SCIENTIFIC NOTE

Propagation of macadamia (*Macadamia integrifolia* Maiden & Betche) by cuttings

Propagación de macadamia (*Macadamia integrifolia* Maiden & Betche) por estacas

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ABSTRACT

Macadamia trees require a long period for flowering when propagated by seeds. To anticipate the yield, maintain cultivar characteristics, and increase the homogeneity of nut quality, it is essential to establish orchards with grafted trees. Although semi-hardwood cutting propagation tests have been performed in Brazil, no method has been shown to be appropriate at a large scale due to the difficulties in implementation of techniques or the high cost. Establishing an effective and affordable protocol may provide great improvement to this productive chain since it will meet the demand of nurseries and stimulate the expansion of orchards. The aim of this study was to develop a protocol for macadamia semi-hardwood cutting propagation. Six cultivars (HAES 344, HAES 816, HAES 660, IAC 4-12B, IAC 9-20, and IAC 4-20) were evaluated and selected because of their ample cultivation in Brazil. The propagation material was collected for four months (October and November 2018, and February and March 2019). The semi-hardwood cuttings were also soaked in indole butyric acid (IBA) at three different concentrations (0, 5, or 10 g L^{-1}) and in the commercial product Clonex®. The experiment was arranged in a two-way completely randomized design with four replicates and data were analyzed by R software. The cultivar IAC 4-12B showed the highest index for root development (37.0%). The treatments did not show significant differences between IBA doses and Clonex® for root development (IBA 10 g L^{-1} - 31.5%, Clonex® - 29.4%, and IBA 5 g L^{-1} - 27.4%). November was the best cutting season for root development of semi-hardwood cuttings (10.0%).

Key words: indole butyric acid, rooting, cultural practices.

RESUMEN

Los árboles de macadamia requieren un largo período para empezar la floración cuando se propagan por semillas. Para anticipar el rendimiento, mantener las características del cultivar y aumentar la homogeneidad de la calidad de las nueces, es esencial establecer huertos con árboles injertados. Aunque se han realizado ensayos de propagación de esta planta por estacas semileñosas en Brasil, ningún método ha demostrado ser el apropiado a gran escala debido a las dificultades en la implementación de las técnicas o al alto costo. El establecimiento de un protocolo eficiente y accesible puede proporcionar una gran mejora a esta cadena productiva ya que satisfará la demanda de viveros y estimulará la expansión de los huertos. El objetivo de este estudio fue desarrollar un protocolo para la propagación de macadamia por estacas semileñosas. Seis cultivares (HAES 344, HAES 816, HAES 660, IAC 4-12B, IAC 9-20, e IAC 4-20) fueron evaluados y seleccionados por su amplio cultivo en Brasil. El material de propagación se recolectó durante cuatro meses (octubre y noviembre de 2018 y febrero y marzo de 2019). Las estacas semileñosas se sumergieron en ácido indolbutírico (AIB) en tres concentraciones diferentes (0, 5, o 10 g L^{-1}), y en el producto comercial Clonex®. El experimento se realizó siguiendo un diseño bidireccional con cuatro repeticiones y los datos fueron analizados por el software R. El cultivar IAC 4-12B mostró el índice más alto para el desarrollo de las raíces (37.0%). Los tratamientos no mostraron diferencias significativas entre las dosis de AIB y Clonex® para el desarrollo de las raíces (AIB 10 g L^{-1} - 31.5%, Clonex® - 29.4%, AIB 5 g L^{-1} - 27.4%). Noviembre fue la mejor temporada de recolección para el enraizamiento de las estacas semileñosas (10.0%).

Palabras clave: ácido indolbutírico, enraizamiento, prácticas culturales.

