v. 4, n. 10, 2024

Journal of Agricultural Sciences Research

ADVENTITIOUS ROOTING OF MINI-CUTTINGS OF AMBURANA CEARENSIS, A TROPICAL SPECIES USED FOR ENVIRONMENTAL, MEDICINAL, AND TIMBER PURPOSES

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Abstract: *Amburana cearensis* (allemão) A. C. Sm. belongs to the Fabaceae family and is primarily used in folk medicine due to its abundance of medicinal compounds, making it a viable option for mixed planting schemes not solely focused on timber exploitation. Its propagation via mini-cuttings could facilitate the multiplication of selected individuals based on desirable traits. However, the rooting percentage of mini-cuttings for this species is low. Thus, this study aimed to evaluate the rooting of mini-cuttings of *A. cearensis* collected from different positions on the shoots (basal, intermediate, and apical) and subjected to treatments with IBA (0, 2000, 4000, 6000, and 8000 mg L^{-1}), with or without the application of H_2O_2 (50 mM). Despite the higher survival rate of basal mini-cuttings, the position of the cuttings did not positively affect the other characteristics of the produced seedlings, except for the dry root mass. At 60 days after planting, the isolated treatments of H_2O_2 and IBA at a concentration of 2000 mg L^{-1} resulted in high survival rates of *A. cearensis* mini-cuttings (> 80%), but did not improve the rooting percentage, which remained below 50%.
Keywords: Amburana; vegetative propagation; mini-cuttings; cloning; plant hormones

INTRODUCTION

The species *Amburana cearensis*, from the Fabaceae family, is widely distributed across several countries in South America. In Brazil, it occurs in the Atlantic Forest, Cerrado, and Pantanal regions, spanning the North, Northeast, Central-West, and Southeast regions (Flora do Brasil, 2020). Its ecological and economic importance, especially for non-timber uses, justifies its prominence in forest and agroforestry plantings, making the provision of high-quality seedlings crucial for various purposes.

Commercial production of seedlings for this species is typically done through seeds. However, ensuring a sufficient number of parent plants annually to maintain genetic variability in reforestation efforts could be addressed by establishing seed orchards, based on progeny or clones of selected parent trees. Vegetative propagation of the species not only facilitates the establishment of these areas but also allows for the multiplication of selected individuals based on desirable productive traits, ensuring homogeneous commercial plantings.

Among the vegetative propagation techniques used for forest species is mini-cutting. However, for its application, the species must exhibit good sprouting capacity after pruning and the ability to root. Preliminary tests indicated that mini-cuttings from juvenile materials of the species have a low rooting percentage (unpublished data), necessitating further studies to develop an efficient propagation protocol.

Adventitious rooting results from anatomical and physiological processes associated with the dedifferentiation and redirection of pluripotent cell development to form meristematic tissues that give rise to adventitious roots (Alfenas et al., 2009). The formation and development of adventitious roots are controlled by complex hereditary traits that are not yet fully understood. However, it is known that endogenous and environmental regulatory factors play a crucial role in the process. In this regard, some compounds are used to stimulate, induce, and/or increase adventitious rooting, such as auxins (Fagnello et al., 2015; Souza et al., 2013; Hartmann et al., 2011).

Another compound capable of benefiting adventitious rooting is hydrogen peroxide (H_2O_2), which, despite being classified as a free radical, can regulate auxin concentration within cells, thus promoting rooting (Pasternak et al., 2005). It can also act as a signaling molecule for auxins (Li et al., 2007). Literature suggests that the combination of auxins and H_2O_2 can enhance adventitious rooting (Sebastiani & Tognetti, 2004; Li et al., 2009; Li et al., 2007; Rugini et al., 1997). The beneficial effect of H_2O_2 on rooting may also be related to reduced auxin degradation. In this way, auxin moves basipetally and concentrates in the cutting's base (Steffens & Rasmussen, 2016). Besides the positive synergistic effects of combining IBA with H_2O_2, H_2O_2 alone has also been identified as a potential promoter of initial root differentiation, inducing greater callus production at the base of mini-cuttings (Liao et al., 2011; Silva et al., 2022).

