

VEGETATIVE PROPAGATION OF ABOREAL SPECIES OF ECOLOGICAL AND CULTURAL IMPORTANCE IN THE TROPICAL DRY FOREST

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ABSTRACT

The tropical dry forest is an important source of resources for the communities established in its surroundings. However, it is considered among the most threatened ecosystems due to anthropogenic activities. Because of the problems of deforestation and biodiversity loss, it is necessary to implement conservation systems and strategies that include the propagation of native species from cuttings. Since desired characteristics can be maintained, and long growth periods be avoided, thus permitting a greater number of plants in a short period of time. The objective was to establish a method of vegetative propagation for three tree species, *Bursera copallifera*, *Bursera fagaroides* and *Ipomoea murucoides* with different ecological characteristics. The cuttings were obtained from trees of the tropical dry forest of the state of Morelos, Mexico. In order to evaluate the survival of the cuttings, it was related to the diameter, height and presence of leaves for each tree species in a one-year greenhouse experiment. The survival of the cuttings was compared among species with the Cox proportional hazards model. After one year, 79 %, 39 %, and 9 % of the cuttings of *B. fagaroides*, *B. copallifera*, and *I. pauciflora* survived, respectively. The presence of leaves had a significant effect on the survival of the cuttings, but neither the diameter nor the length of the cuttings affected their survival in the greenhouse. This information may help with the conservation of the tropical dry forest and the sustainable management of tree species with ecological and commercial importance.

Keywords: plant conservation, biodiversity loss, *Bursera* sp., *Ipomoea*, copal, cazahuate.

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INTRODUCTION

Plant propagation involves applying biological principles focused on the multiplication of useful plants of a specific genotype, and there are basically two alternatives of propagation: sexual through seeds or asexual through plant tissues (Megersa, 2017). Asexual propagation is possible due to the totipotency of plant cells, which consists of each cell containing the genetic information and capacity necessary for regenerating all of the parts of a plant and their functions such as generating new stems, roots and leaves (George *et al.*, 2008). Methods have been developed for producing plants vegetatively; such as high technology *in vitro* cultivation (tissue cultivation) or low technology (grafting, cuttings) (Deepak *et al.*, 2016). In rural zones, plants are usually propagated by means of cuttings, for species of interest or for live fences (Costa *et al.*, 2017; Megersa, 2017).

The species used for vegetative propagation have growth characteristics and particular requirements of cultivation, from which specific products and benefits are obtained. Some studies mentioned that in different regions of America the botanical families most widely used to propagate through cuttings correspond to those that offer nutritional or ornamental alternatives (Pancel and Köhl, 2016).

Some tree species typical of the tropical deciduous forest (TDF), such as those of the genus *Bursera* (Burseraceae), are propagated by cuttings for their commercial use, since they have economic and cultural interest, given that their resins are used in industry and in traditional medicine (Rzedowski and Calderon, 2013; Vázquez-García *et al.*, 2019). *Bursera simaruba* (L.) Sarg. and *Bursera grandifolia* (Schltdl.) Engl. are vegetatively propagated in tropical zones of the country to establish live fences and other agroforest uses (Castellanos-Castro and Bonfil, 2013). *Bursera linanoe* (Llave) Rzed., is a species of the TDF of commercial importance, given that this plant provides wood and essential oils that are used in the elaboration of handcrafts and in traditional medicine. It has been documented that vegetative propagation in the greenhouse is more effective than propagation from seeds (Rzedowski and Calderon, 2013; Vázquez-García *et al.*, 2019). In the TDF of the state of Morelos, the owners of pastures asexually (branches and stems) propagate tree species as *Erythrina americana* Mill. (Fabaceae), and *Spondias purpurea* L. (Anacardiaceae) which are used as borders for plots, to provide shade to animals, to lower the ambient temperature and to provide a fodder reservoir (Ayala-Enriquez *et al.*, 2020). *Ipomoea murucoides* is widely cultivated in range land and has a great ecological importance given that its flowers are specialized in attracting nocturnal pollinizers, although they can be a food source for other insects and birds (Caballero-Martínez *et al.*, 2017).

In view of biodiversity loss due to changes in land use and the accelerated deforestation, it is necessary to plan some integral conservation strategies.

Within context, the selection of species to be integrated in TDF conservation projects depends on the adequate methods of propagation which would allow a successful, rapid and inexpensive implementation that benefits the local populations.

