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Revista Facultad Nacional de Agronomía

Woody Plant Medium optimizes *in vitro* germination and development of *Calycophyllum spruceanum*

UNIVERSIDAD NACIONAL DE COLOMBIA

Woody Plant Medium optimiza la germinación y el desarrollo de *Calycophyllum spruceanum in vitro*

https://doi.org/10.15446/rfnam.v78n3.119370

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ABSTRACT

Climate change and biodiversity loss pose critical threats to ecosystems, making reforestation with native species an attractive mitigation strategy. However, seedling production systems need to be optimized. This study aimed to evaluate the effect of different culture media and concentrations on the germination and *in vitro* morphological development of *Calycophyllum spruceanum*. A completely randomized design with five treatments was used: 100% Murashige and Skoog (MS), 50% MS, 100% Woody Plant Medium (WPM), 50% WPM, and a control treatment (agar), with seven replicates per treatment. Germination parameters were evaluated for 21 days, and morphological development at 70 days. The main results showed that WPM and agar obtained the highest germination percentages (73.33 and 75.24%, respectively) and germination rate (2.02 and 2.32 seeds per day, respectively), while WPM stood out in terms of final germination time. In morphological development, WPM recorded the highest values for number of leaves (8.18), nodes (5.25), plant height (16.83 cm), and fresh and dry biomass (147.27 and 16.07 mg), compared to the other treatments. In addition, principal component analysis showed that WPM was associated with germination and development variables, with significant correlations. In conclusion, the WPM medium optimized germination and promoted the development of healthy and homogeneous seedlings, positioning it as an efficient alternative for propagation, ecological restoration, and native species conservation programs.

Keywords:
Agar
Capirona
Culture media
Environmental restoration
Rubiaceae

RESUMEN

El cambio climático y la pérdida de biodiversidad representan amenazas críticas para los ecosistemas, por lo que la reforestación con especies nativas surge como una alternativa mitigadora. Sin embargo, se requiere optimizar los sistemas de producción de plántulas. El objetivo de este estudio fue evaluar el efecto de diferentes medios de cultivo y concentraciones en la germinación y el desarrollo morfológico *in vitro* de *Calycophyllum spruceanum*. Se utilizó un diseño completamente al azar con cinco tratamientos: 100% Murashige and Skoog (MS), 50% MS, 100% WPM, 50% WPM y un tratamiento control (agar), con siete repeticiones por tratamiento. Se evaluaron parámetros de germinación durante 21 días y desarrollo morfológico a los 70 días. Los principales resultados demostraron que WPM y agar obtuvieron los mayores porcentajes de germinación (73,33 y 75,24%), índice de velocidad de germinación (2,02 y 2,32 semillas por día), mientras en el tiempo de germinación final, el WPM destacó. En el desarrollo morfológico, WPM registró los valores más altos de número de hojas (8,18), nodos (5,25), altura de planta (16,83 cm) y biomasa fresca y seca (147,27 y 16,07 mg), en comparación con los demás tratamientos. Además, el análisis de componentes principales mostró que WPM se asoció a las variables de germinación y desarrollo, con correlaciones significativas. En conclusión, el medio WPM optimizó la germinación y favoreció el desarrollo de plántulas saludables y homogéneas, lo que lo posiciona como una alternativa eficiente para programas de propagación, restauración ecológica y conservación de especies nativas.

Palabras clave:
Agar
Capirona
Medio de cultivo
Restauración ambiental
Rubiaceae

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Received: June 9, 2025; Accepted: July 27, 2025
Rev. Fac. Nac. Agron. Medellín 78(3): 11353-11363. 2025
ISSN 0304-2847 / e-ISSN 2248-7026

Current global challenges, such as climate change and biodiversity loss, pose critical threats to ecosystems (Blankinship 2024). Given this situation, ecosystem restoration has emerged as a key strategy to mitigate these effects (Zhang et al. 2024). Among the most effective strategies, reforestation plays a central role by facilitating the recovery of degraded areas, promoting land-use neutrality, and improving ecological functionality through the balancing of ecosystem services (Das et al. 2020). A crucial component of successful reforestation initiatives is the incorporation of native species with high ecological value, as these species contribute significantly to soil rehabilitation, biodiversity conservation, and climate change mitigation (da Silva et al. 2024). *Calycophyllum spruceanum* (Rubiaceae), a native tree species of the Amazon Basin found in Peru, Brazil, Colombia, and Ecuador, is notable for its rapid growth, adaptability to degraded and flood-prone soils, and high-quality wood (Saldaña et al. 2022). These characteristics make it a suitable candidate for reforestation and ecosystem restoration programs, particularly in areas severely impacted by degradation. Furthermore, the increasing interest in this species, driven by both its ecological and commercial value, highlights the importance of optimizing its propagation to meet market demand without compromising biodiversity (Santos et al. 2016).