Thus, the objectives of this study were to determine whether there are differences in survival and adventitious rooting among basal, intermediate, and apical mini-cuttings of *A. cearensis* and to increase the rooting percentage through the exogenous application of H_2O_2, with or without IBA.

MATERIALS AND METHODS

The experiments were conducted in a greenhouse at the State University of Northern Rio de Janeiro Darcy Ribeiro (UENF), located in Campos dos Goytacazes, RJ, Brazil (latitude 21º 19' 23" S and longitude 41º 19' 41" W). Microclimatic conditions, both in the mist chamber and in the greenhouse, were monitored using an Akso datalogger, model AK 147.

The study was carried out in two stages. The first stage compared seedling production from apical, intermediate, and basal mini-cuttings. The second stage evaluated the rooting capacity of the mini-cuttings with treatments involving Indole-3-Butyric Acid (IBA), with or without Hydrogen Peroxide (H_2O_2) (Figure 1).

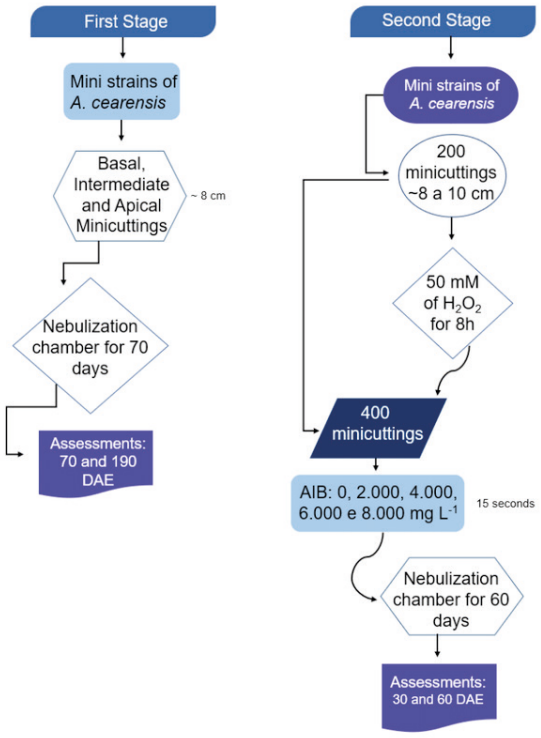

Figure 1. Experimental procedure for the mini-cutting propagation of *Amburana cearensis* - positioning of mini-cuttings on the shoot and rooting induction with IBA combined with H_2O_2.

FIRST STAGE - ROOTING OF MINI-CUTTINGS BASED ON SHOOT POSITION

To produce the mini-cuttings, a multiclonal mini-garden was used, created from seedlings obtained from seeds. At 260 days after sowing, mini-cuttings were collected, each 8 cm in length with a leaf reduced to 75%, from different segments of the shoots (basal, intermediate, and apical). After preparation, the material was planted in 280 cm^3 tubes containing a commercial forest substrate mixture based on peat, with amendments, vermiculite, charcoal, and pine bark (Basaplant®), enriched with Osmocote® controlled-release fertilizer in the NPK formulation (15-09-12), with an action time of eight months at a concentration of 8 g kg^{-1} of substrate.

The material was kept for 70 days under intermittent misting (30 seconds every 20 minutes). The average temperature and relative humidity recorded during this period were 25°C and 94%, respectively. The experiment was arranged in a completely randomized design, with three treatments (apical, intermediate, and basal mini-cuttings), replicated nine times with 15 plants per plot.

Survival of the mini-cuttings was assessed at 70 days after planting (DAP), when they were removed from the rooting sector, and the final evaluation of the seedlings occurred at 190 DAP. Measurements taken included height (H), collar diameter (CD), main root length (MRL), leaf area (LA) using an electronic bench meter (LI-COR model LI-3000), and dry mass of the aerial part (DMAP) and root system (DMRS). For dry mass determination, samples were placed in labeled paper bags and dried in a forced-air oven at 65°C for 72 hours before being weighed on an analytical balance.

