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Scientific Article

Propagation of native plants with ornamental potential from Serra do Oratório, Santa Catarina State, Brazil

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Abstract

The use of native plants enriches the landscape projects, conserves the local flora, creates a local identity and promotes the symbiosis between native fauna and flora. The objective of the present study was to evaluate the vegetative propagation of the native species *Calibrachoa sellowiana*, *Tibouchina dubia* and *Verbena rigida*, using cuttings submitted to different concentrations of indolebutyric acid (IBA), naphthaleneacetic acid (NAA) and substrates. From the stock plants, the cuttings were excised from the apex, with leaves number varying according to the species. In the propagation of *Calibrachoa sellowiana*, *Tibouchina dubia* and *Verbena rigida* under different concentrations of IBA, 4 treatments were carried out, consisting of IBA at concentrations of 0 mg L^{-1} (control), 500 mg L^{-1}, 1000 mg L^{-1} and 2,000 mg L^{-1}. In the propagation of *Tibouchina dubia* under different concentrations of NAA, the experiment was developed using four treatments, NAA was diluted in acetone + talc at concentrations of 0 mg L^{-1} (control), 2,000 mg L^{-1}, 4,000 mg L^{-1} and 6,000 mg L^{-1}. The evaluated variables for all the experiments were: percentage of rooted cuttings, number of roots per rooted cutting, average root length (cm) and number of shoots. In the substrate experiment, the treatments were sand, vermiculite, Plantmax® and mixture of soil + sand, for all the species from the previous experiments. *Calibrachoa sellowiana* and *Verbena rigida,* rooted with low concentration of IBA, 0 e 500 mg L^{-1}, respectively, but the PGR was not essential to rooting phase, since the control also developed roots. For *Tibouchina dubia* the IBA and NAA did not promoted rooting. *Calibrachoa sellowiana* rooted better with soil + sand, differing from *Verbena rigida,* which rooted better with Plantmax®.

Keywords: *Calibrachoha sellowiana,* floriculture, *Tibouchina dubia*, *Verbena rígida.*

Resumo

Propagação de plantas nativas da Serra do Oratório, Santa Catarina State, Brazil com potencial ornamental

O uso de plantas nativas enriquece os projetos paisagísticos, conserva a flora local, cria uma identidade local e promove a simbiose entre fauna e flora autóctones. O objetivo do presente estudo foi avaliar a propagação vegetativa das espécies nativas *Calibrachoa sellowiana, Tibouchina dubia* e *Verbena rigida*, por meio de propagação utilizando diferentes concentrações de ácido indolbutírico (AIB), ácido naftalenoacético (ANA) e com diferentes tipos de substratos. Das plantas-matrizes, as estacas caulinares foram retiradas da parte apical, com número de folhas variando entre as espécies. Nos experimentos de propagação de *Calibrachoa sellowiana, Tibouchina dubia* e *Verbena rigida* sob diferentes concentrações de AIB foram realizados quatro tratamentos, consistindo de estacas tratadas com AIB nas concentrações de 0 mg L^{-1} (testemunha), 500 mg L^{-1}, 1000 mg L^{-1} e 2,000 mg L^{-1}. No experimento de propagação de *Tibouchina dubia* sob diferentes concentrações de ANA foram realizados 4 tratamentos, onde o ANA foi diluído em acetona + talco, com concentrações de 0 mg L^{-1} (testemunha), 2,000 mg L^{-1}, 4,000 mg L^{-1} e 6,000 mg L^{-1}. As estacas de todos os experimentos foram avaliadas com relação às seguintes variáveis: porcentagem de estacas enraizadas, número de raízes por estaca enraizada, comprimento médio de raiz (cm) e número de brotações. No experimento de substratos os tratamentos utilizados foram areia, vermiculita, Plantmax® e mistura de solo + areia. *Calibrachoa sellowiana* e *Verbena rigida,* enraízam com pequenas concentrações de AIB, porém o regulador de crescimento não é essencial à fase, pois a testemunha também desenvolveu raízes. Para *Tibouchina dúbia,* AIB e ANA não promoveram enraizamento. *Calibrachoa selowiana* obteve melhor enraizamento em areia + solo, diferentemente da espécie *Verbena rigida* que obteve melhor resultado com o uso do substrato Plantmax®.

