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MINI-CUTTING TECHNIQUE FOR VEGETATIVE PROPAGATION OF *Paratecoma peroba*

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HIGHLIGHTS

Rooting percentage was positively related to lower substrate density.

More vigorous root system was obtained with leaf area maintenance.

The apical mini-cutting of 10 cm is more adequate in the production of clones.

The protocol will enable mass propagation and recovering the *P. peroba* population.

Keywords:
Adventitious rooting
Indole-3-butyric acid
Clonal forestry
Mini-cut type
Foliar architecture

ABSTRACT

Paratecoma peroba is a native Brazilian forest species of great economic and ecological interest, which is currently at risk of extinction owing to excessive wood exploration. The use of the mini-cutting technique could facilitate the vegetatively propagation of this species. The objective of the present study was to establish a vegetative propagation protocol for *P. peroba* using the mini-cutting technique. In the first experiment, were tested five concentrations of indolbutiric acid (IBA; 0, 1, 2, 4 and 8 g·L^{-1}) and two types of substrates (sand and a commercial substrate based on ground Pinus bark, coconut powder, and mineral additives). In the second experiment, were tested three types of mini-cuttings (apical of 6 cm and 10 cm, and intermediary of 4 cm) and two leaf area sizes (total leaf area and reduced to 50% of leaf area). The results showed that P. peroba could be vegetatively propagated using the mini-cutting technique with up to 82.5% rooting. There was only 5.6% of mini-cutting rooted in sand. High concentrations of IBA promotes decrease in rooting and number of roots. The 10 cm apical mini-cuttiing tend to form clones with better quality standards. The maintenance of the leaf area promotes the attainment of roots with greater root surface area, volume and dry mass of *P. Peroba* plants.

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INTRODUCTION

The evolution of Brazilian environmental legislation together with initiatives such as an international commitment to reforest 12 million hectares until 2030 as promised in the Paris Agreement in 2015 has increased the scientific interest in the production of seeds and seedlings of arboreal Brazilian flora species (Brasil, 2018). Peroba amarela, peroba do campo, and ipê-peroba are popular names for *Paratecoma peroba* (Record and Mell) Kuhlm (Bignoniaceae), which is a native species from Atlantic Forest biome that occurs naturally in a restricted coastal area in eastern Brazil, from the southern state of Bahia to the north of Rio de Janeiro (Lins and Nascimento, 2010; Lorenzi, 2009). Described as being a late secondary species (Archanjo et al., 2012) and having a large size, adult trees can reach up to 40 m in height with diameters varying from 40 to 80 cm (Lorenzi, 2009).

In the past, this species was the target of intense extractivist exploitation owing to the technological qualities and natural beauty of its wood, and has been used to build coatings and luxury furniture (Lins and Nascimento, 2010). Although protected by several conservation factions, the fragmentation of the natural habitat of this species in addition to a lack of reforestation programs has placed *P. peroba* at the risk of extinction. It is estimated that there are just over 8500 adult individuals of this species left in nature (CNCFlora, 2012).

Besides the difficulty of locating plus tree, *P. peroba* undergoes supra-annual flowering, with some with high seed production, followed by years of scarcity (Lins and Nascimento, 2010; Lorenzi, 2009). The seeds are recalcitrant and seedling growth is slow during the nursery phase, which can last up to eight months (Lorenzi, 2009). For these reasons, the search for alternative methods of propagation of this species is justified and important, such as the mini-cutting technique.

The mini-cutting is the main technique for propagating clones of *Eucalyptus* species in Brazil and is one of the factors, together with genetic improvement and hybridisation, which has contributed to an increase in the productivity of forests of this genus throughout the country (Batista et al., 2014; Brondani et al., 2014; Rocha et al., 2015). The mini-cutting technique has been successfully used for the propagation of native forest species, e.g. *Cedrela fissilis* Vell (Xavier et al., 2003), *Anadenanthera macrocarpa* (Benth.) Brenan (Dias et al., 2015), *Handroanthus heptaphyllus* (Vell.) Mattos (Freitas et al., 2016), *Plathymenia foliolosa* Benth. (Pessanha et al., 2018), *Tibouchina selowiana* (Cham.) Cogn (Fragoso et al., 2017), *Peltophorum dubium* (Spreng.) Taub (Mantovani et al., 2017), and *Cabralea canjerana* (Vell.) Mart (Burin et al., 2018).

The formation of new roots from the tissues at the base of the mini-cuttings, called adventitious rooting, is essential for carrying out the clonal propagation. Variables such as the percentage of rooting, number of roots, and length and dry mass of roots can be influenced by a number of factors and their interactions, e.g. the age of the donor plant, hormonal balance, carbohydrate content, mini-cut type, sheet maintenance, substrates, and the application of growth regulators (Denaxa et al., 2012; Hartmann et al., 2011; Xavier et al., 2013). Therefore, the improvement of the mini-cut protocol for *P. peroba* would provide an alternative method of propagation for this species, overcome the difficulties associated with seminal propagation, and increase the availability of seedlings for restoration projects in the Atlantic Forest and commercial plantations of the species.

Therefore, the objective of the present study was to establish a protocol of vegetative propagation for *P. peroba* using the mini-cutting technique. To achieve this, the following hypotheses were tested: (i) *P. peroba* has the fitness for vegetative propagation by the mini-cutting technique; (ii) substrate influences the rooting of mini-cuttings; (iii) higher concentrations of indolbutiric acid (IBA) cause the indution of adventitious roots; and (iv) there is a relationship between the pattern of mini-cuttings and the rooting of *P. peroba* mini-cuttings.

MATERIAL AND METHODS

Study Area

Experiments were undertaken from July 2018 to January 2019 at the Forest Nursery at Universidade Federal do Espirito Santo (DCFM-CCA-UFES), in Jerônimo Monteiro, Espirito Santo State (ES), at 20° 47′ S and 41° 23′ W and an altitude of 120 m. The climate of the Jerônimo Monteiro region based on the Köppen classification is the CWA type, i.e. dry winter and rainy summer (Eugenio et al., 2016).

Establishment of the mini-clonal hedge

The *P. peroba* mini-clonal hedge, was formed from seedlings propagated by seeds collected from 20 matrix trees established in natural occurance in Linhares-ES municipality, situated at 19° 23′ S and 40° 04′ W and at 43 m altitude. Registration of sisgen for access to genetic patrimony, A796E13. The seeds were placed in polypropylene tubes, with a capacity of 280 cm^3, containing commercial substrate based on ground

Pinus bark, coconut powder, and mineral additives. A total of 8.0 kg m^{-3} of controlled release fertiliser (CRF) was added, which formulation was NPK 13-6-16, with a release time of 5 to 6 months. The productivity of the mini-clonal hedge at the time of beginning of the experiment was 4.7 shoots per mini-stump. The nutritional characterisation of the mini-stumps and the physicochemical characterisation of the substrate are presented in Tables 1 and 2 respectively using the methodology described in the study by Silva (2009).

