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Influence of the degree of lignification and use of indole acetic acid on the development and rooting of *Libidibia ferrea* cuttings

Influência do grau de lignificação e uso de ácido indol-acético no desenvolvimento e enraizamento de estacas de *Libidibia ferrea*

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Libidibia ferrea (Mart. ex Tulle.) L.P. Queiroz, commonly known as pau-ferro and belonging to the Fabaceae family, is a species valued in traditional Brazilian medicine, promoting research in pharmacological and biotechnological products. However, seed propagation faces obstacles due to seed coat dormancy, which reduces germination rates, and genetic variability, which can lead to biochemical differences in compounds of interest, complicating standardization for the pharmaceutical industry. Thus, vegetative propagation emerges as a promising alternative to overcome these challenges. In this context, this study evaluated the effect of juvenility and indole acetic acid (IAA) on the rooting of *L. ferrea* cuttings, in addition to anatomical characterization of the cuttings to identify potential barriers to rooting. The experiment was conducted at INPA, Manaus – AM, using regrowth and juvenile cuttings prepared with one or two leaves cut in half and treated with IAA at concentrations of 0, 500, 1000, and 2000 mg L^{-1}. After 60 days in a rooting greenhouse, the results show that regrowth cuttings had higher leaf retention (78.8%), callus formation (84.6%), rooting (65.4%), and average root number (3.0), underscoring the influence of tissue rejuvenation on rooting success. Juvenile cuttings exhibited greater bud formation (59.6%) and survival rate (86.5%), indicating that juvenility favors budding and survival. Therefore, the propagation of *L. ferrea* by cutting is viable and is enhanced when combined with rejuvenation techniques and the application of IAA, especially at a dose of 1000 mg L^{-1}.
Keywords: regrowths, juvenile material, anatomy.

Libidibia ferrea (Mart. ex Tulle.) L.P. Queiroz, conhecida como pau-ferro e pertencente à família Fabaceae, é uma espécie valorizada na medicina tradicional brasileira, incentivando pesquisas em produtos farmacológicos e biotecnológicos. Contudo, a propagação por sementes enfrenta obstáculos devido à dormência tegumentar, que reduz a taxa de germinação, além da variabilidade genética, que pode gerar diferenças bioquímicas nos compostos de interesse, dificultando a padronização destes para a indústria farmacêutica. A propagação vegetativa, assim, é uma alternativa promissora, para superar estas dificuldades. Diante isto, este estudo avaliou o efeito da juvenilidade e do ácido indol-acético (AIA) no enraizamento de estacas de *L. ferrea*, além de caracterizar anatomicamente as estacas para identificar possíveis barreiras ao enraizamento. O experimento foi realizado no INPA, Manaus – AM, com estacas de rebrotas e material juvenil, preparadas com uma ou duas folhas reduzidas pela metade e tratadas com AIA nas concentrações de 0, 500, 1000 e 2000 mg L^{-1}. Após 60 dias em estufa de enraizamento, os resultados gerais mostram que as estacas de rebrota apresentaram maior retenção foliar (78,8%), formação de calos (84,6%), enraizamento (65,4%) e número médio de raízes (3,0), destacando a influência do rejuvenescimento do tecido no sucesso do enraizamento. As estacas juvenis tiveram maior emissão de brotos (59,6%) e taxa de sobrevivência (86,5%), indicando que a juvenilidade favorece a brotação e a sobrevivência. Portanto, a propagação de *L. ferrea* por estaquia é viável e é favorecida quando combinada com técnicas de rejuvenescimento e aplicação de AIA, principalmente na dose de 1000 mg L^{-1}.
Palavras-chave: rebrotas, material juvenil, anatomia.

1. INTRODUCTION

Libidibia ferrea (Mart. ex tulle.) L.P. Queiroz, from the Fabaceae family, is a species popularly known as pau-ferro that occurs throughout Brazil. It is widely known for its use as a source of wood, forage, in the recovery of degraded soils, and as an ornamental tree [1]. It is also used in folk medicine, which is why it was included in the list of Medicinal Plants of Interest to the SUS (Renisus), a list of 71 species with therapeutic potential used to guide the production of herbal medicines and the development of research [2].

The importance of *L. ferrea* in traditional medicine has led to the development of several studies focused on ethnobotanical, phytochemical and pharmacological aspects of the species. In a review by Macêdo et al. (2020) [3], a total of 87 studies were reported, examining both the chemical composition of the plant extract and its biological effects. These studies indicated several pharmacological properties of the species, such as antibacterial, antifungal, anti-inflammatory, antioxidant, anticancer, antileishmanial, and healing properties, among others.

