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Article

Propagación vegetativa del mangle (*Laguncularia racemosa* (L.) C.F. Gaertn.) bajo condiciones de vivero

Vegetative propagation of mangrove (*Laguncularia racemosa* (L.) C.F. Gaertn.) under nursery conditions

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Resumen

El presente estudio se realizó en la Unidad de Manejo para la Conservación de la Vida Silvestre denominada Vivero El Manglar en el municipio Acapulco, Guerrero, México. El objetivo fue evaluar la eficiencia de propagación asexual por medio de material vegetativo (estacas) como propágulos de *Laguncularia racemosa*. Se utilizó la técnica de enraizamiento de estacas para la propagación asexual de manglares; de las plantas madre, se recolectaron 500 estacas entre 50 y 100 cm de longitud y un diámetro de 5 a 10 cm. Se obtuvo 54 % de supervivencia en la propagación, pero con escasa formación de brotes apicales y de raíces adventicias. Se comparó el número de brotes con el de raíces adventicias mediante la prueba de correlación de *Tau* de *Kendall* de lo que resultó un valor de 0.062 y significancia de p= 0.200 mayor que $p > 0.05$, lo que indica correlación positiva muy débil y poco aceptable; se confirmó que la propagación vegetativa por estacas no es las más eficiente para reproducir ejemplares de *L. racemosa* en condiciones de vivero. Para dar soporte al análisis anterior, se realizó la prueba de *Friedman*, que dio un valor de significancia de 0.000 inferior a $p \leq 0.05$, y se observaron diferencias significativas de interacción entre ambas variables. El presente estudio demostró que la propagación asexual de *L. racemosa* tuvo una eficiencia regular, por lo que será necesario dar continuidad con experimentos de reproducción asexual y sexual.

Palabras clave: Enraizamiento, estacas, manglares, producción de brotes, propágulos, reproducción asexual.

Abstract

This study was conducted in the Wildlife Conservation Management Unit called *Vivero El Manglar* located in *Acapulco* municipality, state of *Guerrero*, Mexico; the aim was to assess the asexual propagation efficiency by means of vegetative material through stakes of *Laguncularia racemosa* propagules at the nursery. The methodological technique of rooting stakes for the asexual propagation of mangroves was used; from the mother plants, 500 stakes between 50 and 100 cm long and of 5 to 10 cm in diameter were collected. 54 % survival was obtained in the propagation by this technique, with a small yield formation of apical shoots and adventitious roots. The number of buds was compared to the number of adventitious roots by the Kendall's Tau correlation test, obtaining a value of 0.062 and significance of p= 0.200 higher than $p > 0.05$, which suggests a very weak and little accepted positive correlation; which confirms with this statistical analysis that vegetative propagation through cuttings is not the most efficient for reproducing *L. racemosa* specimens under nursery conditions. To support the above analysis, the Friedman test was performed, with a 0.000 less than $p \leq 0.05$ as significance value, observing significant differences in interaction between the two variables. The present study showed that the asexual spread of *L. racemosa* had a regular efficiency, which makes it necessary to continue with both asexual and sexual reproduction experiments.

Key words: Rooting, stakes, mangroves, production of shoots, propagules, asexual reproduction.

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Introduction

Mangrove ecosystems in Mexico have great forest importance, as they provide innumerable goods and services, in addition to being the support for the productive activities of riverside communities; they have are about of 905 086 ha, 7 730 ha of which belong to the state of *Guerrero* (Conabio, 2020).

Mangrove nursery production is an alternative sustainable development option to conventional productive activities (Castillo, 2007); however, most of the existing mangrove nurseries are family or community units that produce plants to reforest *ejido* areas.

In some coastal areas of the world, community-managed mangrove nurseries have been established where restoration and reforestation activities are carried out; viable possibilities are developed with them to improve the socioeconomic level of the region, and products derived from mangroves such as firewood, charcoal, wood are used, the use of various aquatic species is promoted, technical training is received and employment opportunities are provided in the establishment and management of plantations (Álvarez-León, 2003).

In Mexico, aspects regarding the propagation techniques of mangrove species in nurseries have been documented. Benítez *et al.* (2002) and Hernández *et al.* (2012) used the vegetative reproduction technique through stakes and aerial layering in the state of *Veracruz.*

Studies such as that made by Carmona-Díaz (2010) cite learning experiences in the reforestation of mangroves in Mexico; while Hernández *et al.* (2016) carried out a documentary diagnosis of the reforestation process in mangroves on the *Tabasco* Coast. On the other hand, Castillo and Gervacio (2009) designed a methodological proposal for mangrove nursery production. Teutli-Hernández *et al.* (2020) wrote community mangrove restoration manuals, in which they included methodological processes for the production of mangrove plants both *in situ* and *ex situ* in rustic *ejido* nurseries.