After 120 days sowing, when the height of the seedlings was approximately 30 cm, seedlings were transplanted in 3.8 L polyethylene pots filled with commercial substrate added with 8.0 kg·m^{-3} of CRF. The density of plants was 30 pots per square meter, with an average productivity of 141 shoots m^{-2}. After transplantation, seedlings were placed in a shad house with 50% shading. Irrigation was performed by microaspertion that was triggered for 10 min, two times a day with an 8 mm·m^{-2}. During the experimental period, mean maximum temperatures of 28.3 ° C and minimum of 17.1 ° C, with relative humidity of 24.83%, obtained in an automatic station installed in the nursery. At 15 days after transplantation into the vessels, the stems of the seedlings were broken at a height of 10 cm from the base, which aimed to stimulate the occurrence of shoots, according to the procedure described in a study by Xavier et al. (2013). Fifteen days later, when mini-stumps had already produced new shoots, the previously broken part was removed using scissors. Thus, 30 days after transplantation, a mini-clonal hedge was formed with a total of 150 mini-stumps, which produced enough shoots in quantity and size to be used for the following experiments.

Experiment 1 – Substrates and IBA in the rhizogenesis of *Paratecoma peroba*

Experiment 1 was conducted in a completely randomised design in a 5 × 2 factorial arrangement, corresponding to five concentrations of IBA (0, 1, 2, 4, and 8 g·L^{-1}) and two substrates (sand and commercial substrate based on ground *Pinus* bark, coconut powder, and mineral additives) with four replications and 10 mini-cuttings per plot, totalling 400 experimental units.

For the preparation of the liquid solution, 0.1, 0.2, 0.4, and 0.8 g of the regulator was weighed on an analytical balance with a precision of 0.001g. The solutions were then diluted in 2 mL of 1 M potassium hydroxide and placed in a test tube, where distilled water was added until a volume of 100 mL was obtained.

The commercial substrate used in this experiment was the same that was used for the growth of forest clones (Table 2), whereas the sand substrate is the main material used in Brazil as sowing or as beds for seed germination and rooting of cuttings. The sand was sterilised at 127 °C in an autoclave for 60 min. Before the beginning of the experiment, the sand was had the followed chemical and physical attributes: pH in water: 8.2; electrical conductivity: 0.28 mS cm^{-1}; and density: 1.48 g·cm^{-3}.

The *P. peroba* shoots used for the preparation of the mini-cuttings were collected in the morning, owing to the lower temperature present at this time, using manual pruning shears and were kept in styrofoam boxes with water to maintain the ideal vigour and turgescence conditions. Subsequently, they were taken to the Laboratory for Propagation and Analyzes of Seedling Quality in preparation for the mini-cutting procedure.

The mini-cuttings were cutted to a size of 6 cm, in which the terminal bud and leaves with 100% of the original leaf area were maintained. After the mini-cuttings collected, they were disinfected by immersion for 2 min in 0.5% sodium hypochlorite, with subsequent washing under running water, and were then treated with a non-systemic fungicide (0.2%) as preventive action. Then, approximately 1.0 cm of the mini-cuttings were immersed in the corresponding concentrations of

TABLE 1 Nutritional characterisation of the mini-stumps of Paratecoma peroba.

N	P	K	Ca	Mg	S	B	Zn	Mn	Fe	Cu
g·kg^{-1}						mg·kg^{-1}				
29.40	4.75	34.52	7.60	3.45	1.41	51.42	18.60	53.15	300.67	5.21

TABLE 2 Physicochemical characterisation of the substrates.

pH	N	P	K	Ca	Mg	S	EC	Density
Commercial substrate								
H_2O	g·kg^{-1}						mS·cm^{-1}	g·cm^{-3}
6.2	11.5	9.0	5.2	15.0	7.2	4.5	0.8	0.35
Sand substrate								
H_2O	g·kg^{-1}						mS·cm^{-1}	g·cm^{-3}
8.2	-	-	-	-	-	-	0.28	1.48

pH =Hydrogenionic potential; EC = Electrical conductivity.

IBA for 5 s and inserted in 55 cm^3 tubes that contained the different substrates. Subsequently, the mini-cuttings were placed trays in a greenhouse under intermittent nebulisation, which was commanded by a digital controller, and connected to a temperature sensor and a relative humidity air sensor. The irrigation was triggered when the air temperature inside the greenhouse exceeded 30 °C or when the relative humidity was less than 80%.

After 60 days of stay in the greenhouse, the mini-cuttings were removed from the tubes and the substrate was completely removed by washing under running water for root observation. The following variables were measured: survival percentage, S (%), by counting the number of live mini-cuttings at the end of the experiment as a function of the number of staked mini-cuttings; rooting percentage, R (%), by counting the number of mini-cuttings rooted based on the number of live mini-cuttings; calli percentage, C (%), by counting the number of mini-cuttings with calli as a function of the number of live mini-cuttings; number of shoots (NS); number of roots, counting only the roots emitted directly from the base (NRB); and length of the root system (RL, cm) using a ruler.

The roots were then placed on a scanner model Epson Perfection 750 Pro at a resolution of 400 pixels. Once the images were scanned, they were submitted to the SAFIRA version 1.1 software program (Jorge and Silva, 2010) to quantify the superficial area of the roots (SA, cm^2) and root volume (RV, mm^3). To determine the dry mass of the aerial part (DMA, g) and dry mass of the roots (DMR, g) of the mini-cuttings, the plant material was divided into the aerial part and the roots, packaged separately in paper bags, and placed in a forced air circulation oven at a temperature of 65 °C for 72 h to reach a constant dry mass. DMA, DMR and total dry mass (TDM, g) were weighed on a precision balance with an accuracy of 0.001 g.

Experiment 2 - Types of mini-cuttings and leaf area in the rhizogenesis of *Paratecoma peroba*

In this experiment, three types of mini-cuttings were evaluated: 1. apical with length of 6 cm (6 AM); 2. intermediary with length of 4 cm (4 IM); and 3. complete apical with length of 10 cm (10 AM, which refers to the apical portion of 6 cm + the intermediary portion of 4 cm) and two leaf area sizes: 1. maintenance of the total leaf area (LA 100%) and 2. reduction of the leaf area to half of its total size (LA 50%). The experiment was conducted in a completely randomised design under a 3 × 2 factorial arrangement, with four replications and 10 mini-cuttings per plot, totalling 240 experimental units.

