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Cutting propagation technique of mahogany (*Swietenia macrophylla*) in microtunnels from the Peruvian Amazon

Técnica de propagación de esquejes de caoba (*Swietenia macrophylla*) en microtúneles de la Amazonía peruana

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ABSTRACT

Swietenia macrophylla is a forest species of great commercial value that is categorized as vulnerable in Peru. Therefore, the objective of this study is to optimize a protocol for cutting propagation of *S. macrophylla* using microtunnels in the San Martín, Peruvian Amazon. Three experiments were conducted: sterilization, which tested ethyl alcohol (EA), Tween-80 (T), carbendazim (CZ), and combinations; a rooting experiment with different substrates and doses of indole-3-butyric acid (IBA); and an acclimation experiment of rooted cuttings, with different shade coverage and relative humidity conditions. The lowest contamination of *S. macrophylla* cuttings (9.75 %) was achieved with the combined EA-CZ treatment; this treatment resulted in the lowest necrosis (9.1 %) and survival of 86.50 %. The best responses in rooting, root biomass, and cutting survival were presented by the combination of sterilized sand with 3,000 mg L^{-1} of IBA, with averages of 73.89 %, 0.036 g, and 2.22 cm, respectively. The best acclimation was obtained under 80 % shade and 60 % relative humidity, with an average survival rate of 91.67 %. The general results were successful; therefore, they could be a valuable tool for the rescue, conservation, and restoration of ecosystems with cloned *S. macrophylla* trees that are resilient to climate change.

Keywords: microtunnels, vegetative propagation, necrosis, cuttings survival.

RESUMEN

Swietenia macrophylla es una especie forestal de gran valor comercial, clasificada como vulnerable en Perú. Por esta razón, el objetivo de este estudio es optimizar un protocolo para la propagación de esquejes de *S. macrophylla* a través de microtúneles en San Martín, Amazonía Peruana. Se realizaron tres experimentos: esterilización, en el que se probaron alcohol etílico (EA), Tween-80 (T), Carbendazim (CZ) y combinaciones; un experimento de enraizamiento con diferentes sustratos y dosis de Ácido Indol Butírico (AIB); y un experimento de aclimatación de esquejes enraizados, con diferentes coberturas de sombra y condiciones de humedad relativa. La menor contaminación de esquejes de *S. macrophylla* (9,75 %) se logró con el tratamiento combinado EA-CZ; este tratamiento tuvo la menor necrosis (9,1 %) y una supervivencia de los esquejes del 86,50 %. Las mejores respuestas en enraizamiento, biomasa de raíces y supervivencia de esquejes las presentó la combinación de arena esterilizada con 3.000 mg L^{-1} de AIB con promedios de 73,89 %, 0,036 g y 2,22 cm, respectivamente. La mejor aclimatación se obtuvo con 80 % de sombra y 60 % de humedad relativa, con una supervivencia promedio de 91,67 %. Los resultados en general son exitosos; por lo tanto, podrían ser una herramienta valiosa para el rescate, conservación, y restauración de ecosistemas con árboles clonados de *S. macrophylla* resilientes al cambio climático.

Palabras clave: microtúneles, propagación vegetativa, necrosis, supervivencia de esquejes.

INTRODUCTION

Mahogany (*Swietenia macrophylla* King), which belongs to the Meliaceae family, is a tree of great economic interest worldwide. Its wood has good physical and mechanical characteristics for the manufacture of high-quality furniture and cabinets (Colares *et al*. 2016). However, deforestation and climate change are the main threats to this species (Herrera-Feijoo *et al.* 2023). *S. macrophylla* is a shade-tolerant tree found in the drylands of the Peruvian and Brazilian Amazon regions (Dolacio *et al*. 2020). It has a low natural population owing to the high demand for wood and its high commercial value (Herrera-Feijoo *et al.* 2023). Likewise, the way its management is practiced makes it more difficult to explore the species in natural forests, as they require better ecological management and silvicultural understanding (Free *et al.* 2017).

Swietenia macrophylla is one of the world's most commercially important plant species (Grogan *et al.* 2014). It is priced between USD $1,700 and USD $11,000 per m^3 of wood (Jhou *et al*. 2017). Its great commercial interest has increased its overexploitation (Myster 2021, Herrera-Feijoo *et al.* 2023). This species has been extensively exploited since the early 1970s and the 1980s, leading to a sharp decline in its population in naturally occurring areas (Degen *et al.* 2013). As a result, *S. macrophylla* has been included in the list of threatened species (CITES 2019). Likewise, in Peru, mahogany has been categorized as a vulnerable species since 2006 by Supreme Decree N°043-2006-AG (SERFOR 2019). Despite this categorization, very little or perhaps nothing has been done to generate technologies that enable its recovery and repopulation. This species is propagated commercially using seeds. However, few seed trees lower the quantity and quality of seeds, leading to limited production of mahogany plants. The natural populations of this species have been particularly affected by the overexploitation of large individuals, which prevents regeneration of the species because the seed sources are extracted. Most mahogany wood is obtained from natural populations in South and Central America, often through illegal extraction and trade, compromising the availability of its genetic resources (Whitmore 2003, Degen *et al.* 2013). Therefore, selective logging in tropical forests and poor propagation techniques have severely affected the recovery and sustainability of mahogany populations.

