Vegetative propagation of *Richeria grandis* Vahl. by mini-cutting

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ABSTRACT: Due to deforestation in Gallery Forests, the current environmental law requires their restoration. Using only seminal propagation for native species of this environment has limited the production of seedlings as the seeds of some species often have limiting factors. *Richeria grandis* Vahl., besides being a characteristic species of this environment, has high value in phytosociological importance; however, it has recalcitrant seeds. In view of this, the objective was to evaluate the rooting of *R. grandis* Vahl. mini-cuttings under the influence of indolebutyric acid (IBA) concentrations. Apical mini-cuttings were prepared with length of 8 ± 2 cm and two pairs of leaves with their leaf area reduced by 50%. For rooting, a mixture of 70% vermiculite and 30% carbonized rice husk with fertilizer was used as substrate. As treatments, four IBA concentrations were tested: 0; 2,000; 4,000; and 6,000 mg L^{-1}, liquid in solution, for 10 seconds. The rooting of mini-cuttings was conducted in a greenhouse, for a period of 90 days. According to the results, this technique is feasible, since there was 100% survival and a high percentage of rooting, with the control treatment having the lowest value (85%). Calluses were found in non-rooted mini-cuttings and, using IBA, there was a tendency to increase the mean number of roots, sprouting percentage and mean sprouts per treatment.

Key words: Gallery Forests; indolebutyric acid; recalcitrant; rooting; seedling production

Propagação vegetativa de *Richeria grandis* Vahl. por miniestaquia

RESUMO: Devido ao desmatamento em Matas de Galeria, a lei ambiental vigente exige a restauração destas áreas. Apenas o uso da propagação seminal para espécies nativas deste ambiente tem limitado a produção de mudas, visto que sementes de algumas espécies muitas vezes possuem algum fator limitante. *Richeria grandis* Vahl. além de ser uma espécie característica deste ambiente, apresenta alto valor de importância fitossociológica, no entanto, suas sementes são recalcitrantes. Diante disto, objetivou-se avaliar o enraizamento de miniestacas de *R. grandis* Vahl. sob influência de concentrações de ácido indolbutírico (AIB). As miniestacas apicais foram preparadas com comprimento de 8 ± 2 cm e dois pares de folhas reduzidas em 50% a área foliar. Para o enraizamento, foi utilizado como substrato, a mistura de 70% de vermiculita e 30% de casca de arroz carbonizada com adição de fertilizante. Como tratamentos, foram testadas quatro concentrações de AIB: 0; 2.000, 4.000 e 6.000 mg L^{-1}, via líquida, durante 10 segundos. O processo de enraizamento das miniestacas foi conduzido em casa de vegetação, por um período de 90 dias. De acordo com os resultados, esta técnica é viável, uma vez que houve 100% de sobrevivência e alta porcentagem de enraizamento, sendo o tratamento controle com o menor valor (85%). Observou-se a presença de calos em miniestacas não enraizadas e, com o uso de AIB, houve tendência de aumento do número médio de raízes, porcentagem de brotação e média de brotos por tratamento.

Palavras-chave: Matas de Galeria; ácido indolbutírico; recalcitrante; enraizamento; produção de mudas

Introduction

Tropical forests have already lost much of their original covered area due to the great exploratory process over the years, in especially those that are along springs and watercourses, such as the Gallery Forests (Silva, 2015). This happens even though these said forests are protected by Brazilian legislation such as the Permanent Preservation Areas – APP (initials in Portuguese) (Law No. 12,651 of 25/05/2012; amended by Law No. 12,727 of 17/10/2012; CONAMA Resolutions 302/02 and 303/02).

The Gallery Forests have high genetic diversity of plant species, with rare species predominating over few conspicuous species, well adapted to this stress environment (Dietzsch et al., 2006). In addition to this, they also stand out for contributing to the maintenance, protection and quality of water resources and assist the conservation of wild fauna acting as ecological corridors (Oliveira et al., 2015; Nogueira, 2016).

