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Clonal propagation of *Gmelina arborea Roxb* grown in southeastern Mexico

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ABSTRACT

Objective: To evaluate the clonal behavior of *Gmelina arborea Roxb* from mother plant cuttings in southeastern Mexico.

Design/Methodology/Approach: A completely random design of three treatments with four repetitions was established; the treatments were concentrations of IBA (4000, 6000 and 3000 ppm). The following were evaluated: percentage of rooting, days until root formation, type of cutting, number of roots, length of roots and absorbent roots. Analysis of variance and Tukey's test were performed with Statistix 9.0.

Results: In the analysis of variance (ANOVA), significant factors were observed (callus and number of roots), and also, a fluctuation was observed in the percentage of rooting from 84% to 92%. Regarding the number of roots and cm of roots, there were significant differences in the presence of number of roots of the 6000 ppm treatment.

Conclusions: The concentration of IBA in the rooting generates good results in different concentrations, depending on the cutting implemented, the factors callus and number of roots; significant records were obtained between treatments, favoring the acceleration of presence of roots.

Keywords: Vegetative propagation, *Gmelina arborea Roxb*, growth regulators.

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INTRODUCTION

The application of biotechnology to forest species constitutes bases and principles of research for tropical species. The tropical zones in Mexico are considered of great potential for the establishment and management of commercial forest plantations; considering their geographic position, they receive large amounts of solar energy. *Gmelina arborea roxb*

(Melina) is characterized by being a species of rapid growth and opportunistic in rainforests, classified as long-life pioneer, its capacity for regrowth presents a fast and vigorous growth.

Project A3-S-131410 of the CONACyT-CONAFOR sectorial Fund developed a clonal propagation protocol of selected *Gmelina arborea* trees, early establishment and evaluation of progeny trials, and clonal trials for commercial forest plantations in southeastern Mexico. It was published on October 8, 2018, in the call for projects "2018-2", which had the objective of establishing the first stages of an operative strategy for genetic improvement of *Gmelina arborea*, species used in commercial forest plantations of FYTEIA CAPITAL in southeastern Mexico. Therefore, this study has the objective of evaluating the clonal behavior of *Gmelina arborea* from cuttings obtained from mother plants in southeastern Mexico.

MATERIALS AND METHODS

This project was conducted with the support of resources from the Sectorial Fund for Development Research and Forest Technological Innovation in the "La Huerta" plot, located in the Ranchería el Corralillo on Km 3.28 of the Fraccionamiento Pomoca-El Tigre Highway, Nacajuca, Tabasco. This study had the purpose of determining the optimal concentrations of IBA hormone (Indole-3-butyric acid) in solid solution under three concentrations, 3000 ppm, 4000 ppm and 6000 ppm, and to determine the concentration that generated roots in the shortest time; for this purpose, primary and secondary cuttings of *Gmelina arborea* were used as plant material.

Background of clones evaluated

The genetic material evaluated is part of the Project A3-S-131410 of the CONACYT-CONAFOR sectorial Fund. Early establishment and evaluation of progeny trials and clonal trials of *Gmelina arborea* for commercial forest plantations in southeastern Mexico, published on October 8, 2018, call for projects "2018-2", which had the objective of establishing the first stages of an operative strategy for genetic improvement of *Gmelina arborea*, species used in commercial forest plantations belonging to FYTEIA CAPITAL in southeastern Mexico.

The genetic material to be propagated massively was obtained through the selection of candidate trees and then, after complying with dasometric and phenotypic traits, they were identified as plus trees. The technique of rough hewing (complete tree cutting) was applied, consisting in cutting the tree horizontally, leaving stumps of an average of 32 cm height (Ramos, 2016; Quispe, 2019). Later, at 11 weeks the harvest of reshoots was made (Figure 1), produced from each stump (Figure 1); for this purpose, the following was used: gps garmin etrex 10, map of the site, plastic bags (10 kg), permanent marker, pruning scissors, ice, thermal cooler.

Experimental design

A completely random design of three treatments with four repetitions was established; the treatments were the IBA concentrations (Table 1):

Figure 1. Visualization of resprouts on stumps (a) and harvesting of resprouts emitted by stumps, activity carried out from 10 p.m. to 4 a.m.

Table 1. Completely random design of three treatments with four repetitions.

Treatment	Repetition	Concentration in PPM
1	R1	4000
2	R1	6000
3	R1	3000
4	R2	3000
5	R2	6000
6	R2	4000
7	R3	3000
8	R3	4000
9	R3	6000
10	R4	6000
11	R4	3000
12	R4	4000

Variables to be measured

Percentage of rooting, Days until root formation Type of cutting (primary, secondary), Number of roots, Cm of roots, Absorbent Bloom. The values measured from each variable were analyzed parametrically using Statistix 9.0, Tukey's test, ANOVA.