The data were tested for adherence to the assumptions of analysis of variance (ANOVA). Data that did not meet the basic assumptions for ANOVA were transformed. Specifically, survival data at 70 and 190 DAP were transformed using the square root of x + 0.5. Subsequently, the results were subjected to ANOVA and differences compared using the Tukey test ($P < 0.05$). The statistical software used was R (R Core Team, 2019).

SECOND STAGE - ROOTING OF MINI-CUTTINGS BASED ON THE APPLICATION OF IBA WITH OR WITHOUT H_2O_2

From 200 seedlings derived from seed propagation, at 143 days after sowing, mini-cuttings measuring 7 to 8 cm in length with leaves reduced to 75% were collected, excluding apical cuttings based on the results from the first stage.

After preparing 200 mini-cuttings, they were placed in plastic trays containing water to prevent dehydration of the material. Subsequently, the bases of the mini-cuttings were immersed in 0.5% sodium hypochlorite solution for five minutes to disinfect the material, followed by washing with deionized water.

Next, the mini-cuttings for the treatment with pre-immersion in hydrogen peroxide were placed in trays with perforated Styrofoam plates containing a 50 mM H_2O_2 solution for eight hours (Silva et al., 2022; Neill et al., 2002).

IBA solutions were prepared by dissolving 4 g of IBA in 10% sodium hydroxide (0.5 M) (Pereira et al., 2021), followed by the addition of deionized water to make a total volume of 500 mL. Serial dilutions from the highest concentration (8000 mg L^{-1}) were made with deionized water to obtain the lower concentrations used (2000, 4000, and 6000 mg L^{-1}).

One hour before removing the material from the H_2O_2 solution, another 200 mini-cuttings were collected and prepared using the same disinfection process.

Thus, the bases of the mini-cuttings, with or without pre-immersion in H_2O_2, were immersed in their respective IBA solutions (0, 2000, 4000, 6000, and 8000 mg L^{-1}) for 15 seconds. The cuttings were then planted in 180 cm^3 tubes containing Basaplant® and Osmocote® controlled-release fertilizer, as described in the previous stage.

The experimental design was a completely randomized design with a 2x5 factorial scheme, consisting of pre-immersion or no pre-immersion in hydrogen peroxide and immersion in five IBA concentrations for 15 seconds, totaling ten treatments with four replications, each consisting of ten mini-cuttings per plot.

The mini-cuttings were placed in the rooting sector as described in the previous stage, but the misting time was reduced to minimize material deterioration observed in the previous stage. Survival of the mini-cuttings was assessed at 30 and 60 days after planting (DAP).

At 60 DAP, after washing the roots of the seedlings over a set of sieves, the following parameters were determined: rooting percentage (%ROO), number of roots (NR), and root length (RL). The aerial part and root system of the seedlings were then placed separately in labeled paper bags and dried in a forced-air oven at 65°C ± 2°C for 72 hours to determine the dry mass of roots (DRM) and the dry mass of the aerial part (DMAP) by weighing the material on an analytical balance.

The data were tested for adherence to the assumptions of analysis of variance (ANOVA). Data that did not meet the basic assumptions for ANOVA were transformed. Rooting percentage and number of roots were transformed using log (x); root length and dry mass of the aerial part were transformed using square root; and dry mass of roots was transformed using 1/x. The results were subjected to ANOVA, and differences were compared using Tukey's test ($P < 0.05$) or regression adjustments. The statistical software used was R (R Core Team, 2019).

RESULTS

FIRST STAGE - ROOTING OF MINI-CUTTINGS BASED ON THE POSITION OF THE CUTTINGS ON THE SHOOT

The survival of seedlings produced from basal mini-cuttings was higher compared to those from intermediate and apical cuttings at both 70 and 190 days after planting (DAP) (Figure 2). Among all mini-cuttings that survived to 190 DAP, 28% had two tuberous roots, while 72% had only one tuberous root.

