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# Efficiency of indolebutyric acid and different substrates in yerba mate cuttings

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**ABSTRACT** - *Ilex paraguariensis* A.St.-Hil. is an arboreal species whose leaves are used in the preparation of mate and in the cosmetics and food industries. Due to difficulty found in the sexual propagation of the species, this paper aimed to test efficiency of indolebutyric acid (IBA) in saplings production from semi-woody cuttings. On the first experiment, cuttings were taken from young plants, immersed in different concentrations of IBA and established in substrate. On the second experiment, cuttings were established in four different substrates previously submersed in IBA. A concentration of 3.0 mg $L^{-1}$ of IBA and a substrate composed of biostabilized pine bark, limestone and vermiculite + carbonized rice hull, have shown a greater efficiency in rooting of young *I. paraguariensis* saplings, and could be used to facilitate yerba mate propagation.

**Keywords:** phytoregulators, rooting, vegetative propagation

**RESUMO** - **Eficiência do ácido indolbutírico e de diferentes substratos na estaquia de erva-mate**. *Ilex paraguariensis* A.St.-Hil. é uma espécie arbórea cujas folhas são empregadas no preparo do mate e na indústria de cosméticos e alimentos. Devido à dificuldade encontrada para a propagação sexuada da espécie, o presente trabalho teve como objetivo testar a eficiência do ácido indolbutírico (AIB) na produção de mudas a partir de estacas semilenhosas. No primeiro experimento, as estacas foram obtidas de plantas jovens, imersas em diferentes concentrações de AIB e estabelecidas em substrato. No segundo experimento, estacas foram estabelecidas em quatro diferentes substratos e com imersão prévia em diferentes concentrações de AIB. A concentração de 3.0 mg $L^{-1}$ de AIB e o substrato composto por casca de pinus bioestabilizada, calcário e vermiculita + casca de arroz carbonizada, mostraram ser mais eficientes para o enraizamento de estacas jovens de *I. paraguariensis*, e poderia ser utilizada para facilitar a propagação de erva-mate.

**Palavras-chave:** enraizamento, fitorreguladores, propagação vegetativa

## INTRODUCTION

*Ilex paraguariensis* A.St.-Hil. (Aquifoliaceae), commonly known as yerba mate, is native to temperate weather regions in Brazil, Paraguay, and Argentina (Oliveira & Rotta 1985, Murakami *et al*. 2013). According to Passionato (2003), the exploitation of this species is important economically, socially and environmentally important throughout the South Brazil. It constitutes an agricultural activity which generates jobs and income in rural settings and prevents soil erosion (Bendlin 2003). Backes & Irgang (2002) have emphasized its environmental importance due to the fruit consumption by avifauna, constituting a potential seeds disperser. Additionally, it is an ornamental tree that may be used in landscaping (Lorenzi 2002). Among its numerous applications, its use in the preparation of beverages such as chimarrão, tererê, and mate is predominant (Coelho *et al*. 2002, Vidor *et al*. 2002, Resende *et al*. 2012).

Currently, the species is gaining attention from the scientific community due to its beneficial effects on human health, especially in terms of its antioxidant and protective effects against degenerative processes, such as those that lead to the development of heart disease and to DNA damage (Machado *et al*. 2007). As stated by Bragagnolo *et al*. (1980), yerba mate is a stimulant and presents diuretic, stomachic and sudorific actions. Besides that, it may be utilized in the manufacturing of dyes, food preservatives, toiletries and cosmetics (Maccari Júnior & Mazuchowski 2000).

The greatest part of its production originates in native yerba mate farms or in plants cultivated from seeds, resulting in high heterogeneity in cultivation. Such situation brings great difficulty to the establishment of culture management standards and product processing (Wendlind 2004). Furthermore, due to embryo immaturity, the rate of seed germination is low and uneven (Carvalho 1994, Fowler & Sturion 2000). There is also an unsatisfactory genetic seeds variability (Sturion 1988), dormancy and longer period of time for stratification (Menna 1995, Prat Krikun 1995), resulting in lengthy periods for the saplings production (Grigoletti Júnior *et al*. 1999).