The propagation of this species via seeds is possible but presents challenges due to seed mechanical dormancy [4]. In this type of dormancy, seeds are viable but do not germinate, even under favorable conditions, due to a hard seed coat that prevents water uptake and seedling development, known as coat-imposed dormancy [4, 5]. Pre-germination treatments are thus necessary to break dormancy. Additionally, seed availability throughout the year is variable, and genetic diversity in the generated seedlings may lead to biochemical differences in the compounds of interest, impacting product standardization for the pharmaceutical industry.

Faced with the growing demand for plant material for biotechnological studies and the establishment of commercial plantations, vegetative propagation techniques emerge as a viable alternative for producing seedlings with great uniformity and good genetic quality annually [6]. The in vitro culture technique in *L. ferrea* has been explored for the multiplication of healthy seedlings and the induction of somatic embryogenesis in explants [7], highlighting the species' potential for vegetative propagation. However, studies assessing the propagation of this species through cutting techniques are still lacking.

The vegetative propagation of forest plantations via adventitious rooting of cuttings is one of the most interesting emerging technologies in forestry [8]. In addition, to preserving the genetic characteristics of the parent plant, it offers the advantage of producing numerous seedlings from a single plant and can even reduce the time required for seedling production [8]. However, there are some factors that reduce the success of this technique in woody plant species, such as the lack of efficient methods of rejuvenation of adult material, difficulty in obtaining vegetative material with adequate juvenility, lack of techniques for managing the propagation environment and the scarcity of studies that deal with factors relevant to rooting [9].

In some species, the rooting capacity of cuttings is related to their anatomical structure and the degree of lignification [10]. Thus, it is essential to use treatments that maintain the juvenile phase of the mother plant to prevent a decrease in rooting potential [8] and overcome anatomical barriers, such as lignified tissues that may decrease rooting. Rejuvenation can be considered a method to revert plants from a mature to a juvenile stage for clonal propagation purposes [11, 12]. The most common methods include serial propagation, successive pruning, and coppicing [11].

Given the difficulty involved in rooting, which includes factors related to the plant itself or environmental conditions, the use of plant regulators is advisable [13]. Exogenous auxins have been successfully used to increase the survival and rooting of cuttings of forest species, and they are considered the main substances for inducing adventitious rooting [9]. Some auxins, such as indole acetic acid (IAA), are produced naturally by plants, but they can also be produced synthetically [14].

IAA is responsible for cell elongation, cell division, vascular tissue differentiation (phloem and xylem), root formation (cuttings, nodal segments, etc.), among other processes [15]. Its effect as a promoter of adventitious roots in cuttings has been tested in woody species such as *Vaccinium meridionale* (agraz), *Citrus limon* (lemon), and *Ilex paraguariensis*, where its application stimulated cutting development [16-18].

Despite the importance of *L. ferrea*, the lack of information and protocols to overcome species-specific barriers still limits the clonal propagation of this species under nursery conditions. Given this context, the objective of this study was to evaluate and compare the effects of juvenility and

the use of IAA on the rooting of *L. ferrea* cuttings, as well as to anatomically characterize the cuttings to identify potential impediments to rooting.

2. MATERIAL AND METHODS

2.1 Study area

The experiment was conducted in the nursery of the Laboratory of Forestry and Digital Technologies (LASTED), at the National Institute for Amazonian Research (INPA), Manaus, Amazonas. According to the Köppen classification, the climate corresponds to the "Ami" type and is characterized by a high annual rainfall index of about 2,286 mm, an average annual temperature of 26.7 ºC and relative humidity oscillating around 80%. The rainy period extends from December to May and the dry period from June to November [19].

2.2 Methods

2.2.1 Origin of the cuttings and implementation of the experiment

For the preparation of the cuttings, two types of *L. ferrea var. ferrea* plant material were used. The first type consisted of cuttings taken from two-month-old epicormic shoots (regrowth cuttings) collected from six-year-old plants. The second type included cuttings taken from two-year-old *L. ferrea* plants (juvenile cuttings). These cuttings were collected early in the morning from the apical part (excluding the apex) and middle of the branches, with a length of approximately 15 cm and a diameter of about 0.4 cm. The basal end was beveled, and the apex was cross-cut. All cuttings were made with one or two halved leaves. During the preparation process, the cuttings were kept in trays with water to avoid drying them out.

In total, 52 cuttings from regrowth shoots and 52 juvenile cuttings were obtained. Then, their bases of the cuttings were immersed in the following treatments for 10 minutes: a control treatment and three aqueous solutions of IAA at concentrations of 500 mg L^{-1}, 1000 mg L^{-1} and 2000 mg L^{-1}. To prepare IAA solutions at different concentrations, the acid was initially dissolved in sodium hydroxide (NaOH) and subsequently brought to a final volume of one liter with distilled water. After the application of the treatments, the material was stacked in polypropylene trays (30 x 15 cm) containing black soil that had been previously sterilized for one hour in an autoclave at 121 °C, with pressure adjusted to 1 atm. Once the cuttings were planted, the remaining IAA solutions were applied to the substrate.