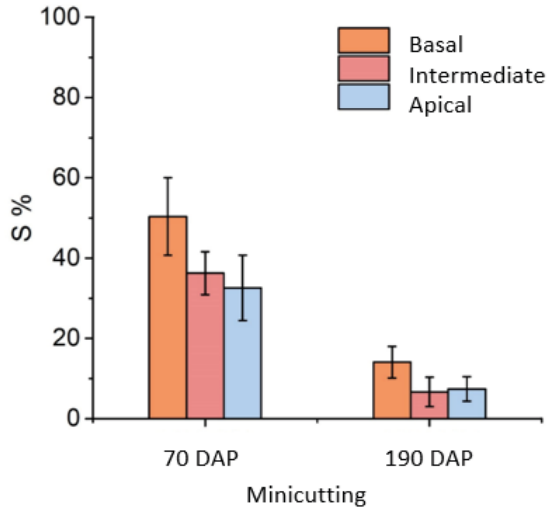

Figure 2. Average survival (S) of *A. cearensis* mini-cuttings at 70 and 190 days after planting (DAP), based on the position of the cuttings on the shoot.

The dry mass of roots (DMR) was the only variable that differed statistically in the experiment, being higher in apical mini-cuttings (Table 1). The other evaluated parameters (height, collar diameter, main root length, leaf area, and dry mass of the aerial part) did not vary based on the position of the mini-cuttings on the shoots.

SECOND STAGE - ROOTING OF MINI-CUTTINGS BASED ON THE APPLICATION OF IBA WITH OR WITHOUT H_2O_2

At 30 days after planting (DAP), there was no effect of the treatments with IBA and H_2O_2, either alone or in combination, on the survival of the mini-cuttings, which averaged 82.75% across the treatments (Table 2).

SOB 30 DAP (%)						
H_2O_2 (mM)	0	2000	4000	6000	8000	Mean
	----------mg L^{-1}----------					
0	82,50	95,00	67,50	70,00	82,50	79,50 ns
50	92,50	75,00	90,00	92,50	80,00	86,00 ns
Mean	87,50	85,00	78,75	81,25	81,25	82,75

Table 2. Survival at 30 days after planting (SOB30) of *A. cearensis* mini-cuttings based on different concentrations of IBA with and without the addition of H_2O_2.

At 60 days after planting (DAP), the presence of hydrogen peroxide increased the survival percentage of the mini-cuttings. However, even the lowest concentrations of IBA diminished the positive effect of H_2O_2, showing a negative linear correlation (Figure 3). The presence of H_2O_2 resulted in a 39.6% reduction between the 0 and 8000 mg L^{-1} IBA concentrations.

Without pre-immersion in H_2O_2, a cubic regression model provided a significant fit for the survival percentage of the mini-cuttings based on increasing IBA concentrations. It was observed that with 2000 mg L^{-1} of IBA, there was an increase in survival compared to the control, reaching values similar to those obtained with only hydrogen peroxide. However, higher concentrations of IBA resulted in a reduction of this percentage.

Position of the Mini-Cuttings	H (cm)	CD (mm)	MRL (cm)	LA (cm^2)	DMAP (g^{-1})	DMR (g^{-1})
Apical	7.32 a	3.66 a	15.74 a	78.92 a	0.79 a	2.23 a
Intermediate	7.43 a	3.94 a	15.04 a	75.35 a	0.78 a	0.98 b
Basal	5.34 a	3.97 a	12.21 a	39.29 a	0.51 a	0.64 b
CV(%)	29,66	15,40	25,00	54,54	48,34	47,21

Table 1. Mean values of height (H), collar diameter (CD), main root length (MRL), leaf area (LA), dry mass of the aerial part (DMAP), and dry mass of the root system (DMR) of *A. cearensis* mini-cuttings, 190 days after planting.

Means followed by the same letter within a column do not differ significantly from each other at the 5% probability level according to the test.

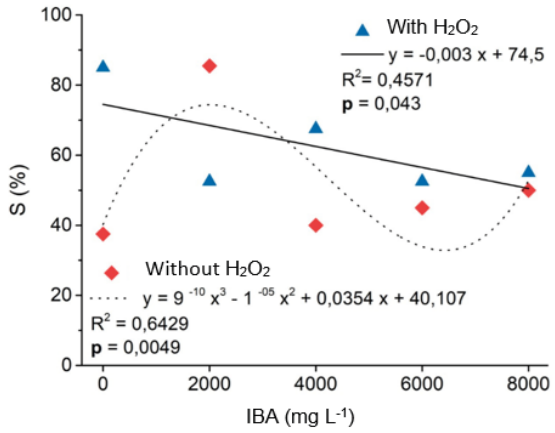

Figure 3. Survival (S) of *A. cearensis* mini-cuttings at 60 days after planting, treated with different concentrations of IBA in association with 50 mM of H_2O_2.

