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ROOTING OF GUANANDI (*Calophyllum brasiliense* CAMBESS) CUTTINGS USING INDOLE-BUTYRIC ACID

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ABSTRACT: Commercial reforestation of Brazilian native species to produce hardwood for sawmills has been recently intensified in the country. Among the potential species planted by the logging industry is guanandi (*Calophyllum brasiliense* Cambess) because it is widely distributed in the country, highly adapted to different soil and climate conditions, good bole form and high quality timber. The development of genetic improvement programs should prioritize gains in productivity and yields in the medium and long term. For such programs to be successful, the study of vegetative propagation techniques to abbreviate steps in forest improvement and allow its mass production is fundamental. To assess the viability of vegetative propagation of the species, two successive experiments were carried out during two years testing the best type of cutting, hormone concentration and management. Different cuttings types submitted to increasing doses of indole-butyric acid (IBA) were tested to evaluate survival, sprouting, rooting and callus formation. Results indicate that the species is viable for vegetative propagation with 85 to 90% rooting of cuttings from seedlings in the IBA concentrations of 3000 to 7000 mg·L⁻¹. For the cuttings, sprouting from the base of adult trees 3000 mg·L⁻¹ was the best concentration of IBA.

ENRAIZAMENTO DE ESTACAS DE GUANANDI (*Calophyllum brasiliense* CAMBESS) UTILIZANDO O ÁCIDO INDOL-BUTÍRICO

RESUMO: O reflorestamento comercial de espécies nativas brasileiras, visando à produção de madeira nobre para serraria, tem se intensificado nos últimos anos em diversas regiões do Brasil. Dentre as espécies nativas potenciais que veem sendo plantadas por empresas do setor florestal, o guanandi (*Calophyllum brasiliense* Cambess.) é destaque, uma espécie com ampla distribuição natural, alta adaptabilidade a diferentes condições de solo e clima, boa forma fuste e ótima qualidade madeira. O desenvolvimento de um bom programa de melhoramento genético requer prioridade para a obtenção de ganhos de produtividade e rendimento no médio e longo prazo. Nesta linha de pesquisa, o estudo das técnicas de propagação vegetativa, visando abreviar etapas no melhoramento florestal e viabilizar a produção maciça de material melhorado, tem importância fundamental no sucesso do programa de melhoramento. Com objetivo de avaliar a viabilidade da propagação vegetativa da espécie, foram realizados dois experimentos sucessivos, instalados e avaliados ao longo de dois anos, a fim de identificar os melhores tipos de estacas, doses de hormônio e manejo. Foram testados diferentes tipos de estacas, submetidas a doses crescentes de ácido indolbutírico (AIB), avaliados principalmente os dados de sobrevivência, emissão de brotos e formação de raiz. Os resultados indicaram que a espécie se mostrou viável a propagação vegetativa, obtendo índices de 85% a 90% de enraizamento de estacas provenientes de mudas, para as doses de ácido indolbutírico (AIB) nas concentrações entre 3.000 a 7.000 mg·L⁻¹. Para as estacas de brotações da base de árvores adultas 3.000 mg·L⁻¹ foi a melhor dose de AIB.

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INTRODUCTION

In Brazil, forestry is an activity with economic and social importance due to the production of a wide variety of commercial products such as resins, essential oils, fruits and honey (CARPANEZZI, 2000), and mainly paper, cellulose, timber, hardboard, veneer, plywood, and particle board (TZFIRA et al., 1998; MORA & GARCIA, 2000). Such products play important roles in the life quality of people, directly and indirectly, by generating more than two million jobs (FERREIRA & GALVÃO, 2000).

According to the Brazilian Association of the Mechanically Processed Wood Industry – ABIMCI (2008), plantation forests covered 5.98 million ha in 2007, of which 62.7% were eucalyptus, 30.2% pine, and 7.1% other species such as acacia, rubber trees, paricá, and teak, among others. The development of the industry was focused on the production of exotic species, which corresponded to 97% of the area planted in the country in 2007 and only 3% with commercial plantation of native species.

Interest in the production of hardwood with high aggregated value has grown in the last decade in Brazil, especially for the plantation of teak (*Tectona grandis*), Australian cedar (*Toona ciliata*), and African mahogany (*Khaya* sp.) among the exotic species, and guanandi (*Calophyllum brasiliense* Cambess) and mahogany (*Swietenia macrophylla*) in the native species group.

