

## **Minicutting technique and IBA application in vegetative propagation of *Schinus terebinthifolia* Raddi**

**Técnica de miniestaquia e aplicação de AIB na propagação vegetativa de *Schinus terebinthifolia***

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### **Felipe Bueno Dutra**

ORCID: <https://orcid.org/0000-0002-6280-7307>

Universidade Federal de São Carlos, Brazil

E-mail: [fbdutra@estudante.ufscar.br](mailto:fbdutra@estudante.ufscar.br)

### **Bruno dos Santos Francisco**

ORCID: <https://orcid.org/0000-0001-8586-3750>

Universidade Federal de São Carlos, Brazil

E-mail: [brunofrancisco@estudante.ufscar.br](mailto:brunofrancisco@estudante.ufscar.br)

### **Lausanne Soraya de Almeida**

ORCID: <https://orcid.org/0000-0001-8689-580X>

Universidade Federal de São Carlos, Brazil

E-mail: [lausannesoraya@gmail.com](mailto:lausannesoraya@gmail.com)

### **Ivonir Piotrowski Santos**

ORCID: <https://orcid.org/0000-0001-6697-8093>

Universidade Federal de São Carlos, Brazil

E-mail: [ivonir@ufscar.br](mailto:ivonir@ufscar.br)

### **Emerson Viveiros**

ORCID: <https://orcid.org/0000-0002-0215-0585>

AES Brasil, Brazil

E-mail: [emerson.viveiros@aes.com](mailto:emerson.viveiros@aes.com)

### **Rafael Paranhos Martins**

ORCID: <https://orcid.org/0000-0003-4264-3736>

AES Brasil, Brazil

E-mail: [rafael.paranhos@aes.com](mailto:rafael.paranhos@aes.com)

### **Raquel Passaretti**

ORCID: <https://orcid.org/0000-0001-6975-1505>

AES Brasil, Brazil

E-mail: [raquel.passaretti@aes.com](mailto:raquel.passaretti@aes.com)

### **Fatima Conceição Márquez Piña-Rodrigues**

ORCID: <https://orcid.org/0000-0001-8713-448X>

Universidade Federal de São Carlos, Brazil

E-mail: [fpinarodrigues@gmail.com](mailto:fpinarodrigues@gmail.com)

### **José Mauro Santana da Silva**

ORCID: <https://orcid.org/0000-0003-0662-4132>

Universidade Federal de São Carlos, Brazil

E-mail: [josemauro@ufscar.br](mailto:josemauro@ufscar.br)

### **Abstract**

Vegetative propagation is an alternative for the production of seedlings of native tree species such as *Schinus terebinthifolia* that presents a high predation rate on its seeds. In order to develop vegetative propagation technology, different concentrations of indolebutyric acid (IBA) were tested in the rooting of apical and intermediate minicuttings of *Schinus terebinthifolia*. The 2x4 factorial experimental design involved two types of minicuttings (apical and intermediate) and four dosages of the hormone IBA (0, 1000, 2000, and 4000 mg. L<sup>-1</sup>). The percentages of survival and rooting were evaluated. The intermediate minicuttings (37% and 35%) showed significantly greater survival than the apical cuttings (22% and 9%) respectively at 30 and 60 days, regardless of the IBA concentration. Among the rooting averages at 60 days obtained in the different concentrations of IBA, the treatment of 4000 mg. L<sup>-1</sup> generated the highest results (9% for apical and 35% for intermediate). The vegetative propagation by minicuttings of *Schinus terebinthifolia* demonstrated the viability of intermediate minicuttings with high rooting associated with the highest concentration of IBA.

**Keywords:** Brazilian peppertree; Cloning; Forestry.

## Resumo

A propagação vegetativa é uma alternativa para a produção de mudas de espécies arbóreas nativas como *Schinus terebinthifolia* que apresenta uma alta taxa de predação de suas sementes. Com o objetivo de desenvolver a tecnologia de propagação vegetativa, diferentes concentrações de ácido indolbutírico (AIB) foram testadas no enraizamento de miniestacas apicais e intermediárias de *Schinus terebinthifolia*. O delineamento experimental fatorial 2x4 envolveu dois tipos de miniestacas (apical e intermediária) e quatro dosagens do hormônio IBA (0, 1000, 2000 e 4000 mg. L<sup>-1</sup>). Foram avaliadas as porcentagens de sobrevivência e enraizamento. As miniestacas intermediárias (37% e 35%) apresentaram sobrevivência significativamente maior do que as estacas apicais (22% e 9%), respectivamente, aos 30 e 60 dias, independentemente da concentração de AIB. Dentre as médias de enraizamento aos 60 dias obtidas nas diferentes concentrações de AIB, o tratamento de 4000 mg. L<sup>-1</sup> gerou os resultados mais elevados (9% para apical e 35% para intermediário). A propagação vegetativa por miniestacas de *Schinus terebinthifolia* demonstrou a viabilidade de miniestacas intermediárias com alto enraizamento associadas à maior concentração de AIB.