The variables ENR, NR, RL, and DMR did not vary based on the combination or absence of IBA (0, 2000, 4000, 6000, and 8000 mg L^{-1}) with 50 mM of H_2O_2 (Table 3).

ENR (%)						
H_2O_2 (mM)	0	2000	4000	6000	8000	Mean
	----------mg L^{-1}----------					
0	35,00	42,50	37,50	42,50	47,50	41,00 ns
50	45,00	45,00	57,50	52,50	47,50	49,50 ns
Mean	40,00	43,75	47,50	47,50	47,50	45,25
NR						
H_2O_2 (mM)	0	2000	4000	6000	8000	Mean
	----------mg L^{-1}----------					
0	1,52	1,63	1,48	1,62	1,67	1,58 ns
50	1,64	1,60	1,73	1,69	1,66	1,66 ns
Mean	1,58	1,62	1,61	1,66	1,67	1,62
RL						
H_2O_2 (mM)	0	2000	4000	6000	8000	Mean
	----------mg L^{-1}----------					
0	7,15	5,50	8,00	6,62	6,74	6,80 ns
50	8,40	6,15	6,54	6,31	6,59	6,80 ns
Mean	7,78	5,82	7,27	6,47	6,67	6,80
DMR (g)						
H_2O_2 (mM)	0	2000	4000	6000	8000	Mean
	----------mg L^{-1}----------					
0	0,03	0,02	0,03	0,02	0,03	0,03 ns
50	0,02	0,03	0,02	0,04	0,04	0,03 ns
Mean	0,02	0,02	0,02	0,03	0,03	0,03
DMAP (g)						
H_2O_2 (mM)	0	2000	4000	6000	8000	Mean
	----------mg L^{-1}----------					
0	1,13 ns	1,10 ns	1,14 ns	1,10 ns	1,12 ns	1,12 ns
50	1,12 ns	1,11 ns	1,10 ns	1,12 ns	1,11 ns	1,11 ns
Mean	1,13	1,11	1,12	1,11	1,12	1,12

Table 3. Rooting percentage (ENR), number of roots (NR), root length (RL), dry mass of roots (DMR), and dry mass of the aerial part (DMAP) of *A. cearensis* mini-cuttings based on different concentrations of IBA with and without the addition of H_2O_2, at 60 days after planting (DAP).

Figure 4B shows the rooting of mini-cuttings treated solely with 50 mM of H_2O_2, while Figure 4C displays rooting of mini-cuttings treated with 2000 mg L^{-1} of IBA without H_2O_2. These treatments exhibited a higher survival percentage compared to the control shown in Figure 4A.

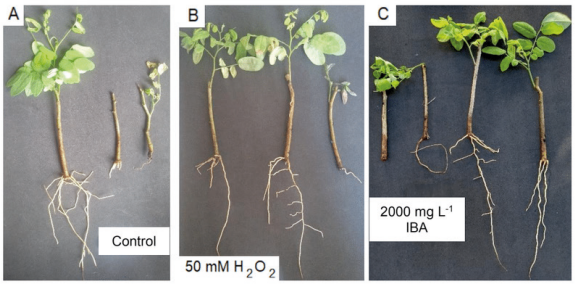

Figure 4. Rooting of *A. cearensis* mini-cuttings observed in the control treatment (A) and when treated with 50 mM of H_2O_2 (B) and 2000 mg L^{-1} of IBA (C).

DISCUSSION

FIRST STAGE - ROOTING OF MINI-CUTTINGS BASED ON THE POSITION OF THE CUTTINGS ON THE SHOOT

Located at the basal portion of the branches are larger in diameter, which may have favored the higher survival percentage. According to Pacheco and Franco (2008), thicker mini-cuttings have higher reserves of carbohydrates and photoassimilates available, which contributes to successful rooting, as the availability of energy and structural carbon are fundamental elements for cell multiplication (Rapaka et al., 2007). These two components are crucial for the biosynthesis of proteins and nucleic acids, which are also directly involved in rhizogenesis (Fachinello et al., 1995). Therefore, the greater availability of carbohydrate and photoassimilate reserves may have been decisive for the higher survival percentage of the basal mini-cuttings of *A. cearensis*, despite the low production rate.

