PAB
Pesquisa Agropecuária Brasileira

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

Forestry/ Original Article

Rooting of mini-cuttings and quality of plantlets of *Cordia trichotoma*

Renata Smith Avinio[(1)],
Angélica Costa Malheiros[(2)],
Luciane Grendene Maculan[(1)],
Denise Gazzana[(1)],
Kelen Haygert Lencina[(3)],
Thaíse da Silva Tonetto[(1)] and
Dilson Antônio Bisognin[(1 ✉)]

(1) Universidade Federal de Santa Maria, Avenida Roraima, nº 1.000, Cidade Universitária, Camobi, CEP 97105-900 Santa Maria, RS, Brazil. E-mail: renataavinio@gmail.com, lucianemaculan@hotmail.com, denygazzana@hotmail.com, thaisetonetto@hotmail.com, dilson.bisognin@ufsm.br

(2) Universidade de São Paulo, Escola Superior de Agricultura Luiz de Queiroz, Avenida Pádua Dias, nº 11, CEP 13418-900 Piracicaba, SP, Brazil. E-mail: angelcmalheiros@gmail.com

(3) Universidade Federal de Santa Catarina, Rodovia Ulysses Gaboardi, s/nº, Km 3, CEP 89520-000 Curitibanos, SC, Brazil. E-mail: kelen.lencina@ufsc.br

✉ Corresponding author

Received
April 26, 2022

Accepted
August 02, 2022

How to cite
AVINIO, R.S.; MALHEIROS, A.C.; MACULAN, L.G.; GAZZANA, D.; LENCINA, K.H.; TONETTO, T. da S.; BISOGNIN, D.A. Rooting of mini-cuttings and quality of plantlets of *Cordia trichotoma*. **Pesquisa Agropecuária Brasileira**, v.57, e02970, 2022. DOI: https://doi.org/10.1590/S1678-3921.pab2022.v57.02970.

Abstract – The objective of this work was to evaluate the effects of indolebutyric acid (IBA) concentration, substrate composition and volume, and propagule type on the adventitious rooting and quality of *Cordia trichotoma* plantlets produced by mini-cuttings. Mini-cuttings were evaluated for rooting percentage and root number and length at 30 and 60 days of cultivation. Plantlets were evaluated for shoot height and stem diameter at 30, 60, 90, and 120 days of cultivation. The Dickson quality index, number of leaves, and fresh and dry weights of shoots and roots were also determined at 120 days of cultivation. The experiments were carried out in a completely randomized design, with six replicates, in a greenhouse, evaluating different concentrations of IBA, substrate composition and volume, and propagule type for adventitious rooting. Plantlets of *C. trichotoma* can be produced with quality and proper rooting from mini-cuttings with one or two buds or multiple buds (apical) when treated with 3,000 mg L^{-1} IBA and planted in 110 cm^3 tubes containing equal proportions of substrate and vermiculite.

Index terms: cordia, indolebutyric acid, mini-cuttings, native species, propagule type, substrate composition.

Enraizamento de miniestacas e qualidade de mudas de *Cordia trichotoma*

Resumo – O objetivo deste trabalho foi avaliar a concentração de ácido indolbutírico (AIB), a composição e o volume do substrato, e o tipo de propágulo no enraizamento adventício e na qualidade de mudas de *Cordia trichotoma* produzidas por miniestaquia. As miniestacas foram avaliadas quanto à percentagem de enraizamento e número e comprimento de raízes aos 30 e 60 dias de cultivo. Já as mudas foram avaliadas quanto à altura da parte aérea e diâmetro do colo aos 30, 60, 90 e 120 dias de cultivo. O índice de qualidade de Dickson, o número de folhas, e a massa fresca e seca da parte aérea e das raízes também foram determinados aos 120 dias de cultivo. Os experimentos foram conduzidos em delineamento inteiramente casualizado, com seis repetições, em casa de vegetação, tendo-se avaliado diferentes concentrações de AIB, composição e volume do substrato, e tipo de propágulo no enraizamento adventício. As mudas de *C. trichotoma* podem ser produzidas com qualidade e enraizamento adequado a partir de miniestacas de uma ou duas gemas ou de múltiplas gemas (apicais) quando tratadas com 3.000 mg L^{-1} de AIB e plantadas em tubetes de 110 cm^3 contendo igual proporção de substrato comercial e vermiculita.

Termos para indexação: louro-pardo, ácido indolbutírico, miniestacas, espécie nativa, tipo de propágulo, composição do substrato.

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Introduction

The demand for new and high-quality timber products is boosting the forestry sector (McEwan et al., 2020), increasing the planted area worldwide (Silva et al., 2019). This justifies the exploration of alternatives for the *Pinus* and *Eucalyptus* genera, for which the techniques for commercial management and production are already well established (Eloy et al., 2013; De Araujo et al., 2017).

