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Scientific Article

Can indolebutyric and fulvic acids induce adventitious rhizogenesis on mini-cuttings from Brazilian native tibouchinas?

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Abstract

The use of Brazilian native species for ornamental purposes is a promising alternative for local floriculture. Seeking to contribute with new information in this context, the present study aimed to evaluate the rooting performance of mini-cuttings from three *Tibouchina* species (*T.* aff. *fothergillae*, *T. heteromalla* and *T. moricandiana* var. *vinacea*) as affected by the use of indolebutyric acid (IBA) and fulvic acid (FA). Mini-cuttings with 5 cm in length were prepared with plant material from clonal mini-hedges and submitted to different treatments as follows: control treatment (T1); 2,000 mg L^{-1} IBA (T2); 2,000 mg L^{-1} FA (T3) and 2,000 mg L^{-1} IBA + 2,000 mg L^{-1} FA (T4). Planting was carried out in plastic containers filled with vermiculite, and, after 26 days under greenhouse conditions, the following variables were evaluated: mini-cuttings rooting percentage (RP), roots number (RN), roots length (RL), initial leaves maintenance (ILM) and sprouting (SP). The experiment was conducted under a completely randomized design in a 3 x 4 factorial scheme, with four replications and 20 minicuttings per plot. Rooting percentages were higher than 90% in all three species, regardless of IBA or FA treatments. RP, ILM and SP did not show statistically significant interaction between the treatments. For RN, T2 and T4 promoted the best results on *T.* aff. *fothergillae* (12.62 and 14.92, respectively) and T2 resulted in maximum values for *T. heteromalla* (15.65). For RL, T2 and T4 were statistically superior on *T. heteromalla* (9.52 and 8.20 cm, respectively). The use of IBA and FA is dispensable for rhizogenesis induction on mini-cuttings from the studied species.

Keywords: *Tibouchina* aff. *fothergillae* Cogn., *Tibouchina heteromalla* Cogn., *Tibouchina moricandiana* var. *vinacea* Baill., plant propagation.

Resumo

Os ácidos indolbutírico e fúlvico podem induzir rizogênese adventícia em miniestacas de quaresmeiras nativas?

O uso de espécies nativas brasileiras para fins ornamentais é uma alternativa promissora para a floricultura local. Buscando contribuir com novas informações neste contexto, objetivou-se avaliar o desempenho de enraizamento de miniestacas de três espécies de *Tibouchina* (*T.* aff. *fothergillae, T. heteromalla* e T. *moricandiana* var. *vinacea*) com o uso de ácido indol butírico (AIB) e ácido fúlvico (AF). Miniestacas com 5 cm de comprimento foram preparadas com material vegetal oriundo de minijardins clonais e submetidas a diferentes tratamentos: tratamento controle (T1); 2.000 mg L^{-1} de AIB (T2); 2.000 mg L^{-1} de AF (T3); 2.000 mg L^{-1} de AIB + 2.000 mg L^{-1} de AF (T4). O plantio foi realizado em tubetes plásticos preenchidos com vermiculita e, após 26 dias em casa de vegetação, foram avaliadas as seguintes variáveis: porcentagem de enraizamento das miniestacas (RP), número de raízes (RN), comprimento de raízes (RL), manutenção das folhas iniciais (ILM) e brotação (SP). O experimento foi conduzido num delineamento inteiramente casualizado, em esquema fatorial 3 x 4, com quatro repetições e 20 miniestacas por parcela. Os percentuais de enraizamento foram superiores a 90% para as três espécies, independentemente dos tratamentos com AIB ou AF. RP, ILM e SP não apresentaram interação estatisticamente significativa entre os tratamentos. Para RN, T2 e T4 promoveram os melhores resultados em *T.* aff. *fothergillae* (12,62 e 14,92, respectivamente) e T2 resultou em valores máximos para *T. heteromalla* (15,65). Para RL, T2 e T4 foram estatisticamente superiores em *T. heteromalla* (9,52 e 8,20 cm, respectivamente). O uso de AIB e AF é dispensável para indução de rizogênese em miniestacas das espécies estudadas.

Palavras-chave: *Tibouchina* aff. *fothergillae* Cogn., *Tibouchina heteromalla* Cogn., *Tibouchina moricandiana* var. *vinacea* Baill., propagação vegetal.

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Introduction

Species of the Melastomataceae family are widely distributed throughout the Brazilian territory, being present in all biomes, with the exception of Caatinga. The species present different population levels according to biome characteristics in each specific region (Goldenberg et al., 2012).