Faced with the problems involved in sapling production from seeds, vegetative propagation offers an excellent alternative not only for obtaining saplings, but also for improvement and preservation, being important for the recovery of adult genotypes selected in the field (Brondani *et al*. 2009). This technique, besides being simple and low-cost, guarantees uniformity in plants generated and a greater number of saplings per matrix (Higa 1983). However, Wilkins *et al*. (2005) affirm that cuttings in forest species is difficult, and in some cases, impracticable, due to the fact that rooting quickly decreases as the matrix plant ages.

Based on what has been stated, this paper aims to test the efficiency of indolebutyric acid (IBA) hormone and to verify the best substrate for the production of yerba mate (*Ilex paraguariensis*) saplings from semi-woody cuttings.

## MATERIAL AND METHODS

### Experiment location and period

The experiments were conducted in Universidade do Vale do Taquari greenhouses (Lajeado, Rio Grande do Sul, Brazil), with a temperature and humidity of approximately 27°C and 60%, respectively, controlled by an automatic ventilation and nebulization system. The duration of the experiments was 180 days. Both experiments were carried out with the use of semi-woody cuttings obtained from young plants. The first experiment was initiated in April 2011, and the second in April 2012.

### Vegetative material collection

*I. paraguariensis* matrix plants of approximately two years of age, cultivated in greenhouses, were utilized. The cuttings were prepared with an average length of seven centimeters (first experiment) and 12-15 cm (second experiment), with leaves reduced to half and a bevel-cut apex to prevent water accumulation and, consequently, cuttings deterioration.

### Cutting bioessays

Experiment 1: Four treatments were established, constituted in 30-second immersions of the cuttings into a solution containing IBA in the following concentrations: zero (T1 - control treatment); 1.0 mg $L^{-1}$ (T2); 3.0 mg $L^{-1}$ (T3) and 5.0 mg $L^{-1}$ (T4). Each treatment was composed of three repetitions containing ten cuttings each. After the immersion in the IBA solution, the cuttings were established in plastic packs containing a biostabilized pine bark, limestone and vermiculite substrate (1:1:1 – substrate S1), with an electric conductivity (mS/cm) of 0.37 ± 0.3; density 650 Kg/m³; water retention capacity 45%; and pH 7.5 ± 0.5.

Experiment 2: Substrates S1; S2 (a combination of S1 with carbonized rice hull in a proportion of 1:1); S3 (a combination of S1 with vermiculite in a proportion of 1:1), and S4 (composed of soil coming out of a yerba mate farm in the township of Ilópolis, Rio Grande do Sul, Brazil) were tested. For each substrate, two concentrations of IBA were tested (zero and 3.0 mg $L^{-1}$), totalizing eight treatments (T1= S1 not immersed in IBA; T2= S1 with an immersion of the cuttings into 3.0 mg $L^{-1}$ of IBA; T3= S2 not immersed in IBA; T4= S2 with an immersion into 3.0 mg $L^{-1}$ of IBA; T5=S3 not immersed in IBA; T6= S3 with an immersion into 3.0 mg $L^{-1}$ of IBA; T7= S4 not immersed in IBA; T8= S4 with an immersion into 3.0 mg $L^{-1}$ of IBA). Each treatment was composed of six repetitions containing ten cuttings each. The bases of the cuttings were immersed for 30 seconds into the IBA solutions and then established in the substrates in plastic packs.

### Statistical analysis

Experiment 1: Saplings were evaluated after 180 days, at which the root number, longest root length, shoot height and leaf number (over 1 cm long) were considered. The data obtained were analyzed by One-Way ANOVA, followed by Tukey Test ($p \leq 0,05$).

Experiment 2: The monitoring of surviving cuttings was performed every 30 days and went on for up to 180 days, at which moment the following variables were taken into consideration: bud and leaf number, root and shoot fresh and dry weights. The data from the latter assessment (180 days) were analyzed by One-Way ANOVA, followed by Tukey Test ($p \leq 0.05$).

