ROOTING OF *Inga edulis* Mart. (LEGUMINOSAE-MIMOSOIDEAE) MINI-CUTTINGS

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Resumo

Enraizamento de miniestacas de Inga edulis Mart. (Leguminosae-Mimosoideae). As sementes da espécie *Inga edulis* são recalcitrantes e perdem a viabilidade rapidamente, o que restringe a produção de mudas em apenas uma determinada época do ano. Desta forma, objetivou-se com este trabalho analisar o potencial da miniestaquia e a influência de diferentes concentrações de ácido indol-3-butírico (AIB) na propagação vegetativa de *Inga edulis*. O material vegetativo utilizado na pesquisa foi coletado de plantas juvenis de minijardim clonal. O experimento foi realizado em delineamento inteiramente casualizado, cujos tratamentos foram as diferentes concentrações de AIB (0, 1000, 2000, 4000 e 8000 mg Kg^{-1}), com cinco repetições e cada unidade experimental contendo oito estacas. Após 45 dias em casa de vegetação foi analisada a porcentagem de miniestacas vivas e enraizadas, o número de raízes emitidas a partir da base, o comprimento da maior raiz, número total de raízes, comprimento de raízes finas, comprimento total das raízes, área superficial das raízes, diâmetro médio ponderado das raízes, volume radicular, massa seca da parte aérea, massa seca de raízes, massa seca total, comprimento específico radicular, área superficial específica da raiz e densidade da raiz. Todas as miniestacas de *Inga edulis* sobreviveram e o enraizamento foi superior a 85%. As concentrações de AIB não promoveram efeito significativo para a maioria das variáveis analisadas, porém a auxina exógena entre 2000 e 4636,96 mg Kg^{-1} proporcionou miniestacas com maior número de raízes, área superficial e volume radicular. O enraizamento de miniestacas juvenis de *Inga edulis* pode ocorrer sem a utilização do AIB.

Palavras-chave: miniestaquia; ácido indol-3-butírico; enraizamento adventício; produção de mudas; silvicultura clonal.

Abstract

Seeds of the species *Inga edulis* are recalcitrant and lose viability quickly, which restricts seedling production at only a certain time of the year. Thus, this study aimed to analyze the potential of mini-cutting and the influence of different indole-3-butyric acid (IBA) concentrations on the vegetative propagation of *Inga edulis*. The vegetative material was collected from juvenile plants from a clonal mini-garden. The experiment was carried out in a completely randomized design, with five replications and each experimental unit with eight cuttings. Treatments consisted of different IBA concentrations (0, 1000, 2000, 4000, and 8000 mg kg^{-1}). The percentage of live and rooted mini-cuttings, number of roots emitted from the base, longest root length, total number of roots, fine root length, total root length, root surface area, weighted average root diameter, root volume, shoot dry matter, root dry matter, total dry matter, specific root length, specific root surface area, and root density were analyzed after 45 days in a greenhouse. All *Inga edulis* mini-cuttings survived, and rooting was over 85%. IBA concentrations had no significant effect on most of the analyzed variables. However, exogenous auxin concentration between 2000 and 4636.96 mg kg^{-1} provided mini-cuttings with a higher number of roots, surface area, and root volume. Rooting of juvenile *Inga edulis* mini-cuttings may occur without the use of IBA.

Keywords: mini-cutting; indole-3-butyric acid; adventitious rooting; seedling production; clonal forestry.

INTRODUCTION

Faced with increasing legal requirements and worsening environmental problems, the demand for forestry information regarding the multiplication of forest species of the Brazilian flora has intensified. The species *Inga edulis* Mart. (Leguminosae-Mimosoideae) is a widespread fruit tree in Brazil and South and Central America. It has the potential for reforestation due to its rusticity to adverse environmental conditions and biological nitrogen fixation capacity. This species is commonly used as a shade tree in intercropped plantations of coffee and cocoa (NYGREN *et al.*, 2013). *I. edulis* still has the potential for the management of non-timber products, as its leaves have anti-inflammatory properties, especially flavonoids (DIAS *et al.*, 2010; SILVA *et al.*, 2013). This versatility makes *I. edulis* interesting for use in forest restoration programs and commercial plantations.