Calophyllum brasiliense was used in the present study because it is a native species known worldwide for timber quality, widely distributed in the tropical region, and commercially planted in Latin America with good growth results in experimental plots (PIOTTO et al., 2003a; PIOTTO et al., 2003b). The vegetative propagation of this species is being studied and comproved by Santos et al (2010) and Kalil Filho et al (2010), presenting good rooting results.

The economic uses of vegetative propagation for seedling production in the forestry sector is justified when it has availability of high productive genotypes and, or, the seed is a limited resource (Xavier et al. 2003). For *Calophyllum brasiliense*, seeds are not a limitation for commercial seedling production, since many commercial plantations are producing seeds in quantity, regularly and with high quality. In this study, the focus was to develop the vegetative propagation technics for *Calophyllum brasiliense*, to produce clonal seedling of high productive genotypes.

MATERIAL AND METHODS

The study was part of the *Calophyllum brasiliense* Genetic Improvement Program of the Tropical Flora Reforestation Company in partnership with the Faculty of Agrarian Sciences (FCA/UNESP), Botucatu campus, Department of Plant Production and the graduate program in Forestry.

The study included two rooting trials between 2007 and 2009 in the greenhouse of the Plant Production Department, FCA/UNESP. The cuttings used in the study were produced in the nursery and in the commercial plantation sites of Tropical Flora Reforestation Company, in the city of Garça, SP.

Different concentrations of indole-butyric acid (IBA) were tested to identify the most adequate concentration for the species. Other parameters assessed were the provenance of the cuttings (from seedlings or from different parts of the tree top, crown or base), either lignified or young.

Materials used in the experiments, such as cartridges, trays and substrates were provided by the nursery from the Department of Natural Resources, FCA/UNESP. The same commercial substrate was used for all rooting trials, and it was composed by pine bark, vermiculite, acidity corrector, and mineral fertilizers with a cation exchange capacity of 200 mmol c.kg⁻¹.

First rooting trial

In the first rooting trial (Table 1) several possibilities were tested to find a direction for the following trials. Thus, different types of cuttings from trees were used to test rooting of mature material, cuttings 10 cm long were collected from three positions on the trees: base (0 to 50 cm height), crown (0.5 to 2.0 m height), top (> 3.0 m height), and divided in two groups, lignified and young. The trees that provided the material for the first trial came from a productive plot of Tropical Flora, in Garça/SP, with four years of age, of mixed provenances, with good nutrition conditions and free of pests or diseases. In all types of cuttings, five doses of IBA were tested, including a control: 0, 100, 1000, 5000 and 10000 mg.L⁻¹. Cuttings were planted in polyethylene cartridges of 45 mm diameter and 68-cm³ volume. The cuttings were removed from the plastic tubes to assess rooting and callus growth. The test included 30 treatments, in triplicate, with 4 cuttings per treatment, starting on September 19, 2007, and assessed on January 20, 2008.

Table 1 Description of treatments (1 to 30), portion of the tree from where the cuttings was collected (tree base, crown, top), types of cuttings (lignified or young) and the concentration of IBA used (0, 100, 1000, 5000 and 10000 mg·L⁻¹), with *Calophyllum brasiliense*.

Tabela 1 Descrição do tratamentos (1 a 30), proporção de árvores onde foram coletadas os brotos (base da árvore, copa, topo), tipo de brotos (lignificado ou jovem) e a concentração de AIB usado (0, 100, 5000 e 10000 mg·L⁻¹), em *Calophyllum brasiliense*.

1° Rooting trial / GUANANDI - Date : 19/09/2007							
Treatments	Portion	Type	Dose (AIB) ppm	Treatments	Portion	Type	Dose (AIB) ppm
1	Top	Lignified	0	16	Crown	Young	0
2	Top	Lignified	100	17	Crown	Young	100
3	Top	Lignified	1000	18	Crown	Young	1000
4	Top	Lignified	5000	19	Crown	Young	5000
5	Top	Lignified	10000	20	Crown	Young	10000
6	Top	Young	0	21	Tree base	Lignified	0
7	Top	Young	100	22	Tree base	Lignified	100
8	Top	Young	1000	23	Tree base	Lignified	1000
9	Top	Young	5000	24	Tree base	Lignified	5000
10	Top	Young	10000	25	Tree base	Lignified	10000
11	Crown	Lignified	0	26	Tree base	Young	0
12	Crown	Lignified	100	27	Tree base	Young	100
13	Crown	Lignified	1000	28	Tree base	Young	1000
14	Crown	Lignified	5000	29	Tree base	Young	5000
15	Crown	Lignified	10000	30	Tree base	Young	10000

