Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Histology and induction of rooting in rubber tree cuttings (*Hevea brasiliensis* (Willd. Ex A.Juss.) Müll.Arg.)

Histologia e indução ao enraizamento em estacas de seringueira (*Hevea brasiliensis* (Willd. Ex A.Juss.) Müll.Arg.)

L. F. Rocha[a], B. S. Francisco[b]*, F. B. Dutra[b], L. S. Souto[c], F. C. M. Pinã-Rodrigues[d] and J. M. S. Silva[d]

[a]Universidade Federal de São Carlos – UFSCar, Graduação em Engenharia Florestal, Sorocaba, SP, Brasil

[b]Universidade Federal de São Carlos – UFSCar, Programa de Pós-graduação em Planejamento e Uso de Recursos Renováveis, Sorocaba, SP, Brasil

[c]Universidade Federal de São Carlos – UFSCar, Departamento de Biologia, Laboratório de Diversidade Vegetal, Sorocaba, SP, Brasil

[d]Universidade Federal de São Carlos – UFSCar, Departamento de Ciências Ambientais, Sorocaba, SP, Brasil

Abstract

The rubber tree (*Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg.), a native Amazonian species, is responsible for more than 50 thousand products and stands out as the world's main source of natural rubber. Commercial production is carried out by grafting, however, the technique has gaps in terms of time and quality for seedling production. Vegetative production via cuttings is an alternative, however, the species is difficult to root. Thus, the study tested the rooting induction, through a chemical method, with the hormonal regulator indolbutyric acid (IBA) of 5000 ppm, and a mechanical method, with the strangulation of stems, and the interaction between the methods, to analyze the survival and sprouting of rubber tree (*Hevea brasiliensis*) cuttings, as well as verifying the efficiency of breaking the sclerenchyma ring by strangulation. A randomized block design was used, with four treatments (control, with strangulation, with IBA, strangulation x IBA) distributed in six blocks with 36 cuttings. Data were submitted to ANOVA test and Tukey's post-test ($p>0.05$). The results obtained 12.5% of live cuttings, without rooting, during 68 days, being the combination of strangulation and IBA with greater survival and sprouting. No breakage of the sclerenchyma ring was observed by histological analysis. The data indicate strategic gains in combining chemical and mechanical techniques for species of difficult rooting in vegetative propagation, however, the test was not enough to affirm an answer in relation to each technique, the deepening of the technique on the behavior of the species remains the biggest challenge.

Keywords: cuttings, seedling production, seedling.

Resumo

A seringueira (*Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg.), espécie nativa da Amazônia, é responsável por mais de 50 mil produtos e se destaca como a principal fonte mundial de borracha natural. A produção comercial é realizada por enxertia, porém, a técnica apresenta lacunas em termos de tempo e qualidade para produção de mudas. A produção vegetativa via estaquia é uma alternativa, porém a espécie é de difícil enraizamento. Assim, o estudo testou a indução do enraizamento, por método químico, com o regulador hormonal ácido indolbutírico (AIB) de 5000 ppm, e método mecânico, com estrangulamento de hastes, e a interação entre os métodos, para analisar a sobrevivência e brotação de estacas de seringueira (*Hevea brasiliensis*), bem como verificar a eficiência da quebra do anel esclerenquimático por estrangulamento. Utilizou-se o delineamento em blocos ao acaso, com quatro tratamentos (testemunha, com estrangulamento, com AIB, estrangulamento x AIB) distribuídos em seis blocos com 36 estacas. Os dados foram submetidos ao teste ANOVA e pós-teste de Tukey ($p>0,05$). Os resultados obtiveram 12,5% de estacas vivas, sem enraizamento, durante 68 dias, sendo a combinação de estrangulamento e IBA com maior sobrevivência e brotação. Nenhuma quebra do anel esclerenquimático foi observada pela análise histológica. Os dados indicam ganhos estratégicos em combinar técnicas químicas e mecânicas para espécies de difícil enraizamento na propagação vegetativa, entretanto, o teste não foi suficiente para afirmar uma resposta em relação a cada técnica, resta o aprofundamento da técnica sobre o comportamento da espécie maior desafio.

Palavras-chave: clonagem, produção de mudas, mudas.

*e-mail: brunofrancisco@estudante.ufscar.br

Received: April 18, 2023 – Accepted: August 14, 2023

1. Introduction

The rubber tree (*Hevea brasiliensis* (Willd. ex A. Juss.) Müll.Arg.) is native to the Amazon region and stands out in the economy mainly as a world source of natural rubber (Hurley, 1981). The economy of the species revolves around latex, a renewable raw material that impacts the world's industrial, economic, and social development. It is a component of more than 50,000 products, ranging from the tire industry for all types of transportation to the manufacture of flooring, footwear, adhesives, gloves, and surgical materials (Embrapa, 2022).

To supply the global demand, currently, the production is formed mainly by the grafting system, of the budding type (Borelli, 2016). Unselected seeds are used in the rootstock and in six months the seedlings are ready to receive the portion of the scion, responsible for the bleeding, and provided with selected hybrids (Masson and Monteuuis, 2017). The grafted seedlings are suitable for planting in about 24 to 48 months (Peres-Junior, 2019).

Rubber tree seeds express high genetic and phenotypic variability for characters of economic interest, such as low fruit production and recalcitrant seeds, making homogeneous production difficult (Gonçalves et al., 1990). The use of techniques to overcome the variability of the species was already noted since the beginning of production in the 1910s, mainly in Southeast Asia (Galbiati Neto and Guglielmetti, 2012). Grafting ensured large-scale development, however, its large-scale production remained long-term and high investment.

