	0.085670	8.40 $\pm$ 0.74 *	0.00709
4000	Basal	9.10 $\pm$ 1.10 ns		9.45 $\pm$ 0.87 ns		14.02 $\pm$ 1.55 *	
6000	Apical	4.72 $\pm$ 0.58 *	1.40427E-06	9.14 $\pm$ 0.85 ns	0.668322	9.61 $\pm$ 1.17 *	0.002197
6000	Basal	9.62 $\pm$ 0.64 *		11.38 $\pm$ 0.38 ns		15.48 $\pm$ 1.30 *	

Within each IBA concentration, means followed by an asterisk (*) indicate significant differences between apical and basal mini-cuttings according to the Mann–Whitney test ($p < 0.05$). The effect of IBA concentration was evaluated by regression analyses (Fig. 4) and is not tested in this Table

Regarding rooting performance, apical mini-cuttings exhibited a mean rooting percentage of 88.10 $\pm$ 5.00% in the absence of IBA, increasing to 95.24 $\pm$ 3.00% at 2000 mg L⁻¹, remaining at 92.86 $\pm$ 4.00% at 4000 mg L⁻¹, and decreasing to 78.57 $\pm$ 6.00% at 6000 mg L⁻¹ (Fig. 4a; Table S2). In contrast, basal mini-cuttings showed 100.00 $\pm$ 0.00% rooting in the control treatment and maintained high rooting percentages across all evaluated concentrations, with 95.24 $\pm$ 3.00% at 2000 mg L⁻¹, 80.95 $\pm$ 6.00% at 4000 mg L⁻¹, and 97.62 $\pm$ 2.00% at 6000 mg L⁻¹.

The number of roots per mini-cutting varied according to mini-cutting position and IBA concentration (Fig. 4b). In apical mini-cuttings, the mean number of roots decreased from 11.17 $\pm$ 0.86 in the control treatment to 9.67 $\pm$ 0.78 at 2000 mg L⁻¹ and 8.40 $\pm$ 0.74 at 4000 mg L⁻¹, with values close to 9.61 $\pm$ 1.17 roots per mini-cutting at 6000 mg L⁻¹. Conversely, basal mini-cuttings showed a progressive increase in root number with increasing IBA concentration, rising from 11.17 $\pm$ 0.49 in the control to 13.19 $\pm$ 0.95, 14.02 $\pm$ 1.55, and 15.48 $\pm$ 1.30 roots per mini-cutting at 2000, 4000, and 6000 mg L⁻¹, respectively (Table S2). Significant differences between apical and basal mini-cuttings were detected at 4000 and 6000 mg L⁻¹ (Table 1).

Similarly, mean root length was influenced by both mini-cutting position and IBA concentration (Fig. 4c). In apical mini-cuttings, mean root length decreased from 10.67 $\pm$ 0.72 cm in the control to 9.14 $\pm$ 0.85 cm at 6000 mg L⁻¹, whereas basal mini-cuttings showed an increase in root length with increasing IBA concentration, reaching 11.38 $\pm$ 0.38 cm at the highest dose evaluated (Table S2). Significant differences between positions were observed only at 6000 mg L⁻¹ (Table 1).

Shoot length also differed significantly between mini-cutting positions across all IBA concentrations (Table 1). Basal mini-cuttings consistently exhibited higher mean shoot length than apical mini-cuttings, with values ranging from 10.99 $\pm$ 0.56 cm in the control to 9.62 $\pm$ 0.64 cm at 6000 mg L⁻¹. In apical mini-cuttings, mean shoot length ranged from 7.57 $\pm$ 0.55 cm in the control to 8.65 $\pm$ 0.55 cm at 2000 mg L⁻¹, decreasing sharply to 4.72 $\pm$ 0.58 cm at 6000 mg L⁻¹ (Fig. 4d; Table S2).

In terms of biomass accumulation, shoot fresh mass in apical mini-cuttings decreased progressively with increasing IBA concentration, from 17.11 ± 2.56 g in the control to 10.42 ± 4.16 g at 6000 mg L^{-1} , whereas basal mini-cuttings maintained high shoot fresh mass values across all concentrations, ranging from 22.44 ± 7.18 g to 29.30 ± 4.74 g (Fig. 4e; Table S2). The highest shoot dry mass values were observed at 2000 mg L^{-1} of IBA, reaching 4.38 ± 1.41 g in apical mini-cuttings and 11.13 ± 1.47 g in basal mini-cuttings (Fig. 4f).