**Palavras-chave:** Aroeira pimenteira; Clonagem; Silvicultura.

## Resumen

La propagación vegetativa es una alternativa para la producción de plántulas de especies arbóreas nativas como *Schinus terebinthifolia* que presenta una alta tasa de depredación sobre sus semillas. Para desarrollar la tecnología de propagación vegetativa, se probaron diferentes concentraciones de ácido indolbutírico (IBA) en el enraizamiento de minicortes apicales e intermedios de *Schinus terebinthifolia*. El diseño experimental factorial 2x4 involucró dos tipos de minicortes (apical e intermedio) y cuatro dosis de la hormona IBA (0, 1000, 2000 y 4000 mg. L<sup>-1</sup>). Se evaluaron los porcentajes de supervivencia y enraizamiento. Los minicortes intermedios (37% y 35%) mostraron una supervivencia significativamente mayor que los cortes apicales (22% y 9%) respectivamente a los 30 y 60 días, independientemente de la concentración de IBA. Entre los promedios de enraizamiento a los 60 días obtenidos en las diferentes concentraciones de IBA, el tratamiento de 4000 mg. L<sup>-1</sup> generó los resultados más altos (9% para apical y 35% para intermedio). La propagación vegetativa por minicortes de *Schinus terebinthifolia* demostró la viabilidad de minicortes intermedios con alto enraizamiento asociado a la mayor concentración de IBA.

**Palabras clave:** Aroeira pimenteira; Clonación; Silvicultura.

## 1. Introduction

*Schinus terebinthifolia* is a native Brazilian tree commonly known as aroeira-vermelha, aroeira-pimenteira or pimenta-rosa, belonging to the Anacardiaceae family. Its fruits are valued in cuisine (Silva et al., 2017) as a seasoning. It also has a great role in ecological restoration (Butiri et al., 2015) and draws attention because of its pharmacological properties (Uliana et al., 2016; Rocha et al., 2017; Rocha et al., 2019; Liden et al., 2020).

Despite its importance, the species is intensely predated by the wasp *Megastigmus transvaalensis* (Hymenoptera: Torymidae) which attacks its fruit, damaging its seeds and compromising its germination potential (Ferreira-Filho et al., 2015). For this reason, techniques of vegetative propagation can be an alternative for the production of this and other forest species for use in the ecological restoration of degraded areas, as well as for silvicultural purposes in food production, and in the rescue and conservation of genetic resources (Ledig et al., 1992; Eriksson and Fröborg, 1996; De Oliveira et al., 2020; Viveiros et al., 2021).

Minicutting is one of the most widely used techniques for vegetative propagation and consist of using plant shoots propagated by cuttings or seedlings produced by seeds (Xavier et al., 2003; Alfenas et al., 2004; Ferriani et al., 2010; Joaquina et al., 2021). It is a process of great interest, presenting an alternative in the production of seedlings for several species because it allows reducing the productive area (adoption of the mini-garden), reducing the rooting and acclimatization period (Xavier et al., 2003; Wendling et al., 2005). In addition, the technique can be used when the availability of seeds from a matrix is low or presents difficulties in storage and germination (Jesus et al., 2020).