Within this framework, plant tissue culture techniques have gained attention as a biotechnological tool for the rapid and large-scale propagation of plant species. These techniques offer advantages such as space-efficient multiplication, improved pathogen control, and enhanced nutrient management, ensuring the production of healthy and uniform seedlings, an essential aspect for effective ecological restoration and commercial cultivation (Indacochea et al. 2018).

Although several studies have explored the ecological and silvicultural aspects of *C. spruceanum* (Guerra-Arévalo et al. 2024), biotechnological research has primarily focused on its pharmacological, biochemical, and genomic properties (Saldaña et al. 2022). Tissue culture studies; however, remain limited, with only two investigations addressing *in vitro* establishment and callus induction (Olivas 2014; Figueiredo et al. 2014).

Given the fundamental role of culture media in germination and seedling development, it is essential to identify formulations that optimize germination rates and promote the development of genetically and phytosanitarily sound, uniform seedlings. To date, no studies have examined the influence of culture media on the germination and morphological development of *C. spruceanum*, which is crucial for enhancing the efficiency and quality of seedling production in support of reforestation efforts. According to Sudheer et al. (2022), culture media significantly influence plant growth by supplying essential nutrients, providing structural support, and maintaining optimal conditions for morphological development. Previous *in vitro* studies on *C. spruceanum* have employed Murashige and Skoog (MS) medium (Olivas 2014; Figueiredo et al. 2014). However, recent findings suggest that Woody Plant Medium (WPM) may offer superior outcomes in terms of germination and development (Gonzales-Alvarado et al. 2024). WPM has been reported to enhance shoot proliferation, rooting, and overall growth in species such as *Durio dulcis* and *Guazuma crinita*, outperforming conventional media under *in vitro* conditions (Hardarani et al. 2023; Gonzales-Alvarado et al. 2024). Based on this evidence, it is hypothesized that the WPM culture medium will result in higher germination rates and improved morphological development in *C. spruceanum* compared to other commonly used media. This study aims to evaluate the effect of different culture media and concentrations on the germination and *in vitro* morphological development of *C. spruceanum*, to maximize seedling productivity, quality, and uniformity. The findings will contribute to the scientific understanding of this species' biology and support its application in reforestation and genetic improvement programs. In this way, the research seeks to provide innovative and sustainable solutions to face global challenges, promoting the recovery of degraded areas and strengthening conservation efforts.

MATERIALS AND METHODS

Plant material and sterilization

The experiment was conducted at the Plant Tissue Culture Laboratory of the National University of Ucayali, located in Pucallpa, Peru. As plant material, seeds of *Calycophyllum spruceanum*, which were acquired in commercial seed markets, were used. Seed sterilization was performed in a laminar flow chamber. The seeds were immersed in a

Benlate® solution (30 g L^{-1}) for 30 minutes. Subsequently, they were immersed in 70% alcohol and immediately treated with 1% sodium hypochlorite (NaClO), adding three drops of Tween® 20. The solution was kept in constant agitation for 10 minutes. Finally, the seeds were rinsed five times with sterilized distilled water to ensure the elimination of residues.

Culture media, treatments, and variables

For *in vitro* germination, Murashige and Skoog (MS) and Woody Plant Medium (WPM) at different concentrations were used as base culture medium. Agar alone was used as a control. The experiment consisted of five treatments: T1: 100% MS, T2: 50% MS, T3: 100% WPM, T4: 50% WPM, and T5: control (agar only). Additionally, in each of the treatments, 6.5 g L^{-1} of agar (PhytoTech®), 30 g L^{-1} of sucrose, and pH was adjusted to 5.8. However, for the control (T5), only 30 g L^{-1} sucrose was added, and the pH was adjusted to 5.8, because it already contained agar.

Each treatment consisted of seven replicates, and five flasks (13 cm height and 6 cm mouth) were used in each replicate. Each flask contained 45 mL of medium, which were autoclaved at 121 °C and 1.1 atm for 20 min. Subsequently, the flasks were cooled and three seeds per flask were inoculated, being 105 seeds per treatment, which were placed randomly. The flasks were incubated at a temperature of 23±2 °C, with a relative humidity of 80% and a photoperiod of 16 hours.