Native species are usually considered for the reforestation of degraded areas and for commercial purposes, depending on the availability of high-quality plantlets and on silvicultural management practices (Carvalho et al., 2020), which involves genetic improvement. In Brazil, *Cordia trichotoma* (Vell.) Arrab. Ex Steud (Boraginaceae) stands out, also being widely distributed in Argentina, Bolivia, Paraguay, and Uruguay (Fleck et al., 2019). The native species is highly demanded and accepted in the market, mainly for the manufacture of luxury furniture due the following characteristics of its wood: good density, high durability, and good resistance to bending, which ensure the quality and added value of the made products (Zimmermann et al., 2017).

The mini-cutting technique has been used for the production of plantlets of native species of economic importance, such as *Cabralea canjerana* (Vell.) Mart. (Burin et al., 2018) and *Ilex paraguariensis* A.St.-Hil. (Pimentel et al., 2020). However, the produced plantlets are affected by several factors, including propagule type for adventitious rooting, the application or not of a growth regulator, and substrate composition and volume; the type of used propagule is key for determining the best form of propagation to enhance plantlet production.

For *I. paraguariensis*, for example, the size of the mini-cuttings did not affect their rooting or the quality of the resulting plantlets, whose production can be maximized when using single-bud mini-cuttings (Pimentel et al., 2020). However, since the rooting of single-bud cuttings decreases with the increase of shoot diameter, genotypes with a lower rooting capacity require the application of indolebutyric acid (IBA) when induced by the coppicing of adult plants (Gazzana et al., 2020). An adequate substrate composition is also necessary to provide good humidity and aeration for plantlet support during the rooting process (Costa et al., 2017), as well as a suitable substrate volume for an adequate development of the roots and aerial part of the plantlets, improving their quality (Vargas et al., 2011).

For *C. trichotoma*, IBA application has been shown to increase rooting percentage, number of rooted mini-cuttings from mini-stumps of seed origin, and number of roots from the radicular cuttings of young plants (Kielse et al., 2013; Faganello et al., 2015). When associated with hydrogen peroxide (H_2O_2), it also improves the rooting capacity of the mini-cuttings from the lateral shoots of pruned seedlings (Silva et al., 2022). However, under humidity chamber conditions, the root cuttings of adult plants treated with IBA did not root until 180 days of cultivation (Bisognin et al., 2020).

For the success of a forest plantation aiming commercial purposes, not only the production but also the quality of the plantlets is important (Dutra et al., 2016). Plantlet quality is commonly characterized by morphological traits (Eloy et al., 2013), as shoot height, stem diameter, root mass, and shoot-to-root ratio, which increase the probability of plant establishment (Grossnickle & MacDonald, 2018). Shoot height and stem diameter are easily measured traits, giving growth advantage to the plants during field development (Pinto et al., 2015) and showing correlation with seedling weight and root system size (Grossnickle & MacDonald, 2018), respectively. The Dickson quality index is used to determine this correlation, which has been shown to be higher between stem diameter and dry matter in forest seedlings (Binotto et al., 2010).

The objective of this work was to evaluate the effects of IBA concentration, substrate composition and volume, and propagule type on the adventitious rooting and quality of *C. trichotoma* plantlets produced by mini-cuttings.

Materials and Methods

Different experiments were carried out from November 2018 to June 2020 at the Center for Plant Breeding and Propagation of Universidade Federal de Santa Maria, located in the municipality of Santa Maria, in the state of Rio Grande do Sul, Brazil. The effects of IBA concentrations, substrate composition and volume, and propagule type on the adventitious rooting of *C. trichotoma* mini-cuttings, as well as plantlet quality, were evaluated. The mini-cuttings planted in all experiments were obtained from shoots, with leaves reduced to 50% of their original area. A commercial substrate, consisting of a mixture of pine bark and carbonized rice hull, was used.

The cloning hedge was previously established with plantlets originated from rooted cuttings (Kielse et al., 2013). Mini-stumps, spaced at 10×10 cm, were grown in a closed soilless system, containing coarse sand as a substrate, and then placed in a greenhouse whose top and sides were covered with 30% shading. The mini-stumps were fertigated two (during autumn and winter) or three (during spring and summer) times a day for 15 min, with the aid of a timer and a submerged low-flow pump. The nutrient solution was prepared as described by Pimentel et al. (2021). The pH of the solution was maintained between 5.5 and 5.8, and the electrical conductivity at 1.5 dS m^{-1}; both were adjusted weekly.

The mini-cuttings were rooted for 30 days in an automated mist chamber at a maximum temperature of 34°C. Misting was carried out every 20 min for 10 s, between 7:00 a.m. and 7:00 p.m. After this period, the mini-cuttings were transferred to the greenhouse for 30 days for acclimatization and adventitious root growth. Manual irrigation was performed daily as necessary.