Root biomass followed a similar pattern. In apical mini-cuttings, root dry mass decreased from 1.64 ± 0.10 g in the control to 0.99 ± 0.48 g at 6000 mg L^{-1} , whereas basal mini-cuttings showed the highest root dry mass at 6000 mg L^{-1} (2.84 ± 1.13 g) (Fig. 4h; Table S2). Root fresh mass was also higher in basal mini-cuttings, particularly in the control treatment (7.97 ± 3.75 g) and at 6000 mg L^{-1} (8.93 ± 6.20 g), while apical mini-cuttings exhibited a progressive reduction with increasing IBA concentration (Fig. 4g).

Overall, basal mini-cuttings exhibited superior morphological performance compared to apical mini-cuttings, particularly with respect to shoot length, number of roots, and biomass accumulation (Table 1; Table S2). The response to IBA concentration was dependent on mini-cutting position, with intermediate concentrations generally favoring biomass accumulation, whereas higher concentrations negatively affected several traits in apical mini-cuttings.

Adventitious Rooting Dynamics

The temporal dynamics of rooting in mini-cuttings showed similar patterns between apical and basal positions when treated with 2000 mg L^{-1} of IBA (Fig. 5; Fig. 6). Swelling at the base of the mini-cuttings (Fig. 5a) was high during the initial evaluation periods and progressively declined toward the end of the experimental period for both positions. Callus formation (Fig. 5b) also occurred predominantly at the early stages and decreased over time.

Rooting percentage (Fig. 5c) increased progressively throughout the evaluation period, reaching 100% in apical mini-cuttings and 94.4% in basal mini-cuttings at 25 days (Table S3). This increase was accompanied by a gradual rise in the number of roots per mini-cutting (Fig. 5d), which reached mean values between 28 and 31 roots per mini-cutting at the final evaluation, as well as by an increase in mean root length (Fig. 5e), which approached values of approximately 11 cm.

When the data were analyzed globally, considering the entire rooting period, no statistically significant differences were detected between apical and basal mini-cuttings for rooting percentage, number of roots, or mean root length, as indicated by the Mann–Whitney test ($p > 0.05$; Supplementary Table 4). Nevertheless, subtle differences in rooting dynamics were visually observed (Fig. 5), with apical mini-cuttings showing earlier tissue responses during the initial evaluation stages, whereas basal mini-cuttings exhibited slightly higher rooting intensity at later stages. From 15 days onward, both positions displayed similar trends in root number and root length.

Spearman's correlation analysis (Fig. 5f) revealed clear relationships among the morphophysiological variables. Callus formation showed a strong negative correlation with rooting percentage, number of roots, and mean root length, whereas rooting percentage was strongly and positively correlated with both number of roots and mean root length. In

