VIABILITY OF THE MINI-CUTTING TECHNIQUE IN THE PRODUCTION OF *Drimys brasiliensis* Miers SEEDLINGS

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Received for publication: 03/03/2018 – Accepted for publication: 17/09/2019

Resumo

Viabilidade da técnica de miniestaquia na produção de mudas de Drimys brasiliensis Miers. Drimys brasiliensis, conhecida popularmente como cataia ou casca-d'anta, é uma espécie arbórea nativa da Mata Atlântica e pertencente à família Winteraceae. Por suas sementes possuírem dormência por imaturidade embrionária, a presente pesquisa objetivou avaliar a viabilidade da técnica de miniestaquia em *D. brasiliensis* em diferentes épocas de coleta dos propágulos. Miniestacas provenientes de brotações de mudas produzidas previamente por meio da estaquia convencional foram coletadas em seis diferentes momentos (dezembro/2015, fevereiro/2016, abril/2016, junho/2016, agosto/2016 e outubro/2016), confeccionadas com 6-8 cm de comprimento, com corte em bisel na base e reto no ápice, mantendo duas folhas com sua área reduzida à metade. O experimento foi realizado seguindo um delineamento inteiramente casualizado contendo 4 repetições e 14 miniestacas por unidade experimental. As miniestacas foram plantadas em tubetes com substrato vermiculita e casca de arroz carbonizada (1:1) e mantidas em casa de vegetação climatizada. Após 120 dias, avaliou-se a porcentagem de miniestacas enraizadas, número de raízes por miniestaca, comprimento das três maiores raízes por miniestaca, porcentagem de miniestacas com calos, vivas, mortas, com brotações e que mantiveram suas folhas originais. As porcentagens de enraizamento das miniestacas foram superiores a 75% independente da estação do ano em que os propágulos foram coletados. A formação de raízes adventícias de *D. brasiliensis* ocorre de forma indireta, a partir de tecido caloso formado na base das miniestacas. A técnica de miniestaquia é recomendada para produção de mudas da espécie, em qualquer estação do ano.
Palavras-chave: Propagação vegetativa, cataia, rejuvenescimento, espécie nativa, espécie medicinal.

Abstract

Drimys brasiliensis, popularly known as cataia or casca-d'anta, is a tree species native to the Atlantic Forest and belonging to the family Winteraceae. Due to its seeds have dormancy due to embryonic immaturity, the present work aimed to evaluate the viability of the mini-cutting technique in *D. brasiliensis* in different seasons of collection of the propagules. Mini-cuttings from seedlings sprouts previously produced by conventional cutting were collected at six different times (December/2015, February/2016, April/2016, June/2016, August/2016 and October/2016), made 6-8 cm long, with bevel cut at the base and rectum at the apex, keeping two leaves with their area halved. The experiment was carried out in a completely randomized design with 4 replicates and 14 mini-cuttings per experimental unit. The mini-cuttings were planted in tubes with substrate containing vermiculite and carbonized rice husk (1:1) and kept in a heated greenhouse. After 120 days, the percentage of rooted mini-cuttings, number of roots per mini-cuttings, length of the three largest roots by mini-cuttings, percentage of mini-cuttings with callus, live, dead, with shoots and leaves that maintained their original leaves were evaluated. Rooting percentages of mini-cuttings were greater than 75% regardless of the season of the year in which the propagules were collected. The adventitious root formation of *D. brasiliensis* occurs indirectly, from the callus tissue formed at the base of the mini-cuttings. The mini-cuttings technique is recommended for the production of seedlings of the species, at any seasons of the year.
Keywords: vegetative propagation; cataia; rejuvenation; native species, medicinal species.

INTRODUCTION

Drimys brasiliensis Miers (Winteraceae) is a native species of Brazil, popularly known as cataia or casca-d'anta. Found from Bahia to the Rio Grande do Sul, it is represented by trees and large bushes, with phytochemical importance, being used, among others, as raw material for the manufacture of a biochemical product called drimanial, which posesses active action against migraine (SIMÕES *et al*., 1986; LORENZI; MATOS, 2008).