The use of the hormone indolebutyric acid (IBA) to promote adventitious rooting in miniaturized cuttings has shown good results for forest species (Wendling et al., 2005; Pescador et al., 2007; Cunha et al., 2008; Valmorbidia et al., 2008). Studies have shown that there is a wide variation in rooting rates in native forest species. Santos et al. (2011) studied the rooting potential of 20 forest species with the use of different concentrations of IBA showed that for species of different families, the rooting rate ranged from 0.5% to 88%. Studies with *Combretum leprosum* Mart (Oliveira et al., 2014) and

*Plathymenia reticulata* Benth (Pessanha et al., 2018) showed rooting rates of 0% to 16%, respectively. These variations have been observed even within the same family, as found for the Fabaceae *Hymenaea courbaril* L. and *Apuleia leiocarpa* (Vogel) J. F. Macbr., which, with the use of IBA with different minicuttings (basal, intermediate, and apical), showed rooting variation of 0% for *H. courbaril* and 40% for *A. leiocarpa* (Freire et al., 2019). These results indicate the need to generate more studies on the vegetative propagation of native forest species, as responses vary according to species, growth hormone dosage, and type of minicuttings.

That it is necessary to develop and improve this technique in order to increase survival and rooting rates as well as contribute to consolidate the minicutting technique as an applicable strategy in the forestry of native species. Thus, our aim was to evaluate the effect of applying different concentrations of indolebutyric acid (IBA) on the rooting of apical and intermediate minicuttings of *Schinus terebinthifolia*.

## 2. Material and Methods

### 1.1 Study Area

The experiment was implemented in January 2021, in a nursery located in Sorocaba, State of São Paulo (23° 34'40.02" S and 47°31' 17.80" W). According to the climate classification of Köppen (1948), the predominant climate in the region is Cwa in the peripheral depression and Cwb in the higher areas, classified as hot and humid tropical with a dry winter and a rainy summer. In the summer, average temperatures are above 23°C and average rainfall is about 224 mm and, in the winter, the average temperature is below 17°C (Climate-Data.org, 2021).

### 1.2 Clonal Mini-Garden

The clonal mini-garden of the species studied was of the virtual type, located in the area in full sun consisting of the seedlings (ministumps) that gave rise to the minicuttings. The seedlings were produced via sexual propagation (seminal) and kept in 280 cm<sup>3</sup> tubs, placed in their trays already alternated at 50% occupancy. The cultural treatments of the ministumps consisted in fertilization, selective pruning for maintenance and cleaning, application of fungicide (every 15 days), and minicutting collection for the experiment. The irrigation blade of the virtual clonal mini-garden was 14 mm/day and fertilization with nutrient solution was performed every 5 days, consisting of simple super phosphate (0.16 g/L calcium, 0.08 g/L sulfur, and 0.18 g/L phosphorus oxide) calcium nitrate (0.46 g/L nitrogen and 0.57 g/L calcium), covering NPK (20-0-20 with 0.6 g/L nitrogen and 0.6 g/L potassium) and fertigation NPK (8-16-41 with 0.08 g/L nitrogen, 0.16 g/L phosphorus, and 0.41 g/L potassium) with micronutrients.

### 1.3 Staking Process and Application of Indolebutyric Acid (IBA)

We selected 100 young *Schinus terebinthifolia* seedlings up to 15 cm tall. From these, we collected 100 apical minicuttings and 100 intermediate minicuttings from the ministumps, standardizing the size of 5 cm in length for all minicuttings. Immediately after collection, the minicuttings were taken to the staking stage in the greenhouse, placed in Styrofoam boxes with cold water and humidified with a manual sprayer to ensure good conditions for the vegetative material. Staking took place in the greenhouse (properly prepared and sterilized) in 280 cm<sup>3</sup> tubes with Carolina Soil (type XVI) substrate and with 6 mm/day of irrigation, with the residence period in the greenhouse was 60 days.

To verify the efficiency of the growth regulator IBA (indolebutyric acid) on the minicuttings, was evaluated the survival and rooting (presence and absence of roots) in the two types of minicuttings (primary and intermediate) at dosages of 0, 1000, 2000, and 4000 mg L<sup>-1</sup> prepared in the laboratory by dissolving in distilled water.

The application of the IBA preparation was performed by submerging the base of the minicuttings for 10 seconds in the solution inside the greenhouse. After 30 days of staking, was assessed survival, and at 60 days we evaluated survival and rooting of apical and intermediate minicuttings.

### 1.4 Sampling and Data Analysis

The experimental design was a 2x4 factorial with two types of minicuttings (apical and intermediate), four doses of hormones (0, 1000, 2000, and 4000 mg. L<sup>-1</sup>), with five minicuttings per replication, totaling 25 minicuttings per treatment. We calculated the percentage of survival of the minicuttings at 30 days and 60 days, as well as the rooting percentage of the apical and intermediate minicuttings. The Kruskal-Wallis test complemented by Dunn's post-test was applied to verify the differences in survival and rooting of the minicuttings between the concentrations of IBA per analysis period.