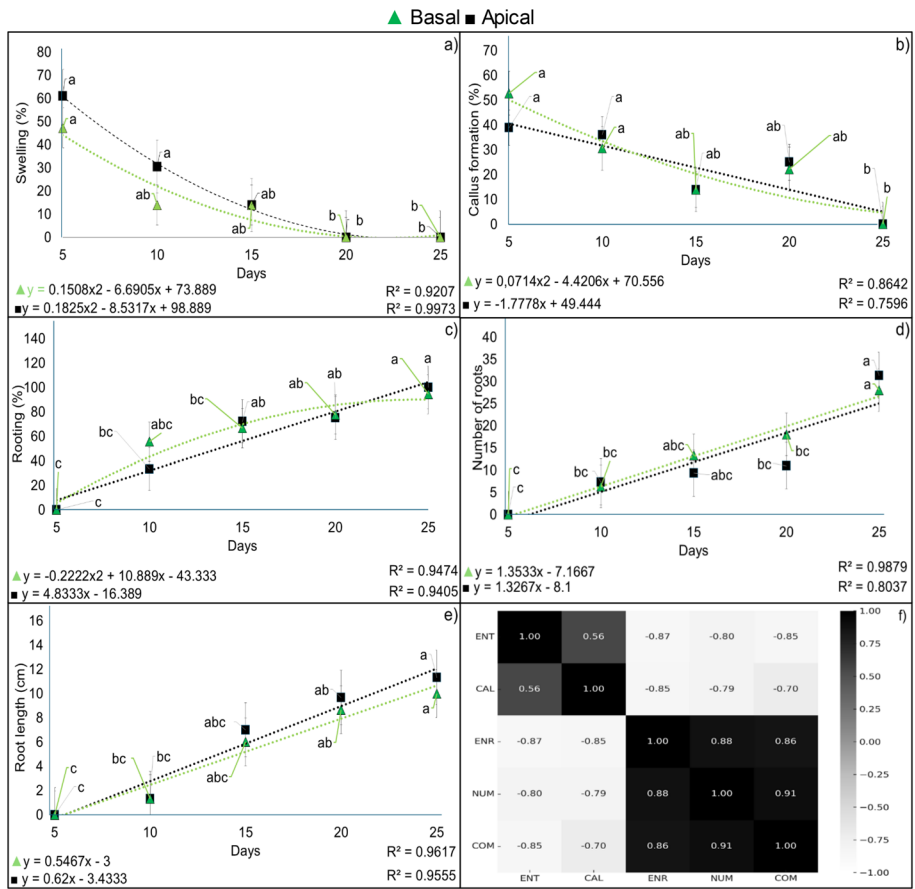


Fig. 5 Temporal dynamics of adventitious rooting of apical (black filled square) and basal (green filled triangle) mini-cuttings of *Mimosa ophthalmocentra* treated with indole-3-butyric acid (IBA) at 2,000 mg L⁻¹, over 25 days of cultivation. The figure shows: **a** swelling (%), **b** callus formation (%), **c** rooting (%), **d** number of roots per mini-cutting, **e** mean root length (cm), and **f** Spearman correlation matrix among morphophysiological variables. Dashed lines correspond to the fitted regression models (linear or polynomial, depending on the best fit for each variable), and vertical bars indicate the standard error of the mean. In the correlation matrix (f), coefficients range from -1 to 1, with darker tones indicating stronger positive or negative correlations among the analyzed variables. The first regression equation in each graph corresponds to basal mini-cuttings (green filled triangle), and the second to apical mini-cuttings (black filled square). Source: Authors

addition, the number of roots and mean root length were strongly and positively associated, indicating coordinated development of the root system throughout the rooting process.

The Fig. 6 shows the rooting dynamics and associated anatomical changes in mini-cuttings of *M. ophthalmocentra* treated with 2000 mg L⁻¹ of IBA (indole-3-butyric acid). Panels (a) and (b) correspond to apical and basal mini-cuttings, respectively, photographed at successive evaluation times (5, 10, 15, 20, and 25 days after planting). In both mini-cutting positions, the first visible roots were detected between 5 and 10 days, followed by progressive root elongation and emergence after 15 days. These observations are consistent with the quantitative results, which showed a decline in callus occurrence and a concomitant