Preparation of the substrate

Trays with 42 cavities were used (34 cm × 37 cm) with a capacity of 175 cm^3, they were filled with substrate made up of 80% bark and 20% Peat Moss and slow-release fertilizer was added (Figure 2).

Process of obtaining cuttings

Performing cuts during the whole process of obtaining cuttings (Figure 3) was done with a scissor (Truper, stainless steel), disinfected before and after each cut, and performing the change in management of the genetic material. The cut of the reshoot was done in the basal part, the closest to the principal stalk.

Sectioned

To select the primary and secondary cuttings (Figure 4), the cuts were performed in the inferior part of each cutting, the closest to the axillar bud; then, the withdrawal or cut of the leaf part of 50% was done, reducing the transpiration of those cuttings.

Figure 2. Substrate preparation: 80% bark and 20% Peat Moss.

Figure 3. Shoot preparation in mother plants of *Gmelina arborea*, the handling of the prepared shoots is done with latex gloves to avoid contamination of the material to be propagated.

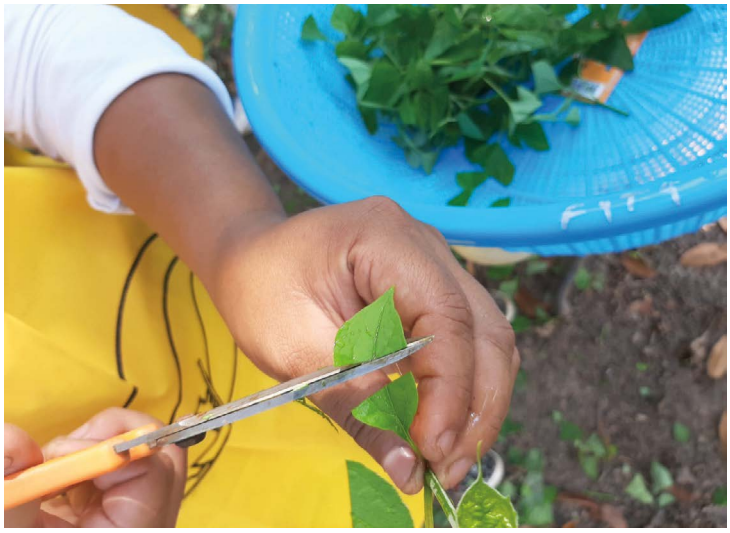

Figure 4. Sectioning process to obtain primary and secondary cuttings, after which 50% of the leaf part of each cutting obtained is removed.

Application of stimulant

For the establishment of the cuttings, the IBA growth hormone, the preparation of the hormone and its concentration of 3000, 4000 and 6000 mg kg^{-1} was applied (Figure 5); industrial talcum powder was mixed with indole-3-butyric acid (impregnable powder as active ingredient).

Later, the cuttings were impregnated with IBA, following the quick immersion method, which consists in the inferior part of the cutting being introduced into the impregnable powder; therefore, it is established in the cavities with inert substrate, and pressured to the margin of the inferior part of the cutting with the aim of avoiding mobility and eliminating air bags. Once the trial is finished, they are introduced into the area where they will remain

Figure 5. Application of AIB for stimulation and reaction of secondary and primary stake cofactors.

until starting the rooting and at the same time the temperature, irrigation and relative humidity is regulated and controlled.

RESULTS AND DISCUSSION

Table 2 shows the results from the ANOVA (Value of F and degree of significance) during the trial in minimum time of rooting and optimal concentrations of root stimulating hormones in southeastern Mexico, through primary and secondary cuttings of Melina (*Gmelina arborea Roxb*), where significant statistical differences were found between the treatments evaluated and the variables measured.

The variables that presented degrees of significance ($P<0.05$) were the presence of callus with a mean of 1.38 and number of roots, which indicates that the variable measured of IBA concentration did not affect the percentage of rooting, later reflected in the type of cutting used during the trial (Primary and Secondary). Table 3 shows that the results obtained of the variable percentage of rooting are higher than those found in the study conducted by Ruiz *et al.* (2005), who obtained 71.8% with concentrations of 1000 ppm to 2000 ppm.

Table 3 shows the means of the variables of each variable evaluated during the trial, according to the results shown in Table 2, and shows two groups in the variable number of roots where each concentration of IBA presents a mean of 4.66 (4000 ppm), 3.4286 (6000 ppm), and 3.2857 for concentration of 3000 ppm; this variable was significant.

