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BIOLOGY

Shoot segment and substrate composition in rooting of juvenile ipe-roxo mini-cuttings

Tipo de segmento e composição do substrato no enraizamento de miniestacas juvenis de ipê-roxo

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ABSTRACT

*This study aimed to evaluate the rooting capability of apical and nodal mini-cuttings of ipe-roxo (**Handroanthus heptaphyllus**) in different substrates. Shoots produced from mini-stumps were fractioned into mini-cuttings 2-cm long apical or nodal mini-cuttings and treated with 1000 mg L^{-1} of indolebutyric acid (IBA). Four substrate combinations were evaluated: commercial substrate composed of pine bark and vermiculite in proportions of 1:1, 2:1 and 1:2 (v /v) and commercial substrate, vermiculite and sand in the proportion of 1:1:1 (v/v). The factorial experiment utilized a completely random design, with five replicates of four mini-cuttings. Rooting, shooting and survival percentage, number and length of shoots and roots were evaluated at 60 and 90 days of growth. Commercial substrate and vermiculite are a good combination for rooting mini-cuttings of ipe-roxo. Apical and nodal mini-cuttings exhibit similar rooting behavior and both are feasible for production of ipe- roxo plantlets.*

Key words: ***Handroanthus heptaphyllus***, *tree species, vegetative propagation and mini - clonal hedge.*

RESUMO

*O objetivo deste estudo foi avaliar o enraizamento de miniestacas apicais e nodais de ipê-roxo (**Handroanthus heptaphyllus**) em diferentes substratos. Brotos coletados de minicepas foram seccionados em miniestacas de 2cm de comprimento, classificadas em apical ou nodal e tratadas com 1000mg L^{-1} de ácido indolbutírico (AIB). Foram também testadas as composições de substrato comercial à base de casca de pinus e vermiculita nas proporções de 1:1; 2:1 e 1:2 (v/v) e de substrato comercial, vermiculita e areia grossa (1:1:1 v/v). O experimento foi um fatorial no delineamento inteiramente casualizado, com cinco repetições de quatro miniestacas. A porcentagem de enraizamento, de brotação e de sobrevivência, o número e comprimento de brotos e raízes foram avaliados aos 60 e 90 dias. Miniestacas de ipê-roxo cultivadas em substrato comercial e vermiculita apresentam maior capacidade de enraizamento. Miniestacas apicais e nodais apresentam similar comportamento de enraizamento, podendo ambas serem utilizadas para a produção de mudas de ipê-roxo.*

Palavras-chave: ***Handroanthus heptaphyllus***, *espécie arbórea, propagação vegetativa e minijardim clonal.*

INTRODUCTION

Ipe-roxo (***Handroanthus heptaphyllus***) is a native deciduous arboreal species of the family ***Bignoniaceae***, which possesses valuable wood in addition to ornamental and medicinal value. It presents a wide geographical distribution, occurring in the South and West of the states of Bahia, Espirito Santo, Minas Gerais, Mato Grosso do Sul, Rio de Janeiro, Sao Paulo, Santa Catarina and Rio Grande do Sul (CARVALHO, 2003). Ipe-roxo seeds present short viability (PINTO et al., 1988), which hinders the production of seedlings throughout the year and vegetative propagation may be an alternative for the multiplication of this species.

Minicutting is a vegetative propagation technique widely used for commercial production of ***Eucalyptus*** plantlets with high genetic quality and plant health. There are few studies on the use of this technique in native tree species; however, promising results have been obtained for cedro-rosa (***Cedrela***

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fissilis), mogno (***Swietenia macrophylla***) and angico-vermelho (***Anadenanthera colubrina***) (XAVIER et al., 2003; SANTOS, 2002). To our knowledge, there are no studies that address the production of plantlets by minicutting for the ipe-roxo species.

A number of factors may influence adventitious rooting in vegetative propagation, notably the type of shoot segment used and the substrate composition. For cedro-rosa (***Cedrela fissilis***), apical segments showed greater rooting competence (XAVIER et al., 2003), while in cuttings of ***P. glomerata***, apical segments presented lower rooting competence compared to nodal segments (NICOLOSO et al., 1999). Another important aspect related to substrate composition is that arboreal species have specific nutritional and physiological needs, making it important to perform studies to define the best substrate for propagation of each species (WENDLING & GATTO, 2002).

Considering the lack of information and the importance of vegetative propagation for the production of ipe-roxo plantlets, this study aimed to evaluate the rooting of apical and nodal mini-cuttings grown in different substrate compositions.