The Mann-Whitney non-parametric test was used to verify if there was a difference in survival between the minicutting type (apical and intermediate) at 30 days, and the same analysis was done at 60 days. We verified with simple linear regression analysis, the relationship between IBA concentrations with the number of surviving minicuttings at 30 days, at 60 days, and rooted, classifying in high ( $R^2 > 0.70$ ), medium ( $0.50 < R^2 \leq 0.70$ ), and low ( $R^2 < 0.50$ ) correlation. All analyses were performed using the software R (R Core Team 2021).

## 3. Results

The highest average survival and rooting rates at 60 days were for the intermediate minicuttings with rates of 35±31.5% for both, and the rates for the primary minicuttings were 9±18% for both survival and rooting. At 30 days, we obtained the highest survival rate of *S. terebinthifolia* in the intermediate minicuttings at the concentration of indol-butyric acid (IBA) 4000 mg. L<sup>-1</sup>, followed by 2000 mg. L<sup>-1</sup>, 1000 mg. L<sup>-1</sup>, and zero (Table 1).

For apical cuttings the highest survival rate at 30 days also occurred at the highest IBA concentrations; however, the survival rates of intermediate cuttings were higher for all IBA concentrations (Table 1). The highest average rooting occurred in the intermediate minicuttings with IBA concentration 4000 mg. L<sup>-1</sup>, followed by the apical minicuttings with IBA concentration 4000 mg. L<sup>-1</sup>, and intermediates at concentrations of 1000 mg. L<sup>-1</sup> and 2000 mg. L<sup>-1</sup>.

**Table 1.** Percentage of mean survival and rooting of apical and intermediate *Schinus terebinthifolia* (Brazilian peppertree) minicuttings at 30 and 60 days after planting with application of indol-butyric acid (IBA) at concentrations of 0, 1,000, 2,000, and 4,000 mg. L<sup>-1</sup>.

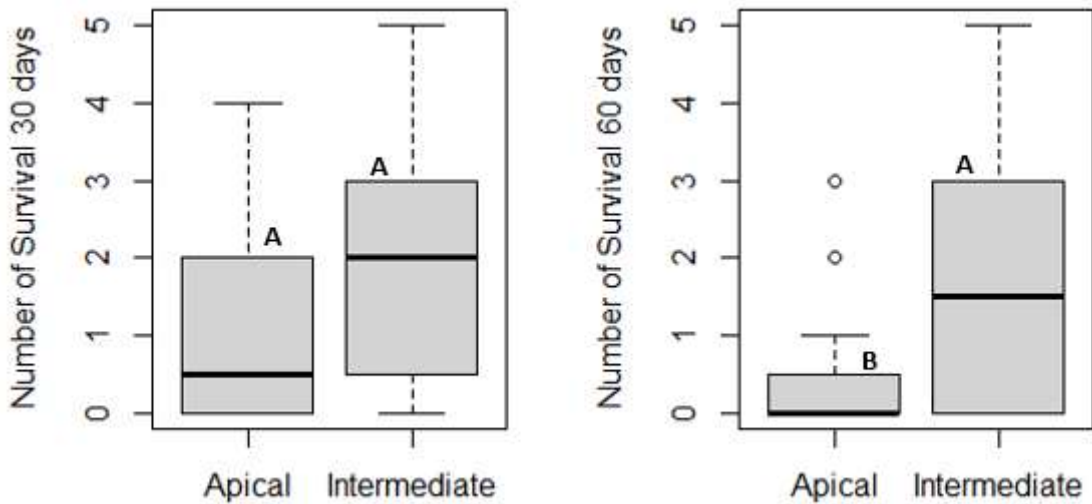
<i>Schinus terebinthifolia</i>																
		Survival (%)					Rooting (%)									
		30 days			60 days		60 days									
		IBA Concentration (mg. L <sup>-1</sup> )														
Minicuttings	0	1000	2000	4000	Mean	0	1000	2000	4000	Mean	0	1000	2000	4000	Mean	
Apical	0bA	8bA	16abA	64aA	22A	0bA	0bB	0bB	36aB	9B	0a	0a	0a	36b	9A	
Intermediate	4aA	32abA	32abA	80bA	37A	0aA	32abA	28abA	80bA	35A	0a	32ab	28ab	80b	35B	

Means followed by equal letters, lowercase in the row (between IBA levels on each date) did not differ by the Dunn's post hoc test at 5% probability. Averages followed by capital letters in the column (between type of minicuttings for each IBA combination on each date) do not differ by the Mann-Whitney test at 5% probability. Source: Authors.