Palavras-chave: *Calibrachoha sellowiana,* floricultura, *Tibouchina dubia*, *Verbena rígida.*

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Introduction

The Brazilian market for ornamental plants is based in exotic species and little is known about the native species in the national urban areas. The knowledge, identification and propagation of these species can reduce the risk of their extinction and modify the production chain of ornamental plants. The interest to identify native plants that can be produced commercially is increasing among ornamental plant growers, but the plants must propagate uniformly and grow well in containers to become a successful commercial crop (Lubell and Griffith-Gardner, 2017). Propagation by cuttings is a rapid method of vegetative propagation, that allows to maintain characteristics of interest (Villa et al., 2017), promoting the *ex situ* conservation of native species.

The genus *Calibrachoa* contain about 25 species, originated from South America. In Brazil are found 23 species, most of them inhabiting the Southern States. The species richness is found on South Brazilian Plateau, at altitudes ranging above 900m (Stehmann, 2019). *Calibrachoa sellowiana* has infundibuliform corola, externally hairy, internally papillae near anthers, tube with 9-15(20) mm of length, yellow with purple striated-reticulated venation, yellow fauce, blade with 15-30 mm of diameter, magenta or purple color (Stehmann, 2019).

The genus *Verbena* has more than 100 species; the *Verbena rigida* is native and occur in the three states of South Brazil, as well as in the states of São Paulo and Minas Gerais, in anthropic areas and in high altitude fields, showing great adaptability to environmental and climatic adversities (O'Leary, 2019). This species *Verbena rigida* can be recognized by the oblong-elliptical leaves, with incise-serrated margin, ciliate and purple flowers gathered in corymbous inflorescences (Cardoso et al., 2018).

The genus *Tibouchina* have more than 350 species, and *Tibouchina dubia* is a native and endemic species of Brazil, occurring in South and Southern Brazil, is present in high altitude fields and Ombrophilous Forest, and can be recognized by its medium height, approximately 5 meters, and purple flowers that turn into white as they become older (O'Leary, 2019).

Most of these ornamental species spread naturally by seeds, but are difficult to propagate in nurseries. Therefore, to know the best propagation method for native plants with ornamental potential is of great importance and one of the primary aspects for agronomic exploitation of plants. The best viable propagation technique is vegetative propagation by cuttings that guarantees a progeny identical to the mother plant and also decreases time for seedlings use of several ornamental species (Loss et al., 2015). Then, it is necessary to study and standardize adequate procedures to each species (Gomes and Krinski, 2017). Plant cuttings can be woody and/or herbaceous, made from stems, tubers, bulbs, leaves, rhizomes and roots. The choice of plant material will be related to the plant conditions, however, regardless of the cutting typed, to induce adventitious roots it may be necessary the use of exogenous growth regulators, such as auxins (Hartmann et al., 2010).

For a long time in agriculture, growth regulators have been used, being widely exploited in some commercial species of ornamental plants. Auxins are used to induce development of roots, though the time can vary between 30 to 60 days, depending on the species and the growth regulators used. According to Hartmann et al. (2010) the indolebutyric acid (IBA) auxin has become the best choice to promote rooting, since it presents low toxicity to explants, low mobility and high chemical stability. Though there are other auxins that can promote rooting in cuttings, such as indoleacetic acid (IAA) and naphthalene acetic acid (NAA), their cost is more expensive, so their use is commonly limited for plants that present difficulties to develop adventitious roots.

The IBA auxin has a non-toxic effect to most plants, even in high concentrations, it is light stable and has low susceptibility to enzyme degradation. The NAA auxin is also light stable and non-degraded by the IAA-oxidase system, although presents some toxicity to plants in comparison to IBA (Epstein and Lavee, 1984). Therefore, it is difficult to make specific recommendations, since the appropriate growth regulator and concentration vary according to the species or cultivar, type of cutting used, collection period of the cutting, and others (Loss et al., 2015).