Regarding the high deforestation rate of these APPs, the Federal law ensure their control and inspection, establishing recovery goals and guidelines through the restoration of these degraded areas. One of the restoration techniques used is the planting of native seedlings occurring in the areas to be restored (Martins, 2015), and for that matter, bettering the propagation techniques for native species will contribute in advancing the forestry in several different sectors, including the restoration of degraded areas and environmental preservation (Fonseca, 2016).

However, most studies concerning the propagation of native forest species are related to the sexual propagation, yet its use has limited the seedlings commercial production, since the seeds of most woody species have some limiting factors (Ferriani et al., 2010), mainly for recalcitrant and late secondary species. It is worth mentioning that the species that produce desiccation-sensitive seeds correspond to from 7.5 to 19.6% of the plant species in the world, and in tropical forests, which includes the Gallery Forests, the contribution to this percentage is expressive (Wyse & Dickie, 2017).

Despite the diversity of woody species in the Brazilian flora, there are few studies with vegetative propagation (Hernández et al., 2013; Fonseca, 2016). Vegetative propagation techniques, including the mini-cuttings, are a promising alternative for overcoming any difficulties in seedling production (Oliveira & Ribeiro, 2013), and are one of the main processes for producing good quality seedlings (Oliveira & Ribeiro, 2013).

The species studied was the *Richeria grandis* Vahl. (Phyllanthaceae), an evergreen tree, non-pioneer, widely geographical distributed and having a unique environmental specificity (Rabinowitz, 1981). Considered as one of the most representative species of riparian environments, the species is characterized by hydromorphic and dystrophic soils (Costa, 2017), mainly by Ciliary and Gallery Forests (Cerqueira et al. 2016; Costa, 2017; Moura, 2017). It has morphophysiological adaptations to withstand the physical conditions and low oxygenation of the soil in these said locations (Bambi et al.,

2016), and is responsible for contributing of over 50% of the litter biomass, thus acting as determining for the stability of these ecosystems (Rezende et al., 2017; Calderón et al. 2019). Furthermore, it is an accumulator species of Al^{3+} (Cuenca et al., 1991), a metal regarded as toxic, when in high concentrations, to both plant growth and development.

Bearing this in mind, the need of incorporating the said species in programs for restoring this phytocenosis is justified. However, its seeds, in addition to their recalcitrant nature, which means losing their total viability only in a few days of storage, are also not produced annually (Moura, 2017).

In light of the foregoing, the objective of this study was to evaluate the viability of the vegetative propagation of *Richeria grandis* Vahl. mini-cuttings under the influence of different indolbutyric acid (IBA) concentrations.

Materials and Methods

The study was conducted in a greenhouse (covered with a 150-μ-thick plastic film and a 50% shading screen) located at the Integrated Center of Forest Species Propagation CIPEF) of the Forestry Department, at the Federal University of Vales do Jequitinhonha e Mucuri, Diamantina, Minas Gerais, Brazil (18°12'S; 43°34' W and altitude of 1370 m).

The used plant material was from 80 young seedlings of *Richeria grandis* Vahl. brought in by Oliveira (2014) in 2012, from areas cleared for vegetation suppression in remnants of Atlantic Forest destined for implementing mining areas, tailings dam and other facilities belonging to the Anglo American mining company, in the municipality of Conceição do Mato Dentro, MG. These seedlings were transplanted into 10 x 15 x 0.20 cm plastic bags with capacity for 0.03 dm³, containing substrate composed of 70% subsoil, 15% tanned manure, 15% Bioplant® and 7 g L^{-1} of Osmocote® in the N-P-K proportion (15-9-12), slow releasing from 5 to 6 months (Oliveira, 2014). They were allocated in a semi-acclimatized greenhouse for 210 days in order to develop and recover from the stress caused by the transport and, later, to the hardening area, remaining there until the present study.