Therefore, the objective of this study was to establish a protocol for rapid and economic vegetative propagation of three tree species of the TDF (*Bursera copallifera*, *Bursera fagaroides* and *Ipomoea murucoides*) with different ecological characteristics and of economic importance for the rural communities.

MATERIALS AND METHODS

The collection of plant material was done in April 30, 2019 in the tropical dry forest of San Andrés de la Cal, Tepoztlán, Morelos. The trees were previously identified and randomly selected. Trees were found in growth dormancy, as they had lost their leaves. Ten adult trees of each species were selected, with a height of over 4.5 m and a DAP \geq 20 cm in at least one of their trunks. Secondary terminal branches were selected from each tree, which resulted from the last period of growth (Castellano-Castro and Bonfil, 2013). The collection of plant material was made during the morning and each cutting was placed in plastic bags and carried to the greenhouse of the Centro de Investigación en Biodiversidad y Conservación (CIβγC). Once they were in the greenhouse, to avoid dehydration and as a preventative treatment against diseases and pathogens, branches were placed in a fungicide solution (BYOPROTEK) at a 1.8 mL L⁻¹ dose. The diameter and length of each one were registered (Table 1). To induce root production the growth hormone Indole-3-butyric acid (IBA) was applied because it is the most utilized auxin for stimulating root formation. The commercial brand RADIX was applied in the powdered presentation at 1500 ppm. The cuttings were submerged in water in the basal portion and then in the powder, ensuring to cover the surface of the base (Figure 1).

To establish the cuttings a wooden tool was used to remove a portion of substrate and thus avoiding damages to the cuttings and loss of rooting substance. The cuttings were planted in plastic bags of 13 × 25 cm previously labelled (to maintain the identity of the tree of origin). As the substrate, a mixture of peat moss and agrolite 1:2 was used (Flores-Palacios *et al.*, 2014). Once the cuttings

Table 1. Diameter and length of cuttings of *Bursera copallifera*, *Bursera fagaroides* and *Ipomoea murucoides* at greenhouse condition.

Species	N [†]	Diameter (mm) [‡]	Length (cm) [§]
<i>Bursera copallifera</i>	100	15.70 ± 3.55	85.57 ± 14.44
<i>Bursera fagaroides</i>	100	18.76 ± 4	79.12 ± 14.35
<i>Ipomoea murucoides</i>	100	15.01 ± 3.28	92.28 ± 14.83

[†]N: size of the sample, [‡]Mean ± standard deviation.



Figure 1. Transplanting process for cuttings in the greenhouse; A: application of auxin IBA in powder, B: mixture used as substrate, C: cuttings transplant.

were transplanted, water was supplied through an automated drip irrigation system, three times per week (Figure 1).

One month after planting, a treatment of control for pests and diseases was implemented; along with a fertilization treatment, for this purpose the cuttings were sprayed monthly with fertilizer (RAICIN) in a concentration of 30 g L^{-1} . This solution contains auxin (400 ppm), nitrogen (16%), phosphorus (45 %), potassium (11 %), sulfur (0.8 %), magnesium (0.6 %), and zinc (0.4%). After a year of growth, the cuttings were transplanted to plastic bags of $40 \times 40 \text{ cm}$. Before transplant, in order to strengthen the plant and reduce possible mortality from the movement of the cuttings, powdered fertilizer was applied (Agrylap triple 20), a 20 g L^{-1} concentration. This formula contains nitrogen (20 %), phosphorus (20 %), potassium (20 %), aminoacids and vitamins. The cuttings were maintained under greenhouse conditions with an average temperature of $20 \text{ }^\circ\text{C}$ (max. $38 \text{ }^\circ\text{C}$ and min. $10 \text{ }^\circ\text{C}$), the relative humidity was 73.7 % (max. 100 % and min. 5.9) which was registered daily at intervals of 30 minutes with a data logger (HOBO PRO H08-030-08).

