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Sexual and asexual propagation of *Brosimum alicastrum* Swartz in Campeche, Mexico

Propagación sexual y asexual de *Brosimum alicastrum* Swartz en Campeche, México

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Abstract

Brosimum alicastrum is a tree species in Mexico with wide potential for animal and human food, which is distributed naturally with no silvicultural management, so there is little information on the propagation methods of the species. The objective of this work was to analyze the scientific research published on *B. alicastrum*, through literature review to know the techniques that exist on its propagation. In addition, the quality of the seedling obtained by sexual propagation and asexual methods (cuttings, layers and grafts) was evaluated in the nursery, by means of experimental designs. 550 scientific articles on *B. alicastrum* were found, the disciplines where they were published were: Ecology (44.18%), Botany (13.27%), Forest Sciences (11.27%, of which 2.54% worked propagation in the nursery), Zoology (11.09%), Agriculture (9.64%), Anthropology (5.45%) and others (5.10%). Regarding the seed propagation method, the best seedling quality was associated with low porosity substrates (bush soil) and containers with large diameters (36 cm). In the case of asexual propagation, with the layering method when peat was used as the substrate 90% survival was obtained, and by lateral grafting technique 75% yield was found. Due to the little research that exists on the propagation of the species, it is recommended that the selection of the propagation technique is based on the purpose of the seedling; if it is required to shorten the seed production cycles of *B. alicastrum* the asexual techniques grafting and layering can be more efficient.

Keywords: Ramon, forestry, forest nursery, graft, rooting of cuttings, air layering.

Resumen

Brosimum alicastrum es una especie arbórea en México con amplio potencial para la alimentación animal y humana, que se distribuye de manera natural con nulo manejo silvícola, por lo que existe poca información sobre los métodos de propagación de la especie. El objetivo de este trabajo fue analizar la producción científica reportada sobre *B. alicastrum* mediante minería de textos para conocer las técnicas que existen sobre su propagación; y evaluar en vivero la calidad de plántulas obtenidas por métodos de propagación sexual y asexual (estacas, acodos e injertos) mediante diseños experimentales. Se encontraron 550 artículos científicos sobre *B. alicastrum*, las disciplinas donde se publicaron fueron: Ecología (44,18%), Botánica (13,27%), Ciencias Forestales (11,27%, de los cuales el 2,54% trabajó propagación en vivero), Zoología (11,09%), Agricultura (9,64%), Antropología (5,45%) y otras (5,10%). Respecto al método de propagación por semilla, la mejor calidad de plántula se asoció con sustratos de baja porosidad (tierra de monte) y con contenedores con diámetros grandes (36 cm). Para el caso de la propagación asexual por acodos, cuando se empleó turba como sustrato se obtuvo 90% de sobrevivencia, y por injerto de enchape lateral se encontró 75% de prendimiento. En virtud de la poca investigación que existe sobre la propagación de la especie se recomienda que la selección de la técnica de propagación esté en función de la finalidad de la plántula. Las técnicas asexuales de injerto y acodo pueden ser más eficientes en caso de requerir acortar los ciclos de producción de la semilla de *B. alicastrum*.

Palabras clave: Ramón, silvicultura, vivero forestal, injerto, enraizamiento de estacas, acodo aéreo

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1 Introduction

Brosimum alicastrum Swartz, commonly known as Ramon or breadnut, is a tree native from Mesoamerica and the Caribbean, and it is widely distributed in Mexico (Peters and Pardo, 1982). It is known by having high nutrient content foliage, mainly for cattle and goats and also for its availability during the dry season (Hernández et al., 2014; Rojas et al., 2017). Ramon represents an ecologically important element in the floristic composition of the low and medium forests of southern Mexico (Gutiérrez and Dirzo, 2009).

Ramon produces four times more food and 10 times more protein per hectare than maize, without causing damage to the environment. In addition, its seed, foliage, latex and wood have high economic potential for food (animal and human) and for medicinal and cultural uses (Ramírez et al., 2017; Domínguez et al., 2019). Even though its importance, the species is distributed mostly naturally, with no forest management (Vergara et al., 2014).

The establishment of plantations requires trees with outstanding phenotypic and genotypic characteristics, thus the propagation method for obtaining germplasm is key to good silvicultural management (Hernández et al., 2015). Its dissemination can be sexual or asexual (Molina et al., 2015).

