Vegetative multiplication of the Atlantic Rainforest species Eugenia involucrata

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Vegetative multiplication of the Atlantic Rainforest species *Eugenia involucrata*

Abstract – The objective of this work was to evaluate air layering, cutting, and grafting, with different indolebutyric acid (IBA) rates, for the vegetative propagation of *Eugenia involucrata*. Air layering was carried out for two consecutive years with five different IBA rates. Cutting was tested for woody and herbaceous cuttings, using four IBA rates, in three seasons. The grafting methods – cleft graft and splice graft – were tested in two seasons. For the air layering, cutting, and grafting trials the experimental designs were randomized complete blocks, completely randomized in a 3x4 factorial arrangement, and completely randomized, respectively. Plants were evaluated for rooting, survival percentage, and shoot development. In air layering, low rooting rates of 0 to 50% were observed, the use of IBA showed no consistent results, and no resulting propagules survived after being transplanted. The cutting technique showed low survival, rooting, and leaf retention rates, with no consistent effect of IBA rates. Grafting showed 35 to 50% graft fixation, with no significant difference between the splice and cleft grafting techniques. The air layering and cutting techniques are not efficient for the propagation of *E. involucrata*, as they do not favor the survival and rooting of seedlings. Grafting, by both techniques, is the most efficient method for *E. involucrata* propagation.

Index terms: Myrtaceae, cherry of the Rio Grande, cutting, grafting, air layering, vegetative propagation.

Multiplicação vegetativa da espécie *Eugenia involucrata*, nativa da Mata Atlântica

Resumo – O objetivo deste trabalho foi avaliar a alporquia, a estaquia e a enxertia, com diferentes doses de ácido indolbutírico (AIB), para a propagação vegetativa de *Eugenia involucrata*. A alporquia foi realizada por dois anos consecutivos, com cinco diferentes doses de AIB. A estaquia foi testada com estacas lenhosas e herbáceas, quatro doses de AIB, em três estações do ano. Os métodos de enxertia – garfagem em fenda cheia e inglês simples – foram avaliados em duas estações do ano. Para os ensaios de alporquia, estaquia e enxertia, os delineamentos experimentais foram em blocos ao acaso, inteiramente casualizado em arranjo fatorial 3x4 e inteiramente casualizado, respectivamente. As plantas foram avaliadas quanto ao enraizamento, à percentagem de sobrevivência e ao desenvolvimento da parte aérea. Na alporquia, foram observadas baixas taxas de enraizamento foram observadas na alporquia de 0 a 50%, o uso de AIB não apresentou resultados consistentes e nenhum propágulo resultante sobreviveu após o transplante. A técnica de estaquia mostrou baixas taxas de sobrevivência, enraizamento e retenção foliar, sem efeito consistente das doses de AIB. A enxertia mostrou de 35 a 50% de fixação do enxerto, sem diferença significativa entre as técnicas de enxerto de emenda e de fenda. As técnicas de alporquia e estaquia não são eficientes para a propagação de *E. involucrata*, pois não favorecem a

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sobrevivência e o enraizamento das mudas. A enxertia, por ambos os métodos, é a técnica mais eficiente para a propagação de *E. involucrata*.

Termos para indexação: Myrtaceae, cereja-do-Rio-Grande, estaquia, enxertia, alporquia, propagação vegetativa.

Introduction

Eugenia involucrata DC. (Myrtaceae) is a native fruit tree from the Brazilian Atlantic Rainforest displaying potential for agricultural exploitation, landscaping, urban afforestation and restoration of forest ecosystems (Tokairin et al., 2018a; Stuepp et al., 2018). Its fruit, in addition to being highly appreciated both in natura and processed forms (Lorenzi et al., 2014), contain phenolic and bioactive compounds (Girardelo et al., 2020) with antioxidant activity (Infante et al., 2016), which shows its economic exploitation potential.

However, as other Myrtaceae species, many obstacles concerning the establishment and production of *E. involucrata* commercial orchards are observed, especially due to the dependence of sexual multiplication for seedling production and the scarcity of knowledge on the agronomic management of this species (Cassol et al., 2017; Tokairin et al., 2018b; Santoro et al., 2021).

Sexual multiplication does not guarantee the maintenance of agronomic traits of interest, leading to genetic progeny variability (Hartmann et al., 2017) and juvenile plants (Cassol et al., 2017). Furthermore, *E. involucrata* seed are recalcitrant and loses viability when the water content is reduced (Gallon et al., 2018; Stefanel et al., 2021a), which jeopardizes the seedling production.

