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GROWTH RESPONSE OF COFFEE (*Coffea arabica* L.) TO MYCORRHIZAL INOCULATION (*Glomus* sp.) DURING THE NURSERY STAGE

Carlos Alberto Garzón-Pajoy[1a], Jordan Alexis Castillo-Coronado[1b]*, Juan Gonzalo Ardila-Marín[1c], Diana Carolina Polanía-Montiel[1d], and Jean Carlos Acosta-Vargas[1e]

[1a] Faculty of Engineering, Universidad Surcolombiana, 410001, Neiva, Colombia
https://orcid.org/0009-0005-0710-5977
[1b] Faculty of Engineering, Universidad Surcolombiana, 410001, Neiva, Colombia
https://orcid.org/0000-0003-2742-5095
[1c] Faculty of Engineering, Universidad Surcolombiana, 410001, Neiva, Colombia
https://orcid.org/0000-0003-4461-7195
[1d] Faculty of Engineering, Universidad Surcolombiana, 410001, Neiva, Colombia
https://orcid.org/0000-0001-5306-6431
[1e] Faculty of Engineering, Universidad Surcolombiana, 410001, Neiva, Colombia
https://orcid.org/0009-0003-5266-1556
* Corresponding author: lubey98@hotmail.com

ABSTRACT

Coffee is one of the most representative agricultural products of Colombia and worldwide, particularly in the Huila Department, due to its high production and excellent quality. As coffee is a crop of high economic value and is increasingly affected by the impacts of climate change, it is essential that the nursery stage contributes to improved crop development and resilience. In order to promote and encourage new practices among coffee growers in the region, this study aimed to evaluate the effects of arbuscular mycorrhizal fungi inoculation (*Glomus intraradices*) in a coffee nursery located in the municipality of Hobo, Huila. The experiment included two treatments with different doses of a commercial liquid arbuscular mycorrhizal inoculant (Biofermex®), based on *G.* sp.: T1, with 5 cm^3 L^{-1} of water, and T2, with 10 cm^3 L^{-1} of water, as well as a non-inoculated control treatment (T0). Evaluations were conducted from 30 to 105 days after transplanting (DAT), assessing primary root length (PRL), number of secondary roots (NSR), stem diameter (SD), stem length (SL), total leaf area (TLA), and total dry mass (TDM). Statistical analysis was performed using analysis of variance (ANOVA) and graphical methods, whereas a technical and economic assessment was carried out to compare production costs during the nursery period. The results showed that T1 and T2 presented statistically significant increases in NSR (44.4 and 27.4%) and TLA (84.5 and 24.7%) compared with the control. In addition, the implementation of temporary nurseries with appropriate mycorrhizal management resulted in 36.34% higher profitability compared with the purchase of seedlings from external nurseries.

Keywords: Mycorrhizal agents, fungal sporulation, infection, seedbed.

INTRODUCTION

Coffee is one of the main agricultural products in the Huila Department, Colombia, providing the economic livelihood for over 84,000 rural families (FNC, 2025). However, limited technical assistance and the low incorporation of research findings into production systems have led many producers to remain unaware of sustainable agronomic practices that increase yield without compromising environmental health (Franco et al., 2021; Valbuena et al., 2021).

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In Colombia, approximately 96% of coffee growers are smallholders managing less than five hectares, with production systems operating as family enterprises (UPRA, 2024). Most agricultural tasks are performed directly by family members to reduce production costs (Bavorová et al., 2025). One practice where farmers seek to optimize resources is through the production of their own seedlings for planting, which offers advantages over purchasing commercial plant material and reduces transportation costs (Castro-Toro et al., 2008).