For the state of *Guerrero*, Tovilla and Orihuela (2002) described the in situ sexual propagation of *Rhizophora mangle* L. in *Barra de Tecoanapa*; Castillo (2007) proposed the methodology of implementation and operation of mangrove production nurseries for *L. racemosa* (L.) C.F. Gaertn.) in the *Tres Palos* lagoon in the same state.

In this context, the present study focused on *L. racemosa*, as it is one of the overexploited mangrove species in the study region; based on the above, the study of vegetative propagation through stakes was proposed as a method to rescue the phylogenetic resource of the species and generate the production of germplasm.

The objective of the present research was to evaluate the efficiency of asexual propagation by means of vegetative material through stakes such as propagules of *Laguncularia racemosa*, in a nursery.

The following research hypotheses were raised:

H_i: Vegetative propagation through stakes is not the most efficient to produce *L. racemosa*.

H_0: Vegetative propagation through stakes is the most efficient to produce *L. racemosa*.

Materials and Methods

Study area

The study was carried out from March 2018 to April 2019 at the facilities of the Wildlife Conservation Management Unit called *UMA-Vivero El Manglar* with registration code No. SEMARNAT-UMA-VIV-002-GRO, located in the town of *Barra Vieja, Acapulco* municipality, *Guerrero*, close to the *Tres Palos* Lagoon, between the geographic coordinates 16°41'33'' N and 99°37'33'' W (Figure 1).

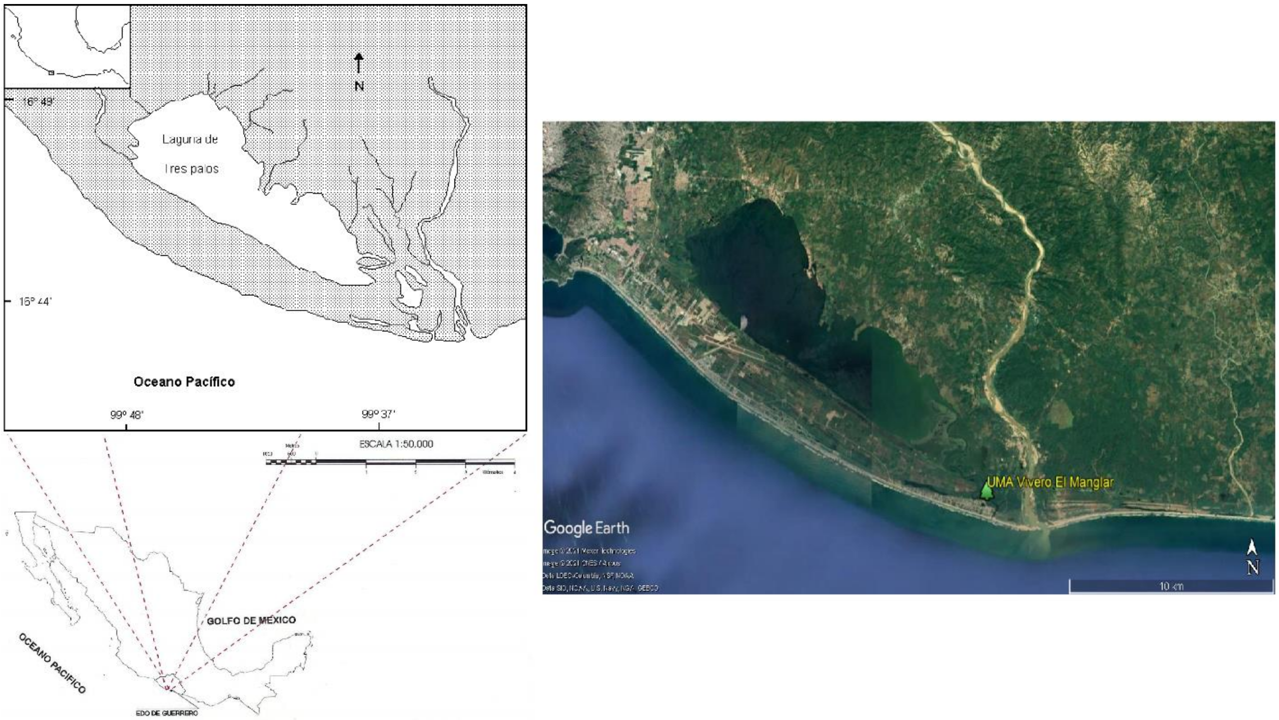

Figure 1. Location of the study area.

