Scientific Article

VEGETATIVE RESCUE OF *Azadirachta indica* BY CUTTINGS

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ABSTRACT – Finding forest species adapted to different soil and climatic conditions and, that provide favorable attributes to commercial use is a challenge. *Azadirachta indica* is a potential species to attend to this goal. Thus, this paper aims to carry out the vegetative rescue of different *Azadirachta indica* Juss adult trees by cuttings. For that, *Azadirachta indica* individuals were randomly selected in a plantation of species set ten years ago and the cut was carried out at 30 cm in height. In sequence, verification was carried out on: (a) the stem cut ability to regrowth (collections and measurements of the shoot length on the 15th, 30th, 45th, and 60th days); (b) the need to use exogenous auxin for rooting induction [testing 0 (control), 2000, 4000, and 6000 mg.l^{-1} of Indole-3-butyric acid (IBA)]; (c) the ideal of the propagule size (with 8 cm and 13 cm in height); and (c) the rooting dynamics (every seven days for thirty-five days; the propagules were evaluated for callus formation, oxidation, and rooting). The species showed high regrowth ability, as well as easy rooting with or without the use of IBA; it was found that the ideal size for the propagule is 13 cm and that the process of rooting and root elongation is completed after thirty-five days of staking. The conclusion is: (a) using cuttings for the species vegetative rescue is interesting; (b) 13 cm is considered appropriate for the propagule size, and; (c) the aid of IBA at a dose of 2,000 mg L^{-1} is ideal to standardize rooting.

Keywords: Cloning; Neem; Plant propagation.

RESGATE VEGETATIVO DE Azadirachta indica *POR ESTAQUIA*

RESUMO – Encontrar espécies florestais adaptadas a diversas condições edafoclimáticas, e que forneçam atributos favoráveis ao uso comercial é um desafio. A ***Azadirachta indica*** *é uma espécie potencial para amenizar este problema. Assim, o objetivo deste trabalho foi realizar o resgate vegetativo de diferentes árvores adultas de* ***Azadirachta indica*** *Juss por estaquia. Para tanto, indivíduos de* ***Azadirachta indica*** *foram selecionados aleatoriamente em um plantio da espécie com 10 anos de implantação e realizada a decepa à altura de 30 cm. Em sequência, verificamos: a capacidade de rebrota das cepas (coletas e medições do comprimento das brotações aos 15, 30, 45 e 60 dias), a necessidade de usar auxina exógena para indução do enraizamento (testando 0 (controle), 2000, 4000 e 6000 mg.l^{-1} de AIB), o tamanho ideal do propágulo (propágulos com 8 cm e 13 cm de altura) e a dinâmica de enraizamento (cada sete dias, em um período de 35 dias, os propágulos foram avaliados quanto a: formação de calos, oxidação e enraizamento). A espécie demostrou alta capacidade de rebrota, bem como facilidade de enraizamento com ou sem a utilização de AIB; verificou-se que o tamanho ideal para o propágulo é 13 cm e que o processo de enraizamento e alongamento das raízes se completa após 35 dias do estaqueamento. Concluímos que o uso da estaquia para o resgate vegetativo da espécie é interessante,* *recomendamos que 13cm é adequado para o tamanho do propágulo e o auxílio do AIB na dose de 2.000 mg L^{-1} é ideal para uniformizar o enraizamento.*

Palavras-Chave: Clonagem; Nim; Propagação de plantas.

1. INTRODUCTION

Azadirachta indica Juss from Asian origin, distributed in Asia and in the Americas, popularly known as neem, presents rapid growth and multiple uses (Azevedo et al., 2015). It is a species adapted to tropical and semi-arid climates, resisting low fertility soils (Braga et al., 2021). Its best development is found in drained and porous soils with rainfall from 800 mm to1800 mm, which does not make its cultivation impossible in regions with rainfall below 800 mm (Soulé et al., 2021).

Azadirachta indica wood has good physical and mechanical characteristics and can be used as raw material for making musical instruments (Hassan and Tippner, 2019), cladding panels (Nagamadhu et al., 2020), furniture manufacturing and charcoal production (Antwi-boasiako and Galah, 2021). In addition, its chemical compounds are used in many of industry areas such as oil to control phytopathogens and pests in agriculture (Kala et al., 2019), as a biofuels source (Dhanalakshmi and Madhu, 2019), and for pharmacological purposes in many medicine areas. Recently, the potential of this species in the production of inhibitors of the structural protein sars-cov-2 was verified (Borkotoky and Banerjee, 2020).

Due to the adaptive potential of the species and its multiple uses, commercial planting of *Azadirachta indica* is recommended in many countries; however, seedlings with good morphophysiological quality and productive genotypes are needed. The *Azadirachta indica* propagation occurs through seeds but attention should be paid to the genetic and the seed recalcitrance viability when there is interest in the commercial seedlings production. In addition, the neem heterozygous nature is a significant trouble for selecting uniform plants aiming to produce azadirachtin, the main product of the species to control diseases and insects, as well as in the wood production (Bisht et al., 2021).