The genus *Tibouchina* was first described in 1775 by Aublet and was officially recognized around 1875, by Baillon. Currently, the genus comprises approximately 350 species, and, among them, *Tibouchina fothergillae* Cogn., *Tibouchina heteromalla* Cogn. and *Tibouchina moricandiana* var. *vinacea* Baill. stand out for their ornamental potential (Aublet, 1775; Lorenzi, 2008).

T. fothergillae shows remarkable ornamental potential, with abundance of purplish flowers at different times of the year. As it grows as a shrub, 1 to 2.5 meters height, it is ideal for landscape projects in reduced spaces (Lorenzi, 2002; Silva and Affonso, 2005).

T. heteromalla is widely used for ornamentation in squares and gardens. This species produces flowers with white base lilac petals, which become reddish after anthesis. Morphologically, it is similar to *Tibouchina carvalhoi* Wurdack (Freitas et al., 2016).

T. moricandiana var. *vinacea* is characterized by vigorous branching and production of dark red flowers, especially in times of favorable inductor photoperiod (Lorenzi, 2008).

Species from Melastomataceae family usually present relatively large amounts of aborted seeds and low germination rates (Cézar et al., 2009), which makes their sexual propagation inefficient, considering commercial exploitation. Therefore, the use of vegetative propagation techniques for these species can be a suitable approach.

Vegetative propagation is widely used for ornamental species propagation (Hartmann et al., 2011). This type of propagation comprises diverse techniques such as grafting, layering, air layering, cuttings and mini-cuttings. Vegetative propagation is one of the most important techniques for the regeneration of plants with agronomic interest (Chapman, 1989; Hartmann et al., 2011).

The mini-cutting technique is derived from traditional cuttings propagation. The main advantages of this method in comparison to the use of conventional stem cuttings are: (1) reduction of the required area to obtain viable propagules (adoption of mini-hedges or clonal mini-hedges); (2) shorter period of time for propagules rooting, since it consists of rejuvenated material; and (3) reducing the use of plant growth regulators for rhizogenesis induction (Ferriani et al., 2010). The use of mini-hedges in the vegetative propagation by mini-cuttings presents, additionally, the possibility to control mini-stumps mineral nutrition.

Mineral nutrition influences the availability of several rooting co-factors and its correct management is a fundamental feature for a satisfactory rooting performance of stem mini-cuttings. Zinc (Zn) is one example of an important micronutrient that works as a rooting co-factor, since it contributes to the biosynthesis of tryptophan, the precursor amino acid for indoleacetic acid (IAA) production in plant tissues. Manganese (Mn), in turn, together with boron (B), promotes the activation of the enzyme IAA-oxidase, whose function is the control of IAA biosynthesis, maintaining its concentration at an adequate level (Hartmann et al., 2011).

The use of plant growth regulators in vegetative propagation aims to provide higher rooting percentages and rhizogenesis speed, quality and uniformity (Hartmann et al., 2011). The regulation of roots elongation exerted by auxin is somewhat paradoxical, since this hormone induces ethylene biosynthesis, which in turn inhibits cell division. However, if ethylene biosynthesis is reduced or blocked, low concentrations of auxin (10^{-10} and 10^{-9} *M*) will promote perfect stem tissue differentiation in adventitious roots (Blazich, 1987; Stout et al., 2013).

Besides the use of synthetic auxins, humic substances (HS) with biostimulating action on the growth and development of plants can also be a viable approach to improve vegetative propagation. The benefits of using HS derive from water soluble organic acids from different organic sources that stimulate nutrients absorption (Marchi et al., 2008).

Humic substances can be divided into three operational sub-categories: humic acids, fulvic acids (FA) and humin, main components of soil organic matter (Silva and Mendonça, 2007). The effects on plant metabolism and growth, however, are mainly attributed to fulvic acids (Nardi et al., 2002). Humic substances can regulate the activity of H^+ pumps, and increase the synthesis of enzymes such as H^+ ATPases (Canellas et al., 2006). Therefore, it can be assumed that humic substances may present IAA-like activities in plant tissues.

The efficient propagation of species with ornamental potential may directly influence the economy of the floristic and ornamental sectors. Considering the above, the objective of this study was to evaluate the rooting and roots development on mini-cuttings from three *Tibouchina* species (*T.* aff. *fothergillae*, *T. heteromalla* and *T. moricandiana* var. *vinacea*) according to IBA and FA application, alone and in combination, thus recommending a protocol for the species propagation.

