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Effect of indol-3-butiric acid (IBA) on rooting of cuttings of *Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby

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Abstract

The propagation of *S. amazonicum* by seeds can result in uneven seedlings that are subject to poor quality, which is detrimental to uniformity and productivity. The absence of an efficient method of vegetative propagation of the species may be a limitation for genetic improvement, and consequently the increase in productivity of plantation. This study aimed to evaluate the effect of indole-3-butyric acid (IBA) in the rooting of cuttings of paricá - *Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby. The cuttings obtained from the stems of the seedlings then were treated with IBA in the hydro-alcoholic solutions at 1000, 2000, 3000 and 4000 ppm; commercial product with 2000 ppm IBA powder; commercial product composed of IBA and nutrients; and treatment control (0 ppm). The cuttings were planted in plastic trays with vermiculite and coconut fiber (1:1 v/v) and placed in a box lined with plastic to control moisture. The experimental design was completely randomized with five replications and 10 cuttings per plot. After 60 days, the percentage of rooted cuttings, living and dead, and number and length of roots were analyzed. The analysis of variance at 5% of probability indicated statistical differences for all variables. The best results were observed when we used IBA at 2000 and 3000 ppm, diluted in hydro-alcoholic solutions. These treatments showed statistically equal and produced the highest percentage of rooting, average number and length of roots and lowest percentage of dead cuttings. The polynomial regression analysis indicated that higher rooting rates can be obtained in 2550 ppm of IBA diluted in hydro-alcoholic solutions.

Keywords: auxin, adventitious roots, fabaceae, vegetative propagation.
Abbreviations: IBA_indole-3-butyric acid; PPM_ part per million; RC_Percentage of rooted cuttings; NMR_Average root number per stem; LLR_Length of the largest root; LC_Percentage of live cuttings; DC_Percentage of dead cuttings.

Introduction

Schizolobium parahyba var. *amazonicum* (Huber ex Ducke) Barneby, popularly known as paricá, is a forest species native to the Amazon, with high potential for timber production, which is widely cultivated by reforestation companies in the state of Pará (Rosa, 2006). The increase of plantations with this species is mainly due to accelerated growth, good adaptability to adverse soil conditions and the physicochemical properties of its wood, which allow multiple uses, especially for the manufacture of slides and compensated panel (Reis et al., 2007).

Although paricá gives satisfactory responses to sexual propagation, it only requires methods of dormancy breaking for the germination of its seeds. However, there are certain characteristics that can only be maintained through asexual reproduction. In addition, to meet an increasingly demanding market this plant will require technological and silvicultural advances that maximizes forest production with more desirable genetic material.

Xavier et al. (2003) emphasized that vegetative propagation is one of the main processes of seedling production, being the basis of clonal silviculture, mainly for its effectiveness in capturing the genetic gains obtained from breeding programs.

The clonal production of seedlings offers innumerable benefits, among them the conservation of genotypic characteristics observed in higher individuals, the possibility of dissemination of species with low fertility or seed shortage problems, and the attainment of genetic gains greater than in seed reproduction in one a shorter period of time, becoming an alternative for the production of seedlings in less time and throughout the year (Iritani and Soares, 1983).

Among the various vegetative propagation techniques, cuttings have been recognized as greatest economic viability for the establishment of clonal plantations (Paiva et al., 1996). However, for the rooting studies, several factors must be considered for success in the process. Among these factors, it is worth mentioning the use of growth regulators, which in many species can accelerate rooting and improve the quality of the root system formed. Higashi et al. (2000) emphasized that the use of growth regulators reduces the time of permanence of the cuttings for rooting, thus accelerating the time of formation of the plantlets, providing the appearance of better root system. Thus, seedlings will have a greater chance of survival and more vigorous and rapid development (REIS et al., 2000). According to Pires and Biasi (2003), the indol-3-butyric acid (IBA) is probably the best general-purpose plant regulator because it is non-toxic to most plants, even at high concentrations. The IBA is quite effective for a large number of species and relatively stable to the action of auxin degradation enzymes. Rosa and Pinheiro (2001) studied different concentrations of IBA on juvenile material of paricá and obtained significant results for the rooting of the cuttings. However, studies with the use of exogenous auxins for rooting are still necessary for the optimization of the cutting technique and consequently success in the production of vegetatively propagated seedlings. The aim of this study was to evaluate the effect of different concentrations of indole-3-butyric acid (IBA) on the rooting of cuttings produced from paricá seedlings (*Schizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby).