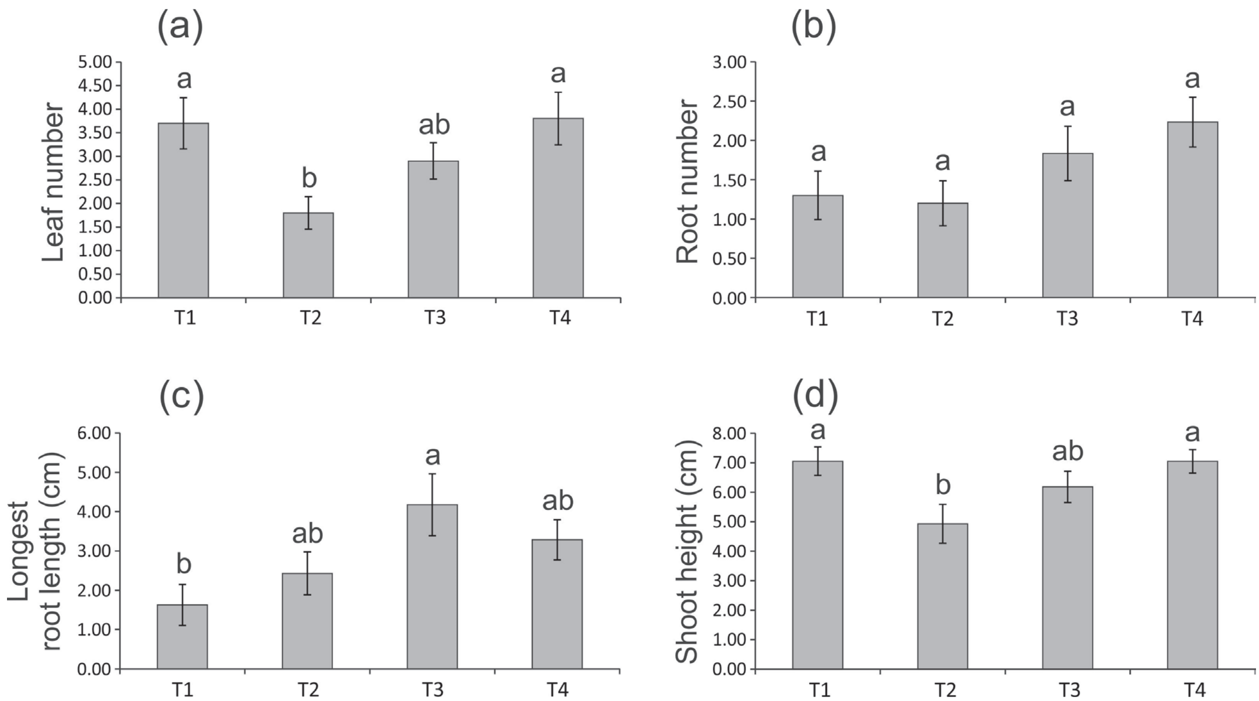
For both experiments, the Levene's test (for homogeneity of variance) was used prior to ANOVA.

## RESULTS

### Experiment 1

At 180 days, the 5.0 mg $L^{-1}$ IBA treatment resulted in 93% of saplings formed from cuttings, followed by the control treatment (90%). There was a smaller percentage (67%) of saplings when cuttings were immersed in the 1.0 mg $L^{-1}$ IBA solution (data not shown). Considering the total amount of saplings obtained, it is assumed that the substrate, along with greenhouse humidity and temperature, were adequate for yerba mate cutting.

Along with the percentage of saplings, the leaf number over 1 cm long (LN), the root number (RN), the longest root length (LRL) and the shoot height (SH) were also assessed at the end of the experiment 1 (180 days). For LN and SH analyses, T2 treatment (immersed in 1.0 mg $L^{-1}$ of IBA) presented the smallest values (Fig. 1a and 1d). In the LRL analysis, T1 treatment (not immersed in IBA) presented the smallest values (Fig. 1c). It is likely that the IBA application may have influenced the rooting of yerba mate cuttings because the length of the roots increased in consonance with IBA concentration, showing that in T3 treatment (immersed in 3.0 mg $L^{-1}$) the root length averages were superior, however, with insignificant differences when compared to T2 and T4 treatments. In the 5.0 mg $L^{-1}$ (T4) concentration, the size of the roots presented a slight decrease. Although the difference was not significant, it is possible that, in higher concentrations, the hormone may have had a negative effect.




**Figs. 1a-d. a.** Leaf number; **b.** root number; **c.** longest root length; **d.** shoot height of *Ilex paraguariensis* cuttings submitted to different concentrations of IBA (indolebutyric acid). T1: control treatment (no IBA added); T2: 1.0 mg $L^{-1}$ IBA; T3: 3.0 mg $L^{-1}$ IBA; T4: 5.0 mg $L^{-1}$ IBA. Values are the average of 30 samples ± standard error. Mean values with different letters are different by the Tukey test ($p \leq 0.05$).