S. macrophylla is a tree species that is spread by seeds. However, they usually lose their viability in a short time, which means that alternatives that allow trees to be propagated in quantity and quality of seedlings throughout the year without depending on seeds are sought (Herrera-Feijoo *et al.* 2023). All the above-mentioned reasons highlight the necessity of vegetative propagation of high commercial value tree species from cuttings and making use of rooting microtunnels under aseptic conditions, the purpose of which is to produce cloned plants with similar characteristics to the selected plant free of diseases (Mohammed *et al.* 2022). Microtunnels play an essential role in plant propagation, as shown in several previous studies (Vallejos-Torres *et al.* 2020, Vásquez-Zamora *et al.* 2022). This technique employs substrates and auxins such as indole-3-butyric acid (IBA) (Solís *et al.* 2019, Vallejos-Torres *et al.* 2021). The optimization of plant propagation technology reduces production costs without compromising quality (Vallejos-Torres *et al.* 2020).

Clonal propagation proposes the prospects, changes, and opportunities to generate a reliable and sufficient supply of better-quality planting material locally in a timely and rapid manner (Azad & Matin 2015). Several tropical tree species have been successfully propagated using branch cutting (Solis *et al*. 2019, Vallejos-Torres *et al*. 2021, Vásquez-Zamora 2022). However, few technologies have been developed for the propagation of *Swietenia macrophylla*. Therefore, it is evident that there is a need to establish a vegetative propagation protocol for mahogany that will not only aid in maintaining qualitative and quantitative traits, but also create a platform for more efficient plant breeding and propagation programs (Farooq *et al.* 2021). To diversify technologies to spread the species for production purposes, the present study aimed to optimize a protocol for vegetative propagation using the cutting technique of mahogany (*Swietenia macrophylla*) in microtunnels in the Peruvian Amazon.

METHODS

Study site. The present study was conducted in the nursery of the Centro de Investigación e Innovación Agroforestal, CIIA, located in the city of Tarapoto, province, and the region of San Martin, between January and April 2023. The area is located at 06° 35' 28″ S and 76° 18' 47″ W, at an altitude of 230 m a.s.l., with an average temperature and monthly precipitation of 24.6 °C and 63.1 mm, respectively, and an average relative humidity of 45 %.

Sterilization and establishment of vegetative material. Actively growing shoots (10 cm) of *S. macrophylla* were collected from a clonal garden established at CIIA under greenhouse conditions (figure 1A). This vegetative material originates from seedlings propagated from different seed trees originating from the San Martin region, Peru, and has outstanding characteristics of straightness, health, crown type, and stem quality. The clonal hedge is located inside a greenhouse with controlled environmental conditions, such as relative humidity, temperature, sun radiation, and fertilization. The collected material was cut and reduced to a length of 7 cm with a leaf area of 50 cm^2. This experiment consisted of 2 × 2 × 2 factorial arrangements with the application of ethyl alcohol (EA) for 10 min (0 and 70 %), addition of Tween-80 (T) for 10 min (0 and 20 drops L^{-1}), or addition of carbendazim (CZ) for 5 min (0 and 0.01 %) (Farooq *et al.* 2021), arranged in a completely randomized design with ten replications (10 cuttings) per experimental unit, for a total of

80 cuttings, and containing sterilized medium sand as a substrate installed in microtunnels with controlled temperature and relative humidity conditions. The following eight combinations were used in the experimental setup: control (CT), CZ, T, T-CZ, EA, EA, CZ, EA-T, and EA-T-CZ. Contamination, necrosis, and survival percentages of the explants were recorded 35 days after the experiment was established.

Substrates and IBA concentration in adventitious rooting. Rooting was carried out in a 108 m^2 greenhouse area, distributed in three micro-tunnels constructed with galvanized metal covered with white transparent plastic that allowed the passage of diffuse sunlight (figure 1B). The microtunnels have three nebulizers to prevent desiccation and water stress, operate with automated irrigation/cooling, maintain relative humidity above 75 %, and maintain temperature between 28 and 35 °C. Automated irrigation allows 30-second watering every 4 h (from 9:00 am to 5:00 pm) with a frequency of 3 times day^{-1}, supplying a total of 3.6 liters of water day^{-1} per microtunnel.

This experiment presented a 2 × 6 factorial arrangement in a completely randomized design with 12 replications, using 10 cuttings per experimental unit. The substrates used for rooting cuttings of *S. macrophylla* were I) white medium sand (SMS) collected from the road quarry, sieved through a 2 mm sieve, washed with abundant water and sodium hypochlorite (diluted in 2 % sterile water), rinsed four times, sun-dried, and placed in an autoclave for sterilization at 131 °C × 15 lb. pressure for 2 h, and ii) specialized substrate (Jiffys) (SS) (Solis *et al*. 2019). Six concentrations of auxin (0; 1,000; 2,000; 3,000; 4,000; and 5,000 mg L^{-1}). The following twelve combinations were used in the experimental setup: SMS, SS, SMS -1,000, SS -1,000, SMS -2,000, SS-2,000, SMS -3,000, SS-3,000, SMS -4,000, SS-4,000, SMS -5,000, and SS-5,000. After IBA application, 7 cm long cuttings with 50 cm^2 of leaf area 50 cm^2 were allowed to stand for 15 s to allow alcohol volatilization and were then deposited in growth trays (52 cm × 28 cm; 180 g) containing the respective substrates. After 32 days, the percentage of rooting, root biomass, root length, and survival of the cuttings were recorded (figure 1C).