Second rooting trial

In the second rooting trial (Table 2), was tested cuttings from the tree base and cuttings from seedlings was included. Two more doses of IBA were added, with a total seven doses, including control treatment. Six-months cuttings originated from the same seed batch from nursery was used (40 cm height, good health and ready for planting).

Statistics analyses

For the statistical analysis, data from the first trial were $(X+0.5)^{-2}$ transformed to fulfill the assumption of data normality and also to maintain coefficient of variation within limits to perform analysis of variance (ANOVA). No data transformation was required for the results from the second trial. Mean values of survival, rooting, sprouting and callus growth were compared with Tukey test at 5% probability (PIMENTEL-GOMES, 1978) using the statistic software Sisvar (FERREIRA, 2003) for the first and second rooting trials.

RESULTS AND DISCUSSION

First rooting trial

Survival, rooting, sprouting and callus growth were assessed 120 days after the implementation of the trial. Mean survival of the treatments was of 60.2%, and mortality was mainly from fungi attack on the cuttings, probably caused by

Table 2 Second rooting trial with *Calophyllum brasiliense*. Treatments (1 to 14), position of the cuttings (from the tree base or seedlings top), type of cutting (tree or seedlings) and doses of IBA (0, 100, 1000, 3000, 5000, 7000 and 10000 mg·L⁻¹).

Tabela 2 Segundo teste de enraizamento com *Calophyllum brasiliense*. Tratamentos (1 a 14), posição dos brotos (base árvore ou topa da muda), tipo de broto (árvore ou muda) e doses de AIB (0, 100, 1000, 3000, 5000, 7000 and 10000 mg·L⁻¹).

2 nd ROOTING TRIAL / GUANANDI - DATE : 01/03/2009			
Treatments	Portion	Type	Dose (IBA) ppm
1	Tree Base	Tree	0
2	Tree Base	Tree	100
3	Tree Base	Tree	1000
4	Tree Base	Tree	3000
5	Tree Base	Tree	5000
6	Tree Base	Tree	7000
7	Tree Base	Tree	10000
8	Seedling Top	Seedling	0
9	Seedling Top	Seedling	100
10	Seedling Top	Seedling	1000
11	Seedling Top	Seedling	3000
12	Seedling Top	Seedling	5000
13	Seedling Top	Seedling	7000
14	Seedling Top	Seedling	10000

the long period inside the greenhouse and/or by the humidity excess throughout the 120 days of experiment. No fungicide was applied during the experiment.

Table 3 presents the results for survival rate, rooting, sprouting, and callus growth of the first trial. The best results both for young and lignified cuttings were from the tree bases (treatments 21 to 30).

Results from the analysis of variance (ANOVA) and Tukey test at 5% probability are presented in Table 4. No significant differences for survival, sprouting and callus

growth were observed, whereas for rooting there was significant differences in treatments 18, 23, 25, 27, 28 and 29, all from cuttings from the tree base, except treatment 18. The best performance was with treatment 28, with 83.3% rooting and 100% survival (Table 4). The coefficient of variation for rooting, the main objective of the present study, was 18.17%, below the desirable 20% limit.

Comparing the three portions (top, crown, base) of the trees from where the cuttings were taken, those from the base of the trees (0 to 50 cm) resulted in

Table 3 Percentage of survival, rooting, sprouting and callus growth (treatments 1 to 30) with the five doses of indole-butyric acid (IBA) tested (0, 100, 1000, 5000 and 10000 mg/L) for the two types of cuttings (young and lignified) obtained from the top, crown and base of *Calophyllum brasiliense* and the coefficient of determination (R^2).

Tabela 3 Porcenta de sobrevivência, enraizamento, brotação e calo de crescimento (tratamentos de 1 a 30) com cinco doses de ácido indolbutílico (AIB) testado (0, 100, 1000, 5000, 10000 mg·L⁻¹) para os dois tipos de estacas (jovem e madura) obtida do topo, copa e base de *Calophyllum brasiliense* com coeficiente de determinação (R^2).