MATERIALS AND METHODS

This study was conducted in a greenhouse at the Plant Science Department of the Universidade Federal de Santa Maria, Rio Grande do Sul. Mini-clonal hedge was established in a closed soilless cultivation system with coarse sand substrate and fertigation adapted following BANDINELLI et al. (2013). Mini-clonal hedge was fertirrigated three times daily for 15min until complete soaking of the substrate and formation of a surface layer of nutrient solution. The nutrient solution consisted of the following amounts of macronutrients (mmol L^{-1}): 4.09 of potassium nitrate; 6.66 of calcium nitrate (Calcinit);11.18 magnesium sulfate; 0.96 ammonium nitrate; 0.92 potassium monophosphate and 1.12 5% iron chelate. Micronutrients were added to the nutritive solution (7.5 mL) in a previously prepared mixture containing (mmol L^{-1}): 0.10 sodium molybdate; 0.60 boric acid; 0.83 copper sulfate; 0.82 manganese sulfate and 0.18 zinc sulfate. The pH of the nutrient solution was 5.5 and conductivity (1.0 dS m^{-1}) was adjusted weekly.

In each tray, 12 seedlings grown from 30 day-old seeds were planted with 10 x 10cm spacing. After 60 days, seedlings underwent drastic pruning, leaving a bud to form mini-stumps for producing the shoots used to prepare the mini-cuttings Collection of shoots was carried out 60 days after pruning and shoots were sectioned into two segment types: apical mini-cuttings being the branch segment containing the apical buds and nodal mini-cuttings those with a pair of lateral buds.

For rooting, apical and nodal mini-cuttings, 2cm in length with two leaves with leaflets reduced to 50% of the original area, which were treated with a 1000mg L^{-1} indolebutyric acid (IBA) water-alcohol solution for 10 s. They were grown in polyethylene trays (55 x 34 x 15cm) with different substrate compositions (v/v): 1) commercial and vermiculite (1:1); 2) Commercial and vermiculite (2:1); 3) Commercial and vermiculite (1:2) and 4) Commercial, vermiculite and sand (1:1:1). Vermiculite in the form of fine powder (particles of between 0.3 and 0.5 mm diameter) and H. DECKER® commercial substrate pine bark and coarse sand (particles between 1 and 3 mm in diameter) were used for substrate compositions. A sample of each substrate combination was sent to the Substrates Laboratory of the Universidade Federal do Rio Grande do Sul for physical analysis according to the methodology described in Norm No. 14 of the Brazilian Ministry of Agriculture, Livestock and Food Supply. Rooting of the mini-cuttings was carried out in a wet chamber with relative humidity of approximately 85%, supplied by an climate control automatically triggered 8 times a day for 1 minute. The average temperature inside the wet chamber was 27°C.

The 4 x 2 factorial experiment (substrate composition and mini-cutting segment type) utilized a completely random design with 5 repetitions of 4 shoots. The percentage of survival, rooting and shooting of mini-cuttings and the number and length of roots and shoots were evaluated after 60 and 90 days of growth. The mini-cuttings were considered rooted when they presented at least one adventitious root of length equal to or greater than 0.1cm. Mini-cuttings rooted at 60 and/or 90 days of growth were used to study the relationship between mini-cutting volume, length and number of roots and shoot length. The mini-cutting diameters were measured at the central portion to calculate volume (cm^3), using the following equation: $v = \left(\pi . \frac{d^2}{4}\right) . L$, where d = diameter (cm) and L = length, in this case 2cm.

To account for assumptions of normality, percentage data were transformed to arc-sine ($\sqrt{x/100}$) and count data to $\sqrt{x+0,5}$ and submitted to analysis of variance. Treatment means were compared by Tukey test (P<0.05).

RESULTS AND DISCUSSION

Dry density (DD) of the substrates evaluated for rooting in ipe-roxo mini-cuttings ranged between 255.31 and 770.74 kg m^{-3} and lower DD was reported for commercial and vermiculite in a proportion of 1:2 (Table 1). Vermiculite is a low-density substrate, which contribute to a lower density in the final composition in mixtures with other substrates (FERMINO, 2014). In this case, the low DD of commercial substrate and vermiculite (1:2) is probably related to the higher amount of vermiculite, compared with the proportion used in the other substrate compositions. A higher DD was reported in the composition of commercial substrate, vermiculite and sand (Table 1) since this composition contains two high-density components (commercial substrate and sand), which does not occur in the other substrates evaluated. DD is related to total porosity (TP) and it has been observed that the lower the TP, the higher the DD will be in the substrate (DE BOODT & VERDONCK, 1972), which was observed in the commercial substrate, sand and vermiculite (1:1:1 v/v) composition used in this study (Table 1). According to RIVIERE (1980), 75% TP is optimal in substrates. Therefore the commercial substrate and vermiculite compositions in ratios of 1:1, 2:1 and 1:2 v/v presented values nearest to the optimal TP range, while the composition of commercial substrate, vermiculite and sand (1:1:1 v/v) presented TP below the optimal range for plantlet development (Table 1).