Material and Methods

The experiment was carried out in an artificilly climatized greenhouse (25 ± 2 °C and about 85% air relative humidity) located in the Biological Sciences Division, Federal University of Paraná (UFPR), Curitiba, Parana State, Brazil.

Mini-cuttings from three *Tibouchina* species (*T.* aff. *fothergillae*, *T. heteromalla* and *T. moricandiana* var. *vinacea*) were collected from shoots of mini-stumps approximately two years old The mini-stumps were part of a mini-hedge from cutting technic conducted at open sunlight at the GEPE (Research and Study Group on Cuttings) nursery, located at UFPR, Curitiba, Paraná State, Brazil. The climate of the region is characterized as Cfb, according to the Köppen classification. The mini-cuttings were collected during the morning period in the summer of 2017.

During mini-hedge management phase, the mini-stumps were submitted to fertigation every 15 days with 50 mL each of the following nutrient solution: 4 g L^{-1} ammonium sulfate, triple superphosphate and potassium chloride and 1 g L^{-1} FTE BR-10 (7% Zn, 4% Fe, 4% Mn, 0.1% Mo, 2.5% B and 0.8% B).

Mini-cuttings were made 5±1 cm long and with different basal diameter according to the species: 1.5 mm (*T.* aff. *fothergillae*), 2 mm (*T. heteromalla*) and 1.3 (*T. moricandiana* var. *vinacea*). The propagules were made with a bevel (diagonal) cut at the base and a straight cut at the apex. Two leaves reduced to half their area were kept at the apical region of each mini-cutting.

Mini-cuttings bases were submerged during 10 seconds in 50% (v/v) hydroalcoholic solutions containing different treatments as follows: control (T1), 2,000 mg L^{-1} IBA (T2), 2,000 mg L^{-1} FA (T3) and 2,000 mg L^{-1} IBA + 2,000 mg L^{-1} FA (T4). Fulvic acid (10%) from leonardite was used to perform this study.

After treatments, mini-cuttings were planted in 53 cm^3 polypropylene containers with six interior vertical ribs, filled with pre-moistened fine-granulumetry vermiculite. The cone-tainers were kept for 26 days under previously described greenhouse conditions until evaluation.

The following variables were assessed: rooting percentage (percentage of living mini-cuttings with roots at least 2 mm long [RP]), average number of roots per minicutting (RN), average length of the 3 longest roots per minicutting (RL), percentage of minicuttings that maintained the apical leaves (ILM) and sprouting percentage (SP).

The experimental design was completely randomized in a 3x4 factorial scheme (3 species x 4 treatments), with four replications and 20 mini-cuttings per experimental unit, totaling 320 mini-cuttings per species and 960 mini-cuttings in the experiment. Data were submitted to variance homogeneity analysis by the Bartlett test, variance analysis (ANOVA), and the means were compared by the Tukey test at the 5% probability level.

Results and Discussion

According to variance analysis, there was no interaction between species and treatments for RP, SP and ILM. Analyzing the factors in isolation, both the species and treatments with rooting stimulants did not differ among themselves for RP and ILM. *T.* aff. *fothergillae*, *T. heteromalla* and *T. moricandiana* var. *vinacea* can be considered as easy rooting species, since they all presented rooting rates superior than 90% regardless the use of IBA or FA (Table 1).

Table 1. Average rooting (RP), sprouting (SP) and initial leaves maintenance (ILM) on mini-cuttings from different *Tibouchina* species (*T.* aff. *fothergillae*, *T. heteromalla* and *T. moricandiana* var. *vinacea*) submitted to different treatments with indolebutyric acid (IBA) and fulvic acid (FA), after 26 days in artificially climatized greenhouse.

Species		**RP (%)**	**SP (%)**	**ILM (%)**
T. aff. *fothergillae*		95.83 a	82.81 a	85.62 a
T. heteromalla		95.08 a	75.00 a	97.94 a
T. moricandiana var. *vinacea*		90.62 a	75.00 a	80.62 a
Rooting stimulants		**RP (%)**	**SP (%)**	**ILM (%)**
Control		92.85 a	84.04 a	85.35 a
2,000 mg L^{-1}	IBA	96.07 a	67.44 b	89.46 a
2,000 mg L^{-1}	FA	92.20 a	85.35 a	82.73 a
2,000 mg L^{-1}	IBA + FA	94.40 a	73.57 ab	81.36 a
Coefficient of variation (%)		7.24	14.76	12.93

Means followed by the same lowercase letter in the columns do not differ statistically from each other according to the Tukey test at 5% probability.