Seeds of species of the genus *Inga* are recalcitrant, not tolerating small reductions in water content, and present low natural longevity during storage, making them non-viable few days after collection and processing (BILIA; BARBEDO, 1997). The vegetative propagation of *I. edulis* using mini-cuttings meets the overcoming of the difficulties of the seminiferous propagation, thus increasing seedling availability.

Rhizogenic potential or root formation ability depends on several factors and differs among tree species. Synthetic auxins have been widely used in vegetative propagation. IBA has been effective in stimulating rooting in cuttings, such as the case of *Schizolobium parahyba* var. *amazonicum* (DIAS *et al.*, 2015) and *Cordia trichotoma* (FAGANELLO *et al.*, 2015), which were dependent on auxin to promote higher rooting rates.

The literature shows that IBA is not a condition for adventitious rooting in mini-cuttings of native woody species unless propagules from young materials are used, as shown by Mantovani *et al.* (2017) with cana fistula (*Peltophorum dubium* (Spreng.) Taub.) and Oliveira *et al.* (2016) with pink trumpet tree (*Handroanthus heptaphyllus* (Vell.) Mattos). Although IBA did not promote gains in the rhizogenesis process in *Peltophorum dubium* mini-cuttings, it stimulated dry matter production and the number, size, and diameter of roots in the mini-cuttings at 40 days. Moreover, IBA was not necessary for rooting of *Handroanthus heptaphyllus* mini-cuttings, but its use promoted an increase in the number and length of seedling roots at 120 days. It indicates that IBA may promote an increase in dry matter and the number, volume, and length of roots in already formed seedlings.

Thus, this study aimed to analyze the rooting and root growth of *I. edulis* stem mini-cuttings submitted to different IBA concentrations. The following hypotheses were tested: (i) it is possible to propagate vegetatively the species *I. edulis* using mini-cuttings; (ii) higher IBA concentrations influence the adventitious rooting and increase the length, volume, and root biomass production of *I. edulis* mini-cuttings and; (iii) there is a relationship between root dry matter and the number, length, and volume of roots in *I. edulis* mini-cuttings.

MATERIAL AND METHODS

The experiment was set up and conducted at a university forest nursery located in the experimental area of the Department of Forestry and Wood Sciences belonging to the Federal University of Espírito Santo (DCFM–CCA–UFES), in Jerônimo Monteiro, ES, Brazil, from September 2016 to May 2017. The experimental area has a latitude of 20°47′ S and a longitude of 41°23′ W, with an altitude of 120 m. The regional climate is classified by Köppen as Cwa (dry winter and rainy summer), with an average annual temperature of 24.1 °C and average annual precipitation of 1104 mm (LIMA et al., 2008).

Step 1: Multiclonal mini-garden formation

The *I. edulis* mini-garden, which originated the propagules, was formed from seedlings propagated by seeds collected from parent trees of the region of Jerônimo Monteiro, ES, and sown in polypropylene tubes with a capacity of 280 cm^3 containing a commercial substrate of ground and decomposed pine bark, coconut fiber, and organic compost, with pH of 6.2, density of 0.5 g cm^{-3}, moisture of 55%, and electrical conductivity of 0.4 mS cm^{-1}.

Seedlings were transplanted into 10-L pots with a dimension of 25 cm in height, 32 cm in diameter at the top, and 24 cm at the base at four months after sowing when they were about 30 cm high. These pots were filled with a mixture of 60% Oxisol subsurface samples and 40% commercial pine bark substrate and 150 g N, 700 g P_2O_5, and 100 g K_2O per m^3 of soil, as recommended by Gonçalves (2005). These seedlings were placed in an area with 50% shading polypropylene screen after transplanting. Irrigation was performed by micro-sprinklers triggered for 10 minutes two times a day and flow of 8 mm m^{-2}.