Parallel to production, research with other methods of vegetative propagation, such as cutting, was used (Castro et al., 1987). Starting in the 1970s, priority was given to in vitro methods that were booming at the time, but after years of investment, alternative techniques of rubber tree clones could not be propagated efficiently for commercial scale (Masson and Monteuuis, 2017). Although numerous researches have been conducted (Alves et al., 2019; Antwi-Wiredu et al., 2018; Cardoso, 1961; Lemos-Filho et al., 1994; Masson and Monteuuis, 2017; Medrado et al., 2000; Muzik, 1953; Muzik and Cruzado, 1958; Muzik and Cruzado,1956), few published studies have achieved rooting success, however, none of them used the combination of techniques tested in our study (Castro et al., 1987; Medrado et al., 1995; Mendes, 1959; Monteiro et al., 2015; Silva et al., 2020; Vallejos-Torres et al., 2021).

To guarantee the success of the techniques, it is necessary to have knowledge of the species and its endogenous factors for propagation, as well as control over local environmental factors. These factors may act in an isolated or integrated way and may benefit or not the induction of rooting (Cuco, 1997).

Some internal factors to consider are tissue maturation, anatomical barriers, hormonal balance, the vigor of the mother plant and type of cuttings, and external factors such as time of collection of cuttings, relative humidity, and climatic conditions of the propagation site (Dias et al., 2012). These factors can be achieved with the aid of chemical or mechanical systems for inducing the rooting of cuttings (Borges et al., 2011).

Among the chemical system, there are the hormonal regulators, such as indol butyric acid (IBA), which has been one of the most used in research and commercial use, since it has low mobility and greater chemical stability in the cuttings, but the toxicity may vary depending on the species and concentration (Khan et al., 2024). The exogenous application acts in auxin control, interfering in the meristematic activity of the cuttings (Cuco, 1997), as well as in carbohydrate transport and increase in DNA synthesis, which implies a greater photosynthetic performance and survival of the cuttings (Vallejos-Torres et al., 2021).

Among the mechanical system, there is strangulation, mentioned Medrado et al. (1995) for rubber trees. The method consists of partially blocking the flow of nutrients, just above the strangulated point, forcing cell differentiation and thus aiding rooting. The system is also efficient in breaking the sclerenchyma ring, characteristic of the species, and leads to greater cell differentiation for rooting.

The mastery of vegetative propagation for the rubber tree contributes to advances in forestry. Studies with rubber trees date back more than a century, including attempts at vegetative propagation (Muzik, 1953). This issue is gaining more and more space in view of developing techniques for seedling production and optimizing resources adapted for clonal production, ensuring greater productivity and seedlings with high added value.

In this work we evaluated the survival, sprouting and root formation of rubber tree (*Hevea brasiliensis* (Willd. ex A.Juss.) Müll.Arg.) clones RRIM 600, using two methods of rooting induction. We also tested the strangulation technique as an inducer of sclerenchyma ring breakage of the cuttings.

2. Methods

Our experiment was conducted in the greenhouse located at Universidade Federal de São Carlos (UFSCar), municipality of Sorocaba, state of São Paulo, Brazil (23°35'09.4 "S 47°31'10.4 "W), from April to July 2021. The climate classification is Cfa (humid subtropical climate), with a mean temperature of 20.5 °C and mean annual rainfall of 1219 mm (Climate-Data, 2021).

We collected two-year-old and six-month-old rubber tree cuttings of the RRIM 600 clones, from grafts produced via sexual propagation (seminal), cultivated under field conditions, with 3 m x 3 m spacing, at the experimental plantation of the UFSCar, Sorocaba campus. 30 days before planting, 36 rubber matrices were identified and throttled three stems per matrix, with 0.77 mm steel wire (Figure 1), and the fourth stem was not throttled because it was the control. The position of the strangulation was ten cm from the apical stem, between the buds, according to Medrado et al. (1995).

We collected four cuttings per matrix, totaling 144 cuttings, which were then placed in Styrofoam boxes with cold water and humidified with a manual sprayer to ensure good conditions for the vegetative material, until the staking site. We prepared the apical cuttings with a length of ten cm, leaving three or four leaves cut in half, no disinfection treatment was performed on the cuttings before staking (Figure 1).

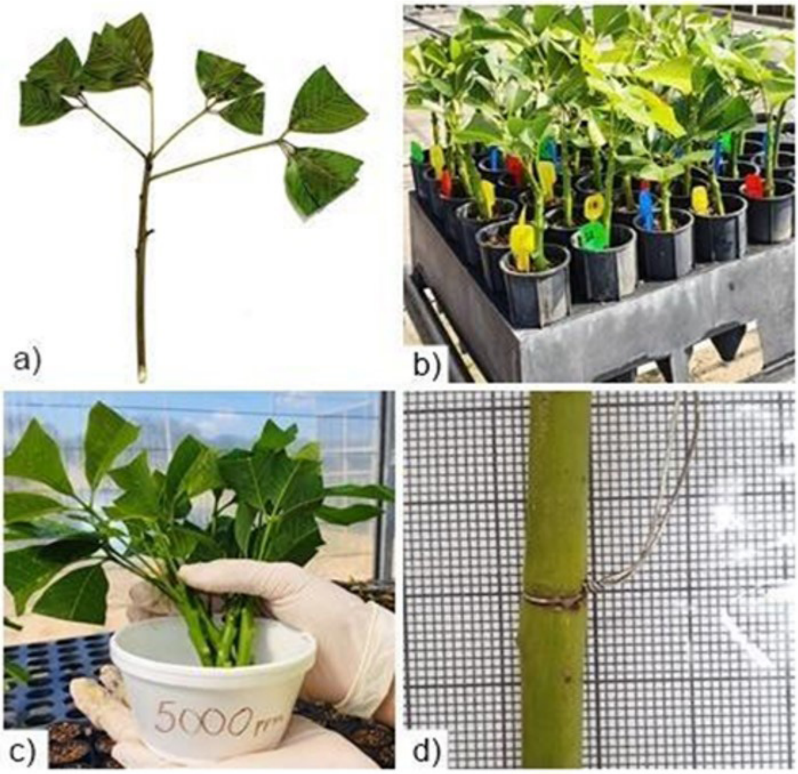

Figure 1. a) Stake without strangulation prepared for planting in a tube. b) Tray representing a block with all the treatments, with treatments identified with colored spoons. c) Immersion of stakes in IBA 5000ppm solution. d) Stake with strangulation technique performed between the buds.