The satisfactory results reported in the present study, together with the published literature regarding Tibouchina species propagation, reinforce that vegetative propagation can be a valuable approach to exploit economically important species.

Corroborating the results from the present study, rooting rates above 85% were reported on *T. moricandiana* var. *vinacea* (Pereira et al., 2015; Latoh et al., 2018) and *T. fothergillae* (Nicknich et al., 2013; Latoh, et al., 2018) stem cuttings regardless IBA application or absence.

The so-called 'easy rooting plants', are those plants whose tissues contain appropriate levels of endogenous substances to induce adventitious rooting, and, therefore, do not need any treatment with exogenous substances, such as synthetic auxins or rooting co-factors, to induce cuttings rhizogenesis (Hartmann et al., 2011). The native species investigated in the present study fit into this definition, and possibly presented ideal levels of auxins and rooting co-factors, since high rooting rates were observed on mini-cuttings.

The fact that mini-cuttings presented high percentages of original leaves maintenance (above 80%) may also be an important factor responsible for high rooting rates, since the leaves are a source of auxins and carbohydrates, among other compounds, which are translocated from the apex to the base of mini-cuttings, stimulating rhizogenesis (Hartmann et al., 2011). It can be inferred that mini-cuttings from the studied species had high auxin levels due to their reduced rate of foliar abscission. Such a behavior demonstrates maybe that auxin is in significantly higher levels than ethylene (i.e. auxin/ethylene ratio is high), at least in the basal region of leaf petiole.

For *Rubus* spp cv. Guarani, Tupy and Xavante, leaves are indispensable structures for vegetative propagation, since they provide a significant increase in stem cuttings rooting (Vignolo et al., 2013). Similarly, leaf maitenance is a fundamental feature for rooting and roots growth in *Prunus serrulata* Lindl. (Fragoso et al., 2015) and *Piper amalago* L. (Nunes Gomes and Krinski, 2016).

Regarding mini-cuttings sprouting, analyzing the factors in isolation, no difference was observed among the different species. For the treatments with rooting stimulants, however, statistical difference at 5% probability was detected. T1 (control) and T3 (2,000 mg L^{-1} FA) promoted better results than T2 (2,000 mg L^{-1} IBA) (Table 1).

The use IBA may have caused alterations in the mini-cuttings hormonal balance, increasing the auxin/cytokinin ratio, which is known to cause sprouting reduction on vegetative propagules (Hartmann et al., 2011). For *Stevia rebaudiana* Bert. Stem cuttings, treatment with 2,000 mg L^{-1} IBA dramatically reduced sprouting percentage, a fact that, according to the authors, was caused because the synthetic auxins in excessive concentrations can have a phytotoxicity effect and inhibit the development of roots and shoots (Pigatto et al., 2018).

Despite the fact some reports show sprouting increase in cuttings with biofertilizers application (Smitha and Umesha et al., 2012; Hakim et al., 2018), in the present study FA did not promote significant improvement of shoots growth on mini-cuttings. This may be attributed to the mini-cuttings appropriate hormonal balance for propagation, since the control treatment presented high levels of both rooting and sprouting. Therefore, the use of FA or any other exogenous substance showed to be unnecessary to stimulate sprouting.

For roots number and length, there was significant interaction between species and treatments with rooting stimulants. Treatment of *T. heteromalla* mini-cuttings with 2,000 mg L^{-1} IBA promoted longer roots when compared to the treatment with 2,000 mg L^{-1} FA and the control. The use of 2,000 mg L^{-1} IBA + 2,000 mg L^{-1} FA was superior to the control treatment but did not differ from treatments with FA or IBA alone. For the other studied species, the treatments did not influence roots length. Among the species, regardless of the treatments, *T. moricandiana* var. *vinacea* showed lower results, demonstrating relative slow adventitious roots growth (Table 2).

Table 2. Average roots length (RL) and roots number (RN) on mini-cuttings from different *Tibouchina* species (*T.* aff. *fothergillae*, *T. heteromalla* and *T. moricandiana* var. *vinacea*) in interaction with different treatments with indolebutyric acid (IBA) and fulvic acid (FA), after 26 days in artificially climatized greenhouse.