Seedlings were submitted to adequate maintenance pruning to obtain the mini-cuttings used in the rooting experiment. For this, seedlings were broken at the height of 10 cm from the collar at 15 days after transplanting to pots, and the previously broken part was removed with scissors 30 days later when they had already sprouted. The clonal mini-garden was formed with 120 mini-cuttings at 75 days after transplantation, with sufficient size and amount of sprouts to be used in the experiments.

Step 2: Mini-cutting rooting

The vegetative material of *I. edulis* used for mini-cutting preparation was collected in the morning due to the lower temperature. Sprouts were collected with manual pruning shears. Immediately after harvesting, sprouts were packed in Styrofoam boxes, being sprayed with water using a manual sprayer to maintain vigor and turgidity conditions, and then taken to the Laboratory of Quality Seedling Propagation to prepare the mini-cuttings.

Mini-cuttings were made with a size of approximately 10 cm, in which the apical bud and two leaves with two pairs of half-sized leaflets were maintained. After preparation, mini-cuttings were subjected to disinfection by immersion for 10 minutes in 0.5% sodium hypochlorite, with subsequent washing in running water and treatment with the fungicide Captan® at 0.2%. Subsequently, the bases of the mini-cuttings were immersed in the different concentrations of indole-3-butyric acid prepared with industrial talcum powder ($3MgO.4SiO_2.H_2O$) for 10 seconds and transplanted into 55-cm^3 tubes containing medium-grained vermiculite.

Mini-cuttings were then placed in trays, which were allocated in a hanging bed inside a greenhouse under intermittent misting, with 10-second sprayings every 5 minutes, thus maintaining the humidity above 80%. Daily measurements of air temperature and relative air humidity inside the greenhouse were performed using a weather mini-station. Measurements were always taken at 14:00 h. The averages of temperature and humidity during the experiment was 28.7 °C and 82.6%, respectively.

The experimental arrangement was a completely randomized design, with five replications composed of eight experimental units. Treatments consisted five concentrations of indole-3-butyric acid (0, 1000, 2000, 4000, and 8000 mg kg^{-1}).

Step 3: Analysis

Mini-cuttings were removed from the tubes after 45 days in the greenhouse when the substrate was completely removed by washing in running water to collect the roots. Subsequently, the following variables were determined: percentages survival (S%) and rooting (R%) of mini-cuttings, number of roots emitted from the base (NRB), and longest root length of mini-cuttings (RL, cm), using a millimeter ruler.

Roots were placed in properly identified plastic bags and kept in a freezer for later digitization. For image digitalization, the roots were removed from the freezer, placed on absorbent paper to dry, and scanned on an Epson Perfection 750 Pro scanner at a resolution of 400 pixels. Once digitized, the images were submitted to the software SAFIRA version 1.1 (JORGE *et al.*, 2010) to quantify the total number of roots (TNR); fine root length, ≤ 2 mm (FRL, cm); total root length (TRL, cm); root surface area (SA, cm^2); weighted average root diameter (ARD, mm), and root volume (RV, mm^3).

Shoot dry matter (SDM) and root dry matter (RDM) of mini-cuttings were determined by separating the shoot from the roots, being the material separately packed in properly identified paper bags, dried in a forced-air circulation oven at 65 °C until constant weight, and weighed on a precision scale. The total dry matter (TDM) was obtained by the sum of SDM and RDM.

The specific root length (SRL, cm g^{-1}, length of digitized roots divided by root dry matter), specific root surface area (SRSA, cm^2 g^{-1}, area of digitized roots divided by root dry matter), and root density (RD, mg mm^{-3}, root dry matter divided by root volume) were determined from the morphological variables. The methodological flowchart of the steps required for the study of *I. edulis* mini-cutting is shown in Figure 1.