The apical mini-cuttings were removed from these seedlings after 4 years of being taken from the field, at the end of the rainy season (April/2016), and prepared with length of 8 ± 2 cm in height and 5 ± 1 mm in diameter, keeping the minimum of two pairs of leaves reduced to half their original size. In order to maintain the conditions of vigor and turgidity of the vegetative material, immediately after collection, the mini-cuttings were packed in Styrofoam boxes with water, with irrigation held by means of a manual spray, in intervals of less than 10 minutes until the plantation.

As for the rooting, hard conical plastic tubes with capacity for 180 cm³ were used, containing as substrate the mixture of 70% vermiculite and 30% rice husk ash with the addition of fertilizer at 5 g L^{-1} of the Osmocote® controlled-release formulation at the N-P-K proportion (15-9-12). The mini-cuttings were placed on the substrate, paying attention to its centering, straightness, depth (2 cm) and firmness.

As treatments, four indolbutyric acid (IBA) concentrations were tested: 0; 2,000; 4,000; and 6,000 mg L⁻¹. The IBA solution was prepared by dissolving the acid in sodium hydroxide (NaOH) and then diluting it in distilled water. The mini-cutting bases were immersed in the IBA solution for 10 seconds.

The mini-cuttings rooting process was performed in a semi-acclimated greenhouse, for a period of 90 days, with monitored environment conditions, aiming at obtaining a temperature of around 28 °C and relative humidity of over 80%. The luminosity inside was reduced by 50% of natural light due to the shading at the top of the structure.
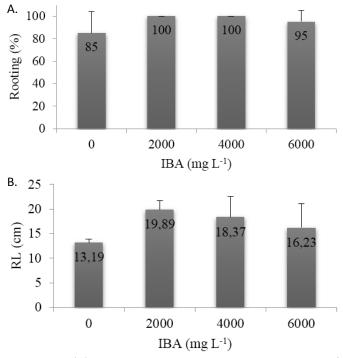
At 90 days, the percentage of survival (%) and rooting (%), mean number of roots (NR) (total number of roots (≥ 2 mm in length) (TNR) by the number of rooted mini-cuttings (RM), and the mean root length (RL) (total root length (TRL) (sum of the length values of each root) by the mean number of roots (NR)). The percentage of callogenesis (%) and the mean number of calluses (NC) (total number of calluses (TNC) were determined by the number of hardened cuttings (C)), when cell differentiation occurred at the base of the mini-cutting. It was also evaluated the percentage of shoots emitted (%), the mean number of shoots (NS) (total number of shoots (TNS) by the number of mini-cuttings that sprouted (S) and the increase in height (IH) and diameter of the mini-cuttings (ID).

The randomized blocks experimental design was used, consisting of four treatments (IBA concentrations), in four blocks with five cuttings per plot (totaling 80 mini-cuttings). For statistical analysis, the assumptions of normality (Shapiro-Wilk test) and homoscedasticity (Bartlett test) were calculated and tested, at a level of 5% significance. The variances were compared by using the F test at the 5% significance level. Regression analyzes were held using the treatment means. For all analyzes, the statistical software R (R Development Core Team, 2020) and the package "ExpDes.pt" (Ferreira et al., 2018) were employed. As for the results that did not show any significant differences (p > 0.05), descriptive analysis by graphs means was used.

Results

For all analyzed variables, the statistical assumptions were met (p > 0.05), with residuals and variance of normal and homogeneous residuals, respectively. However, the analyzes of variance from the regressions were not significant (p > 0.05) for the following variables: rooting (%), root length (RL), number of shoots (NS), increase in height (IH) and diameter (ID). To that end, the data were analyzed descriptively from the means, standard errors of the means and coefficients of variation, and displayed in bar graphs.