During the sexual reproduction of trees, parents may inherit desirable and undesirable characteristics (Molina et al., 2015). Meanwhile during vegetative propagation, germplasm with genetic information and traits of economic importance can be obtained in shorter periods, with the limitation that the genetic diversity of the species is reduced by the uniformity of the offspring (Bailey et al., 2009). In both cases, nursery management is observed in the quality of the seedlings produced, through their morphological characteristics and their ability to adapt to the area (Rueda et al., 2014).

Traditional techniques of asexual propagation in forest species are cuttings, layers and grafts (Pardo et al., 2002). Thanks to biotechnology, *in vitro* propagation has become an alternative for those species with high commercial value and difficulty to disseminate by traditional techniques (Bailey et al., 2009). However, the main limitation of vegetative propagation is the low multiplication percentage, hence it is important to expand the number of species to be propagated and improve the technique, since plants reproduced vegetatively have better silvicultural management (Sampayo et al., 2016).

There is almost no research on propagation techniques in *B. alicastrum*, and the one reported does not have any scientific rigor (Gillespie et al., 2004; Molina et al., 2015). Due to the importance of the species in the Yucatan peninsula as an alternative plant resource for animal and human feeding for food security and climate change (Ramírez et al., 2017), research that develops around the forest management of the species will be very useful and important (Hernández et al., 2015).

In this context, the aim of this paper is to analyze the scientific production reported on *Brosimum alicastrum* Swartz, using text mining to know the techniques that exist on its propagation; and to evaluate in nursery conditions the quality of seedlings obtained by sexual and asexual propagation methods through experimental designs (cuttings, layers and grafts).

2 Materials and Methods

2.1 Study area

The research was carried out in the experimental nursery located in the facilities of the Campus Campeche Graduate School (Champotón, Campeche). Vegetative material of *B. alicastrum* was collected in different areas of the research center (Figure 1). The region is characterized by having a cover of medium subperennifolia forest and high perennifolia forest, with clay soils and precipitation of 600 to 4000 mm with estimated periods from three to seven months, average annual temperature of 18 °C to 27 °C and altitudes of 20 to 1000 masl. The main economic activities of this region are the cattle raising and crop of maize (*Zea mays* L.), beans (*Phaseolus vulgaris* L.), chihua (*Cucurbita argyrosperma* H.) and sugar cane (*Saccharum officinarum* L.) (White and Hood, 2004). It is noted that the main feed for cattle during drought is Ramon tree (Góngora et al., 2016).

2.2 Text mining and bibliometric analysis

In order to know the techniques used in the propagation of *B. alicastrum*, the scientific articles on the species available in the main publishing houses (Elsevier, Springer and Scopus) and websites (Latindex, SciELO, Redalyc, Thomson-Reuters, Periodica, DOAJ, Google Scholar and Conricyt) were analyzed. The keyword used in the search was *Brosimum alicastrum*, identifying it in the titles and keywords of the publications.

The variables that were analyzed for each article were: year to know the time of the research; discipline of study to determine the area of knowledge where more research has been conducted; and authors to know the actors involved in the research. The information was systematized in a spreadsheet. Using the plugin RcmdrPlugin.temis of the R statistical software (Bouchet and Bastin, 2013), the time of the research and the frequency of the publications by study discipline were obtained.

The network of authors was created with the Sci2tool software (Börner, 2011). Authors with more publications on the network were associated with the area of knowledge. The syntax used in the Sci2tool software was Extract bipartite Network, for its visualization the software Gephi was used (Jacomy et al., 2014).

2.3 Sexual propagation

In March 2019, Ramon seed was collected in the location called 20 de noviembre (Calakmul, Campeche). The methodology described by Vallejos et al. (2010), and the trees with the best phenotypic (dasometric) characteristics with a minimum distance between selected trees of 100 m were selected. The germplasm was stored in sterilized plastic bags that were moved to the facilities of the College of Postgraduates, Campeche campus (Champotón, Campeche), Mexico.