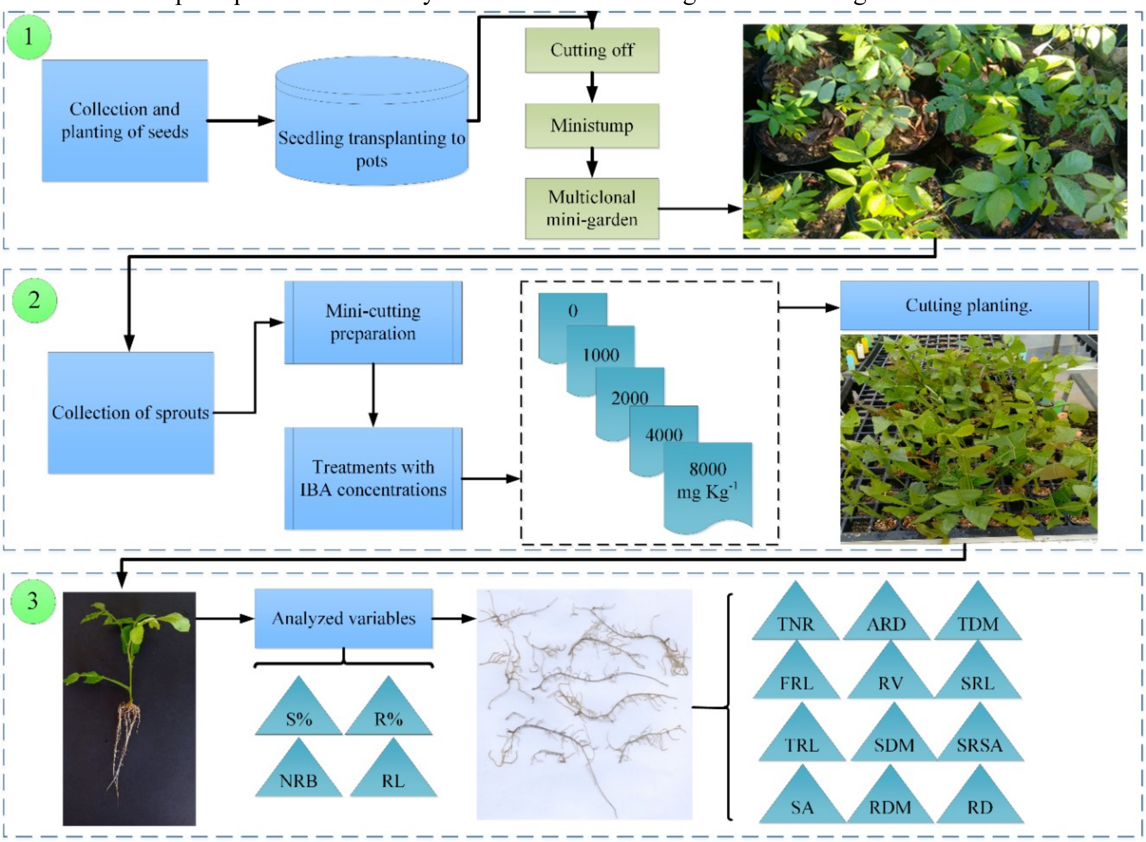

Figura 1. Fluxograma ilustrando os três passos metodológicos dos passos necessários para o estudo da miniestaquia do Inga edulis. Etapa 1: Formação do minijardim seminal; Etapa 2: Enraizamento de miniestacas e; Etapa 3: Avaliação do enraizamento e crescimento radicular. Sobrevivência (S%), enraizamento (E%), número de raízes emitidas da base (NRB), comprimento da maior raiz das miniestacas (CR), número total de raízes (NTR), comprimento de raízes finas (CRF), comprimento total das raízes (CTR), área superficial das raízes (AS), diâmetro médio ponderado das raízes (DMR), volume radicular (VR), massa seca da parte aérea (MSPA) e massa seca de raízes (MSR), massa seca total (MST), comprimento específico radicular (CER), área superficial específica da raiz (ASER) e densidade da raiz (DR) das miniestacas de *Inga edulis* submetidas à diferentes concentrações de ácido indol-3-butírico.