Treatment	Portion	Type	Dose (ppm)	Survival	Rooting	Sproutin	Callus Formation
1	Top	Lignified	0	41,7%	0,0%	0,0%	0,0%
2	Top	Lignified	100	16,7%	0,0%	8,3%	0,0%
3	Top	Lignified	1000	58,3%	0,0%	25,0%	41,7%
4	Top	Lignified	5000	50,0%	0,0%	16,7%	0,0%
5	Top	Lignified	10000	66,7%	8,3%	41,7%	25,0%
R^2	Top	Lignified	-	0,47	0,77	0,70	0,05
6	Top	Young	0	75,0%	0,0%	0,0%	16,7%
7	Top	Young	100	75,0%	0,0%	16,7%	0,0%
8	Top	Young	1000	66,7%	16,7%	33,3%	16,7%
9	Top	Young	5000	58,3%	0,0%	25,0%	25,0%
10	Top	Young	10000	66,7%	0,0%	25,0%	33,3%
R^2	Top	Young	-	0,32	0,08	0,16	0,71
11	Crown	Lignified	0	16,7%	0,0%	8,3%	0,0%
12	Crown	Lignified	100	25,0%	0,0%	0,0%	25,0%
13	Crown	Lignified	1000	25,0%	8,3%	16,7%	8,3%
14	Crown	Lignified	5000	50,0%	0,0%	25,0%	41,7%
15	Crown	Lignified	10000	25,0%	8,3%	16,7%	16,7%
R^2	Crown	Lignified	-	0,11	0,23	0,33	0,11
16	Crown	Young	0	83,3%	0,0%	0,0%	8,3%
17	Crown	Young	100	100,0%	0,0%	0,0%	25,0%
18	Crown	Young	1000	91,7%	25,0%	8,3%	25,0%
19	Crown	Young	5000	91,7%	8,3%	8,3%	25,0%
20	Crown	Young	10000	91,7%	8,3%	8,3%	8,3%
R^2	Crown	Young	-	0,00	0,01	0,45	0,14
21	Tree Base	Lignified	0	8,3%	0,0%	8,3%	0,0%
22	Tree Base	Lignified	100	33,3%	8,3%	33,3%	16,7%
23	Tree Base	Lignified	1000	58,3%	25,0%	41,7%	16,7%
24	Tree Base	Lignified	5000	33,3%	16,7%	25,0%	16,7%
25	Tree Base	Lignified	10000	91,7%	66,7%	83,3%	16,7%
R^2	Tree Base	Lignified	-	0,60	0,80	0,64	0,17
26	Tree Base	Young	0	91,7%	8,3%	8,3%	0,0%
27	Tree Base	Young	100	91,7%	33,3%	33,3%	16,7%
28	Tree Base	Young	1000	100,0%	83,3%	66,7%	0,0%
29	Tree Base	Young	5000	58,3%	33,3%	25,0%	0,0%
30	Tree Base	Young	10000	66,7%	8,3%	0,0%	16,7%
R^2	Tree Base	Young	-	0,63	0,14	0,27	0,15

Table 4 Analysis of variance (ANOVA) of the parameters assessed (survival, rooting, sprouting and callus growth) in cuttings of *Calophyllum brasiliense* Cambess.

Tabela 4 Análise de variância (ANOVA) dos parâmetros avaliados (sobrevivência) enraizamento, brotação e crescimento de calo) em brotos *Calophyllum brasiliense* Cambess.