Consequently, the lower survival rate of the apical mini-cuttings may have been caused by the low carbohydrate concentration. It is likely that the endogenous reserves do not meet the rooting process needs, or the mini-cuttings were unable to direct them in time for root emergence (Nicoloso et al., 1999). Santos et al. (2016), working with cuttings of *Lippia gracilis* Schauer, observed that for all evaluated variables, median and basal cuttings had higher values than apical ones. Still, it should be noted that the plants obtained from apical mini-cuttings showed a greater root dry mass, which is essential for rapid post-planting growth.

SECOND STAGE - ROOTING OF MINI-CUTTINGS BASED ON THE APPLICATION OF IBA WITH OR WITHOUT H_2O_2

The reduction in the survival of mini-cuttings in the presence of H_2O_2, when combined with increasing concentrations of IBA, suggests a possible phytotoxic effect of IBA on this species when associated with H_2O_2. H_2O_2 is constantly produced by cells, and antioxidant enzymes are responsible for its internal regulation. This balance is vital as it protects cells from oxidative damage and also influences some intrinsic cellular physiological processes (Smirnoff & Arnaud, 2019).

Auxins, due to their antioxidant properties, also protect cells from this type of damage (Li et al., 2019). To elucidate how antioxidant substances act in these cases, further cellular-level studies are important.

The positive results of treatments with 50 mM hydrogen peroxide alone align with studies that highlight the importance of peroxide for adventitious rooting (Li et al., 2009; Silva et al., 2022). When catalase and ascorbic acid, inhibitors of hydrogen peroxide synthesis, are present at high levels, adventitious rooting does not occur (Li et al., 2009). Some time after collecting the mini-cuttings, there is a significant increase in H_2O_2, which also occurs in the presence of auxin, suggesting an interaction between them (Li et al., 2009).

When the cut is made on the branch to form the mini-cutting, the stress caused induces an

increase in auxin concentration at the base of the cutting, derived from the aerial part via basipetal polar transport, which will induce the dedifferentiation of cells at this base, to initiate the root formation process. Hydrogen peroxide acts by preventing auxin levels from stabilizing, either by being degraded or having its transport impeded. By maintaining elevated auxin levels, H_2O_2 aids in promoting adventitious rooting (Steffens & Rasmussen, 2016). Thus, the presence of H_2O_2 increased the survival rate of mini-cuttings when no IBA concentration was applied.

The positive interaction between H_2O_2 and IBA occurs when hydrogen peroxide reduces auxin degradation, allowing it to concentrate at the base of the cuttings, thereby inducing the formation of the adventitious root system (Steffens & Rasmussen, 2016). The interaction occurs when auxin signaling is detected by peroxide and IBA stimulates peroxide biosynthesis (Li et al., 2009; Kang et al., 2018).

The survival percentage data based on IBA concentrations in this study are similar to those found by Valeri et al. (2012) regarding the rooting percentage of *Paubrasilia echinata* cuttings, although with lower concentrations than those used in this research. The cubic behavior of the results based on concentrations directs future studies to investigate intervals closer to the 2000 mg L^{-1} IBA concentration, aiming to find the most effective concentration for species propagation through mini-cuttings with this inducer.

The marked reduction in the survival of *A. cearensis* mini-cuttings between 30 and 60 days after planting in the mist chamber suggests the importance of studies to determine the necessary duration of intermittent misting for rooting induction.

CONCLUSION

It was concluded that apical mini-cuttings exhibited greater root dry mass compared to the others, although with a lower survival rate. There was an increase in the survival rate at 60 days after planting (DAE) for mini-cuttings whose bases were immersed in a 50 mM H_2O_2 solution, as well as for those immersed in a 2000 mg L^{-1} IBA solution for 15 seconds.

There was no effect of IBA and H_2O_2 on the rooting of the mini-cuttings.

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