Introduction

The macadamia (*Macadamia integrifolia* Maiden & Betche) is a nut tree belonging to the Proteaceae family, native to tropical and subtropical Australian forests (Peace *et al.*, 2005). It was introduced into Brazil in 1931 (Dierberger & Marino Netto, 1985) and in 1940 the Agronomic Institute began the only macadamia breeding program in the

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country (Sobierajski *et al.*, 2006). Orchards are generally established by using grafted trees to anticipate yield, maintain cultivar characteristics, and increase the homogeneity of nut quality (Melo *et al.*, 2019). The technique usually used for vegetative propagation is grafting (Campo-Dall'Orto *et al.*, 1988). It takes 18 months for the seedling to be ready for planting in the orchard (Russell *et al.*, 2016). In previous studies, the rate of grafting success reached 80% (Campo-Dall'Orto *et al.*, 1983). However, this rate is lower than 50% in commercial nurseries. These factors increase the cost of seedlings and reduce their availability to growers making it difficult to expand the culture in Brazil (Melo *et al.*, 2019).

The macadamia tree produces a nut of great acceptance and high value in the international market (Penoni *et al.*, 2011; Maro *et al.*, 2012). In recent years, there has been an increase in the demand for seedlings of macadamia in Brazil (Perdoná & Soratto 2015; 2016).

The propagation by semi-hardwood cuttings can reduce the time and costs of seedling production (Bell, 1993). Therefore, establishing an effective and affordable protocol of seedling production by semi-hardwood cuttings will provide an advance for macadamia in Brazil, stimulating orchard expansion.

Pereira *et al.* (1987) studied the rate of rooting in cuttings of five cultivars from two seasons. The authors observed significant differences for cultivars and seasons but no interactions between them. The emission of roots in cuttings of macadamia is the major difficulty for vegetative propagation. Therefore, the use of synthetic growth regulators, such as auxin can be an alternative to overcoming this problem (Garbelini, 2009). Another option is the use of plant growth regulators, e.g., indole butyric acid (IBA) that, when compared to a synthetic auxin, shows lower sensitivity to biological degradation (Fachinello *et al.*, 2005). These plant growth regulators also stimulate root growth (Alvarenga, 1990). However, the ideal IBA concentration for cutting immersion varies among different species and cultivars (Hartmann *et al.*, 2002), and it is still unknown for macadamia. On such a background, the aim of this study was to identify the macadamia cultivars with the best rooting performance of semi-hardwood cuttings and test different IBA concentrations and seasons for seedling production by semi-hardwood cuttings.

Materials and methods

Six macadamia cultivars were propagated by semi-hardwood cuttings. The percentage of survival, callus formation, and rooting were evaluated. The cultivars were selected for their characteristics of yield and nut quality. The cultivars HAES 344, HAES 816, and HAES 660 were developed by the Hawaii Agricultural Experiment Station (HAES) and the Agronomic Institute (IAC) developed the cultivars IAC 4-12B, IAC 9-20, and IAC 4-20.

The semi-hardwood cuttings were collected from 12-year-old trees in Dois Córregos, São Paulo, Brazil, for four months (October and November 2018, and February and March 2019). The materials were cut from the medium treetop with 15-20 cm of length and 3-5 cm of diameter; three leaves were kept for all cuttings, as recommended by Russell *et al.* (2016). The semi-hardwood cuttings were soaked in IBA (0, 5, or 10 g L^{-1}) (NEON®, Suzano, Brazil) for 15 min, followed by planting in a propagation tube (120 ml) filled with substrate (peat-vermiculite-limestone, Carolina Soil®, Pardinho, Brazil). In addition, the tips of cuttings collected in November, February and March were also soaked in Clonex® (IBA 3 g L^{-1}, for 15 min) (Growth Technology, Taunton, UK). To prevent the cuttings from drying out, they were planted on the same day of collection in a mist-house.

The humidity in the mist-house was maintained using a controller (Fascitec NTI 12 - AC, São Bernardo do Campo, Brazil) for intermittent watering and to avoid accumulation of water on the leaves. The experimental design was a two-way completely randomized design with four replicates and five cuttings by plot/month, of which 20 cuttings were the control treatment (distilled water). Thus, 1,800 cuttings were evaluated.