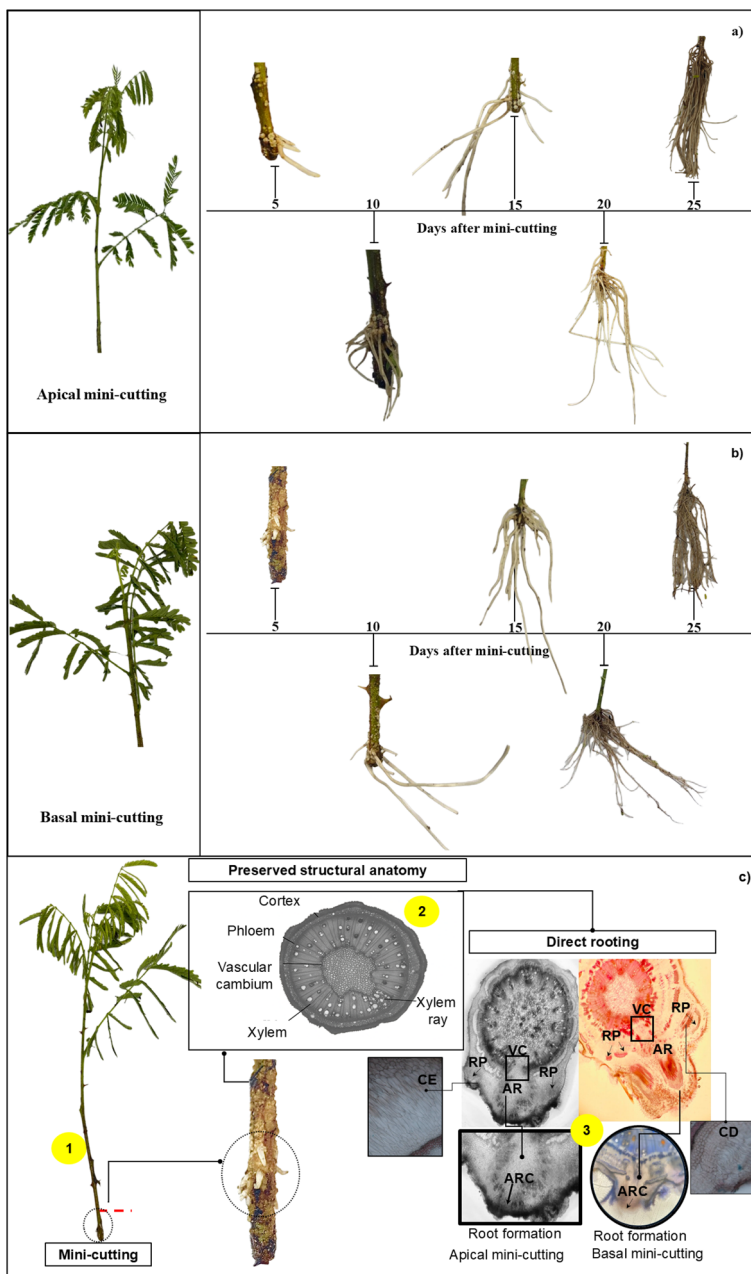


Fig. 6 Rooting dynamics and anatomical structure of *Mimosa ophthalmocentra* mini-cuttings treated with indole-3-butyric acid (IBA) at 2.000 mg L^{-1} . **a** Apical mini-cuttings and **b** basal mini-cuttings evaluated at 5, 10, 15, 20, and 25 days after insertion, showing progressive development of adventitious roots. **c** Anatomical and physiological representation of the rooting process: (1) basal region of the mini-cutting, (2) preserved anatomical structure of the stem, including cortex, phloem, vascular cambium, xylem, and xylem rays, and (3) anatomical changes associated with direct root formation, showing localized tissue reorganization with cell differentiation (CD) and cell elongation (CE), root primordia (RP), vascular connection (VC), adventitious root (AR), and adventitious root cap (ARC). Source: Authors

increase in rooting percentage, root number, and root length over time. Apical mini-cuttings exhibited earlier production of thinner roots, while basal mini-cuttings developed thicker and more branched roots at later stages (20 and 25 days); however, these differences were morphological and not statistically significant, as indicated by the quantitative analyses.

Panels (c) highlight the anatomical aspects of the rooting process: (1) the basal region of the mini-cutting where root induction occurs, (2) the preserved stem anatomy prior to root emergence, and (3) anatomical changes associated with adventitious root formation. The preserved stem anatomy prior to root emergence is shown, including the cortex, phloem, vascular cambium, xylem, and pith. Arrowed areas indicate regions where root primordia (RP) initiation was observed, characterized by localized tissue reorganization, evidenced by changes in cell arrangement with cell differentiation (CD) and elongation (CE) adjacent to conductive tissues, suggestive of increased meristematic activity. Enlarged details emphasize the position of root primordia adjacent to conductive tissues with vascular connection (VC) and the emergence of roots crossing the epidermis with a clearly defined root cap (ARC). These images allow the characterization of the cellular and structural events involved in ontogeny of adventitious rooting.

At early stages, tissues at the base of the mini-cuttings retained their structural organization, with well-defined vascular tissues. As rooting progressed, localized cellular proliferation (CE and CD) was observed in regions adjacent to the phloem and vascular cambium, coinciding with the appearance of root primordia (RP). These anatomical observations are consistent with the quantitative data, which showed high frequencies of swelling during the initial evaluation period (5 days), followed by a progressive decline over time. The emergence of root primordia directly from conductive tissues, without an intermediate callus phase, corresponds to the marked increase in rooting percentage, number of roots, and mean root length observed after 15 days.