After planting the cuttings, the trays with the experiments were placed in the rooting greenhouse. This greenhouse was built with PVC pipes (1/2") and completely covered with polyethylene. The greenhouse was placed under a shading screen with 50% light intensity and remained closed to guarantee the humidity of the air inside. Irrigation was done every day manually. Every 15 days foliar fertilization was applied with 15-5-5 fertilizer at a concentration of 5 mL L^{-1}, and NATIVO® systemic fungicide (3 mL L^{-1}) was used to control fungi and pests that might attack the cuttings.

Temperature and humidity were monitored using a digital thermohygrometer (Tomate®, PD-003). Figure 1 shows the daily average values of these variables throughout the experiment, with temperature ranging from 24.3°C to 31°C and relative humidity varying from 91% to 99%.

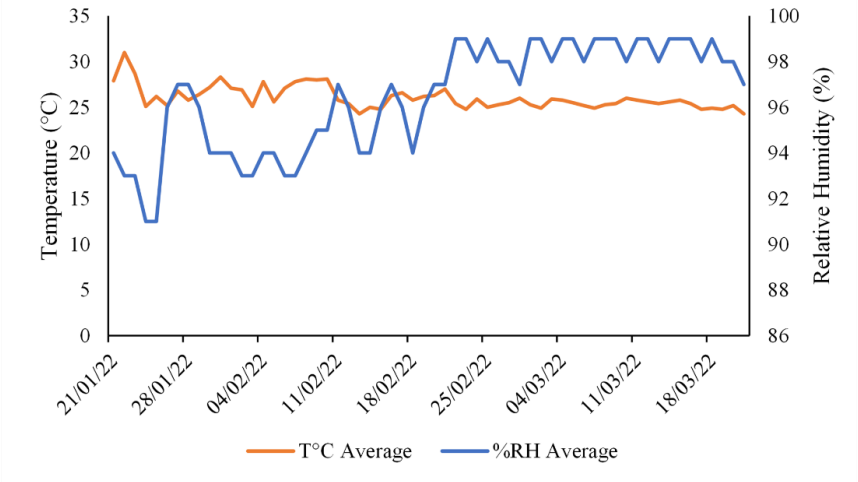

Figure 1. Record of the daily average values of temperature (°C, left axis) and relative humidity (%, right axis) in the rooting greenhouse during the course of the experiment.

2.2.2 Experimental design and evaluation

The experimental design was completely randomized, in a 2 x 4 factorial arrangement, with two types of material (regrowth shoots and juvenile cuttings), four treatments using indole-acetic acid (0, 500, 1000, and 2000 mg L^{-1}), and 13 repetitions each. The evaluation period lasted 60 days, during which the number of cuttings with leaves and new shoots was counted each week. After this period, the experiment was disassembled and evaluated using the following parameters:

- Leaf retention percentage (%): proportion of cuttings with retained leaves relative to the total number of cuttings in the replication, multiplied by 100.
- Sprouting percentage (%): cuttings with new leaves in normal physiological conditions. Calculated by dividing the number of cuttings with new leaves by the total number of cuttings in the replication, multiplied by 100.
- Callus percentage (%): live or dead cuttings with undifferentiated cell mass formation at the base. Calculated by dividing the number of cuttings with callus by the total number of cuttings in the replication, multiplied by 100.
- Rooting percentage (%): cuttings that developed one or more differentiated roots. Obtained by dividing the number of rooted cuttings by the total number of cuttings in the replication, multiplied by 100.
- Average number of roots per treatment: total number of roots in the cuttings divided by the number of cuttings in the replication.
- Survival percentage (%): live cuttings with normal physiological conditions. Calculated by dividing the number of live cuttings by the total number of cuttings, multiplied by 100.

The variables evaluated were previously transformed using arc sine, except for the root number variable, which was transformed using the square root ($\sqrt{x+1}$). The statistical analysis was performed by estimating generalized linear models ($p<0.05$). Subsequently, Fisher's test ($p<0.05$) was applied to the significant treatments to determine those that presented differences between them. The statistical program used was Minitab 18.

2.3. Anatomical analysis

The qualitative anatomical analysis of the cuttings was carried out in the Thematic Laboratory of Microscopy and Nanotechnology at INPA. Two cuttings were selected per type of material (regrowth and juvenile), which, 60 days after planting, showed root development. A sample was taken from the stem and adventitious root emission zone of each cutting.