TABLE 3 Characterisation of the dry mass of the shoots, starch content, and starch accumulation in shoots of *Paratecoma peroba* (n = 30).

Mini-cuttings pattern	Dry mass of shoots (g)	Starch content (%)	Starch accumulation (mg)
6 AM - LA 100%	0.32	16.90	53.91
6 AM - LA 50%	0.13	14.00	18.20
4 IM - LA 100%	0.36	18.80	68.06
4 IM - LA 50%	0.21	17.10	35.40
10 AM - LA 100%	0.37	15.10	56.78
10 AM - LA 50%	0.32	17.30	56.40

6 AM - apical with length of 6 cm; 4 IM - intermediary with length of 4 cm; 10 AM - complete apical with length of 10 cm; LA 100% - maintenance of the total leaf area; LA 50% - reduction of the leaf area to half its total size.

Before the experiment was undertaken, 30 shoots of each treatment were collected to determine the dry mass of the shoots, the total starch content, and the starch accumulation in the mini-cuttings based on the different treatments. The total starch was determined according the methodology described in the study by Koakuzu et al. (2015). The accumulation was calculated by multiplying the dry mass of the shoots by the starch content (Table 3).

The mini-cuttings inserted planted in tubes with 55 cm^3 capacity, which had been previously disinfected with a 2% sodium hypochlorite solution, and that contained a commercial substrate based on *Pinus* bark (Table 2), without the application of IBA, based on the results obtained in Experiment 1. The measurments taken after 90 days (end of the experiment) were as follows: S (%), R (%), and C (%). Based on the average data of the productivity of the mini-clonal hedge (141 shoots m^{-2}) and the rooting data, the productivity index (PI = yield × rooting in decimal) was determined based on the methodology described by Rocha et al. (2015). NS, NRB, RL (cm), SA (cm^2), RV (mm^3), DMA (g), DMR (g), and TDM (g) were all determined.

Statistical analysis

Data were submitted to normality presupposition verification tests (Shapiro Wilk) and homogeneity (Bartlett test). The assumptions were met, and there was no need for data transformation. Then the data from both experiments were subjected to analysis of variance and, when verifying significant differences by the F test 5%. Variables means of substrate (Experiment 1), type of mini-cuttings and leaf area (Experiment 2) were compared by Tukey's test at the 5% and 1% probability levels. The concentrations of IBA (Experiment 1) were subjected to regression analysis to verify the optimum concentration for each variable, by means of the first derivative of the estimers β_0 and β_1. For the choice of equations, the significance of the betas was considered, the meaning and biological realism of the models (linear and quadratic), and the coefficient of determination (R^2). The analyses were performed using the statistical program R Core Team (2018), version 3.5.0., and package ExpDes.pt.

RESULTS

Substrates and IBA in the rhizogenesis of *Peratecoma peroba*

The *P. peroba* mini-cuttings had average of survival (S), calli (C), and number of shoots (NS) higher than 90%, 62%, and 2, respectively, with no influence from the different substrates or IBA concentrations. However, there was an isolated effect ($p \leq 0.01$) of the substrate type and IBA concentrations for rooting (R) in the mini-cuttings.

The R (%) of the *P. peroba* mini-cuttings that were grown in washed sand had lower values (5%) than that of the commercial substrate based on *Pinus* bark (64%) after 60 days (Figure 1a). The few individuals that were rooted in the washed sand prevented the continuity of the radicial system evaluations. There was a significant difference ($p \leq 0.01$) for IBA concentrations for R (%),

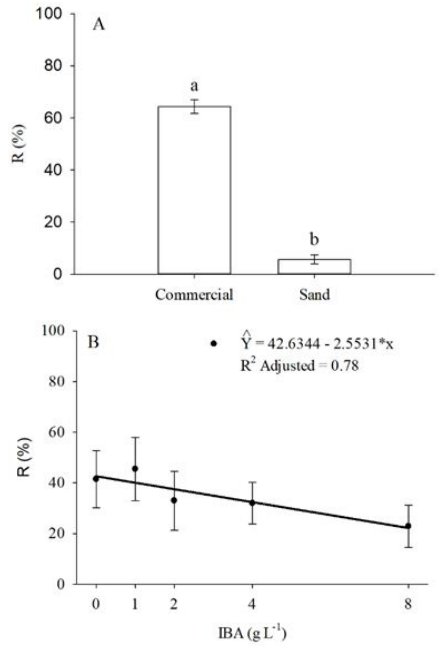

FIGURE 1 Rooting (R) of mini-cuttings of *Paratecoma peroba* after 60 days as a function of substrate and indole-3-butyric acid (IBA) concentration. Vertical bars stand for standard error.

showed that an increase in the concentrations on this growth regulator linearly decreased the rooting, with a maximum value of 43% obtained without any IBA application (Figure 1b).

For the mini-cuttings rooted in the commercial substrate, there was a significant effect ($p \leq 0.05$) for different IBA concentrations on number of roots (NRB). However, there was no significance in the regression models using the means test. The highest IBA concentrations were ineffective in promoting an increase in RL and alterations in root morphology as increase in

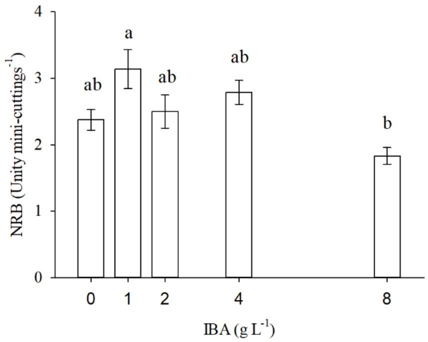

FIGURE 2 Number of roots emitted from the base (NRB) of the mini-cuttings of *Paratecoma peroba* after 60 days based on the concentration of indole-3-butyric acid (IBA). Vertical bars stand for standard error.

the the superficial area of the roots (SA) and root volume (RV) of the mini-cuttings, with no significant differences. There was no influence of IBA concentration on dry mass of the roots (DMR), dry mass of the aerial part (DMA), and dry mass of the roots (TDM) in *P. peroba* mini-cuttings.

The IBA concentrations of 0, 1, 2, and 4 $g \cdot L^{-1}$ presented the best results regarding the NRB of the *P. peroba* mini-cuttings; however, there was no significant differences among these different concentrations (Figure 2).

