

VEGETATIVE PROPAGATION OF *Mimosa Caesalpinifolia* BY MINI-CUTTINGS TECHNIQUE

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ABSTRACT – This study aimed to establish a methodology for vegetative propagation via mini-cuttings technique for *Mimosa Caesalpinifolia*. For such, three independent experiments were conducted: the first one evaluated survival and production of mini-stumps; the second tested the interaction between mini-cuttings types (apical and intermediate) and different concentrations of indolbutyric acid (IAB; 0, 2,000, 4,000 and 6,000 mg.L⁻¹) on adventitious rooting; and the third analyzed the effect of leaf area reduction (0%, 25%, 50%, 75%, and 100%) on mini-cuttings. Mini-stumps survival at 180 days was 80%, with an average yield of 5 sprouts per mini-stump at 120 days. Apical mini-cuttings demonstrated a higher rooting percentage, without IBA application, higher than 80%. On the other hand, IBA application promotes increased rooting percentage in intermediate mini-cuttings. Treatments without leaf reduction and with reduction of 25% promoted better results concerning rooting and plant development. Results support the hypothesis that mini-cuttings technique is viable for the propagation of *Mimosa Caesalpinifolia*.

Keywords: Plant propagation; Adventitious rooting; Indole-3-butyric acid.

PROPAGAÇÃO VEGETATIVA DE *Mimosa Caesalpinifolia* PELA TÉCNICA DE MINIESTAQUIA

RESUMO – Este trabalho teve como objetivo estabelecer uma metodologia de propagação vegetativa via técnica de miniestaquia para *Mimosa Caesalpinifolia*. Para tanto, foram conduzidos três experimentos independentes: o primeiro avaliou a sobrevivência e produção das minicepas; o segundo testou a interação entre tipos de miniestacas (apical e intermediária) e diferentes concentrações de ácido indolbutírico (AIB; 0, 2.000, 4.000 e 6.000 mg.L⁻¹) no enraizamento adventício; e o terceiro analisou o efeito da redução da área foliar (0%, 25%, 50%, 75% e 100%) nas miniestacas. A sobrevivência das minicepas aos 180 dias foi de 80%, com rendimento médio de 5 brotos por minicepa aos 120 dias. Miniestacas apicais apresentaram maior porcentagem de enraizamento, sem aplicação de AIB, superior a 80%. Por outro lado, a aplicação de AIB promove aumento da porcentagem de enraizamento em miniestacas intermediárias. Os tratamentos sem redução foliar e com redução de 25% promoveram melhores resultados quanto ao enraizamento e desenvolvimento das plantas. Os resultados confirmam a hipótese de que a técnica de miniestaquia é viável para propagação de *Mimosa Caesalpinifolia*.

Palavras-Chave: Propagação de plantas; Enraizamento adventício; Ácido indol-3-butírico.



1. INTRODUCTION

Native forests of tropical countries such as Brazil have been target of frequent exploitation of timber and non-timber resources in order to meet the domestic demand or to generate subsidies for foreign trading. In Brazil, the caatinga is a dry forest located largely in the Northeastern region of the country. There, mainly due to climatic and edaphic characteristics, forest implementation is incipient and there is a shortage of timber and non-timber forest products to meet the local demand. Management of native forests and in many cases irregular deforestation supply that demand in the absence of wood from forest plantations. As a result, native tree species of the caatinga are used in an intensive and disorderly manner for obtaining firewood and charcoal (IBGE, 2020), with the lack of forest replacement in these exploited areas, in turn, limiting the availability of these resources.

However, the pressure from environmental agencies, concern with restitution of exploited areas and significant losses of genetic material of great ecological and economic importance have guided the study of native and exotic species while aiming at forest implementation and marketing of timber and non-timber forest products (Dias, et al., 2015). Among native caatinga species, Sabiá (*Mimosa caesalpinifolia* Benth.), stands out for its rustic and fast-growing nature, being recommended for shading, to act as living fence, forage, recovery of degraded areas, and source of wood for stakes, fencepost, firewood and charcoal (Balbinot et al., 2010; Araujo and Paes, 2018; Batista et al., 2020).

A species of multiple boles and with adaptation to the most diverse site conditions, is cultivated in commercial plantations in some Brazilian Northeastern states such as Ceará, Rio Grande do Norte, and Pernambuco. However, this species is still in process of domestication, as different silvicultural systems having been tested with it used in pure and intercropped plantations (Balbinot et al., 2010) while also evaluating its potential in recovery of degraded areas (Ribeiro et al., 2021). Nevertheless, the species multiplication is still a process requiring further studies in order to increase the quantity of seedlings offered as well as to improve their morphological and genetic qualities.

Production of Sabiá seedlings is commonly via seedlings; that they present tegumentary dormancy,

hindering absorption of water and oxygen and consequently delaying germination and producing uneven seedlings (Costa et al., 2018). Methods proposed for breaking the dormancy of *Mimosa caesalpinifolia* seeds involve mechanical, chemical or physical scarification (Medeiros et al, 2020). Such methods require time, produce uneven seedlings, may offer risks to the operator, and may present low efficiency. To this end, propagation by cuttings was tested for the species vegetative propagation, but the rooting percentage was very low (less than 5%) (Holanda et al., 2012). *In vitro* propagation of *Mimosa caesalpinifolia* has shown promise (Bezerra et al., 2014), however, production costs restricted the applicability of this technique on an operational scale.