The importance of the species is mainly linked to the use of leaves and barks in the folk medicine because it presents stimulant action, anti-convulsant, aromatic, anti-diarrheic, anti-fever, against abnormal uterine bleeding and in certain affection of the digestive tract (SIMÕES *et al*., 1986).

The seeds of the *D. brasiliensis* have dormancy because of embrionary immaturity, that is to say, the embryo needs a additional time to sprout even under favorable environmental conditions, which is a problem due to the the long sprouting time that delays the development of the seedlings, complicating the generation and consequently propagation of the species (SANTOS *et al*., 2004). Thus, studies related to the plant spread by cutting of *D. brasiliensis* were accomplished in order to obtain a larger production of seedlings, cultivation uniformity and low cost of manufacture. Although, the percentage are still low, approximately 50%, obstructing the manufacture in comercial scale (MACHADO *et al.*, 2011; ZEM *et al.*, 2015; ZEM *et al.*, 2016).

The plant dissemination by the mini-cutting subsidized the evolution of the clonal forestry, with increase of productivity, planting pradonization and, especially, qualified forestry products (WENDLING *et al*., 2010; KRATZ *et al*. 2015). This technique exhibits quite a few strengths compared to the convencional cutting, such as the increase of productivity in the sprouting per area, beyond the exploitation of the juvenal aspects of the propagating material that benefit the adventitious rooting (DIAS *et al.*, 2012; STUEPP *et al.*, 2015).

The process of root formation implicates various factors such as physiological condition of the mother plants, juvenility of the planting material, hormonal balance, planting material retrieval season, among others (ZHANG *et al.*, 2016). The seasonality of the rootings of mini-cuttings, due to variation in the evironmental conditions, such as temperature and photoperiod, which can influence the physiological condition of the mother plant, with variations in the endogenous hormonal balance over the seasons of the year (ZUFFELLATO-RIBAS; RODRIGUES, 2001).

Thus, given the importance of the species and the necessity of the development of a eficient production system of *D. brasiliensis*, this research aimed to evaluate the viabilility of the mini-cutting technique of cataia, as well as the influence of different harvest seasons in the rooting of the mini-cuttings, besides the characterizaton of the species root formation.

MATERIAL AND METHODS

The experiment was conducted in a greenhouse at the Macropropagation Laboratory, of the GEPE (Research and Study Group on Cuttings), located at Federal University of Paraná (UFPR) in Curitiba (PR). The vegetative material was collected from ~7 month old mini-stump shoots (Figure 1A and 1B) that were previously produced through conventional cutting, conducted by a clonal mini-garden system in full sun, with controlled irrigation (three times a day for 8 minutes). Each mini-stump was fertilized every 15 days with 50 ml of solution: containing 4 g of ammonium sulfate, 4 g of triple superphosphate, 4 g of potassium chloride and 1 g of FTE BR-12 per liter of water.

To this paper, the harvesting of the sproutings were conducted, approximately, each 60 days, as the growth of the sproutings, constituing six harvests (december/2015; february/2016; april/2016; june/2016; august/2016 e october/2016). From this branches, stemmed mini-cuttings were prepared with approximately 6-8 cm of length (1C Figure), with a bevel cut in the base and straight at the tip, mantaining two leaves in the apical portion with its area reduced to half. During the preparation process, the mini-cuttingswere kept in a recipient with water to avoid the dehidration of the material.

Mini-cuttings were planted in 53 cm^3 polypropylene tubes filled with fine-grained vermiculite and carbonized rice husk (1:1), kept in a greenhouse with intermittent mist (temperature of 24ºC ± 2ºC and relative humidity equal to or higher than 80%) (Figure 1D).