After 120 d, the semi-hardwood cuttings were removed from the tubes, washed, and evaluated for survival, callus, and root development. The data were transformed by the BoxCox method (Box & Cox, 1964) to meet the normality assumption. The data were analyzed using the R software (R Development Core Team, 2019), and the cluster analyses and threshold were calculated using the R-package 'factoextra' (Kassambara & Mundt, 2017).

Results and discussion

From the 1,800 semi-hardwood cuttings, 9.8% showed callus, 5.7% developed roots, and 84.5% did not survive. This high mortality caused the data to be unbalanced, which prevented the use of an analysis of variance. The statistical analysis was performed by descriptive and multivariate analysis.

The best season for removing semi-hardwood cuttings for the presence of callus was October 2018 (22.8%), considering all cultivars and doses of IBA and Clonex® (Fig. 1A). However, the best results for root development were observed for those plants collected in November 2018 (10.0%; Fig. 1B). The lowest semi-hardwood cutting mortality was observed when the removals occurred in October 2018 (71.9%), while the highest rates occurred in February 2019 (90.2%) and March 2019 (92.3%; Fig. 1C).

The control treatment (without IBA application) showed the highest rate for the presence of callus (42.4%), considering all cultivars and collecting seasons (Fig. 2A). However, this treatment showed the lowest rooting rate (11.8%). The other treatments showed no statistical differences for root development with 31.4% for 10 g L^{-1} IBA, 29.4% for Clonex®, and 27.4% for 5 g L^{-1} IBA (Fig. 2B). The mortality was statistically lower in semi-hardwood cuttings treated with Clonex® (19.5%) than with the other doses of IBA (Fig. 2C).