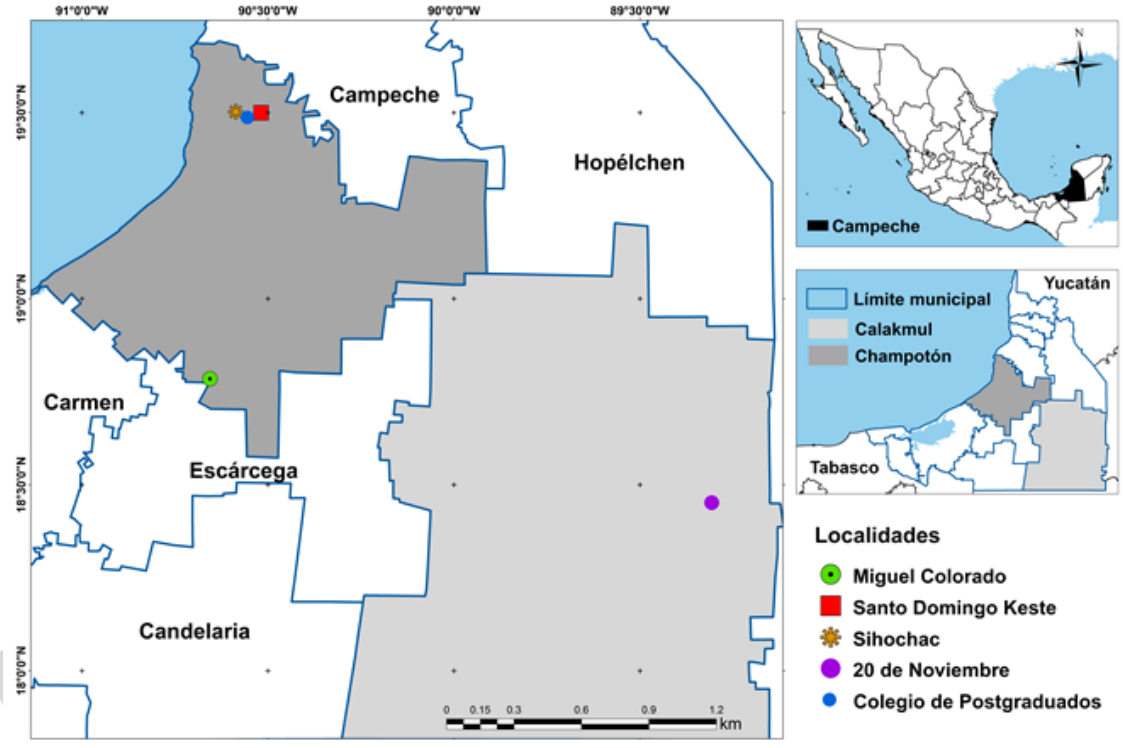

Figure 1. Spatial location of the localities where the vegetative material was collected and where experiments were conducted to evaluate the propagation of *Brosimun alicastrum* Swartz in Campeche, Mexico.

Seeds were subjected to a purity analysis and viability test with tetrazolium chloride® at 0.5%, according to the methodology described by Orantes et al. (2013). It was evaluated in a rustic forest nursery from April to June 2020, where a completely randomized experimental design with factorial arrangement was established. The factors analyzed were: 1) type of container: Tubes (36 and 21 cm in diameter) and plastic bag (36 and 21 cm in diameter), and 2) substrate: (peat moss 50% + agrolite 25% + perlite 25%) and (bush soil 60% + lumbricompost 40%).

The mixture (peat moss 50% + agrolite 25% + perlite 25%) was characterized by a slightly acidic pH with high porosity and moisture retention capacity; while the mixture (bush soil 60% + lumbricompost 40%) showed a neutral pH with low porosity and high-water retention capacity (Pérez de la Cruz et al., 2012). For the eight treatments, a manual watering was applied every three days to the experimental units according to the moisture retention of the substrates used (Del Amo et al., 2002).

30 days after the experimental design was established, the germination percentage per treatment was evaluated. A variance analysis and Tukey mean tests ($\alpha = 0.05$) were used to determine the statistical differences per treatment three months after the experiment was established for the variables: height (cm), number of leaves, stem diameter (cm), dry stem biomass (kg), root width (cm), root length (cm) and dry root biomass (kg). Dry biomass of the root and stem was obtained on an analytical scale after extracting the vegetative material from a stove with forced air circulation at 70 °C for 24 hours.