No difference was found in the first 30 days between the number of survivors for apical and intermediate minicuttings; however, at 60 days there was a significant reduction in the survival of apical minicuttings ( $p < 0.05$ ) (Figure 1).

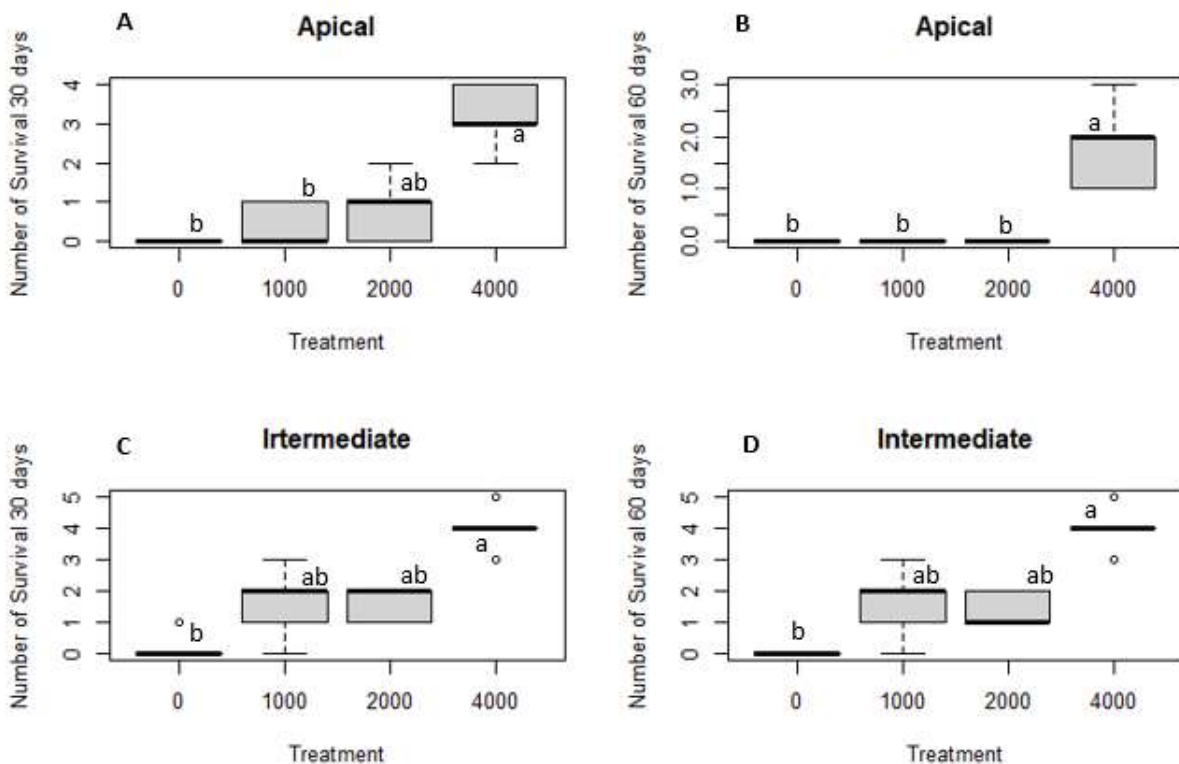
For the survival of the apical minicuttings at 30 days among the different IBA concentrations, the treatment with the concentration of 4000 mg. L<sup>-1</sup> did not differ from the treatment of 2000 mg. L<sup>-1</sup>; however, at 60 days, the treatment with 4000 mg. L<sup>-1</sup> was the only one that showed survivals with apical minicuttings (Figure 2).

**Figure 1.** Box-plot of median and quartiles of survival in number of apical and intermediate minicuttings at 30 and 60 days of planting.



Equal letters have no significant differences by Mann-Whitney test ( $p < 0.05$ ). Source: Authors.