The experiment was implanted in a entirely designed delineation, with four repetitions, containing 14 mini-cuttings per experimental unity, totalizing 56 mini-cuttings by harvest season, except the harvest conducted in august/2016, in which the development of the ministumps sproutings was smaller, enableling to collect only 10 mini-cutting per experimental unity.

The data were submited to the Bartelett test to verify the homogeneity and aferwards submetid to analyses of variancy (ANOVA). The varibles that presented significative difference by the F test had their averages compared by the Tukey test to the 5% probality level.

After the period of 120 days of estabelishment of the experiment, the following variables were analysed: percentage of rooted mini-cuttings (mini-cuttings that output roots of at least 1 mm of length) (1E Figure); number of roots by mini-cutting; length of the three bigger roots by mini-cutting (cm); percentage of mini-cutting with callus (mini-cuttings, without roots, forming non-differentiated celular mass in the base); percentage of live mini-cuttings (mini-cutting with no presence of callus and no roots); percentage of dead mini-cuttings (mini-cuttings with rotted tissue); percentage of sprouted mini-cuttings (live mini-cuttings, with or without roots and callus, that presented sprouting of new leaves); percentage of mini-cuttings that maintained

their original leaves (live mini-cuttings, with or without roots and callus, that mantained their original leaves in the moment of the evaluation).