We immersed the base of all cuttings for 20 seconds (Figure 1) in a concentration of Indolbutyric Acid (IBA) prepared in the laboratory, at a dose of 5000 ppm, as described by Castro et al. (1987). Staking was performed in the greenhouse, in 280 cm^3 tubes with Carolina Soil (type XVI) substrate, both sterilized (Figure 1). To identify the treatments, we used colored spoons. The trays were conditioned on suspended benches. The humidity and temperature in the greenhouse were maintained at 95% relative humidity (RH) by means of a misting system, with a blade of 6 mm/day, and a flow rate of 64 l/h, divided into 12 sets of 2 minutes each. The residence period of the cuttings in the greenhouse was 68 days.

Whenever we noticed leaf abscission of the cuttings, these leaves were removed from the trays to avoid contamination. We quantified the number of live cuttings; we considered alive those cuttings that presented more than 70% of the green branch. We sampled the number of sprouts per cutting, being considered sprouting the elongation at the apex or laterals of the branch (Deus et al., 2020). Furthermore, we checked the presence of roots per cutting, the presence of lenticels, and the presence of callus (Deus et al., 2020).

2.1. Anatomy

We collected three samples of strangulated cuttings from three different matrices, the same was done with three non-strangulated cuttings, for posterior comparison. We fixed all samples in FAA 50 (formaldehyde: glacial acetic acid: 50% ethanol, at 5:5:90 v/v/v) (Johansen, 1940) for 48h, and then stored them in 70% ethanol.

Samples were dehydrated in graded ethyl series, included in cold (2-hydroxyethyl)-methacrylate Leica TM (Paiva et al., 2011). We cut on a Leica® rotary microtome 8 µm thick in transverse and longitudinal positions. The slides were stained with 0.05% Toluidine Blue in acetate buffer pH 4.7 (O'Brien et al., 1964, modified) and mounted in water for observation. All samples were analyzed and photographed on an Olympus® BX53 light microscope with an Olympus® digital camera attached.

2.2. Experimental design in a vegetation greenhouse

The experimental design was randomized block design (BDC), where six blocks were randomized in the greenhouse, with nine cuttings from each of the four treatments per block, totaling 36 cuttings per block.

Treatment 1 or control treatment (cuttings without any rooting induction method), Treatment 2 (cuttings strangled for 30 days in the matrix), Treatment 3 (cuttings without strangulation and with an application of 5000 ppm of IBA), and Treatment 4 (cuttings strangled for 30 days in the matrix and application of 5000 ppm of IBA).

2.3. Data analysis

We tested the data for normality and homoscedasticity. After verifying that our data were normal, we used the analysis of variance through the ANOVA test to check if there was a difference between the survival of the cuttings in the four treatments. In addition, we checked whether there was a difference in the number of sprouts per cutting between treatments using the ANOVA test. Both analyses were subjected to Tukey's post-test ($p<0.05$). All analyses were processed in Python language 3.8.12, in Jupyter Lab interface, using pandas and statsmodels libraries (Python Software Foundation, 2022).

3. Results

3.1. Cuttings

Leaf abscission in the cuttings occurred from day 20 (the twentieth day) of testing and continued until day 68 (the last day of sampling). In no treatment with live cuttings, we did observe callus and root formation. We observed the presence of two lenticels, one in treatment 1 (cuttings without any rooting induction method) and another in treatment 4 (cuttings strangled for 30 days in the matrix and application of IBA at 5000ppm). All the cuttings with necrotic bases (Figure 2).