Results and discussion

Effect of IBA concentrations on rooting of cuttings

The analysis of variance found significant differences between the treatments (Table 2) for all the evaluated parameters, with the values of the F-test significant at 5% of probability. The coefficients of variation showed values ranging from low (8.89%) to high (22.61%) according to the classification of Pimentel-Gomes (1990), but this author considered relevant factors at the moment of interpretation of such measurement. Thus, there is a need to accumulate experimental data for these types of variables and for the species to infer the magnitude of this parameter. Regarding the treatments, it was possible to detect that the IBA in hydroalcoholic solution had a great inductive effect on rooting and root growth on paricá cuttings. The comparison of means of the treatments (Table 2) indicated that IBA solutions diluted in alcohol at 2000 and 3000 ppm were more efficient in promoting rooting and root growth, being statistically equal to each other and with the highest values for RC (Percentage of rooted cuttings), lower DC (Percentage of dead cuttings), higher values for NMR (Average root number per stem) and LRL (Length of the largest root) and with permanence of live cuttings with later rooting capacity. The treatments with IBA at 1000 and 4000 ppm diluted in alcohol presented statistical equal values for RC (%), DC (%) and NMR and were able to induce rooting of the cuttings. However, application of IBA at 2000 and 3000 ppm diluted in alcohol in a smaller proportion showed statistically higher values when compared to commercial products and the control. These results demonstrate the effect of IBA in promoting the rooting of paricá cuttings. Studies with different forest species have evidenced the positive contribution of growth regulators, especially IBA in rooting and root growth in cuttings, such as those reported by Sampaio et al. (2010) on juvenile mini-cuttings of *Aniba canelilla*; Bortolini et al. (2009) on *Tibouchina Aubl*; Gratieri-Sossella et al. (2008) on cuttings of *Erythrina crista-galli*; Endres et al. (2007) on cuttings of *Caesalpinia echinata*; Oliveira et al. (2015) on mini cuttings of *Handroanthus heptaphyllus*; in many researches with *Eucalyptus spp*. (Goulart et al., 2008; Almeida et al., 2007).

Influence of commercial products on rooting of cuttings

The commercial products we used had little influence on the induction of rooting of cuttings. The cuttings treated with the commercial product 1, which contains 2000 ppm of IBA in talc, induced rooting. However, it did not differ statistically from the solution of lower concentration of IBA (1000 ppm) in a percentage lower than the treatment with the alcoholic solutions of IBA. The commercial product 2, containing small amount of IBA and nutrients, did not differ statistically from the untreated growth regulator (0 ppm), presenting the lowest percentage of rooting. This indicates the need for IBA application, concentration and form of delivery. The IBA delivery form may have different results for each species. Bitencourt et al. (2010) verified that application of IBA in the form of solution and talc promoted the highest percentage of rooted cuttings in *Acacia mearnsii*. Ferreira et al. (2009), observed that the talc-treated treatments were not efficient in *Sapium glandulatum* semi-woody cuttings. Therefore, they did not recommend it for the increase of the root induction in this species. While Bortolini et al. (2008) recommended the application of 3000 ppm for the rooting of cuttings of *Tibouchina sellowiana* in both liquid and talc form. According to Endres et al. (2007), one of the justifications for the distinct behavior of the species is that the solution acts more effectively in the rooting, while the powder may present variations in the amount of auxin and be less soluble, which may result in the adhesion to the base of the stake and damages in the uniformity of rooting. However, this is a factor that may vary by species. The non-application of IBA and the use of commercial products presented the highest LC (%) and non-rooted DC (%). According to Nachtigal (1999), the non-formation of roots is considered one of the factors for the death of the cuttings, since this causes a depletion of the nutrient reserves contained in the cuttings, causing cuttings to death. For the variable mean number of roots, it was observed that the four solutions of IBA diluted in alcohol did not differ statistically from each other. However, they differed from the other treatments. The two commercial products did not differ from each other, and compared to control treatment (0 ppm).