Acclimation of rooted cuttings. Rooted cuttings were extracted from the microtunnels and transferred to 500 mL plastic pots for acclimatization purposes (Vallejos-Torres *et al.* 2021); these pots contained an agricultural soil substrate mixed with sand (3:1) (figure 1D). This experiment presented a 2 × 3 factorial arrangement (shade cover and relative humidity conditions) in a completely randomized design with 12 cuttings per treatment, for a total of 72 cuttings. The levels for each factor were medium shade at 50 % (MS), high shade at 80 % (HS), low relative hu-

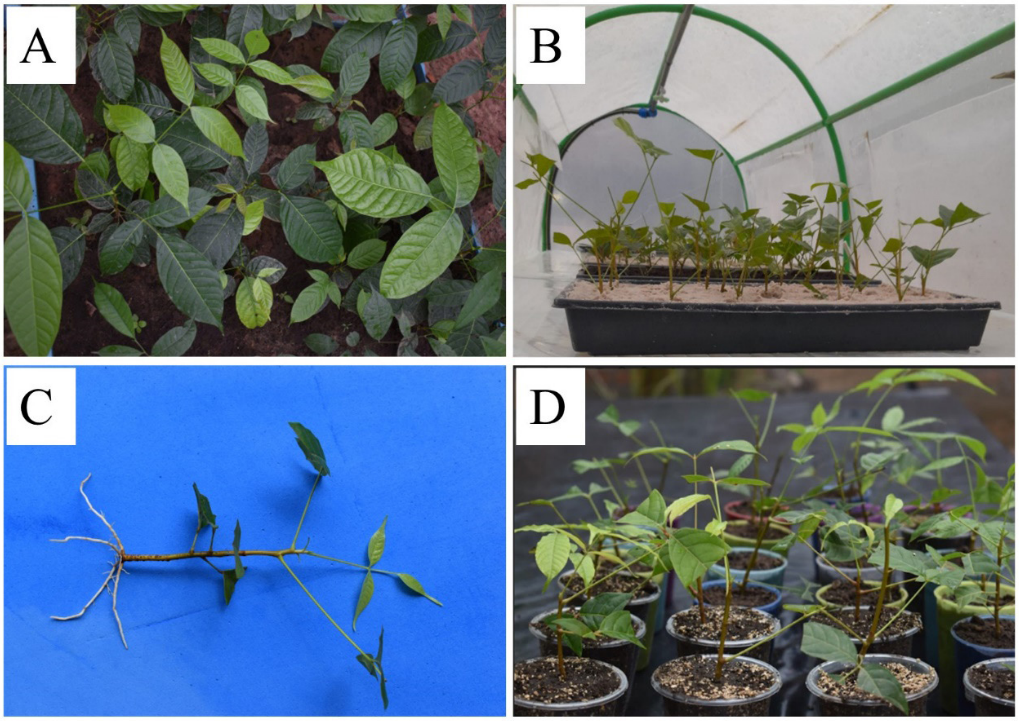

Figure 1. Optimized protocol for vegetative propagation of *S. macrophylla* in microtunnels. A) Clonal mini hedge; B) Microtunnel rooting; C) Rooted shoot; and D) Acclimation of rooted mahogany shoots.

Protocolo optimizado para la propagación vegetativa de *S. macrophylla* en microtúneles: A) Mini seto clonal; B) Enraizamiento en microtúnel; C) Brote enraizado; y D) Aclimatación de brotes enraizados de caoba.

midity at 30 % (LRH), medium relative humidity at 60 % (MRH), and high relative humidity at 80 % (HRH), using the following six combinations in the experimental setup: MS-LRH, MS-MRH, MS-HRH, HS-LRH, HS-MRH, and HS-HRH. Relative humidity was maintained in a nursery environment with sprinkler irrigation three–five times per day to maintain the turgor of the cuttings. After 90 d, the percentages of survival and shoot length were recorded.

Statistical analyses. For sterilization, rooting, and acclimation experiments of rooted cuttings, the normality and homoscedasticity of the data were tested using Shapiro-Wilk and Breush-Pagan tests ($P < 0.05$). At each stage of the process, the effects of the factors and their interactions on the response variables were analyzed. For this purpose, all data were subjected to an analysis of variance (ANOVA) and Duncan's mean comparison test with a 5 % probability of error. All analyses were performed using the R Studio (R Core Team 2023). Before analysis, the number of roots was transformed using ecuation [1]. Likewise, the contamination, necrosis, rooting, and survival percentages of the cuttings were transformed using ecuation [2], where x represents the response variables.

$$\sqrt{x+1} \qquad [1]$$

$$arcsen\ \sqrt{x} \qquad [2]$$

RESULTS

Sterilization and establishment of the propagules. Table 1 shows that treatments with EA, T, CZ, and a combination of EA-T and EA-CZ significantly influenced the contamination of mahogany cuttings. In contrast, EA, T, and EA-T treatments significantly influenced the necrosis of these explants. Almost all treatments significantly influenced survival, except treatment T (table 1). The lowest contamination of the mahogany cuttings was achieved with the combined EA-CZ treatment, with an average of 9.75 %. In comparison, the highest contamination was observed in the control treatment (CT), with a value of 29.13 %, showing significant differences between the treatments (figure 2A). Similar results were obtained for the necrosis indicator, where the lowest percentage (9.1 %) was achieved with the combined EA-CZ treatment. In contrast, the highest contamination was observed in the CT treatment (23.33 %), showing significant differences between treatments (figure 2B). Higher survival of mahogany cuttings was achieved with EA-CZ treatment, with an average of 86.50 %. In contrast, a lower survival rate was observed with CT treatment (43.25 %), showing significant differences (figure 2C).