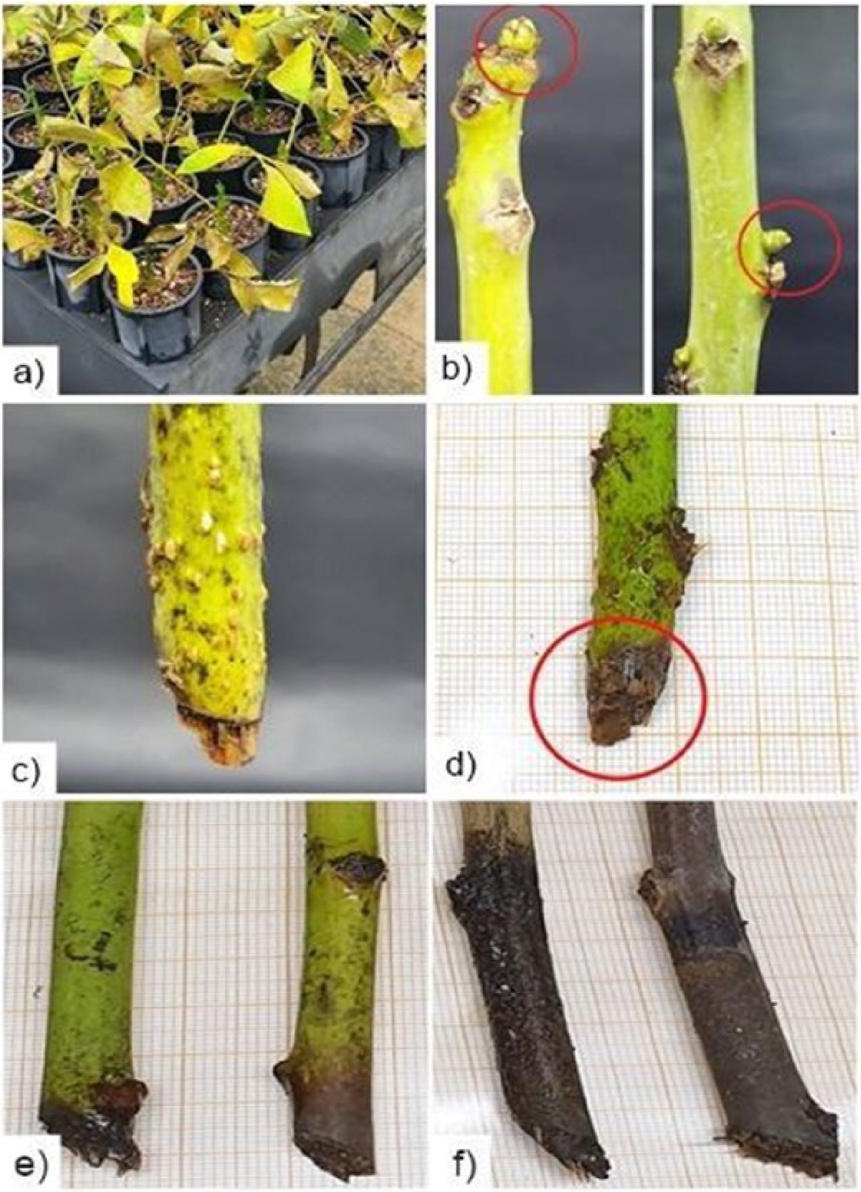

Figure 2. a) Yellowing and leaf abscission of the cuttings; b) Bud formation in the cuttings at 68 days; c) Lenticels formation in the basal part of the cut; d) Basal necrosis of the cut; f) Identification of a live cut with more than 70% green and g) Identification of a dead cut with total necrosis.

After 68 days of testing, only 12.5% of the total number of cuttings survived, i.e., only twenty-seven cuttings with at least 70% of green branches, being six cuttings (22.2%) in treatment 1, four cuttings (14.8%) in treatment 2 (cuttings strangled for thirty days in the matrix), no cuttings (0%) in treatment 3 (cuttings without strangulation and with IBA application at 5000ppm) and 17 cuttings (63.0%) in treatment 4.

We observed that 66.7% (18 cuttings) of all live cuttings sprouted, 4 cuttings (22.2%) in treatment 1, 3 cuttings (16.7%) in treatment 2 (cuttings strangled for thirty days in the matrix) sprouted, no cuttings in treatment 3 (cuttings without strangulation and with an application of IBA at 5000ppm) sprouted, and 11 cuttings (61.1%) in treatment 4 sprouted.

We found that treatment 4 was the only one that showed a difference between the studied treatments, being the treatment with the highest number of surviving cuttings (mean=2.8 per block; standard deviation =± 1.3) and with the highest number of sprouts (mean=2.0; standard deviation ± 1.0) (Figure 3).

The combination of strangulation and IBA techniques showed greater survival and sprouting, differing from the other treatments. Thus, indicates that the rooting induction techniques tested in the present study could have beneficial effects, but are not decisive in guaranteeing the rooting of the cuttings.

3.2. Anatomy

After 30 days of strangulation, no structural changes were observed between control plants and those that underwent strangulation (Figure 4a-g). The epidermis of the cuttings is uniseriate, formed by cells with a thick outer periclinal wall (Figure 4e). The epidermis of the cuttings is uniseriate, formed by cells with a thick outer periclinal wall (Figure 4e). The cortex is externally composed of fundamental parenchyma where phenolic idioblasts, crystalliferous idioblasts, and sclereids occur (Figure 4c-e). In the median region of the cortex, there is a sclerenchyma ring (Figure 4c-g) formed by fibers and sclereids (Figure 4f), this ring presents about two layers of thick cells being interrupted in some regions by fundamental parenchyma (Figure 4g). The inner region of the cortex is composed of fundamental parenchyma, with phenolic idioblasts, but in smaller quantities, if compared to the outer region of the cortex (Figure 4e). The vascular system is formed by the secondary phloem and xylem, and internally to the xylem it is still possible to observe the parenchyma pitch (Figure 4a-b).

4. Discussion

4.1. Cuttings

The leaf abscission observed in our study may be related to water stress, because in cuttings with a larger leaf area, as is the case of the rubber tree, water loss through transpiration is greater, with a greater possibility of suffering water stress, closing the stomata and limiting photosynthesis, thus possibly causing leaf abscission (Vallejos-Torres et al., 2021). As well as from suffering the "umbrella" effect, preventing intermittent water from entering the substrate (Beyl and Trigiano, 2016).

For the rubber tree, the results of Vallejos-Torres et al. (2021) suggest the use of five leaves, the study also observed lower survival at twenty-nine days with two leaves, but even so, studies are needed to establish an ideal range, considering that our study tested three to four leaves per cuttings, and we did not get positive results, which leads to considering that the amount of leaves is a beneficial factor but not limiting one for the rooting of the species.

The balance between the number of leaves and leaf area per cutting depends on the morphology of each species (Leakey, 2014), as well as on the environmental conditions. The greenhouse can be a favorable place for greater control of external factors and greater air

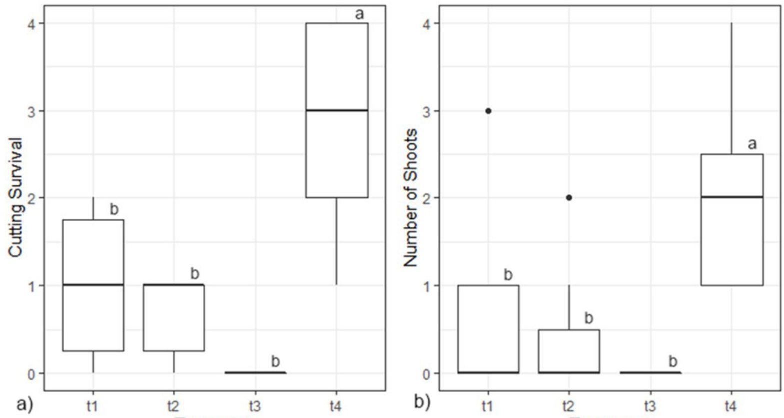

Figure 3. Box-plot of median and quartiles of the analysis of variance among treatments for a) survival of cuttings (p<0.0001) and b) number of sprouts (p=0.0003). t1 = cuttings without any rooting induction method, t2 = cuttings strangled for 30 days in the matrix, t3 = cuttings without strangulation and with an application of 5000 ppm of IBA, t4 = cuttings strangled for 30 days in the matrix and application of 5000 ppm of IBA. ANOVA test (P<0,05) and Tukey's post-test p<0,05.