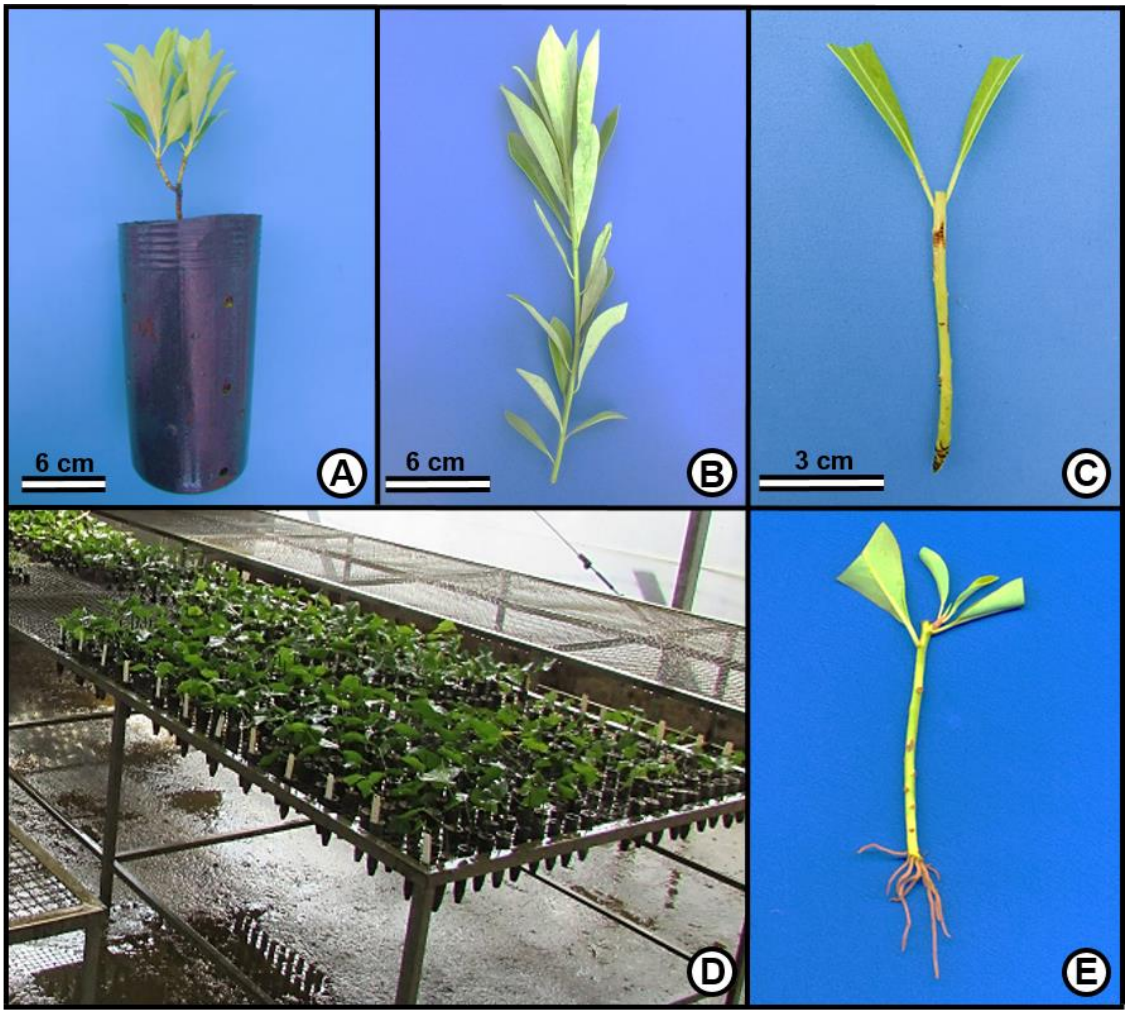

Figure 1. Schematic sequence of *D. brasilienesis* mini-cuttings technique: general characterization of mini-stumps (a), collected sprout (b), prepared mini-cutting (c), mini-cutting planted in greenhouse (d), rooted mini-cutting (e).

Figura 1. Sequência esquemática da técnica de miniestaquia de *D. brasilienesis*: caracterização geral de minicepa (a), ramo coletado (b), miniestaca preparada (c), experimento instalado em casa de vegetação climatizada (d), miniestaca enraizada (e).

RESULTS

A significative difference occurred to the level 5% of probability to the variable percentage of rooting, average number of roots by mini-cutting, average length of root by mini-cutting, percentage of mini-cuttings with caluses, percentage of dead mini-cuttigs, with sproutings and that mantained their original leaves. Although, there was no significative difference to the variable percentage of live mini-cuttings. (Table1).

The harvest conducted on august/2016 presented a bigger percentage of rooting (87.50%), but with no statistic difference from the harvests of december/2015 (75%), april/2016 (76.79%) e october/2016 (76.79%). That way, it is possible to notice that there was no relation between the season the plant material were collected and the rooting of the mini-cuttings, to the extent that even if noted 55.36% of rooting on two harvest dates (february and june), figures superior than 75% of rooting were noted in every season of the year (december = summer; april = autumn; august = winter; october = spring).

With regard to the number of roots by mini-cutting, the dates of harvest presented lower quantities of rootes were on april/2016 (3.92) and june/2016 (4.08). For average length of the three bigger roots by mini-

cutting, the harvests conducted on october/2016 (2.25 cm) and february/2016 (1.78 cm) were statistically superior to the others (Table 1).

Table 1. Mean comparisons of *D. brasiliensis* mini-cuttings at the six collection times in Curitiba, PR.
Tabela 1. Comparação de médias das variáveis de miniestacas de *D. brasiliensis* em diferentes datas de coleta, Curitiba, PR.

Harvest date	RM	NR	MRL	MC	LM	DM	MS	MML
	%		cm	%	%	%	%	%
December/2015	75.00 ab	4.74 ab	0.87 c	14.29 b	3.57 a	7.14 ab	83.93 ab	85.71 b
February/2016	55.36 b	6.09 ab	1.78 ab	17.86 ab	12.50 a	14.29 a	58.93 bc	82.14 b
April/2016	76.79 a	3.92 b	0.91 c	16.07 b	7.14 a	1.79 b	67.86 bc	91.07 ab
June/2016	55.36 b	4.08 b	1.08 c	37.50 a	7.14 a	0.00 b	46.43 c	98.21 a
August/2016	87.50 a	7.06 a	1.50 bc	7.50 b	5.00 a	0.00 b	100.00 a	100.00 a
October/2016	76.79 a	6.96 a	2.25 a	10.71 b	1.78 a	7.14 ab	85.71 ab	91.07 ab
CV (%)	12.41	23.03	21.18	54.09	87.96	86.45	17.59	5.60

