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Research article

Assessment of culture media in the *in vitro* multiplication of mahogany (*Swietenia macrophylla* King)

Evaluación de medios de cultivo en la multiplicación *in vitro* de caoba (*Swietenia macrophylla* King)

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Abstract

Swietenia macrophylla is a species of high economic value and has been subject to high timber extraction over decades. It is included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Cites). Its low germination rate and the lack of genotypic and phenotypic selection programs hinder its propagation, resulting in heterogeneous plantations. Thus, plant tissue culture is an option for multiplying forest species. In this study, Murashige and Skoog (MS) and Woody Plant Medium (WPM), both supplemented with 2 mg L^{-1} of 6-benzylaminopurine (BAP), were evaluated as a reproducible method to facilitate the multiplication of mahogany plants, focusing on shoot length and number of nodes. The results indicate that the interaction between the culture medium and the BAP concentration influences both variables. In MS medium, shoot length and the number of nodes decreased with increasing BAP concentration from 0 to 2 mg L^{-1}, while the opposite occurred in WPM medium. For multiplication (each node giving rise to a new plant), MS medium without growth regulators produced shoots of 11.18 mm with an average of 4.08 nodes, resulting in a multiplication rate of 4.08. WPM medium, on the other hand, produced shoots of 11.82 mm with an average of 3.29 nodes. These results suggest that *in vitro* multiplication via nodes (buds) in a medium without growth regulators is possible.

Keywords: Mahogany, culture media, micropropagation, Generalized Linear Model, multiplication, *Swietenia macrophylla* King.

Resumen

Swietenia macrophylla es una especie de alto valor económico con una alta extracción maderable a lo largo de décadas. Está incluida en el Apéndice II de la Convención sobre el Comercio Internacional de Especies Amenazadas de Fauna y Flora Silvestres (Cites). La baja tasa de germinación y la ausencia de programas de selección genotípica y fenotípica desafían su propagación, lo que deriva en plantaciones heterogéneas. Así, el cultivo de tejidos vegetales es una opción para la multiplicación de la especie. En este estudio se evaluaron los medios *Murashige* y *Skoog* (MS) y *Woody Plant Medium* (WPM), ambos suplementados con 2 mg L^{-1} de 6-bencilaminopurina (BAP) como método reproducible que facilite la multiplicación de plantas de caoba, sobre las variables longitud de brote y número de nudos. Los resultados indicaron que la interacción entre el medio de cultivo y la concentración de BAP tuvieron incidencia en ambas variables. En el medio MS, la longitud de brote y la cantidad de nudos disminuyeron al pasar de 0 a 2 mg L^{-1} de BAP, y con WPM ocurrió lo contrario. Para obtener multiplicación (cada nudo da origen a una nueva planta), el medio MS sin reguladores de crecimiento generó brotes de 11.18 mm con 4.08 nudos en promedio; se generó una tasa de multiplicación de 4.08, mientras que el medio WPM dio origen a brotes de 11.82 mm con 3.29 nudos en promedio. Estos resultados permiten multiplicar a *S. macrophylla in vitro* a través de nudos (yemas) en medio sin reguladores de crecimiento.

Palabras clave: Caoba, medios de cultivo, micropropagación, Modelo Lineal Generalizado, multiplicación, *Swietenia macrophylla* King.

Introduction

Swietenia macrophylla King, known as mahogany, is a monoecious tree belonging to the Meliaceae family, which can reach up to 60 m in height (Bakewell-Stone, 2023; Ochoa-Gaona et al., 2008; Pennington, 2002). It is naturally distributed throughout Tropical America, from the *Yucatán* Peninsula in Mexico to the Amazon rainforests of Brazil, Peru and Bolivia. It has a narrow crown that retains its leaves for most of the dry season, making it suitable for intercropping with crops such as coffee, cacao, vanilla and grassland (Corea-Arias et al., 2020; de Souza-Schottz et al., 2007; Maruyama et al., 1997).