**Figure 2.** Box-plot of median and quartiles of survival in number of cuttings in different IBA concentrations (Treatments) (0, 1000, 2000, 4000 mg. L<sup>-1</sup>). In A apical minicuttings at 30 days of planting and B at 60 days of planting. C intermediate minicuttings at 30 days of planting and D at 60 days of planting.

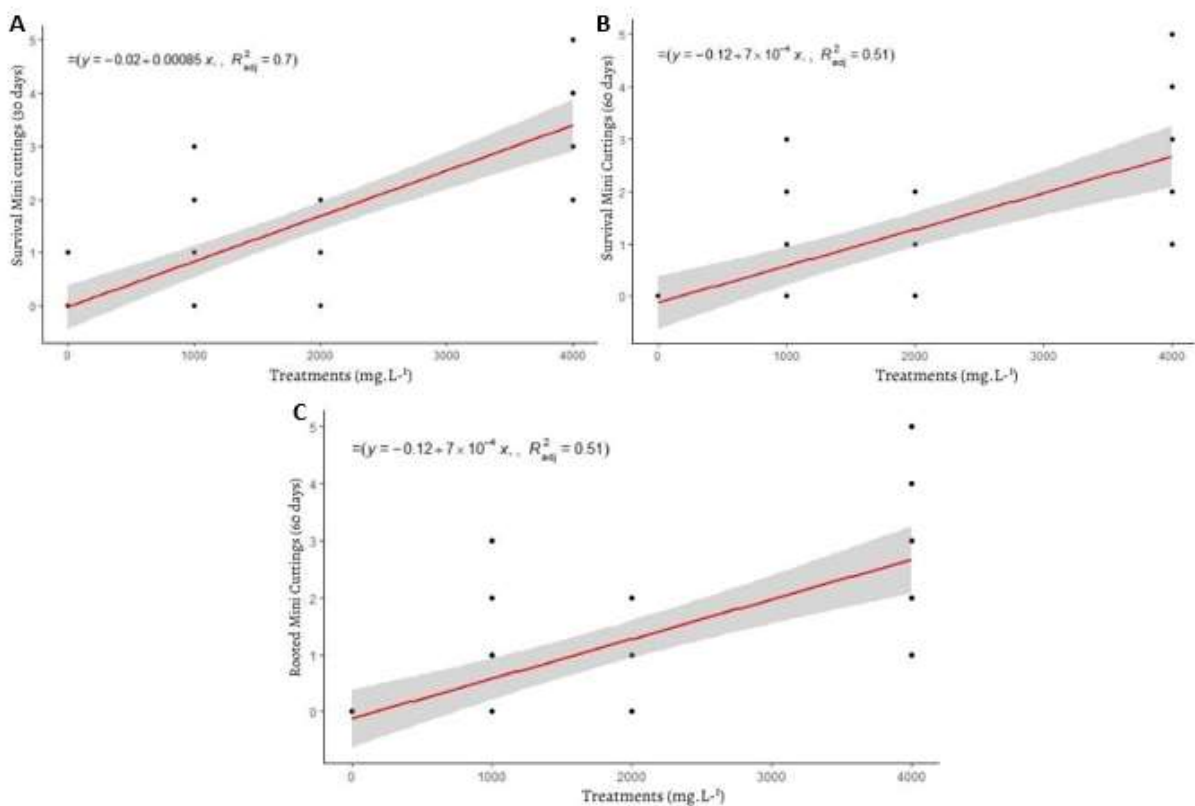


Equal letters have no significant differences by Dunn's post-hoc test ( $p < 0.05$ ). Source: Authors.

For intermediate minicuttings, we observed no differences in survival between the IBA concentrations 4000 mg. L<sup>-1</sup>, 2000 mg. L<sup>-1</sup>, and 1000 mg. L<sup>-1</sup>, in any of the time intervals analyzed (Figure 2C and 2D); nevertheless, the average survival rate for the 4000 mg. L<sup>-1</sup> was higher with 80% (Table 1).

Survival showed an increasing linear trend of the minicuttings as IBA concentration increased. In the first 30 days of the experiment, the minicutting survival obtained an average correlation with R<sup>2</sup> = 0.70 (Figure 3A) (F=92.91 and p < 0.05) with the highest IBA concentration. At 60 days, this correlation remained average with R<sup>2</sup> = 0.51 (Figure 3B) (F=41.36 and p < 0.05) and was maintained (F=41.36 and p < 0.05) in the minicuttings' rooting (Figure 3C).