The tissues were processed for histology using the paraffin technique as follows: to preserve the tissues with minimal alterations, they were placed in a 15 mL glass bottle with FAA fixative (formaldehyde 36%-40%) for 24 hours. These were subsequently dehydrated in a series of ethyl

alcohols, in descending order (100%, 90%, 70%, 50%), for three minutes in each and again hydrated for five minutes with xylol I and xylol II. Then, they were included in liquid paraffin and placed in a refrigerator for hardening.

Once the samples were fixed in the paraffin block, they were cut longitudinally (root) and transversely (stem), using a microtome for paraffin. The slices were then placed on slides for microscopy. Three plates per type of material were obtained, each composed of a sample of the stem and the root growth point.

For the staining of the samples, it was necessary to remove the paraffin using xylol I and xylol II and ethyl alcohol at concentrations of 100%, 90%, 70% and 50%. Methylene blue-safranin dye was subsequently applied for five minutes. After staining, the samples were placed under a tap and again dehydrated with 70%, 90% and 100% ethyl alcohol, then again at 100% for three minutes for each. Finally, the samples were rehydrated with xylol I and xylol II for five minutes each, then they were sealed with a drop of glue and a coverslip the size of the tissue.

The preparations were then placed to dry in an oven at 30 ºC. Subsequently, the tissues were analyzed using a microscope with an integrated digital camera (Pixera Wiender Pro) and the Zeiss ZEN microscope software (blue edition). The best samples were selected and photographed with 2.5 x, 10x and some with 20x objectives.

3. RESULTS

3.1. Rooting of the cuttings

The type of material significantly influenced the percentage of shoots and calluses ($p<0.05$). However, it was observed that, in general, the regrowth material (RM) had higher rates of leaf retention (78.8%), callus formation (84.6%), root formation (65.4%) and number of roots (3.0). The shoot emission and survival rates were higher for cuttings from juvenile material (JM), with 59.6% and 86.5%, respectively (Table 1, Figure 2).

Table 1. Analysis of variance of percentage of leaf retention, shoot emission, callus formation, root formation, number of roots and survival in cuttings from regrowth material (RM) and cuttings of juvenile material (JM) from Libidibia ferrea var. ferrea after 60 days of cultivation under the effect of different concentrations of the plant regulators IAA.

	ANOVA F-test											
Variation Factor	**Leaf retention (%)$^{+}$**		**Shoots (%)$^{+}$**		**Calluses (%)$^{+}$**		**Rooting (%)$^{+}$**		**No. of roots (Mean)$^{++}$**		**Survival (%)$^{+}$**	
	MS	**F-Value**	**MS**	**F-Value**	**MS**	**Value F**	**MS**	**F-Value**	**MS**	**F-Value**	**MS**	**F-Value**
Material	1.16	2.58$^{(ns)}$	12.55	31.12*	5.33	12.62*	1.16	2.21$^{(ns)}$	1.57	3.54$^{(ns)}$	0.024	0.08$^{(ns)}$
IAA	0.72	1.6$^{(ns)}$	2.62	6.49*	1.54	3.64*	2.49	4.74*	3.47	7.82*	0.846	2.97*
Material* IAA	1.67	3.7*	0.34	0.84$^{(ns)}$	1.04	2.45$^{(ns)}$	1.04	1.97$^{(ns)}$	0.96	2.16$^{(ns)}$	0.593	2.08$^{(ns)}$

$^{(ns)}$ not significant at the level of 5% probability, using Fisher's test, (*) Significant at the level of 5% probability, using Fisher's test. +Mean square of data transformed to arc sine. ++ Data transformed with ($\sqrt{x+1}$). MS – mean square.

The application of IAA significantly benefited the evaluated variables, except for the percentage of leaf retention, while the interaction between the type of material and the IAA dosage only stimulated the leaf retention variable (Table 1). For the regrowth material, a significant response to the application of IAA was observed in the variables shoot emission, callus formation, and number of roots of the cuttings (Figure 2). For all variables, the best results were obtained with the highest dosages (1000 and 2000 mg L^{-1}).

For the cuttings from the juvenile material, it is noted that all variables presented a significant response to the application of the treatments. The concentration of 1000 mg L^{-1} presented the best results for the evaluated parameters, increasing the percentages of emission of shoots, calluses

formation, root formation, number of roots, and survival compared with the control treatment. These same parameters decreased when the dosage was increased to 2000 mg L^{-1} (Figure 2).