**Experiment 2**

At 30 days, buds were found in the cuttings of every treatment. At 60 days the amount of live cuttings decreased drastically in all treatments, wherein for T7 (soil from a yerba mate farm in Ilópolis, Rio Grande do Sul, Brazil, not immersed in IBA) and T8 (soil from a yerba mate farm in Ilópolis, Rio Grande do Sul, Brazil, immersed in 3.0 mg $L^{-1}$ of IBA), less than 10% of the cuttings survived. In the analysis of day 90 it was found that in T6 (combination of S1 with vermiculite in a 1:1 proportion, immersed in 3.0 mg $L^{-1}$ of IBA), T7 and T8 treatments, none of the cuttings had survived. The same happened in T5 (combination of S1 with vermiculite in a 1:1 proportion, not immersed in IBA) on day 120, with remaining live cuttings only left in the other treatments (T1, T2, T3 and T4) at 150 days (Fig. 2). Even though not statistically significant, throughout the experiment a progressive decrease in live cuttings was observed, which also occurred in the research undertaken by Wilkins *et al*. (2005), evidencing the difficulty in obtaining saplings from forest species.

In relation to the saplings obtained, the assessment made at 180 days showed that there was no significant difference as to the amount of buds formed in cuttings which received the four surviving treatments (Fig. 3a). It was the T4 treatment (combination of S1 with carbonized rice hull in a 1:1 proportion, immersed in 3.0 mg $L^{-1}$ of IBA) which generated the higher number of leaves (Fig. 3b). In relation to fresh and dry weights of buddings, treatments T3 (combination of S1 with carbonized rice hull in a 1:1 proportion, not immersed in IBA) and T4 presented the greatest values (Fig. 3c and 3e), what was repeated in the analyses of root fresh and dry weights (Fig. 3d and 3f). The positive results in T3 and T4 indicate that the S2 substrate (combination of a substrate composed of biostabilized pine bark, limestone and vermiculite with carbonized rice hull in a proportion of 1:1) had a greater influence in the development of saplings because, regardless of the cuttings having been immersed in IBA hormone or not, the fresh and dry weights of the shoots and roots were superior in both treatments with the same substrate.

## DISCUSSION

The smaller amount of cuttings rooted in T1 (biostabilized pine bark, limestone and vermiculite, not immersed in IBA) in experiment 1 may be an indication that *I. paraguariensis* does not have endogenous auxin in sufficient amount for the promotion of the rooting process, even when concerning younger plant matrixes, and that the IBA treatment is necessary. In accordance with Iritani (1981), cuttings from stems between one and two years of age treated with IBA present up to 75% of rooting, a similar result to that obtained in this research. Iritani & Soares (1981) suggest that the most efficient hormone in the induction of yerba mate roots is indolebutyric acid (IBA) due to its low mobility and chemical stability, and due to its rooting time reduction effect. Graça *et al*. (1988) have also reported the importance of this phytoregulator by stating that there had been no rooting in yerba mate cuttings which had not been treated with IBA. Moreover, Zerbielli & Nienow (2008)