Type of mini-cuttings and leaves in the rhizogenesis rhizome of *Paratecoma peroba*

After 90 days, there was a significant interaction ($p \leq 0.05$) between the mini-cuttings and leaf area for the MSPA variable. There was an individual effect of the type of mini-cuttings for S (%), C (%), PI, NS, NRB, and TDM. An isolated effect of leaf area was also observed for SA, RV, DMR, and TDM. The RL variable was not influenced by the isolated factors or by any interactions among them.

The 4 IM and 10 AM mini-cuttings presented higher averages of S (%), R (%), and C (%) than 6 AM mini-cuttings after 90 days (Figure 3a, b, c). Considering the productivity of the mini-clonal hedge and R (%) for

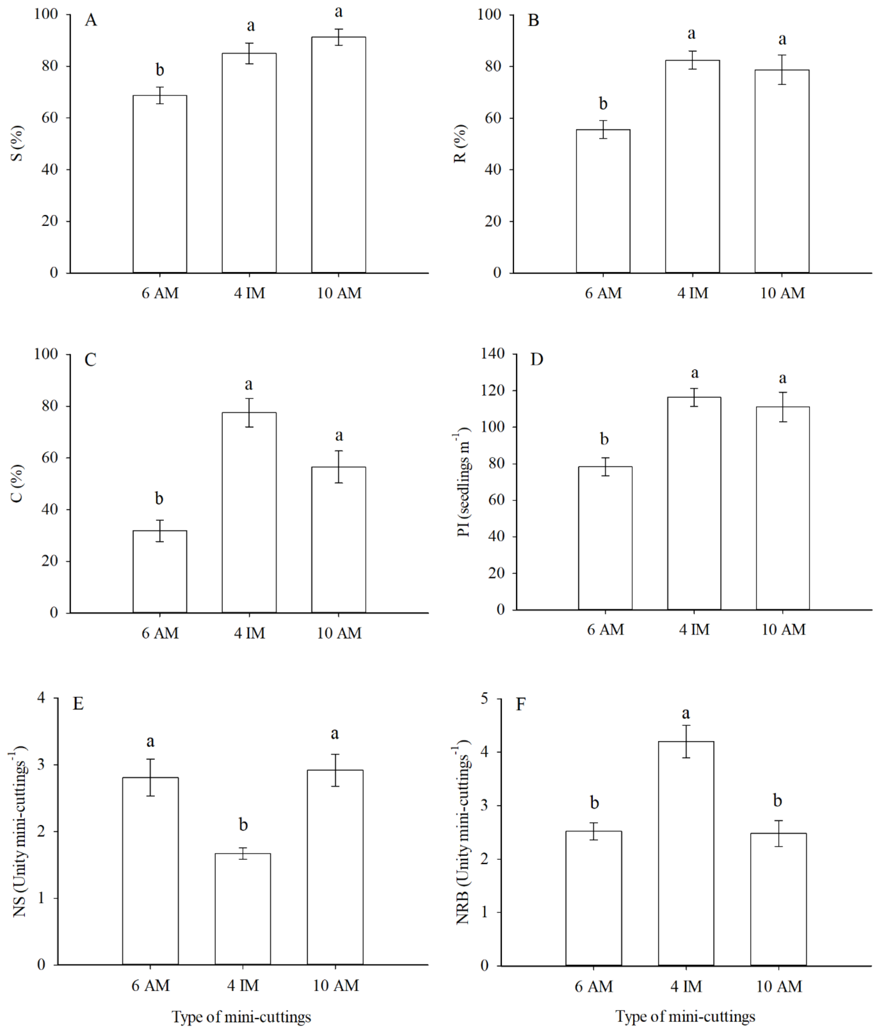

FIGURE 3 N(A) S - survival; (B) R - rooting; (C) C - calli; (D) IP - productivity index; (E) NS - number of shoots; and (F) NRB - number of roots of *Paratecoma peroba* mini-cuttings after 90 days. 6 AM - apical with length of 6 cm; 4 IM - intermediary with length of 4 cm; 10 AM - complete apical with length of 10 cm. Vertical bars stand for standard error.

each type of mini-cutting, the PI of the rooted clones presented higher values when using 4 IM and 10 AM mini-cuttings. In relation to NS, the 4 IM mini-cuttings had lower averages (Figure 3e).

In the evaluation of the root system, NRB was influenced by the size of the mini-cutting, with the highest average shown in the 4 IM mini-cuttings (Figure 3f). The *P. peroba* mini-cuttings with an intact leaf area showed higher growth and vigour for radicial system, which was verified by the higher averages of SA, RV, and DMR (Figure 4a, b, c).

For DMA, the 10 AM and 4 IM mini-cuttings with LA 100% had the highest averages (Figure 5a). In relation to TDM, there was an isolated treatment effect, where the 4 IM and 10 AM mini-cuttings showed the highest TDM production of the *P. peroba* mini-cuttings (Figure 5b). The LA 50% mini-cuttings had the lowest TDM, which was the same trend as that shown for DMR (Figure 5c).

DISCUSSION

Substrates and IBA in rhizogenesis of *Paratecoma peroba*

The first hypothesis tested was validated, i.e. *P. peroba* can propagate vegetatively by the mini-cutting technique, with satisfactory percentages of survival and rooting, and crucial variables, and considered as the most important in the study of mini-cutting technique. The S (%) and R (%) of the *P. peroba* mini-cuttings were similar to other species of the family Bignoniaceae such as *Handroanthus heptaphyllus* (Freitas et al., 2016) and *Tecoma stans* (Biondi et al., 2008), which showed greater than 80% survival and 50% rooting of mini-cuttings.

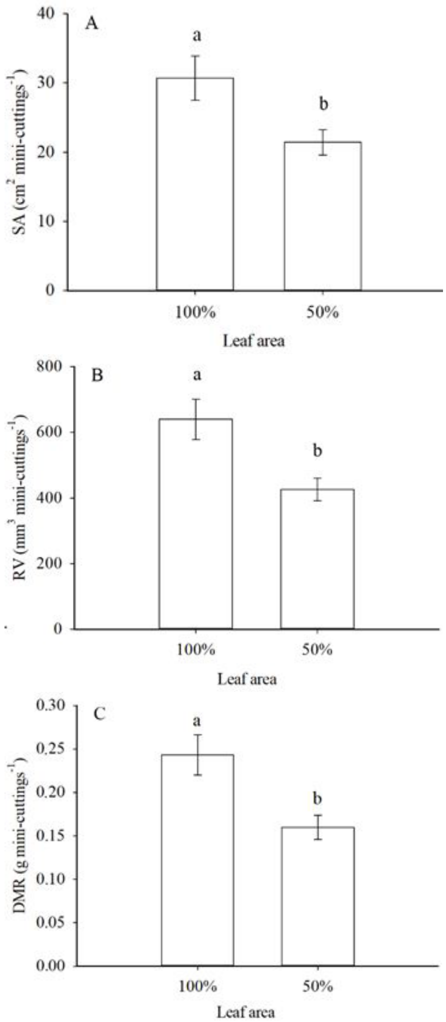

FIGURE 4 (A) SA - Root surface area; (B) RV - root volume; and (C) DMR - root dry mass of *Paratecoma peroba* mini-cuttings after 90 days as a function of leaf area. LA 100% - maintenance of the total leaf area; LA 50% - reduction of the leaf area to half its total size. Vertical bars stand for standard error.