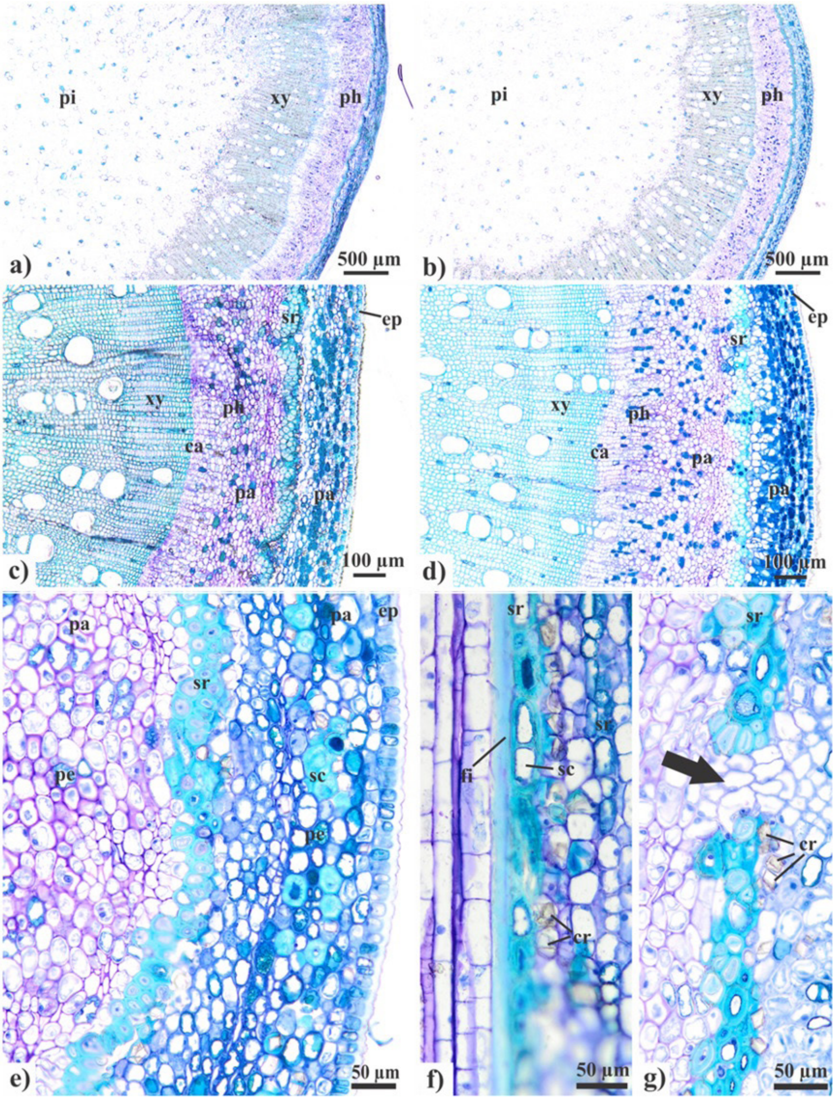

Figure 4. Transverse (a-e, g) and longitudinal sections (f) and *Hevea brasiliensis* cuttings from the control treatment (a, c, e-g) and submitted to strangulation (b, d). (a-b) General view of the stem. c-d) Detail of the cortex region and vascular system, note the sclerenchyma ring in the cortex. e) Detail of the cortex with a sclerenchyma ring, phenolic idioblasts, and sclereids. f) sclerenchyma ring in the longitudinal section, showing the presence of fibers and sclereids. g) Detail of the region where the sclerenchyma ring is interrupted by parenchyma (arrow). Abbreviation: ca, exchange; cr, crystals; ep, epidermis; fi, fibers; pa, parenchyma; eg, phenolic idioblast; ph, phloem; pi, pith; sc, sclereid; sr, sclerenchyma ring; xy, xylem; pe; phenolic compounds.

circulation in the environment for rubber tree propagation (Smart et al., 2002). However, the site may have partial control of edaphoclimatic conditions.

We found a wide presence of cutting mortality, with 87.5% dying early after leaf abscission, and of the live cuttings, all presented necrosis at the base. Although the utensils used in the greenhouse were sterilized before the implementation of the experiment, the cuttings were not sterilized, being the main source of inoculum. In the in vitro propagation of rubber trees, contamination control is also complex, especially when mature plant material is used, with a predominance of endophilic propagules (Cuco, 1997). However, pilot studies with fungicides are emerging to control the species' propagules and understand whether it is an internal or external cause of the plant (Silva et al., 2021).

In our work, there was no rooting of the cuttings, even using the proposed set of mechanical and chemical techniques. Some authors have already reported that the rubber tree is a difficult species to root (Medrado et al., 1995; Mendes, 1959; Muzik and Cruzado, 1958). The first attempts date back to 1870 and only in 1950, with the help of techniques, were the first promising studies achieved in young plants (Cuco, 1997). Muzik (1953) reported one of the first recorded attempts, and only seven years later Tinley and Garner (1960) stated that the species could have potential through propagation techniques.

The difficulty in rooting may be related to several factors, internal to the material, such as age, levels of endogenous phytoregulators, presence of carbohydrates, bud dormancy, and the emergence of sprouts, or external such as the time of collection of the material, temperature, and humidity conditions for harvest and in the greenhouse (Smart et al., 2002). Even in vitro rubber tree tests, the difficulty of rooting is present (Cuco, 1997). In mature plant material, the loss of the rhizogenic layer, referring to the meristematic activities of the lateral roots, is related to the reduction of IAI (indolacetic acid) and increase of ABA (abscilic acid) (Cuco, 1997). As for rubber tree latex, we did not find studies that indicate that it is a factor that inhibits rooting.

The results of the treatments with IBA showed that the isolated use of the hormone regulator was not able to guarantee the survival of any cutting. However, when associated with the strangulation technique, it proved positive. This indicates that the use of hormonal regulators, such as IBA, is a propitious technique for vegetative propagation until a certain peak is reached, if exceeded, toxic effects may occur (Vallejos-Torres et al., 2021).