Collection of vegetative propagules

For the present investigation, the implementation of the propagule technique of *L. racemosa* by means of propagules by means of the staking technique was considered important, which, according to Sisaro and Hagiwara (2016), is a reliable means of asexual or vegetative reproduction, from which a new plant genetically identical to the mother plant is obtained.

Prior to the establishment of the experiment, the work area in the nursery was conditioned, and the methodologies proposed by Eganathan *et al.* (2000), Benítez *et al.* (2002), Clarke and Johns (2002), Castillo (2007), Hernández *et al.* (2012) and Silva *et al.* (2016) for the management of the plant in the nursery.

Establishment of the experiment and treatments

The substrate used was taken from the same collection site of the stakes and its silty-clay consistency was determined by sieving granulometric analysis and a slightly acidic pH measured with a HI 991300 Hanna pH multiparametric. The water used to irrigate the germination beds came directly from the lagoon and from artisanal wells attached to the nursery; the stakes were watered every other day, with manual sprinklers.

Plastic seedlings were constructed (15 cm × 15 cm × 20 cm); The substrate mixture was prepared in the nursery in a proportion of 30 % sand, 30 % clay from the site and 40 % compost made on site. Magic Root® rooting agent was added as well as Organodel® natural organic fertilizer; 500 reusable polyethylene terephthalate (PET) plastic bottles were used with a capacity of 1, 2, 4 and 6 L with perforations in their lower part for water drainage and reusable conventional wooden bars for the accommodation and transfer of PET bottles. According to Clough (1984), for irrigation a mixture of water with a salinity of 15 to 17 pus (practical units of salinity) was prepared in a proportion of 50 % salt water for 50 % fresh water; Clarke and Johns (2002) point out that the salinities of 17 pus optimize the growth of mangrove species produced in the nursery. Salinity was maintained at a homogeneous level when monitored with a manual multiparametric probe type meter for pH, conductivity, salinity and temperature of the HI 991300 Hanna. A black polyethylene mesh-shade cover, 80 % shade (Easy Gardener®) was used to protect cuttings from heat stroke and prevent dehydration.

The collection of vegetative material of *L. racemosa* was carried out during the spring of March 2018. 10 adult mangrove trees were selected and without apparent damage by pests and diseases, with an average height of 20 m, as mother plants; With its secondary branches, 500 semi-woody wood stakes were made (50 stakes per tree), with an average length of between 50 to 100 cm and an average diameter of 5 to 10 cm; later they were placed on wooden bars for transport to the nursery facilities. It should be noted that both the length and the average diameter were measured with a 3 m 13 mm Pretul tape.

There, and according to Sisaro and Hagiwara (2016), the stakes were subjected to hormonal treatment to stimulate the production of roots, which consisted of immersing them for five seconds through the basal part in a solution with a Magic Root® rooter with concentrations of 500 at 10 000 ppm; later one stake was planted per PET container.

The maintenance pruning of the shoots that emerged on the lateral part of the stakes was carried out for three weeks in order to favor the growth of the apical shoots. With this last step, it was necessary to wait for the development of the apical shoots, whose number varied from one to eight, to reach an average height of 25 to 35 cm. Such variable was measured with a 3 m 13 mm Pretul tape.

During the propagule propagation and maintenance process, and in accordance with the recommendation of González *et al*. (2018), a single dose of 1 kg m^{-3} of natural organic fertilizer substrate Organodel® was directly applied to the substrate two months after the stakes were planted in order to strengthen the development of shoots and roots. Likewise, inside the nursery, once a month the ambient temperature and the substrate temperature were monitored in the seedlings, determined with a Brannan brand mercury thermometer, ranging from -20 to 150 °C and the pH of the substrate determined with a. HI 991300 Hanna digital potentiometer; the survival percentage of the number of stakes and the apical shoot formation count and adventitious roots were also determined.

Assessed variables and data analysis

The assessed variables during the experiment were exclusively the production of apical shoots and adventitious roots generated by each stake.

Root production records were obtained at 40 days in 400 stakes of the 500 *L. racemosa* that were sown, and a scarce establishment was achieved in the formation of apical shoots, these cuttings were followed for 4 months; after which only 270 survived and remained in optimal development.

With the field data, statistical significance tests were performed that quantify the association or independence between quantitative variables; the statistical program SPSS (Statistical Package for the Social Sciences) v. 22 (Castañeda *et al*., 2010), so it was verified whether both quantitative variables complied with the normal distribution in the groups being compared (Zar, 1996), using the Kolmogorov-Smirnov test (Juárez *et al.,* 2002), whose non-compliance leads to the need to resort to non-parametric statistical tests. Failing the normality test, the Wilcoxon T test was performed for two related numerical variables.