The rooting of the mini-cuttings was evaluated on the basis of rooting percentage. The number of roots and length of the three longest roots (cm) were determined at 30 days of cultivation in the mist chamber and at 30 days of cultivation in the greenhouse, totaling 60 days of rooting. The mini-cuttings were considered to have rooted when they had at least one adventitious root that was at least 1.0 mm long.

One experiment, carried out from February to June 2020, evaluated the effect of IBA concentrations (0, 1,500, and 3,000 mg L^{-1}) on the adventitious rooting of two-bud mini-cuttings and apical multiple-bud mini-cuttings, both between 2.0 and 4.0 cm long. The basal portion of the mini-cuttings was immersed for 10 s in the hydroalcoholic solution (1:1 v/v) of IBA representing each treatment. After treated, the mini-cuttings were planted in 50 cm^3 polypropylene tubes, containing equal proportions of substrate and vermiculite (v/v). The experimental design was completely randomized, with six replicates. The number of mini-cuttings per replicate varied between 100 and 120, depending on their availability, but was the same for each mini-cutting type in each replicate.

Another experiment, from November 2018 to March 2019, evaluated the effects of different substrate compositions and volumes on the adventitious rooting of apical multiple-bud mini-cuttings, 2.0 to 4.0 cm long, when treated with 3,000 mg L^{-1} IBA. The tested compositions were substrate and vermiculite (1:1 v/v) and subsoil and sawdust (1:1 v/v) in 50, 110, and 280 cm^3 polypropylene tubes. The experimental design was completely randomized, in a factorial arrangement, with six replicates. The number of mini-cuttings per replicate varied from 100 to 120 according to their availability.

The other experiment, performed from February to June 2019, evaluated the effects of propagule types (apical multiple-bud, one-bud, and two-bud mini-cuttings) on the adventitious rooting of mini-cuttings treated with 3,000 mg L^{-1} IBA and planted in 110 cm^3 polyethylene tubes containing substrate and vermiculite (1:1 v/v). The experimental design was completely randomized, with six replicates. The number of mini-cuttings per replicate ranged from 50 to 60 according to their availability.

The originated plantlets were transferred to the greenhouse, where they were irrigated twice a day for 15 min with an automated irrigation system and fertilized monthly via topdressing with 1,000 g ammonium sulfate and 300 g potassium chloride per 100 L water.

In the final experiment, from February to July 2019, the quality of the plantlets produced by the apical multiple-bud mini-cuttings treated with 3,000 mg L^{-1} IBA was evaluated under two substrate compositions – substrate and vermiculite (1:1 v/v) and subsoil and sawdust (1:1 v/v) – in 50, 110, and 280 cm^3 polypropylene tubes. The experimental design was completely randomized, in a factorial arrangement, with six replicates. The number of plantlets ranged from 100 to 120 per replicate according to the number of rooted mini-cuttings.

To determine plantlet quality, stem diameter (mm) and shoot height (cm) were measured at 30, 60, 90, and 120 days of cultivation in the greenhouse. At 120 days, the number of leaves was determined and destructive evaluations were performed on five plantlets from each replicate. The plantlets were separated into shoots and roots, which were both washed in tap water. Each part of the plantlet was then identified and weighed to obtain the fresh weight of shoots and roots, being subsequently dried in a forced-air circulation oven, at 60°C, until reaching a constant weight. Afterwards, the total dry weight and the ratio between shoot and root dry weight were quantified to determine the seedling quality index by the Dickson quality index.

Data were subjected to the analysis of variance, and means were compared by Duncan's test, at 5% probability. To determine normality requirements, percentage data was transformed to arccosine $(x/100)^{0.5}$, whereas number and length data were transformed to $(x+0.5)^{0.5}$. The analyses were performed using the Rbio software (Bhering, 2017).

Results and Discussion

Although both the application of 1,500 and 3,000 mg L^{-1} IBA increased the number of roots, the latter resulted in the highest rooting percentages and number of roots both in the mist chamber and greenhouse, increasing rooting percentage in 37.4 and 53.8%, respectively (Figure 1). IBA application, however, did not affect the length of the three longest roots, which was, in average, 2.88 cm in the mist chamber and 7.65 cm in the greenhouse.

Similarly, Faganello et al. (2015) found that IBA was more efficient in promoting the adventitious rooting of forest species due to its lower mobility and greater chemical stability. For *C. trichotoma*, the application of 6,000 mg L^{-1} IBA improved the adventitious rooting of root cuttings (Kielse et al., 2013), whereas 8,000 mg L^{-1} IBA led to the highest rooting percentage (28.9%) of stem cuttings (Faganello et al., 2015). However, the application of high concentrations of rooting promoters can cause plant toxicity, which is why they are used in woody cuttings or recalcitrant species (Faganello et al., 2015). In *Cariniana estrellensis* (Raddi) Kuntze, for example, the application of 6,000 mg L^{-1} IBA increased the rooting speed and percentage of cuttings, although apical cuttings rooted easily without IBA (Hernandez et al., 2013). This could be explained by the fact that juvenile propagules are more responsive to adventitious rooting, often dispensing the use of growth regulators (Hartmann et al., 2011).