In terms of aeration space (AS), the commercial substrate and vermiculite (2:1) and commercial substrate, vermiculite and sand compositions presented values of 20.22 and 25.31% respectively (Table 1). An AS between 20 and 40% is considered optimal (DE BOODT & VERDONCK, 1972); however, AS determination may be influenced by the type of plant material used, as well as the species to be propagated (FERMINO, 2014). For water retention capacity (WRC), the compositions of commercial substrate and vermiculite, in all proportions, were superior to that of commercial substrate, vermiculite and sand. This result is due to the small particle size of the vermiculite and pine bark (present in the commercial substrate), causing less water mobility in the substrate and consequently, increased volume of water retained (HANDRECK & BLACK, 1999). Conversely, the composition of commercial substrate, vermiculite and sand presented the lowest water retention (Table 1) probably due to the high density and fast drainage of sand (KAMPF, 2000).

There was no significant interaction ($P<0.05$) between substrate composition and mini-cutting segment type for all variables evaluated at 60 and 90 days of growth. Survival percentage was influenced by the different compositions of substrates at 90 days of growth, with commercial substrate and vermiculite (1:2) resulting in the highest percentage of survival in mini-cuttings, although with no significant difference from the same composition at the other proportions (1:1 and 2:1) (Table 2).

Percentage of rooting and number and length of roots were influenced by the substrate composition at 60 and 90 days of growth (Table 2). At 60 days, greater rooting capacity occurred in mini-cuttings grown in the compositions of substrates with a greater proportion of vermiculite, i.e. commercial substrate and vermiculite in proportions of 1:2 and 1:1 (v/v). Vermiculite, besides reducing the DD of the substrates, presents a high water retention capacity, which probably favored the rooting of ipe-roxo mini-cuttings. In addition, vermiculite presents a high cation exchange capacity and may retain nutrients that will later be used by plants depending on their nutritional requirements (HARTMANN et al., 2010). At 90 days, the compositions containing commercial substrate and vermiculite, in all proportions, presented higher rooting (57.5%) compared to the composition of commercial substrate, vermiculite and sand (20%). Physical properties of the substrates containing

Table 1 - Physical Features of the compositions of substrate used for rooting of ipe-roxo mini-cuttings.

Substrate composition	DD * (kg m^{-3})	TP (%)	AS (%)	WRC 10 (%)	WRC 50 (%)	WRC100 (%)
CS + V (1:1 v/v) **	277.78	77.06	19.86	57.20	46.34	44.31
CS + V (2:1 v/v)	296.88	78.13	20.22	57.91	44.86	43.15
CS + V (1:2 v/v)	255.31	74.57	19.96	54.61	45.54	44.04
CS + V + S (1:1: 1 v/v)	770.74	64.05	25.31	38.74	31.13	29.95

*DD = dry density; TP = total porosity; AS = aeration space; WRC 10 = water retention capacity under 10cm suction in water column; WRC 50 = water retention capacity under 50cm suction in water column; WRC 100 = water retention capacity under 100cm suction in water column. ** CS = pine bark commercial substrate, V = vermiculite and S = coarse sand.

Table 2 - Survival and rooting percentage, number and length of roots in ipe-roxo mini-cuttings grown in different substrate compositions at 60 and 90 days.

Substrate composition	Survival (%)	Rooting (%)	Number of roots	Root length (cm)
	-----60 days of growth-----			
CS + V (1:1)**	70.00 a*	20.00 ab	0.67 ab	6.03 ab
CS + V (2:1)	72.50 a	17.50 b	0.55 ab	3.23 ab
CS + V (1:2)	80.00 a	45.00 a	1.22 a	8.49 a
CS + V + S (1:1:1)	57.50 a	0.00 b	0.00 b	0.00 b
Mean	70.00	20.60	0.61	4.44
CV (%)	34.70	74.40	28.12	75.61
-----90 days of growth-----				
CS + V (1:1)	60.00ab	52.50 ab*	1.62 a	15.46 a
CS + V (2:1)	57.50ab	47.50 ab	1.25 ab	10.33 ab
CS + V (1:2)	75.00 a	72.50 a	2.17 a	14.61 a
CS + V + S (1:1:1)	25.00 b	20.00 b	0.45 b	5.51 b
Mean	54.30	48.10	1.37	11.48
CV (%)	47.60	50.00	67.20	61.15

*Values followed by the same letter do not differ by Tukey test at 5% probability of error.
**CS = Pine bark commercial substrate, V = vermiculite and S = coarse sand.

vermiculite also favored the rooting of mini-cuttings of guanandi (***Calophyllum brasiliense***) with 95.8% rooting at 60 days of growth (SILVA et al., 2010).