Discussion

The productivity of mini-stump gardens is influenced by intrinsic species characteristics, as well as by substrate conditions, nutrient availability, and the physiological adjustment of plants to pruning and repeated shoot harvests (Wendling et al. 2014a, 2014b; Brondani et al. 2014; Stuepp et al. 2018; Freitas et al. 2018). In species from seasonally dry environments such as the Caatinga, resprouting represents an important adaptive trait that provides a favorable biological basis for the establishment of vegetative propagation systems based on pruning (Sampaio et al., 1998; Araújo et al. 2025).

In the present study, the temporal variation observed in mini-stump productivity (Fig. 3) exhibited a non-linear pattern, with an initial reduction followed by a marked increase in the later evaluation cycles. This response is consistent with the physiological adjustment and stabilization phase typical of seminal mini-gardens (Dias et al. 2012a, b; Silva et al., 2022; Freire et al. 2025). The increase observed in the final evaluation periods indicates the progressive recovery and stabilization of shoot emission and elongation capacity after the initial establishment phase. Although shoot production in mini-garden systems is known to be sensitive to environmental conditions such as temperature and relative humidity (Freire et al. 2025), these variables were not monitored as covariates in the present study and are

therefore considered here as potential background influences rather than as factors directly driving the observed temporal patterns.

The patterns observed in Fig. 3 indicate that shoot elongation was primarily driven by evaluation time rather than constrained by shoot density. This interpretation is supported by the correlation analysis, which showed a moderate positive association between days and shoot length, whereas the relationship between the number of shoots and shoot length was weak. Together, these results indicate that higher shoot density did not impose a restriction on shoot elongation and that shoot growth progressed concomitantly with shoot emission during the establishment phase of the mini-garden.

Despite the slower increase in shoot length during intermediate evaluation periods, mean shoot length exceeded 20 cm, which can be considered adequate for the production of mini-cuttings. In the present study, mini-cuttings with a length of 13 cm were successfully used, demonstrating that the observed shoot elongation was sufficient to support operational mini-cutting production. Shoot density followed a non-linear temporal pattern during the establishment phase, ultimately reaching nearly 100 shoots m^{-2} at 90 days. This response reinforces the operational feasibility of using *M. ophthalmocentra* in vegetative propagation systems based on mini-cuttings. These findings suggest that evaluations restricted to the initial establishment phase may not fully capture the subsequent stabilization and recovery of shoot productivity.

It is also important to consider the contribution of genetic heterogeneity to the observed variability in shoot production. As the mini-garden was established from seeds collected from 11 mother trees, the resulting mini-stumps represent a genetically heterogeneous population. Such heterogeneity may contribute to variation in shoot emission and elongation over time (Dias et al. 2012b), particularly during the stabilization phase of the mini-garden, without compromising overall productivity.

Similar temporal dynamics have been reported for other woody Fabaceae managed under mini-garden systems, in which shoot emission and elongation increase concurrently during the transition from establishment to stabilization phases (Dias et al. 2012b; Silva et al., 2022; Souza et al. 2024; Freire et al. 2025). In the present study, mini-stump survival remained at 100% throughout the evaluation period, demonstrating the high tolerance of *M. ophthalmocentra* to pruning and repeated shoot harvests. Together, these results indicate that the species responds positively to the management conditions adopted for the seminal mini-garden system, maintaining shoot growth despite increasing propagule production.

The rooting results demonstrate that *M. ophthalmocentra* exhibits intrinsic rhizogenic competence, as indicated by high rooting percentages even in the absence of exogenous IBA, particularly in basal mini-cuttings. In apical mini-cuttings, rooting percentage increased at intermediate IBA concentrations (2000–4000 mg L^{-1}) but declined at the highest dose, indicating that exogenous auxin modulates rooting performance rather than being essential for root induction in this species.