As an alternative to seminiferous propagation of the species, vegetative propagation allows the multiplication of selected genotypes, producing uniform seedlings with higher physiological and morphological quality (Sahoo et al., 2021). Vegetative rescue is a technique used in the genotype propagating of different forest species and can be performed by canopy shoots and basal epicormic shoots (Stuepp et al., 2017). Among the vegetative rescue techniques, cuttings can be used for selected genotypes at the rotation age (Hu et al., 2020), helping to produce clonal seedlings (Dias et al., 2015). Thus, cuttings are an alternative that should be tested in vegetative rescue and, later, in the production of *Azadirachta indica* seedlings aiming to multiply selected genotypes. Vegetative propagules with high vigor and juvenility have shown greater potential for adventitious rooting by cuttings. Therefore, the induction of basal epicormic shoots has been used to promote the propagule invigoration, such as the cutting (Dias et al., 2015; Stuepp et al., 2017).

However, obtaining young shoots with high physiological vigor is the first step in the success of vegetative rescue in the propagules to induce adventitious rooting (Xavier et al., 2021). Thus, for the vegetative rescue of matrices, techniques that favor the propagule invigoration are used, such as the stem cutting and girdling. However, the use of auxin is needed, when adventitious rooting is low (Araújo et al., 2019). Indole-3-butyric acid (IBA) is the main synthetic auxin for this purpose, due to its low toxicity and greater stability (Xavier et al., 2021).

Some papers investigating vegetative propagation through cuttings with IBA application on *Azadirachta indica* were carried out (Gehlot, 2017). However, the results are not conclusive regarding the vegetative rescue application using cuttings in the seedlings production on a large scale; therefore, in-depth studies, this procedure is indicated to determine the genotype interference on the adventitious rooting process, on the propagule proper size, on the need or not of auxin for root induction, and on the rooting dynamics of propagules collected from genotypes to vegetative rescue, among other factors. To answer these questions, this paper has aimed to carry out the vegetative rescue from different adult trees of *Azadirachta indica* Juss by cuttings, testing different IBA doses, propagule sizes, and characterizing the rooting dynamics.

2. MATERIAL AND METHOD

2.1 *Vegetative rescue*

The vegetative rescue followed the methodology indicated by Dias et al. (2015). *Azadirachta indica* individuals were randomly selected in a species plantation made ten years ago. Weed control by hand was carried out within a range of 50 cm around the twenty trees to avoid weed competition. Subsequently, the cut was performed at 30 cm above the ground with a chainsaw to induce epicormic shoots (Figure 1). The harvesting was carried out in the rainy season, January 2019, in Mossoró municipality, Rio Grande do Norte State, Brazil. The climate in the municipality is classified as BSh, according to the Köppen classification, that is, a dry semi-arid climate, an average temperature of 27.4 °C, an average relative humidity of 68.9%, irregular annual precipitation of 673.9 mm average, and a dry period of six to eight months (Melo et al., 2020).

Collections and measurements of the shoot length were carried out on the15^{th}, 30^{th}, 45^{th}, and 60^{th} days to evaluate the species regrowth ability (Figure 1B). The shoot length was measured with a ruler graduated in centimeters. The stem cut diameter was taken with a tape measure. The information found was submitted to the estimation of Pearson's correlation coefficients (r).

For evaluating the adventitious rooting in the next experiments, the cuttings were kept in a mini greenhouse, sized 3 m long, 1 m wide, and 0,60 m high with an irrigation system using micro sprinklers with a flow rate of 10 L/h, propagate over three irrigation lines, two on the sides and one in the center, with a distance of 0,30 m between micro sprinklers. The irrigation system was automatically activated every 30 min for 30 sec for 12 h. A shading screen (70% retention) was installed on the external surface to maintain the air temperature inside the mini greenhouse and a thermohygrometer was used to measure the temperature and internal humidity. The temperature inside the mini greenhouse was maintained from 26 °C to 30 °C and the humidity above 80%. The container used in the experiment was a polyethylene tube with a capacity of 55 cm3, filled with commercial substrate consisting of peat, vermiculite, and roasted rice husk with 5.5 hydrogenionic potential (pH), 0.7 mS/cm electrical conductivity (EC), 130 kg/m^3 density, 300 water holding capacity (WHC) (% w/w), and 60% maximum humidity.