	Species/ Treatments	**Control**	**2,000 mg L^{-1}**	**2,000 mg L^{-1}**	**2,000 mg L^{-1}**
			IBA	**FA**	**IBA/FA**
RL(cm)	*T.* aff. *fothergillae*	7.72 aA	8.25 aA	7.14 aA	7.71 aA
	T. heteromalla	6.08 aC	9.52 aA	6.98 aBC	8.20 aAB
	T. moricandiana var. *vinacea*	3.30 bA	3.01 bA	2.81 bA	3.76 bA
	Coefficient of variation (%)	17.41			
RN	*T.* aff. *fothergillae*	8.00 aB	12.62 bA	8.37 aB	14.91 aA
	T. heteromalla	6.81 aC	15.65 aA	8.41 aC	11.86 bB
	T. moricandiana var. *vinacea*	8.18 aA	10.21 bA	8.71 aA	10.97 bA
	Coefficient of variation (%)	15.96			

Means followed by the same capital letters in the rows and lowercase letters in the columns do not differ statistically from each other according to the Tukey test at 5% probability.

This internal regulation of roots elongation by auxin is highly variable, depending on the endogenous hormone concentration and sensitivity of each species/genotype and, therefore, the appropriate concentration of plant growth regulators to stimulate rooting and roots growth, if necessary, should be studied for each species/genotype. In *P. serrulata* stem cuttings, for example, Fragoso et al. (2017) reported that concentrations of 750 and 1,000 mg L^{-1} IBA promoted longer roots. For stevia propagation, in turn, roots number and length reached maximum values at concentrations of 1,250 and 700 mg L-1 IBA, respectively (PIGATTO et al., 2018).

Regarding roots number, *T.* aff. *fothergillae* had better results when submitted to treatments with 2,000 mg L^{-1} IBA and 2,000 mg L^{-1} IBA + FA. *T. heteromalla* presented superior results when submitted treated exclusively with 2,000 mg L^{-1} IBA and *T. moricandiana* var. *vinacea* did not show variations in roots number as a function of treatments with rooting stimulants. *T.* aff. *fothergillae* presented the highest RN among the studied species when treated with 2,000 mg L^{-1} IBA + FA, whereas *T. heteromalla* was superior to the others when IBA was applied alone (Table 2).

It is important to note that producing lateral roots will increase nutrient uptake by increasing root surface area (Zonta et al., 2006). Therefore, the higher the number of roots per mini-cuttings, the greater the efficiency of the plant in absorbing minerals available in the soil nutrient solution.

IBA effects on roots number can vary dramatically according to the species to be studied. For *Varronia curassavica* Jacq. stem cuttings, 3,000 mg L^{-1} IBA promoted better results than the control treatment (Bischoff et al., 2017), whereas to other species such as *Drimys brasiliensis* Miers (Zem et al., 2015) and *Annona crassiflora* Mart. (Pimenta et al., 2017), IBA had no effects on this variable. This reinforces the demand for specific studies in agronomic important and/or potential plant species.

Nomura et al. (2012), affirm that the use of biofertilizer based on humic substances improves the acclimatization of micropropagated *Musa* spp. plantlets, considerably increasing roots fresh and dry biomass. The increase in H^+ pumps activity elicited by humic substances resembles the IAA mode of action, favoring the induction of lateral roots and, therefore, increasing the roots system surface area (Canellas et al., 2006).

Despite the positive effects reported in scientific literature, in the present study, the use of FA on *Tibouchina* species mini-cuttings did not differ from the control treatment for none of the analyzed variables. Because it is a relatively new technology in vegetative propagation and species tend to have different tissue sensibility to rooting stimulants, future studies with on different FA concentrations and modes of application are necessary to state their activity in the studied species.

Conclusions

T. aff. *fothergillae*, *T. heteromalla* and *T. moricandiana* var. *vinacea* can be considered easy rooting species, since mini-cuttings from these plants reach rooting rates superior than 90% without the need of IBA or FA application.

The use of 2,000 mg L^{-1} IBA or 2,000 mg L^{-1} IBA + 2,000 mg L^{-1} FA promotes higher roots number in *T.* aff. *fothergillae.* For *T. heteromalla,* 2,000 mg L^{-1} IBA is recommended to obtain higher roots number and length. *T. moricandiana* var. *vinacea* mini-cuttings are not affected by the use of IBA or FA.

Author contribution

L.P.L. 0000-0002-6450-2347**:** study conception and design, acquisition, analysis and interpretation of data, manuscript draft and review. **E.N.G.** 0000-0002-7999-070X**:** Interpretation of data, manuscript draft and critical review. **K.C.Z.R.** 0000-0001-6320-5773**:** study conception and design, manuscript critical review.

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