Likewise, to determine if there is a correlation between the number of apical shoots with the number of adventitious roots and to determine if the propagation technique by cuttings is efficient through vegetative propagation, the correlation test of Kendall's Tau was carried out. applicable to quantitative variables that do not present the normality test (Kendall, 1938), this correlation coefficient establishes values between 0 and 1, the closer it is to 1 indicates a good correlation, which are applicable to negative values.

To validate the Kendall correlation, the Friedman test was performed, which is equivalent to the ANOVA test, to calculate the significance value of the interaction between shoots and roots, which does not exist when $p > 0.05$, or does exist when $p \leq 0.05$ (Villarroel, 2018).

Results and Discussion

After four months, the results indicate that only 270 stakes maintained optimal development. The survival efficiency was 54 % by this rooting technique, which presented apical shoots at a rate of 1 to 8 per stake and a minimum of 1 to a maximum of 5 adventitious roots, which corresponds to one root per stake on average. Therefore, it was considered a poor generation of structures.

Table 1 shows the frequency of apical shoots and adventitious roots per stake and their appearance in groups. Such data show that the highest frequencies were verified in the samples with 2, 3 and 4 shoots, with 23 %, 16 % and 22 %, respectively; In the case of adventitious roots, the highest frequencies occurred in stakes 1, 2 and 3 with 1, 2 and 3 roots, with 20 %, 26 % and 27 %, respectively.

Table 1. Frequency of shoots and adventitious roots.

Number of apical shoots	Frequency	%	Number of adventitious roots	Frequency	%
1	27	10	0	6	2
2	62	23	1	55	20
3	43	16	2	70	26
4	61	22	3	72	27
5	16	6	4	40	15
6	29	11	5	27	10
7	10	4			
8	22	8			
Total	270	100	Total	270	100

Source: Own elaboration with SPSS software

Statistical analysis

The value obtained from the Kolmogorov-Smirnov test for an independent sample was 0.000 if $p \leq 0.05$, which indicates that the variables do not follow a normal distribution (Table 2).

Table 2. Normality tests.

Variable	Kolmogorov-Smirnov		
	Statistical	**df**	**Sig.**
Number of shoots	0.174	270	0.000
Number of aadventitious roots	0.168	270	0.000

Source: Own elaboration with SPSS software

A non-parametric statistical test was applied by means of the Wilcoxon test, resulting in a value of 0.000, therefore, $p \leq 0.05$ indicates that the related samples present significant differences (Table 3).

Table 3. Wilcoxon test.

	Number of adventitious roots-Number of shoots
Z	-7.258
Asymptotic Sig. (bilateral)	0.000

Source: Own elaboration with SPSS software

To determine the statistical correlation, the number of apical shoots was compared with the number of adventitious roots with the Kendall's Tau correlation test; A value of 0.062 was calculated, less than 1; likewise, this test is reinforced with the significance value of $p = 0.200$ greater than $p > 0.05$ (Table 4), which indicates that there is a very weak and poorly accepted positive correlation between the relationship of the presence of apical shoots and adventitious roots with a 95 % confidence level.

Table 4. Correlation coefficient between shoots and adventitious roots.

			Number of adventitious roots
Number of shoots	Tau de Kendall correlation	1	0.062
	Sig. (bilateral)		0.200
	N	270	270

Source: Own elaboration with SPSS software

Therefore, the vegetative propagation technique by cuttings was considered with a low propagation efficiency under nursery conditions, consequently and based on this statistical analysis, the research hypothesis (H_i) raised at the beginning of this study is accepted, in which it was established that vegetative propagation through cuttings is not the most efficient to reproduce specimens of *L. racemosa.*

The Friedman test was used to contrast the interaction between apical shoots and adventitious roots; from it, a significance value of 0.000 resulted, which is lower than $p \leq 0.05$, and, therefore, there are significant interaction differences between both variables (Table 5).

Table 5. Friedman test.

N	270
Chi-square	33.927
df	1
Sig. asymptotic	0.000

Source: Own elaboration with SPSS software

The analysis of the results of this study is similar to that cited by Hernández *et al*. (2012) who compared the number of shoots with the number of adventitious roots for *Conocarpus erectus* L. using a Pearson correlation test (0.001); the relationship between apical shoots and adventitious roots was very low and not widely accepted, for which they concluded that the propagation technique by layering has little viability.

De Silva and Amarasinghe (2013) determined that there are no significant differences in the growth rates of shoots obtained from mangrove seedlings when they are grown in different growth media, opposite to the results described herein in which significant interaction differences were obtained between the number of apical shoots with the number of adventitious roots.