On this basis, studies using vegetative propagation techniques as an alternative method for multiplying *Mimosa caesalpinifolia* are thereby recommended. Among these techniques, mini-cuttings has proven to be efficient for propagation of different forest species, such as *Ilex paraguariensis* (Pimentel et al., 2021), *Khaya anthotheca*. (Barbosa Filho et al., 2018), *Plathymenia reticulata* (Pessanha et al., 2018) and *Paratecoma peroba* (Araújo et al., 2019). This vegetative propagation method allows propagule-donor plants to be grown within a controlled environment with nutrition and irrigation management, and is considered efficient and economically viable for easy and rapid multiplication of genotypes on a commercial scale (Kuppasamy et al., 2019).

Roots formation is essential for propagation by mini-cutting technique and characters such as the percentage of rooting, surviving, dynamic of rooting, number of roots, length and dry mass of roots can be influenced by a number of factors and their interactions, as follow the age of the donor plant, hormonal balance, carbohydrate content, mini-cut type, sheet maintenance, substrates, and the application of growth regulators (Neubert et al., 2017; Araújo et al, 2019; Rasmussen et al., 2020; Saha et al. 2020; Pimentel et al., 2021; Xavier et al., 2021). Thereby, the establishment of the mini-cut protocol for *Mimosa Caesalpinifolia* would provide an alternative method of propagation for this specie, overcome the difficulties associated with seminal propagation, and increase the possibility to get more plants for restoration projects in Caatinga Biome and commercial plantations of these specie.

In light of the importance of the vegetative propagation of *Mimosa Caesalpinifolia*, the present study aimed to establish a protocol of vegetative propagation via mini-cuttings technique for this species. For such, the following hypotheses were tested: (i) *Mimosa Caesalpinifolia* has aptitude for vegetative propagation by the mini-cuttings technique, because this technique retain juvenility and produce propagules with better nutritional, hydric and physiological conditions which increase rooting; (ii) Indolbutyric acid (IAB) reflect in greater induction of adventitious roots in apical and intermediate mini-cuttings, due to the adventitious root regeneration being a highly complex regenerative process that is influenced by numerous internal and external factors, including auxin level; (iii) *Mimosa Caesalpinifolia* apical mini-cuttings have higher rooting rate than the intermediate ones, because the amount of hormone produced internally is sufficient, since the ontogeny speed is higher and most transport vessels are functional with low lignification, which increases hormone transport, carbohydrates and unloading; and (iv) leaf area influences rooting and survival of *Mimosa Caesalpinifolia* mini-cuttings, since the leaves support photosynthesis and carbohydrate accumulation, which are related to successful of adventitious roots.

2. MATERIALS AND METHODS

2.1 Implantation and management of the clonal mini-garden

Experiments were undertaken from January to March 2019 at the forest nursery at Universidade Federal Rural do Semi-árido, in Mossoró, Rio Grande do Norte state. According to Köppen's classification, the climate of the municipality is classified as BSh, that is, dry semiarid climate, the average temperature of the region is 27.4 °C, with average relative humidity of 68.9%, very irregular annual rainfall, with an average of 673.9 mm and a drought period of 6 to 8 months (Melo et al., 2020).

According to the mini-cuttings technique described in Xavier et al. (2021), the mini-garden consisted of mini-stumps, obtained by seeds propagation of *Mimosa caesalpinifolia*. Three seeds were sown per tube, with thinning performed at 30 days when more than one seed germinated. When the

seedlings reached an average height of 15 cm, they were transferred to a trough with sand and, after 50 days (seedlings adaptation and growth period), their apices were pruned to a height of 12 cm from the base, aiming to stimulate sprouting on the mini-stumps, thus providing the mini-cuttings for the experiments.

Using a suspended bed kept under full sun, the mini-garden was established in a semi-hydroponic system. The used trough was a masonry trough 7.5 m long, 0.8 m wide and 25 cm deep, containing medium grain-size sand to support the mini-stumps placed at the spacing of 15 x 15 cm, as described by Dias et al. (2012).

The nutrient solution used for fertilization consisted of the following: 117.0 mg L⁻¹ of N in nitrate form; 15.75 mg L⁻¹ of N in ammonium form; 14.63 mg L⁻¹ of P; 131.62 mg L⁻¹ of K; 84.0 mg L⁻¹ of Ca; 25.21 mg L⁻¹ of Mg; 73.28 mg L⁻¹ of S; 0.01 mg L⁻¹ of B; 0.02 mg L⁻¹ of Cu; 69.73 mg L⁻¹ of Fe; 0.03 mg L⁻¹ of Mn; 0.008 mg L⁻¹ of Zn; 0.0016 mg L⁻¹ of Mo (Pimentel et al., 2021). The electrical conductivity of the nutrient solution was maintained at 1.5 dS m⁻¹ at 28 °C and the pH kept between 5.5 and 5.8.