It is a highly valued forest species for its high-quality, hard, and durable wood. Its economic importance makes mahogany the most relevant species among those native to Latin America (Campos-Ruiz et al., 2020). According to the State of Plant Conservation in Costa Rica study (Estrada, 2005), it is classified as critically endangered and is included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Cites) due to the high rate of timber exploitation caused by intensive extraction over decades (Convención sobre el

Comercio Internacional de Especies Amenazadas de Fauna y Flora Silvestres [Cites], 2017; Rojas-Vargas & Hine-Gómez, 2019).

Furthermore, because it is a species whose seeds lose viability quickly, it exhibits low germination rates (10 to 70 %), which reduces its sexual propagation (Quinto et al., 2009; Sampayo-Maldonado et al., 2021). Furthermore, the lack of selection trials based on phenotypic and genotypic characteristics results in highly heterogeneous plantations (Prado et al., 2010).

The use of *in vitro* culture techniques in reforestation programs is a viable alternative for plant production and species conservation, which can reduce deforestation pressure, since, in principle, it allows for the rapid propagation of elite individuals and genetically stable plant material from nodal segments (Delgado et al., 2008; Uribe et al., 2008).

Several protocols have evaluated the *in vitro* multiplication of mahogany through direct organogenesis. Tacoronte et al. (2004) reported the greatest stimulation of longitudinal growth in 25 mm mahogany shoots when using modified Murashige and Skoog (MS) medium supplemented with 2.56 mg L^{-1} of 6-benzylaminopurine (BAP) and 0.44 mg L^{-1} of naphthaleneacetic acid (NAA). Collado et al. (2004), working with different concentrations of BAP in the same medium, noted that shoot length increased as the concentration of this growth regulator decreased or was eliminated. In contrast, Carranza-Patiño et al. (2013) found no significant differences in shoot length when evaluating different combinations of BAP and indole-3-butyric acid (IBA), also in modified MS medium.

Subsequent studies have employed other culture media and hormonal combinations that have also shown varying effects on shoot length. Campos-Ruiz et al. (2020) found that the use of 1 mg L^{-1} of BAP in Woody Plant Medium (WPM) promoted the formation of longer shoots, reaching 8 mm. Mona (2012) obtained the greatest shoot length (39 mm) using MS medium supplemented with 2 g L^{-1} of activated carbon (AC), 4 mg L^{-1} of BAP, and 0.4 mg L^{-1} of 2ip (6-γ-γ dimethyl-1-allylaminopurine).

Due to the difficulties of sexual propagation of *S. macrophylla*, its high economic value, the high rate of exploitation, and its inclusion in Appendix II of Cites, it is a priority to develop efficient strategies that contribute to its conservation. Unlike previous research that has focused its efforts on generating shoots by testing different concentrations of BAP or hormonal combinations, this study proposes a direct comparison between MS and WPM media with a dose of 2 mg L^{-1} of BAP on the variables shoot length and number of nodes, as a reproducible strategy to facilitate the multiplication of mahogany plants through *in vitro* culture techniques, via direct organogenesis, for conservation and improvement purposes

Materials and Methods

The initial plant material consisted of 90 seeds from a composite sample of 15 *S. macrophylla* trees, collected in the *Triunfo de Madero ejido*, *Cintalapa* municipality, state of *Chiapas*. The preparation of this material, as well as the experiment, were carried out at the Forest Biotechnology Laboratory of the *Centro Nacional de Investigación Disciplinaria en Conservación y Mejoramiento de Ecosistemas Forestales* (National Center for Disciplinary Research in Conservation and Improvement of Forest Ecosystems), located in the *Coyoacán* borough of Mexico City, from April 19th to October 17th, 2024.

The seeds were deseeded and soaked in sterile water for 24 hours. Subsequently, the seeds underwent a disinfection process under aseptic conditions in a laminar flow hood. This process consisted of washing with Cascade® commercial liquid soap (5 mL L^{-1}) for 5 minutes, immersion in 50 % Merk® hydrogen peroxide for 10 minutes, and finally, immersion in Cloralex® 30 % (v/v) sodium hypochlorite for 30 minutes. After each step, continuous rinses were performed with sterile water.