Substrates, IBA concentration, and microtunnel environment for adventitious rooting. Table 2 shows that the rooting medium contributed significantly to root biomass. At

Table 1. ANOVA multifactorial effect of sterilant types on the vegetative propagation of mahogany.
Efecto multifactorial ANOVA de los tipos de esterilizantes en la propagación vegetativa de la caoba.

Factors		Variables evaluated		
		Contamination (%)	Necrosis (%)	Survival (%)
EA	*P*	< 0.001***	< 0.001***	< 0.001***
	F	346.627	682.243	138.397
T	*P*	0.048*	< 0.001***	0.817 ns
	F	4.064	18.203	0.054
CZ	*P*	< 0.001***	0.067 ns	0.001**
	F	44.100	3.446	11.432
Interaction EA x T	*P*	< 0.001***	< 0.001***	< 0.001***
	F	119.025	81.386	163.409
Interaction EA x CZ	*P*	0.027*	0.974 ns	< 0.001***
	F	5.077	0.001	25.021
Interaction T x CZ	*P*	0.555 ns	0.948 ns	< 0.001***
	F	0.352	0.004	16.992
Interaction EA x T x CZ	*P*	1.000 ns	0.245 ns	0.003**
	F	0.000	1.375	9.344

F: Calculated; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: $P > 0.05$. Factors: EA: Ethyl alcohol for 10 min (0 and 70 %), T: Tween-80 (0 and 20 drops L^{-1}), CZ: Carbendazim (0 and 0.01 %).

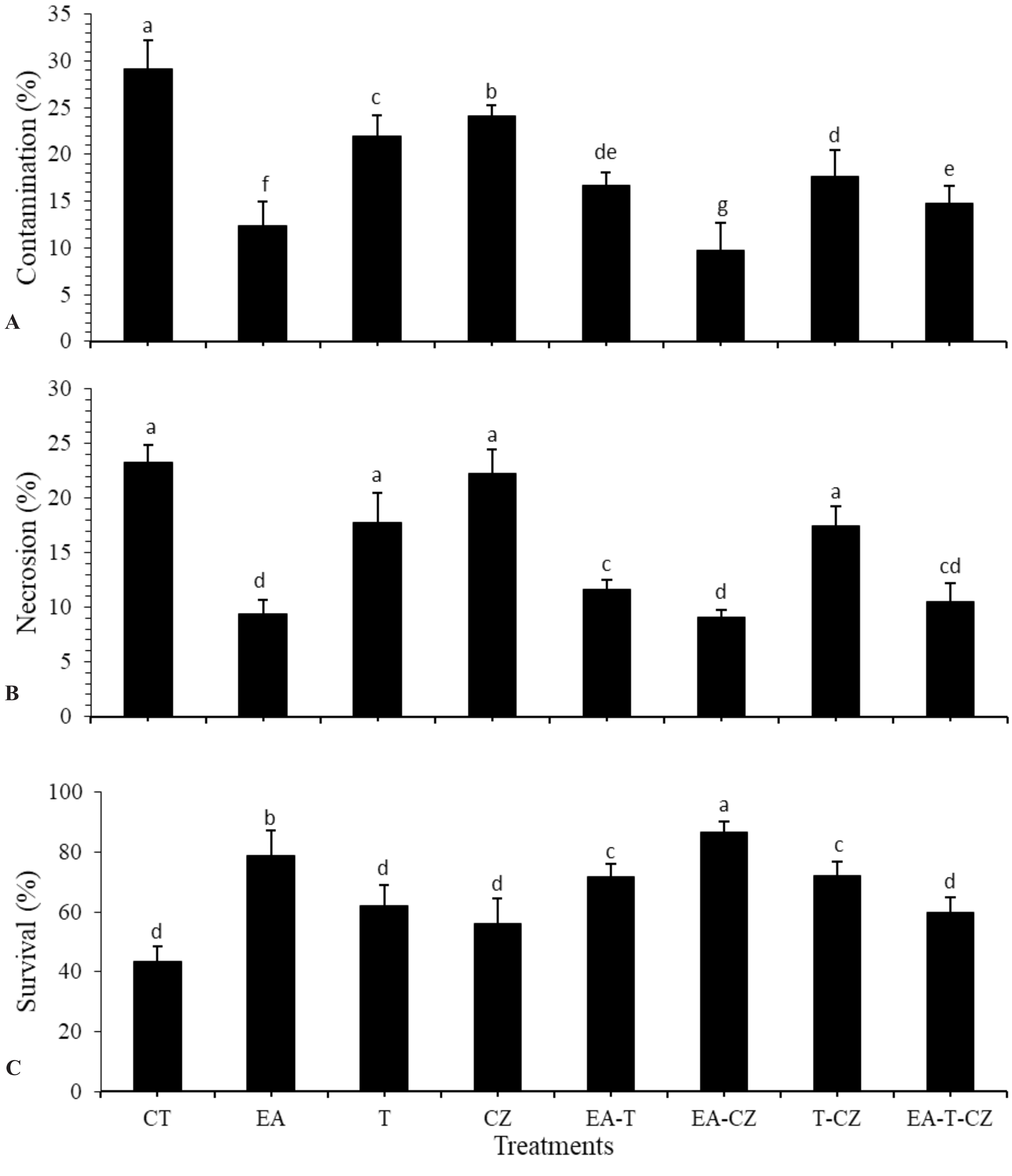

Figure 2. Comparison (after 35 days) of means for sterilization of cuttings with application of Ethyl Alcohol (EA: 0 y 70 %), Tween 80 (T: 0 y 20 gotas L^{-1}) and Carbendazim (CZ: 0 y 0.01 %); A) Contamination; B) Necrosis and C) Survival. Letters above bars indicate significant differences according to Duncan's test ($P < 0.05$).