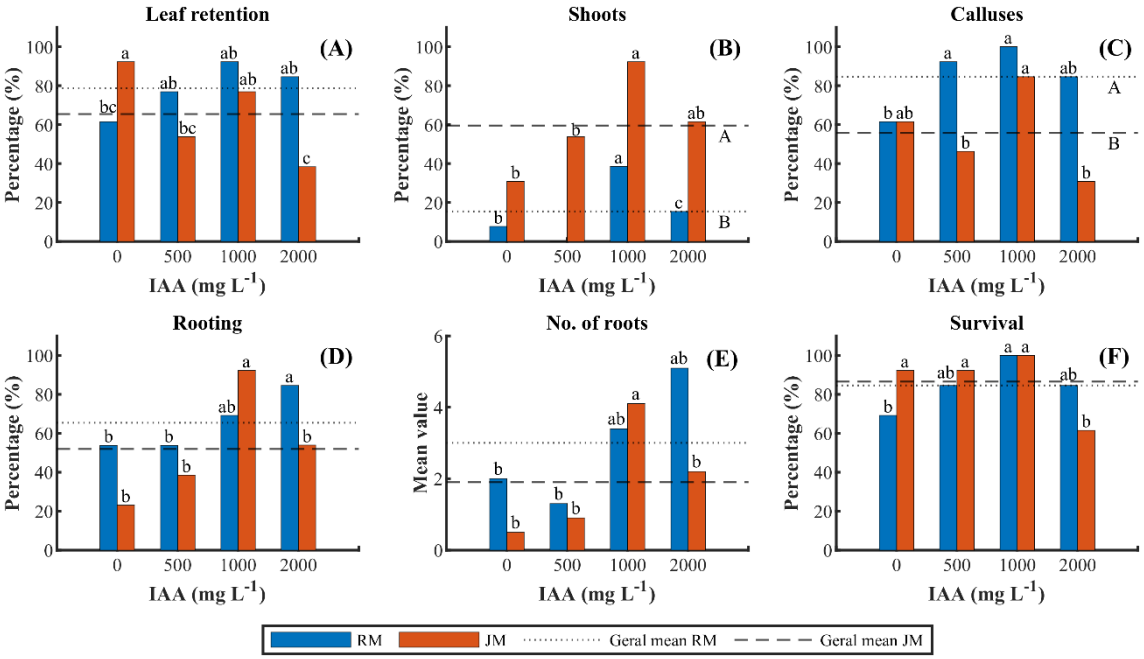

Figure 2. Means of percentage of leaf retention (A), shoot emission (B), callus formation (C), root formation (D), number of roots (E), and survival in cuttings (F) from regrowth material (RM) and cuttings from juvenile material (JM) of Libidibia ferrea var. ferrea after 60 days of cultivation under the effect of different concentrations of the plant regulators IAA. Means followed by the same letter within the column do not differ significantly by Fisher's test ($p< 0.05$). On the other hand, solid and dashed lines with different uppercase letters indicate significant differences in the overall means by Fisher's test ($p< 0.05$).

3.2. Anatomical analysis

In the anatomical characterization of the cuttings from regrowth, a periderm formation was observed, composed of 5-6 layers of irregularly shaped cells in the initial phase of lignification, providing external protection to the stem (Figures 3A, 3C and 3E). Following this was the cortex, composed mainly of poorly lignified pericyclic fibers forming a continuous and regular ring around the vascular cylinder. In some regions, this also presented cortical parenchyma cells, contributing to nutrient storage. The vascular cylinder is growing and presents areas with secondary phloem formation with active vascular exchange, followed by xylem characterized by isolated or grouped vessel elements, tracheas, fibers, and reserve parenchyma. Inside, the medulla occupies approximately one-third of the stem and is formed by reserve parenchyma cells, where starch grains can be observed.

The cuttings taken from juvenile material have a similar structure to the regrowth cuttings; however, it is observed that these have more remarkable development. Both also have a periderm with a layer of 6-7 elongated and lignified cells (Figures 3B, 3D and 3F). The cortex presents cortical parenchyma cells and lignified pericyclic fibers that form a continuous band around the vascular cylinder and providing structural strength. Next is the vascular cylinder where uniseriate rays, vessels, trachea, and lignified fibers are observed, indicating structural maturity. The medulla is smaller and formed by reserve parenchyma with a smaller amount of starch grains, which may suggest a lower accumulation of reserves for initial growth.