The variables related to germination, such as germination percentage (G), germination speed index (GSI), germination initiation day (GID), final day of germination (FDG), germination energy (GE), germination energy period (GEP), germination energy period percentage (% GEP) were evaluated for 21 days after *in vitro* inoculation (Bewley and Black 1994). Equation 1 was used for the germination percentage G (%):

$$G(\%) = \frac{\sum n_i}{N} \times 100 \quad (1)$$

Where, $\sum$ ni is the total number of germinated seeds and N is the total number of inoculated seeds. The germination speed index (GSI) was calculated using Equation 2:

$$GSI = \frac{G_1}{N_1} + \frac{G_2}{N_2} + ... + \frac{G_n}{N_n} \quad (2)$$

Where G1, G2, ... G_n refer to the number of germinated seeds and N1, N2, ... Nn, to the number of days after *in vitro* inoculation. Germination energy (GE) was determined using Equation 3:

$$GE = \frac{G_n}{N} \times 100 \quad (3)$$

Where G_n is the number of seeds germinated in a given day period (seven days), and N is the total number of inoculated seeds. The germination energy period (GEP) was used through Equation 4:

$$GEP = \frac{PG}{N} \times 100 \quad (4)$$

Where PG is the number of seeds where the highest percentage of non-accumulated germination occurs and N is the total number of inoculated seeds.

The morphological development variables, such as plant height (PH), number of nodes (NN), number of leaves (LN), fresh biomass (FB), and dry biomass (DB), were analyzed 70 days after inoculation. To determine the fresh biomass, all seedlings from each treatment were used, cleaned, and weighed on a digital analytical balance accurate to 0.1 mg. Subsequently, the same seedlings were dried in an oven at 70 °C for 72 hours to determine the total dry biomass (Gonzales-Alvarado and Cardoso 2024).

Experimental design and statistical analysis

The experiment was carried out using a completely randomized design. For all variables evaluated, the assumptions of normality were verified using the Shapiro-Wilk test and homogeneity of variances using the Levene test. When these assumptions were not met, the Kruskal-Wallis nonparametric test was applied.

The variables associated with germination and morphological development complied with the assumptions of normality and homoscedasticity, and were therefore subjected to an analysis of variance (ANOVA). In cases where significant differences were found, Tukey's test of means was used with a significance level of 5% (α=0.05). However, the variable number of nodes (NN) did not meet the assumptions, so it was analyzed using the Kruskal-Wallis nonparametric test. To analyze the seed germination percentage as a function of the treatments, a second-degree polynomial regression was applied.

In addition, principal component analysis (PCA) was performed, including all the variables analyzed, using the ggplot2 function and confidence ellipses (level=0.95) in the biplot (Wickham 2016). For the correlation analysis between variables, Spearman's correlation coefficient was used by corrplot, considering $-0.6 \leq P \geq 0.6$, as strong correlation (Wei et al. 2024). All statistical analyses were performed using R Studio software, version 4.3.2.

RESULTS AND DISCUSSION

Effect of culture media on *in vitro* germination parameters of *Calycophyllum spruceanum*

The germination behavior (G) of *C. spruceanum* shows a trend of progressive increase, stabilizing around 15 to 18 days after *in vitro* inoculation (Figure 1). The mathematical models fitted to the data correspond to quadratic equations with high coefficients of determination ($R^2>0.94$), indicating an adequate representation of germination behavior in each treatment.

It is observed that the culture medium significantly influences germination, with notable differences in the germination rate and the final percentage reached. The treatment with agar (T5) presents the most negative quadratic coefficient ($-0.3613x^2$), suggesting a faster initial germination, but with an earlier stabilization. In contrast, the WPM and 50% WPM treatments (T3 and T4) show a more sustained germination rate over time, reaching higher values compared to the MS and 50% MS treatments (T1 and T2).

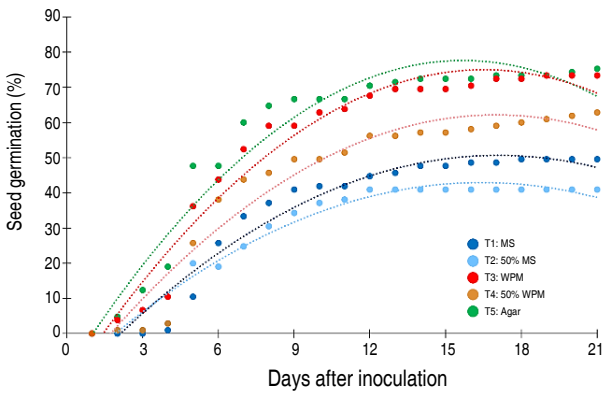

Figure 1. Germination percentage of *C. spruceanum* during 21 days post-inoculation in different culture media. **T1**: $y = -0.2262x^2 + 7.7312x - 15.946$. $R^2 = 0.9474$, **T2**: $y = -0.2041x^2 + 6.7035x - 12.13$. $R^2 = 0.961$, **T3**: $y = -0.33x^2 + 10.899x - 15.022$. $R^2 = 0.9598$, **T4**: $y = -0.2668x^2 + 9.0709x - 14.946$. $R^2 = 0.9413$, and **T5**: $y = -0.3613x^2 + 11.339x - 11.335$. $R^2 = 0.9449$.