All mini-cuttings survived (100%) keeping their initial leaves for 90 days (the entire experimental period), having a high rooting percentage, with the control treatment retaining the lowest mean value (85%) (Figure 1A), with a coefficient data variation (CV) of 12.15%. The mean root length (RL) ranged from 13.19 cm, for the control, to 19.89 cm, for the treatment that had 2000 mg L⁻¹ of IBA added to it (CV= 20.47%) (Figure 1B).
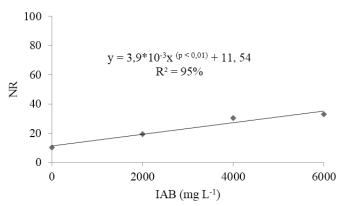

Figure 1. (A) Relation between the concentration of indolbutyric acid (IBA) and the mean rooting rate (%) of mini-cuttings of *Richeria grandis* Vahl. (B) Relation between the concentration of indolbutyric acid (IBA) and the mean root length (RL) of *Richeria grandis* Vahl. mini-cuttings in a greenhouse at 90 days. Lines above the bars indicate the standard error of the mean.
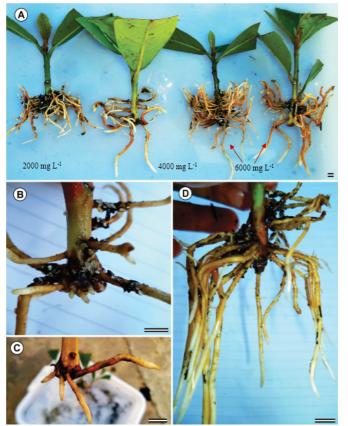
Significant differences (p < 0.05) found by the F test between IBA concentrations for were regarding the following characteristics: mean number of roots (NR), percentage of callogenesis, mean number of calluses (NC), and percentage of sprouts.

There is a tendency to increase the mean number of roots (NR) as the concentration of the plant regulator rises (Figure 2), which is explained by a simple linear regression with the coefficient of variation of 15.77%.

Figure 3 illustrates these results; where the mini-cuttings treated with 2000, 4000 and 6000 mg L⁻¹ IBA (Figure 3A, D) have

$$y = 3.9 \times 10^{-3} x^{\,(p < 0,01)} + 11,54$$
$$R^2 = 95\%$$

Figure 2. Relation between the concentration of indolbutyric acid (IBA) and the mean number of roots (NR) of *Richeria grandis* Vahl. rooted mini-cuttings in a greenhouse at 90 days.
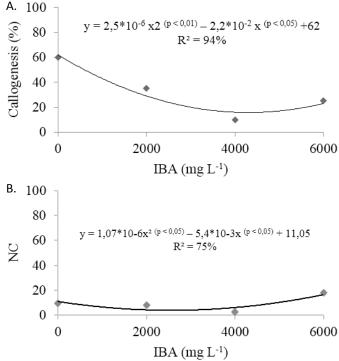
Figure 3. Rooting of *Richeria grandis* Vahl. mini-cuttings in a greenhouse at 90 days, in which: (A) Mini-cuttings subjected to the indolbutyric acid concentrations of 2000, 4000 and 6000 mg L^{-1}; (B) (C) Rooted mini-cuttings without addition of indolbutyric acid and (D) Rooted mini-cuttings with addition of 4000 mg.L^{-1} of indolbutyric acid. The lines in the figure are equivalent to 1 cm.

a higher mean number of roots than the control treatment (Figure 3B, C).

The calluses formation occurred at the base of the mini-cuttings in all treatments (Figure 5B-D). The treatments that had a rooting rate below 100% (0 and 6000 mg L^{-1}), had their highest callogenesis percentage ($p < 0.05$) (Figure 4A). The control treatment demonstrated a higher callogenesis percentage, with it gradually decreasing in the concentrations of 2000 and 4000 mg L^{-1}, and increasing once again in the IBA concentration of 6000 mg L^{-1}, thus composing a quadratic regression with a variation coefficient of 47 % (Figure 4A).