Comparación (después de 35 días) de medias para la esterilización de esquejes con aplicación de Alcohol Etílico (EA: 0 y 70 %), Tween 80 (T: 0 y 20 gotas L^{-1}) y Carbendazim (CZ: 0 y 0,01 %); A) Contaminación; B) Necrosis y C) Supervivencia. Las letras sobre las barras indican diferencias significativas según la prueba de Duncan ($P < 0,05$).

the same time, the IBA treatment significantly influenced all the variables studied. The same was true for the interaction between the rooting medium and IBA (table 2). These results confirmed a strong influence of the two factors on the rooting capacity of mahogany cuttings.

In this sense, the best rooting treatment of mahogany cuttings was the interaction of 3,000 mg L^{-1} and 2,000 mg L^{-1} IBA with 73.89 and 67.22 %, respectively. The substrate with the lowest rooting percentage (32.78 %) was SMS without IBA application (figure 3A). Similar results were obtained for the root biomass of the cuttings, which showed significant differences among the five treatment groups (figure 3B). Cuttings treated with SMS and 3,000 mg L^{-1} IBA and 2,000 mg L^{-1} IBA presented the highest root biomass values of 0.035 g and 0.034 g, respectively (figure 3B). Likewise, the highest root length was obtained with the treatment of sand with 3,000 mg L^{-1} and 2,000 mg L^{-1} of IBA, with an average of 2.22 and 2.14 cm, respectively, showing significant differences with the treatment with sand without IBA application, which showed a value of 0.53 cm (figure 3C). The highest cutting survival (73.89 %) was observed with the specialized substrate and 3,000 mg L^{-1} IBA application, with significant differences compared with the other treatments (figure 3D).

Acclimation of the propagules. Table 3 shows significant differences between shade and relative humidity in the survival of mahogany cuttings in the acclimatization process (table 3). In this sense, the best survival during the acclimatization process of the rooted mahogany cuttings was achieved with 80 % shade and 60 % relative humidity, with an average of 91.67 % (HS-MRH). The treatment with 50 % shade and 30 % relative humidity with an average of 41.67 % (MS-LRH) presented the lowest percentage of survival in acclimatization, showing significant differences between treatments (figure 4A). The highest shoot length was achieved with HS-MRH (80 % shade and 60 % relative humidity), with an average of 3.57 cm. In comparison, the shortest length of cuttings was presented by the MS-LRH treatment (80 % shade and 30 % relative humidity) with 1.06 cm, showing significant differences between treatments (figure 4B).

Table 3. Multifactorial ANOVA showing the effects of cover types and humidity conditions on the vegetative propagation of mahogany.

ANOVA multifactorial mostrando los efectos de los tipos de cubierta y las condiciones de humedad sobre la propagación vegetativa de la caoba.

Factors		Variables evaluated	
		Survival (%)	Shoot length (cm)
S	*P*	0.007**	< 0.001***
	F	15.976	92.213
H	*P*	0.034*	0.004**
	F	6.246	13.000
Interaction S x H	*P*	0.111 ns	0.005**
	F	3.246	12.148

*: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: $P > 0.05$. Factors: S: Shade cover (50 and 80 %) and H: Relative humidity (30, 60 %, and 80 %).

DISCUSSION

Sterilization and establishment of the propagules. It is essential to select the optimum explant for the success of a mahogany propagation protocol, especially with higher cleanliness, that is, with high survival and low contamination and necrosis of the cutting. The findings of this study show that treatment with ethyl alcohol (70 %) and a fungicide (carbendazim 0.01 %) efficiently resulted in higher sterilization in *Swietenia macrophylla* cuttings. Alcohols

Table 2. ANOVA multifactorial effect of substrates and IBA concentrations on the vegetative propagation of mahogany.

Efecto multifactorial ANOVA de sustratos y concentraciones de IBA en la propagación vegetativa de la caoba.

Factors		Variables evaluated			
		Rooting (%)	Radicular biomass (g)	Root length (cm)	Survival of cuttings (%)
RM	*P*	0.199 ns	0.041*	0.292 ns	0.346 ns
	F	1.743	4.729	1.114	0.926
IBA	*P*	< 0.001***	< 0.001***	< 0.001***	< 0.001***
	F	15.078	29.479	42.430	31.550
Interaction RM x IBA	*P*	0.019*	0.002**	< 0.001***	0.012*
	F	3.357	7.443	13.506	3.847

F: Calculated; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; ns: $P > 0.05$. Factors: RM: Rooting Medium (sand and jiffy) and IBA: Indole-3-Butyric Acid (0, 1,000, 2,000, 3,000, 4,000, and 5,000 mg L^{-1}).