Germination is a fundamental aspect for proper management in plant development (Gonzales-Alvarado et al. 2022). Germination of *C. spruceanum* was influenced by the type of culture medium and its concentration. The use of agar and Woody Plant Medium (WPM) resulted in the highest germination percentages within 21 days, reaching 75.24 and 73.33%, respectively (Table 1 and Figure 1). Similar results were reported by Gonzales-Alvarado et al. (2024) and Marwein and Vijayan (2024), who observed that 100% agar and WPM enhanced the germination of *Guazuma crinita* and *Adinandra griffithii*, respectively. In contrast, Guerra-Arévalo et al. (2024) evaluated organic substrates for the germination of *C. spruceanum* and achieved 62.3% germination. When compared with these previous results, the application of agar and WPM in the present study increased the germination percentage by 17.7 and 20.77%, respectively. According to Pereira (2023), agar is a polysaccharide derived from algae that does not provide nutrients to plants and facilitates seed hydration, activating metabolic pathways that lead to the synthesis of proteins and enzymes essential for germination. In the present study, no statistically significant differences were found between agar (T5) and WPM (T3) regarding germination performance in *C. spruceanum* (Table 1). This result can be attributed to the early developmental stages of the embryo, during which external nutrients are not essential, as the embryo utilizes seed reserves to generate energy and the necessary chemical compounds for development (Meneses et al. 2022). According to Gonzales-Alvarado et al. (2022), they also emphasized the

importance of medium composition in seed germination. For instance, Kirillov et al. (2024) reported that MS medium enhanced germination in *Aflatunia ulmifolia*, achieving over 50% germination compared to other media. However, in the present study, WPM promoted higher germination percentages than MS treatments. This effect may be associated with the lower concentrations of nitrogen, potassium, and ions in WPM, as well as its reduced ammonium content—approximately 25% lower than that of MS medium (Munthali et al. 2022).

Regarding the germination speed index. (GSI) and germination energy (GE), agar (T5) and WPM (T3) produced the highest values, with GSI reaching 2.32 and 2.02 seeds per day, and GE values of 60 and 52.38%, respectively. Germination was initiated at two days post-inoculation in all treatments except T1 (MS). Furthermore, during the Germination Energy Period (GEP), T1 (MS) exhibited the slowest response, reaching GE at six days with only 15.24%, the lowest value recorded (Table 1). In contrast, T5 and T3 presented GEP values of 28.57 and 25.71%, respectively.

Table 1. Effect of culture medium on *in vitro* germination parameters (mean ± standard error) of *C. spruceanum*.

Culture medium	(%)	GSI (seeds per day)	GID	FDG	GE (%) (7 days)	GEP (days)	GEP (%)
T1: MS	49.52±2.46^{BC}	1.10±0.07^{C}	4	16	33.33±4.11^{BC}	6	15.24
T2: 50% MS	40.95±5.13^{C}	1.03±0.15^{C}	2	12	24.76±4.04^{C}	5	17.14
T3: WPM	73.33±4.11^{A}	2.02±0.21^{AB}	2	19	52.38±3.97^{A}	5	25.71
T4: 50% WPM	62.86±5.22^{AB}	1.50±0.10^{BC}	2	21	43.81±4.08^{AB}	5	22.86
T5: Agar	75.24±2.80^{A}	2.32±0.11^{A}	2	21	60±5.25^{A}	5	28.57
CV	18	22.7			26.64		

Different capital letters indicate significant differences between treatments according to the Tukey test at the 5% significance level. G: germination percentage, GSI: germination speed index, GID: germination initiation day, FDG: final day of germination, GE: germination energy, GEP: germination energy period, and GEP (%): percentage of germination energy period.