Variation Source	Survival	Rooting	Sprouting	Callus Formation
	Value f (significance)			
Treatment	0,0000	0,0000	0,0015	0,5069
Repetition	0,1219	0,3741	0,1219	0,0471
CV	19,23	18,17	31,43	27,25
1	1,33 a	1,00 a	1,00 a	1,00 a
2	1,00 a	1,00 a	1,00 a	2,00 a
3	2,00 a	1,00 a	1,00 a	1,67 a
4	1,67 a	1,00 a	1,00 a	1,00 a
5	1,67 a	1,00 a	1,67 a	1,00 a
6	2,00 a	1,00 a	1,00 a	1,33 a
7	2,00 a	1,00 a	1,00 a	1,00 a
8	2,00 a	1,00 a	1,67 a	1,33 a
9	1,67 a	1,00 a	1,33 a	1,33 a
10	2,00 a	1,00 a	1,67 a	1,33 a
11	1,00 a	1,00 a	1,00 a	1,00 a
12	1,33 a	1,00 a	1,00 a	1,33 a
13	1,00 a	1,00 a	1,00 a	1,00 a
14	1,67 a	1,00 a	1,33 a	1,67 a
15	1,33 a	1,00 a	1,00 a	1,33 a
16	2,00 a	1,00 a	1,00 a	1,00 a
17	2,00 a	1,00 a	1,00 a	1,33 a
18	2,00 a	1,33 ab	1,00 a	1,33 a
19	2,00 a	1,00 a	1,00 a	1,33 a
20	2,00 a	1,00 a	1,00 a	1,00 a
21	1,00 a	1,00 a	1,00 a	1,00 a
22	1,33 a	1,00 a	1,33 a	1,33 a
23	1,67 a	1,33 ab	1,33 a	1,33 a
24	1,33 a	1,00 a	1,33 a	1,00 a
25	2,00 a	2,00 b	2,00 a	1,00 a
26	2,00 a	1,00 a	1,00 a	1,00 a
27	2,00 a	1,33ab	1,33 a	1,00 a
28	2,00 a	2,00 b	2,00 a	1,00 a
29	2,00 a	1,33 ab	1,33 a	1,00 a
30	2,00a	1,00 a	1,00 a	1,00 a

*CV: Coefficient of variation; Tukey test at 5% probability

higher survival, rooting and sprouting. Only callus growth rate was lower for young or lignified cuttings.

When treatments are analyzed separately,

lignified cuttings, presents the best results in the highest concentration of IBA tested (10000 mg·L⁻¹). Best results for young cuttings were in the intermediate concentrations of IBA (1000 and 5000 mg·L⁻¹), as shown in Table 3.

The treatments with the best performances were for cuttings from the base of the trees, both young and lignified, in the IBA concentrations of 1000 and 10000 mg·L⁻¹, respectively.

Lignified cuttings achieved higher capacity of sprouting, lignified cuttings presented best rooting results with higher IBA doses. Xavier et al. (2009) related gains from growing regulators applications in materials with more difficult rooting, or for genetic questions, or in function of the cuttings maturity. It could explain the best rooting for lignified cuttings observed in the higher IBA doses.

Davis et al. (1986) relates that young sprouts generally present lower lignification, which gets higher from the top to the base of the branches, where the tissue presents a higher grade of differentiation, with lower capacity to recover the meristem conditions, fundamental to start rooting, due to it the young cutting obtained a higher level of rooting. The low rate of rooting observed in the mature material from the planted trees tested confirm this affirmation, and when compared with 85% of average rooting achieved with cuttings of *Calophyllum brasiliense* of young material achieved by Silva et al (2010).

Second rooting trial

The second trial aimed at verifying the best performance of cuttings from the tree base, which presented the best results in the first trial. This trial also tested cuttings from seedlings, a younger material with higher probability of rooting, and reduced the interval between the IBA concentrations (inclusion of 3.000 and 7.000 mg·L⁻¹). Trial evaluation was carried out after 120 days of experiment, removing the cuttings from the tubes.

Cuttings from seedlings presented better results in comparison to cuttings from trees, which demonstrate higher survival and rooting of the young material (Table 5). Roots were not scanned to evaluate the quantity and quality, but visually cuttings from seedlings presented larger volume of roots, as well as faster rooting.

As shown in Table 5, the introduction of two more test-concentrations of IBA (3000 and 7000 mg·L⁻¹) demonstrated the importance of reducing the

Table 5 Percentages of survival, rooting, sprouting and callus formation according to cutting provenance and concentrations of indole-butyric acid (IBA) concentrations (0, 100, 1000, 3000, 5000, 7000 and 10000 mg·L⁻¹) for *Calophyllum brasiliense*.**Tabela 5** Porcentagem de sobrevivência, enraizamento, brotação e formação de calo, de acordo com a origem da estação e concentração de (AIB) (0, 100, 1000, 3000, 5000, 7000 and 10000 mg·L⁻¹) para *Calophyllum brasiliense*.