In addition to the use of growth regulators, there is also the need to use adequate substrates for each species. Among the common and accessible products for plantlets and seedlings production, commercial substrate and vermiculite are the most used by farmers and nurseries, being important to evaluate them in the rooting process of species of interest. Since a substrate must provide support for the plant, aeration, conditions for root development, proper water and nutrient absorption (Gomes and Krinski, 2017) it was chosen to evaluate substrates easy and cheap to obtaining. Among the environment factors that influence the cuttings rooting, the substrate has great importance supporting the cuttings (Zanao et al., 2016), justifying the soil use as a treatment, in order to promote stability for the cuttings.

Thus, the objective of this study was to evaluate the vegetative propagation of the native species *Calibrachoa sellowiana, Verbena rigida* and *Tibouchina dubia*, found in Serra do Oratório, Santa Catarina State, using different concentration of indolebutyric acid (IBA), naphthaleneacetic acid (NAA) and different substrates.

Material and Methods

Plants of *Calibrachoa sellowiana, Verbena rigida* and *Tibouchina dubia* were collected during the spring and summer, at Serra do Oratório, Catarinense Plateau region, located in the county of Bom Jardim da Serra-SC. After collection, they were placed in thermal boxes to preserve temperature and humidity. These plants were planted in plastic vases (10 liters), filled with Plantmax® to become stock plants, and placed in greenhouse with sprinkling irrigation, made 3-5 times a day for 1-minute period. From these stock plants, cuttings were made for the elaboration of experiments. Were collected 40 plants of *Verbena rigida* and *Calibrachoa sellowiana* and 5 (approximately 40 cm high) of *Tibouchina dubia* shrubs.

Experiment 1 - Propagation of cuttings of *Calibrachoa sellowiana, Tibouchina dubia* and *Verbena rigida*, with different IBA concentrations

The experiments were developed using herbaceous cuttings of *Calibrachoa sellowiana, Tibouchina dubia* and *Verbena rigida* (from apical part of the plants), collected between December and March, when the plants were in a vegetative and/or flowering stage. The cuttings were collected during the floral period because during autumn and winter the aerial part of the plants dies. The cuttings were collected from the stock plants one day after the stock plants were allocated in a greenhouse. During the vegetative period, cuttings were collected every 15 days for experiments. Were used 200 cuttings standardized at 10 cm in length (40 leaves, 20 internodes and 10 buds in *C. sellowiana*; 14 leaves, 7 internodes and 4 buds in *T. dubia* and 8 leaves and 4 internodes for *V. rigida*). The cuttings were planted with four internodes below the substrate for *C. sellowiana* and *T. dubia*; and one internode below the substrate for *V. rigida* (with straight cut below the substrate and a downward linear cut on the top of the cutting). Four treatments were carried out, consisting of cuttings treated with IBA diluted in 96° ethyl alcohol, concentrations of 0 mg L^{-1} (distilled water + alcohol 96° - control), 500 mg L^{-1}, 1000 mg L^{-1} and 2,000 mg L^{-1}, by immersing 2 cm of the base for 15 seconds and placed to root in polystyrene trays (40 cm^3 volume $cell^{-1}$), with Plantmax®, settled in a greenhouse with nebulization (timer controlled) permitting 97% RH and 27 ± 2 ºC temperature. After 60 days, plants were removed from the greenhouse and evaluated the following variables: rooting percentage of cuttings, number of roots per rooted cutting, average length of root (cm) and number of shoots. The experimental design used was completely randomized, with 4 treatments (IBA concentrations) and 5 replicates composed of 10 cuttings, totaling 200 cuttings per species. The data were submitted to analysis of variance (ANOVA) and when significant, means were compared by Tukey's test ($p < 0.05$) and/or regression analysis.