Mini-cuttings with lengths between 5 and 10 cm were obtained from mini-stumps at regular periods of 26 days. Mini-cuttings in the rooting phase were kept in an acclimatized greenhouse with 50% shading, relative humidity above 85% and temperature between 25 and 30 °C. Plastic tubes with capacity for 55 cm³ were used as containers, with EcoFertil[®] commercial substrate. Basic mineral nutrition used in the substrate was composed of simple superphosphate (8.00 kg.m⁻³) and osmocote in the formulation 16-06-10 (0.3 kg.m⁻³), as indicated by Dias et al. (2015).

The physical and chemical characteristics of substrate composition were pH in H₂O = 5.70; electrical conductivity = 0.5 mS cm⁻¹; dry density = 878.84 kg m⁻³; total porosity = 60%; aeration space = 30.00%; remaining water = 25 % and available water = 8%.

Thirty days was the time mini-cuttings remained in the greenhouse, after which they were acclimated to a 50% shading house for 10 days and transferred to a full sun area to grow for 70 days. When leaving the greenhouse, a top-dressing fertilization was carried out with 2 mL seedling⁻¹ of monoammonium phosphate (2.0 g L⁻¹), and when leaving the shading house, 5 mL seedling⁻¹ of NPK (10-05-30) (6 g L⁻¹) was also applied, as indicated by Dias et al. (2015).

2.2 Survival and production of mini-stumps

Every 26 days, period determined by the existence of sprouts with minimal size for producing mini-cuttings, survival and production evaluations were performed on existing mini-stumps, observing the number of sprouts per unit and the number of survivors as a function of six successive prunings. Mini-stumps were distributed over the trough in an entirely randomized experimental design with four repetitions of 60 mini-stumps each one. Statistical analysis was performed by comparing characteristics in the six sprout collections and when the variables were significant, regression equations were generated prior to the analysis of variance. The production of mini-cuttings was evaluated per m² per month. The yield of mini-cuttings was calculated from the ratio between the number of mini-cuttings produced per m² in each month.

2.3. IBA influence

In order to evaluate the influence asserted by the growth regulator IBA on rooting of *Mimosa caesalpiniiifolia* mini-cuttings, two types of mini-cuttings were used: one from the apical section, containing two pairs of leaves; and other from the intermediate section, containing one pair of leaves, reduced to 25% of their original size and with 8 cm in size.

After preparation, apical and intermediate mini-cuttings had their bases (2 cm) immersed in the IBA solution for 10 seconds. IBA was used at concentrations of 0; 2000; 4000 and 6000 mg.L⁻¹, dissolved in potassium hydroxide at 1 mol.L⁻¹ and then diluted in distilled water.

A randomized block experimental design was employed, with a 2 x 4 factorial design, consisting of two mini-cutting types (apical and intermediate) and four IAB doses (0; 2000; 4000; and 6000 mg.L⁻¹), having three repetitions of 14 mini-cuttings per plot. Concerning the significant characteristics in the variance analysis, regression equations were generated when the IBA factor was significant, while a test of means using the Tukey test at 5% probability was performed when the mini-cutting type factor was significant.

2.4 Leaf area effect

To evaluate the reduced leaf area influence on rooting of *Mimosa caesalpiniiifolia* mini-cuttings,

8-cm tall apical propagules were used, as well as two pairs of leaves and the treatments defined as E1: 0% leaf reduction (all leaves intact); E2: 25% leaf reduction; E3: 50% leaf reduction; E4: 75% leaf reduction; E5: 100% leaf reduction (no leaves).

Randomized block design was the experimental design used, with three repetitions composed of 14 mini-cuttings per plot. During rooting, these mini-cuttings were evaluated weekly for their percentage of survival and rooting (carried out by observing exposed roots on the bottom opening of the tube). Statistical analysis was performed by comparing leaf reduction levels and, when variables were significant and prior to the analysis of variance, regression equations were generated for survival and rooting in the greenhouse, as well as test of means by using the Tukey test at 5% probability for evaluated characteristics in other phases.

2.5 Experimental evaluations and data analysis

During the experiments assessing IBA influence and leaf area reduction, when leaving the greenhouse, the percentages of survival (SOB, %) and root occurrence observed at the lower end of the tube (RFT, %) were evaluated. After 30 days under full sun, the following were evaluated: rooting percentage (%), seedling height (H, cm), collar diameter (DC, mm), survival percentage (SOB, %), number of roots (NR), largest root length (cm), shoot dry weight (MSPA, g) and root dry weight (RFT, %). (MSRA, g).

3. RESULTS

3.1 Survival and production of *Mimosa caesalpiniiifolia* mini-stumps

The mini-stumps demonstrated variation when producing sprouts over the evaluation period, with quadratic trend and the highest mean of 5 shoots per unit, at 90, 120 and 150 days after establishing the mini-garden (Figure 1). The highest yield of mini-cuttings was 222.22 sprouts per m² per month. At the sixth collection, the number of sprouts per mini-stump decreased, reaching 3.5 propagules per unit, yet this value was higher than the observed during the first and second evaluation. Survival of mini-stumps had a linear decreasing trend, varying by 20% between the first and the last evaluation, with the lowest mean found at 180 days (80% survival).