It has already been reported in studies prior to this one that rubber trees have not been shown to have adverse effects at IBA doses up to 5000 ppm (Castro et al., 1987; Monteiro et al., 2015; Vallejos-Torres et al., 2021). In addition, IBA doses may be able to affect root length and not interfere with plant survival (Vallejos-Torres et al., 2021).

We verified that the use of IBA alone was not effective for the survival and sprouting of the cuttings, as well as, just the strangulation, but it is important to highlight that the combination of methods was the only one to provide the greatest survival and production of sprouts in the cuttings. Castro et al. (1987) observed in rubber tree cuttings, after 77 days of evaluation, that the formation of shoots could be mainly associated with the survival of the cutting, and that its formation did not necessarily imply rooting. Tosta et al. (2012) reported that the use of IBA can induce the number and length of shoots, but that the emission of shoots in cuttings is not determinant for rooting.

4.2. Anatomy

The analysis showed no anatomical difference between the control plants and those that suffered strangulation, and in both cases, the sclerenchyma ring is interrupted. The strangulation technique in rubber trees had already been successfully used by Mendes (1959) and Medrado et al. (1995), and both observed that besides the breaking of the sclerenchyma ring, the strangulation technique can also cause the accumulation of hydrocarbons just above the strangulated point, as also auxins, interfering in the meristematic activity of the cutting, which explains the greater survival of the cuttings, without effectively inducing rooting.

Cox (2018) verified that some species continue to have difficulty in rooting, even with the break in the continuity of the sclerenchyma ring, due to meristematic activity, and Beakbane (1961), already mentioned that it is difficult to explain rooting solely based on the mechanical barrier, however, the decrease in the proportion of sclerenchymatic tissues by the strangulation technique, can promote conditions for the formation of root primordia (Medrado et al., 1995).

Root primordia are formed through the meristem resulting from the differentiation of cambium cells, therefore, any tissue with a primary cell wall of the cellular content can become meristematic again (Taiz and Zeiger, 2009). However, as the secondary wall forms and the material matures, the lignified tissue may become an anatomical barrier (Dias et al., 2012).

Phenolic compounds were also observed, which are part of secondary metabolism and are considered natural inhibitors against injuries and abiotic stresses and may be associated with allelopathic actions, as seen potential in the species in initial trials with *Lactuca sativa* L. (Rocha et al., 2021).

In our study, we identified that the sclerenchyma ring formed by fibers and sclereids has a supporting role by having secondary walls as proposed by Taiz & Zeiger (2009). Despite being related to the degree of lignin in the tissue, these cells are not necessarily related to cell maturity, being possible to form in any period, not only in mature tissues (Apezzato-da-Gloria & Carmello-Guerreiro, 2003). Ontogenesis studies would be convenient to verify the cellular behavior during tissue maturation, in addition to verifying whether root formation occurs in regions near the interruptions of the sclerenchyma ring, or independently of it since there are several studies showing that rooting was granted without arising from mechanical techniques (Castro et al., 1987; Silva et al., 2020; Monteiro et al., 2015; Medrado et al., 1995; Mendes, 1959; Vallejos-Torres et al., 2021).

5. Conclusion

There was no induction of the root system, however, the combination of techniques (strangulation for 30 days and IBA at a dosage of 5000ppm) was the best option for the survival and production of shoots in the cuttings, which indicates that combining chemical and mechanical techniques is a good option for species that are difficult to root in vegetative propagation.

The mechanical and chemical techniques for rooting induction used for staking of *Hevea brasiliensis* obtained 12.5% of live cuttings, during 68 days. Of the total number of live cuttings, 66.7% sprouted. The strangulation technique is not necessary for the rupture of the sclerenchyma ring, since it was possible to observe the discontinuity of the ring even in the control treatment.

We suggest further studies with other techniques and variations in rooting doses, as well as in the morphological and physiological study of the species so that ideal protocols and conditions can be established for the production of seedlings via vegetative propagation.

Acknowledgements

The authors thanks for GRIPMASTER and CAPES for granting research grants and the Laboratory of Seeds and Forest Seedlings (LASEM) of UFSCar (Federal University of São Carlos) for providing all human and material resources for the field experiment.

References

ALVES, V.C.D., FERREIRA, S.E., BORGES, R.S. and MARUYAMA, W.I., 2019 [viewed 10 October 2021]. Enraizamento de estacas de seringueira com uso de ácido bórico [online]. In: *Anais do ENIC*, 2019, Mato Grosso do Sul. Dourados: UEMS, no. 11. Available from: https://anaisonline.uems.br/index.php/enic/article/view/6448

ANTWI-WIREDU, A., AMITEYE, S., DIAWUOH, R.G. and KLU, G.Y., 2018. Ex vitro propagation of rubber tree (*Hevea Brasiliensis*) using stem cuttings. *International Journal of Environment, Agricultural Biotechnology*, vol. 3, no. 3, pp. 846-854. http://dx.doi.org/10.22161/ijeab/3.3.20.

APEZZATO-DA-GLÓRIA, B. and CARMELLO-GUERREIRO, S.M., 2003 [viewed 10 October 2021]. Anatomia vegetal [online]. 4. ed. Viçosa: Editora da Universidade Federal de Viçosa. 438 p. Available from: https://uab.ufsc.br/biologia/files/2020/08/Anatomia-Vegetal.pdf

BEAKBANE, A.B., 1961. Structure of the plant stem in relation to adventitious rooting. *Nature*, vol. 192, no. 4806, pp. 954-955. http://dx.doi.org/10.1038/192954a0.

BEYL, C.A. and TRIGIANO, R.N., 2016. Plant propagation concepts and laboratory exercises. Boca Raton: CRC Press.

BORELLI, K., 2016. Produção de mudas de seringueira em viveiro suspenso. Piracicaba: Universidade de São Paulo, 87 p. Dissertação de Mestrado em Ciências, Programa de Recursos Florestais. http://dx.doi.org/10.11606/D.11.2016.tde-28032016-123413.