Experiment 2 - Propagation of cuttings of *Calibrachoa sellowiana* and *Verbena rigida*, with different substrates

The experiment was elaborated with herbaceous cuttings of *Calibrachoa sellowiana* and *Verbena rigida*. Were used 200 cuttings standardized at 10 cm in length (40 leaves, 20 internodes and 10 buds in *C. sellowiana*, 14 leaves and 8 leaves and 4 internodes for *V. rigida*).

The treatments used were different substrates: sand, vermiculite, Plantmax®, and a mixture of 50% soil + 50% sand (1:1). The cuttings were placed in polystyrene trays (40 cm^3 volume $cell^{-1}$), and settled in a greenhouse with nebulization, 27 ± 2 ºC temperature and 97% RH. It was not used any type of growth regulator in this experiment. After 60 days, plants were removed from the greenhouse and evaluated the following variables: mortality of cuttings, rooting percentage of cuttings, number of shoots per cutting, callus formation on non-rooted cuttings, number of roots per rooted cuttings and root volume per rooted cutting. The experimental design was completely randomized, with 4 treatments (substrate types) and 5 replicates composed of 10 cuttings, totaling 200 cuttings per species. The data were submitted to analysis of variance (ANOVA) and when significant, means were compared by Tukey's test ($p < 0.05$).

Experiment 3 - Propagation of *Tibouchina dubia* cuttings with different NAA concentrations

This experiment was developed using 100 semi-woody cuttings of *Tibouchina dubia*. The cuttings were standardized to 10 cm length, 14 leaves, 7 internodes and 4 buds. Four treatments were carried out, consisting of cuttings treated with NAA diluted in a mixture of acetone and talc, concentrations of 0 mg L^{-1} (talc + acetone), 2,000 mg L^{-1}, 4,000 mg L^{-1} and 6,000 mg L^{-1}, by immersing 2 cm of the base for 15 seconds, placed to root in polystyrene trays (40 cm^3 of volume/cell), with Plantmax®, settled in a greenhouse with nebulization, 27 ± 2 ºC temperature and 97% RH. After 60 days, plants were removed from the greenhouse and evaluated the following variables: rooting percentage of cuttings, number of roots per rooted cutting, average length of root (cm) and number of shoots. The experimental design used was completely randomized, with 4 treatments (NAA concentrations) and 5 replicates composed of 5 cuttings, totaling 100 cuttings. The data were submitted to analysis of variance (ANOVA) and when significant, means were compared by Tukey's test ($p < 0.05$) and/or regression analysis.

Results and Discussion

***Calibrachoa sellowiana* (sendtn.) Wijsman**

With the regression adjustment for the quantitative factor, for *C. sellowiana* species, it was observed that the increase of IBA concentration was inversely proportional to rooting percentage, root length and number of roots (Figure 1). Feliciana et al. (2017) stated that this behavior may be related to endogenous amounts of hormones the cuttings have, which can promote or inhibit rooting. The authors Ahkami et al. (2013) quantified the endogenous IAA found in several types of cuttings for *Petunia hibrida* and stated that apical shoots, with younger leaves, have the highest levels of IAA, and rooting occurs naturally in these cuttings, without the need to apply exogenous auxins. The role of auxins in root formation consider its transport, synthesis and cell conjugation in a plant, so, the same auxin level can inhibit cell elongation in favor of cell division in the root meristem (Overvoorde et al., 2010). Therefore, the results found in this study suggest that higher levels of exogenous IBA combined with the endogenous IAA of the *C. sellowiana* cuttings decreased the root formation.