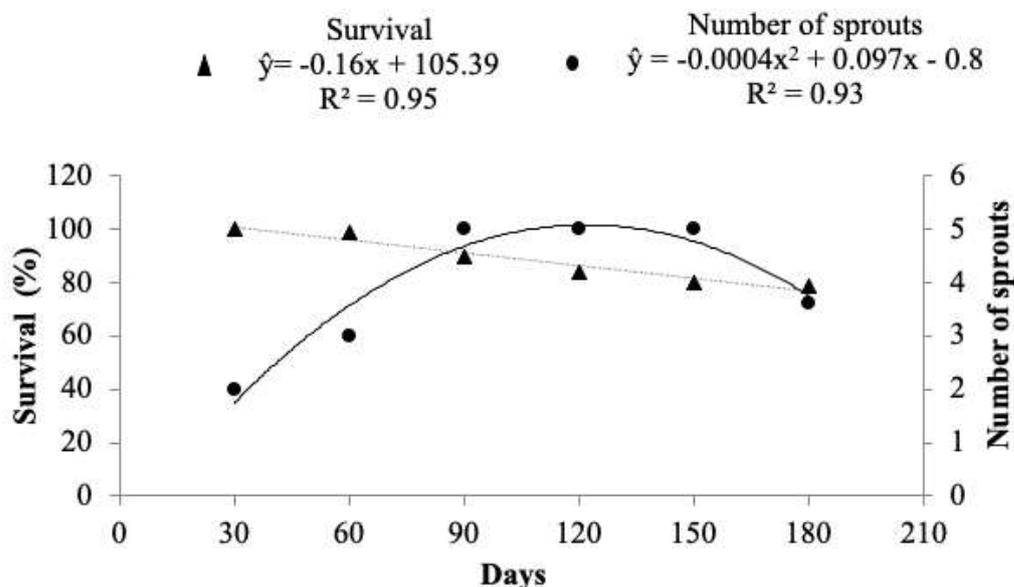


Figure 1 – Survival and number of sprouts per mini-stump in *Mimosa caesalpinifolia* Benth., as a function of successive collections at 30, 60, 90, 120, 150 and 180 days after establishing the mini-garden.

Figura 1 – Sobrevivência e número de brotos por minicepa em *Mimosa caesalpinifolia* Benth., em função das coletas sucessivas aos 30, 60, 90, 120, 150 e 180 dias após a implantação do minijardim.

3.2 IBA influence

According to the analysis of variance, interaction between the factors mini-cutting type and IBA doses was significant for the survival (%) ($p = 0.021$) and rooting (%) ($p = 0.021$), evaluated after the full sun phase. IBA doses of and the apical and intermediate mini-cuttings did not influence the characteristics roots observed at the tube lower end (ROEIT) and survival (%) after rooting phase, as well as the largest root length (g), number of roots (h) and root dry weight, all evaluated after the full sun phase. On the other hand, the factor mini-cutting type influenced collar diameter, shoot height and dry weight.

The mean percentage of propagules with exposed roots on the tube bottom opening was of 78.6%, after 30 days in the rooting environment; survival was 100% during this phase. At the experiment end, 50 days after planting, the mean largest root length was 12 cm, averaging six roots per rooted propagule and root dry weight of 3.8 g.

IBA doses for survival and rooting after the full sun phase and considering apical mini-cuttings,

illustrated by Figure 2a and 2b, demonstrate a linear decreasing behavior while the intermediate mini-cuttings show a polynomial trend of second degree, as seen in Figure 2a and 2b. For apical mini-cuttings, IBA application tends to reduce both survival and rooting. In contrast to that, survival and rooting of intermediate mini-cuttings tended to increase when IBA was applied. Without IBA application, apical mini-cuttings stand out in relation to intermediate ones regarding the characteristics survival and rooting. However, for these same characters, IBA presents a positive effect for intermediate mini-cuttings and a negative effect for apical mini-cuttings.

Mini-cuttings have increased height when apical propagules are used, with these being superior to the intermediate ones (Figure 3a). On the other hand, intermediate mini-cuttings demonstrated a superior behavior to the apical mini-cuttings concerning collar diameter and shoot dry weight (Figures 3a and 3b).