Germination under aseptic conditions. The 90 seeds were sown in 100×10 mm flat-bottomed glass tubes, one seed per tube. Each tube contained 7 mL of MS medium supplemented with 1 mL L^{-1} of Plant Preservative Mixture (PPM, PhytoTech Labs®), 8.5 g L^{-1} of agar, and 30 g L^{-1} of sucrose, at a pH of 5.7. The tubes were sealed with plastic caps and parafilm tape. The tubes were taken to the incubation room under controlled conditions of temperature (25 °C), light intensity of 50 μmol m^{-2} s^{-1}, and a photoperiod of 16 hours of light.

Shoot induction. After 66 days of culture, the resulting plantlets were sectioned into nodal segments of 1 to 1.5 cm and subcultured in a 2×2 factorial design: factor 1, "culture medium," included MS and WPM levels, while factor 2, "BAP concentration," included 0 mg L^{-1} (control) and 2 mg L^{-1} levels; the latter determined by the authors in a previous experimental phase. Therefore, the resulting number of treatments was four (Table 1). The experimental unit consisted of one tube with one explant; in total, the experiment consisted of 62 tubes, as the number of replicates varied for each treatment.

Table 1. Description of factors and levels.

Factor 1	Factor 2	Treatment
MS	0	MS0
MS	2	MS2
WPM	0	WP0
WPM	2	WP2

MS = Murashige and Skoog; WPM = Woody Plant Medium.

Shoot growth. Explants were cultured for 66 days, the time it took for the shoot to reach the top of the test tube, under the previously mentioned conditions. After this time, the following variables were evaluated in each experimental unit: shoot length, measured with a model HER-441 Steren® digital vernier caliper with a resolution of 0.2 mm, and number of nodes, counted from the base of the shoot. The corresponding

data were analyzed using a Generalized Linear Model considering a Lognormal distribution and an identity link function, which, according to Gbur et al. (2012) and Stroup (2015), presents the following predictor:

$$n_{z,jk} = \mu + \alpha_j + \beta_k + (\alpha\beta)_{jk}$$

Where:

$Z_{ijk}=log(Y_{ijk})$; $\eta_{Z,jk}=\mu_{Z,jk}=E[Z_{ijk}]$ and $\varphi=var[Z_{ijk}]$; Y_{ijk} = Shoot length or number of nodes in the i^{-th} experimental unit (tube) of the j^{-th} culture medium of the k^{-th} BAP concentration

μ = Mean value for the reference level.

α_j = Mean difference for the j^{-th} culture medium with the reference level

β_k = Mean difference for the k^{-th} BAP concentration with the reference level

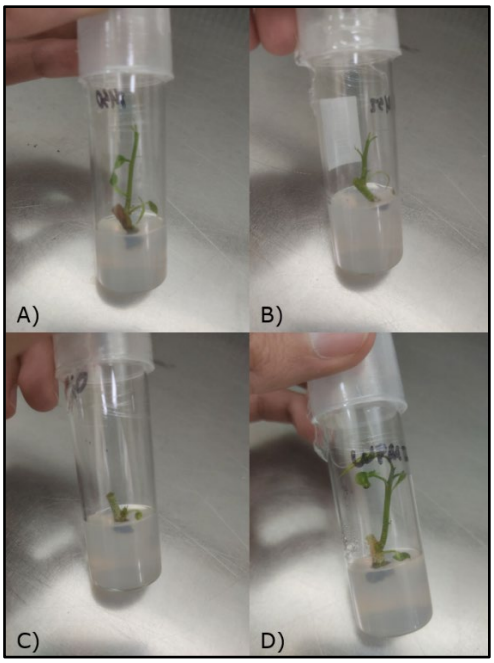
$(\alpha\beta)_{jk}$= Interaction between the j^{-th} culture medium and the k^{-th} BAP concentration

Data analysis, including residual modeling, was performed using PROC GLIMMIX of the Statistical Analysis System (SAS) software, version 9.4 (SAS Institute Inc., 2025). The variable type and its empirical probability distribution (right skewness) determined the statistical model in each case; furthermore, under these conditions, normality and the assumption of constant variances are not justified.