2.2 *IBA influence on adventitious rooting*

Eight matrices with the highest number of shoots were chosen among the twenty ones submitted to harvesting to evaluate the IBA influence on adventitious rooting. The propagules were 10 cm tall, containing a pair of leaves, reduced to 25% from their original size. The treatments to which the cuttings were submitted included 0 (control), 2000, 4000, 6000 $mg.l^{-1}$ of IBA dissolved in 1% potassium hydroxide (KOH) and later diluted in water. The base of the cuttings was immersed in the IBA solution for 10

Source: The author (2021).
***Fonte**: O autor (2021).*

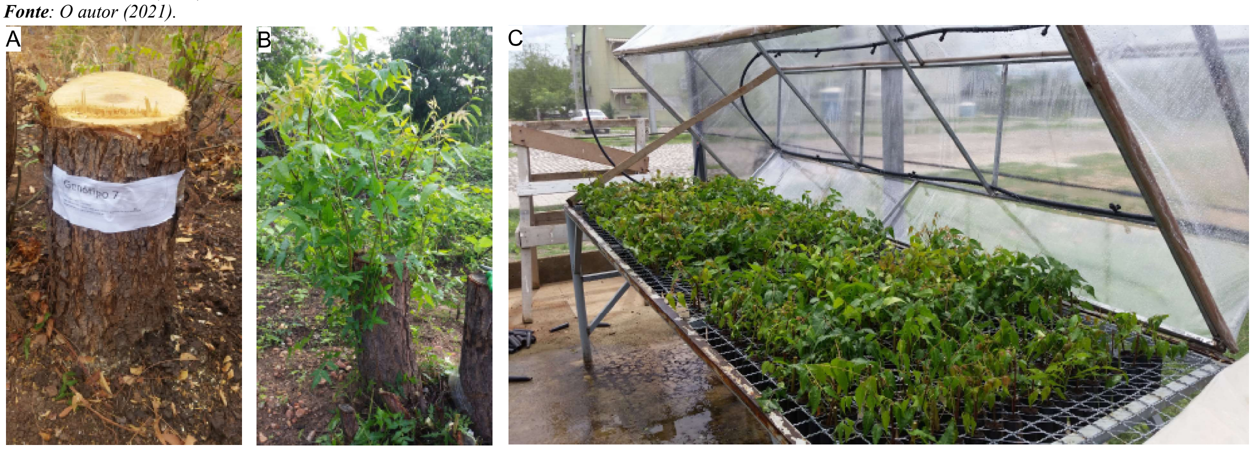

Figure 1 – Shoots reinvigoration of *Azadirachta indica*: (A) pruning, (B) shoots, (C) staking.
***Figura 1** – Revigoração de brotos de **Azadirachta indica**: (A) poda, (B) brotos, (C) estaqueamento.*

sec in each treatment; the base of the propagules was immersed in water for 10 sec in the control treatment, being later staked in containers filled with substrate. The cuttings remained in the mini greenhouse for thirty days; after this period, they were transferred to a shade house with 50% shading for approximately fifteen days and, finally, to an area of full sun to growth until completing seventy days.

The experimental design used in this step was in randomized complete block design (CBD), 8x4 factorial scheme (8 matrices x 3 IBA doses + control), with 3 replicates and 8 measurement units per treatment. After the full sun phase, the survival was evaluated by counting individuals with formed root system and aerial part with leaves; adventitious rooting was evaluated observing roots formed in cuttings. Data were submitted to analysis of variance (ANOVA) at 95% probability, and their results were compared using Tukey's test ($p<0.05$).

2.3 *Influence of propagule size on rooting*

Twenty matrices were used in this experiment as a propagule source, sectioning them by sizes from 8 cm to 13 cm in length, containing a pair of leaves reduced to 25% from their original size. Subsequently, the base of the cuttings was immersed in an IBA solution at a concentration of 2000 $mg.l^{-1}$ for ten sec and stacked. The cuttings remained in a greenhouse for thirty days, being transferred to a house with 50% shading for fifteen days and, finally, to a full sun area until completing seventy days.

The experimental design was in randomized complete block design in a 2x20 factorial scheme (propagule sizes 8 cm and 13 cm, and 20 matrices), with 3 replicates consisting of 8 measurement units per treatment. After the propagules have been removed from the mini greenhouse and from the shade house, rooting percentage, survival, length of the largest root, number of roots, collar diameter, and root dry mass and root wet mass were evaluated. Data were submitted to analysis of variance (ANOVA) at 95% probability, and results were compared using the Tukey test ($p<0.05$).

2.4 *Dynamics of adventitious rooting*

The adventitious rooting dynamics was carried out adapting the methodology described by Ferreira et al. (2004). Eight matrices were randomly selected, and the