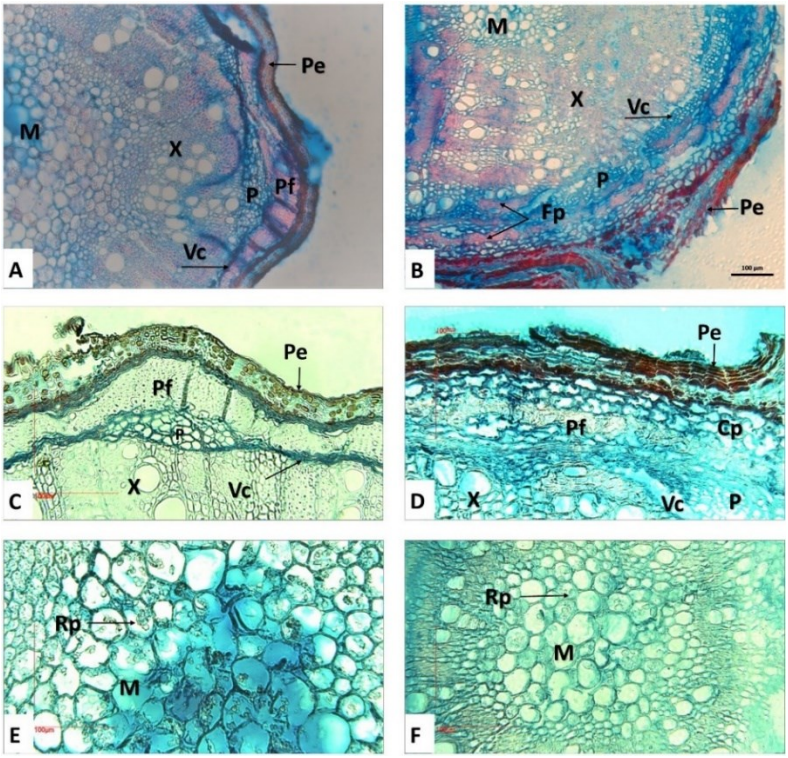

Figure 3. A-C-E: cross-section of the cuttings from Libidibia ferrea var. ferrea regrowth; B-D-F: cross-section of the cuttings from juvenile L. ferrea. M: medulla; X: xylem; P: phloem; Pf: pericyclic fibers; Cp: cortical parenchyma; Pe: periderm; Rp: reserve parenchyma; Vc: vascular cambium (10x-20x).

At 60 days after planting, the rooted cuttings already had a well-developed root system (Figure 4). This development occurs in the vascular cylinder, in the outer region of the phloem, indicating that the origin of the adventitious roots in both types of cuttings possibly occurred from the cambium. It is possible to observe that the roots begin their growth outward by breaking the barrier of the pericyclic fibers and the periderm, while the xylem remains intact.

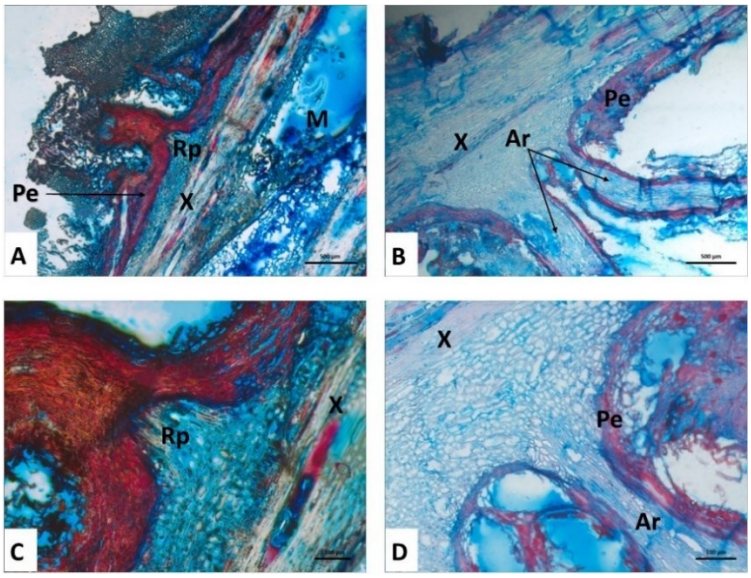

Figure 4. Longitudinal section of Libidibia ferrea var. ferrea cuttings at 60 days after planting. A-C: root primordium of cuttings from L. ferrea regrowth; B-D: adventitious root of cuttings from juvenile L. ferrea. M: medulla; X: xylem; P: phloem; Pf: pericyclic fibers; Pe: periderm; Rp: root primordium; Ar: adventitious root (10x-20x).

4. DISCUSSION

4.1. Influence of juvenility and use of IAA on the rooting of L. ferrea cuttings

Root formation is a prerequisite for successfully propagating cuttings from plants [8]. In this study, it was possible to obtain good results in rooting and survival of the *L. ferrea* cuttings, thus obtaining new seedlings via this cloning technique (Figure 5). However, the cuttings of rejuvenated material showed better root development, and it can be inferred that this process increased the vigor of the cuttings. Younger tissues have a higher endogenous content of auxins and carbohydrate reserves and no mechanical barriers, which are factors that favor rooting [20, 21].