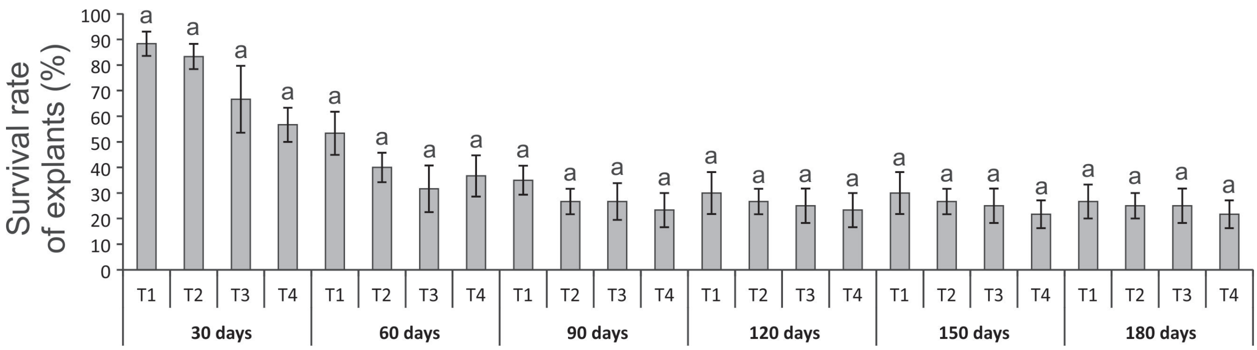
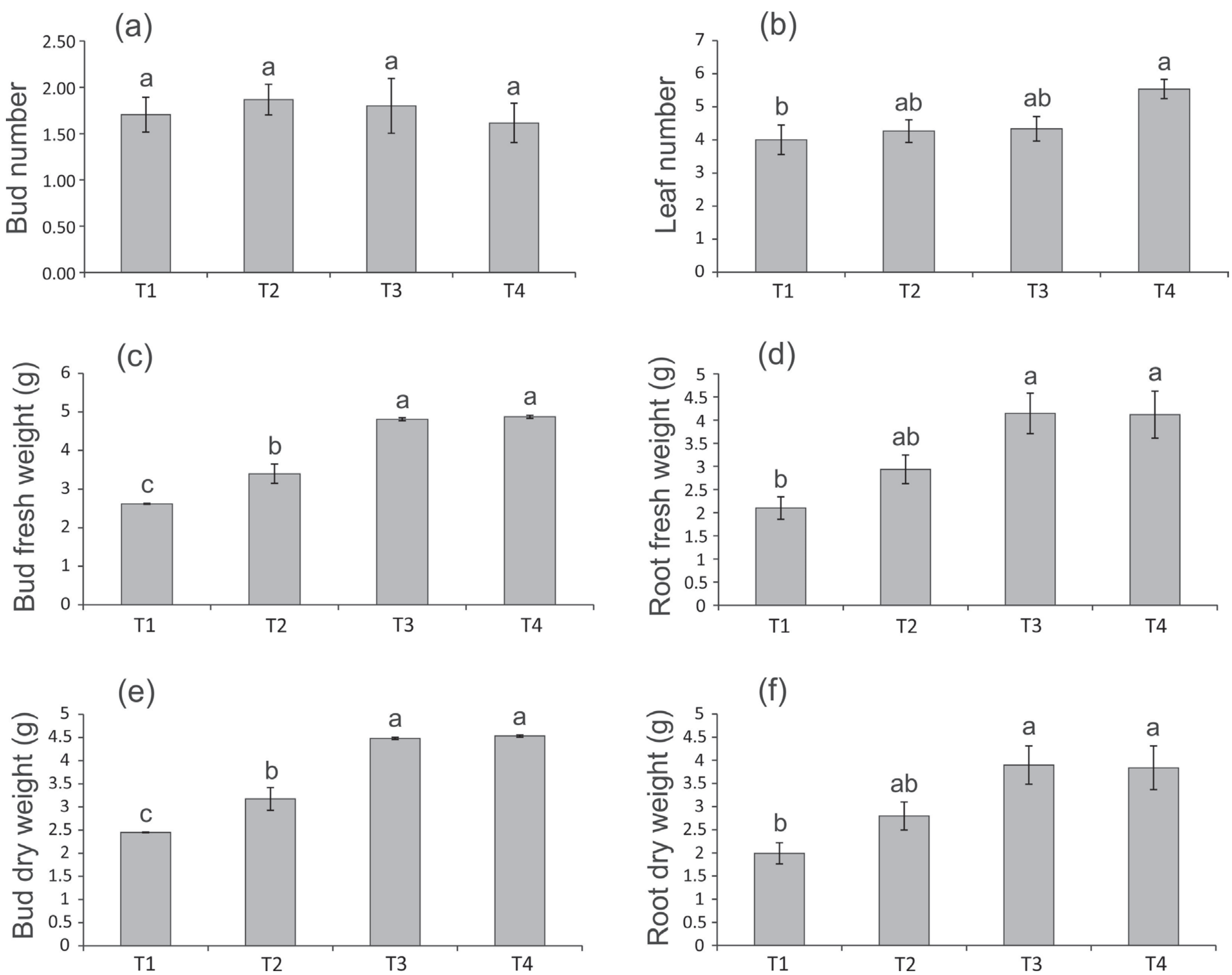



**Fig. 2.** Periodical analysis (up to 180 days) of survival rate (%) of *Ilex paraguariensis* cuttings, considering only the treatments with survivor explants (T1: S1 without IBA; T2: S1 + 3.0 mg $L^{-1}$ IBA; T3: S2 without IBA; T4: S2 + 3.0 mg $L^{-1}$ IBA). S1: substrate composed of biostabilized pine bark, limestone and vermiculite; S2: S1 + carbonized rice hulls (1:1). All the treatments initiated with sixty cuttings. Mean values with different letters are different by the Tukey test ($p \leq 0.05$).