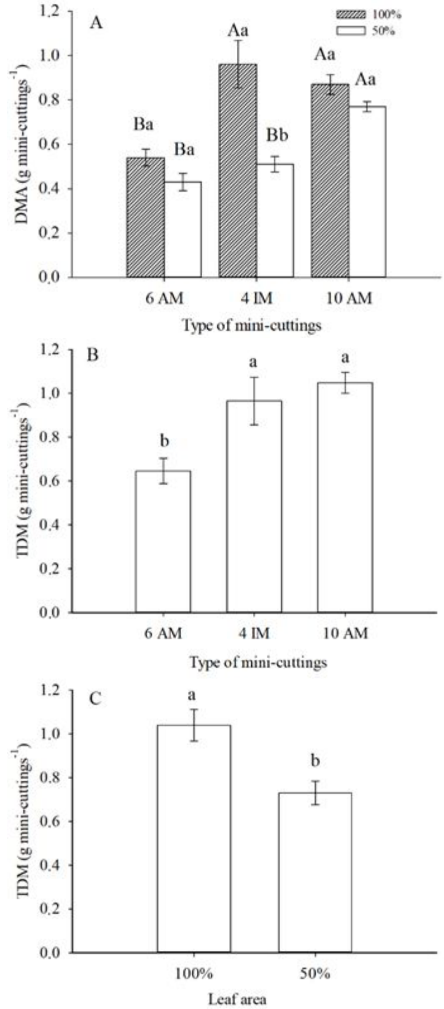

FIGURE 5 (A) DMA - Aerial part dry mass; (B-C) TDM - total dry mass of *Paratecoma peroba* mini-cuttings after 90 days based on the type of mini-cutting and leaf area. Uppercase letters compare the types of mini-cutting by Tukey's test ($p \leq 0.05$). Lowercase letters compare the leaf area within the mini-cutting type. 6 AM - apical with length of 6 cm; 4 IM - intermediary with length of 4 cm; 10 AM - complete apical with length of 10 cm. LA 100% - maintenance of the total leaf area; LA 50% - reduction of the leaf area to half its total size. Vertical bars stand for standard error.

The hypothesis that the substrate influences on the rooting of the *P. peroba* mini-cuttings was also confirmed. The discrepancies among the rooting of *P. peroba* mini-cuttings in different substrates might be linked to the different physical characteristics of the substrates. The washed sand had an apparent density that was four times higher (1.48 $g{\cdot}cm^{-3}$) than that of the commercial substrate (0.35 $g{\cdot}cm^{-3}$), which may have exerted a mechanical resistance or impediment for the formation and penetration of the root system. The inadequate rooting of *P. peroba* in sand might pose a problem for clones producers of this species. Pimentel et al. (2016) demonstrated that substrates with lower densities showed higher porosity and lower water retention capacity, which allowed greater rooting capacity of *Handroanthus heptaphyllus* mini-cuttings. Mendoza-Hernández et al. (2014) stated that the rooting substrate should have an apparent density between 0.3 and 0.8 g cm^{-3}. For the validation of this hypothesis, other materials used as substrates should be evaluated, with different densities and chemical attributes that are easy for the producer to acquire, are inexpensive, have high quality, and are easy to use.

Contrary to the third hypothesis, IBA did not increase the formation of adventitious roots of the

P. peroba mini-cuttings cultivated in the commercial substrate. Higher concentrations of this growth regulator promoted a decrease in R (%). With the exception of NRB, there were no changes in the growth and quality of the root system formed from the mini-cuttings grown in the different IBA concentrations.

Quantification of auxin is not a widely calculated in rooting studies owing to its low concentration in tissues and the interference of other compounds during the analysis. However, studies have shown a correlation between endogenous auxin levels and rooting capacity (Stuepp et al., 2017). It can be inferred that the linear reduction of R (%) owing to the concomitant increase of IBA concentrations might be linked to the degree of juvenility of the matrix plant, where the concentration of endogenous auxin (indol-3-acetic acid) of the shoots might be at sufficient levels to stimulate the emission of adventitious roots. The fact that the shoots came from mini-stumps that originated from seedlings produced by seeds (juvenile material) also confirms this hypothesis, as reported by Wendling et al. (2015).

Auxin is an essential hormone for the induction and formation of roots (Atangana et al., 2006; Daskalakis et al., 2018). However, its exogenous application has been questioned in previous studies of the mini-cutting technique of native species, using juvenile propagules, where the rooting of mini-cuttings with IBA is not favoured, and might actually be harmful to the induction of root beginnings, as for *P. peroba*. This tendency has been corroborated for *Cedrela fissilis* (Xavier et al., 2003), *Anadenanthera macrocarpa* (Dias et al., 2012), *Araucaria angustifolia* (Pires et al., 2013)*este trabalho objetivou avaliar a influência de concentrações de ácido indolbutírico (AIB*, *Tibouchina sellowiana* (Fragoso et al., 2017), *Peltophorum dubium* (Mantovani et al., 2017), and *Plathymenia reticulata* (Pessanha et al., 2018). The application of high concentrations of synthetic auxin may lead to toxicity owing to hormonal imbalance (Rinaldi et al., 2018). This imbalance leads to many propagules reserves not being directed to the formation of adventitious roots (Lima et al., 2006). Therefore, given the negative effect on rooting and the association with the purchase, preparation, application, and storage of IBA. The use of IBA in the concentrations utilised for the rooting of *P. peroba* mini-cuttings in the present study is not recommended.

Vegetative propagation is an important tecniques means for conserving the germplasm of native and threatened species (Azad et al., 2018; Guo et al., 2017), and is the first stage that must be overcome for the domestication of species that have high economic potential (Atangana et al., 2006; Guimarães et al., 2019) stakes with 50% of leaflets cut from their original size showed 2.5% of rooting whereas those with 6 leaflets showed 22.5% of rooting. Stakes from pruned apical branches exhibited 21.2% of rooting. Both callogenesis and rooting of pequi stakes demonstrated the potential of this particular species from the Cerrado (Brazilian savanna. Thus, the mini-cutting is a viable technique to overcome the difficulties of producing *P. peroba* clones from seeds. This technique allows seedling to be obtained for restoration projects in the Atlantic Forest, agroforestry systems, and homogeneous commercial plantations, so long as important factors regarding the maintenance of genetic variability are observed, such as the use of sprouts from a larger number of plants possible, so that they are representative of the population.