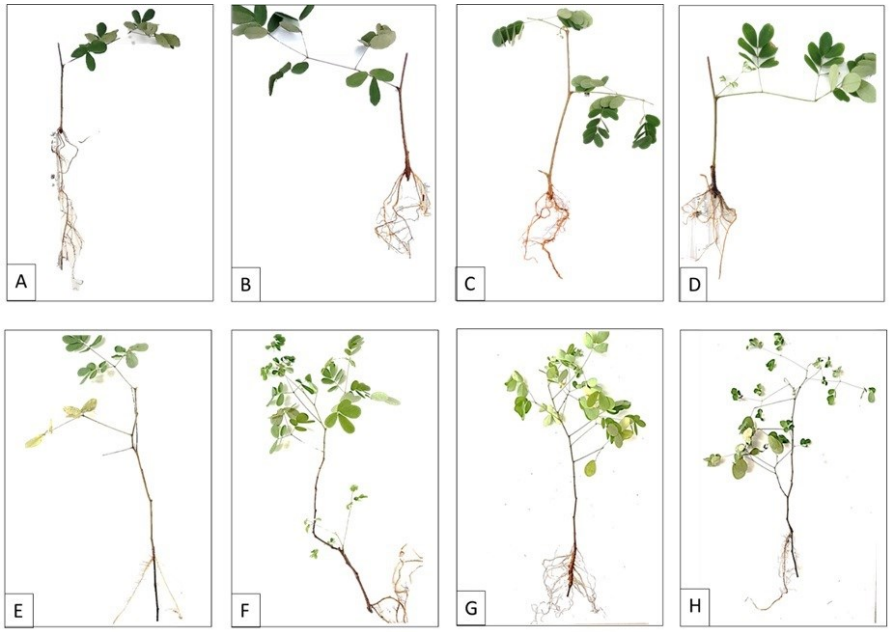

Figure 5. Libidibia ferrea var. ferrea regrowth cuttings under treatments: (A) control; (B) 500 mg L^{-1}; (C) 1000 mg L^{-1}; (D) 2000 mg L^{-1}. Juvenile L. ferrea cuttings under treatments: (E) control; (F) 500 mg L^{-1}; (G) 1000 mg L^{-1}; (H) 2000 mg L^{-1}.

Similar results were obtained for *Aniba rosaeodora*, and show that cuttings of rejuvenated material present a higher percentage of rooting when compared to cuttings of juvenile to adult material [22]. Other species of the same family have also shown the importance of the juvenility of the material for the rooting of cuttings. For *Mimosa caesalpiniifolia* and *Prosopis alba*, it is observed that the mini-cuttings with a lower degree of lignification favored higher percentages of rooting when compared to the more lignified cuttings [10, 23].

The vigor of the cuttings from regrowth favored leaf retention, which led to a lower emission of new shoots when compared to juvenile cuttings, which showed greater leaf fall and more significant emission of shoots. Taiz and Zeiger (2013) [24] indicate that, in young leaves, fall is not induced despite adverse environmental conditions, since the high level of auxin in the leaf reduces the sensitivity of the abscission zone and prevents fall, while in mature or old leaves, once environmental changes occur, they can respond to the senescence signal, which is what was observed in our study.

We observed that leaf retention was an essential factor in the development of the root system. The cuttings from regrowth showed a lower leaf fall and a higher root formation, and the opposite behavior was observed in the cuttings of juvenile material. The synthesis of carbohydrates, auxin, and rooting cofactors occurs in the leaves [8], which may explain this result.

Other species also show the importance of leaves. *Piptocarpha angustifolia* presented the mortality of cuttings after leaf fall [13]; in *Litchi chinensis,* the total absence of leaflets on the

cuttings results in their death [25]; in mini-cuttings of *Mimosa caesalpiniifolia*, without leaf reduction and with a 25% reduction, rooting and plant development increased [23] and, in *Myrceugenia exsucca*, the presence of the leaves favored the survival and rooting of the cuttings [26].

The *L. ferrea* cuttings from regrowth and juvenile material showed a high percentage of callus formation in the absence of exogenous auxin (61.5%) and a satisfactory percentage of rooted cuttings (53%-23% respectively), thus evidencing the capacity of the material for cell differentiation. In some species, the formation of calluses is a precursor to the formation of adventitious roots; however, in some cases, they are independent processes due to external factors [8].

It can be inferred that callus formation favored root development since, in regrowth material, 100% of the rooted cuttings showed callus formation, while, in juvenile material, this was 81.5%. For some species, such as *Calliandra tweedii*, callus formation prior to rooting seems to be a prerequisite [27]. Others, such as *Vochysia bifalcata*, show the ability to form roots without the formation of calluses [28].

The use of IAA favored the development and rooting in both types of materials used. However, it is noted that the application of the highest dosage (2000 mg L^{-1}) can impair the development and survival of the cuttings. The material from regrowth showed an increase in the percentage of rooting and number of roots with the application of the maximum dosage; however, the other parameters evaluated decreased with the application of IAA.

As for the juvenile material, all evaluated parameters decreased when subjected to a dosage greater than 1000 mg L^{-1} of IAA. Plants contain a natural concentration of auxins that favor growth and induce root formation in the cuttings [8], but exceeding this dosage can cause toxicity, leading to the death of the cuttings [29].