On the Final Day of Germination (FDG), WPM completed the germination process in 19 days, while agar did so in 21 days. These results support the hypothesis that WPM enhances germination. The favorable outcomes observed with agar can be attributed to its stable surface, which supports seed germination and seedling development, despite its lack of nutrients. In contrast, WPM, characterized by lower salt concentrations (Delgado-Paredes et al. 2023), did not negatively impact germination parameters such as G, GSI, GID, GE, GEP, and GEP (%), indicating no phytotoxic effects on *C. spruceanum*. Conversely, the high salt concentration in MS medium appeared to limit the development of these germination parameters *in vitro* (Cabral-Miramontes et al. 2022).

Effect of culture media on the morphological development and biomass of *Calycophyllum spruceanum*

The results of morphological development showed significant differences in all the variables analyzed (Figure 2). Initial plant development encompassed key morphological parameters that influenced subsequent growth and adaptation to different cultivation conditions. Understanding these parameters contributed to the efficient production of seedlings and optimized propagation under *in vitro* conditions the efficient production of seedlings and optimizes their propagation in *in vitro* systems.

The application of Woody Plant Medium (WPM) significantly enhanced the morphological development of *C. spruceanum*, yielding the highest values for number of leaves (LN), number of nodes (NN), and plant height (PH), with averages of 8.18 leaves per plant, 5.25 nodes per plant, and 16.83 cm in height, respectively (Figure 2). Similar results were reported by Marwein et al. (2024), Lima et al. (2024), and Meneses et al. (2022), in which WPM demonstrated superior efficiency compared to other culture media, promoting the morphological development of *in vitro* seedlings of *Rhododendron inaequale Hutch, Vismia japurensis,* and *Vaccinium floribundum*, respectively. According to Krasnoperova and Bukharina (2020), indicated that WPM enhances both plant survival and development, supporting the hypothesis that this medium optimizes the morphological development of *Calycophyllum spruceanum*.

To date, no previous studies have evaluated this specific aspect, suggesting that the present findings may contribute to the large-scale propagation of the species with improved morphological traits, suitable for meeting market demand in ecological restoration programs and industrial applications.

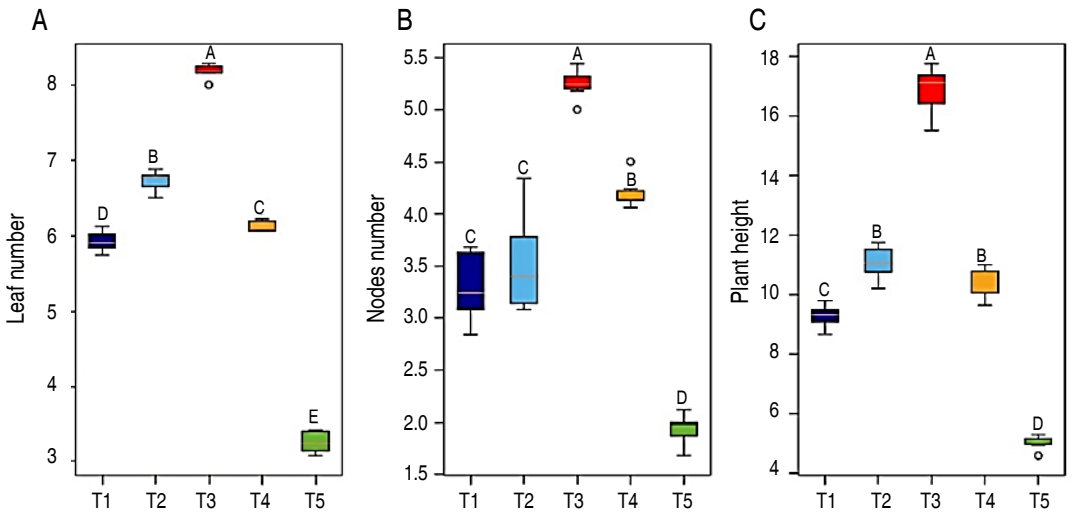

Figure 2. Effect of culture media on *in vitro* morphological development of C. spruceanum. **A**. number of leaves (LN). **B**. number of nodes (NN), and **C**. plant height (PH). **T1**: MS; **T2**: 50% MS; T3: WPM; T4: 50% WPM; and T5: Agar. Different letters indicate significant differences between treatments according to Tukey's test at 5% significance level.

According to Stachevski et al. (2013), WPM has 50% of the ionic strength of Murashige and Skoog (MS) medium, as well as higher levels of sulfur (S) and lower concentrations of ammonium (NH_4^+) and nitrate (NO_3^-), which reduce the risk of ionic toxicity and improve the adaptability of woody species. Additionally, its balanced composition of macro- and micronutrients, including adjusted concentrations of nitrogen, phosphorus, and potassium, was essential for regulating plant metabolic functions (Delgado-Paredes et al. 2023). Previous studies on *C. spruceanum* under *in vitro* conditions employed MS medium for bud establishment and callus induction, with promising results (Olivas 2014; Figueiredo et al. 2014), obtaining promising results. The findings of the present study indicated that WPM represents a more effective alternative for enhancing morphological development and could complement previous efforts in tissue culture research on this species.