2.4 Asexual propagation

Three asexual propagation techniques were tested: cuttings, laters and grafts. In the case of cuttings, plant material was collected in March 2019 at Miguel Colorado community (Champotón, Campeche). Through a field tour, healthy, vigorous, pest-free trees were selected with fruits at the time of harvest and straight trees without bifurcation. The plant material was wrapped in wet newspaper to prevent it from becoming dehydrated during its transfer to the nursery of the Graduate School, Campeche campus.

Cuttings were placed in polythene bags with traditional nursery substrate (peat moss 50%, agrolite 25% and perlite 25%). Shoots and roots in cuttings were evaluated at three months (April-June) using a fully randomized experimental design with factorial arrangement. The factors considered were: type of rooting powder (Auxin, Fortimax, Raidzone Plus and Magic Plus), and stake position (basal, intermediate, and apical). For the 12 treatments, manual irrigation was applied every three days to the experimental units. The variables analyzed were number and length (cm) of aerial shoots, and dry and fresh biomass of roots and stems (kg), vertical height (cm) and horizontal height (cm) of the root.

In May 2019, *in vivo* propagation by layers to young Ramon trees (under 5 years) was conducted in the town of Sihochac (Champotón, Campeche). Through a field trip, healthy, vigorous, pest-free and straight trees without bifurcation were selected. A completely randomized experimental design was considered with three treatments (T) and 30 replications per treatment: T1 (Auxin + peat moss), T2 (Auxin + vermiculite) and T3 (Auxin + Perlite). The variables analyzed were: survival rate at three months, and number, length (cm) and dry biomass (kg) of the roots.

In July 2019, in the town of Santo Domingo Keste (Champotón, Campeche), *in vivo* propagation by graft to Ramon trees under one year old was carried out with vegetative material of trees of the same species not less than five years old and with the capacity to produce fruits. For the selection of the standard trees and from which the scion wood to be grafted were obtained, healthy, vigorous, pest-free and straight trees without bifurcation were selected.

The graft techniques used were: cleft, bud, lateral and simple English. A completely randomized experimental design was established, where each graft technique was taken as a treatment with 20 replications per treatment. The variables analyzed were growth percentage at three months, height (cm), diameter (cm), number of leaves and number of shoots of grafted vegetative material. To establish statistical differences by treatments and variables analyzed, a variance analysis and Tukey mean tests ($\alpha = 0.05$) were performed for cuttings, layers and grafts propagation method.

3 Results and Discussion

3.1 Text Mining and Bibliometric Analysis

From 1970 to 2019, 550 scientific articles were published, in which *B. alicastrum* appeared in the title or keywords. According to De Granda et al. (2005) the title indicates the precise subject matter of the work and the keywords allow to place the topic studied in the article. The disciplines that published themes related to *B. alicastrum* were: Ecology (44.18%), Botany (13.27%), Forestry (11.27%, of which 2.54% worked in nursery propagation), Zoology (11.09%), Agriculture (9.64%), Anthropology (5.45%) and others (5.10%).

Figure 2 shows the network of authors of the 550 papers analyzed. The authors with the highest number of contributions per area were highlighted. Ecology turned out to be the discipline where most researches have been conducted. This aspect has been reported by Vergara et al. (2014), who agreed that there is little research on the silvicultural management of Ramon. For this reason, it is an area of research opportunity, since according to Ramírez et al. (2017) the species has broad potential for the livestock food, particularly in the following sectors: pig, bovine, sheep, poultry and aquaculture for food security and climate change.