Mini-cutting position influenced root system attributes and biomass accumulation. Basal mini-cuttings showed increased root number and longer roots with increasing IBA concentrations. In contrast, apical mini-cuttings exhibited a progressive reduction in root number, root length, and biomass as IBA concentration increased, particularly at higher doses. Taken together, these contrasting responses indicate that root induction and root elongation respond differently to exogenous auxin depending on the developmental condition of the propagule. IBA application was associated with increased root initiation, whereas higher

concentrations were accompanied by reductions in root extension growth. This pattern may be associated with hormonal imbalance or shifts in assimilate allocation between induction and elongation phases, as reported for other woody species (Stuepp et al. 2018; da Luz et al. 2025).

Responses reported for other Caatinga species support this interpretation and illustrate the variability among taxa. In *M. caesalpiniifolia*, apical mini-cuttings root efficiently (>80%) even without exogenous regulators, whereas concentrations between 2000 and 6000 mg L⁻¹ improve rooting (Silva Aguiar et al. 2022). In *Tabebuia aurea*, intermediate IBA doses promote root formation and biomass accumulation, while higher concentrations reduce performance (Souza et al. 2023). Similarly, contrasting outcomes reported for *Spondias tuberosa*, *Croton heliotropiifolius*, and *Bauhinia cheilantha* (De Lima et al. 2020; Souza et al., 2019; Vêras et al. 2018) demonstrate that both the requirement for IBA and the optimal dose depend on species-specific physiology and on the developmental condition of each propagule. In *M. ophthalmocentra*, the application of IBA proved useful for optimizing specific root traits, particularly in basal mini-cuttings, but was not essential for rhizogenesis. This relative independence from hormonal supplementation represents an operational advantage, enabling the development of lower-cost and more flexible propagation protocols for large-scale propagation.

The lower rooting percentage, reduced root number, shorter sprout length, and decreased shoot and root biomass observed in apical mini-cuttings may be associated with their predominantly herbaceous tissue condition. Apical tissues can present higher water content and lower reserve accumulation, which may increase susceptibility to dehydration and limit the availability of carbohydrates required for the formation and subsequent growth of the root system and the aerial part (Wendling et al. 2014a; Brondani et al. 2014; Stuepp et al. 2018). Conversely, highly lignified tissues, commonly found in more basal stem portions, may impose mechanical and physiological constraints on root emergence (Steffens & Rasmussen 2015; Gonin et al. 2019). Thus, an intermediate developmental condition, such as the propagule used to basal mini-cutting in this study, corresponding to semi-lignified tissues, may represent a more favorable balance between cellular plasticity, water status, and reserve availability for adventitious rooting. This conceptual framework is consistent with classical interpretations of cutting maturation effects on rooting performance in woody species (Wendling et al. 2014a; Hartmann et al. 2018; Stuepp et al. 2018).

The contrasting effects of IBA concentration and mini-cuttings position on specific rooting attributes discussed above indicate that exogenous auxin modulates quantitative aspects of root system development in *M. ophthalmocentra*, without being essential for rhizogenesis. To further explore the morphogenetic pathway underlying these responses, the temporal dynamics and the anatomical origin of adventitious roots were examined using mini-cuttings treated with 2000 mg L⁻¹ of IBA. This approach allowed the characterization of whether rooting occurred predominantly via direct rhizogenesis or involved an intermediate callogenetic phase under a representative and operationally relevant auxin dose. Although the anatomical and cellular processes were not experimentally compared across different IBA concentrations, integrating these morpho-anatomical observations with the quantitative rooting data provides a more comprehensive interpretation of the of adventitious root development of the species under the propagation conditions evaluated.

In the adventitious rooting dynamics, swelling at the base of the mini-cuttings was observed five days after insertion, particularly in apical mini-cuttings. This early morpho-

logical response likely reflects initial cellular and tissue reorganization events preceding rhizogenic induction activated by the synthesis and basipetal transport of endogenous auxins (Díaz-Sala 2020; Sá et al. 2021; Liu et al. 2024), which drive the first morphophysiological changes required for rhizogenic induction. Although endogenous auxin synthesis and basipetal transport are widely recognized as key regulators of early phases of adventitious root formation, and disruption of polar auxin transport can inhibit rhizogenesis (Garrido et al. 2002; Stuepp et al. 2017; Hartmann et al. 2018), these processes were not directly assessed in the present study and are therefore discussed here as plausible physiological mechanisms underlying the observed swelling.