The method by stakes in the study described here was not very feasible since few suckers were produced, which appeared in a period of 20 to 40 days. This was due to the use of commercial rooting for growth, which, in this case, was not very effective in vegetative propagation. In accordance with the above, Hernández *et al.* (2012) recommend the use of rooters based on auxins and indolebutyric acid such as Raizone-Plus®, which favors the activation of rooting and is efficient in the vegetative propagation of *R. mangle* L., *L. racemosa* and *A. germinans* L. Benítez *et al.* (2002) and Hernández *et al.* (2012) argue that the best asexual reproductive means in *L. racemosa* is through air layering, which has recorded up to 90 % of rooting.

Eganathan *et al.* (2000) and Thatoi *et al.* (2001), who worked in India with the mangrove species *Excoecaria agallocha* L., *Heritiera fomes* Buch.-Ham. and *Intsia bijuga* (Colebr.) O. Ktze. proposed the use of auxins and the seasonal climatic condition to accelerate the rooting potential through cuttings; being a viable and applicable condition for the mangrove species of the present study. On the other hand, Perera *et al.* (2020) suggested applying a broader range of rooting hormones and hormone combinations to mangrove stem cuttings to induce adventitious root formation.

Benítez *et al.* (2002) recorded 50 % success rate of propagation by cuttings of *L. racemose*. This result of vegetative reproduction was due to the seasonal time during the propagation process, which was summer, and suckers could be reproduced asexually in an extended period of three months from sowing, and they remained alive for 6 to 12 months, without be successful in issuing roots. These data are similar to those of the present work, in which the propagation of *L. racemosa* did not have the desired success with the propagation technique by cuttings, with a percentage of 54 %, considered as low efficiency. In contrast, Hernández *et al.* (2012) reached results of vegetative production by higher layering, as they achieved that *L. racemosa* produced a greater number of roots by this route during the summer.

The emergence of roots produced in the cuttings was influenced by environmental factors such as humidity and rainfall during the year, as well as by the physicobiological conditions of the *L. racemosa* species, a question that coincides with that reported by Hernández *et al*. (2012) with the understanding that the success of vegetative propagation will depend on the particular characteristics of the mangrove species.

Based on the above, the results of the present study showed that the length of up to 1 m of the stakes did not favor the induction of roots in the species of interest, so it is convenient to select longer cuttings of up to 3 m and of 10 to 15 cm in diameter. Despite the fact that the species generated roots in the propagules by cuttings, the success of vegetative reproduction by cuttings was regular, because in most of them they were not able to produce suckers, which differs from that recorded by Hernández *et al*. (2012), who described a greater induction capacity, and thus, to form several roots through asexual propagation through layering.

The success in this technique of vegetative propagation of the mangrove by means of stakes will depend on the correct selection of trees as breeding stock, from which the vegetative material will be obtained; thus, they must be mature, vigorous and healthy, in addition to preselecting the size and thickness of the propagule to guarantee better results; in this study, the stakes of *L. racemosa* did not exceed one meter in length. In contrast, Hernández *et al.* (2012) identified that in *L. racemosa* the selected layers measured 4 m in length and with this it was possible to have a greater ability for root formation.

In regard to the above and according to these last cited authors, it is necessary to consider the monitoring of biotic factors such as pests, weeds, possible infections and abiotic factors such as climatic variations (humidity, temperature, light and rain), in order to have a control that guarantees a satisfactory vegetative propagation in the generation of mangrove plants at any time of the year.

Conclusions

In the present study, it was shown that the vegetative propagation of *L. racemosa* by the staking technique is not efficient and a well-structured methodological process is required, an economic investment for the purchase of necessary accessories for the infrastructure that allows the process of reproduction of the species.

It was shown that the success of the implementation and vegetative propagule of *L. racemosa* propagules by means of cuttings was not as expected, even when a propagation efficiency of 54 % was obtained, but the analysis and statistical tests showed that the efficiency was considered short.

From the vegetative propagation of this same species and other mangroves in different parts of Mexico and the world, and the results obtained, the correct selection of propagules, the weather conditions and the seasonal period have a positive or negative influence on the process. Therefore, it is concluded that in the study that was carried out the experimental conditions did not favor the emission of roots in this mangrove species.

Conflict of interest

The authors declare no conflict of interest.

Contribution by author

Benjamín Castillo Elías: planning and supervision of research, direction, development, design of the experiment, statistical analysis and writing of the manuscript; Herlinda Gervacio Jiménez: writing and revision of the manuscript, supervision of the investigation; José Ángel Vences Martínez: financial support management.

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