The negative effect of using higher dosages of exogenous auxins has also been reported for other species. Mini-cuttings of *Apuleia leiocarpa*, treated with IBA had no significant influence on rooting success and survival, and the concentrations used reduced the number, length, and dry mass of the roots [30]. *Piptocarpha angustifolia* cuttings treated with IBA interfered in the increased mortality of the cuttings, suggesting a phytotoxic effect for the species [13].

4.2. Anatomy of the rooting of *L. ferrea* cuttings

The main anatomical differences observed between cuttings from regrowth and juvenile material were the degree of lignification and development of tissues. The cuttings from regrowth showed juvenile morphological characteristics, with a ring of pericyclic fibers with a lower degree of lignification, a greater reserve of starch grains, and a less lignified periderm. In contrast, the cuttings of juvenile material presented more lignified cells in both the periderm and pericyclic fibers and fewer reserve parenchyma cells.

The formation of roots is the main objective in propagation using cuttings; however, some internal conditions of the cuttings can decrease the success of the process. For some difficult-to-root species, it has been reported that the presence of a continuous ring of lignified tissue can act as a mechanical barrier to root emergence [8], as is the case of *Maytenus muelleri*, *Pictocarpa angustifolia,* and *Calliandra twedii* [13, 27, 31].

L. ferrea also presented the ring of pericyclic fibers, but this did not represent a barrier to the emission of roots. Nonetheless, it was found that the lower the degree of lignification, the greater the rooting success of the cuttings, thus evidencing the importance of the juvenility factor in the tissues. For *Calliandra brevipes*, *Vochysia bifalcata,* and *Ilex paraguariensis*, the ring of pericyclic fibers or sclerenchyma was also reported, though this ring did not impede the emission of roots [27, 28, 32].

The application of IAA may also have stimulated the rupture of the barrier formed by the pericyclic fibers and increased the percentage of rooted cuttings. Studies carried out on cuttings of *Malus domestica* that were treated with IAA showed that the use of this auxin promoted the hydrolysis of starch grains, leading to cell division and favoring rooting [33]. According to Hartmann et al. (2014) [8], treatments with auxins and rooting under high humidity levels cause

considerable cell expansion and proliferation in the cortex, phloem, and cambium, which result in breaks in continuous sclerenchyma rings, as was observed in our experiment.

The carbohydrate reserve also influenced the rooting success of the cuttings. According to Hartmann et al. (2014) [8], a higher rooting rate is associated with a higher carbohydrate content, which is consistent with our results. After 60 days of planting the cuttings, starch was still visible in the pith, with a higher percentage in the sprout cuttings, indicating that they had a high carbohydrate reserve at the time of cutting. Similar results were reported by Lischka et al. (2008) [27], who observed that *Calliandra brevipes* cuttings containing starch grains in the pith rooted better than *Calliandra tweedii* cuttings, which did not contain these grains.

Furthermore, the success in rooting rejuvenated cuttings compared to juvenile material may be explained by the metabolic "reprogramming" that occurs during rejuvenation [34]. This process promotes increased cellular activity and the accumulation of energy reserves, such as starch and soluble carbohydrates, which are essential for the growth and development of new tissues [34].

The formation of adventitious roots from the vascular cambium is considered direct, and those formed from callus tissue are known as indirect [8]. However, for the *L. ferrea* cuttings, it was not possible to determine the initiation type of the new root system, as no intermediate collections of cuttings were made during the experiment. Nevertheless, with the results obtained, it is plausible to infer that growth was direct, occurring from the vascular cambium, since the new tissues are adjacent to the xylem, which remains unchanged.

Previous studies on species from the same family as *L. ferrea* and other species, report that the formation of adventitious roots in cuttings occurs from the vascular cambium or from nearby regions, as observed in *Calliandra brevipes, Calliandra tweedii, Eucalyptus clones, and Maytenus muelleri* [27, 31, 35]. Thus, our findings confirm the importance of carbohydrate reserves and rejuvenation conditions for the successful rooting of cuttings and suggest that the vascular cambium is a critical origin point for the formation of adventitious roots in this context.

5. CONCLUSIONS

We concluded that the cutting technique, combined with material rejuvenation and the use of IAA, is an effective strategy for the vegetative propagation of L. ferrea, enabling commercially viable rooting and survival rates. The optimal IAA dosage was 1000 mg L^{-1}, as higher concentrations reduced the values of the parameters evaluated. Rejuvenation of the plant material contributed to reducing the degree of lignification in cuttings and increased starch grain concentration, which enhanced rooting and survival rates. Anatomical analyses also indicated that adventitious roots originate from the vascular cambium and that the presence of pericyclic fibers did not act as an anatomical barrier in the rooting process of the cuttings.

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