BORGES, S.R., XAVIER, A., OLIVEIRA, L.S.D., MELO, L.A.D. and ROSADO, A.M., 2011. Enraizamento de miniestacas de clones híbridos de Eucalyptus globulus. *Revista Árvore*, vol. 35, no. 3, pp. 425-434. http://dx.doi.org/10.1590/S0100-67622011000300006.

CARDOSO, M., 1961. Conservação de hastes de seringueira destinadas à enxertia. *Bragantia*, vol. 20, no. unico, pp. 63-66. http://dx.doi.org/10.1590/S0006-87051961000100062.

CASTRO, P.R., MORETI, A.C., TOLEDO-FILHO, M.R., BERNARDES, M.S., SILVA FILHO, N.L. and PERES-FILHO, O., 1987. Estimulação do enraizamento de estacas de seringueira (*Hevea brasiliensis* Muell. Arg.) pela aplicação de reguladores vegetais. *Anais da Escola Superior de Agricultura Luiz de Queiroz*, vol. 44, no. 2, pp. 1025-1035. http://dx.doi.org/10.1590/S0071-12761987000200007.

CLIMATE-DATA, 2021 [viewed 10 October 2021]. Clima Sorocaba (Brasil) [online]. Climate-data.org. Available from: https://pt.climate-data.org/america-do-sul/brasil/sao-paulo/sorocaba-756/

COX, D.A., 2018. Hartmann and Kester's plant propagation principles and practices. *HortScience*, vol. 53, no. 5, pp. 741-741.

CUCO, S.M., 1997. Caracterização citomorfológica da seringueira [Hevea brasiliensis (Willd. ex Adr. de Juss.) Muell. Arg.]. Piracicaba: Universidade de São Paulo, 137 p. Dissertação de Mestrado em Agronomia. http://dx.doi.org/10.11606/T.11.2020.tde-20200111-143556.

DEUS, R.R.P., COUTINHO, G. and MELO, E.T., 2020 [viewed 10 January 2022]. Ácido indolbutírico como indutor de enraizamento em estacas de pequizeiro. *Magistra* [online], vol. 31, pp. 611-619. Available from: http://docplayer.com.br/204143976-Acido-indolbutirico-como-indutor-de-enraizamento-em-estacas-de-pequizeiro.html

DIAS, P.C., DE OLIVEIRA, L.S., XAVIER, A. and WENDLING, I., 2012. Estaquia e miniestaquia de espécies florestais lenhosas do Brasil. *Pesquisa Florestal Brasileira*, vol. 32, no. 72, pp. 453-462. http://dx.doi.org/10.4336/2012.pfb.32.72.453.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA - EMBRAPA, 2022 [viewed 10 January 2022]. Cientistas usam nanotecnologia para avaliar novos clones de seringueira [online]. Brasília: EMBRAPA. Available from: https://www.embrapa.br/instrumentacao/

GALBIATI NETO, P. and GUGLIELMETTI, L.C., 2012. Heveicultura, a cultura da seringueira. São José do Rio Preto, SP: Grafisa -Santos Gráfica e Editora. 344 p.

GONÇALVES, P. S., CARDOSO, M. and ORTOLANI, A.A., 1990. Origem, variabilidade e domesticação da *Hevea*: uma revisão. *Pesquisa Agropecuária Brasileira*, vol. 25, no. 2, pp. 135-156.

HURLEY, P.E., 1981. History of natural rubber. *Journal of Macromolecular Science. Chemistry*, vol. 15, no. 7, pp. 1279-1287. http://dx.doi.org/10.1080/00222338108056785.

JOHANSEN, D.A., 1940 [viewed 10 January 2022]. Plant microtechnique [online]. New York: McGraw-Hill Publishing Company, Ltd. Available from: https://www.scirp.org/(S(lz5mqp453edsnp55rrgjct55.))/reference/referencespapers.aspx?referenceid=1848276

KHAN, M.A., WANG, Y., MUHAMMAD, B., UDDIN, S., SAEED, A., KHAN, D., ALI, M., SAEED, S., and JIA, Z., 2024. Morpho-physiological and phytohormonal changes during the induction of adventitious root development stimulated by exogenous IBA application in *Magnolia biondii* Pamp. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 24, pp. e255664. https://doi.org/10.1590/1519-6984.255664.

LEAKEY, R.R.B., 2014. Plant cloning: macropropagation (Clonación de plantas: macropropagación). *Encyclopedia of Agriculture and Food Systems*, vol. 4, pp. 349-359. http://dx.doi.org/10.1016/B978-0-444-52512-3.00223-0

LEMOS-FILHO, J.P., PEREIRA, J.D.P. and MEDRADO, M.S., 1994 [viewed 10 January 2022]. Mini-enxertia da seringueira (*Hevea* Spp.) Ii. problemas e avanços na técnica. *Pesquisa Agropecuária Brasileira* [online], vol. 29, no. 5, pp. 779-784. Available from: https://seer.sct.embrapa.br/index.php/pab/article/view/4115/1406

MASSON, A. and MONTEUUIS, O., 2017. Rubber tree clonal plantations: grafted vs self-rooted plant material. *Bois et Forêts des Tropiques*, vol. 332, pp. 57-68. http://dx.doi.org/10.19182/bft2017.332.a31333.

MEDRADO, M.J.S., APPEZZATO-DA-GLÓRIA, B. and COSTA, J.D., 1995. Alterações anatômicas em estacas de seringueira (*Hevea brasiliensis* clone RRIM 600) em resposta a diferentes técnicas de indução ao enraizamento. *Scientia Agrícola*, vol. 52, no. 1, pp. 89-95. http://dx.doi.org/10.1590/S0103-90161995000100016.