These results shows that the first hypothesis tested was validated, that is, *Mimosa caesalpinifolia* can be vegetatively propagated by mini-cuttings, with

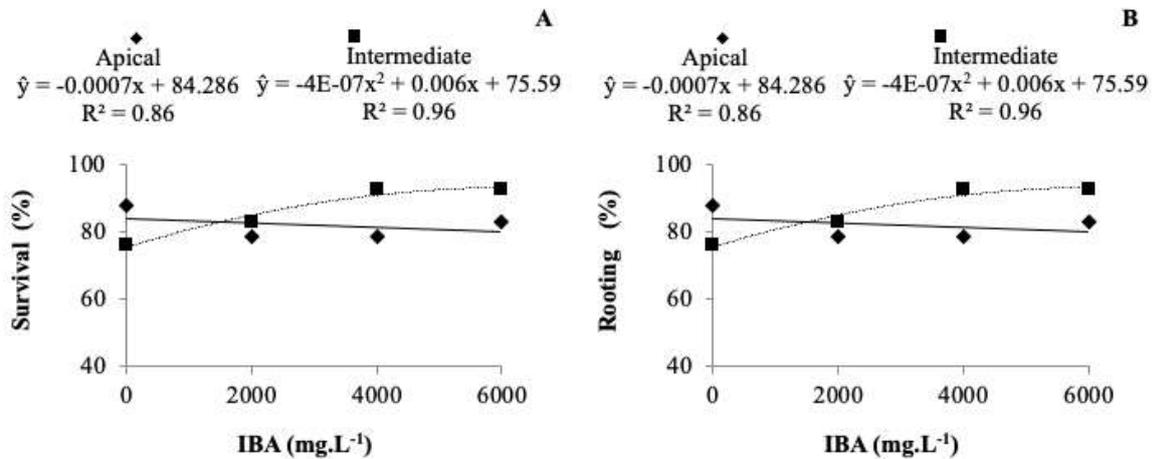


Figure 2 – Survival (A) and rooting (B) of *Mimosa caesalpinifolia* Benth. mini-cuttings, under full sun conditions, as a function of IBA concentration (0 mg.L⁻¹; 2000 mg.L⁻¹; 4000 mg.L⁻¹ and 6000 mg.L⁻¹) and mini-cutting type.

Figura 2 – Sobrevivência (A) e enraizamento (B) de miniestacas de *Mimosa caesalpinifolia* Benth. a pleno sol, em função da concentração de AIB (0 mg.L⁻¹; 2000 mg.L⁻¹; 4000 mg.L⁻¹ e 6000 mg.L⁻¹) e tipo de miniestaca.

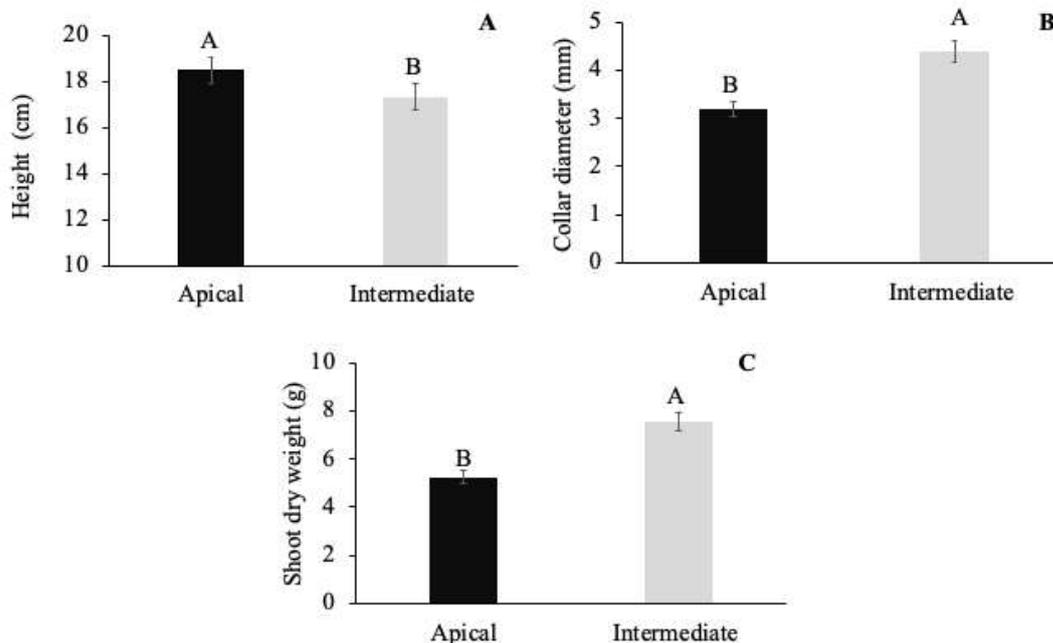


Figure 3 – Height (A), collar diameter (B) and shoot dry weight (C) of *Mimosa caesalpinifolia* Benth. mini-cuttings, as a function of the mini-cutting type (apical and intermediate).

Figura 3 – Altura (A), diâmetro do colo (B) e massa seca da parte aérea (C) de miniestacas de *Mimosa caesalpinifolia* Benth., em função do tipo de miniestaca (apical e intermediária).