Results

Shoot length

The shoots formed in treatments MS0, WP2, and MS2 reached mean lengths of 13.85, 13.35, and 12.50 mm, respectively; while WP0 barely exceeded 5.60 mm (Figure 1). It was also observed that the greatest variability (with respect to the mean) occurred in MS2, which reached a Coefficient of variation greater than 85 %, while WP2 only approached 50 %. The other treatments had coefficients of variation between these extremes (Table 2).



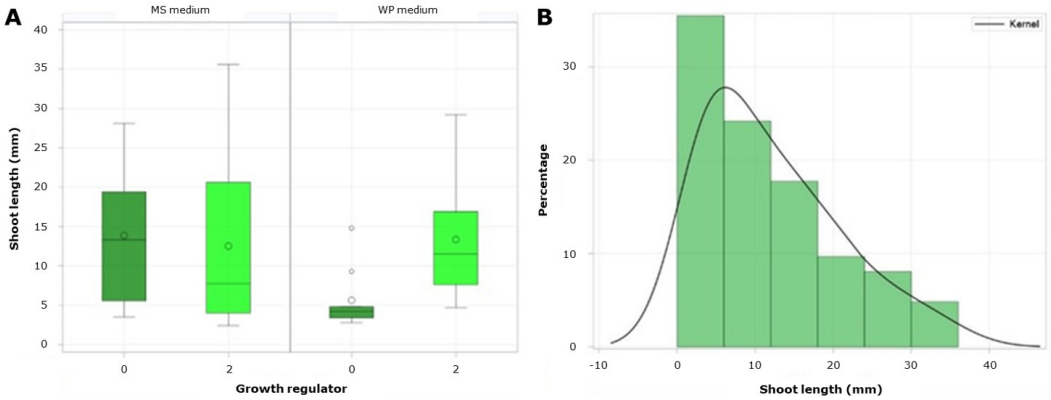
A = MS0; B = MS2; C = WP0; D = WP2.

Figure 1. Experimental unit per treatment for shoot length and number of nodes at 66 days.

Table 2. Descriptive statistics for the shoot length variable.

Treatament	Mean (mm)	Standard deviation (mm)	Coefficient of variation (%)
MS0	13.8500	8.3218	60.0850
MS2	12.5045	10.7079	85.6322
WP0	5.6333	3.9456	70.0397
WP2	13.3474	6.6456	49.7894

In three treatments, the segment above the interquartile range was significantly larger than the segment below it. In particular, the variability of WP0, reflected in its standard deviation, was quite low compared to the other treatments (Figure 2A). Even in this case, extreme values were observed. Therefore, neither normality nor homogeneity of variances is justified.



A = Variability by treatment; B = Empirical distribution of shoot length.

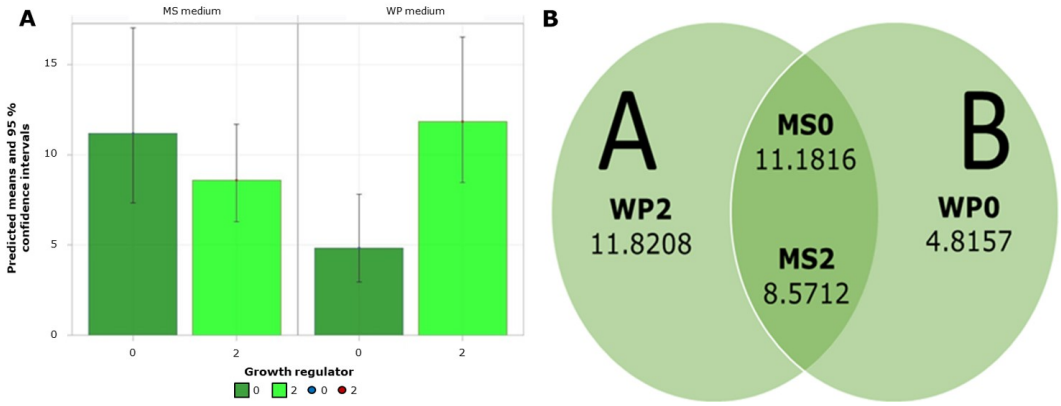
Figure 2. Dispersion and distribution of shoot length.