Data analysis

The survival of the cuttings was compared among species with the Cox proportional hazards model, through the '*survival*' library (Therneau, 2022). In this analysis the only factor was the tree species, and the covariables were the presence of leaves (number of weeks with leaves), diameter and length of the cuttings. This analysis measures the frequency of individuals that die and the time until the event of interest occurs. The survival of the cuttings of each species was graphed with survival curves of Kaplan-Meier (Kleinbaum and Klein, 2012) with the library '*survminer*' (Kassambara *et al.*, 2019). To isolate the differences among the species multiple comparisons were made by contrasts with the library '*multcomp*' (Hothorn *et al.*, 2008). All analyses were made in R version 3.6.3 (R Core Team, 2020).

RESULTS AND DISCUSSION

The tropical dry forest in Mesoamerica is one of the most threatened and least protected ecosystems, due in part to the increase of the human population (Sotelo-Caro *et al.*, 2015). Therefore, it is necessary for its protection to establish traditional propagation methods that help *in situ* conservation.

One of the goals was establishing a propagation protocol for tree species with ecological importance of the tropical dry forest (*Bursera copallifera*, *Bursera fagaroides* and *Ipomea murucoides*) by asexual reproduction, to obtain a higher number of individuals in a shorter period of time. After 366 d since the cuttings transplant, survival of the three selected tree species was different (Table 2).

The best response after one year of observation corresponded to *B. fagaroides* with 79 % of survival of the cuttings, followed by *B. copallifera* which presented 39 % survival (Figure 2). The lowest percentage was for *I. murucoides*, after 114 d of observation only 9 % of the cuttings survived, the highest mortality occurred at 78 d after the cuttings were transplanted (Figure 2). Among the covariables, our results showed that the presence of leaves had a significant effect on the survival of the cuttings, but neither the diameter nor the length of the cuttings affected survival in the greenhouse, the results did not show significant differences (Table 2).

Regarding leaves production of the cuttings, 99 % of the plants of *B. fagaroides* developed leaves two months after transplant (Figure 3). In *B. copallifera*, 72 % of the plants produced leaves three months after transplant; after this, cuttings began to linearly lose leaves (Figure 3). Only the cuttings of *B. fagaroides* maintained and developed leaves throughout the experiment. In contrast to the behaviour of the two species of *Bursera*, all of the cuttings of *I. murucoides* developed leaves in the first months of transplant; however, in the following months only 9 % of the cuttings kept their leaves and survived. All of the surviving cuttings developed roots (Figure 3).

The cuttings of *Bursera fagaroides* had the highest percentage of survival and root formation under greenhouse conditions. This coincides with the results of Castellanos-Castro and Bonfil (2013), who reported percentages of over 70 % in

Table 2. The Cox-proportional Hazards model proves the effect of the species and three covariables in the survival of cuttings of *Bursera copallifera*, *Bursera fagaroides* and *Ipomoea murucoides*.

Source of variation	Degrees of freedom	χ^2	P
Species	2	209.11	< 0.001
Leaves production	1	199.07	< 0.001
Cutting diameter	1	0.79	0.375
Cutting length	1	0.43	0.512

χ^2 : Chi-squared, P = value of probability associated with χ^2 , significant differences at $p \leq 0.05$.