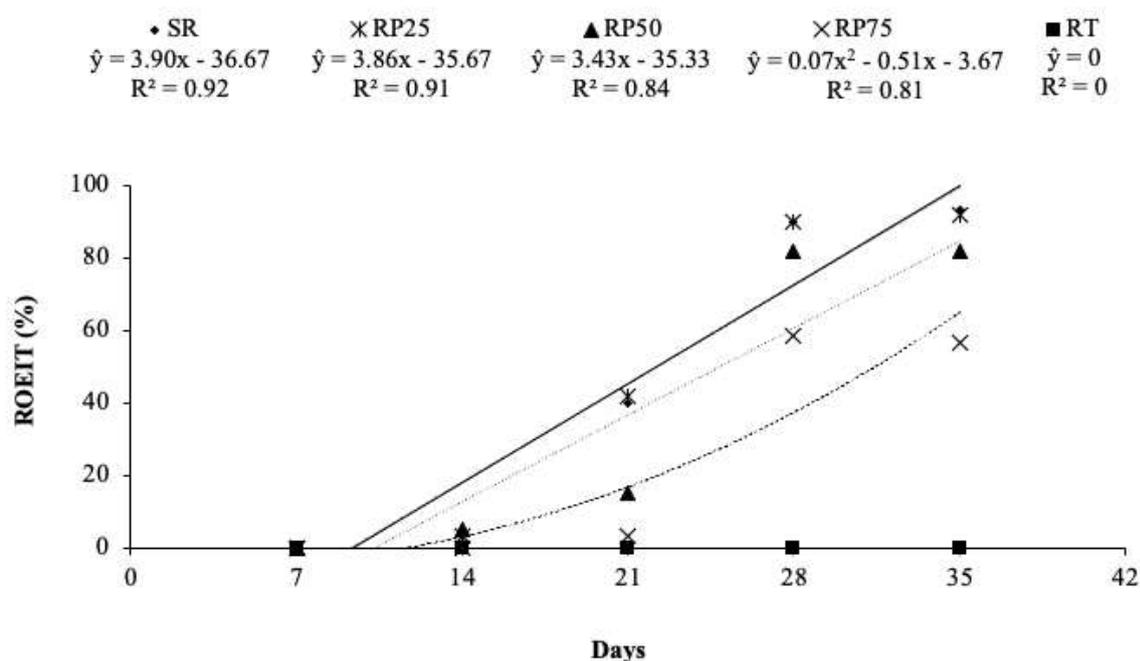


Figure 4 – Roots observed on the tube lower end (ROEIT) in *Mimosa caesalpinifolia* Benth. mini-cuttings, as a function of leaf reduction (SR: leaf without reduction; RP25: 25% leaf reduction; RP50: 50% leaf reduction; RP75: 75% leaf reduction and RT: mini-cuttings with no leaves).

Figura 4 – Raízes observadas na extremidade inferior do tubo (ROEIT) em miniestacas de *Mimosa caesalpinifolia* Benth., em função da redução foliar (SR: folha sem redução; RP25: redução foliar de 25%; RP50: redução foliar 50%; RP75: redução foliar 75% e RT: miniestacas sem folhas).

percentages of survival and rooting above 80% of the mini-cuttings and a good percentage of survival and sprout production of the mini-stumps. The second hypothesis was validated only for intermediate mini-cuttings, where IBA increased rooting percentage and mini-cutting survival. The third hypothesis was confirmed for the control treatment, without IBA application, where apical mini-cuttings had a higher

rooting rate. However, in presence of IBA, intermediate mini-cuttings had a higher rooting percentage.

3.3 Leaf reduction influence

As for observation of exposed roots on the tube bottom opening (Figure 4), the increment projection tended toward a linear increase for treatments with no leaf reduction, 25% leaf reduction and 50% leaf

Table 1 – Survival (SOB), rooting (ENR), height (ALT), collar diameter (DC), number of roots (NR), root and shoot dry weights of *Mimosa caesalpinifolia* Benth mini-cuttings, under full sun conditions, as a function of leaf reduction (SR: leaf without reduction; RP25: 25% leaf reduction; RP50: 50% leaf reduction; RP75: 75% leaf reduction and RT: mini-cuttings with no leaves).

Tabela 1 – sobrevivência (SOB), enraizamento (ENR), altura (ALT), diâmetro do colo (DC), número de raízes (NR), massa seca de raiz e parte aérea de miniestacas de *Mimosa caesalpinifolia* Benth, em condições a pleno sol, em função da redução foliar (SR: folha sem redução; RP25: redução foliar 25%; RP50: redução foliar 50%; RP75: redução foliar 75% e RT: miniestacas sem folhas).

Leaf reduction	SOB.(%)	ENR.(%)	DC(mm)	ALT(Cm)	NR	dry weights	
						root (g)	shoot (g)
SR	93.33 A	93.33 A	4.01A	19.66A	4.49 AB	5.73A	9.38A
RP25	91.67A	91.67A	3.88A	19.33A	5.31A	4.85A	8.03A
RP50	81.67A	81.67A	3.85A	14.75B	4.37AB	1.9B	4.78B
RP75	56.67B	56.67B	4.85A	13.45B	3.53B	1.5B	4.67B
RT	0.00C	0.00C	0.00B	0.00C	0.00C	0.00C	0.00C

In which: Means followed by the same lowercase letter, in the column, do not differ in leaf reduction levels by the Tukey test at 5% probability.