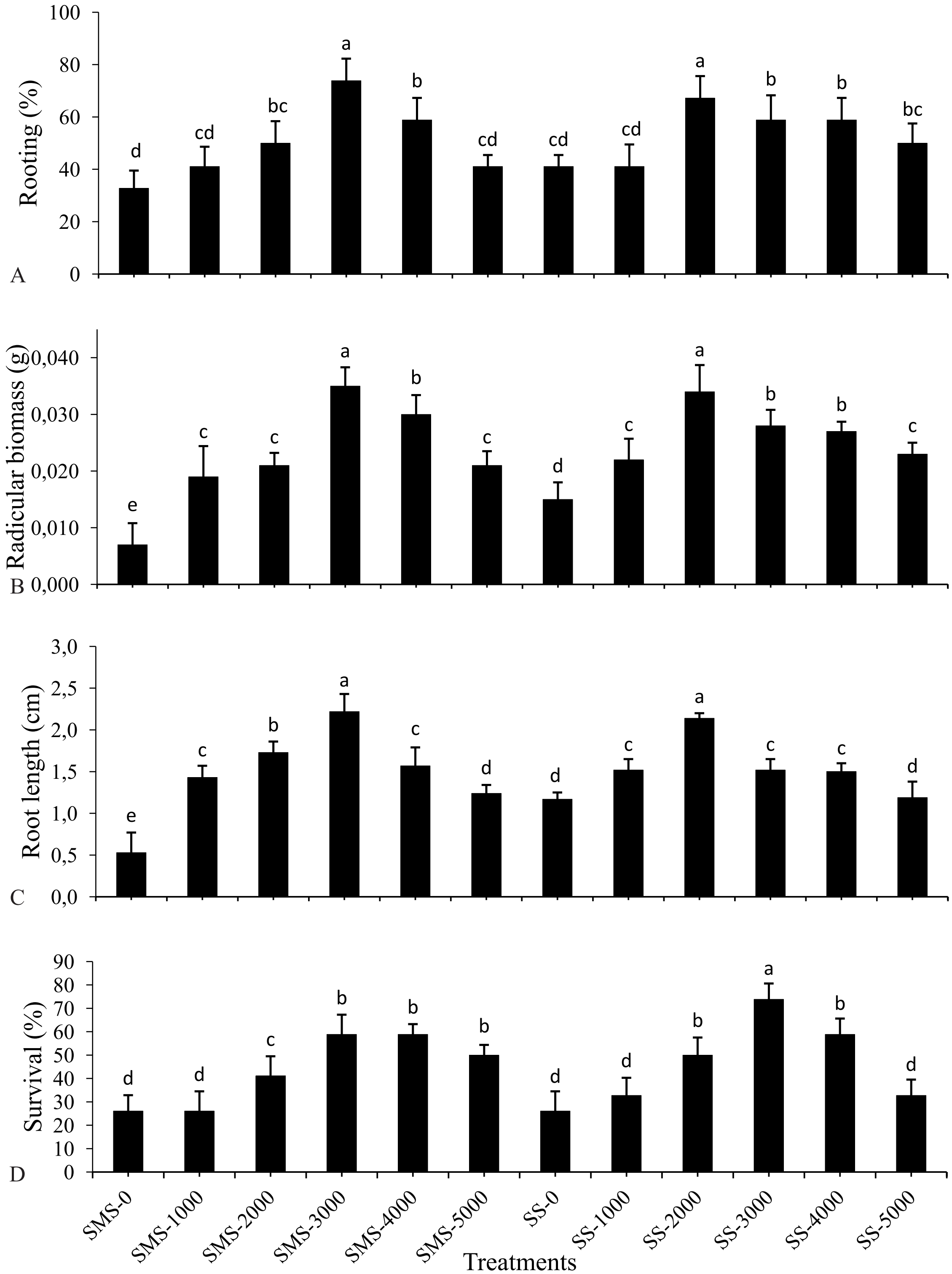

Figure 3. Comparison (after 32 days) of means for rooting of cuttings with application of rooting medium (SMS: Sterilized Medium Sand and SS: Specialized Substrate) and Indole-3-Butyric Acid (0; 1,000; 2,000; 3,000; 4,000; and 5,000 mg L^{-1}); A) Rooting; B) Root biomass; C) Root length and D) Survival. Letters above the bars indicate significant differences according to Ducan's test ($P < 0.05$).

Comparación (después de 32 días) de medias de enraizamiento de esquejes con aplicación de medio de enraizamiento (SMS: Medio Esterilizado Arena y SS: Sustrato Especializado) y Ácido Indol-3-Butírico (0; 1.000; 2.000; 3.000; 4.000; y 5.000 mg L^{-1}); A) Enraizamiento; B) Biomasa radicular; C) Longitud radicular y D) Supervivencia. Las letras sobre las barras indican diferencias significativas según la prueba de Ducan ($P < 0,05$).