However, one of the main limitations when acquiring seedlings from nearby nurseries is damage associated with vehicular transit, as dust and emissions from unpaved roads can negatively affect germination and vigor of young plants (Afsharnia and Moosavi, 2021; Lee et al., 2012; Zeeshan et al., 2024). Additionally, vehicles can act as vectors for pests and pathogens between nurseries and farms (Carlin et al., 2025; Lee et al., 2012), which, in Colombian coffee regions –where most crops are established on slopes with limited road access– worsens logistical and phytosanitary conditions, further hindering the acquisition of high-quality seedlings (Ovalle-Castiblanco, 2022; Rivillas, 2022). For this reason, temporary nurseries are established near cultivation plots, where farmers explore different techniques and resources to enhance seedling vigor and resistance to pests and diseases (Castro-Toro et al., 2008; World Coffee Research, 2021). Various studies have demonstrated that inoculation with beneficial microorganisms can promote coffee plant development, resulting in more vigorous and healthier growth through symbiotic interactions under nursery conditions (Urgiles-Gómez et al., 2021; Asad et al., 2023; Martins et al., 2024).

Mycorrhizae, symbiotic associations between fungi of the phylum Glomeromycota and the roots of over 80% of plant species, provide mutual benefits: the fungus obtains carbon from the host plant, while the plant gains enhanced access to essential soil nutrients (Kalamulla et al., 2022; Razack et al., 2024). Specifically, arbuscular mycorrhizal fungi penetrate the cortical root cells, promoting phosphorus uptake, stimulating root formation, and enhancing plant growth (Lu et al., 2023; Nie et al., 2024; Razack et al., 2024). Their establishment depends on the presence of fungal spores (natural or induced), and the optimal inoculation timing varies according to fungal species and spore density applied (Paré et al., 2022; Liu et al., 2025; Pérez-Redondo et al., 2025). Additionally, mycorrhizae contribute to plant defence against root pathogens through phytohormone production and help mitigate water stress by improving water uptake and evapotranspiration efficiency (Guo et al., 2024; Nie et al., 2024).

Species of the genus *Glomus* are widely used to enhance plant productivity and soil quality (Roussis et al., 2022; Deja-Sikora et al., 2023). In Brazil, França et al. (2014) evaluated growth in coffee seedlings inoculated with *G. clarum* and *Gigaspora margarita*, observing significant increases in height, leaf area, and dry biomass compared to uninoculated controls during the nursery stage. Similarly, in Medellín, Colombia, González-Osorio (2022) reported that inoculation with *Rhizophagus fasciculatum* increased total dry mass in coffee seedlings under greenhouse conditions, highlighting the role of mycorrhizae in early crop growth. Furthermore, Castro-Toro et al. (2008) demonstrated in Chinchiná, Colombia, that the use of *Trichoderma harzianum* reduced seedling losses by 10–15% due to pests through biological control supported by phytohormone production from the host fungus. Silveira et al. (2023) also reported positive growth responses in coffee seedlings following mycorrhizal inoculation in two substrate types in Brazil, with significant increases in aboveground biomass.

In this context, and with the aim of promoting sustainable practices for improved coffee crop establishment, the present study evaluated the effect of arbuscular mycorrhizal inoculation on coffee seedling growth and analyzed the technical and economic aspects of the treatments during the nursery stage in a temporary experimental nursery located in Hobo, Huila, Colombia.

MATERIALS AND METHODS

Study area

The present study was conducted in 2023 in the El Batán village, municipality of Hobo, Huila, Colombia (2° 29′ 32.33″ N, 75° 26′ 0,44″ W) (Fig. 1). The experimental site, registered in the Coffee Information System (SICA) as "El Santuario," is located at an elevation of 1,554 meters above sea level. The soil type is classified as sandy loam, and the site has an average temperature of 23 ºC, relative humidity of 53%, and an annual precipitation ranging between 900 and 1,000 mm $year^{-1}$.

Crop establishment

Initially, a 0.36 m^2 germination bed was prepared, consisting of a 0.10 m layer of gravel at the bottom and a 0.20 m layer of sand (disinfected with Tricho-D®) as the upper substrate layer. A total of 600 coffee seeds of the Cenicafé 1 variety, with a germination rate of 75%, were sown in the