The most appropriate probabilistic model was identified as a Lognormal distribution with an *AICC* (Corrected Akaike Information Criterion) value of 149.49; this value corresponds to the lowest compared to other skewed continuous distributions of the exponential family. Shoot length is characterized by a skewed histogram, which coincides with the behavior of the Lognormal distribution (Figure 2B).

Under the postulated statistical model and with a significance level of 0.05, the ANOVA showed that the main effects of the factors "culture medium" and "BAP concentration" were not significant. However, the interaction between both factors was highly significant (F=8.76, p-value=0.0045) (Table 3). In fact, while in MS the mean decreases when moving from concentration 0 to 2, in WP the opposite occurs (Figure 3A).

Table 3. Analysis of variance for shoot length (Lognormal model).

Factors	*F* value	*Pr>F*
Medium	1.76	0.1904
Regulator	2.58	0.1134
Medi×Regu	8.76	0.0045



A = Predicted means and 95 % confidence intervals (mm); B = Venn diagram for treatment grouping by Tukey-Kramer.

Figure 3. Predictions and comparison of means based on the Generalized Linear Model for the shoot length variable.

The means predicted by the model, except for MS2, include the observed means. This may be due to high variability (Tables 2 and 4). Among the four treatments, WP2 stood out with a higher estimated mean (11.82 mm), followed by MS0 (11.18 mm), while the lowest value was recorded in WP0 (4.82 mm).

Table 4. Estimated Means and Confidence Limits (95 % confidence intervals) for shoot length (mm).

Treatment	Predicted mean	Standard error	*DF**	Predicted lower mean	Predicted higher mean
MS0	11.1816	1.2336	58	7.3460	17.0199
MS2	8.5712	1.1677	58	6.2848	11.6894
WP0	4.8157	1.2742	58	2.9647	7.8224
WP2	11.8208	1.1815	58	8.4654	16.5061

*Degrees of Freedom: total number of observations for the four treatments minus the number of estimated model parameters including the intercept, *i. e.*, 62-4=58. The total of 62 is derived from considering the number of observations for the treatments: MS0=12, MS2=22, WP0=9, WP2=19.

The Tukey-Kramer multiple comparison test (a=0.05) identified two general non-disjoint groups (Table 5). The first group (A) consists of treatments WP2, MS0, and MS2, while the second group (B) consists of MS0, MS2, and WP0; consequently, WP2 and WP0 were statistically different (Figure 2B).

Table 5. Comparison of Tukey-Kramer treatments for shoot length (mm).

Treatment	Predicted mean	Group	
WP2	11.8208		A
MS0	11.1816	B	A
MS2	8.5712	B	A
WP0	4.8157	B	

The normality analysis of the Pearson residuals, considering the Kolmogorov-Smirnov and Cramer-von Mises statistics, indicated that they have a normal distribution (mean 0 and standard deviation of 0.9751). In both cases, the p-value was greater than

0.05. Likewise, the histogram of the residuals, resulting from the postulated statistical model, was symmetrical (Figure 4).