In contrast, vegetative multiplication methods, such as cutting, air layering, and grafting (Hartmann et al., 2017), preserve the characteristics of interest of selected canopies (Fachinello et al., 2005) and ensure the formation of uniform orchards (Trueman et al., 2013). However, the current literature lacks studies involving these methods for *E. involucrata*, despite their importance for the exploitation of this fruit tree (Lorenzi et al., 2014).

It is widely known that the use of plant growth regulators – such as exogenous auxins (IBA, for instance) – for difficult-to-root species can increase the percentage of rooting of cuttings and air layers. These substances enhance the chances of rooting and stimulate the formation of adventitious roots and better quality roots.

In vitro propagation has been reported for the vegetative multiplication of this species. Although showing some favorable multiplication rates (Stefanel et al., 2021a), this technique is expensive, and adjustments to the protocol are still necessary (Gallon et al., 2018).

Air layering, cutting, and grafting may ensure, especially in the early stages of the domestication process, a cheaper alternative for the vegetative rescue of native tree species (Stuepp et al., 2018).

The objective of this work was to evaluate air layering, cutting, and grafting, with different indolebutyric acid rates, for the vegetative propagation of *E. involucrata*.

Materials and Methods

For the air layering propagation method, ten mature Rio Grande cherry plants in good sanitary conditions, located in a private property in the municipality of Paraibuna, in the state of São Paulo (SP), Brazil (23°23'10"S, 45°39'44"W, at 635 m altitude), were selected for the experiments carried out in the spring of 2018 and 2019. The local climate is humid mesothermal with no defined dry season (Cfb), according to the Köppen-Geiger's climate classification (Alvares et al., 2013). The plants were under rainfed conditions and no other crop management procedure, such as pruning, were performed during the experimental period.

Girdling and bark removal were performed using a grafting knife on five branches of each selected plant. Lanolin paste was manually mixed with the exogenous auxin IBA and, then, applied at the exposed area of each air layer. IBA rates at 0, 1,500, 3,000, 4,500 and 6,000 mg kg^{-1} were used. The girdled areas were then covered with moistened coconut fiber substrate (Amafibra Ltda., Arthur Nogueira, SP, Brazil), closed with transparent plastic and tied with string at the ends (Hartmann et al., 2017). The air layers were evaluated every month for water content, which was replaced when necessary, using a syringe.

After 150 days, the rooting in the air layers was evaluated as follows: rooting percentage (RP, %), by counting the number of air layers showing roots; number of roots, by counting the number of roots emitted from each air layer; and the length of the largest root (LLR, cm). After the evaluations, the air layers were transplanted.

The experimental design was the randomized complete blocks consisting of five treatments (IBA rates) and 10 blocks, totaling 50 air layers each year. The data were subjected to a generalized linear mixed model, and the means were compared by the Tukey-Kramer's test, at 5% probability, using the SAS v.9.4 statistical software (SAS Institute Inc., Cary, NC, USA).

The experiments for cutting and grafting methods were carried out in an experimental area belonging to the Escola Superior de Agricultura Luiz de Queiroz (ESALQ), Universidade de São Paulo (USP), Crop Science Department in the municipality of Piracicaba, SP, Brazil (23°31'27"S, 45°27'12"W, at 546 m altitude), whose climate is tropical humid with dry winter and hot summer (Cwa), according to the Köppen-Geiger's classification (Alvares et al., 2013).

For the cutting method, three trials were conducted, using both woody and herbaceous cuttings collected in three different seasons (autumn/2018, spring/2019, and summer/2020), which were subjected to IBA applications (0, 1,500, 3,000, and 4,500 mg L^{-1}). During the autumn and spring trials, cuttings were collected from plants in the Fazenda Areão (22º42'30"S, 47º38'00"W, at 576 m altitude), belonging to the ESALQ/USP, while the summer trial cuttings were collected from plants in Sítio do Belo, in the municipality of Paraibuna, SP, Brazil (23º27'49"S, 45º42'40"W, at 745 m altitude). Plants from which the cuttings were collected, in both areas, were under rainfed conditions, and in good sanitary and nutritional status.