Effect of IBA on the length of the largest root and mean number of roots

As for the length of the largest root, treatments with IBA at concentrations of 2000, 3000 and 4000 ppm diluted in alcohol provided the highest values for this variable without

Table 1. Treatments and formulations used for rooting of *Schizolobium parahyba* var. *amazonicum* cuttings.

Products and Solutions	Formulation*
IBA concentrated	Concentrated IBA with 99.9% of purity (Sigma) carried in alcoholic solution at 1000, 2000, 3000 e 4000 ppm
Comercial product 1 (CP1)	IBA 2000 ppm carried in talcum powder
Comercial product 2 (CP2)	Nutrient solution containing: Boron (B) – 0.5%; Iron (Fe) – 0.15%; Sulfur (S) – 0.5%; Chlorine (Cl) – 0.3%; Zinc (Zn) – 1.12%; Thiamine; IBA (without specification)

* As guarantees on the packaging of products (g/100mL).

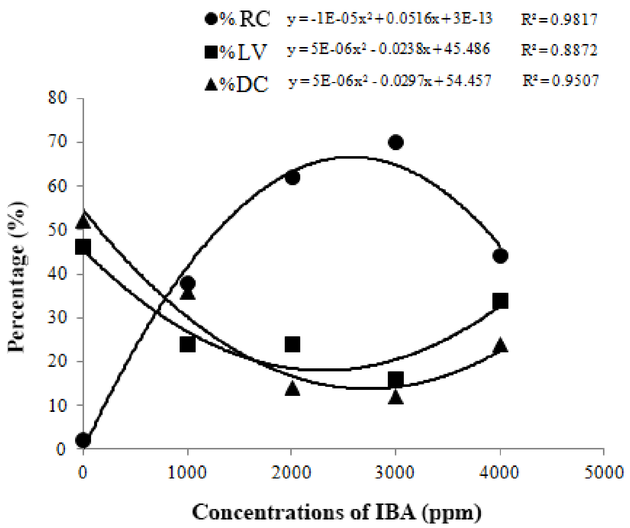

Fig 1. Rooting of paricá - S*chizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby cuttings - 45 days after treatment with IBA concentrations (0, 1000, 2000, 3000 and 4000 ppm). Percentage of rooted cuttings (RC); Percentage of live cuttings (LV) and Percentage of dead cuttings (DC).

Table 2. Comparison of means test, the overall mean, coefficient of variation and F-value calculated for the evaluated parameters percent rooted cuttings, percentage of live cuttings, percentage of dead cuttings, average number of roots and longer root length obtained paricá - S*chizolobium parahyba* var. *amazonicum* (Huber ex Ducke) Barneby treated with IBA at different concentrations, 60 days after the experiment implantation.

Treatments	Rooted cuttings (RC%)	Live cuttings (LC, %)	Dead cuttings (DC, %)	Mean Number of Root (MNR)	Length of the largest root (LLR)
3000 ppm	70.00 a	17.00 c	13.00 c	3.13 a	2.32 a
2000 ppm	62.00 a	24.00 bc	14.00 c	3.16 a	2.30 a
4000 ppm	44.00 b	33.00 b	23.00 c	3.25 a	2.21 a
1000 ppm	39.00 bc	24.50 bc	36.50 b	3.27 a	2.03 b
PC1[1]	26.00 c	34.00 ab	40.00 ab	1.99 b	1.87 bc
PC2[2]	7.00 d	41.50 a	51.50 a	1.87 b	1.76 c
0 ppm	3.50 d	45.00 a	51.50 a	1.57 c	1.81 c
Mean	35.93	31.29	32.79	2.61	2.04
CV%	22.61	19.67	18.83	10.86	8.89
F	23.22*	36.89*	14.10*	23.55*	6.13*