Rooting, associated with the hormonal gradient of the branch, causes apical mini-cuttings to present higher concentrations of endogenous auxins, which promote rooting (Ramos et al., 2003). However, tissue lignification can prevent the formation of roots due to the presence of peroxidase, an enzyme responsible for the synthesis of lignin and that degrades auxins (Hartmann et al., 2011). Despite this, it was shown that H_2O_2 and peroxidase were positively correlated with adventitious root formation, indicating that H_2O_2 can be used, for example, to improve the regeneration capacity of *Linum usitatissimum* Griseb. by regulating the level of endogenous auxins (Takáč et al., 2016). In mini-cuttings of *C. trichotoma*, a positive synergic effect was observed between the application of H_2O_2

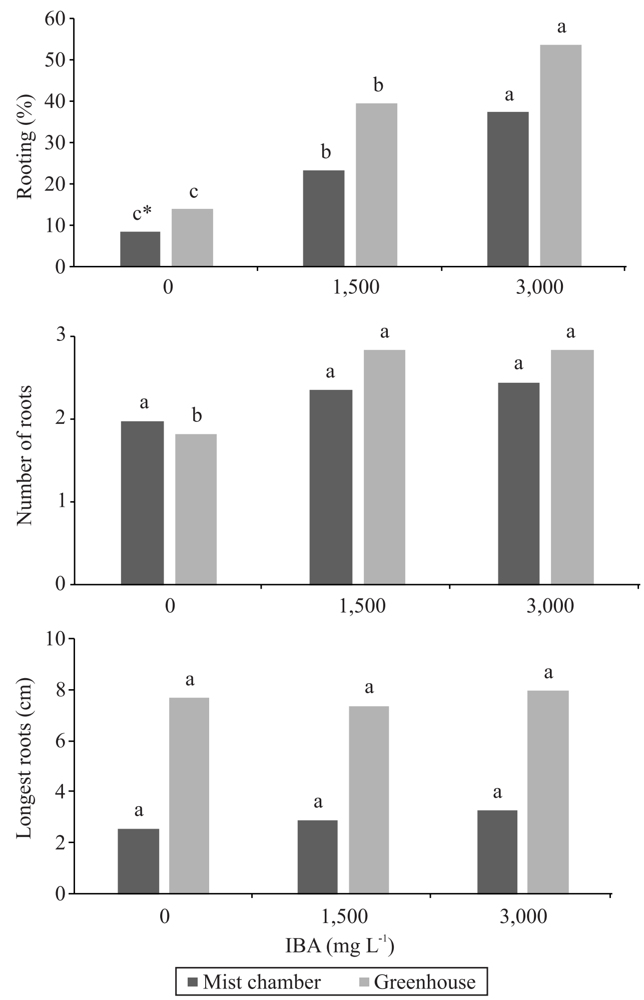

Figure 1. Rooting percentage, root number, and length of the three longest roots of *Cordia trichotoma* mini-cuttings treated with 0, 1,500 and 3,000 mg L^{-1} indolebutyric acid (IBA), planted in 50 cm^3 polyethylene tubes filled with equal proportions of substrate and vermiculite, and cultivated for 30 days in a mist chamber and 30 days in a greenhouse. Means followed by equal letters for each local cultivation do not differ by Duncan's test, at 5% probability.

and IBA, showing an increase in rooting percentage and root number, length, and dry matter (Silva et al., 2022).

In the process of mini-cutting rooting, no significant interaction was found between substrate composition and volume (Figure 2). However, under greenhouse conditions, the combination of equal proportions of substrate and vermiculite increased the percentage of rooting, as well as the number of roots and the length of the three longest roots; the longest three longest roots were those of the mini-cuttings cultivated in 280 cm^3 tubes. Vermiculite is being increasingly combined with substrate for rooting because of its high porosity and water retention capacity, as well as other desirable characteristics for root formation (Tavares et al., 2012). Microporosity is responsible for water retention in the substrate, whose low-porosity components can hinder the gaseous exchange of roots and the movement and drainage of water, negatively influencing the development of roots and plants, and whose high-porosity components can provide a better root aeration, although potentially leading to a low water retention and consequent water deficiency (Zorzeto et al., 2014).