Types of mini-cutting and leaves in the rhizogenesis of *Paratecoma peroba*

In general, vegetative propagation using the mini-cutting technique for *P. peroba* is possible regardless of the pattern adopted for the type of the mini-cuttings. The segmentation of the 10 AM mini-cuttings into two additional mini-cuttings of 4 IM and 6 AM, in theory, should increase the production of rooted clones of *P. peroba*; however, this depends on the degree of rooting of each type of propagule. Using the PI as a reference, from all the apical 10 AM mini-cuttings, it would be possible to produce 111 clones m^{-2}, whereas the production of rooted clones originating from the 4 IM and 6 AM groups would create 194 m^{-2} clones; thus, optimising the productivity of the mini-garden of *P. peroba*.

However, the pattern of the mini-cuttings influenced the quality of the root system and the growth of the different components, which confirmed the fourth hypothesis being tested. Mini-cuttings of 10 AM and 4 IM showed superior performance of S (%) and R (%), and were prone to the formation of more vigourous clones, thus presenting better TDM results, compared to treatment 6 AM. The better rooting found in the 4 IM and 10 AM mini-cuttings is related to the greater accumulation of starch in these portions (Table 3).

The rooting process is slow, demanding days and even months for completion, which promotes high energy expenditure and consumption of propagule reserves. Thus, insoluble carbohydrates, such as starch, potentiate rooting and improve the effect of auxins (Hartmann et al., 2011). The positive effect of sugars on rooting has also been attributed to the fact that glucose-

6-phosphate and glucose are capable of gluing (chemical reaction in which a carbohydrate is added to another molecule) in the DNA, and thus alter the transcription, performing an important role in regulating root initiation (Denaxa et al., 2012).

The intermediary mini-cuttings presented higher C (%), but they did not have a negative influence on R (%). The calli is a tumour tissue, which usually appears on wounds of differentiated organs and tissues, and has the ability to differentiate forming tissues as roots (Mayer et al., 2018). According to a study by Hartmann et al. (2011), the formation of calli and roots are independent processes for most plants, although in some plants, calli formation may or may not be a precursor to root formation.

Although the intermediary mini-cuttings presented lower NS, they were well developed by the end of the 90-day experiment, reflecting the increase of DMA and TDM. These showed a strong tendency of bifurcation in view of the prominent growth of these shoots of lateral buds just below the upper cut of the mini-cuttings. This characteristic is undesirable for the production of quality clones for timber production in commercial plantations, where there is a preference for individuals with a straight trunk. However, for use in recovering degraded areas, this is not a limiting factor. To establish commercial forests of *P. peroba*, apical mini-cuttings of 10 cm in length from clones with straight stems that have better morphological conformity and a higher-quality standard should be use.

The types of mini-cutting did not influence the rooting of *Handroanthus heptaphyllus* (Freitas et al., 2016) and *Toona ciliata* (Ferreira et al., 2012). Yet, in *Anadenanthera macrocarpa* (Dias et al., 2015), *Melaleuca alternifolia* (Oliveira et al., 2012), and *Peltophorum dubium* (Mantovani et al., 2017) apical mini-cuttings were superior for rooting.

The maintenance of 100% of the leaf area positively influenced the growth and radicial architecture, presenting higher averages of SA, RV, and DMR. A well-formed and robust root system can increase the chances of survival and clones establishment under field conditions (Barroso et al., 2018). The presence of leaves is essential for the rooting of mini-cuttings because the young leaves and buds are synthesis sites for auxins and cofactors that are translocated via phloem to the base of the stalk, stimulating the exchange activity, differentiation of the xylem, and root initiation (Hartmann et al., 2011). The maintenance of leaves, especially the basal or subapical leaves, promote root formation. This positive effect is caused by the higher photosynthetic rate provided by the larger leaf area and a higher accumulation of carbohydrates (Batista et al., 2014; Souza et al., 2013). Given the superiority of the quality of the root system, it is recommended to maintain the entire leaf for *P. peroba* mini-cuttings.

A reduction of leaf area is adopted to reduce transpiration and avoid the 'umbrella effect', which can reduce irrigation efficiency (Alfenas et al., 2009; Xavier et al., 2013). Previous studies have shown that the maintenance of whole leaves provided maximisation of the rooting of mini-cuttings of *Eucalyptus dunni* clones, *E. saligna* and *E. urophylla* × *E. globulus* (Batista et al., 2014); *Eucalyptus grandis* × *E. urophylla* (Fernandes et al., 2018) and *Eucalyptus benthamii* (Mayer et al., 2018). Dias et al. (2015) found that the presence of whole leaves led to significant gains in survival, rooting, and number of mini-cutting roots of Anadenanthera macrocarpa. These authors affirm that this practice reduces the risk of lesions and contamination in the leaves and also increases the operational yield, because there is a decrease in repetitive movements for leaf cutting, increasing daily production per person.

Therefore, in view of the ease of rooting of *P. peroba* due to the factors evaluated in the present study, the mini-cutting technique for clone production is recommended as an alternative form to seed propagation. For the vegetative production of *P. peroba* clones to be technically feasible and environmentally responsible for restoration projects in the Atlantic Forest, it is recommended to use as large a number of matrices as possible in the mini-clonal hedge to cover the highest possible genetic variability. For commercial purposes, mini-cuttings of 10 cm in length with 100% leaf area are recommended, in this case, harvested in mini-clonal hedge originating from cloning of upper trees.

CONCLUSION

The mini-cutting technique using shoots originating from seedlings produced from seeds is technically feasible for the vegetative propagation of *P. peroba* independently of the mini-cutting pattern and without the addition of IBA. The substrate exerts influence on rooting, not being takeout sand. The entire apical mini-cuttings of 10 cm in length are adequate for clone's production and the maintenance of the total leaf area promotes a greater vigour of the root system of *P. peroba* clones.

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REFERENCES

ALFENAS, A.C.; ZAUZA, A.A. V.; MAFIA, R.G.; FRANCISCO DE A, T. **Clonagem e doenças do eucalipto**. Viçosa, MG: Universidade Federal de Viçosa, 2009. 500p.

ARCHANJO, K.M.P. DE A.; SILVA, G.F. DA; CHICHORRO, J.F.; SOARES, C.P.B. Estrutura do componente arbóreo da reserva particular do patrimônio natural cafundó, Cachoeiro de Itapemirim, Espírito Santo, Brasil. **Floresta**, v. 42, n. 1, p. 145-160, 2012.