As observed for the percentage of callogenesis, the mean number of calluses per treatment ($p < 0.05$) (CV = 58.78%) demonstrated a quadratic trend (Figure 4B). However, it is worth emphasizing that for the treatment with concentration of 6000 mg L^{-1}, the mean number of calluses was higher in relation to the other dosages. This can be explained by the fact that even the percentage of mini-cuttings that had calluses occurring in this concentration was lower than the control treatment, so the 6000 mg L^{-1} concentration had a higher number of calluses per callused mini-cuttings, when compared to the other treatments.
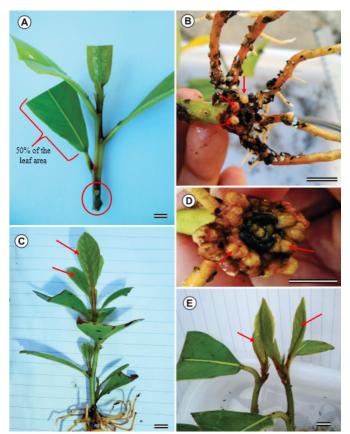

Figure 4. (A) Relation of the concentration of indolbutyric acid (IBA) and percentage of callogenesis of mini-cuttings of *Richeria grandis* Vahl. (B) Relation between the concentration of indolbutyric acid and the mean number of calluses (NC) present in the base of *Richeria grandis* Vahl. cuttings in a greenhouse at 90 days.

Furthermore, shoots were found in rooted mini-cuttings (Figure 5C, E). Regarding the sprouting percentage ($p < 0.05$) and the number of sprouts, the treatment with the highest IBA concentration also had the highest mean (Figure 6A). There is an increase in sprouts (%) as the IBA concentration rises ($p < 0.05$) (Figure 6A), explained by a linear regression with a coefficient of variation of 51.8%, and means varying between 0.75 and 2.67 sprouts per treatment ($p > 0.05$) (CV = 63.24%), for the control and the treatment with concentration of 6000 mg L^{-1}, respectively (Figure 6B).

In general, an increase in height (IH) and diameter (ID) is observed for all mini-cuttings, even though it is not statistically significant ($p > 0.05$). The control resulted in the lowest height growth and the largest diameter growth (Figure 7), whereas the mini-cuttings treated with 4000 mg L^{-1} of IBA had the opposite results from the control, that is, a greater growth in height and a smaller growth in diameter (Figure 7). Even so, standard errors of high means are observed for both characteristics, with a variation coefficient of 128.89% and 131.44% for height and diameter growths, respectively.
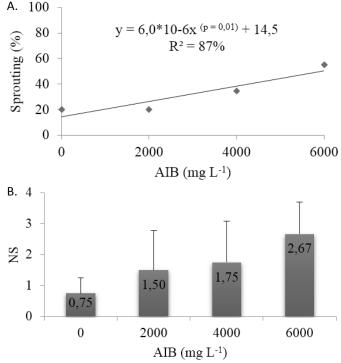
Discussion

The survival of all mini-cuttings and the high rooting percentage obtained in this experiment was very expressive, even with the absence of IBA plant regulator, since according to Xavier et al. (2013), rooting is one of the most critical aspects of the propagation.

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Figure 5. (A) Preparation of *Richeria grandis* Vahl. mini-cuttings to be subjected to the treatments, at time 0; (B) (D) Presence of calluses on the base of *Richeria grandis* Vahl. cuttings subjected to different indolbutyric acid concentrations, in a greenhouse at 90 days; (C) (E) Presence of sprouting in *Richeria grandis* Vahl. mini-cuttings subjected to the 6000 mg L^{-1} concentration in a greenhouse at 90 days. The lines in the figure are equivalent to 1 cm.
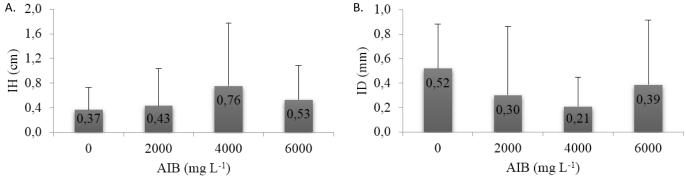
Oliveira (2003) also found positive results for *R. grandis* rooting in cuttings collected from stock trees, located in Gallery Forests, at different periods of the year, which had the ability to emit adventitious roots regardless of the IBA application, although having a lower percentage than the one found in this present study. Other authors have also obtained positive results on the rooting of cuttings without adding IBA for some
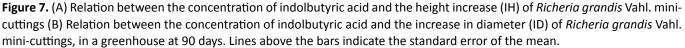