Callus formation was also detected during the early evaluation period and was quantitatively assessed. In this study, callus occurrence showed a negative association with adventitious rooting, indicating that higher callus formation was associated with delayed or reduced root emergence. This pattern suggests that, in *M. ophthalmocentra*, callogenesis does not function as a prerequisite for rhizogenesis but rather represents an alternative wound-healing pathway that may compete with direct root formation, as reported for other woody species (Zhou et al. 2018; Pimentel et al. 2019; Freire et al. 2025; da Luz et al. 2025).

From a morpho-anatomical perspective, *M. ophthalmocentra* predominantly exhibited a direct rhizogenic pattern, with adventitious roots originating from reorganized vascular tissues without an intermediate callus phase. This anatomical configuration is consistent with the quantitative temporal dynamics observed, in which callus occurrence declined while rooting percentage, root number, and root length increased after 15 days. Although the micrographs mainly depict later stages of root development, the spatial organization of primordia adjacent to conductive tissues supports a vascular origin of roots (Bryant & Trueman 2015).

In the present study, apical and intermediate mini-cuttings exhibited similar morpho-anatomical rooting patterns, and the subtle differences observed in rooting performance between propagule types may be associated with differences in tissue water content and carbohydrate reserves. Although reserve dynamics were not directly assessed in this study, the mobilization of carbohydrate reserves has been widely reported as an important energetic component supporting cell division and differentiation during adventitious rooting in woody species (Gonin et al. 2019; Porfirio, et al. 2016). In woody species, differences in adventitious rooting capacity among propagule types are commonly interpreted based on stem maturation gradients, in which less lignified and developmentally younger tissues tend to exhibit higher cellular plasticity and responsiveness to rhizogenic stimuli, whereas more mature tissues often present structural constraints and lower meristematic competence (Wendling et al. 2014a; Stuepp et al. 2018).

Despite the genetic heterogeneity inherent to the propagation material, derived from seeds collected from multiple mother trees, the consistently high rooting rates observed across individuals indicate that *M. ophthalmocentra* presents intrinsically favorable rhizogenic competence. Although genotype-dependent variation is widely reported as an important determinant of adventitious rooting in woody species (Dias et al. 2012b; Ranjan et al. 2022), the overall data recorded here support the operational feasibility of using this species in vegetative propagation and plant propagation programs.

Conclusion

The results demonstrate that *M. ophthalmocentra* exhibits high competence for adventitious rooting, reaching rates above 80% in both apical and basal mini-cuttings, even without the application of exogenous growth regulators. These findings enable the standardization of an efficient vegetative propagation protocol using mini-cuttings, with rooting achieved under greenhouse conditions in approximately 25 days using juvenile propagules. Morphoanatomical analyses confirmed that rooting occurs predominantly through a direct pathway, originating from vascular cambium cells. Overall, the study reinforces the potential of the species for domestication and vegetative multiplication programs, as well as its applicability in conservation and restoration initiatives in semiarid ecosystems such as the Caatinga.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s11056-026-10174-5>.

Acknowledgements The authors express their sincere gratitude to the Northeast Ecology and Management Research Group (NorEMa) for its intellectual collaboration and essential contribution to the conception and development of this study.

Author Contributions All authors made substantial contributions to the conception, execution, and development of this study. Conceptualization and design: Cleyton dos Santos Souza, Poliana Coqueiro Dias Araujo, Rafael Leite Braz, and Eliane Cristina Sampaio de Freitas. Data collection, analysis, and processing: Cleyton dos Santos Souza, Maria Janaina Nascimento Silva, Eliane Cristina Sampaio de Freitas, and Gleydson Vinicius dos Santos Silveira. Manuscript drafting: Cleyton dos Santos Souza, Alan Cauê de Holanda, and Gleydson Vinicius dos Santos Silveira. Critical review and final editing: Poliana Coqueiro Dias Araujo and Rafael Leite Braz.

Funding The Article Processing Charge (APC) for the publication of this research was funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) (ROR identifier: 00x0ma614). This study was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil, under Funding Code 001.

Data Availability The datasets generated and analyzed in this study are not provided with the manuscript as supplementary material. However, all data can be obtained directly from the corresponding author upon request.

Declarations

Conflict of interest The authors declare no competing interests.

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