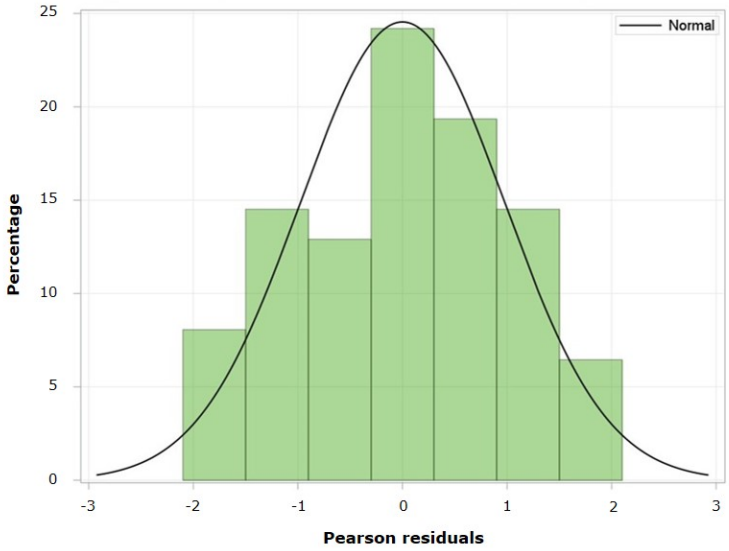


Figure 4. Pearson residual histogram for shoot length.

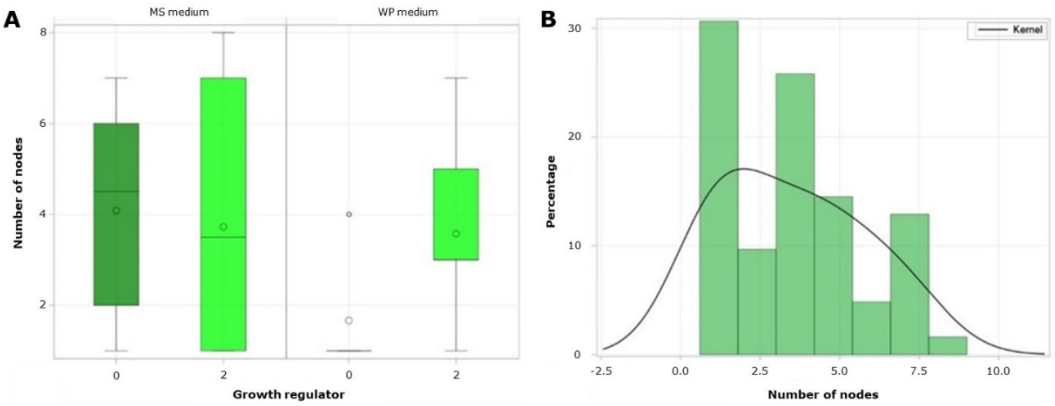
Number of nodes

For the number of nodes variable, treatment MS0 registered the highest value (4.08 nodes), while WP0 had the lowest (1.67 nodes) (Figure 1). The most significant variability was observed in WP0 with a *CV* of 79.37 %, and the lowest in WP2 (39.86 %) (Table 6). In this case, MS2 ranked second in terms of variability. Likewise, WP2 achieved a Coefficient of variation of almost 40 %

Table 6. Descriptive statistics for the number of nodes variable.

Treatment	Mean (mm)	Standard deviation (mm)	Coefficient of variation (%)
MS0	4.0833	2.2344	54.7193
MS2	3.7273	2.6936	72.2670
WP0	1.6667	1.3229	79.3725
WP2	3.5789	1.4266	39.8599

Figure 5A shows that WP0 presents values concentrated below 1, with outliers around 4 nodes. Treatments MS0 and MS2 showed an interquartile range (*IQR*) of 4 and 6, respectively, while WP2's was 2. Since this variable has an asymmetric distribution, the most appropriate model was also the Lognormal, with an *AICC* value of 266.68 (Figure 5B).



A = Variability by treatment; B = Empirical distribution.

Figure 5. Dispersion and distribution of the number of nodes.

Statistical analysis shows that the main factors did not have significant effects. However, the interaction between culture medium and BAP concentration was significant at a 5 % confidence level (F=8.52, p-value=0.0050) (Table 7).

Table 7. Analysis of variance for number of nodes (Lognormal Model).