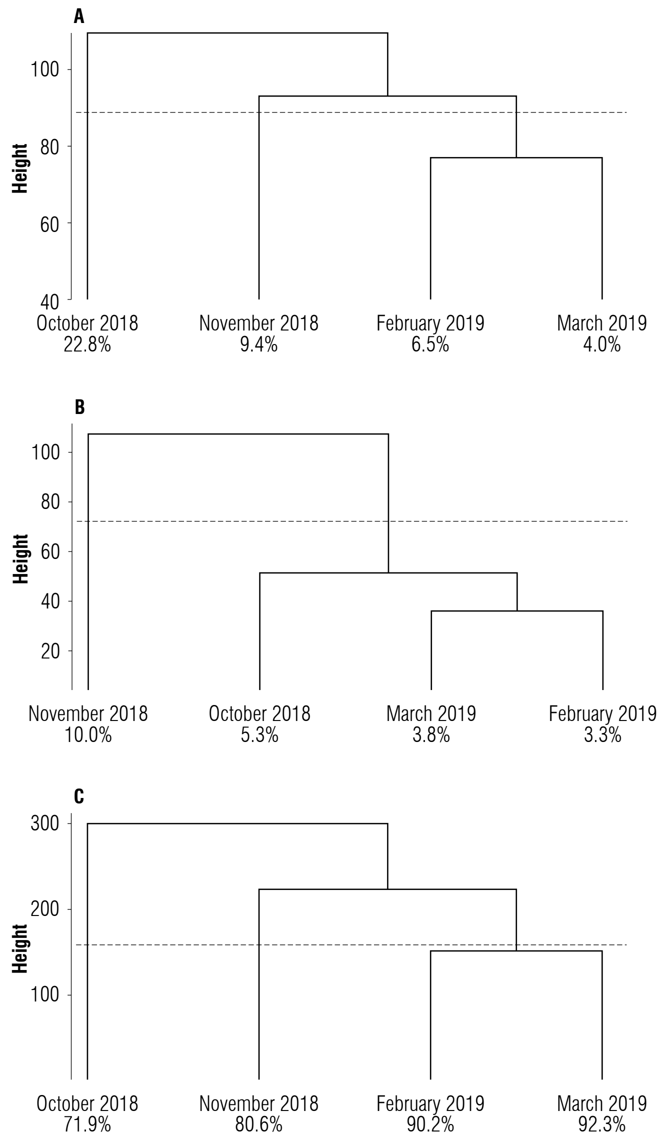

FIGURE 1. Similarity matrix (Euclidean distance) among collection seasons of semi-hardwood cuttings of *Macadamia integrifolia*, considering all cultivars and doses of IBA and Clonex®. The similarity matrix considered the following parameters: A) presence of callus, B) root development, and C) mortality. The grouping method (cluster analyses) was based on the K-means algorithm (Kassambara & Mundt, 2017).

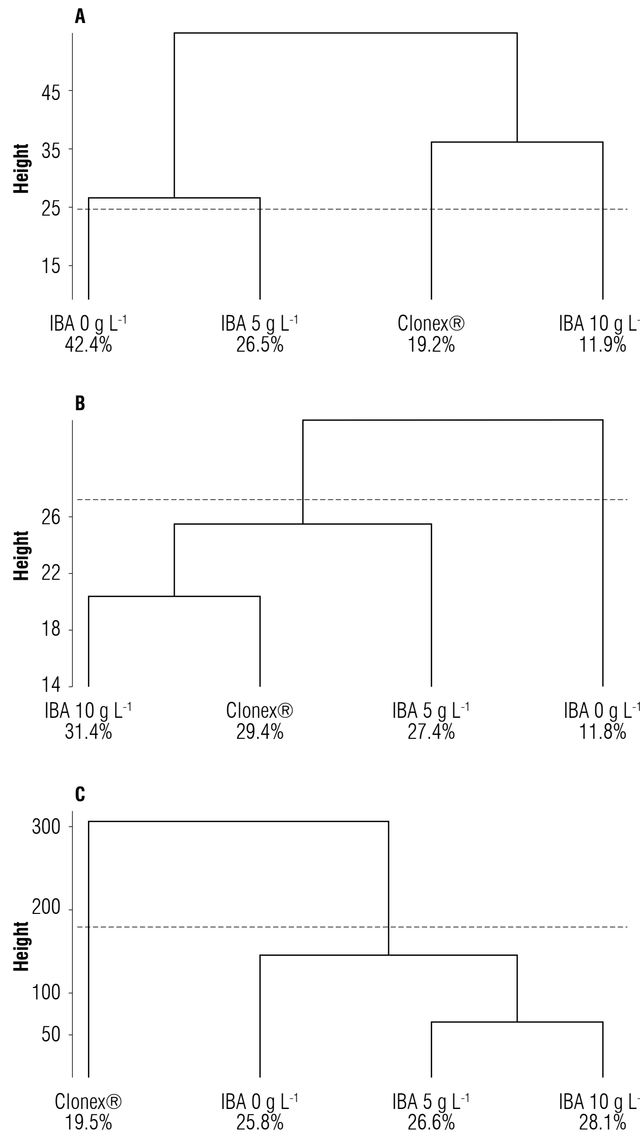

FIGURE 2. Similarity matrix (Euclidean distance) among doses of indole butyric acid (IBA) and Clonex® for semi-hardwood cuttings of *Macadamia integrifolia* treatment, considering all cultivars and collecting seasons. The similarity matrix considered the following parameters: A) presence of callus, B) root development, and C) mortality. The grouping method (cluster analyses) was based on the K-means algorithm (Kassambara & Mundt, 2017).