Cuttings ranged from 8 to 10 cm long and similar diameter in all trials, showing a single pair of leaves which were reduced to half their size to avoid excessive water loss. The cutting bases were cut in a bevel, injured with a scalpel, immersed in an ascorbic acid solution (5 g L^{-1}) for 15 s (Santoro et al., 2022) and in IBA solutions for 10 s, at the aforementioned concentrations. After immersion, the cuttings were immediately placed in 72-cell polystyrene trays containing moistened medium-sized expanded vermiculite, and maintained in an intermittent nebulization chamber, programmed for 2 min nebulization every 15 min, until the end of the experimental period. No further managements (fertilization, crop protection, etc.) were performed for the cuttings.

Evaluations were carried out fortnightly to count the number of leaves per cutting and the number of alive and dead cuttings in each treatment. At 90 days, the final number and percentage of rooted cuttings, number of roots per cutting, and length of the longest root (cm) were determined.

A completely randomized experimental design was adopted, with a 3x4 factorial arrangement consisting of three seasons and four IBA rates, with four replicates with five cuttings, totaling 240 cuttings. The type of cutting, either woody or herbaceous, was analyzed independently. The data were subjected to a generalized linear mixed model, and the Tukey-Kramer's mean comparison test, at 5% of probability was applied, using the SAS v.9.4 statistical software (SAS Institute Inc., Cary, NC, USA).

The grafting methods were evaluated in the autumn and spring of 2018. One-year-old rootstocks grown from seed were acquired at the seedling production nucleus of the Coordenadoria de Desenvolvimento Rural Sustentável (CDRS), in the municipality of São Bento do Sapucaí, SP, Brazil. The semi-woody branches used as scions were collected from privately owned, healthy, vigorous, and productive-age plants in the municipality of Paraibuna, SP, Brazil (23°23'10"S, 45°39'44"W, at 635 m altitude).

The adopted grafting methods were cleft graft and splice graft. Concerning the former, the rootstock was torn off, and a slit was opened, in which the scion whose base was previously cut in a double bevel (wedge shape) was fitted. For the splice graft, the rootstock was subjected to a simple bevel cut, followed by a complementary cut at the base of the scion, joining both materials (Hartmann et al., 2017). Plastic tape was used in both methods, to fix the graft to the rootstock and parafilm 'M' biodegradable tape was employed to cover the scion and the grafting region.

Plants were maintained in greenhouses which were covered with transparent plastic and 50% shading side nets, and watered as needed. No other crop management was performed during the experimental period. Monthly evaluations were carried out for 120 days, to determine the percentage of live (fixed) grafts, the average number of shoots per graft, and the length of the largest shoot, which was measured with a ruler graduated in centimeters.

A completely randomized experimental design was carried out with two treatments (cleft graft and splice graft), and 10 replicates comprising five plants, totaling 100 grafts per season. The data were subjected to the analysis of variance and to the Tukey's test, at 5% probability, using the SAS v.9.4 statistical software (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

For the air layering experiments, the percentage of rooted air layers (RP) in 2019 was the only variable affected by IBA at 6,000 mg kg^{-1} (Table 1). The highest IBA dose resulted in better air layer rooting percentage (50%) in comparison with the control; however, it did not differ from the other rates.

These findings corroborate those reported by Hossel et al. (2017), who did not verify rooting of *E. involucrata*, when using low or no IBA rates. Nevertheless, in the 2018 trial, the treatment without IBA application showed a small percentage of rooting (10%).

Although the scientific literature mentions the benefits of using plant growth regulators, such as IBA, it still lacks a definition for the best dose to be used (Santoro et al., 2021). Especially when it comes to a species that is still in its early steps of domestication and vegetative rescue, such as *E. involucrata* and many others (Stuepp et al., 2018).

As for the species of the Myrtaceae family, the literature shows a wide range of responses to IBA applications. Jabuticaba tree (*Plinia truncifolia*) air layers exposed to 4,000 mg kg^{-1} IBA, for instance, exhibited rooting rates above 80%, while jamboleiro (*Syzygium cumini*), displayed over 60%, even without the application of this plant phytoregulator (Danner et al., 2006; Hossel et al., 2016). Meanwhile, air layers performed in guabirobeira (*Campomanesia xanthocarpa*) and cambucizeiro (*Campomanesia phaea*) showed no root (Teleginski et al., 2018). Not even when treated with IBA rates from 0 to 6,000 mg kg^{-1} (Santoro et al., 2022; Teleginski et al., 2018).