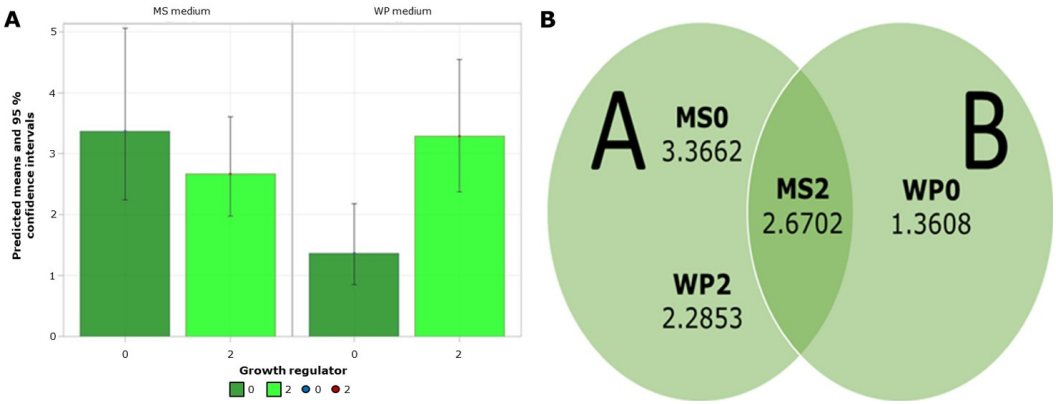
Factors	*F* value	*Pr>F*
Medium	3.35	0.0722
Regulator	2.90	0.0938
Medi×Regu	8.52	0.0050

The predicted means by treatment are shown in Table 8. MS0 and WP2 stand out for having means greater than 3 nodes, with MS2 having a value greater than 2.5; while WP0 did not reach 1.5 (Figure 6A). These findings demonstrate that when WP0 does not include a growth regulator, the number of nodes is also affected.

Table 8. Estimated means and confidence limits (95 % confidence intervals) for number of nodes.

Treatment	Predicted mean	Standard error	*DF**	Predicted lower mean	Predicted upper mean
MS0	3.3662	1.2257	58	2.2397	5.0593
MS2	2.6702	1.1622	58	1.9763	3.6077
WP0	1.3608	1.2650	58	0.8501	2.1783
WP2	3.2853	1.1756	58	2.3766	4.5416

*Degrees of Freedom: total number of observations of the four treatments minus the number of estimated model parameters including the intercept, *i. e.*, 62-4=58. The total of 62 arises from considering the number of observations of the treatments: MS0=12, MS2=22, WP0=9, WP2=19.



A = Predicted means and 95 % confidence intervals; B = Venn diagram for treatment grouping by Tukey-Kramer.

Figure 6. Predictions and comparison of means based on the Generalized Linear Model for the variable number of nodes.

The multiple comparison analysis using Tukey-Kramer (α=0.05) showed two significantly different groups (Table 9). MS0 and WP2 form group A, while WP0 belongs to group B. Treatment MS2 is present in both groups (Figure 6B).

Table 9. Tukey-Kramer comparison of treatments for number of nodes.

Treatament	Predicted mean	Group	
MS0	3.3662		A
WP2	3.2853		A
MS2	2.6702	B	A
WP0	1.3608	B	

Under the statistical model considered, the distribution of the residuals tends toward normality, with a mean of 0 and a standard deviation of 0.9751. Thus, the *p*-value of the Kolmogorov-Smirnov statistic was 0.034, while the corresponding Cramer-von Mises statistic exceeded 0.05 (0.074) (Figure 7).