Figure 1. Flowchart illustrating the three methodological steps necessary for the study of *Inga edulis* mini-cutting. Step 1: Multiclonal mini-garden formation; Step 2: Mini-cutting rooting and; Step 3: Rooting and root growth evaluation. Survival (S%), rooting (R%), number of roots emitted from the base (NRB), longest root length (RL), total number of roots (TNR), fine root length (FRL), total root length (TRL), root surface area (SA), weighted average root diameter (ARD), root volume (RV), shoot dry matter (SDM), root dry matter (RDM), total dry matter (TDM), specific root length (SRL), specific root surface area (SRSA), and root density (RD) of *Inga edulis* mini-cuttings submitted to different concentrations of indole-3-butyric acid.

The data were submitted to the test of the normality assumption (Shapiro-Wilk test). The Variables NRB, ARD, RD, SRL, and SRSA presented p-values below 0.05, without normal distribution of residuals, which led to data transformation using the function $Y = log\ (x + 1)$ but presented in the original version. Subsequently, the data were subjected to analysis of variance, and when significant differences were found by the F-test at 5%, the means were subjected to regression analysis to verify the optimal dose of indole-3-butyric acid for each variable by the first derivative of the estimators β0 and β1. Equations were chosen considering the significance of parameters, the t-test, the biological significance, and the coefficient of determination (R^2). The Tukey test at 5% probability was used for variables that did not meet these criteria. Pearson correlation coefficients were also calculated between root growth variables. The analyses were performed using the software SISVAR 5.4 (FERREIRA, 2014).

RESULTS

Mini-cuttings of *I. edulis* had survival close to 100%, and rooting was above 85% in all treatments, with no influence of indol-3-butyric acid (Table 1). The originated root system was well-formed with thin main roots and presence of secondary roots. According to the results of the analysis of variance of the variables related to root system growth of *I edulis* mini-cuttings, a significant difference ($p \leq 0.05$) was observed in the indol-3-butyric concentrations for number of roots emitted from the base (NRB), root surface area (SA), and root volume (RV).

Tabela 1. Resumo da análise de variância para as variáveis de crescimento do sistema radicular das miniestacas de *Inga edulis* submetidas à diferentes concentrações de ácido indol-3-butírico, aos 45 dias após estaqueamento.

Table 1. Summary of the analysis of variance for root system growth variables of *Inga edulis* mini-cuttings submitted to different concentrations of indole-3-butyric acid at 45 days after cutting planting.

SV	DF	Mean square			
		S (%)	R (%)	NRB (un)	RL (cm)
Doses	4	7.8125 ^{ns}	11.718 ^{ns}	0.063*	4.45 ^{ns}
Residual	20	7.8125	182.291	0.016	4.15
CV (%)		2.81	15.21	12.20	21.20
Mean		99.37	88.75	10.91	9.61
SV	DF	Mean square			
		TNR (un)	FRL (cm)	TRL (cm)	SA (cm^2)
Doses	4	171.84 ^{ns}	0.024 ^{ns}	160.62 ^{ns}	1.661*
Residual	20	346.68	0.034	163.42	0.53
CV (%)		43.26	12.99	38.82	33.37
Mean		43.04	1.42	32.92	2.19
SV	DF	Mean square			
		ARD (mm)	RV (mm^3)	RDM (g)	SDM (g)
Doses	4	0.000077 ^{ns}	124.48*	0.017 ^{ns}	0.554 ^{ns}

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Residual	20	0.000068	38.92	0.012	0.566
CV (%)		12.44	39.67	42.55	22.24
Mean		0.164	15.72	0.26	3.38
SV	DF	Mean square			
		TDM (g)	SRL (cm g^{-1})	SRSA (cm^2 g^{-1})	RD (mg mm^{-3})
Doses	4	0.54 ns	0.0166 ns	0.024 ns	0.062 ns
Residual	20	0.67	0.0302	0.026	0.053
CV (%)		22.44	8.27	16.75	18.14
Mean		3.64	135.95	9.12	20.43