Treatment	Stump Provenance	Dose IBA (ppm)	Survival	Rooting	Sprouting	Callus Formation
1	Tree Base	0	75,0%	35,0%	5,0%	0,0%
2	Tree Base	100	75,0%	20,0%	30,0%	10,0%
3	Tree Base	1000	60,0%	15,0%	15,0%	5,0%
4	Tree Base	3000	75,0%	75,0%	0,0%	5,0%
5	Tree Base	5000	55,0%	20,0%	10,0%	5,0%
6	Tree Base	7000	65,0%	35,0%	5,0%	10,0%
7	Tree Base	10000	75,0%	25,0%	30,0%	5,0%
R ²	Tree Base	-	0,01	0,00	0,03	0,05
8	Seedling Top	0	85,0%	50,0%	20,0%	5,0%
9	Seedling Top	100	100,0%	20,0%	45,0%	10,0%
10	Seedling Top	1000	85,0%	15,0%	30,0%	10,0%
11	Seedling Top	3000	90,0%	90,0%	0,0%	15,0%
12	Seedling Top	5000	95,0%	85,0%	5,0%	10,0%
13	Seedling Top	7000	95,0%	90,0%	0,0%	0,0%
14	Seedling Top	10000	90,0%	70,0%	15,0%	20,0%
R ²	Seedling Top	-	0,02	0,42	0,33	0,09

interval between the concentrations tested in the first trial, as the IBA 3000 mg·L⁻¹ presented the best results for the two types of cuttings.

Survival and sprouting did not show significant differences between treatments (ANOVA; $p > 0.05$), whereas rooting and callus formation presented significant differences ($p < 0.05$, Table 6).

High incidence of fungi diseases in the cuttings from the trees was noted, which caused lower survival, when compared to cuttings from seedlings. No fungicide was used throughout the experiment.

The best results for cuttings from trees and from seedlings were with treatments 4 and 11, respectively. Control plants also presented good survival and rooting

Table 6 Analysis of variance (ANOVA) of the parameters assessed (survival, rooting, sprouting and callus growth) in cuttings of *Calophyllum brasiliense*.**Tabela 6** Análise de variância (ANOVA) dos parâmetros avaliados (sobrevivência, enraizamento, brotação e crescimento de calo em brotos de *Calophyllum brasiliense*.

Variation Source	Survival	Rooting	Callus Formation	Sprouting
	Value de f (significance)			
Treatment	0,0408	0,0000	0,0000	0,7043
Repetition	0,5712	0,9276	0,8048	0,9807
CV	23,90	37,38	80,29	160,10
1	3,75 a	1,75 bc	0,25 b	0,00 a
2	3,75 a	1,00 c	1,50 ab	0,50 a
3	3,25 a	0,75 c	0,75 ab	0,25 a
4	3,75 a	3,75 ab	0,00 b	0,25 a
5	2,75 a	1,00 c	0,50 b	0,25 a
6	3,75 a	1,75 bc	0,25 b	0,50 a
7	3,75 a	1,25 c	1,50 ab	0,25 a
8	4,25 a	2,50 abc	1,00 ab	0,25 a
9	5,00 a	1,00 c	2,25 a	0,50 a
10	4,25 a	0,75 c	1,50 ab	0,50 a
11	4,50 a	4,50 a	0,00 b	0,75 a
12	4,75 a	4,25 a	0,25 b	0,50 a
13	4,75 a	4,50 a	0,00 b	0,00 a
14	4,50 a	3,50 b	0,75 b	0,75 a

*CV: Coefficient of variation; Tukey test at 5% de probability

rates, which revealed that the species has potential for natural vegetative propagation, and potential to rooting without use of IBA, as observed by Silva et al (2010). Santos (2002) affirms that there is a maximum level for IBA application that is prejudicial, as can be observed in the rooting results for the 10.000 mg·L⁻¹ (14 treatment) doses of IBA when rooting starts decreasing.

CONCLUSIONS

Calophyllum brasiliense is a feasible species for vegetative propagation through cutting technique and may be widely used in the mass production of improved material. Young cuttings from the base of the trees presented best results for mature material, and the best concentration of indole-butyric acid (IBA) was 3000 mg·L⁻¹, with 75% rooting. Rooting of young material presented the best results, demonstrating good potential for the establishment of clonal plantations.

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