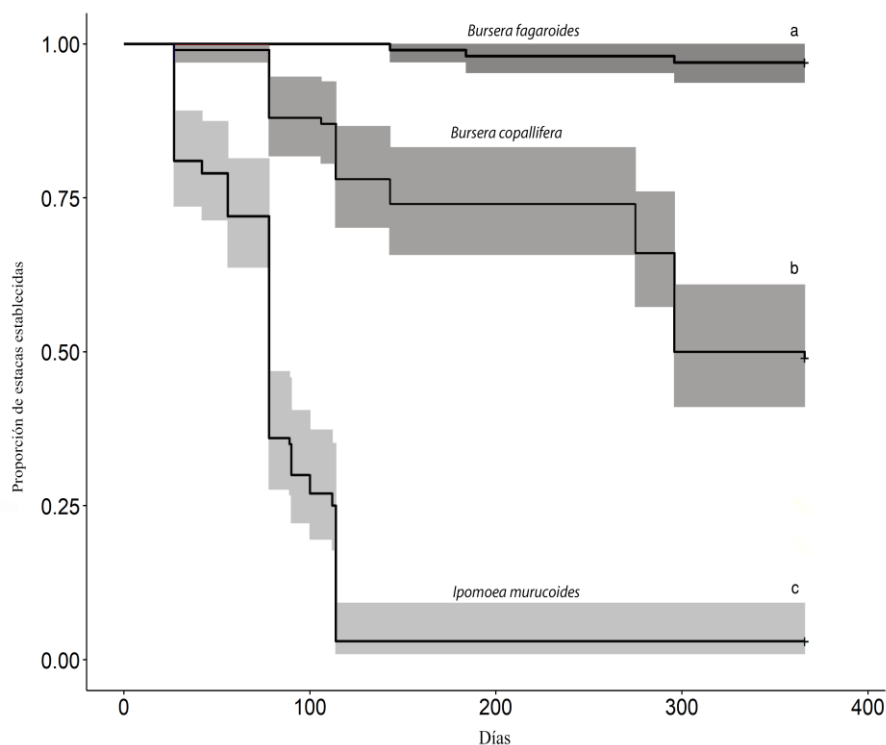


Figure 2. Kaplan-Meier survival curves of cuttings of *Bursera copallifera*, *Bursera fagaroides* and *Ipomoea murucoides* in a greenhouse. The shadow areas are the 95 % confidence intervals.

the capacity to form calli and roots by adding auxins to cuttings of *B. fagaroides*, *B. glabrifolia* and *B. linanoe*; *B. fagaroides* developed calli and roots without needing the addition of growth phytohormones.

In this study good results were obtained using cuttings of *Bursera fagaroides* in a total state of latency, before the initiation of development of new tissues. Since, as it occurs with other species of the TDF, growth is seasonal and is associated with environmental conditions. Following their natural phenology, trees of this species lose their leaves during the dry season and the tissues of stems and branches have accumulated carbohydrates and auxins, which should favoured the formation of roots in the cuttings collected.

It is considered that the development of leaves in the cuttings is a good indicator of root development (Zahawi, 2005, Villegas-Monter *et al.*, 2019). After two months remaining in the greenhouse, all of the cuttings of *B. fagaroides* began the formation of new leaves. Thus, the high percentage of survival of *B. fagaroides* could be explained both by the increase in the rate of photosynthesis and by the early appearance of roots. This increased the plant capacity to use nutrients and water applied to the substrate (Coll *et al.*, 2019).

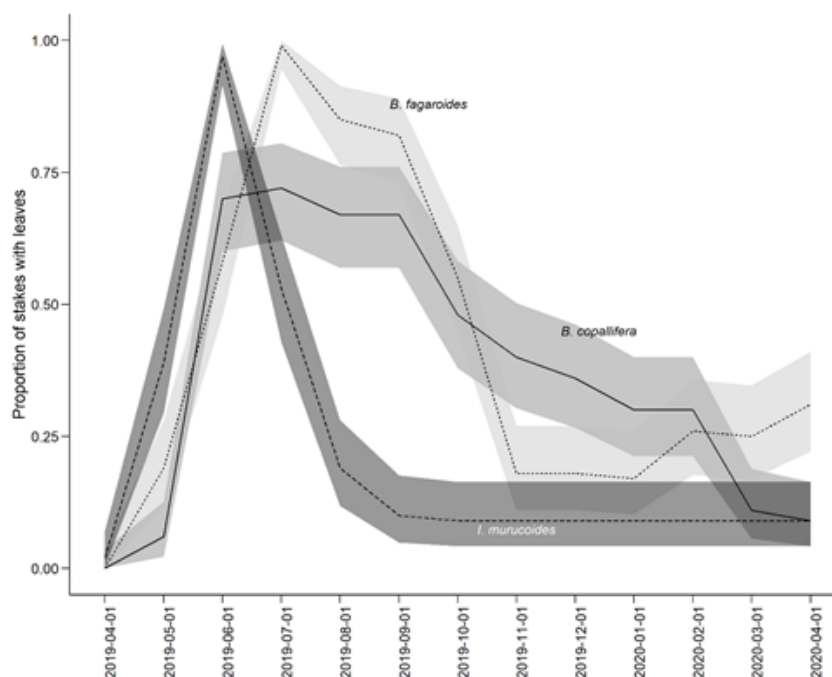


Figure 3. Proportion of cuttings with leaves through time of *Bursera copallifera*, *Bursera fagaroides*, and *Ipomoea murucoides* under greenhouse conditions. The shaded area shows the confidence intervals at 95 %.