SV: source of variation; DF: degrees of freedom; CV: coefficient of variation. * and ** significant at 5% and 1% probability level by the F-test, respectively; ns not significant at 5% probability by the F-test. Survival (S%), rooting (R%), number of roots emitted from the base (NRB), longest root length (RL), total number of roots (TNR), fine root length (FRL), total root length (TRL), root surface area (SA), weighted average root diameter (ARD), root volume (RV), shoot dry matter (SDM), root dry matter (RDM), total dry matter (TDM), specific root length (SRL), specific root surface area (SRSA), and root density (RD) of *Inga edulis* mini-cuttings submitted to different concentrations of indole-3-butyric acid.

The best fit for NRB was an increasing linear model, in which indol-3-butyric acid at a dose of 8000 mg kg^{-1} provided the maximum yield, but this interpretation does not reflect the biological reality of the phenomenon and, therefore, the Tukey test was used. Regarding the control, the dose of 2000 mg kg^{-1} presented the best NRB result (Figure 3a). A quadratic increasing tendency was observed for SA, in which the highest value (2.84 cm^2 $root^{-1}$ mini-$cutting^{-1}$) was recorded when indol-3-butyric acid was used at the estimated dose of 4636.96 mg kg^{-1} (Figure 3b). The maximum RV of *I. edulis* mini-cuttings was 16.1 mm^3, obtained at the estimated dose of 2384 mg kg^{-1} of indole-3-butyric acid (Figure 3c). The decrease in SA and VR values of *I. edulis* mini-cuttings from the estimated optimal concentration may have occurred due to the exogenous auxin phytotoxicity.