MEDRADO, M.J.S., COSTA, J.D., FONSECA FILHO, H., LEAL, R.S. and POMPERMEYR, S.A., 2000 [viewed 10 January 2022]. Influência da época de poda da planta matriz na produção de brotações para formação de estacas do clone GT1, de Hevea brasiliensis [online]. Colombo: Embrapa Florestas. 12 p. Embrapa Florestas. Boletim de Pesquisa, no. 1. Available from: https://www.infoteca.cnptia.embrapa.br/bitstream/doc/290722/1/Boletimdepesquisa01.pdf

MENDES, L.O.T., 1959 [viewed 10 January 2022]. A multiplicação da Seringueira por meio de estacas. *Boletim Técnico do Instituto Agronômico do Estado de São Paulo* [online], vol. 18, no. 17, pp. 245-274. Available from: https://www.scielo.br/j/brag/a/rrZz3YL59ZqRg4ZR8JxnQqt/?format=pdf&lang=pt

MONTEIRO, W.R., MARQUES, J.R.B. and PACHECO, E.R., 2015. Produção de mudas de seringueira por meio do enraizamento de estacas coletadas em plantas adultas. *Agrotropica*, vol. 27, no. 2, pp. 191-198. http://dx.doi.org/10.21757/0103-3816.2015v27n2p191-198.

MUZIK, T.J. and CRUZADO, H.J., 1956. Formation and rooting of adventitious shoots in Hevea brasiliensis. *American*

Journal of Botany, vol. 43, no. 7, pp. 503-508. http://dx.doi.org/10.1002/j.1537-2197.1956.tb10524.x.

MUZIK, T.J. and CRUZADO, H.J., 1958. Transmission of juvenile rooting ability from seedlings to adults of *Hevea brasiliensis. Nature*, vol. 181, no. 4618, pp. 1288-1288. http://dx.doi.org/10.1038/1811288a0.

MUZIK, T.J., 1953. Growth and regeneration in *Heve*a seedling. *Science*, vol. 117, no. 3047, pp. 555-556. http://dx.doi.org/10.1126/science.117.3047.555. PMid:13056611.

O'BRIEN, T., FEDER, N. and MCCULLY, M.E., 1964. Polychromatic staining of plant cell walls by toluidine blue O. *Protoplasma*, vol. 59, no. 2, pp. 368-373. http://dx.doi.org/10.1007/BF01248568.

PAIVA, E.A.S., PINHO, S.Z.D. and OLIVEIRA, D.M.T., 2011. Large plant samples: how to process for GMA embedding?. In: H. CHIARINI-GARCIA and R. MELO, eds. Light microscopy. Totowa: Humana Press, vol. 689, pp. 37-49. http://dx.doi.org/10.1007/978-1-60761-950-5_3.

PERES-JÚNIOR, J.B.R., 2019. Estudo das características do látex e da borracha de Hevea brasiliensis cultivadas e nativas da Amazônia. Brasília: Universidade de Brasília, 99 p. Dissertação de Doutorado em Química.

PYTHON SOFTWARE FOUNDATION, 2022 [viewed 10 January 2022]. Python Language Site: Documentation, 2022 [online]. Available from: https://www.python.org/doc/

ROCHA, L.F., FRANCISCO, B.S., DUTRA, F.B., TERAÇÃO, B.S., ALMEIDA, L.S., VIVEIROS, E., PIÑA-RODRIGUES, F.C.M. and SILVA, J.M.S., 2021. Efeitos alelopáticos de seringueira (*Hevea brasiliensis* (Willd. Ex A. Juss.) Müll.Arg.) na germinação e crescimento inicial da alface (*Lactuca sativa* L.). *Research, Social Development*, vol. 10, no. 14, pp. e70101421712. http://dx.doi.org/10.33448/rsd-v10i14.21712.

SILVA, A.C.L., MANFIO, C.E., LEÃO, J.R.A., DE CARVALHO, J.C., DE CARVALHO GONÇALVES, J.F. and RAPOSO, A., 2021. Indução da calogênese em segmentos foliares de seringueira (*Hevea* spp.) na Amazônia Sul-Ocidental. *Research, Social Development*, vol. 10, no. 9, pp. e17410917639. http://dx.doi.org/10.33448/rsd-v10i9.17639.

SILVA, J.V.D., MARUYAMA, W.I., OLIVEIRA, C.E.D.S., STEINER, F., ZUFFO, A.M. and ZOZ, T., 2020. Zinc-rooting cofactor in rubber tree mini-cuttings. *Bioscience Journal*, vol. 36, no. 6, pp. 1821-1827. http://dx.doi.org/10.14393/BJ-v36n6a2020-48170.

SMART, D.R., KOCSIS, L., ANDREW WALKER, M. and STOCKERT, C., 2002. Dormant buds and adventitious root formation by vitis and other woody plants. *Journal of Plant Growth Regulation*, vol. 21, no. 4, pp. 296-314. http://dx.doi.org/10.1007/s00344-003-0001-3.

TAIZ, L. and ZEIGER, E., 2009. Fisiologia vegetal. 6a. ed. Porto Alegre: Artmed, pp. 848.

TINLEY, G.H. and GARNER, R.J., 1960. Developments in the propagation of clones of *Hevea brasiliensis* by cuttings. *Nature*, vol. 186, no. 4722, pp. 407-408. http://dx.doi.org/10.1038/186407a0.

TOSTA, M.S., OLIVEIRA, C.V.F., FREITAS, R.M.O., PORTO, V.C.N., NOGUEIRA, N.W. and TOSTA, P.A.F., 2012. Ácido indolbutírico na propagação vegetativa de cajaraneira (Spondias sp.). *Semina: Ciências Agrárias*, vol. 33, no. 1, pp. 2727-2740. http://dx.doi.org/10.5433/1679-0359.2012v33Supl1p2727.

VALLEJOS-TORRES, G., RÍOS-RAMÍREZ, O., CORAZON-GUIVIN, M.A., REÁTEGUI, E., MESÉN SEQUEIRA, F. and MARÍN, C., 2021. Effects of leaflets and indole-3-butyric acid in the vegetative propagation by mini-tunnels of rubber tree (Hevea brasiliensis). *Journal of Rubber Research (Kuala Lumpur, Malaysia)*, vol. 24, no. 3, pp. 533-540. http://dx.doi.org/10.1007/s42464-021-00097-5.