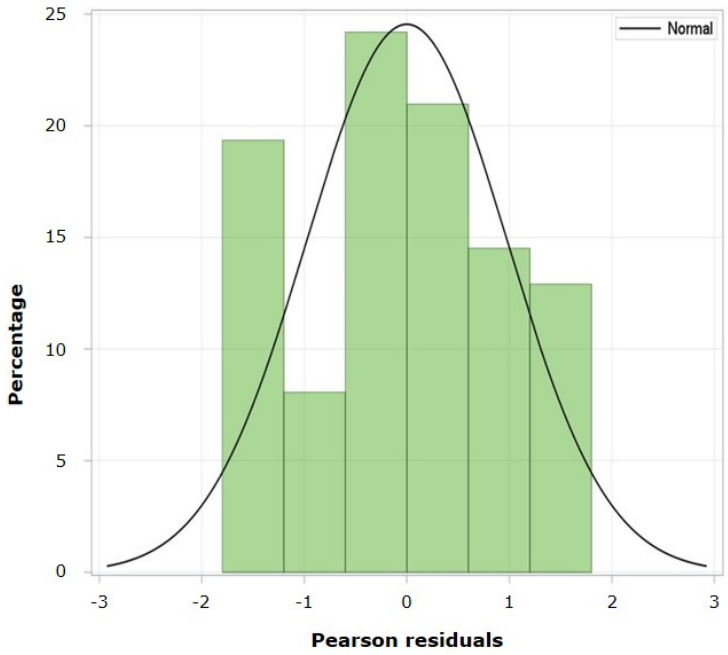


Figure 7. Histogram of Pearson residuals for number of nodes.

Discussion

In contrast to previous research that has focused on shoot generation, this study focuses on shoot length and number of nodes, considering that nodes constitute a source of meristematic tissue (Akin-Idowu et al., 2009), which allows for the medium- and long-term preservation of explants. This characteristic is key to establishing and maintaining an *in vitro* multiplication program. Furthermore, each node represents a potential shoot capable of generating a new plant, enabling exponential multiplication (Putri et al., 2022) beyond the traditional approach focused solely on shoot production.

The results of this study show clear differences between WPM medium with 0 mg L^{-1} and 2 mg L^{-1} of BAP, both in shoot length and number of nodes; the 2 mg L^{-1} concentration yields higher values for both variables. Regarding this, Collado et al.

(2004) and Mona (2012) agree that cytokinins, such as BAP, exert relevant physiological effects by influencing cell division and growth. These findings reinforce the importance of the culture medium and the appropriate use of cytokinins in shoot growth, as noted by Campos-Ruiz et al. (2020), who emphasize that organogenesis is the result of the interaction between the plant material (explant), the culture medium, and the phytohormones that play the main role.

On the other hand, in MS medium, shoot length and the number of nodes decreased as the BAP concentration increased from 0 to 2 mg L^{-1}, which coincides with the findings reported by Collado et al. (2004), who observed an increase in shoot length as the BAP concentration was reduced or eliminated. Conversely, Mona (2012) recorded a greater shoot length (28 mm) with 2 mg L^{-1} of BAP; however, the shoots exhibited hyperhydricity.

Furthermore, in the present study, MS medium, even in the absence of BAP, promoted a greater number of nodes and greater shoot elongation compared to WPM medium without growth regulators. It is worth noting that the MS medium has a higher concentration of nitrates (NO_3^-), ammonium (NH_4^+) and chloride (Cl^-) in relation to WPM, which can be used by the explants to carry out their physiological processes, thus favoring a more pronounced growth (Campos-Ruiz et al., 2020).

In a similar way, the differences observed between treatments could be related to the interaction between ion absorption and endogenous cytokinin levels. In this context, the absorption of nitrogen, phosphorus, and potassium ions can directly influence the plant's hormonal metabolism, indicating that endogenous cytokinins do not act in isolation, but rather their effect is modulated by the nutritional environment of the culture medium (de Souza-Schottz et al., 2007; Ramage & Williams, 2002).

Finally, Madrigal-Villalobos et al. (2025) indicate that *in vitro* propagation methods for mahogany have shown promise, with overcoming challenges such as low regeneration rates and optimizing media formulations being crucial for advancing commercial and conservation applications.

Conclusions

This study establishes a precedent for *in vitro* multiplication from nodes (axillary buds) in MS medium without growth regulators in mahogany (*S. macrophylla*), achieving a multiplication rate of 4.08 (buds per stem), outside the traditional approach of shoot generation, as a reproducible strategy using *in vitro* culture techniques. This approach contributes to the development of efficient protocols that promote the propagation and conservation of the species, minimizing the risk of somaclonal variation due to the use of growth regulators.

Acknowledgments

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Conflict of interest

The authors declare no conflict of interest.

Contribution by author

Kevin Aldahir Salas Salinas: planning and execution of laboratory work and writing of the manuscript; Carlos Román Castillo Martínez: writing and revision of the manuscript; Efraín Velasco Bautista: statistical data analysis, writing and revision of the manuscript.

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