Em que: Médias seguidas por letra maiúscula, na coluna, não diferem quanto aos níveis de redução da área foliar pelo teste de Tukey a 5% de probabilidade.

reduction. On the other hand, the treatment with 75% leaf area reduction had a quadratic tendency for the percentage of roots observed on the tube bottom openings and means, in the evaluations, lower than the three treatments abovementioned. On the contrary, mini-cuttings with total reduction did not survive the rooting process.

Based on results obtained (Table 1), leaf area reduction levels asserted a significant effect ($p < 0.05$) for evaluated characteristics. Treatments SR, RP25 and RP50 had the highest percentage of survival and rooting, as well as the highest number of roots at the base of mini-cuttings (Table 1). Percentage of survival and rooting of SR and RP25 treatments was greater than 90%.

Treatments SR, RP25, RP50 and RP75 did not differ in collar diameter and largest root length, being on average greater than 3.8 mm and equal to 12 cm, respectively. Not reducing the leaf area and reducing it by 25% promoted greater height, shoot and root dry weights, with values greater than 19 cm for height, 4.8 g for root dry weight and 8 g for shoot dry weight. Unlike other treatments, mini-cuttings without leaves did not survive the rooting phase, thus assigned a zero value for evaluated characteristics.

4. DISCUSSION

4.1 Survival and production of *Mimosa caesalpiniiifolia* mini-stumps

Managing the mini-garden is paramount for survival of the plant providing vegetative propagules, namely one of the main factors affecting vegetative propagation of forest species (Xavier et al., 2021). Therefore, the high survival of *Mimosa caesalpiniiifolia* mini-stumps, after six prunings performed at 30-day intervals, demonstrates that the adopted management was adequate in conducting the mini-garden.

Another important factor is the species, also able to interfere in technical and operational viability of the mini-garden. Some species can easily survive at the successive pruning and be responsive to the management adopted in the trough, as observed for *Myracrodruon urundeuva* mini-stumps that had 100% of survival after five pruning (Justino et al., 2017). On the other hand, some genotypes have low survival of mini-stumps, such as some progenies of

Plathymenia foliolosa, with survival less than 25% after four pruning (Neubert et al., 2017). *Mimosa caesalpiniiifolia* proved to be responsive to the management adopted in the trough as well as to the successive pruning, having mini-stump survival of 80% after the sixth pruning.

Moreover, variations in mini-stump survival can be related to the season and to physiological conditions of the seedling-donor parent plant, such as hormone transport, carbohydrates content, starch hydrolysis, free sugars, nitrogen level and tissue hydration (Abarca, 2021). A high percentage of mini-stumps survival demonstrate tolerance of the species to periodic pruning, indicating potential viability of the mini-cuttings technique (Wendling et al. 2015).

The good yield of the mini-stumps during the experimental period, averaging five shoots per unit in the third, fourth and fifth collections, is due to their good nutritional status, which consequently provided an increase in shoot production for the other propagation stages. These results are also related to the ontogenetic stage of the mini-stumps. Juvenile materials show predisposition for growth and development when subjected to different propagation conditions (Wendling et al. 2014, 2015).

Our results are similar to other studies that analyzed the performance of mini-stumps from different forest species (Wendling et al. 2015; Mantovani et al., 2017; Barbosa Filho et al., 2018; Lima et al., 2021), boasting a mini-stump survival rate higher than 80% and yield higher than 50 mini-cuttings per m². It is noteworthy that the low yield during the first regrows of the species is due to the conformation process of the mini-stumps and adaptation to the cultivation system to which they are submitted to (Mantovani et al., 2017), as verified in the present study.

4.2 IBA influence

The linear reduction in rooting and survival of apical mini-cuttings as a function of the concomitant IBA concentration increase may be linked to, among other factors, the juvenility degree of the parent plant, physiological condition of propagules and the endogenous production of auxin and rooting enzymes such as starch phosphorylase, amylases and Kinases (Quan et al., 2017). These enzymes are needed to

support root primordium initiation and development. In general, Adventitious root formation has been investigated and it is considered as a complex multi-step process which is affected by endogenous factors, including phytohormones with a central role of auxin, the influence of carbohydrates and hormonal crosstalk, of nitrogen supply, of free amino acids, of general mineral nutrition, of antioxidative enzymes and environmental factors, such as wounding or light (Wendling et al., 2014; Quan et al., 2017; Diaz-Sala, 2020; Abarca, 2021).

Mini-stumps conducted in the present study are juveniles, coming from seedlings produced by seeds. Hence, the concentration of endogenous auxin, indole-3-acetic acid and root cofactors, as listed above, in the apical sprouts may be at sufficient levels to stimulate adventitious root emission and seedling survival. As reported by Wendling et al. (2015), propagules originating from juvenile mini-stumps are more responsive to adventitious rooting.

Moreover, auxin is mainly synthesized in growing apices and young leaves and its transport is basipetal (Costa et al. 2013), contributing to higher rooting rate of apical mini-cuttings without IBA application. Therefore, results of the present study suggest that endogenous auxin levels may have been optimal for root formation in apical mini-cuttings, and that exogenous applications increased concentrations to supra-optimal levels, which in turn inhibited rooting.