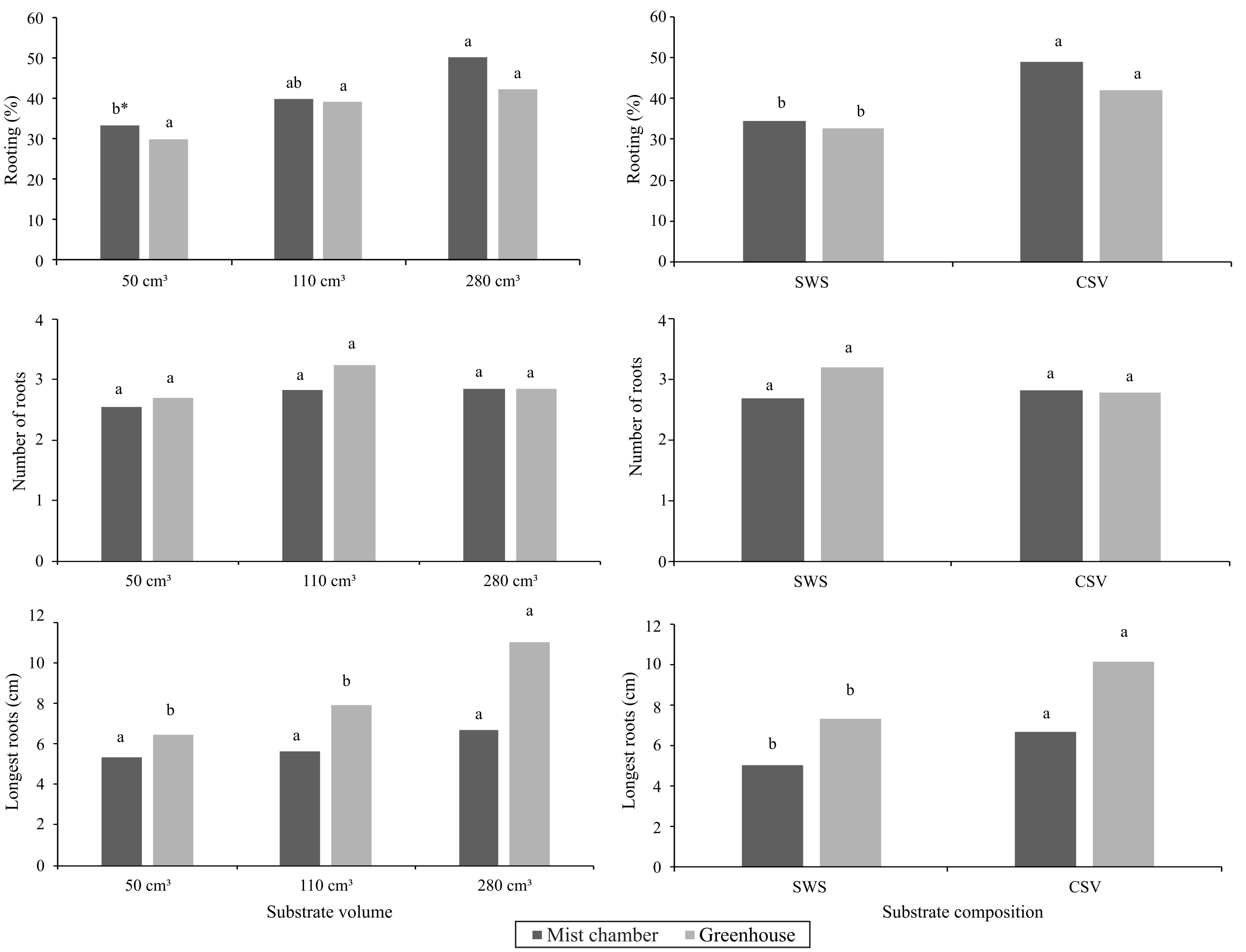

Figure 2. Rooting percentage and root number and length of the three longest roots of *Cordia trichotoma* mini-cuttings treated with 3,000 mg L^{-1} indolebutyric acid and planted in 50, 110, and 280 cm^3 polyethylene tubes filled with different compositions and volumes of substrate at 30 days of cultivation in a mist chamber and 30 days of cultivation in a greenhouse. Treatments: SWS, subsoil and wood sawdust (1:1 v/v); and CSV, substrate and vermiculite (1:1 v/v). Means followed by equal letters for each local cultivation do not differ by Duncan's test, at 5% probability.

In the present study, rooting capacity differed according to mini-cutting type (Figure 3). During cultivation in the mist chamber, a higher rooting percentage was obtained for the two-bud mini-cuttings in comparison with the apical multiple-bud mini-cuttings; however, this difference disappeared when the mini-cuttings were transferred to the greenhouse. Overall, both mini-cuttings types showed a high number of roots; the exception were the apical multiple-bud mini-cuttings in the mist chamber. Moreover, the length of the three longest roots was shorter for the apical multiple-bud mini-cuttings under greenhouse conditions.