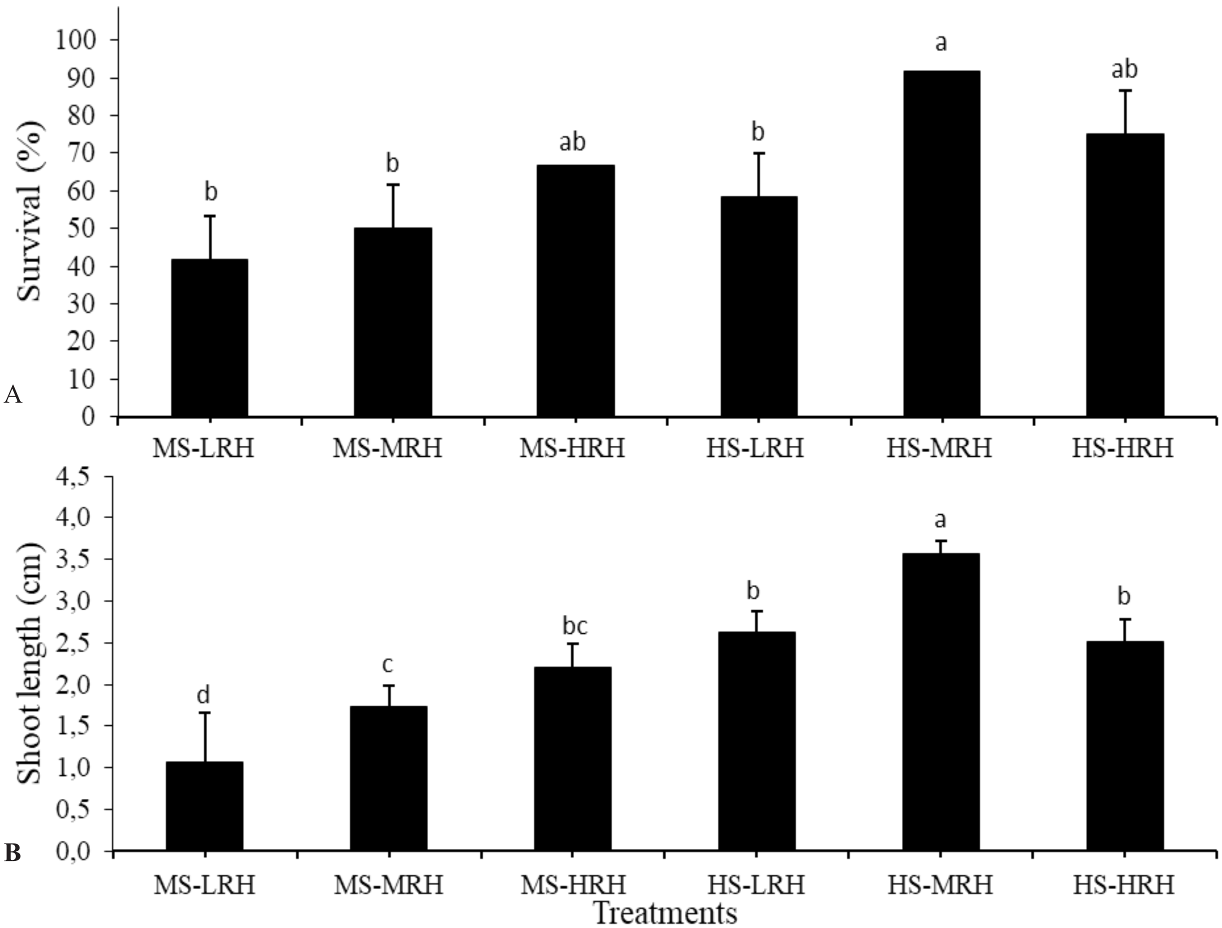

Figure 4. Comparison (after 90 days) of means for acclimation of cuttings with shade application (MS: Medium Shade at 50 % and HS: High Shade at 80 %) and Relative Humidity (LRH: Low Relative Humidity at 30 %, MRH: Medium Relative Humidity at 60 % and HRH: High Relative Humidity at 80 %); A) Survival and B) Shoot length. Letters above bars indicate significant differences according to Ducan's test ($P < 0.05$).

Comparación (después de 90 días) de medias para la aclimatación de esquejes con aplicación de sombra (MS: Sombra Media al 50 % y HS: Sombra Alta al 80 %) y Humedad Relativa (LRH: Humedad Relativa Baja al 30 %, MRH: Humedad Relativa Media al 60 % y HRH: Humedad Relativa Alta al 80 %); A) Supervivencia y B) Longitud del brote. Las letras sobre las barras indican diferencias significativas según la prueba de Ducan ($P < 0,05$).

have been used as disinfectants in several studies. Aslam *et al*. (2013) used a combined sterilization treatment regimen that included 96 % ethanol, 70 % commercial bleach, and 0.1 % mercuric chloride ($HgCl_2$) to develop a protocol for the micropropagation of *Lilium orientalis* L. in addition to *Lilium longiflorum* Thunb. cv. (White fox). Similarly, Delgado-Paredes *et al.* (2021) achieved a 100 % disinfection rate in *Manihot esculenta* buds by applying 70 % ethanol and 0.5 % sodium hypochlorite for three minutes. Similarly, Natalija *et al*. (2015) successfully sterilized the surface of *Petunia* explants using 70 % ethanol for 10–30 s. The findings of this study are consistent with those of Farooq *et al*. (2021), who obtained an 83.33 % survival rate of cuttings of *Petunia hybrida* Vilm. by applying 0.01 % carbendazim for 30 min, 70 % ethyl alcohol for 10 s, or 0.1 % HgCl2 for 5 min. In addition, our results are similar to those of Rafiq *et al*. (2021), who determined that the best sterilization of the oriental *Lilium* scale surface was with 200 mg L^{-1} Carbendazim for 30 min, followed by 0.1 % mercuric chloride for 10 min, and 70 % ethyl alcohol for 30 s, by recording a higher explant survival (86.12 %). Similarly, Farooq *et al*. (2021) obtained an 85.41 % survival rate in *Petunia hybrida* cuttings using carbendazim 0.02 % for 30 min, 0.1 % mercury for 5 min, and 70 % ethyl alcohol for 10 s over four weeks. The results obtained here are explained by the fact that concentrated ethyl alcohol is a potent disinfectant that shows rapid and broad-spectrum antimicrobial activity against vegetative bacteria (including mycobacteria), some viruses, and fungi (Şahiner *et al*. 2019). Carbendazim is a systemic fungicide that has wide applications in fungal control (Singh *et al.* 2016).