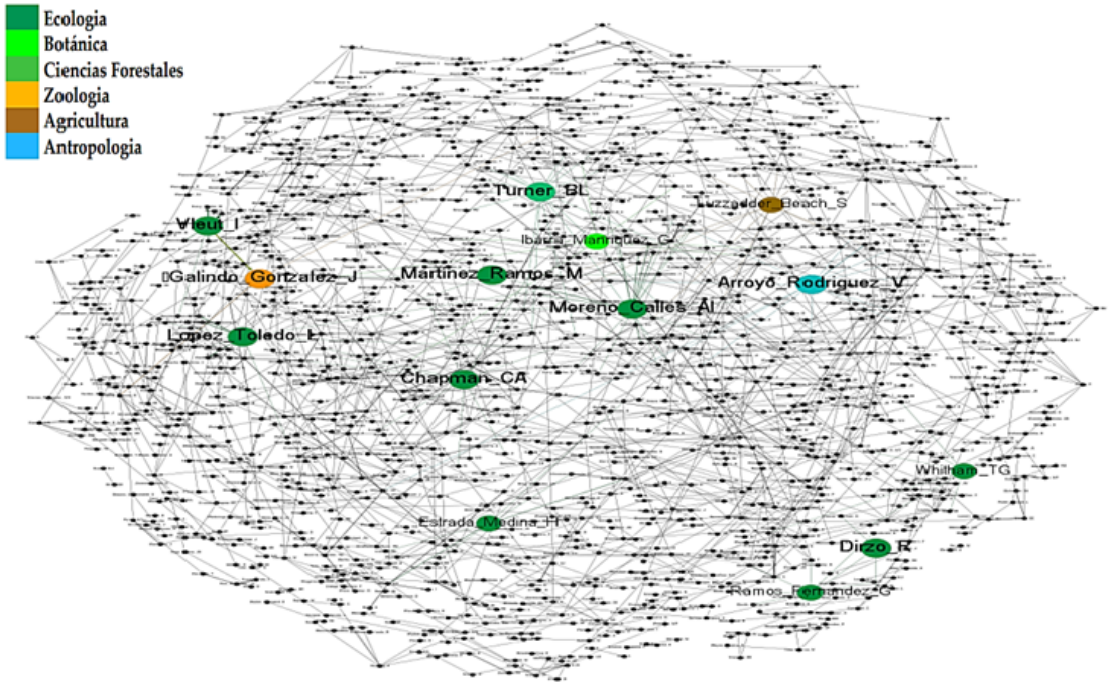

Figure 2. A network of authors from the disciplines where more research has been conducted on *Brosimum alicastrum* Swartz.

Hernández et al. (2014) mentioned the importance that Ramon's plantations in southeastern Mexico would have for the livestock feed agroindustry in guaranteeing the raw material of its operations. According to Vergara et al. (2014) no silvicultural management of the species happens because the industry around *B. alicastrum* is emerging and current research focuses on evaluating its potential properties and uses.

3.2 Sexual propagation

For the purity test, 858 *B. alicastrum* seeds with a total biomass of 2128 g were analyzed. 91% of seeds (785; 1944 g) were not broken, stained and showed no trace of insect attack (holes and/or larvae). However, the high purity percentage contrasted with the viability (germination potential) of the seed. The results of direct contact of the seeds with tetrazolium® solution at room temperature and with

limited light for 24 hours showed on average a high viability in 20% of the seeds, medium viability (27.5%) and low viability (52.5%) for the four replications with 10 seeds per replication.

The low germination potential of *B. alicastrum* seeds has been mentioned by Gillespie et al. (2004) with values lower than 50%, which coincided with the results obtained in this research, but contrasted with the one reported by Del Amo et al. (2002) with germination percentages higher than 75%. These differences were explained because Ramon's seeds are recalcitrant (García et al., 2012; Loría and Larqué, 0015), fact that makes them more sensitive to dehydration and rapid viability loss, limiting their storage for propagation purposes (Magnitskiy and Plaza, 2007). The germination evaluation 30 days after the crop por sow resulted in 59.6%, which coincided with the low viability of the seed obtained in the tetrazolium® test (Table 1). However, it differed from what was reported by Laborde and Corrales-Ferrayola (2012), who found 75% of germination when the seeds fell directly from the tree to the soil. According to Del Amo et al. (2002) the germination percentage of *B. alicastrum* seeds in nurseries was directly related to the collection-planting temporality, due to the recalcitrant property of the seed. Table 1 presents the results of the variance analysis and Tukey mean test ($\alpha = 0.05$) by treatment and evaluated variables. Statistical differences were observed between the treatments used ($P < 0.0001$). Tukey multiple tests indicated that treatment 6 (bag with 36 cm of diameter with substrate 2: bush soil 60% + lumbricompost 40%) presented the highest values for the phenotypic characteristics evaluated.

The type of container (tube or plastic bag) showed no statistical difference. This result coincided with that reported by Luna et al. (2012), who found that the use of plastic bags as a container in nursery occurs by economic reasons. The factor that was statistically significant was the size of the container diameter. The highest values for the phenotypic characteristics evaluated were presented in those containers of higher diameter (36m).