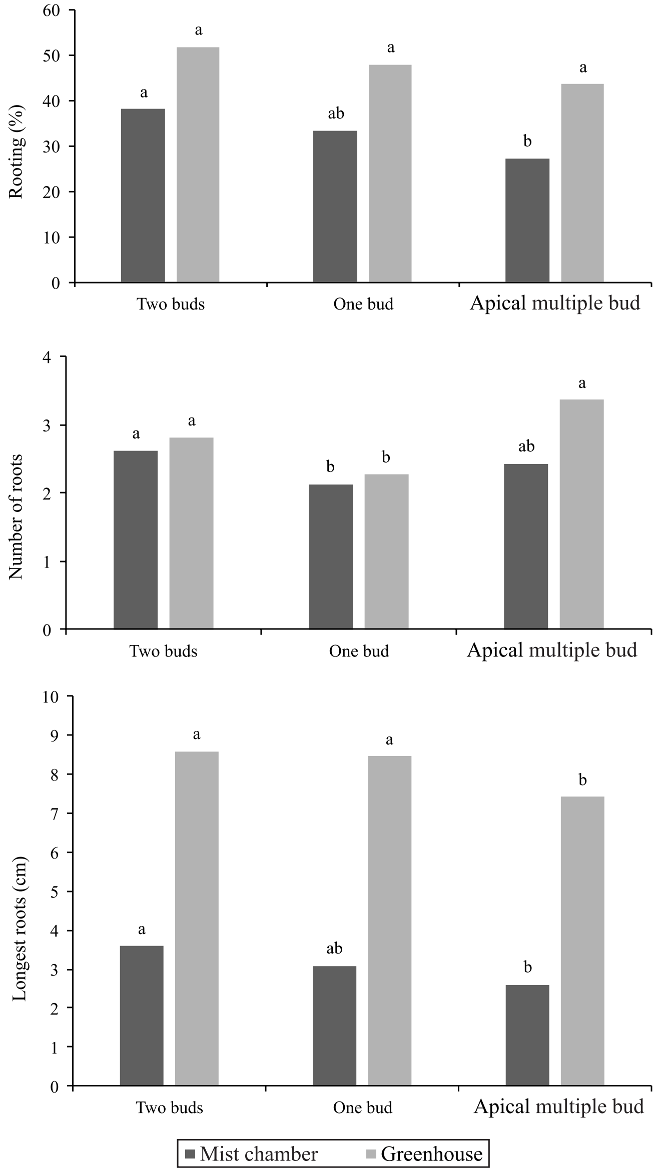

Figure 3. Rooting percentage, root number, and length of the three longest roots of one-bud, two-bud, and apical multiple-bud mini-cuttings of *Cordia trichotoma* treated with 3,000 mg L^{-1} indolebutyric acid and planted in 110 cm^3 polyethylene tubes filled with equal proportions of substrate and vermiculite at 30 days of cultivation in a mist chamber and 30 days in a greenhouse. Means followed by equal letters for each local cultivation do not differ by Duncan's test, at 5% probability.

Although bud type did not significantly affect the rooting of the mini-cuttings (Figure 3), increasing IBA concentrations increased the number of roots (Figure 1). According to Moraes et al. (2014), adventitious rooting can be related to the hormonal gradient of the branch or to the size and number of the leaves – that produce the endogenous auxins necessary for this complex process – left on the mini-cuttings. In addition, the leaves, which are sites of carbohydrate and auxin synthesis, may also have enhanced the survival and rooting of the mini-cuttings, possibly explaining the greater number of roots of the two-bud and apical multiple-bud mini-cuttings (Figure 3), which stored a large amount of energy as insoluble carbohydrates required for the rooting process (Hartmann et al., 2011). In other studies, adventitious rooting was maximized with 2.5 cm long single-bud mini-cuttings of *I. paraguariensis* with halved leaves (Pimentel et al., 2021) and with mini-cuttings of *Anadenanthera macrocarpa* (Benth.) Brenan with whole leaves (Dias et al., 2015).

There was no significant interaction ($p>0.05$) between substrate composition and volume on the quality of the plantlets produced from *C. trichotoma* mini-cuttings (Figure 4). However, the combination of equal proportions of substrate and vermiculite resulted in a better plantlet quality in terms of stem diameter and shoot height at 60 days of cultivation in the greenhouse (Figure 4), as well as of leaf number, shoot and root fresh weight, and the Dickson quality index (Table 1). At 90 and 120 days, the thickest and tallest plantlets were those planted in the 280 cm^3 tubes; however, no differences were observed between the substrate volumes of 50 and 110 cm^3.

Besides providing support and an adequate humidity and porosity to the mini-cuttings, an appropriate substrate must be free of pathogens, have a low density, and be economically viable (Hartmann et al., 2011), as is the case of vermiculite.