Substrates, IBA concentration, and microtunnel environment for adventitious rooting. In the present study, the highest percentages of rooting, root biomass, root length, and survival were obtained with 3,000 mg L^{-1} IBA. Several other studies have supported the use of IBA for rooting agroforestry species. Moreover, the best rooting result (89 %) in *Coffea arabica* L. was obtained with a 2,000 mg L^{-1} IBA dose in microtunnel environments and sterilized sand (Vallejos-Torres *et al.* 2020). Similarly, Solis *et al.* (2019), using specialized substrates (Jiffy), one mist irrigation per day, 8 cm long juvenile cuttings with 75 cm^2 leaf area, and 2,000 mg L^{-1} of IBA, obtained high rooting percentages (93.3 %) and improved root formation during the vegetative propagation of *Plukenetia volubilis* L.. Cuttings obtained from young plants have a greater root capacity owing to a higher rate of cell multiplication. The use of 0.4 % IBA has been suggested for rooting juvenile leafy branch cuttings of *S. macrophylla* (Azad and Matin 2015). Other reports have indicated that IBA can benefit the vegetative propagation of *Veronica dahurica* Steven and *Veronica pusanensis* Y. N. Lee cuttings (Su-Hyun *et al.* 2021). Auxins, especially IBA, are known to significantly improve the rooting percentage and quality. At the same time, they play a key role in cell division and increase cell volume by loosening cell walls, allowing greater water uptake, and thus increasing cell weight (Farooq *et al.* 2021). Vallejos-Torres *et al.* (2021), using medium-sized, sieved, and sterilized sand for the forest species *Manilkara bidentata,* obtained a rooting rate of 75 %, with 3.88 roots of average, 326 cm of major root length, and sprouting percentage of 94 %. Additionally, previous studies have established rooting methodologies for *Plukenetia volubilis* and *Plukenetia polyadenia* Müll.Arg., a wild relative of *Plukenetia volubilis*, using subsurface irrigation propagators and sand as substrates (Solis *et al.* 2017). Sand is a suitable substrate for propagation due to its good aeration and drainage, and it provides adequate support for rooted cuttings.

Acclimation of the propagules. In this study, the highest survival of cuttings (91.67 %) was obtained during acclimatization of *S. macrophylla* under 80 % shade and a relative humidity of 60 %. Shade management as an acclimatization factor has generated promising results, as in the study by Vijayakumar *et al.* (2013), in which 95 % survival was observed with 75 % shade in *Dendrobium* aggregatum seedlings. Similarly, for the propagation of *Coffea arabica*, Vallejos-Torres *et al.* (2020) used Raschel nets for shade management, which allowed 50 % of sunlight to enter. Acclimatization of rooted cuttings in the microtunnels of the forest species *Manilkara bidentata* (A.DC.) A. Chev., an 80 % shade system, was used with irrigation at a rate of four times a day to maintain the turgidity of the rooted cuttings, achieving their hardening for 100 days (Vallejos-Torres *et al*. 2021). Control of adequate relative humidity is fundamental for the acclimatization process. Musharof-Hossain (2014) observed a survival rate of 80 % with a relative humidity between 60 % and 70 % during the acclimatization process in *D. aggregatum* seedlings. It has been shown that adequate control of shade (75 %) and moisture (54.2 %) generates good results for *Fragaria x ananassa* Duch. propagated by cuttings, resulting in 91.9 % survival and a shoot length of 28.1 cm (Valencia *et al*. 2019).

Excessive humidity can cause widespread damage to plants; therefore, it is essential to control and monitor the environment in greenhouses (Rojas-Rishor *et al*. 2022). Fluctuations in humidity adversely affect plant growth and development, which is detrimental to acclimatization (Zhu *et al*. 2021). Hardening medium is important for the *ex vitro* establishment of plants developed *in vitro* (Farooq *et al*. 2021). Challenges occur when these seedlings are transferred to an *ex vitro* climate for acclimatization. Traditional greenhouses are often used; however, they have substantial consequences for mortality (Mohammed *et al*. 2022).

CONCLUSIONS

The vegetative propagation of *S. macrophylla* demonstrated that it is possible to convert cuttings into acclimatized and field-ready seedlings through a vegetative propagation protocol. The lowest contamination of mahogany cuttings was achieved with the combined EA-CZ treatment, with an average of 9.75 %, and the lowest percentage of necrosis was 9.1 %, with a high survival rate of cuttings with an average of 86.50 %, both with the same treatment. The best treatment for rooting of cuttings was SMS-3,000 (73.89 %), with similar results for root biomass, which presented the highest values at 0.036 g. The same treatment favorably influenced root length by 2.22 cm., whereas the survival of cuttings was 73.89 % with the SS-3,000 substrate treatment. In the acclimatization process, the best treatment for cutting survival was the HS-MRH treatment, with an average of 91.67 %. An average shoot length of 3.57 cm was obtained using the same treatment.

AUTHOR CONTRIBUTIONS

GVT, JRBV, NGJ, and CM participated in the conceptualization. LOS, PGG, and WMC conducted data analyses. The preliminary writing of the manuscript was undertaken by GVT, JRBV, NGJ, KR, and CM. All authors have contributed to the integration and revision of the final version of the manuscript.

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