This result contrasted with that reported by Pérez de la Cruz et al. (2012), who found that in the first month *B. alicastrum* seeds in nursery showed faster growth in containers with 21 cm diameters. However, according to Luna et al. (2012) larger diameter containers provided better nursery characteristics to large leaf species because of the space between plants, which allowed them to make better use of light, heat, water and nutrients.

3.3 Asexual propagation

Propagation by cuttings was not significant, and the experimental units evaluated in the different treatments failed to survive. No experimental unit achieved root formation, and only 7 out of 240 experienced aerial shoots (Table 2). Sampayo et al. (2016) found that aerial shoots were common in forest species disseminated by cuttings. However, the success of this technique was measured by the rooting percentage achieved (Peralta et al., 2017).

According to González et al. (2019), the obtaining of non-significant results in the asexual propagation by cuttings of forest species may be due to inadequate management in nurseries, the lignified tissue of the plant material and even the collection date. However, the results were consistent with those reported by Vergara et al. (2014) and Molina et al. (2015) who stated that stake propagation for *B. alicastrum* was a non-viable technique due to the lignified tissue of the species.

Layers and grafting methods were significant, and Tukey mean test showed statistical differences among treatments with 95% of confidence level ($\alpha = 0.05$). Regarding layers, the treatment with peat moss substrate showed the highest results (Table 3). In the case of graft, the lateral technique was the one that was more significant with 75% (15 out of 20 replications), higher than the cleft (20.00%; 4 out of 20 repetitions), bud (0.00%; 0 out of 20 replications) and simple English (10.00%; 2 out of 20 replications) (Table 4).

Molina et al. (2015) when conducting a research in some regions of El Salvador found that for *B. alicastrum* graft is the most viable form of asexual propagation. However, unlike their study, no previous treatments with growth hormones were considered for grafted vegetative materials in this research. In both studies, lateral grafting technique was the one with the highest growth percentage achieved: 75% in this research and 42% for Molina et al. (2015).

Table 1. Phenotypic variables in *Brosimum alicastrum* Swartz seedling propagated by seed.

Treatment*	Replications		Height (cm)	Leaves	Stems		Root		
	Total	% Germination			Diameter (cm)	Biomass (kg)	Width (cm)	Lenght (cm)	Biomass (kg)
T1 (T36+S1)	30	73.33 (22)	26.75 AB	5.77 AB	3.58 AB	0.76 B	10.60 A	12.67 AB	0.65 AB
T2 (T36+S2)	30	80.00 (24)	25.90 BC	6.08 AB	3.37 B	0.54 BCD	12.10 A	12.40 ABC	0.51 BCD
T3 (T21+S1)	30	50.00 (15)	25.70 BC	5.35 BC	3.20 BC	0.54 BCD	4.87 B	10.35 BC	0.39 CDE
T4 (T21+S2)	30	63.33 (19)	19.10 D	3.13 D	2.47 C	0.28 D	2.78 B	9.97 CD	0.24 E
T5 (B36+S1)	30	60.00 (18)	28.35 AB	5.91 AB	3.47 AB	0.66 BC	9.91 A	10.30 BC	0.530 BC
T6 (B36+S2)	30	73.33 (22)	32.50 A	6.96 A	4.18 A	1.18 A	9.56 A	14.17 A	0.83 A
T7 (B21+S1)	30	40.00 (12)	23.71 BC	5.00 BC	3.06 BC	0.43 CD	4.50 B	7.50 DE	0.31 CDE
T8 (B21+S2)	30	36.67 (11)	20.13 CD	4.06 CD	2.49 C	0.31 D	4.69 B	7.22 E	0.28 DE
Total	240	59.58 (143)							

* T36: Tube 36 cm; T21: Tube 21 cm; B36: Bag 36 cm; B21: Bag 21 cm; S1: peat moss 50 % + agrolite 25 % + perlite 25 %; S2: bush soil 60 % + lumbricompost 40 %. Means with the same letter per column are not statistically different (Tukey, $\alpha = 0.05$).