There was no root formation at 60 days in the mini-cuttings grown in commercial substrate, vermiculite and sand and only 20% of rooting was observed at 90 days (Table 2). When root formation is inefficient, the mini-cuttings continue to use only endogenous nutrient reserves, which are limited and allow them to remain alive for a short period of time, hence the lower survival observed in the composition of commercial substrate, vermiculite and sand (Table 2). Low rooting capacity of mini-cuttings grown in this substrate composition may be associated with a high DD and low PT, hindering root penetration and development (GAULAND, 1997). Low water retention of this composition may also have hindered the rooting of ipe-roxo mini-cuttings. A similar result was observed in ***Prunus cerasifera*** cuttings, where those grown in sand presented lower rooting (RAMOS et al., 2003). Conversely, the composition of commercial substrate, coarse sand and carbonized rice husk provided the best rooting responses in mini-cuttings of ***Cabralea canjerana*** (GIMENES et al., 2015). According to WENDLING & GATTO (2002), in addition to the rooting environment, the species to be propagated is an important aspect to be considered when choosing the substrate composition, because of differing nutritional requirements. For ipe-roxo, the segment type did not influence survival and rooting responses, which presented means of 48.12% and 54.37%, respectively, at 90 days of growth (data not shown). However, segment type did influence induction and growth of the shoots, with apical mini-cuttings presenting increased shooting capacity compared with nodal mini-cuttings, regardless of the substrate composition (Table 3).

Table 3 - Shooting percentage, number and length of shoots formed in apical and nodal ipe-roxo mini-cuttings grown in different compositions of substrates at 60 and 90 days.

Mini-cutting	Shooting (%)	Number of shoots	Shoot length (cm)	Shooting (%)	Number of shoots	Shoot length (cm)
	-----60 days of growth-----			-----90 days of growth-----		
Apical	47.60 a*	0.50 a	1.83 a	56.20 a	0.60 a	3.28 a
Nodal	18.80 b	0.21 b	0.26 b	31.20 b	0.33 b	3.81 a
Mean	33.20	0.36	1.05	43.80	0.47	3.55
CV(%)	70.30	18.07	33.16	53.40	63.64	73.95

*Values followed by the same letter do not differ by Tukey test at 5% probability of error.

Apical mini-cuttings also presented increased shoot length compared to nodal mini-cuttings at 60 days, but not at 90 days of growth (Table 3). Apical mini-cuttings normally have advantages over nodal mini-cuttings because they present lower tissue lignification (BORGES et al., 2011) and are produced in younger areas of the plant. These results are due to the fact that the apical and nodal mini-cuttings of ipe-roxo originated from regions of active growth, indicating that they produce high levels of endogenous auxins. Because they present similar volumes (0.03 to 0.31cm³) (Figure 1), they may also have similar amounts of reserve substances, such as carbohydrates, which influence in the emission of roots (FACHINELLO et al., 2005). FERREIRA et al. (2012) also reported that basal, intermediate and apical mini-cuttings of cedro-australiano (***Toona ciliata***) showed no difference in rooting and shoot could be used in its entirety to produce plantlets by mini-cutting.

The volume of the ipe-roxo mini-cuttings did not affect the length of roots and shoots or the number of roots in the mini-cuttings (Figure 1). The lack of a relationship between mini-cutting volume