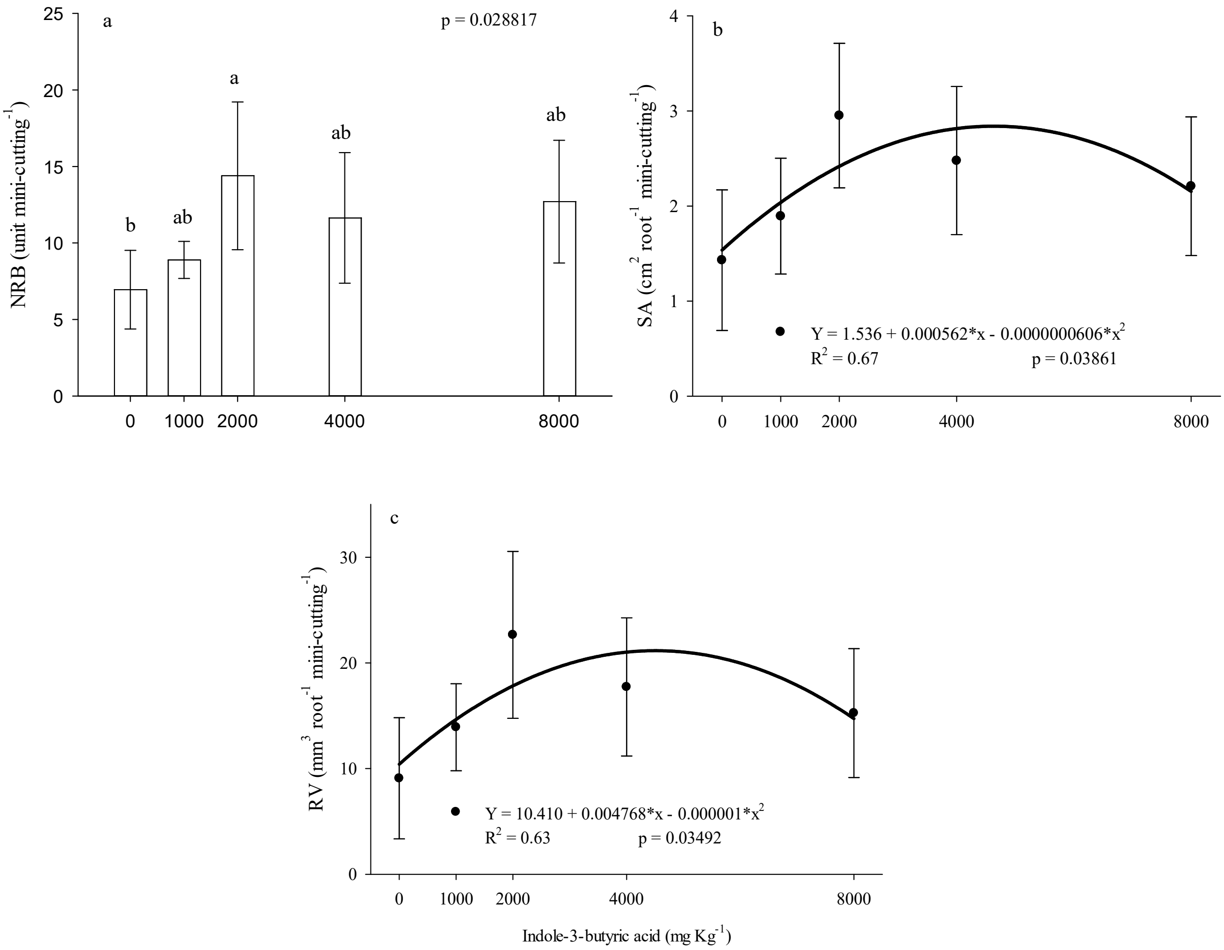

Figura 2. Número de raízes a partir da base (a); área superficial das raízes (b), volume de raízes (c) de miniestacas de *Inga edulis* submetidos a diferentes concentrações de ácido indol-3-butírico, aos 45 dias

após estaqueamento. I = Desvio padrão; * e ** significativo a 5% e 1% de probabilidade, respectivamente.

Figure 2. Number of roots emitted from the base (a), root surface area (b), and root volume (c) of *Inga edulis* mini-cuttings submitted to different concentrations of indole-3-butyric acid at 45 days after cutting planting. I = standard deviation; * and ** significant at 5% and 1% probability, respectively.

The variable RDM showed a strong positive and significant correlation with RL (r = 0.768) and moderate correlation with NRB, TNR, TRL, and SA (Table 2). On the other hand, SA had a strong positive and significant correlation with TRL (r = 0.886) and VR (0.912) of *I. edulis* mini-cuttings.

Tabela 2. Matriz de correlação linear de Pearson entre as variáveis de crescimento radicular das miniestacas de *Inga edulis* submetidos a diferentes concentrações de ácido indol-3-butírico, aos 45 dias após estaqueamento.

Table 2. Pearson linear correlation matrix between root growth variables of *Inga edulis* mini-cuttings submitted to different concentrations of indol-3-butyric acid at 45 days after cutting planting.

Variable	CL	TNR	TRL	SA	ARD	RV	RDM
NRB	0.447*	0.580**	0.421*	0.505*	0.002	0.340	0.640**
RL	1.000	0.404*	0.414*	0.376	−0.200	0.194	0.768**
TNR		1.000	0.476*	0.314	−0.258	0.009	0.679**
TRL			1.000	0.886**	−0.258	0.684**	0.624**
SA				1.000	0.117	0.912**	0.638**
ARD					1.000	0.280	−0.083
RV						1.000	0.410*
RDM							1.000

* and ** significant at 5% and 1% probability, respectively.

DISCUSSION

The first tested hypothesis was proved, as the species *I. edulis* showed aptitude to mini-cutting as a method of vegetative propagation, and can be a viable technique to overcome the difficulties of seedling production of this species via seeds, such as seasonality, storage, viability, and heterogeneity of the seminiferous material. It also allowed obtaining a large number of seedlings of *I. edulis* and other species of the genus for the demand in forest restoration, use in agroforestry systems, and commercial orchards.