In relation to the substrate type, substrate 2 (bush soil 60 % + lumbricompost 40 %) was slightly higher than substrate 1 (peat moss 50 % + agrolite 25 % + perlite 25 %). This result was in accordance with the one reported by Laborde and Corrales (2012), Pérez de la Cruz et al. (2012) and Hernández et al. (2015) who mentioned that *B. alicastrum* had a better performance on local substrates with physicochemical characteristics similar to the areas where it was distributed naturally, rather than on commercial substrates commonly used in nurseries.

Table 2. Phenotypic variables of the vegetative material of *Brosimun alicastrum* propagated asexually by the stake technique.

Treatment	Replications		Aerial shoots		Root (length: Cm)		
	Total	Significant	Number	Length (cm)	Biomass	Vertical	Horizontal
T1 (AU_BA)	30	2	3	0.5	0	0	0
T2 (AU_IN)	30	2	2	0.25	0	0	0
T3 (AU_AP)	30	2	3	3.93	0	0	0
T5 (FO_IN)	30	1	1	2.35	0	0	0
Others **	240	0	0	0	0	0	0

Rooting powder: Auxin (AU), Fortimax (FO), Raidzone Plus (RP), Magic Plus (MP). cutting position: Basal (BA), Intermediate (IN), apical (AP). **T4 (FO_BA), T6 (FO_AP), T7 (RP_BA), T8 (RP_IN), T9 (RP_AP), T10 (MP_BA), T11 (MP_IN), T12 (MP_AP).

Table 3. Phenotypic variables of the vegetative material of *Brosimun alicastrum* Swartz propagated asexually by the bud technique.

Treatment	Replications		Root		
	Total	% Survival	Number	Length (cm)	Biomass (kg)
T1 (Peat moss)	30	90 (27) A	13.56 A	11.99 A	0.61 A
T2 (Vermiculite)	30	80 (24) B	12.44 AB	8.55 B	0.52 AB
T3 (Perlite)	30	80 (24) B	10.78 B	9.05 B	0.31 B

The rooting powder used for all treatments was auxin. Means with the same letter per column are not statistically different (Tukey, $\alpha = 0.05$). The survival % was calculated by treatment, the number of surviving replications of the total per treatment is in parentheses

Table 4. Phenotypic variables of the vegetative material of *Brosimun alicastrum* Swartz propagated asexually by the graft technique.

Type of graft	Replications		Grafted Vegetative Material			
	Total	Growth %	Height (cm)	Diameter (cm)	Leaves (number)	Shoots (number)
Cleft	20	20.00 (4) B	8.75 AB	0.11 B	8.72 B	1.25 B
Layer	20	0.00 (0) C	0.00 C	0.00 C	0.00 D	0.00 C
Lateral	20	75.00 (15) A	10.35 A	0.139 A	15.59 A	2.63 A
Simple English	20	10.00 (2) B	5.54 B	0.06 C	5.28 C	0.74 B

Means with the same letter per column are not statistically different (Tukey, $\alpha = 0.05$). The growth % was calculated by the type of graft. The number of replications that presented growth of the total by type of graft is indicated in parentheses.

The difference in the growth percentages could be explained by the juvenile condition of the vegetative materials used (5 years old with non-lignified tender cuttings) and pattern (1 year old). According to Alba et al. (2017) the growth percentage of the grafted vegetative material was associated with the juvenile condition of the materials, so the younger the individual, the faster and easier its propagation, making graft the most effective asexual propagation method (Bailey et al., 2009).

In relation to the propagation technique by layers, no research was found where the use of this technique was reported in the asexual propagation

of *B. alicastrum*. According to Vergara et al. (2014) the lack of silvicultural management of the species has limited the development of research on its forms of propagation.

4 Conclusions

The scientific production found on *B. alicastrum* focused on analyzing and describing aspects of ecology and botany of the species; research on its propagation was scarce. Therefore, the conduction of research on this topic is a priority area to contribute to the silvicultural management of the species.

In the seed propagation method, the percentage of nursery germination was associated with neutral pH substrates and low porosity, and the quality of seedlings to containers with large diameters (36 cm). Asexual propagation by cuttings was not successful; the best results were obtained with layers with a 90% of survival, and graft with the lateral technique with a 75% increase in growth.

Because of the little research on the dissemination of the species, it is recommended that the selection of propagation technique is based on the purpose of the seedling, and asexual graft and bud techniques may be more efficient if the aim is to shorten the seed production cycles of *B. alicastrum*.

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