Source: The author 2021.
Fonte: *O autor 2021.*

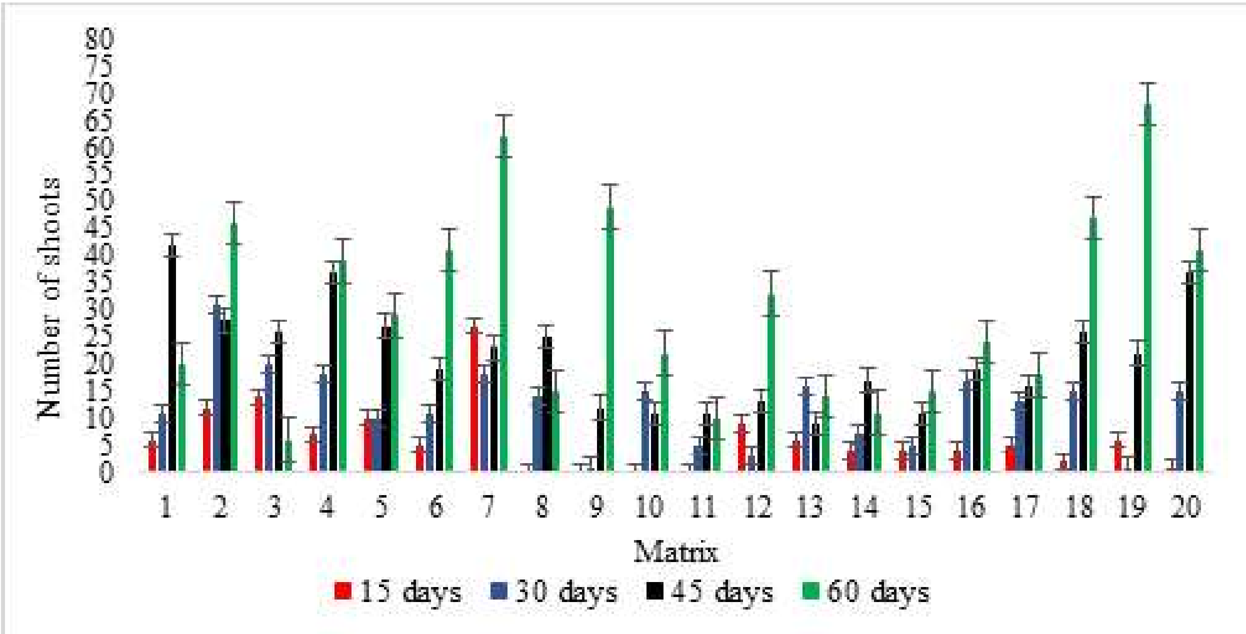

Figure 2 – Number of shoots for 20 matrices of the *Azadirachta indica* species on the 15th, 30th, 45th, and 60th days after cutting.
Figura 2 *– Número de brotações para 20 matrizes da espécie **Azadirachta indica** aos 15, 30, 45 e 60 dias após o corte.*

propagules were sectioned to 13 cm in size (based on the results of experiment 2), containing a pair of leaves reduced to 25% from their original size. The base of the cuttings was immersed in an IBA solution at a concentration of 2000 mg.l^{-1} for ten sec and staked. In sequence, the material was kept in the mini greenhouse; and eight propagules were removed from each treatment every seven days for thirty-five days, and evaluated for callus formation, oxidation, initial rooting (with roots up to 1 cm), and rooting with roots above 1 cm.

The experimental design was in randomized complete block design with eight treatments (different trees) and three replicates with ten measurement units per treatment. Five evaluations were performed on the 7th, 14th, 21st, 28th, and 35th days. Data were submitted to analysis of variance (ANOVA) at 95% probability, and results were compared using the Tukey test ($p<0.05$).

3. RESULTS

3.1 *Regrowth ability*

It is noticeable that the *Azadirachta indica* species has an easy regrowth (Figure 2), even after successive severe cuts.

In the first fifteen days after cutting, 90% of the stems cut had epicormic shoots. Also, variation in the number of shoots between the matrices is found. It is found that some matrices produce more shoots than others with the highest production found for tree7 and tree19 on the 60th day after cutting (Figure 2). Also, there is variation in the amount of shoots produced by the same stem cut during the collections.

Negative and positive interactions at low levels by Pearson's correlation analysis were found between the stem cut diameter and the number of shoots. Thus, it can be stated that the stem cut diameter is not correlated with the shoot production and their size.

3.2 *IBA influencing on adventitious rooting*

There was a difference in response between the IBA dosages applied to the adventitious rooting of the propagules ($p<0.05$). Adventitious rooting without IBA was found (Figure 3). However, the rooting percentage is lower when compared to treatments using IBA. It was also found that IBA dose of 2000 mg.l^{-1} resulted in better propagule adventitious rooting of all trees, and there is a decrease in the rooting percentage with IBA doses greater than

Source: The author (2021.
Note: Different lowercase letters indicate significant differences between genotype combinations within an IBA dose. Different capital letters indicate significant differences between combinations of IBA doses within a genotype (p≤0.05).
***Fonte**: O autor (2021).*
***Nota**: Diferentes letras minúsculas indicam diferenças significativas entre combinações de genótipos dentro de uma dose de AIB. Diferentes letras maiúsculas indicam diferenças significativas entre combinações de doses de AIB dentro de um genótipo (p≤0,05).*