ATANGANA, A.R.; TCHOUNDJEU, Z.; ASAAH, E.K.; SIMONS, A.J.; KHASA, D.P. Domestication of *Allanblackia floribunda*: Amenability to vegetative propagation. **Forest Ecology and Management**, v. 237, n. 1-3, p. 246-251, 2006.

AZAD, M.S.; ALAM, M.J.; MOLLICK, A.S.; KHAN, M.N.I. Rooting of cuttings of the wild Indian almond tree (*Sterculia foetida*) enhanced by the application of indole-3-butyric acid (IBA) under leafy and non-leafy conditions. **Rhizosphere**, v. 5, p. 8-15, 2018.

BARROSO, D.G.; OLIVEIRA, T.P.F.; SIQUEIRA, D.P.; LAMÔNICA, K.R.; CARVALHO, G.C.M.W. Mini-stumps productivity and rooting of *Khaya ivorensis* A. Chev mini-cuttings treated with IBA. **Cerne**, v. 24, n. 2, p. 114-120, 2018.

BATISTA, A.F.; SANTOS, G.A.; SILVA, L.D.; QUEVEDO, F.F.; ASSIS, T.F. Influência da arquitetura foliar de miniestacas na propagação clonal de *Eucalyptus*. **Revista Árvore**, v. 38, n. 5, p. 819-827, 2014.

BIONDI, D.; BREDOW, E.A.; LEAL, L. Influência do diâmetro de estacas no enraizamento de *Tecoma stans* (L.) Juss. ex Kunth. **Semina: Ciências Agrárias**, v. 29, n. 2, p. 277-281, 2008.

BRASIL. MINISTÉRIO DO MEIO AMBIENTE - MMA. **Acordo de Paris**. Available at: http://www.mma.gov.br/clima/convencao-das-nacoes-unidas/acordo-de-paris. Accessed in: March 11th 2019.

BRONDANI, G.E.; BACCARIN, F.J.B.; BERGONCI, T.; GONÇALVES, A.N.; ALMEIDA, M. *Eucalyptus benthamii*: efeito do genótipo, AIB, zinco, boro e coletas de brotações. **Cerne**, v. 20, n. 1, p. 147-156, 2014.

BURIN, C.; BISOGNIN, D.A.; LENCINA, K.H.; GIMENES, E.S. Early selection of *Cabralea canjerana* for propagation by mini-cutting. **Pesquisa Agropecuária Brasileira**, v. 53, n. 9, p. 1018-1024, 2018.

CNCFlora. *Paratecoma peroba* in Lista Vermelha da flora brasileira versão 2012.2 Centro Nacional de Conservação da Flora. Available at: http://cncflora.jbrj.gov.br/portal/pt-br/profile/Paratecoma peroba. Accessed in: January 22th 2019.

DASKALAKIS, I.; BINIARI, K.; BOUZA, D.; STAVRAKAKI, M. The effect that indolebutyric acid (IBA) and position of cane segment have on the rooting of cuttings from grapevine rootstocks and from Cabernet franc (*Vitis vinifera* L.) under conditions of a hydroponic culture system. **Scientia horticulturae**, v. 227, p. 79-84, 2018.

DENAXA, N.-K.; VEMMOS, S.N.; ROUSSOS, P.A. The role of endogenous carbohydrates and seasonal variation in rooting ability of cuttings of an easy and a hard to root olive cultivars (*Olea europaea* L.). **Scientia Horticulturae**, v. 143, p. 19-28, 2012.

DIAS, P.C.; XAVIER, A.; OLIVEIRA, L.S.; CORREIA, A.C.G.; BARBOSA, G.A. Tipo de miniestaca e de substrato na propagação vegetativa de angico-vermelho (*Anadenanthera macrocarpa* (Benth.) Brenan). **Ciência Florestal**, v. 25, n. 4, p. 909-919, 2015.

DIAS, P.C.; XAVIER, A.; OLIVEIRA, L.S.; PAIVA, H.N.; CORREIA, A.C.G. Propagação vegetativa de progênies de meios-irmãos de angico-vermelho (*Anadenanthera macrocarpa* (Benth) Brenan) por miniestaquia. **Revista Árvore**, v. 36, n. 3, p. 389-399, 2012.

EUGENIO, F.C.; SANTOS, A.R; FIEDLER, N.C.; RIBEIRO, G.A.; DA SILVA, A.G.; JUVANHOL, R.S.; SCHETTINO, V.R.; MARCATTI, G.E.; DOMINGUES, G.F.; ALVES DOS SANTOS, G.M.A.D.; PEZZOPANE, J.E.M.; PEDRA, B.D.; BANHOS, A., MARTINS, L.D. GIS applied to location of fires detection towers in domain area of tropical forest. **Science of the Total Environment**, v. 562, p. 542-549, 2016.

FERNANDES, S.J.O.; SANTANA, R.C.; SILVA, E.B.; SOUZA, C.M.P.; SILVA, C.T. Período de enraizamento de miniestacas de eucalipto provenientes de diferentes lâminas de irrigação em minijardim. **Ciência Florestal**, v. 28, n. 2, p. 591-600, 2018.

FERREIRA, D.D.A.; BARROSO, D.G.; SILVA, M.P.S.; SOUZA, J.S.; FREITAS, T.A.S. DE, CARNEIRO, J.G.A. Influência da posição das miniestacas na qualidade de mudas de cedro australiano e no seu desempenho inicial no pós-plantio. **Ciência Florestal**, v. 22, n. 4, p. 715-723, 2012.

FRAGOSO, R.O.; STUEPP, C.A.; SÁ, F.P.; KRATZ, D.; ZUFFELLATO-RIBAS, K.C.; WENDLING, I. Vegetative rescue and ex vitro system production of *Tibouchina sellowiana* clonal plants by cutting and mini-cutting. **Ciência Rural**, v. 47, n. 11, p.1-9, 2017.

FREITAS, T.P.; BARROSO, D.G.; LAMÔNICA, K.R.; CARVALHO, G.C.M.W. Aplicação de AIB e tipo de miniestacas na produção de mudas de *Handroanthus heptaphyllus* Mattos. **Ciência Florestal**, v. 26, n. 1, p. 313-320, 2016.

GUIMARÃES, R.N.; SOUZA, E.R.B.; NAVES, R.V.; MELO, A.P.C.; RUBIO NETO, A. Vegetative propagation of pequi (souari nut) by cutting. **Ciência Rural**, v. 49, n. 2, p. 1-6, 2019.