After 70 days of *in vitro* cultivation of *C. spruceanum*, T3 (WPM) exhibited the highest mean number of leaves (8.18 leaves per plant) and the greatest leaf area. These characteristics are associated with increased survival during the post-*in vitro* acclimatization phase and contribute to more efficient seedling production in a shorter period (Valladares and Niinemets 2008). In contrast, plants under treatment T5 (agar) displayed yellowish leaves with visible signs of stress, likely due to the absence of nutrients in the culture medium (Figure 3). These observations highlight the need for further studies on nutrient accumulation in plant tissues to complement the present findings. Similar results were reported by Gonzales-Alvarado et al. (2022) in *Guazuma crinita*, where plants cultured in phytogel for four months exhibited leaf yellowing and other stress-related symptoms.

Regarding the production of fresh biomass (FB) and dry biomass (DB), it was identified that WPM allowed obtaining the highest values, with 147.27 and 16.07 mg per plant, respectively (Table 2), significantly surpassing the other treatments. The results support the hypothesis that the use of WPM optimizes germination, morphological development, and accumulation of biomass in *C. spruceanum*. In contrast, the agar-only treatment showed a marked limitation in growth, which could be attributed to the deficiency of essential nutrients, since agar alone does not provide the necessary compounds for proper plant development. This had

a negative impact on the physiology of the seedlings, which showed an evident yellowing, as illustrated in Figure 3, possibly due to nutritional deficiencies and alterations in chlorophyll synthesis.

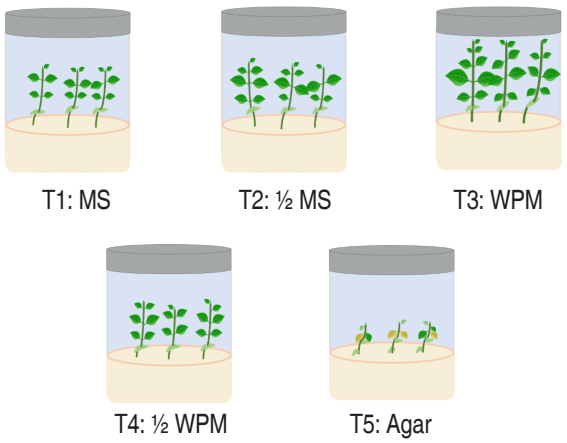

Figure 3. Morphological characteristics of *C. spruceanum* at 70 days post-inoculation *in vitro*.

Table 2. Effect of culture media on biomass of *C. spruceanum*.

Culture medium	FB (mg per plant)	DB (mg per plant)
T1: MS	90.26±0.29^{B}	10.67±0.05^{B}
T2: 50% MS	91.24±0.41^{B}	10.76±0.08^{B}
T3: WPM	147.27±0.30^{A}	16.07±0.08^{A}
T4: 50% WPM	90.49±0.48^{B}	10.51±0.10^{B}
T5: Agar	54.36±0.15^{C}	6.43±0.08^{C}
CV	0.96	2

Different letters indicate significant differences between treatments according to Tukey's test at 5% significance level. **FB**: fresh biomass, and **DB**: dry biomass.

The use of WPM in *C. spruceanum* not only optimizes production times but also yields plants with enhanced morphological characteristics, which is essential for large-scale propagation. From an applied perspective, the methodology implemented in this study has the potential to substantially support the mass production of seedlings, thereby facilitating supply for reforestation programs and the restoration of degraded ecosystems. However, it is important to consider that establishing seeds under controlled and aseptic conditions—such as in specific *in vitro* culture media—may entail higher operational and logistical costs compared to direct sowing in field substrates. Nonetheless, this initial investment is justified by the production of healthy, uniform seedlings with a greater likelihood of survival, which represents a significant advantage for projects requiring high-quality plant material. *In vitro* propagation of *C. spruceanum*, therefore, is recommended as an innovative strategy to promote environmental sustainability and ensure the availability of this species for conservation and forest development initiatives. Furthermore, the findings regarding the application of WPM provide a robust foundation for future research in the *in vitro* culture of *C. spruceanum*, including studies on micropropagation, genetic transformation, and molecular biology. This approach may also prove valuable for research focused on the extraction of bioactive compounds of pharmacological and industrial relevance, enabling the efficient production of high-value secondary metabolites.