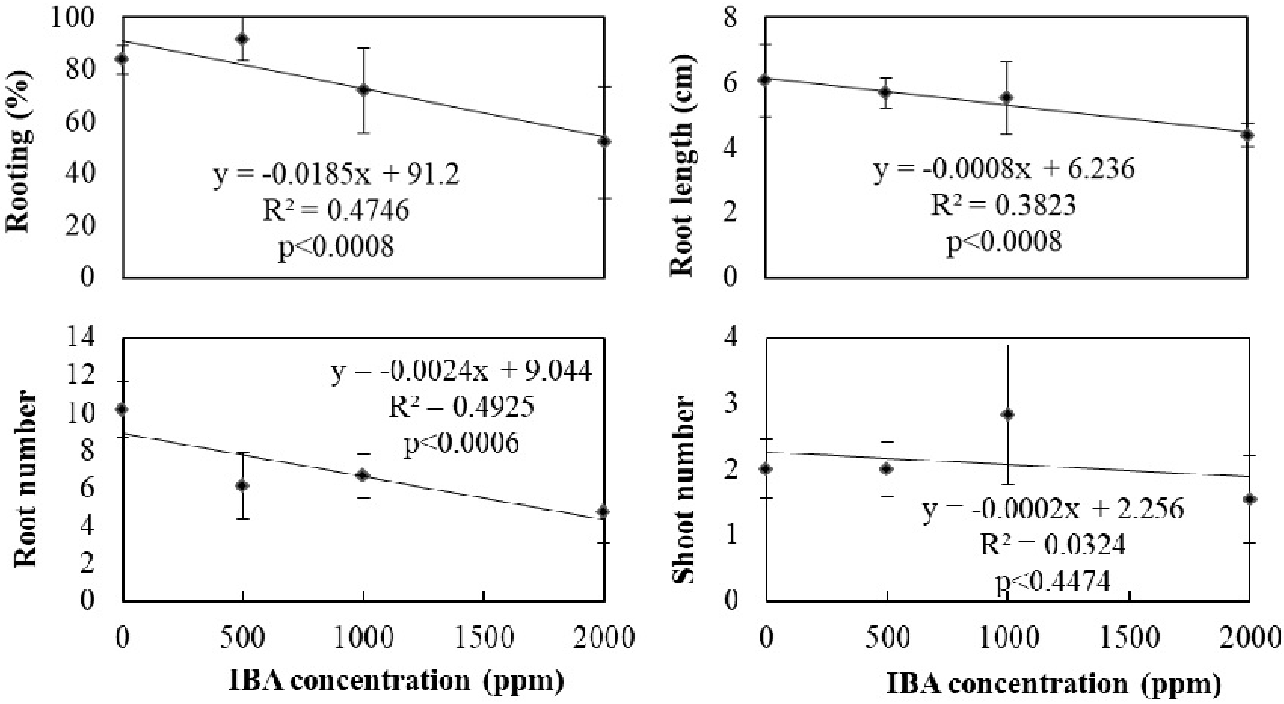

Figure 1. Rooting percentage, average root number per cutting, average root length (cm) and average shoot number per cuttings of the species *Calibrachoa sellowiana* submitted to different concentration of indolebutyric acid (IBA).

Regarding to experiment 2, where different substrates were evaluated, the treatments responded the same for the variable's mortality of cuttings and number of shoots (Table 1). For the rooting percentage the substrate containing soil and sand (1:1) was better when compared to the others. The root length showed difference for the sand substrates, as well as soil and sand (Table 1). The variable number of roots obtained better results for the treatment containing only sand (Table 1). For *Petunia hybrida* and *Calibrachoa hybrids* the authors Dole et al. (2002) affirm that soil is adequate to propagation of these species, with uptake of growth regulators. Substrates with high density, compact particles and low porosity (such as sand and soil, for example) when confined to a limited space (like vases, containers and polyethylene bags) impact the plantlet development, the rooting system architecture and the biological associations between plant and environment, being priority related to nutrition and water flow in the plant-atmosphere system (Cecco et al., 2018).

Table 1. Effect of different substrates on mortality of cuttings, rooting percentage, shoot number, root number and root length of *Calibrachoa sellowiana* cuttings.

Substrates	Survival (%)	Rooting (%)	Shoot number	Root number	Root length
Sand	78^{ns}	48 ab	3.36^{ns}	2.50 a	1.89 a
Vermiculite	66	28 ab	2.65	1.83 ab	0.29 b
Plantmax®	76	16 b	2.48	1.29 b	0.30 b
Soil + sand	68	56 a	2.55	2.18 ab	2.20 a
Means	72	37	2.76	1.95	1.17
C.V. (%)	15.64	43.49	62.06	47.52	79.22

* Averages followed by the same letter do not differ according to Tukey's test at 5% of error.