Vegetative propagation is one of the important means of conservation of undomesticated, native, and threatened tree species. In addition, from the commercial perspective of non-timber products from *I. edulis*, mini-cutting and genetic improvement may be beneficial for increasing yields and maintaining agronomic and medicinal characteristics of the plant, such as secondary metabolites and extracts of its leaves, with antioxidant properties.

The second hypothesis was partially confirmed, with no influence of indole-3-butyric acid on S (%) and R (%) of *I. edulis* mini-cuttings, variables considered crucial in rooting studies. According to Daskalakis *et al.* (2018), auxin is a hormone whose contribution to root induction and formation has been repeatedly documented as essential. However, successful root formation even at low concentrations of indole-3-butyric acid may be linked to the ontogenetic age of the harvested material since the juvenile material has a natural adventitious rhizogenic potential.

Physiologically, the degree of juvenility and internal auxin concentration of propagules (indole-3-acetic acid) were efficient in stimulating the adventitious root emission since no effect was observed after the application of this product. Lateral buds that induce the growth of lateral sprouts, which in turn are used to make mini-cuttings, are precisely the sites of highest auxin biosynthesis in the seedling after the suppression of the stem apex and apical dominance.

This information is in line with several authors who have documented that the application of the growth regulator has little or no detrimental influence on the radial induction of mini-cuttings from juvenile sprouts of forest species. It was found for the species Barbados nut (*Jatropha curcas* L.), manacá (*Tibouchina sellowiana* (Cham.) Cogn.), mate (*Ilex paraguariensis* A. St. Hil.), and cana fistula (*Peltophorum dubium* (Spreng.) Taub.) in studies conducted by Pimenta *et al.* (2014), Fragoso *et al.* (2017), Stuepp *et al.* (2017), and Mantovani *et al.* (2017), respectively.

Although the application of indole-3-butyric acid did not provide higher rooting rates of *I. edulis* mini-cuttings, this growth regulator promoted positive morphological alterations since they presented a more robust root system. Doses between 2000 and 4636.96 mg kg^{-1} provided mini-cuttings with higher NRB, SA, and RV. Above this value, there was a tendency of non-favoritism of indol-3-butyric acid for these variables.

The influence of IBA on rooting success is very species-specific. Endogenous auxin content in different species may vary. Thus, different species need a different IBA concentration for successful rooting (AZAD *et al.*, 2018). Oliveira *et al.* (2015) found that the use of indole-3-butyric acid on pink trumpet tree (*Handroanthus heptaphyllus*) mini-cuttings is not necessary for rooting although it has resulted in seedlings with longer first-order roots and higher number of second-order roots at the maximum studied concentration (8000 mg L^{-1}).

The emission of a higher number of roots, with a higher contact area and root system volume in *I. edulis* plants, is essential to increase the soil area to be explored, favoring water and nutrient absorption. These factors may be useful in recovery programs of degraded areas, as seedlings would need a robust root system to withstand stress conditions in these environments. Invasive and fast-growing tree species (e.g., *I. edulis*) generally have a higher root area and root length and lower root diameter when compared to slow-growing species.

Although RDM is the commonly investigated variable to quantify root development, Smithwick *et al.* (2014) and Koevoets *et al.* (2016) reported that plant performance is predominantly related to length, number, positioning, and angle of root components and size class. Thus, the third hypothesis that there would be a relationship between RDM and other morphological characteristics of the root system of *I. edulis* mini-cuttings was partially validated.

CONCLUSIONS

• Mini-cuttings proved to be technically viable as a means of propagation of *I. edulis*, making it possible to use this methodology for large-scale seedling production.

• Different concentrations of indole-3-butyric acid did not affect survival and rooting of mini-cuttings, but doses between 2000 and 4636.96 mg kg^{-1} provided mini-cuttings with higher number of roots, surface area, and root volume.

• Root dry matter of mini-cuttings was correlated with other morphological characteristics of the root system of *I. edulis* mini-cuttings.

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