Main component analysis and correlation of germination parameters, morphological development, and biomass of *Calycophyllum spruceanum*

The main component analysis (PCA) and correlation of germination parameters and morphological development yielded relevant insights. In the PCA, treatments with agar (T5) and WPM (T3) were grouped and associated with germination percentage (G), germination speed index (GSI), and germination energy (GE) (Figure 4A). However, in the analysis of morphological development, these treatments showed clear differentiation. The WPM treatment (T3) exhibited stronger associations with leaf number (LN), number of nodes (NN), plant height (PH), fresh biomass (FB), and dry biomass (DB) (Figure 5A).

Correlation analysis revealed positive associations within both germination and morphological development parameters (Figure 4B and Figure 5B). Among the germination parameters, correlation coefficients exceeded 0.87, with the strongest observed between GSI and germination percentage (G), at 0.93 (Figure 4B). To date, this is the first *in vitro* study to report such correlations in *C. spruceanum*, emphasizing the importance of further investigations into its germination processes. Additional research should focus on optimizing germination under *ex vitro* conditions to enhance propagation efficiency. Moreover, the inclusion of other germination-related parameters is recommended to refine and improve alternative propagation protocols for both natural and controlled environments.

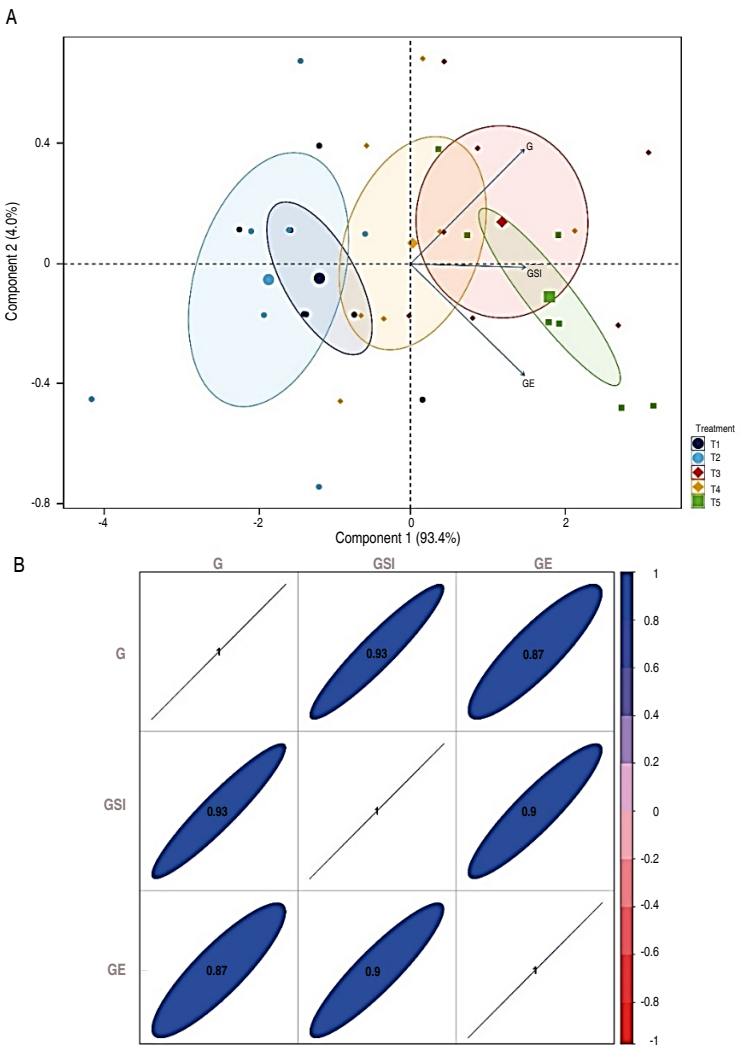

Figure 4. A: Main component analysis of germination parameters of *C. spruceanum*, and **B:** Correlation analysis between germination variables of *C. spruceanum.* **G:** germination percentage, **GSI:** germination speed index, and **GE:** germination energy.