Verbena rigida **Spreng**

Regarding the experiment with *V. rigida* species under different IBA concentrations (experiment 1), the average rooting percentage was 51.3%, but the treatments responded significantly only for the variable number of roots (Figure 2). In studies developed with other species of the *Verbenaceae* family, the IBA is not essential for rooting, but it can promote higher number of roots and optimize its development (Paulus et al., 2014). *Verbena rigida* is a species with rustic characteristics, easy reproduction, decumbent and the stem remaining in contact with the soil, facilitating root development. According to the same authors, the first flowering of *V. rigida* is usually fast, the initial shooting is vigorous and the plant soon spreads, forming new clumps. Therefore, the IBA can be used to optimize the *V. rigida* propagation.

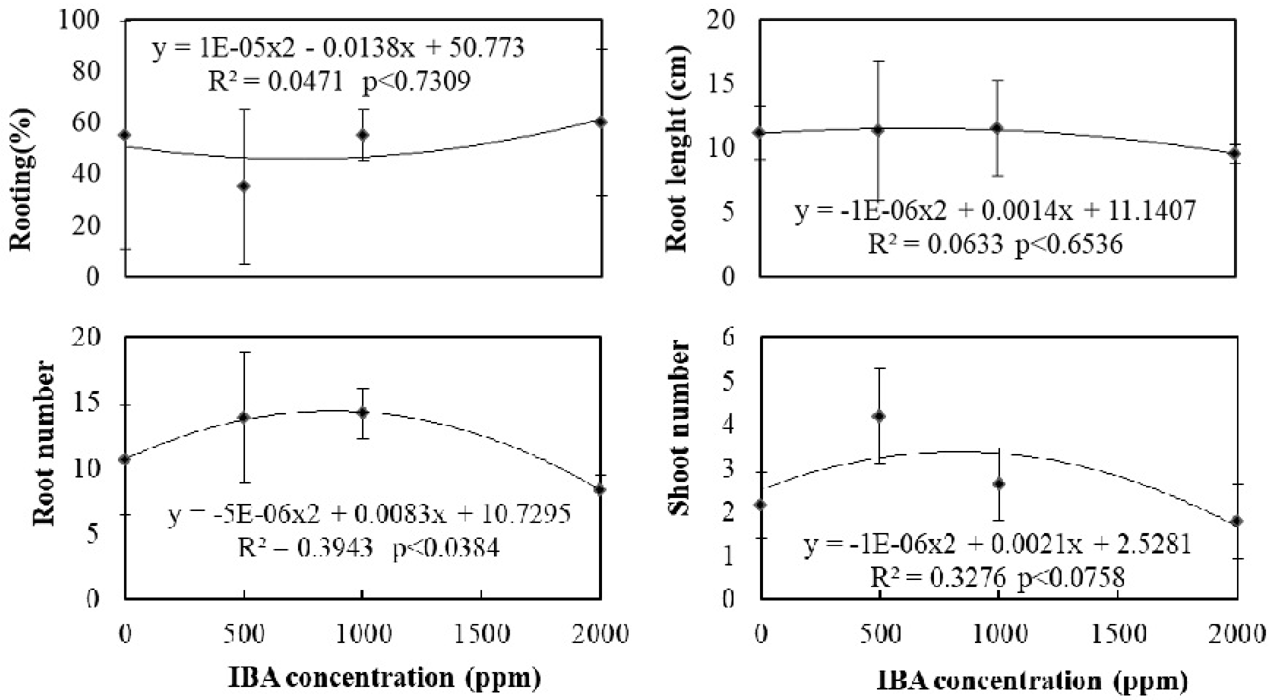

Figure 2. Rooting percentage, average number of roots per cutting, average root length (cm) and average shoot number per cuttings of the species *Verbena rigida* submitted to different concentration of indolebutyric acid (IBA).