**Figs. 3a-f. a.** Bud number; **b.** leaf number; **c.** bud fresh weight; **d**. root fresh weight; **e.** bud dry weight; **f.** root dry weight of *Ilex paraguariensis* cuttings obtained in each treatment (T1: S1 without IBA; T2: S1 + 3.0 mg $L^{-1}$ IBA; T3: S2 without IBA; T4: S2 + 3.0 mg $L^{-1}$ IBA). S1: substrate composed of biostabilized pine bark, limestone and vermiculite; S2: S1 + carbonized rice hulls (1:1). Values are the average of survivor explants (at least thirteen) ± standard error. Mean values with different letters are different by the Tukey test ($p \leq 0.05$).

assert that IBA generates differences in yerba mate rooting potential, and that without the use of the hormone, rooting in a few genotypes was practically null.

Inversely, experiment 2 revealed that the substrate exerted a greater influence in the rooting of the cuttings because, regardless of the presence or absence of IBA, the variables assessed in cuttings under treatments T3 and T4 presented averages that were similar or superior to T1 and T2. The same occurred in the yerba mate cutting research performed by Bitencourt *et al*. (2009), which resulted in a 65% rate in rooting, and in the

*Eucalyptus* sp. cutting, performed by Almeida *et al*. (2007), which obtained over 90% of rooting. In these researches, the utilization of IBA did not influence root formation.

In all surviving treatments of experiment 2 there was a low percentage of rooted cuttings, a fact also verified in other papers, such as in Bitencourt *et al*. (2009), which utilized cuttings from yerba mate buds and was only able to obtain 8.5% rooting. A low percentage of rooting may be influenced by the seasons of the year in which cuttings are collected because endogenous auxin, carbohydrate and protein levels may be different (Ferriani *et al*. 2006). These authors recommend collecting in summer. The relation between the collection period and a higher rooting percentage was shown by Zuffelato-Ribas & Rodrigues (2001), when 75% of yerba mate cuttings rooted in summer and only 27% did so in winter.

Bitencourt *et al*. (2009) verified that yerba mate cuttings originating in rejuvenated (regrowth) material presented a greater percentage of rooting, indicating that the rejuvenation of stems is efficient in the optimization of rooting cuttings of the species. Higa (1983) got a 60% rooting rate in cuttings from saplings and, for that reason, recommends the use of young stage growth material for propagation through cutting, since these propagules root more easily. The same occurred in the work of Carpanezzi *et al*. (2001), in which cuttings from young buds from the aerial parts of young *Erythrina crista-galli* L. plants presented a greater rooting rate than that of adult plants.

As to the substrates, Costa *et al*. (2007) state that the substrate should provide enough porosity to allow for good aeration, have a high capacity of water retention and good drainage, these being determining factors for the rooting success in many species. According to Paiva & Gomes (1995), the substrate may have an influence in the root system quality of cuttings from species that present difficulties in propagation. In experiment 2 the substrate that favored rooting the most was the combination of a biostabilized pine bark, limestone and vermiculite compound with carbonized rice hull in a proportion of 1:1. As stated by Quintero *et al*. (2013), carbonized rice hull is a substrate with great porosity, good drainage and aeration capacities, thus being an excellent substrate for plant cultivation when used either as a standalone substrate or in combination with others (Faria *et al*. 2001, Guerrini & Triguero 2004, Evans 2011).

The length of the longest root presented greater values when immersed in 3.0 mg $L^{-1}$ of IBA in comparison with all other treatments, this being the best indicated concentration for the saplings production from young cuttings. The best substrate available for use is the combination of biostabilized pine bark, limestone and vermiculite with carbonized rice hull in a proportion of 1:1, with or without previous immersion in auxin solution.

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