The cultivar HAES 660 showed the highest presence of callus (35.6%) considering all seasons of collection and doses of IBA and Clonex® (Fig. 3A). However, only 12.0% of the semi-hardwood cuttings of this cultivar effectively developed roots (Fig. 3B). Regarding root development, the cultivar IAC 4-12B showed the highest rate (37.0%), followed by the second group (formed by 'IAC 9-20' and 'IAC 4-20') that showed the second-highest performance (Fig. 3B). The mortality rates by cultivar were similar to each other, but the cultivars HAES 660 (14.8%) and IAC 4-12B (15.6%) showed the lowest values (Fig. 3C).

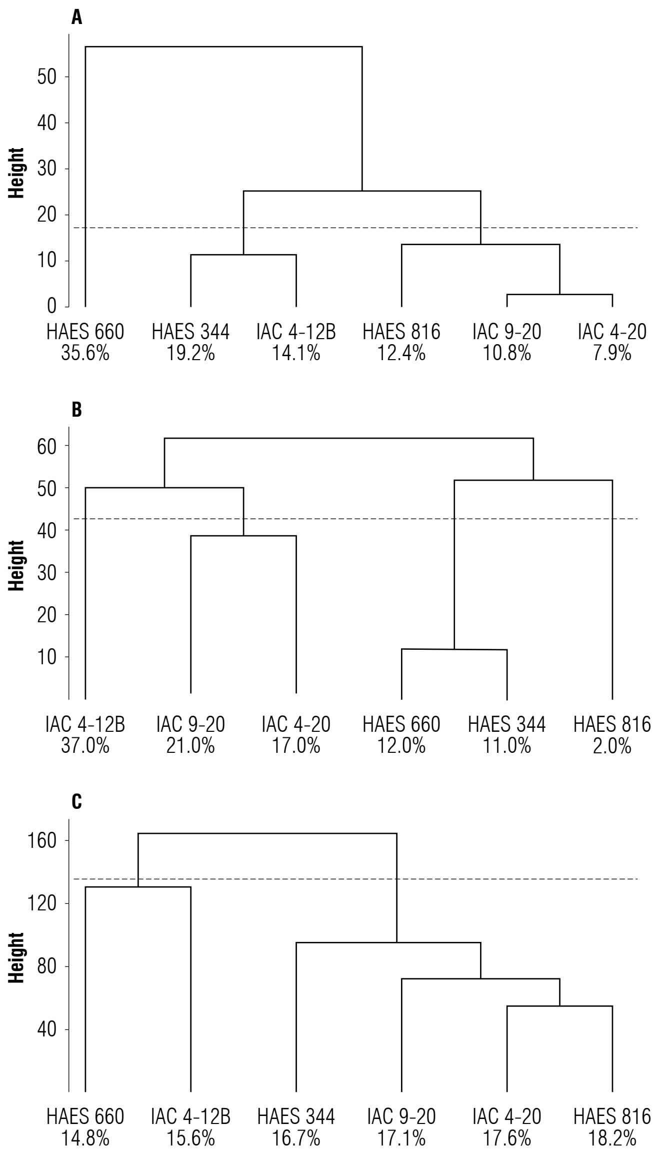

FIGURE 3. Similarity matrix (Euclidean distance) among cultivars of *Macadamia integrifolia* 'IAC 4-12B', 'IAC 9-20', 'IAC 4-20', 'HAES 344', 'HAES 660' and 'HAES 816', considering all collecting seasons and doses of IBA and Clonex®. The similarity matrix considered the following parameters: A) presence of callus, B) root development, and C) mortality. The grouping method (cluster analyses) was based on the K-means algorithm (Kassambara & Mundt, 2017).