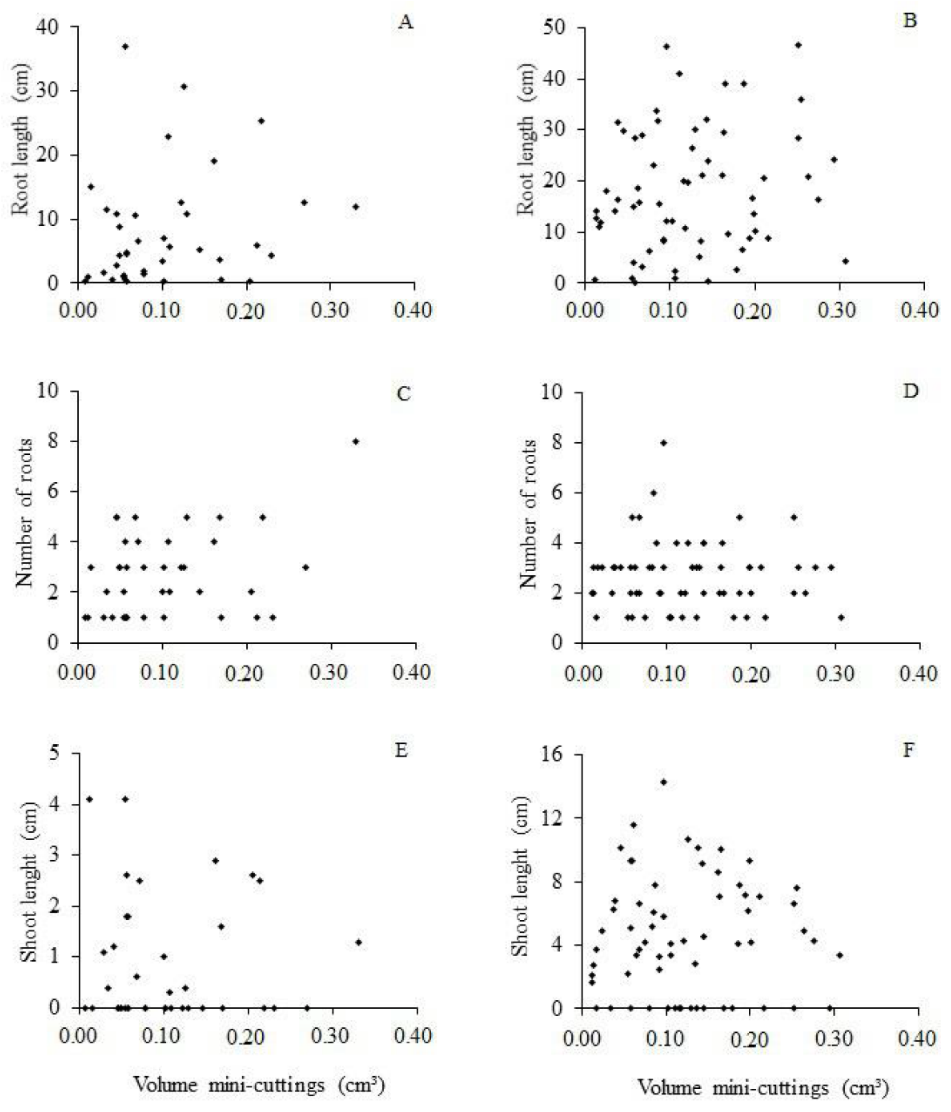

Figure 1 - Relationship between ipe-roxo mini-cutting volume and root length (A and B), number of roots (C and D) and shoot length (E and F) after 60 and 90 days of growth in different compositions of substrate.

and rooting and shooting indicates that the ipe-roxo provides flexibility in terms of mini-cuttings volume and the propagules form roots and shoots regardless of their diameter, maximizing plantlet production. This indicates that the relationship between the amount of reserve carbohydrates, probably related to mini-cuttings volume, and the formation of roots may be controversial. Thus, while carbohydrate quantity and root formation may be positively correlated, carbohydrates do not have regulatory function in these processes (HARTMANN et al., 2010). In louro-pardo (***Cordia trichotoma***), volume of root cuttings affected shooting but not rooting (KIELSE et al., 2013) and in alamo (***Populus tremula*** x ***tremuloides***) it affected rooting, but not shooting (STENVALL et al., 2006).

The results of this study showed that commercial substrate and vermiculite, without the presence of sand in the composition, favor the survival and rooting of ipe-roxo mini-cuttings. The greatest rooting occurred in commercial substrate and vermiculite in ratios of 1:2, 1:1 and 2:1, corresponding to the substrates with lower DD and AS and higher TP and WRC values (Table 1). However, the composition of commercial substrate and vermiculite in the ratio 1:2 produced rapid initiation of roots, since after 60 days of growth, it already presented 45% rooting. Mini-cutting volume did not affect rooting or shooting, suggesting that it is possible to maximize the number of mini-cuttings obtained from mini-stumps, which is advantageous for the commercial production of ipe-roxo plantlets by mini-cutting.

CONCLUSION

Apical and nodal mini-cuttings originatted from juvenile shoots exhibit similar behavior and may be rooted in commercial substrate and vermiculite to produce ipe-roxo plantlets by mini-cutting.

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