In the propagation experiment of *Verbena rigida* with different substrates (experiment 2), for the variable root length there was only significance for the commercial substrate treatment, which was superior to other substrates tested (Table 2). Resende et al. (2015) stated that Plantmax® is ideal for *Lippia rotundifolia* during acclimatization phase of an *in vitro* propagation protocol. The authors Resende et al. (2014) obtained optimal rooting of *Bouchea fluminensis* cuttings with commercial substrate corroborating with the present study.

Table 2. Effect of different substrates on mortality of cuttings, rooting percentage, shoot number, root number and root length of *Verbena rigida.*

Substrates	Survival (%)	Rooting (%)	Shoot number	Root number	Root length
Sand	65ns	65ns	1.30ns	3.35ns	1.73b
Vermiculite	50	50	1.30	2.70	2.02ab
Plantmax®	50	50	2.25	5.55	5.72a
Soil + sand	50	50	1.05	4.90	5.00ab
Means	53.7	53.7	1.47	4.12	3.61
C.V. (%)	36.81	36.81	31.20	20.13	26.70

* Averages followed by the same letter do not differ according to Tukey's test at 5% of error.

***Tibouchina dubia* Cogn.**

Since it was found a small amount of *Tibouchina dubia* plants in its habitat (only five plants), it was decided to perform two different experiments in order to test the rooting capability of this species.

Tibouchina dubia did not respond to IBA or NAA tested in experiments 1 and 3. The cuttings were lush and leafy, and after a few days they showed signs of oxidation, defoliation, and death. The defoliation of cuttings possibly led to the suspension of the auxin production, cofactors and photoassimilates (Hartmann et al., 2010). With the depleted reserves, the cuttings entered a continuous process of senescence and did not have more resources to survive. According to Nienow et al. (2010) the survival and rooting capacity of cuttings is closely related to the maintenance of leaves during the process. It should be noted that the cuttings remained under constant misting, therefore, defoliation did not occur due to any environmental factors.

Although several authors in studies with IBA use in plants of the family *Melastomataceae*, observed increase in the rooting percentage of many species (Engel et al., 2017; Penso et al., 2016; Pizzatto et al., 2011, Suzuki et al., 2015), each species has specific peculiarities about rooting and propagation of cuttings, such as genetic factors and quantity of endogenous auxins. The authors Latoh et al. (2018) tested exogenous auxins in several species of *Tibouchina* and concluded that *T. affinis, T. heteromalla* and *T. moricandiana* do not need exogenous auxins to induce rooting, *T. granulosa* can root when treated with IBA, however, *T. sellowiana* is a hard to root species, and even when treated with IBA, do not develop adequate roots.

The results of these experiments suggest that *Tibouchina dubia* may be a hard to root species, such as *T. sellowiana,* therefore, more studies on the physiology of this species, endogenous levels of hormones and synergistic effects should be performed in order to obtain satisfactory results regarding the vegetative propagation of *Tibouchina dubia*.

Conclusions

In the vegetative propagation of *Calibrachoa sellowiana* and *Verbena rigida,* the rooting phase can be optimized with 500 and 1,000 mg L^{-1} of IBA, respectively. The mixture soil + sand presented better results for the vegetative propagation of *Calibrachoa sellowiana* and the Plantmax® presented better results for the vegetative propagation of *Verbena rigida.*

Tibouchina dubia did not responded to IBA and NAA concentrations tested during rooting phase.

Author Contribution

F.E.A.B.0000-0002-9538-3141: author of the work, contributed to the design and execution of experiments in the field and in the laboratory, data tabulation, preparation of graphs and tables, statistics and writing; **F.G.**0000-0002-2632-9328**:** contributed to the laboratory experiments, statistics, writing and translation of the article into English.; **A.A.K.**0000-0003-0890-8307**:** the author contributed to fundraising for the experiments, planning the experiments and writing the manuscript; **L.F.**0000-0001-9545-7035**:** the author contributed to fundraising for the experiments, planning the experiments and writing the manuscript.

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