**Figure 3.** Effect of IBA concentrations (0, 1000, 2000, and 4000 mg. L<sup>-1</sup>) on survival at 30 days (A), 60 days (B), and rooting at 60 days (C) in minicuttings.



Source: Authors.

#### 4. Discussion

The survival and rooting of *Schinus terebinthifolia* was higher for intermediate minicuttings (35%) compared to apical (9%) after 60 days. The result found was higher than that of Santos et al. (2011), who, when testing the rooting of cuttings of different woody species, found that for *S. terebinthifolia* the rooting percentage was 8% with the use of IBA in lower concentrations (0, 100, 200, and 300 mg. L<sup>-1</sup>). This shows that the species studied presents a good reaction to the application of exogenous IBA, even at high concentrations.

The low survival of apical minicuttings (9%) can be explained by their sensitivity to high temperatures and low relative humidity, which causes the loss of water, making the vegetative material unviable (Hartmann et al., 2011). The conditions of the vegetation house may have caused hydric stress to the minicuttings, where temperatures can reach 50°C in

the afternoon in tropical regions (Beltrão et al., 2002). This suggests that the irrigation conditions in the greenhouse should be improved to obtain apical minicuttings, such as increasing the number of valve starts, their duration, as well as increasing the irrigation blade to provide greater survival and rooting of the apical and intermediate minicuttings. Moreover, other factors may contribute to the low rooting of the minicuttings, such as physiological conditions, temperature, humidity, and the handling to which the minicuttings are submitted during the rooting period (Xavier et al., 2013).

The percentage of survival and rooting of apical minicuttings was also influenced by the IBA concentrations tested. The endogenous auxin level may be insufficient for root formation, and the application of growth regulators with doses of IBA in higher concentrations is vital to stimulate the rooting of apical minicuttings (Xavier et al., 2009). This is evident from the result found for the treatment without IBA application, in which survival was equal to zero, and that only the treatment with the highest IBA concentration provided survival and rooting (36%).

The higher percentage of survival found in the apical and intermediate minicuttings in the treatment with 4000 mg L<sup>-1</sup> IBA corroborates the hypothesis that it is necessary to use high doses of the hormone to express its rooting effect for this species. It has already been evidenced in the literature that minicuttings of *Schizolobium parayba* var. *amazonicum* (Huber ex Ducke) Barneby obtained better rooting results with a dose of 32,000 mg L<sup>-1</sup> of IBA (Dias et al., 2015), as well as reported by Oliveira et al. (2015), in which apical minicuttings of *Handroanthus heptaphyllus* Mattos had a higher number of adventitious roots at the dose of 8,000 mg L<sup>-1</sup> than at lower concentrations (0, 2,000, 4,000, and 6,000 mg L<sup>-1</sup> of AIB).

Although the Mann-Whitney test (Figure 1) showed that there was no significant effect between the different types of minicuttings at 30 days, the highest IBA concentration provided a higher percentage of survival and rooting for the intermediate minicuttings than for the apical minicuttings (Table 1). This study differed from Hernández et al., 2012, who noted higher survival and rooting for apical minicuttings regardless of the IBA doses tested (0, 2000, and 6000 mg L<sup>-1</sup>), where the rooting rate for apical minicuttings was above 90%, and for intermediate minicuttings without IBA application, the rooting rate was 65% (Hernández et al., 2012).

In this study, was observed that the application of the hormone had a relationship with survival and rooting (Figure 3), differing from other studies where the application of the hormone did not influence the survival and rooting of apical and intermediate minicuttings of *Anadenanthera macrocarpa* (Benth.) Brenan in different IBA concentrations (0, 2000, 4000, and 6000 mg L<sup>-1</sup> (Dias et al., 2012). The same result was reported by Fragoso et al. (2017) where rooting rates varied from 42% to 55% for *Tibouchina sellowiana* (Cham.) Cogn, dispensing the use of IBA. For *Paratecoma peroba* (Record & Mell) Kuhl, the highest rooting value recorded (43%) was for the treatment without hormone use (Araújo et al., 2019).

## 5. Conclusion

The vegetative propagation by miniaturization of *Schinus terebinthifolia* with application of the hormone indol-3-butyric acid (IBA) demonstrated its greatest viability with intermediate minicuttings with survival and rooting. Furthermore, we verified that the increase in IBA concentration correlated with the survival and rooting of the minicuttings.

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