Figure 6. (A) Relation of the concentration of indolbutyric acid (IBA) and percentage of sprouting of *Richeria grandis* Vahl. mini-cuttings (B) Relation between the concentration of indolbutyric acid and the mean number of sprouts (NB) present in *Richeria grandis* Vahl. mini-cuttings, in a greenhouse at 90 days. Lines above the bars indicate the standard error of the mean.

species from the Phyllanthaceae family such as the *Phyllanthus sellowianus* (Klotzch) Mull. Arg. (Monteiro et al., 2010) and *Phyllanthus macrocalyx* Mull. Arg. (Kumar et al., 2016).

Studies on the vegetative propagation of other native species that are typical of Gallery Forests such as *Inga laurina* (Sw.) Willd., *Protium almecega* Marchand, *Pseudolmedia laevigata* Trécul, *Xylopia emarginata* Mart. (Oliveira, 2003), *Piper arboreum* Aubl. (Oliveira, 2003; Rios & Ribeiro, 2014) and *Euplassa inaequalis* (Pohl) Engl. (Oliveira, 2003; Oliveira & Ribeiro, 2013), also demonstrate no need for treatment with IBA in the adventitious rooting of cuttings.

Figure 7. (A) Relation between the concentration of indolbutyric acid and the height increase (IH) of *Richeria grandis* Vahl. mini-cuttings (B) Relation between the concentration of indolbutyric acid and the increase in diameter (ID) of *Richeria grandis* Vahl. mini-cuttings, in a greenhouse at 90 days. Lines above the bars indicate the standard error of the mean.

Using propagation material from juvenile seedlings may have favored the rooting, even in the control treatment, as it is a less lignified material with a lower degree of physiological maturation and close to the active growing region, it becomes a more vegetative material responsive to adventitious rooting, often dismissing the application of exogenous auxin (Xavier et al., 2013).

According to the classification by Hartmann et al. (2011), there are species that fall into the category of plants that already possesses all essential endogenous components to initiate the rooting, including auxins. The present result indicates that *R. grandis* is classified within this said category and, thus, it can be classified as a species of easy rooting, since the cuttings had adequate levels of endogenous auxins to stimulate the division of the cambium cells and promote the appearance of adventitious roots in stem cuttings. The auxin naturally formed in these plants with active growth (apical meristem, axillary buds and young leaves), are transported, through the phloem, to the base of the cuttings and, together with other substances and appropriate environmental conditions, are responsible for forming the root system (Hartmann et al., 2011). Oliveira (2003) also points out that, possibly, the endogenous auxins in *R. grandis* increase the rooting capacity of the species, since in their study the control group (no IBA application) had 40% of rooting percentage for cuttings collected at the early dry season.

It is known that the presence of the initial leaves assists rooting and ensures the cutting survival in many plant species, since the stimulus of the cambium activity and cell differentiation starting with the root formation occurs through the carbohydrates production via photosynthesis (Fachinello et al., 2005). Furthermore, it also supply endogenous auxins and other important substances that influence the root formation and the emission of sprouts (Hartmann et al., 2011).

Oliveira & Dorigon (2016) concluded in their study that cuttings that retain the initial leaves of the treatment positively influence survival and rooting. The presence of leaves, then, can be associated with the favorable results for the survival and rooting of *R. grandis*, in addition to exogenous factors such as temperature, incidence of light, quality of the substrate, favorable nutritional and water conditions, absence of injuries (Xavier et al., 2013), and absence of oxidative reactions at the base of the piles, which may also have favored the good results.

However, it was observed that both the mean root length and the mean number of roots were smaller and more uneven for the control treatment, while for the ones that had addition of exogenous auxins, it resulted in higher values and little variation (Figure 3). Oliveira (2003) also noted that even though there were no statistical differences in their results, for treatments with IBA application on *R. grandis* cuttings, there were higher rooting values.