[1] CP1 – Comercial product 1 (IBA 2000 ppm in talcum powder); [2] CP2 – Comercial product 2 (nutrients and IBA solution). CVexp (Coefficient of experimental variation); D.M.S (Significant Mean Deviation): RC = 16,30%; LC =12,35%; DC= 12,85%; NMR= 0,26; LLR= 0,17. Means followed by the same letter in the line do not differ from each other by the Tukey test (p≤0.05); (*) – Significant at the 5% probability level by the F test.

statistical differences between them, differing from the IBA solution at 1000 ppm, which had the lowest value. In turn, this concentration was statistically equal to commercial product 1 (2000 ppm AIB in talc). The two commercial products did not differ with control treatment, which again were less efficient in rooting. The low root development in the paricá cuttings, in both the mean number and the length of the largest root, can be the result of the time that the rooted cuttings remained in the rooting environment (60 days), hampering the development of the cutting, since after the process of root formation, the cutting requires a more adequate nutritional and environmental management to complete its root and shoot development.

The IBA is a product with low toxicity to most plants and has good efficiency, allowing stimulation of rooting in cuttings of several species (Pires and Biasi, 2003). This experiment proved that various concentrations of IBA did not affect on mean number and length of the root. According to the results of all evaluated variables, it is possible to verify the importance of the IBA, not only to induce rooting of the cuttings, but also to improve the quality of the root system in relation to the number and length of the roots. Formation of root in cutting may lead to sustainability of the future plant.

Regression analysis applied to the percentage of rooted cuttings, viable and dead roots as a function of IBA concentration

The regression analysis applied to the percentage of rooted cuttings, viable and dead cuttings (without roots) as a function using different concentrations of IBA. It revealed a quadratic effect for the concentrations (Figure 1).

For the percentage of rooting, there was an increase in rooting, when the IBA concentration increased, but only the concentration increased up to 3000 ppm, from which a reduction in this percentage was occurred. The highest percentages of rooting were obtained between 2000 and 3000 ppm. The polynomial regression analysis also indicated that the estimated maximum rooting point was 65.02 (R^2 = 0.9817), reaching the estimated concentration of 2550 ppm of IBA. Oliveira et al. (2015), evaluated the rooting of mini-leaves of Ipê-Purple (*Handroanthus heptaphyllus*) as function of different concentrations of IBA. They observed that the concentration of 4444 mg L^{-1} promoted a higher percentage of rooting, estimated by the point of maximum technical efficiency.

There is a possibility of increasing the rooting rate, and consequently, greater success in the paricá cutting process, as a result of the 2000 and 3000 ppm concentrations, whereas the highest rooting showed the lowest mortality rates.

These results are similar to those obtained by Rosa and Pinheiro (2001), who reported an increase in the percentage of rooting of cuttings of *Schizolobium parahyba* var. *amazonicum* up to 4000 ppm, after which the reduction was observed. This result can be explained by the fact that the material was juvenile, where the endogenous hormonal balance favored rooting (Xavier et al., 2003), where negative responses to excess exogenous IBA applications may occur in some other species. These results are due to the endogenous concentration of auxin suitable for rooting in juvenile cuttings in the species (Hartmann et al., 2011).

Similar results were also observed in juvenile cuttings of some species. Rickli et al. (2015), Ferriani et al. (2011) and Ferreira et al. (2010), found high rooting percentages in the absence of the synthetic plant regulator for these species minicuts of *Vochysia bifalcata*, *Sapium glandulatum* and *Piptocarpha angustifolia*, respectively. The authors observed that as the concentration of IBA was increased, there was a slight decrease in the rooting rate, pointing this result to the high degree of youthfulness of the material, as the endogenous hormonal balance in these stakes favored rooting.

Materials and methods

Location of the experiment

The experiment was conducted at the Institute of Agricultural Sciences (ICA) of the Federal Rural University of Amazonia (UFRA) - Belém Campus, with a rooting box environment maintained in a greenhouse environment covered with semitransparent tiles. The box was made of wood and covered with transparent plastic on all sides to maintain moisture, aiming to provide conditions favorable to rooting of the cuttings. In the upper part of the box, in addition to the plastic cover, a 50% Sombrite® screen was placed so that the shading would decrease the temperature.