In the cuttings of *B. copallifera*, a low percentage of survival and root formation was recorded (39 %). Bonfil *et al.* (2007) found that between 18 % and 50 % of the cuttings of *B. bipinnata*, *B. copallifera*, *B. glabrifolia* and *B. lancifolia* subsisted under field conditions, in the case of *B. copallifera* and *B. grabifolia*, the survival of a plantation in a site without problems of flooding was 52 %, whereas 32 % of the cuttings survived in another site in which there was water retention (Castellanos-Castro and Bonfil, 2013).

In this study the survival and rooting of *B. copallifera* could be directly linked to the amount of water that was supplied in the substrate. Since it has been reported that the establishment and survival of species of the genus *Bursera* are negatively influenced by the storage of water in the substrate, which generates anoxia, interrupts root growth and favours the appearance of pathogenic fungi that promote cuttings rot (Zahawi, 2005). Therefore, to have more success in the propagation of *B. copallifera* it is recommended to reduce the water supply or to use a substrate that retains less moisture, aiding then to the formation of roots and survival of cuttings from this species.

In a study of the effect of lignification on the root development of species of *Bursera*, it was found that the generation of roots was strongly influenced by

the lignification of the cuttings, which increases with the age of the branch (Castellanos-Castro and Bonfil, 2013). In this study, although no effect was found from the diameter on the survival of the cuttings of *B. copallifera*, it is recommended to use cuttings ≤ 18 mm, given that cuttings of greater diameter are more lignified. Furthermore, in mature branches, the production of resin is high, which diminishes the absorption of the growth regulators (IBA) and reduces the percentage of rooting, and the number and quality of the roots. It is recommended to use younger cuttings, because they have a higher concentration of endogenous auxins, carbohydrates reserves, and are less lignified. These characteristics together increase the probability of root development, and the production of resins is lower (Villegas-Monter *et al.*, 2019).

The species *I. murucoides* presented the lowest percentage (< 10 %) of cuttings survival, which can be explained by the null development of roots, even though the cuttings appeared green and even sprouted buds after three months since planting. Furthermore, when the cuttings were removed from the substrate, it was observed that only a reduced number had begun the formation of callus or roots.

The natural capacity of rooting is the most critical factor for the establishment of cuttings, and is a characteristic that varies among species. One possible explanation for the lack of root formation and higher mortality of the cuttings of *Ipomoea murucoides* is the presence of phenolic compounds, which serve as a defense against various types of stress caused by pathogens or wounds (Devi *et al.*, 2021). The relationship between the phenols and rhizogenesis induced by auxins can have positive or negative effects on the plants. In some occasions the phenols act as rhizogenic cofactors, supporting the root initiation phase; but in others, depending on the quality and quantity in which they are found, phenols can limit root formation, given that in high concentrations they participate as inducers of the oxidation of auxins, interfering with the formation of adventitious roots in stem cuttings or provoking cellular death in the location of the cut (Latsague and Lara, 2003; Devi *et al.*, 2021).

As it was mentioned above, the cuttings of *Ipomoea murucoides*, as with the other species of study, were obtained at the end of the dry season, during the months the trees lose leaves. In contrast to the other species, the trees of *Ipomoea murucoides* flower and fructify between October and November, thus it is possible that the cuttings of these trees had already consumed part of their energy in reproduction. Furthermore, the presence of latex could have impeded the absorption of the exogenous auxins supplied, and the amount of these could have been insufficient to induce the development of roots (Montes, 2021). Therefore, we propose that the selected cuttings of *Ipomoea murucoides* should conserve leaves that help in the autogenous synthesis of auxins, which should favor the formation of roots and survival.

The optimal requirements for the propagation of cuttings of *I. murucoides* are unknown, since there is only information on pre-germinative treatments for propagation through seeds (Martínez-Pérez *et al.*, 2017; Aguilar-Franco *et al.*, 2019).

CONCLUSIONS

This research is among the first attempts on propagation of *Bursera copallifera*, *Bursera fagaroides* e *Ipomoea murucoides*, by means of cuttings under greenhouse conditions. The two species of *Bursera* had greater success in propagation by cuttings than *I. murucoides*; for this latter species it is necessary to establish an adequate propagation protocol.

The low-cost propagation protocols such as the one developed contribute to the integral conservation efforts of the tropical deciduous forest. By using tree species of ecological and economic importance, both diversity maintenance and interactions are promoted, along with the use of these species as part of the needs of the local population.

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