The descriptive analysis regarding the cultivars in all IBA doses and cutting seasons showed 'HAES 660' as the best cultivar regarding the presence of callus (60%), also exhibiting the lowest mortality (40%). These rates were observed for those plants collected in October 2018 and subjected to the control treatment (Tab. 1). The callus is formed by new meristematic cells near the phloem (Hartmann *et al.*, 2002). The presence of calluses in species of hard rooting can precede root emission (Bitencourt *et al.*, 2010). However, the development of callus and roots can be regarded as independent events. For instance, Singh and Ansari (2014) observe high rates for callus development in *Dalbergia latifolia* (91.5%) and *Gmelina arborea* (75.0%) in the control treatment, without these calluses evolving into roots (3.1 and 0%, respectively). The authors suggest that the presence of callus in treatments without hormones is a reaction of the plant to restore the injured cutting and not a prerequisite for root induction. These two events may simultaneously occur, because both processes involve cell division and depend on favorable conditions (Preti *et al.*, 2012).

Regarding root development, 'IAC 4-20' showed the highest rate of rooting (30%) when collected in November 2018 and subjected to treatment with IBA at a dose of 5 g L^{-1} (Tab. 1). For this variable, all cultivars developed by the Agronomic Institute showed higher results than those found for the Hawaiian cultivars. Cultivar IAC 4-12B was the most stable with 20, 20, 15 and 25% of rooting in November 2018 (IBA doses 0, 5, 10 g L^{-1}, and Clonex®, respectively; Tab. 1). This cultivar also showed high values of rooting in the February collection season (20% - Clonex®) and March 2019 (25% for IBA dose of 10 g L^{-1} and 20% for Clonex®; Tab. 1). Cultivar IAC 9-20 also showed a high rooting rate (20%) in the October 2018 collection season in semi-hardwood cuttings treated with 10 g L^{-1} of IBA (Tab. 1). Russell *et al.* (2016) observe a rooting rate of 44% in *M. integrifolia* cultivars. Entelmann *et al.* (2014) obtain rates of 22.9% of rooting when studying the cultivar Aloha 10-14 with an IBA concentration of 3 g L^{-1}.

According to our data, the capability of the cultivars for rootstocks for the presence of callus, rooting, and survival were classified as nine distinct groups by the cluster analyses (Fig. 4). Cluster 3 was formed by 'IAC 4-20' (IBA at a dose of 5 g L^{-1}), 'IAC 4-12B' (IBA at a dose of 5 g L^{-1}) and 'IAC 4-20' (control treatment). These cultivars showed the highest rooting rates and moderate presence of callus. Cluster 7 was formed by the cultivars with lowest mortality rates ('IAC 9-20' - control treatment and 'HAES 660' - IBA at doses of 5 and 10 g L^{-1}). The cultivars grouped into cluster

TABLE 1. Percentage of semi-hardwood cuttings with presence of callus (Callus), root development (Root) and mortality (Mort.) in macadamia (*Macadamia integrifolia*) cultivars collected in four seasons and treated with indole butyric acid (IBA; g L^{-1}) and Clonex®.

Cultivar	Dose IBA	October 2018			November 2018			February 2019			March 2019		
		Callus	Root	Mort.	Callus	Root	Mort.	Callus	Root	Mort.	Callus	Root	Mort.
HAES 344	0	50	0	50	10	15	75	0	0	100	5	0	95
	5	50	0	50	5	0	95	0	0	100	5	0	95
	10	10	5	85	0	15	85	0	5	95	0	5	95
	Clonex®	NA	NA	NA	15	10	75	0	0	100	20	0	80
HAES 660	0	60	0	40	30	0	70	0	0	100	5	0	95
	5	25	15	60	25	10	65	20	5	75	15	0	85
	10	30	10	60	25	1	70	15	0	85	0	0	100
	Clonex®	NA	NA	NA	20	5	75	35	5	60	10	5	85
HAES 816	0	15	0	85	5	0	95	0	0	100	0	0	100
	5	25	5	70	5	0	95	10	0	90	0	0	100
	10	15	5	80	0	0	100	0	0	100	0	0	100
	Clonex®	NA	NA	NA	10	0	95	20	0	80	5	0	95
IAC 4-12B	0	20	0	80	30	20	50	10	0	90	15	5	80
	5	20	15	65	5	20	75	0	5	95	0	5	95
	10	0	0	100	0	15	85	0	10	90	0	25	75
	Clonex®	NA	NA	NA	10	25	65	5	20	75	10	20	70
IAC 4-20	0	25	0	75	15	10	75	10	0	90	0	0	100
	5	20	0	80	0	30	70	0	5	95	0	0	100
	10	0	10	90	0	10	90	0	5	95	0	0	100
	Clonex®	NA	NA	NA	0	15	85	0	0	100	0	0	100
IAC 9-20	0	40	0	60	5	10	85	20	0	80	5	0	95
	5	0	10	90	5	5	90	0	10	90	0	0	100
	10	5	20	75	5	5	90	0	0	100	0	10	90
	Clonex®	NA	NA	NA	0	10	90	10	10	80	0	15	85