GUO, L.; SHAO, X.; XUE, P.; TIAN, Y.; XIAO, Z.; WU, Y. Root sprouting ability and growth dynamics of the rootsuckers of *Emmenopterys henryi*, a rare and endangered plant endemic to China. **Forest ecology and management**, v. 389, p. 35-45, 2017.

HARTMANN, H.T.; KESTER, D.E.; DAVIES, F.T.J.; GENEVE, R.L. **Plant propagation**: principles and practices. 8th ed. New Jersey: Prentice-Hall, 2011. 915p.

JORGE, L.A.C., SILVA, D.J.C.B. **SAFIRA - Software para a análise de fibras e raízes**. Versão 1.1. São Carlos: Embrapa Instrumentação Agropecuária, 2010. https://www.alice.cnptia.embrapa.br/bitstream/doc/884593/1/manualsafira2013.PDF Accessed in: January 5th 2019.

KOAKUZU, S.N.; ARAÚJO, E. J.; BASSINELO., P. Z.; CARVALHO, R. N.; TEIXEIRA, M. C. **Procedimento para determinação de amido resistente, amido não resistente (solúvel) e total**. Comunicado Técnico 228. Santo Antônio de Goiás, GO: Embrapa Informação Tecnológica, 2015. 8 p.

LIMA, R. L.S.; SIQUEIRA, D.L.; WEBER, O.B.; CAZETTA, J.O. Comprimento de estacas e parte do ramo na formação de mudas de aceroleira. **Revista Brasileira de Fruticultura**, v. 28, n. 1, p. 83-86, 2006.

LINS, B.L.A.; NASCIMENTO, M.T. Fenologia de *Paratecoma peroba* (Bignoniaceae) em uma floresta estacional semidecidual do norte fluminense, Brasil. **Rodriguésia**, v. 61, n. 3, 2010.

LORENZI, H. **Árvores brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil.** 3th ed. Nova Odessa: Instituto Plantarum, 2009, 384p.

MANTOVANI, N.; ROVEDA, M.; TRES, L.; FORTES, F.D.O.; GRANDO, M.F. Cultivo de canafístula (*Peltophorum dubium*) em minijardim clonal e propagação por miniestacas. **Ciência Florestal**, v. 27, n. 1, p. 225-236, 2017.

MAYER, E.L.P.; PERES, F.B.; TAMBARUSSI, E.V. Arquitetura foliar de miniestacas e aplicação de biofertilizante à base de aminoácidos na miniestaquia de *Eucalyptus benthamii* Maiden & Cambage. **Scientia Forestalis**, v. 46, n. 118, p. 251-260, 2018.

MENDOZA-HERNÁNDEZ, D.; FORNES, F.; BELDA, R.M. Compost and vermicompost of horticultural waste as substrates for cutting rooting and growth of rosemary. **Scientia Horticulturae**, v. 178, p. 192-202, 2014.

OLIVEIRA, Y.; ALCANTARA, G.B.; GUEDES, I.; PINTO, F.; QUOIRIN, M.; BIASI, L.A. Substratos, concentrações de ácido indolbutírico e tipos de miniestacas no enraizamento de melaleuca (Melaleuca alternifólia Cheel). **Revista Brasileira de Plantas Medicinais**, v. 14, n. 4, p. 611-616, 2012.

PESSANHA, S.E.G.L.; BARROSO, D.G.; BARROS, T.C.; OLIVEIRA, T.P. F.; CARVALHO, G.C.M.W.; CUNHA, M. Limitações na produção de vinhático (*Plathymenia reticulata* Benth) por miniestaquia. **Ciência Florestal**, v. 28, n. 4, p. 1688-1703, 2018.

PIMENTEL, N.; BISOGNIN, D.A.; KIELSE, P.; LENCINA, K.H.; MELLO, U.S. Shoot segment and substrate composition in rooting of juvenile ipe-roxo mini-cuttings. **Ciência Rural**, v. 46, n. 6, p. 996-1002, 2016.

PIRES, P.P.; WENDLING, I.; BRONDANI, G. Ácido indolbutírico e ortotropismo na miniestaquia de *Araucaria angustifolia*. **Revista Árvore**, v. 37, n. 3, p. 393-399, 2013.

R DEVELOPMENT CORE TEAM. **R**: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing, 2011. Available at: <http://www.r-project.org/>. Accessed in: January 22th 2019.

RINALDI, A.R.; VILLA, F.; SILVA, D.F.; YASSUE, R.M. Stem cuttings and substrates in Dovyalis asexual propagation. **Comunicata Scientiae**, v. 8, n. 4, p. 587-595, 2017.

ROCHA, J.H.T.; BACKES, C.B.; BORELLI, K.; PRIETO, M.R.; SANTOS, A.J.M.; GODINHO, T.D.O. Produtividade do minijardim e qualidade de miniestacas de um clone híbrido de *Eucalyptus grandis* x *Eucalyptus urophylla* (I-224) em função de doses de nitrogênio. **Ciência Florestal**, v. 25, n. 2, p. 273-279, 2015.

SILVA, F.C.D.A.S. **Manual de análises químicas de solos, plantas e fertilizantes**. 2. ed. Brasília, DF: Embrapa Informação Tecnológica, 2009. 627 p.

SOUZA, C.C.; XAVIER, A.; LEITE, F.P.; SANTANA, R.C.; LEITE, H.G. Padrões de miniestacas e sazonalidade na produção de mudas clonais de *Eucalyptus grandis* HILL X *E. urophylla* ST Black. **Revista Árvore**, v. 37, n. 1, p.67-77, 2013.

STUEPP, C.; WENDLING, I.; TRUEMAN, S.; KOEHLER, H.; ZUFFELLATO-RIBAS, K. The use of auxin quantification for understanding clonal tree propagation. **Forests**, v. 8, n. 1, p. 27, 2017.

WENDLING, I.; BROOKS, P.R.; TRUEMAN, S.J. Topophysis in *Corymbia torelliana* × *C. citriodora* seedlings: Adventitious rooting capacity, stem anatomy, and auxin and abscisic acid concentrations. **New forests**, v. 46, n. 1, p. 107-120, 2015.

XAVIER, A.; SANTOS, G.A.; WENDLING, I.; OLIVEIRA, M.L. Enraizamento de miniestaca caulinar e foliar na propagação vegetativa de cedro-rosa (Cedrela fissilis Vell.). **Revista Árvore**, v. 27, n. 3, p. 351-356, 2003.

XAVIER, A.; WENDLING, I.; SILVA, R.L. **Silvicultura clonal:** princípios e técnicas. 2ª ed. Viçosa, UFV, 2013, 280p.