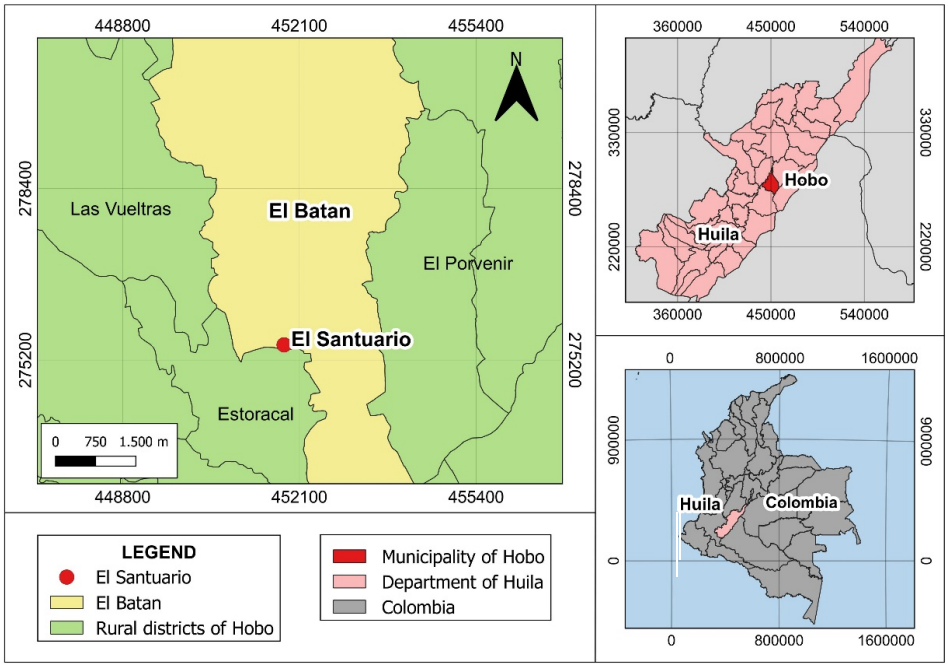

Fig. 1. Geographic location of the experimental nursery in the municipality of Hobo, Huila, Colombia.

bed (Castro-Toro et al., 2008; França et al., 2014; Mejía, 2021). At 70 days after germination (DAG), the seedlings (chapolas) were transplanted into 17 × 23 cm plastic bags filled with selected soil material from the same site, following the recommendations of González-Osorio et al. (2022), Mejía (2021), and Rivera et al. (2021).

Laboratory soil analyses indicated that the substrate contained 2.1% organic matter, 32 ppm of available phosphorus (P) determined using the Mehlich extraction method, a pH of 5.5, and 57% porosity, conditions considered suitable for coffee seedling development (Kobusinge et al., 2023; Mwendwa et al., 2022; Olivas et al., 2023). Subsequently, the seedlings were transferred to a 36 m² nursery constructed with a simple bamboo (guadua) structure and covered with 60% shade netting (dos Reis, 2005).

The fertilization plan was identical for all plants, applying 2.0 g plant^{-1} of diammonium phosphate (DAP 18–46–0) at 60 days after transplanting (DAT). The only factor that differed among treatments was the amount of mycorrhizal inoculum applied (Moreira et al., 2019; Araújo et al., 2020; Mejía, 2021; Pereira et al., 2021; León-Burgos et al., 2022).

Experimental design

The experimental design was completely randomized. A population of 400 coffee seedlings was used, with a calculated sample size of 78 plants per treatment (León-Burgos et al., 2022; Naing et al., 2022). The study included two treatments with different doses of mycorrhizae (*G.* sp.), applied at concentrations of 5 cm³ L^{-1} for T1 and 10 cm³ L^{-1} for T2, with 40 cm³ of solution applied to each plant, as well as a control treatment without mycorrhizae (T0). The commercial inoculant used was Biofermex®, a liquid formulation based on arbuscular mycorrhizal fungi (*G.* sp.). According to the manufacturer's technical information, the product contains external mycelium as the active biological component in an aqueous suspension and is intended to promote root establishment and nutrient uptake during early plant development. The manufacturer's documentation does not specify propagule concentration or spore density; therefore, these parameters could not be reported. The inoculant was applied at transplanting and subsequently reapplied at 15-day intervals as part of the nursery management protocol. For each evaluation, 13 plants were randomly selected per sampling event, with repeated measurements at 30, 45, 60, 75, 90, and 105 DAT, following the methodology described by França et al. (2014) and González-Osorio et al. (2022).