Clonex® - commercial product; NA - not evaluated.

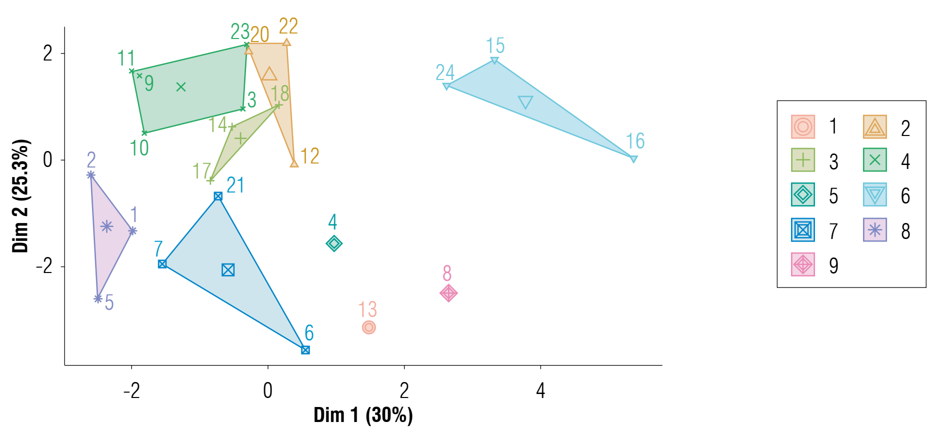

FIGURE 4. Grouping by multivariate features (cultivars, indole butyric acid, and collecting seasons) of semi-hardwood cuttings of *Macadamia integrifolia*. Cultivars (dose of IBA - g L^{-1}): 1 - HAES 344 (0), 2 - HAES 344 (5), 3 - HAES 344 (10), 4 - HAES 344 (Clonex®), 5 - HAES 660 (0), 6 - HAES 660 (5), 7 - HAES 660 (10), 8 - HAES 660 (Clonex®), 9 - HAES 816 (0), 10 - HAES 816 (5), 11 - HAES 816 (10), 12 - HAES 816 (Clonex®), 13 - IAC 4-12 B (0), 14 - IAC 4-12 B (5), 15 - IAC 4-12 B (10), 16 - IAC 4-12 B (Clonex®), 17 - IAC 4-20 (0), 18 - IAC 4-20 (5), 19 - IAC 4-20 (10), 20 - IAC 4-20 (Clonex®), 21 - IAC 9-20 (0), 22 - IAC 9-20 (5), 23 - IAC 9-20 (10), and 24 - IAC 9-20 (Clonex®). The threshold was obtained by the K-means algorithm (Kassambara & Mundt, 2017).