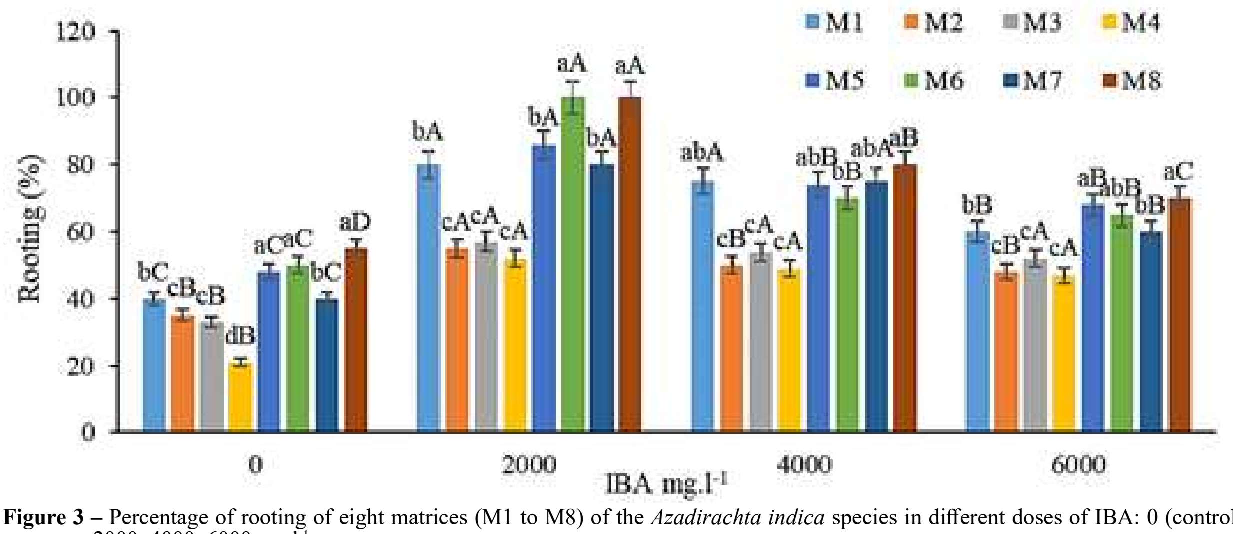

Figure 3 – Percentage of rooting of eight matrices (M1 to M8) of the *Azadirachta indica* species in different doses of IBA: 0 (control), 2000, 4000, 6000 mg.l^{-1}.
***Figura 3** – Percentual de enraizamento de oito matrizes (M1 a M8) da espécie **Azadirachta indica** em diferentes doses de AIB: 0 (controle), 2000, 4000, 6000 mg.l^{-1}.*

Table 1 – Comparison of the analyzes regarding the vegetative propagule size and performance among the matrices.
Tabela 1 *– Comparação das análises quanto ao tamanho do propágulo vegetativo e desempenho entre as matrizes.*

Size	DIAM (cm)	RQTY(cm)	RL(cm)	APWM(g)	RDM(g)	R/SUR (%)
8	4.11 a	7.18 a	8.52 a	6.11 a	0.68 a	64.79 a
13	4.45 b	8.39 b	8.67 a	10.7 b	1.61 b	70.83 b
Matrix	**DIAM (cm)**	**RQTY(cm)**	**RL(cm)**	**APWM(g)**	**RDM(g)**	**R/SUR (%)**
1	3.52 a	8.37 a b	9.35 a	11.8 a	1.48 a b	81.25 c
2	4.16 a b c	4.92 a	8.36 a	8.20 a	1.38 a b	64.58 b
3	4.59a b c	8.34 a b	7.72 a	9.36 a	2.08 b	70,83 b
4	5.90 c	7.64 a b	8.77 a	6,80 a	1.03 a b	64.58 a
5	4.61 a b c	8.87 a b	8,30 a	6.60 a	1.05 a b	77.08 b
6	3.86 a b	9.67 b	7.52 a	8.72 a	0.76 a b	72.92 b
7	4.33a b c	5.87 a b	9.49 a	5.87 a	0.43 a	77.08 b
8	4,24 a b c	9.47 b	8.98 a	8.35 a	1.18 a b	68.75 a
9	5.41 b c	7,94 a b	8.65 a	6.00 a	0.90 a b	52.08 a
10	5.18 a b c	7.25 a b	7.77 a	7.60 a	0.71 a b	58.33 a
11	3.61a b	8.19 a b	9.01 a	9.70 a	1.36 a b	64.58 ab
12	4.83 a b c	7.61 a b	9.25 a	8.87 a	1.33 a b	62.50 ab
13	3.64a b	6.51 a b	8.76 a	9.91 a	1.49 a b	75.00 ab
14	4.73 a b c	8.29 a b	8.79 a	8.11 a	0,76 a b	72,92 b
15	3.69 a b	7.06 a b	8.41 a	10.5 a	2.05 b	79.17 c
16	3.85 a b	9.00 a b	7.54 a	8.52 a	1.06 a b	54.17 b
17	4.10 a b c	8.17 a b	8.57 a	11,0 a	1.31 a b	81.25 c
18	3,63a b	7.24 a b	9.60 a	8.25 a	0.88 a b	62.50 b
19	3.44 a	7.50 a b	8,72 a	7.38 a	0.93 a b	62.50 b
20	4.28 a b c	7.82 a b	8.26 a	6.24 a	0.61 a b	54.17 a