Percentage of rooting

Table 4 shows the factor "Percentage of rooting" and the results from each concentration according to its level, which did not have an impact on the number of cuttings rooted, but did impact the length, something that should be highlighted. With the three concentrations of IBA implemented at the beginning of the period of rooting, an average of 84% of rooting was found for the concentration of 3000 ppm, and likewise it is homogeneous in the concentration of 6000 ppm; and then, 4000 ppm is higher than the previous with 92% of rooting. Villegas *et al.* (2017) reported a percentage of rooting of 98% on average, stressing 100% of rooting at 15 dae (days after established), in addition to the type of material implemented being apical (principal) cuttings.

Authors like Ovalle (2010) obtained a percentage of rooting of 100% in the species *Gmelina arborea* using three treatments (4000, 6000 and 8000 mg kg^{-1}), indicating that the factor that has most influence on rooting is the conditions within the rooting module.

Table 2. ANDEVA Results (F-value and degree of significance).

SOURCE OF VARIATION	CALLUS	VELLOS	STACA	ROOT	NO. OF ROOT	CM MAJOR	CM MINOR
Treatment	4,45	0,65	0,85	1,41	3,81	0,30	1,17
CV	32,14	171,14	35,61	29,57	94,04	85,68	133,93
Media	1,38	6,39	1,34	1,12	3,79	3,38	1,08
Significance	*	N.S	N.S	N.S	*	N. S	N. S

Degrees of significance: $P<0.05$= * Significant, $P<0.01$=** highly significant, N.S.=Not significant.

Table 3. Comparison of means in the minimum rooting times and optimum concentrations of root stimulating hormones with Melina.

COMPARISON OF AVERAGES	TREATMENT	MEDIA	HOMOGENEOUS GROUP
Callus	2	1.4524	A
	1	1.4286	A
	3	1.2637	B
Staca	3	1.3929	A
	1	1.3333	A
	2	1.2976	A
No.of root	1	4.6667	A
	2	3.4286	AB
	3	3.2857	B
CM Minor	2	1.2738	A
	3	0.9810	A
	1	0.9774	A
CM Major	2	3.5807	A
	1	3.3488	A
	3	3.2381	A
Vellos	2	7.4167	A
	1	6.2619	A
	3	5.5119	A
Presence of Root	3	1.1548	A
	1	1.1310	A
	2	1.0714	A

Number of roots/cm of roots

Table 2 shows the analysis of variance (ANOVA), where the factor number of roots obtained was significant, indicating that there is a concentration of IBA that causes the secondary and primary cuttings of the species *Gmelina arborea* to have a higher number of roots. Jovanovic *et al.* (2008), mentioned by Báez (2015), point out that the growth of the root is regulated by endogenous signals which contribute to the pattern of generation of new lateral roots. The analysis of roots of each treatment of the concentrations showed a fluctuation in 4000 and 3000 mg kg^{-1}, with a mean of three.

Table 4. Total number of cuttings, number of cuttings that obtained roots and percentage of rooting during the melina (*Gmelina arborea Roxb*) trial. By means of cuttings.

CONCENTRATION	NO. OF SEEDLINGS	MAIN STAKE	SECONDARY STAKE	ROOTED SEEDLINGS	UNROOTED SEEDLINGS	%
3000	84	56	28	71	12	84
4000	84	59	25	78	3	92
6000	84	51	33	71	12	84

Table 5 and Figure 6 to 9, show a mean of 3.0 cm and a maximum number of roots of 19, with a concentration of 6000 mg kg^{-1}, which explains the concentration applied to secondary and primary cuttings.

Table 5. Ratio of maximum, mean and minimum number of roots in each treatment during the melina (*Gmelina arborea Roxb*) trial. By means of stakes.

CONCENTRATION	MAXIMUM	MEAN	MINIMUM
3000	15	4	1
4000	10	3	1
6000	13	3	1

Figure 6. Visualization of axillary buds (a) secondary and lignified stake at 8 dde and root development (b) at 28 dde.