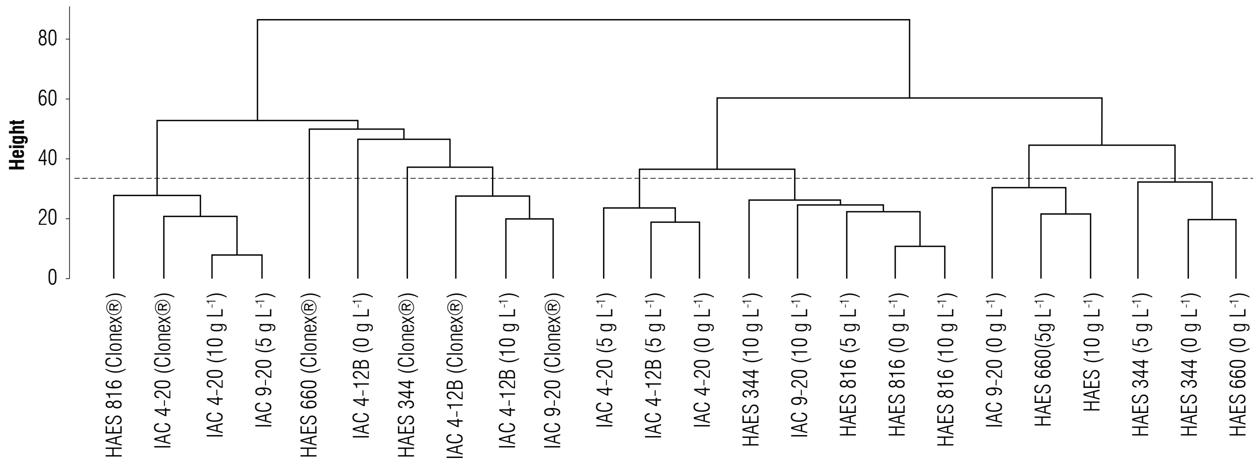

FIGURE 5. Similarity matrix (Euclidean distance) among *Macadamia integrifolia* cultivars HAES 816, HAES 660, HAES 344, IAC 9-20, IAC 4-20, and IAC 4-12 B subjected to treatment with indole butyric acid (0 g L^{-1}, 5 g L^{-1} and 10 g L^{-1}) and Clonex® and collected in different seasons. The threshold was obtained by the K-means algorithm (Kassambara & Mundt, 2017).

8 ('HAES 344' - control treatment and IBA at a dose of 5 g L^{-1}, and 'HAES 660' - control treatment) showed a high rate of presence of callus; however, these cultivars did not develop roots. These clusters are in agreement with the dendrogram obtained by the similarity matrix (Euclidean distance) (Fig. 5).

The high mortality rates observed in this experiment can be related to diseases caused by high environmental moisture, probably due to the identified presence of *Cladosporium* sp. on the leaves of the semi-hardwood cuttings. High mortality rates (46.2%) were also observed by Russell *et al.* (2016) evaluating the rooting ability of 32 macadamia cultivars in Australia. These authors found fungi from *Nectria* spp., seriously affecting *M. integrifolia*.

Conclusions

Considering all the cultivars evaluated in this study, the macadamia cultivar IAC 4-12B showed the highest rooting. This cultivar, along with HAES 660, showed the lowest mortality rate. Therefore, it can be concluded that IAC 4-12B is an interesting option for macadamia producers.

Although there were no statistical differences between indole butyric acid and Clonex® for root development, this last treatment showed the lowest mortality.

Under the conditions of Brazil, November showed the highest rooting values for semi-hardwood cuttings, and October showed the highest values for the presence of callus and low mortality for semi-hardwood cuttings.

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Author's contributions

GRS and MJP formulated the overarching research goals and aims. GRS and GCB implemented the computer code and supporting algorithms. GRS and GCB applied the statistical and computational techniques to analyze the study data. VHDS, MNVM and MJP conducted the research and performed the experiments and data collection. GRS and MJP provided the study materials, reagents, and computing resources for analysis. GRS, VHDS and MNVM managed the activities to annotate and maintain research data for initial use and later re-use. GRS, GCB, and MJP prepared the published work and specifically wrote the initial draft. GRS and GCB carried out the critical review, commentary and revision of the manuscript.

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