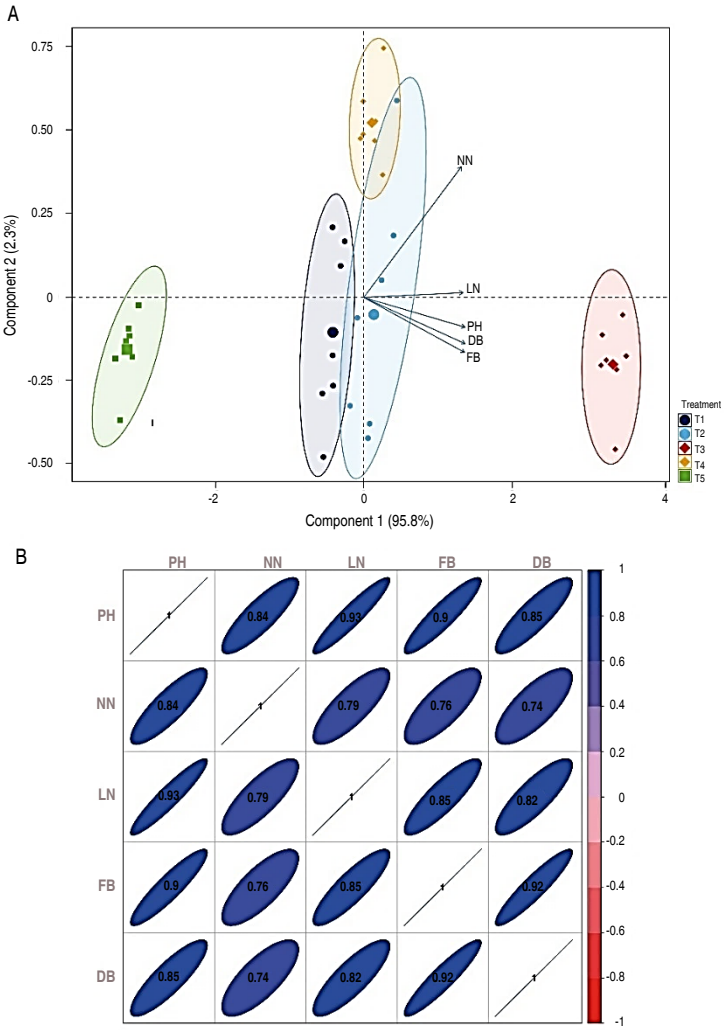

Figure 5. A: Main component analysis of morphological development and biomass of *C. spruceanum*, and **B:** Correlation analysis of morphological development and biomass variables of *C. spruceanum*. **PH:** plant height, **NN:** number of nodes, **LN:** number of leaves, **FB:** fresh biomass, and **DB:** dry biomass.

Regarding morphological development, the correlations of plant height (PH), number of nodes (NN), and number of leaves (LN) were greater than 0.84, suggesting a strong association between these growth factors. The highest correlations were recorded between fresh biomass (FB) and dry biomass (DB), with a value of 0.92, confirming that better morphological development contributes directly to higher biomass accumulation. Similar results were reported by Gonzales-Alvarado et al. (2024) and Mori-Vásquez et al. (2024) in *Guazuma crinita* and *Simarouba amara,* respectively, where strong correlations were evidenced between fresh and dry biomass, as well as between plant height and biomass. These results reinforce the present findings and confirm the interdependence between morphological traits and biomass production.

The outcomes of this study underscore the necessity for further investigations into the responses of *C. spruceanum* under various growth conditions. It is particularly important to evaluate root system interactions, assess physicochemical parameters such as pH, electrical conductivity (EC), and the accumulation of macro and micronutrients. Additionally, future research should explore the influence of biochemical components in the culture media on chlorophyll content and the expression of growth- and development-related genes under *in vitro* conditions. Integrating genetic, physiological,

and molecular approaches would facilitate more accurate correlations between morphological responses and molecular regulation in this species. Such insights could significantly enhance propagation protocols and improve the efficiency of *C. spruceanum* seedling production for both commercial use and conservation or ecological restoration programs.

CONCLUSION

This study demonstrated that culture media and their different concentrations directly influence germination parameters and *in vitro* morphological development. In particular, Woody Plant Medium (WPM) significantly optimized both germination and morphogenetic development of *C. spruceanum*, promoting the production of healthy and uniform seedlings. These results highlight the importance of selecting an appropriate culture medium and its concentrations to obtain high-quality and uniform plants, which is essential for supporting ecological restoration and native species conservation programs. Furthermore, these observations underscore the need for additional studies on nutrient accumulation in plant tissues to complement the findings obtained. It should be noted that this is the first report comparing different culture media for *C. spruceanum*. Further research on this and other species of ecological interest is recommended to strengthen conservation and restoration strategies for degraded ecosystems.

ACKNOWLEDGMENTS

We thank the collaborators who participated in the field and laboratory phases, contributing significantly to the development of this research. Finally, we acknowledge the valuable suggestions of the anonymous reviewers that allowed us to substantially improve the manuscript.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest of any kind in the preparation and publication of the manuscript.

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