In the present work, the lower rates of *E. involucrata* rooting can be associated more to the large variability in its population than to the IBA dose applications (Stefanel et al., 2021b), which may lead to different results along the years'

In addition to stimulating rhizogenesis, IBA has been also widely reported as positively influencing the root system quality, ensuring a greater root volume and length (Hartmann et al., 2017; Emer et al., 2018). However, under the experimental conditions of the present study, the low rates of rooting made it impossible to verify this information (Table 1).

For the cutting method, adventitious rooting was observed in a small number of herbaceous cuttings treated with 3,000 mg L^{-1} IBA in the autumn and spring, and 4,500 mg L^{-1} IBA in the spring, with an overall low rooting rate (1.25%) (Table 2). No rooting was observed for the woody cuttings.

Significant differences were observed for leaf retention and cutting survival, after 90 days, in both herbaceous and woody cuttings (Table 2). Despite the low percentage of cutting rooting, the best leaf retention and survival values of herbaceous cuttings were observed in the spring and autumn, while for woody cuttings this fact was noted in the spring and summer. Interactions between factors were also significant for herbaceous cuttings. An inconsistent IBA performance was observed among seasons, in which no significant differences were observed for the autumn and summer, while harmful effects were observed in the spring. Native species from the Myrtaceae family are not easy-to-root and may present discrepant results in accordance with the season and type of cutting (Silva et al., 2019).

The importance of leaf retention varies in accordance with the type of cutting being used. Usually, more lignified cuttings depend less on leaves presence (Sasso et al., 2010). In the present study, leaf retention in cuttings does not seem to have been decisive for rooting, as very low rates were observed for herbaceous cuttings (1.25%), and no rooting was

Table 1. Rooting percentages (RP), mean number of roots (NR), and length of the longest root (LLR, cm) of cherry of Rio Grande (*E. involucrata*) air layers subjected to different indolebutyric acid (IBA) rates[(1)].

IBA rate	2018			2019		
(mg kg^{-1})	RP	NR	LLR	RP	NR	LLR
0	10	0.30	0.49	0b	0.00	0.00
1,500	30	1.10	1.62	10ab	0.10	0.37
3,000	20	0.60	0.76	20ab	0.70	0.72
4,500	20	0.50	0.58	30ab	0.70	0.56
6,000	20	1.11	1.70	50a	0.80	1.53
Means	20	0.72	1.03	22	0.46	0.64
p-value	0.8187^{ns}	0.6886^{ns}	0.7414^{ns}	0.0332	0.0931^{ns}	0.0986^{ns}

[(1)]Means followed by equal letters in the columns do not differ, by the Tukey-Kremer's test, at 5% probability. [ns]Nonsignificant.

observed in the investigated woody cuttings. However, it is important to note that treatments with higher leaf retention is related to higher cutting survival rates (Table 2).

When it comes to the grafting method, significant differences for the percentage of fixed grafts were observed between the grafting methods after 30 days only in the autumn (Table 3). In general, regardless of the grafting type used, procedures performed in the spring ensured a higher percentage of graft fixation. Similarly, no significant differences were observed between treatments for the mean number of shoots per graft and for the length of the largest shoot.

The results of this experiment exhibited 35 to 50% of graft fixation, in accordance with the season it was performed; *E. involucrata* has an intermediate graft fixation capacity (Table 3). There was no significant difference between splice and cleft graft, except for 30 days in the autumn, when splice graft produced more fixed grafts than the cleft graft. The use of grafting for vegetative propagation of Brazilian native tree species has a wide range of success, from 0 to 100% (Stuepp et al., 2018). However, some authors, such as Lattuada et al. (2010), were not successful in grafting this species. The aforementioned authors used herbaceous branches as grafts; in the present study, the scions collected were semi-woody. Herbaceous grafts show less lignified cells; however, they are significantly more susceptible to dehydration, which can jeopardize the process of graft fixation (Hartmann et al., 2017).

A wide range of graft fixation levels has been reported for other Myrtaceae family species, such as 20, 40, and 50%, for jabuticaba (*Plinia cauliflora*), pitanga (*Eugenia uniflora*) and camu-camu (*Myrciaria dubia*), respectively (Lattuada et al., 2010; Malagi et al., 2012). This variation is also observed within the same species, as reported by Franco et al. (2010) for jabuticaba-sabará [*Plinia jaboticaba* (Vell.) Berg], for which splice grafting guaranteed 96% fixation, while cleft grafting, 69%.