This result can be explained according to Hartmann et al. (2011) and Xavier et al. (2013), since auxin applications provide a higher percentage, speed, quality and rooting uniformity. Using IBA is widely recommended and practiced, due to its

stability to photodegradation, low toxicity and mobility, and high chemical stability (Hartmann et al., 2011). Kumar et al. observed rooting results of cuttings with IBA addition (2016) studying *Phyllanthus macrocalyx* Mull. Arg., a species part of the same family as *R. grandis*.

There are also some species characteristic of Gallery Forests that are only able to emit adventitious roots from cuttings and with using IBA, represented by *Cariniana estrellensis* (Raddi) Kuntze (Hernández et al., 2013), *Copaifera langsdorffii* Undo (Rios & Ribeiro, 2014), *Ilex paraguariensis* A.St.-Hil. (Santin et al., 2015) and by *Calophyllum brasiliense* Cambess. (Oliveira, 2003; Rios & Ribeiro, 2014; Silveira et al., 2016).

According to Hartmann et al. (2011), root formation in some species is preceded by appearance of calluses at the base of the cuttings, serving to indicate the favoring of the rooting conditions. Therefore, the presence of these calluses, mainly in the cuttings of the control treatment of this study, may be an indication that, if the cuttings remained for a longer period in the greenhouse, the percentage of rooting and the number of roots could be increased, not differing from other treatments.

The difference in sprouting percentage and the number of sprouts from treatments that had a significant improvement in rhizogenesis in relation to the control, may be related to a secondary action by the plant regulator (Kotz et al., 2011), mainly regarding the increase in the number of roots, which caused an increase in the absorption of nutrients in the substrate (Taiz & Zeiger, 2013), favoring sprout emission.

The higher height growth in these treatments, even if not statistically proven, besides being related to the greater amount of roots and sprouts, due to the increase in the nutrient absorption and higher photosynthetic rates, may also be related to the other auxin action, which composes the phytohormones group related to cell expansion and stretching (Gastl Filho et al., 2019). Thus, mini-cuttings subjected to exogenous plant regulator attained greater heights when compared to the control group.

Therefore, when comparing the species under study with other forest species that occur in Gallery Forests, the asexually produced *R. grandis* seedlings for programs of restoration of these forests is very advantageous. This is because some studied species, typical to these areas, do not take root, or do so only with presence of plant regulators, such as the following: *Tibouchina stenocarpa* (Schrank & Mart. ex DC.) Cogn. (Rios & Ribeiro, 2014), Virola sebifera Aubl., *Myrcia sellowiana* O.Berg, *Hirtella gracilipes* (Hook.f.) Prance (Oliveira, 2003) which are unable to spread asexually; *Maclura tinctoria* (L.) D. Don ex Steud., *Salacia elliptica* (Mart. Ex Schult.) G. Don, *Emmotum nitens* (Benth.) Miers (Oliveira, 2003) and *Bauhinia rufa* (Bong.) Steud. (Rios & Ribeiro, 2014) which take root only when adding some concentrations of exogenous auxins.

Although asexual propagation can compromise genetic diversity in the environment (Oliveira & Ribeiro, 2013), the mini-cutting technique (propagules from rejuvenated material) is an alternative in overcoming difficulties in propagating native

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species, and can be used for commercial purposes as well as assisting the formation of recovering seedlings, via restoration (Oliveira & Ribeiro, 2013), of the Gallery Forests, since the seeds of some species are recalcitrant (Oliveira, 2003).

Conclusions

Richeria grandis proved to be an easy-rooting species.

All mini-cuttings survived regardless of the application of the IBA plant regulator.

The vegetative propagation technique (adventitious rooting) for *R. grandis*, by mini-cutting, without adding indolbutyric acid (IBA), is feasible.

Using IBA caused a tendency of increasing the mean number of adventitious roots and the sprouting percentage.

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