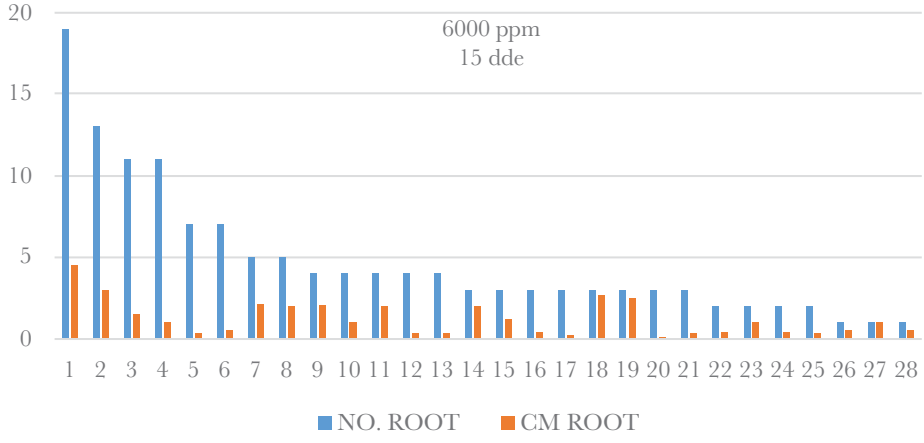

Figure 7. Graph of the relationship between the ratio of major and minor cm of roots during the test of minimum rooting times in melina (*Gmelina arborea Roxb*). Using AIB 6,000 ppm.

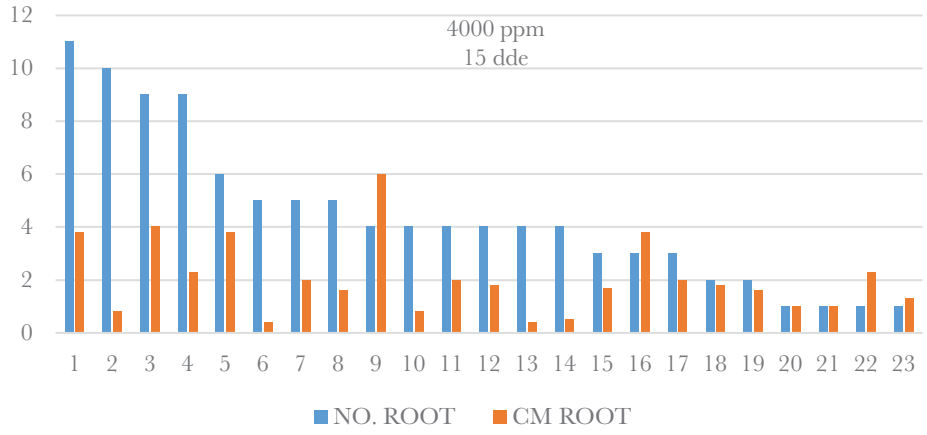

Figure 8. Graph the ratio of major and minor cm of roots during the test of minimum rooting times in melina (*Gmelina arborea Roxb*). Using AIB at 4,000 ppm.

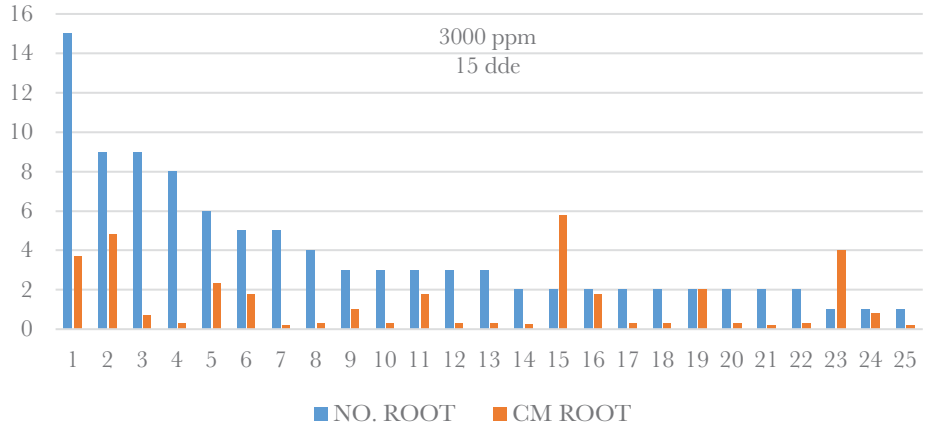

Figure 9. Graph the ratio of major and minor cm of roots during the test of minimum rooting times in melina (*Gmelina arborea Roxb*). Using AIB at 3,000 ppm.

CONCLUSIONS

The factors that intervene in the vegetative propagation of the species *Gmelina arborea* are of utmost importance, with cuttings being a technique of massive clonal propagation that ensures the quality of plants. The concentration of IBA in rooting generates good results in different concentrations based on the type of cutting implemented, with apical cuttings having better quality in the root system with less centimeters and formation of absorbent bloom. In the trial, the factors callus and number of roots obtained significant records between treatments; the use of IBA concentrations favors the acceleration of the presence of adventitious roots.

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