Caption: RM, rooted mini-cuttings; NR, mean number of roots per mini-cutting; MRL, mean root length; MC, mini-cuttings with callus; LM, live mini-cuttings; DM, dead mini-cuttings; MS, mini-cuttings with shoots; MML, percentage of leaf maintenance in mini-cuttings. CV, coefficient of variation. Means followed by the same letter in the column do not differ statistically from each other by the Tukey test at 5% probability.

For the variable mini-cuttings with callus, the harvests conducted on june/2016 and february/2016 presented a increased formation of callus, but no statistics difference from the others. On Figure 2, it is presented the correlation between the total of rooted mini-cuttings (with or without callus), rooted mini-cuttings with the presence of callus and mini-cuttings only with callus.

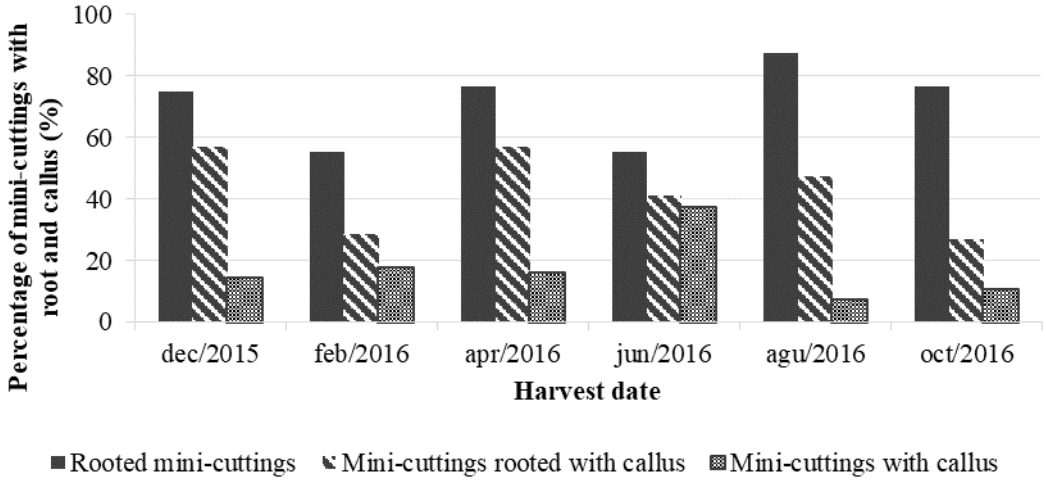

Figure 2 - Influence of callus formation on the rooting of mini-cuttings of *Drimys brasiliensis*, at different collection season.
Figura 2 - Influência da calosidade no enraizamento de miniestacas de *Drimys brasiliensis*, em diferentes épocas de coleta

In relation to the variable percentage of dead mini-cuttings, broadly the mortality can be considered low in all of the harvests with an average higher than 14.29% on the harvest of february/2016, corresponding to the suummer. Observing the percentages of mini-cuttings sprouted and that kept their original leaves, in the harvest

FLORESTA, Curitiba, PR, v. 50, n. 2, p. 1195 - 1202, abr/jun 2020
Maggioni, R. A. *et.al*
Electronic ISSN 1982-4688
DOI: 10.5380/rf.v50 i2. 62020

on august/2016, correspondng to the winter, the figures were 100% to both variables. On this data, a bigger percentage of rooting was noted as well.

DISCUSSION

It is possible to notice, by this paper, that the higher percentage of mini-cutitng rooting, desregarding the season of the year that the plant material were collected, were superior to 75% with no necessity to use a vegetal regulator. This fact frames the *D. brasiliensis* on the category of easy rooting by mini-cutting, with the endogenous presence of auxins and co-factors of the rooting.