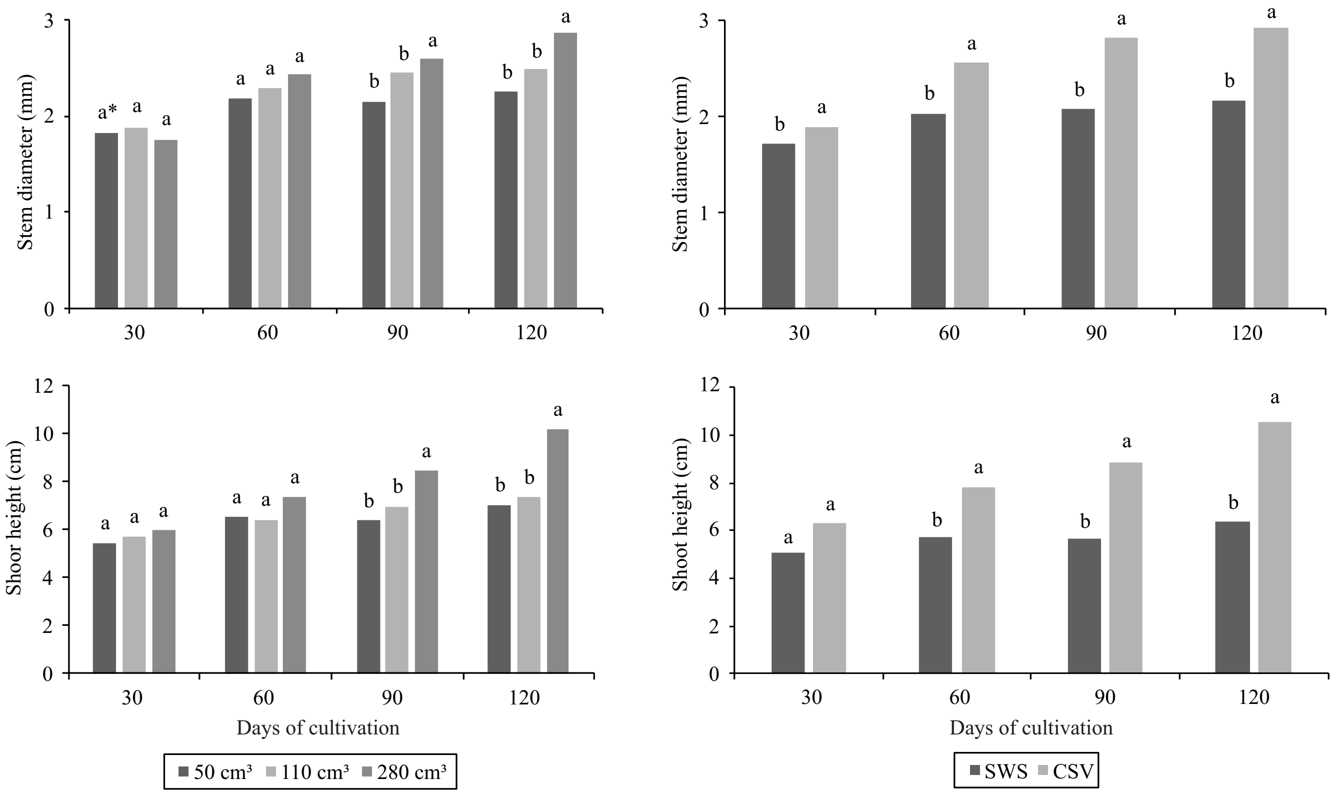

Figure 4. Stem diameter and shoot height of *Cordia trichotoma* plantlets at 30, 60, 90, and 120 days of cultivation in 50, 110, and 280 cm³ polyethylene tubes filled with different compositions and volumes of substrate in a greenhouse. Treatments: SWS, subsoil and wood sawdust (1:1 v/v); and CSV, substrate and vermiculite (1:1 v/v). Means followed by equal letters do not differ by Duncan's test, at 5% probability.

Table 1. Number of leaves, fresh weight of shoots (SFW) and roots (RFW), and the Dickson quality index (DQI) of *Cordia trichotoma* plantlets at 120 days of cultivation in a greenhouse(1).

Treatment(2)	Number of leaves	SFW (g)	RFW (g)	DQI
	Substrate composition for apical mini-cuttings treated with 3,000 mg L^{-1} indolebutyric acid			
CSV	4.31a	1.42a	2.77a	0.21a
SWS	3.07b	0.40b	0.77b	0.07b
	Substrate volume for apical mini-cuttings treated with 3,000 mg L^{-1} IBA			
50 cm³	2.94b	0.50b	1.02b	0.07b
110 cm³	4.00a	0.79ab	1.73ab	0.13ab
280 cm³	4.00a	1.60a	2.74a	0.22a
CV (%)	107.29	117.91	105.18	96.45
	Type of mini-cuttings treated with 3,000 mg IBA and planted in 110 cm³ CSV			
Two buds	1.48a	0.46a	0.90a	0.13a
One bud	1.64a	0.29a	0.58a	0.10a
Apical multiple bud	2.01a	0.47a	1.03a	0.13a
CV (%)	122.19	70.91	57.55	75.30

(1)Means followed by equal letters do not differ by Duncan's test, at 5% probability. (2)One-bud, two-bud, and apical multiple-bud mini-cuttings planted in 50, 110, and 280 cm³ polyethylene tubes, containing: SWS, subsoil and wood sawdust (1:1 v/v); and CSV, substrate and vermiculite (1:1 v/v).