Source: The author (2021.
Caption: collar diameter (DIAM), root quantity (RQTY), root length (RL), root wet mass (RWM), root dry mass (RDM), rooting and survival (R/SUR (%)).
Note: Different letters indicate significant differences among combinations of IBA doses in a genotype ($p \leq 0.05$). Different letters indicate significant differences between genotype/treatment combinations or treatment/genotype combinations ($p \leq 0.05$).
Fonte: O autor (2021.
Legenda: diâmetro do colo (DIAM), quantidade de raízes (RQTY), comprimento da raiz (RL), massa úmida da raiz (RWM), massa seca da raiz (RDM), enraizamento e sobrevivência (R/SUR (%)).
Nota: Letras diferentes indicam diferenças significativas entre combinações de doses de IBA em um genótipo ($p \leq 0{,}05$). Letras diferentes indicam diferenças significativas entre combinações de genótipo/tratamento ou combinações de tratamento/genótipo ($p \leq 0{,}05$).

2000 $mg.l^{-1}$. It is possible to see (Figure 3) that there is rooting variation according to the genotype, with trees presenting propagules that are easier to root than others.

3.3 *Influence of propagule size on rooting*

According to the data presented in this study, vegetative propagules with 13 cm have a better response to adventitious rooting, considering the collar diameter, root quantity, root wet mass, root dry mass, rooting, and survival (Table1).

There was no significant difference among the trees, considering the root length (RL), aerial part wet mass (APWM), root dry mass (RDM), initial rooting (INR), and propagule survival (PSUR) characters. However, there were significant differences between the matrices in the number of roots (NUR), propagules diameter (PDI), aerial part dry mass (APDM), and root dry mass (RDM) variables. The interaction of INR and PSUR is positive, according to Pearson's correlation, and APWM and RDM have presented good interaction.

3.4 *Dynamics of adventitious rooting*

The oxidation of vegetative propagules occurred in all evaluations, being better visualized from seven to fifteen days after staking with subsequent oxidation gradual reduction in the cuttings. The propagule oxidation favors the observation of the cutting rotting, been more evident in the first fifteen days after staking. However, callus formation is evident in all matrices on the 7th day after staking; in the other evaluations, there is a reduction in the number of calluses found on the cuttings (Figure 4).

Regarding the initial rooting, there is a difference among the matrices. The initial cutting rooting occurs from the 15th day after the cuttings go into the greenhouse, except in matrix 2 (Figure 4C). The

Source: The author (2021).
Fonte: *O autor (2021).*

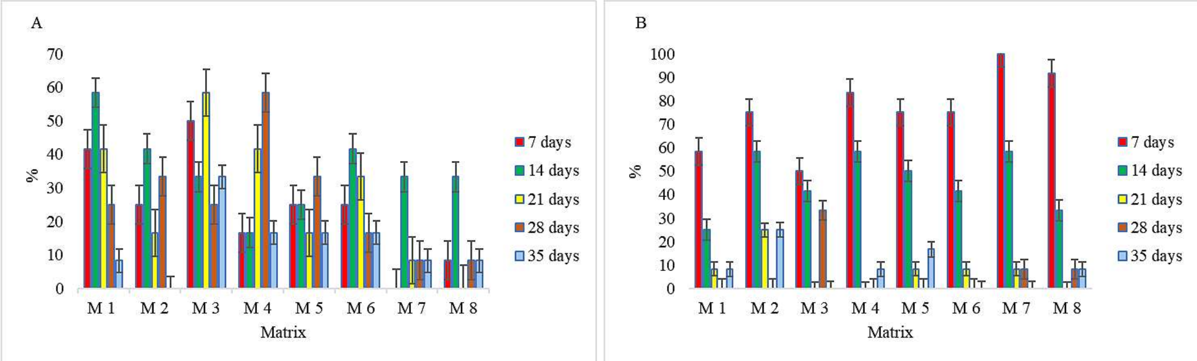

Figure 4 – Rooting speed of *Azadirachta indica*: (A) shows oxidation in the period; (B) shows callus formation in the period; (C) shows the initial rooting in the period; and (D) shows rooting greater than 10 cm in the period.
Figura 4 *– Velocidade de enraizamento de **Azadirachta indica**: (A) mostra a oxidação no período; (B) mostra a formação de calos no período; (C) mostra o enraizamento inicial no período; e (D) apresenta enraizamento superior a 10 cm no período.*

cuttings of all matrices had roots of up to 1 cm on the 21th day after entering the greenhouse. Rooting greater than 10 cm is already possible to be found after twenty-one days, when the roots already appear at the container bottom. It was also found that there is no correlation between rooting and callus formation.