Plant materials

The plant material to obtain the cuttings came from approximately three-month-old paricá seedlings with 50 cm high, produced in polyethylene bags containing black soil as substrate and conducted in an area with 50% shading. From the branches of the seedlings, the cuttings were removed from the basal part, with a mean size of 12 cm in length and 4.0 mm in diameter with two to three buds per cutting.

Stake preparation

Cutting of the base and apex of the cuttings was done in the form of a bevel in order to increase the area of absorption of the growth regulators, as well as to avoid possible excess of water in the upper part of the stake. There was reduction of the leaf area for one or two pairs of leaflets to decrease the transparent surface. During the preparation, the cuttings were arranged provisionally in a vessel with water to minimize the loss of cellular turgor and tissue oxidation.

Treatments and experimental design

The implementation of the test to compare the effect of IBA followed the completely randomized experimental design with seven treatments, five replicates and 10 stakes per plot. The treatments were: alcoholic AIB solution at concentrations of (1000, 2000, 3000 and 4000 ppm); two commercial products inducing rooting (AIB at 2000 ppm conveyed in talc and nutrient solution having AIB as component) and a control treatment (0 ppm), in which there was no application of any product at the base of the cuttings. The products and solutions used in the experiment are listed in Table 1.

Preparation of solutions and application of treatments

Preparation of the alcoholic solution formulation with IBA was done as follows: weighing the IBA in a precision scale in an adequate quantity according to the desired concentration. Subsequently, dilution was carried out in 20 mL of 98° GL ethyl alcohol and addition of 20 mL of distilled water. For the preparation of the commercial product 2, 40 drops of the product were used in 40 ml of distilled water, as indicated on the product packaging.

Treatment with the solutions containing the growth regulators was performed by immersing the stake base into the solution for one (1) minute. The treatment with the commercial product 1, in the form of talc was done by direct contact of the base of the stake with the product. The control treatment (0 ppm) was composed of immersion of the base of the cuttings in solution of water and ethyl alcohol, without addition of IBA.

After treatment with the solutions, the cuttings were placed in plastic trays containing vermiculite and coconut fiber (1: 1 v / v) and placed in the rooting box. The plastic trays and the rooting box were previously washed and disinfested with sodium hypochlorite solution (0.5%).

Stake evaluation

Data collection was performed at 60 days, and later these data were used to obtain the following variables: (a) Percentage of rooted cuttings (RC): cuttings with at least one visible root. The total percentage was calculated by the relation of the rooted cuttings and the total number of rooted cuttings; (b) Average root number per stem (NMR): count of the number of roots that originated directly from the cuttings divided by the total number of cuttings of the plot; (c) Length of the largest root (LLR): measurement made with ruler millimeter considering the largest root formed by cutting (cm); (d) Percentage of live cuttings (LC): live cuttings that did not form calluses or roots. The total percentage was calculated by the relation of viable (live) stakes without callus and without root and the total number of cuttings put to rooting; and (e) Percentage of dead cuttings (DC): cuttings without roots which showed necrosis throughout or for the greater part of their length. The total percentage was calculated by the relation of the dead stakes and the total number of cuttings placed to rooting.

Statistical analysis

For the statistical analysis of the data, the parameters were transformed to guarantee the normality assumptions and the variance equalities required for the application of ANOVA. Percentage values were transformed by the formula arcsen $\sqrt{x/100}$. The mean number of roots and mean length of the largest root were transformed into $\sqrt{x+1}$, where x is the value of the obtained variable. After transformation, we performed analysis of variance (F-Test) and comparison of means by Tukey's test at a level of 5% of error probability. The original data were submitted to polynomial regression analysis. The analysis were carried out using the software Excel 2007 and the statistical program SSGA (System of Statistical and Genetic Analysis) (SAEG, 2007).

Conclusion

Indole-3-butyric acid (IBA) in solution form was efficient to promote development of roots in cuttings of paricá seedlings. Under the conditions of the experiment, the use of IBA diluted in alcohol at an estimated concentration of 2550 ppm provided the maximum rooting potential of the cuttings.

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