Nevertheless, intermediate mini-cuttings need exogenous auxin to achieve higher adventitious rooting rates, mainly due the fact that they present a lower endogenous auxin production added to a higher lignification of the tissues, which is corroborated by the larger collar diameter. Some studies have demonstrated that tissues with higher degrees of lignification and suberization require higher auxin concentrations for inducing adventitious rooting (Wendling et al., 2015; Faganello et al., 2015; Pimentel et al., 2021).

Topophysis effect can exert influence on adventitious rooting, providing reduced rooting rate as a function of maturation of apical tissues (Hung and Trueman, 2011). However, in the present study, the advanced stem development in basal nodes, observed by the intermediate mini-cuttings larger diameter, may explain the observed topophysis gradient, since the apical mini-cuttings had higher rooting rate

without exogenous IBA application. Thus, *Mimosa caesalpinifolia* apical mini-cuttings seem to have physiological conditions that are more favorable to adventitious rooting. New findings support that phytohormone-controlled reprogramming and differentiation of cells near the cut on cuttings and mini-cuttings interact with coordinated reallocation of rooting cofactors, such as carbohydrates, to initiate and conduct the adventitious root formation (Druege et al., 2019).

Concerning mini-cuttings, previous studies have demonstrated that IBA application does not favor adventitious rooting; as the example of *Eucalyptus grandis* x *E. globulus* clones (Borges et al., 2011), *Anadenanthera macrocarpa* (Dias et al, 2012), *Araucaria angustifolia* (Pires et al., 2013), *Tectona grandis* (Badilla et al., 2016), *Tibouchina sellowiana* (Fragoso et al., 2017), *Peltophorum dubium* (Mantovani et al., 2017), *Plathymenia reticulata* (Pessanha et al., 2018) and *Paratecoma peroba* (Araújo et al., 2019). These studies corroborate with the results obtained for *Mimosa caesalpinifolia*. Therefore, given the negative effect on rooting, it is not recommended to use IBA at tested concentrations for rooting of *Mimosa caesalpinifolia* apical mini-cuttings. Meanwhile in intermediate mini-cuttings, to get the rooting rate higher than 80%, it is necessary to use IBA.

4.3 Leaf reduction influence

Maintaining the leaf area in *Mimosa caesalpinifolia* mini-cuttings positively influenced survival, rooting, height, number of roots, and shoot and root dry weights, contributing positively to growth and root architecture, besides providing a faster rooting. Therefore, it is unnecessary to reduce the leaf area when producing seedlings of this species via mini-cuttings, thus validating the fourth hypothesis.

Reducing leaf area is a common step in seedling production for cuttings, employed in order to reduce transpiration and avoid the "umbrella effect" (Xavier et al., 2021). However, mini-cuttings tend to have a lesser leaf area than macro-cuttings, thus reducing the occurrence of these problems, especially at the rooting stage.

Results found here are similar to what was found in other studies on leaf area reduction of mini-cuttings

in different forest species (Santana et al., 2010; Batista et al., 2014; Correia et al., 2015; Dias et al. 2015; Neubert et al., 2017; Fernandes et al., 2018; Mayer et al., 2018; Santana et al., 2018; Araújo et al., 2019). These studies have demonstrated that maintaining whole leaves provides rooting maximization and that reducing leaf area is not a standard procedure in the process of preparing mini-cuttings.

Leaf presence is an essential factor in the propagation of *Mimosa caesalpinifolia* by mini-cuttings, since mini-cuttings without leaves did not survive through the rooting stage. The positive effect of maintaining leaf area in mini-cuttings is related to higher photosynthetic rate that provides, among other factors, increased carbohydrate storage and auxin production, which in turn favor root induction and growth (Hartmann et al., 2011; Batista et al., 2014; Dantas et al. 2016).

In the present study, the leaf area positive effect can also be attributed to the irrigation system quality. Keeping the environment with high humidity inside the greenhouse contributes in reducing vapor pressure deficit, which in turn reduces evapotranspiration and consequently avoids dehydration and death of mini-cuttings before the required period for rooting (Fernandes et al., 2018). Furthermore, not reducing the leaf area increases operational yield and reduces repetitive-strain injuries of workers, seedling production cost and incidence of pathogens on propagules (Santana et al., 2010; Dias et al. 2015).

5. CONCLUSIONS

Mini-cuttings technique employing apical sprouts, without adding IBA and maintaining total leaf area, from mini-stumps produced via seeds, is a strategy technically feasible for propagating *Mimosa caesalpinifolia* seedlings.

AUTHOR CONTRIBUTIONS

Ana Karla Vieira da Silva: Designed the methodology, data collection and organization, bibliographical research, analysis and discussion of results, and article writing.

Thamiris da Silva Aguiar and Márcia Ellen Chagas dos Santos: Designed the methodology, data collection and organization.

Jayane Karine Pereira de Araújo and Álvaro da Costa Freire: Bibliographical research, analysis and discussion of results, and article writing.

Gabriela Salami: Statistics analysis and discussion of results.

Poliana Coqueiro Dias Araujo: Conceived the ideas, designed the methodology, analysis and discussion of results and article writing.

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