The combination of subsoil with other components increases the density and nutrient content of the substrate (Paulino et al., 2011), while also reducing production costs; however, it may contain nematodes and, therefore, not meet phytosanitary requirements due to the risk of contamination, not being recommended for propagation. Commercial substrates, however, usually have an adequate macroporosity and a low density, allowing gaseous exchange and promoting root growth (Abreu et al., 2017). In *A. macrocarpa* plantlets, a vermiculite-based substrate resulted in a higher dry mass of roots, which is directly related to the number of roots produced (Dias et al., 2015). In the present study, the combination of equal proportions of vermiculite and substrate showed the best results.

Regarding the Dickson quality index, which includes the robustness and balance of biomass distribution, the higher the obtained values, the better plantlet quality. The increase in substrate volume improved this index, as well as the number of leaves and the fresh weight of shoots and roots (Table 1); the more developed the shoots were, the greater the photosynthetic capacity of the plant.

Together, the obtained results are indicative that plantlets from mini-cuttings of *C. trichotoma* can be produced both in 110 and 280 cm^3 tubes. However, the use of 110 cm^3 tubes is more advantageous for the operational effectiveness of the nursery due to substrate and fertilizer savings, irrigation, and silvicultural treatments. Berghetti et al. (2016) also concluded that the 110 cm^3 tube was the best container for the production of *C. trichotoma* plantlets.

After 60 days of cultivation, the *C. trichotoma* plantlets produced by the two-bud and one-bud mini-cuttings presented larger diameters (Figure 5). The plantlets produced from apical multiple-bud mini-cuttings showed longer stems, which did not differ significantly from those of the two-bud mini-cuttings, at 120 days of cultivation, when mini-cutting type did not affect leaf number, shoot and root fresh weight, and the Dickson quality index (Table 1). Therefore, *C. trichotoma* plantlets can be produced by one-bud, two-bud, or apical multiple-bud mini-cuttings; however, the latter maximizes the use of the produced propagules.

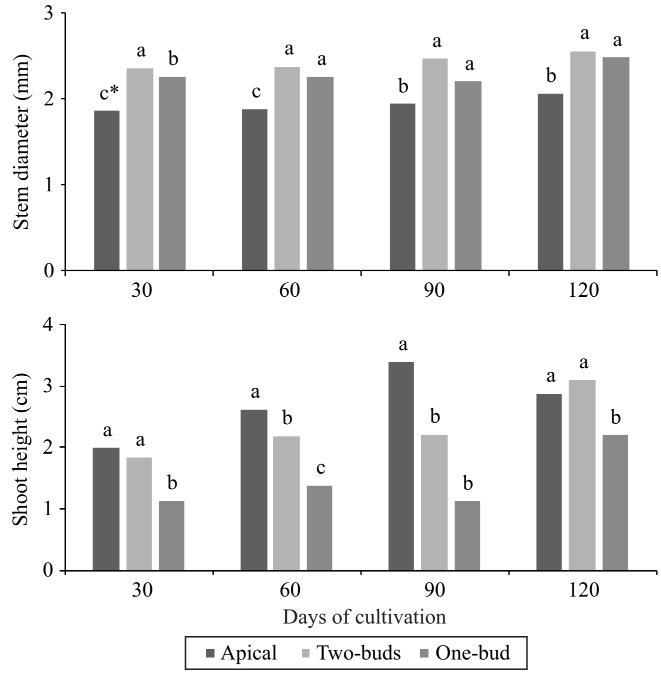

Figure 5. Stem diameter and shoot height of *Cordia trichotoma* plantlets at 30, 60, 90, and 120 days of cultivation in 110 cm^3 polyethylene tubes filled with equal proportions of substrate and vermiculite in a greenhouse. Means followed by equal letters do not differ by Duncan's test, at 5% probability.

Conclusion

Plantlets of *Cordia trichotoma* can be produced with quality and proper rooting with one-bud, two-buds, or apical multiple-bud mini-cuttings treated with 3,000 mg L^{-1} indolebutyric acid and planted in 110 cm^3 tubes containing an equal proportion of substrate and vermiculite.

Acknowledgments

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes, Finance Code 001) and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Grant302388/2019-2, for scholarships.

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