4. DISCUSSION

4.1 *Regrowth abilityy*

On the 15th day after stem cut, practically all the matrices have sprouted, even if in smaller amounts, suggesting that the *Azadirachta indica* species has good regrowth ability. The regrowth of a tree is mainly due to the comprehensive root system and nutritional reserve storage, which provide the needed support for new shoots (Hossain and Caspersen, 2012). It is also facilitated when there are epicormic shoots, favorable geographic conditions, availability of light, and soil with good water retention and well-nourished (Heineman et al., 2021; Lima et al., 2018). The resilience and adaptability potency to environments with extreme climatic variation also favor the regrowth (Montfort et al., 2021), as well as the defense and survival strategies conserved throughout the evolutionary period of the species, allowing the ability to regenerate after the stress factors action, such as stem cut (Heineman et al., 2021). These characteristics can partially explain the good regrowth potential found for *Azadirachta indica* in this study.

Good regrowth ability is interesting in vegetative rescue and vegetative propagation by cuttings. To make a clonal garden, for example, it is needed that the species can resist numerous successive cuts and recover itself, allowing continuous shoot removal cycles, keeping seedling production constant.

Although the *Azadirachta indica* stem cut showed good regrowth ability, it was found that there is no correlation between their diameter and the number of shoots; however, some studies with native species indicate such correlation (Lima et al., 2018). Considering the non-observation of the fact, it is noticed that the *Azadirachta indica's* ability to sprout is not associated with the stem cut diameter, but to the species' natural predisposition to regrowth (Gehlot, 2017; Gehlot et al., 2014).

When verifying the species regrowth ability, it is noticed that some matrices have produced few regrowths or needed more time to do it. This condition can be explained by the individual's genetic characteristics (Oliveira et al., 2015) and by mechanical restrictions caused mainly by tissue lignification (Ross et al., 2021). The increase in the number of shoots throughout the evaluations may be associated with breaking dormancy of the side buds, which stimulates the cell division and the production of more than one shoot per bud (Bogiatzis et al., 2021).

Thus, the difference in the shoot emission speed and the number of shoots in the matrices is possibly related to the genetic factor (Dias et al., 2012), since the matrices, as already informed by this study, were propagated through seeds and were in the same climatic location with the same age and type of soil and nutrition and with no environmental variation. However, it can be stated that the control for shoot formation is complex and involves different mechanisms, such as environmental and developmental signals, as well as hormones and nutrients (Rameau et al., 2015).

4.2 *IBA Influence on adventitious rooting*

In rooting cuttings of economically important plants, the adventitious root formation is a key step. Phytohormones, such as auxin, have been recommended for stimulating and accelerating the adventitious root formation process, increasing the rooting rate, formation speed, and quality/uniformity of the root system (Druege et al., 2019). For this process, the auxin application can interfere on rooting positively or negatively, requiring an appropriate dosage for each species studied (Dias et al., 2012; Druege et al., 2019).

Many studies show that the use of IBA favors the root formation and/or increases their production in species that do not have or have low competence for adventitious rooting. Research carried out with *Azadirachta indica* using semi-woody cuttings found a rooting percentage of 65%, using IBA 500 mg.l^{-1} (Gehlot, 2017). However, a rooting percentage above 90% was observed in this study for some matrices, when IBA 2,000 mg.l^{-1} was used, with a decrease in the rooting percentage at doses higher than this. Auxin is a key phytohormone in adventitious rooting, required in high concentration before root meristemoid formation and in relatively low concentration in root elongation, as it acts as a stimulant during the early stages of adventitious root development and inhibits later stages of rooting development (Amound et al., 2017). Thus, adventitious rooting reduction is found due to the IBA toxic effect in high concentrations. This result reinforces that the optimal IBA dosage for *Azadirachta indica* rooting cuttings is from 0 to 2000 mg.l^{-1}, considering Gehlot's observation (2017).

Confirming the result related to the stem cut shoot production, it was found that there is a difference in the response among the matrices as for adventitious rooting like matrices with rooting greater than 90% at IBA 2,000 mg.l^{-1} and others at the same dosage with less than 60% rooting response. It is clear that the genotype factor has a direct effect on both shoot production and rooting. In *Azadirachta indica* plantations, genotypic variations occur due to heterozygosity and genetic adjustment for the species' adaptation to different locations (Sidhu; Kumar; Behl, 2003). Considering the foregoing, it can be said that the variation found in rooting may be directly associated with the genetic variability of the species, which contributes to the marked difference in adventitious rooting among some evaluated matrices.

For recalcitrant species to adventitious rooting, the specific genotype selection with a higher rooting rate may be a viable alternative to clone propagation (Oliveira et al., 2015). In this context, the superior genotype selection regarding adventitious rooting is possible within a genetic improvement program of *Azadirachta indica*, since there are differences in the matrix response regarding rooting. Therefore, it is extremely important to make the matrix selection in a proper way before starting the species seedling production by cuttings, thus maintaining matrices with high productive potential associated with rooting for clonal propagation.