Conversely, Zem *et al.* (2015), avaluating the of effect of the four seasons of the year over the rooting of cuttings of *D. brasiliensis*, stemmed from adult trees, observed a higher percentage of rooting (46.96%) in cuttings on winter. Rasmussen *et al.* (2015) afirm that temperature can influence on adventitious rooting, mainly concerning the absortion of nutrientes and the metabolism of the plant, since higher temperatures result in a elevated plant development, with higher auxins synthesis and consequently higher adventitious rootings.

To some species, seasons with lower temperatures correspond with the period of rest of the plante, with low metabolism and consequently larger accumulation of supplies, generatibg a bigger root inducement (HARTMANN *et al.*, 2011), corroborating this paper which, on Agosto/2016, corresponding to winter, it was noted a slower development of the ministumps sprouts, although it is possible to note by the Table 1 that the most expresive figures of the rooting percentage (87.50%), percentage of mini-cutting with sprouts (100%) and that mantained the original leaves (100%), were obtained on that date of harvest.

According to Fachinello *et al.* (2005), the formation of roots can be influenced by external factors, such as humidity, temperature, brightness, substrates, types of cutting and period of the year, as well as the genotype of the plant. The environmental factors can influence on the free auxin quantity in mother-plants, determinating insatisfactory levels to the rooting, making necessary the applicaton of exogenous auxins (OSTERC; STAMPAR, 2011; NEGISHI *et al.*, 2014).

The rejuvenescence of the plant material enabled de high percentage of rooting to the *D. brasiliensis* (87.50%), with no necessity to the use of plant regulators. Since it is a native species, this results are promissing and demonstrate the eficiency of the technique of mini-cutting to the species, demonstrated by the inferior percentage of rooting obtained on researchs with convencional cuttings from adult trees of the *D. brasiliensis*, on which Machado *et al.* (2011), evaluating the different effects of plant regulators on rooting of stem cuttings of the species, obtained a higher percentage of rooting (52.53%) when the auxin was used with the caffeic acid conveyed on talcum. To Zem *et al.* (2016), the use of the IBA plant regulator (indole butyric acid) had no nfluence of the rooting of the stem cuttings of the *D. brasiliensis*, even so the better percentage of rooting were only 51.1%. Radomski *et al.* (2013) also observed that the application of the IBA plant regulator had no influence on the rooting of the stem cuttings of the, having the higher percentages of rooting obtained on the study of 46.75%.

Thus, it is possible to notice that even the lower percentage of rooting presented on the study (55.36%) were superior the the higher levels of adventitious rooting on researchs with traditional stem cuttings of the species, with no necessity of application of plant regulators. It appears, this ways, that the juvenility was favorable to the root inducement, unbiased by the period of the harvest of the plant material, which exposes the success of the mini-cutting technique to the generation of sprouts of the species along the whole year.

The influence of rejuvenescence of plant material on the adventitious rooting was already observed by enumerous authors, which related that the fragments collect of plants on juvenile development rooted more easily than the the fragments collected of adult plants (FACHINELLO *et al.*, 2005; DIAS *et al*., 2012; WENDLING *et al*., 2014). Corroborating the obtained results, Pimenta *et al.* (2014), studying the rooting of mini-cuttings of *Jatropha curcas* L., arboreal species, observed viability of the mini-cutting technique on any period of the year to the species. Dias *et al.* (2012), in a tudy on mini-cutting of *Anadenanthera macrocarpa*, afirmed the efficiency of the technique to the species, with no necessity of a IBA plant regulator to the rooting of the mini-cuttings. To the *Paulownia fortunei* var. *mikado*, the mini-cutting technique has shown to be viable, with high rates of survival and rooting of the mini-cuttings (STUEPP *et al*., 2015).