The high variability on grafting success can be associated with several factors, such as the lack of compatibility (physiological and anatomical) between the graft and the rootstock, which are caused by physiological and/or biochemical differences, environmental factors during and after grafting, phytosanitary aspects, and graft craftsmanship (Fachinello et al., 2005; Hartmann et al., 2017).

According to Franzon et al. (2008), when the propagation material has different genetic origins, for

Table 2. Leaf retention and survival of herbaceous and woody cuttings of cherry of Rio Grande (*E. involucrata*), at 90 days after the beginning of the experiment, in accordance with the season, year, and indolebutyric acid (IBA) rates[(1)].

IBA rate (mg kg^{-1})	Herbaceous cuttings			Mean	Woody cuttings			Mean
	Autumn	Spring	Summer		Autumn	Spring	Summer	
	Leaf retention (%)				Leaf retention (%)			
0	26.25Ba	50.00Aa	2.50Ca	26.25	0.00	27.50	30.00	19.16
1,500	31.25Aa	10.00Ab	10.00Aa	17.08	0.00	15.00	7.50	7.50
3,000	33.75Aa	25.00Ab	7.50Aa	22.08	0.00	15.00	12.50	9.16
4,500	23.75Aa	5.00Ab	7.50Aa	12.08	0.00	5.00	7.50	4.16
Mean	28.75A	22.5A	6.87B	19.37	0.00B	15.62A	14.37A	6.94
	Survival (%)				Survival (%)			
0	40.00	50.00	5.00	31.66	0.00	35.00	40.00	25.00
1,500	50.00	15.00	15.00	26.66	0.00	25.00	30.00	18.33
3,000	45.00	25.00	10.00	26.66	0.00	15.00	25.00	13.33
4,500	30.00	5.00	10.00	15.00	0.00	10.00	15.00	8.33
Mean	41.25A	23.75B	10B	25.00	0.00B	21.25A	27.50A	16.25
	Rooting (%)				Rooting (%)			
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1,500	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3,000	5.00	5.00	0.00	3.33	0.00	0.00	0.00	0.00
4,500	0.00	5.00	0.00	1.67	0.00	0.00	0.00	0.00
Mean	1.25	2.50	0.00	1.25	0.00	0.00	0.00	0.00

[(1)]Means followed by equal letters, uppercases in the lines, and lowercases in the columns, do not differ by the Tukey-Kramer's test, at 5% probability.

Table 3. Fixed grafts, number of shoots, and length of the largest shoot of cherry of Rio Grande (*E. involucrata*) grafts, performed in the autumn and spring of 2018, by splice and cleft grafts, at 30, 60, 90, and 120 days after grafting[1].

Treatment	Fixed graft (%)				Average number of shoots	Length of the largest shoot (cm)
	30 days	60 days	90 days	120 days		
			Autumn/2018			
Splice graft	78.0a	46.0	38.0	38.0	1.6	3.3
Cleft graft	48.0b	34.0	32.0	32.0	1.2	2.7
Mean	63.0	40.0	35.0	35.0	1.4	3.0
p-value	0.0064	0.1988[ns]	0.5785[ns]	0.5785[ns]	0.46[ns]	0.59[ns]
			Spring/2018			
Splice graft	75.0	60.0	50.0	48.3	1.46	3.46
Cleft graft	73.3	56.7	51.7	51.7	1.81	4.98
Mean	74.1	58.3	50.8	50.0	1.63	4.22
p-value	0.8199[ns]	0.6823[ns]	0.8513[ns]	0.6893[ns]	0.37[ns]	0.16[ns]

[1]Means followed by equal letters, in the columns, do not differ by the Tukey-Kremer's test, at 5% probability. [ns] Nonsignificant.

instance, seed rootstocks and grafts, as in the present study, the genetic variations may result in different vegetative propagation potentials.

Conclusions

1. Air layer technique used for the propagation of *Eugenia involucrata* shows low rooting and no surviving of seedlings after transplant; the application of IBA does not show consistent effects on rooting.

2. The cutting method results in poor survival, rooting, and leaf retention of *E. involucrata*; there was no consistent effect of IBA on cutting survival and rooting.

3. Both splice and clef grafting constitute alternatives to multiply *E. involucrata* vegetatively.

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