4.3 *Influence of propagule size on rooting*

It was found that propagules with 13 cm have a better response in the *Azadirachta indica* seedling production by way of cuttings. There is uniformity in the cuttings rooting, including maintaining the root amount. The good response of the vegetative propagule with 13 cm is due, in part, to the greater leaf amount and axillary buds. The leaves synthesize soluble sugars, which provide energy for callogenesis and contribute to higher carbohydrate storage in the cutting, which guarantees greater nutritional reserve, contributing to survival, rooting, and increasing the root dry mass system (Shao et al., 2017).

Easier adventitious rooting was also found with *Azadirachta indica* mini-cuttings larger than 10 cm (Fernandes et al., 2017), similar results to those found in this study. Using species as Picea abies (L.) Karst., *Micromeria fruticulosa*, *Ilex paraguariensis a*. St.-hil, other authors who compared the cutting size also found that cuttings larger than 10 cm are more responsive to adventitious rooting (Ouyang et al., 2014; Pimentel et al., 2020; Sabatino et al., 2017). However, this result should not be considered a rule in the plant cutting context, because the species take root with different dynamics. Studies about the adventitious rooting are needed to determine, case-by-case, which is the propagule best size for this purpose. However, it has been verified that propagule sizes higher than 10 cm are more responsive to adventitious rooting for *Azadirachta indica* cuttings; in this study, 13 cm is the most indicated size.

4.4 *Adventitious rooting dynamics*

The propagule oxidation in cuttings is one of the factors that must be avoided to increase the survival and rooting rate. In this study, the greatest loss of cuttings by oxidation occurred mainly in the first week after staking. Propagule oxidation can happen for many reasons, such as water excess, phenolic compound production, pathogen attack, propagule thickness, the stake lignification degree, high temperature, irrigation time, and cutting density. To reduce damage, many alternatives are indicated as controlling the temperature and humidity of the greenhouse, the use of antioxidants, application of ascorbic acid (Oliveira et al., 2021), and foliar reduction to avoid the umbrella effect (Correia et al., 2015).

After staking, the turgidity maintenance of the cuttings is expected, initiating physiological changes which may be part of the adventitious root formation process. One of these processes is the base swelling followed by callogenesis. Calluses are a cluster of non-differentiating cells that can perform a healing function of a particular wound on the plant and, depending on the density, may be indicative that the propagule has the competence to produce adventitious roots (Zhou et al., 2018). Callus formation is also directly affected by the time of immersion in auxin, type of cut, and propagule age, in which there is a tendency to increase callus formation as age increases (Pimentel et al., 2019). However, indirect rooting through callogenesis contributes to the root formation with little vascular connection with the explant tissues, not being ideal for propagation through cuttings and direct rooting formed from vascular cells is preferred (Xavier et al., 2021).

However, adventitious root formation can also occur through xylogenesis, the genetic control of wood formation, and involves the expression of a series of genes with complex molecular regulations that favor xylem differentiation into a root primordium (Pereira and Bandeira, 2016). It was found that there was no correlation between cuttings rooting and callus formation, indicating that the adventitious root formation in *Azadirachta indica* cuttings occurs directly. In this case, the cellular dedifferentiation process must occur to initiate the adventitious root formation, which involves redirecting the development of totipotent cells towards the meristem formation giving rise to adventitious roots.

Considering the cuttings rooting dynamics, it was found that there are differences between the matrices regarding the rooting process, according to the cutting permanence in the greenhouse under different times. It is known that the rooting process in woody species is genotype dependent (Oliveira et al., 2015). As a result, different species, hybrids, and clones from the same parent may require different cultivation conditions and, consequently, different indices and rooting processes (Silva et al., 2021).

Despite the variations found between the matrices, it can be concluded that the cuttings are already rooted after twenty-one days, with root elongation from twenty-one to thirty-five days, the appropriate period to remove the rooted cuttings from the greenhouse.

5. CONCLUSION

Vegetative rescue of *Azadirachta indica* by cuttings is possible with higher rooting rates using cuttings with 13 cm in size, associated with 2,000 mg L^{-1} IBA dose. The period for rooting and the propagule permanence in the greenhouse depends on the genotype, ranging from twenty-one to thirty-five days. Better results in the species large-scale propagation should be preceded by testing and selecting adult material more responsive to adventitious rooting and suitable for commercial purposes.

AUTHOR CONTRIBUTIONS

Cleyton dos Santos Souza: Conceptualization, Data, curation, Formal analysis, Research, Original writing, Writing - review and editing. Poliana Coqueiro Dias Araújo: Conceptualization, Resources, Methodology, Analysis .Writing - review and editing. Dandara Yasmin Bonfim de Oliveira Silva: Statistical analysis, Figures and graphs, review and editing. Geovane de Almeida Nogueira: Data collection, methodology, field, review and editing. Maria Janaina

Nascimento Silva: Methodology, Field, Resources Review and Editing.

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