Contrary to a traditional mini-garden (semi-hydroponic system on sand), the technique presented in this study is simplified (1.7 litre pots containing comercal substrate), with lower frenquecy of fertigation and, therefore, lower substrate salinization, what was already observed as a issue to traditional systems (WENDLING *et al*., 2010; BRONDANI *et al*., 2012). This way, the technique used on this research garanteed the nutricional conditions of the mother plants favorable to root, taking in consideration the high levels of rooting obtained.

Regarding the variables number of roots by cutting and average length of roots, the values obtained can be considered adequate to the survival of sprouts on field. According to Radmann *et al.* (2014), the number and length of the three bigger roots by mini-cutting are important variables to the generation of sprouts, since the

better responses to that variables correspond into a better root development increasing the chances of survival of the sprouts when taken to the field.

In this present work, callosity and rooting happened simustaneously, in every harvest conducted (Figure 2). It is possible to notice that the harvests with lower percentages of mini-cuttings wit rooting also presented higher percentage of mini-cuttings with callus. Other author already noted thatJá foi, to the *D. brasiliensis*, the appearence of tissue with non-differentiated cells is a condition to the formation of adventitious rooting (MACHADO *et al*., 2011; ZEM *et al*., 2015).

There are two distinct ways to the formation of adventitious roots: direct and indirect. On the direct way, it is argumented the existence of two cells responsable to form roots that, after the inducement, celular division begins, leading the formation of the origin root, which arises from cells of the vascular system and of cells nearby to it (HARTMANN *et al*., 2011). The indirect ways occurs when the non-oriented division cells form callus, which are groups of cells of desorganized growth that, when divided in a organized manner, diferentiate in adventitious roots (HARTMANN *et al.*, 2011).

Once the majority of the mini-cuttigs emited roots with previous formation of callus (Figure 2), except the harvest of october/2016, when it presented more rooted mini-cutting and without callluses, and, since the species has a high percentage of rooting, it is possible to affirm that the root formation of this species can be direct such as indirect, and that certainly a longer stay in a greenhouse would provide that the cutting with callus to diferentiate in roots.

The reduced values of dead and live mini-cuttings are consequence of the high rates of rooting, validating the efficiency of the mini-cutting technique to the *D. brasiliensis*. This results exposeEsses resultados emphasize the adequate the environmental conditions (temperature and umidity) of the greenhouse, also justifying by the high numbers of mini-cuttings with sproutingst and that mantained their original leaves (Table 1) (ZUFFELLATO-RIBAS; RODRIGUES, 2001; BORGES *et al*., 2011). Similar results were observed by Stuepp *et al.* (2016) in studies with mini-cuttings of the *Acer palmatum*, a arboreal species, in which the average of survival of the mini-cuttings was of 17.5% and mortality of 18.8%, with rooting of approximately 95%.

The percentages of mini-cuttings with sprouts and that mantained the original leaves were elevated on all the harvest dates. The importance of the mantainance of the leaves is directly related to the root inducement, mainly in relation to the presence of certain compounds in the leaves, such as carbohydrates, auxins and co-factors of rooting, which can be moved via phloems to the base of the cuttings, this way stimulating the formation of roots (BONA; BIASI, 2010; FRAGOSO *et al*., 2015).

As presented in this present work, the rejuvenescence of the plant material garanteed the elevated percentage of rooting and development of the root system, sugesting that the mini-cutting technique is recommended to the manufecture of sprouts in a comercial scale of the species. This technique garantees the planting uniformity, decrease in the use of plant regulators, reduction of cost and cerntainty of sprouts production over the whole year.

CONCLUSION

In the condition that the present experiment was conducted, it is possible to conclude:

- The mini-cutting technique is viable and recomended to the manufacture of sprouts of the *D. brasiliensis* in every season of the year.
- The formation of adventitious roots *D. brasiliensis* is possible to occur in direct and indirect way (from the calloused tissue formed in the base of the mini-cutting).

AKNOWLEGDEMENTS

To the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), for the financial support.

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