Characterization

Plant initial growth was monitored by evaluating primary root length (PRL), number

of secondary roots (NSR), stem length (SL), stem diameter (SD), total plant height (H), and total leaf area (TLA) at each sampling time, as well as total dry mass (TDM) at 105 DAT. Measurements were conducted following the recommendations of Costa et al. (2020) and León-Burgos et al. (2022), using a digital caliper and a precision ruler.

The growth rate (GR) was calculated as the ratio of growth per unit time, as described in Equation 1.

$$GR = \frac{h_2 - h_1}{t_2 - t_1} \qquad \text{Equation (1)}$$

Where: *GR* is the growth rate [cm day^{-1} or mm day^{-1}]; h_2 and h_1 correspond to final and initial growth, respectively [cm]; and t_2 and t_1 represent the final and initial growth times, respectively [days].

Leaf area was determined by measuring the length and width of the leaf blade at the central portion of each leaf (excluding the petiole), as proposed by Unigarro-Muñoz et al. (2015) in Equation 2.

$$TLA = 0.99927 * \left(L * (-0.14757 + 0.60986 * W)\right) \qquad \text{Equation (2)}$$

Where: *TLA* represents the estimated total leaf area [cm^2], *L* represents leaf length [cm], and *W* represents leaf width [cm].

Total dry mass (TDM) was determined by relating the initial and final plant weights, as indicated in Equation 3**.**

$$TDM = \left(\frac{WHI - WSF}{WHI}\right) \qquad \text{Equation (3)}$$

Where: *MST* is total dry mass [Adim], *WHI* is initial wet weight [g] y *WSF* is final dry weight [g].

Statistical analysis

Growth monitoring data were subjected to parametric statistical analysis. The assumptions of normality and homogeneity of variances were verified using the Shapiro–Wilk and Levene tests, respectively. Because plant growth was evaluated repeatedly throughout the nursery stage, each sampling date (30, 45, 60, 75, 90, and 105 DAT) was analysed independently to compare treatment effects at that specific evaluation time. For each sampling date, a one-way analysis of variance (ANOVA) was performed, followed by Tukey's honestly significant difference (HSD) test for mean separation when significant differences were detected ($P < 0.05$) (Pulido and Salazar, 2008). In addition, linear regression analysis was applied to describe growth trends over time for each treatment. Therefore, the temporal structure of the experiment was considered through successive independent evaluations rather than by fitting a repeated-measures statistical model. All statistical analyses were performed using Microsoft Office Excel® and RStudio®.

Technical and Economic analysis

For the technical and-economic analysis, production costs associated with obtaining 400 coffee seedlings at 105 DAT were evaluated, considering material and labor as the main cost components. This value was then compared with the commercial cost of the same number of seedlings in commercial nurseries within the region.

RESULTS AND DISCUSSION

After verifying the assumptions of normal distribution and homoscedasticity in the collected data, the results are presented and discussed as follows:

Root development

Arbuscular mycorrhizal colonization begins with fungal penetration of the root cortex and the subsequent formation of arbuscules, which constitute the primary sites of nutrient exchange between the fungus and the host plant. The extent of root colonization is commonly associated with improvements in phosphorus acquisition, root system development and seedling establishment, although the magnitude of these responses depends on fungal species, host genotype and environmental conditions (Chialva et al., 2023; Nie et al., 2024; Pérez-Redondo et al., 2025). Because root colonization was not quantified in the present study, these mechanisms cannot be confirmed directly and should therefore be interpreted as biological processes commonly associated with arbuscular mycorrhizal symbiosis rather than demonstrated outcomes of the present experiment.

The spatiotemporal analysis of coffee plant root development revealed a progressive increase in PRL across all treatments, with continuous growth throughout the evaluated period (Fig. 2). At all sampling dates, treatment T2 exhibited the highest root growth rate (GR; 0.09 cm day^{-1}), followed by T1 (0.08 cm day^{-1}), while the control treatment (T0) consistently showed the lowest values (0.07 cm day^{-1}). These results are consistent with those reported by León-Burgos et al. (2022), who obtained an average growth rate of 0.087 cm day^{-1}.

Differences between T1 and T2 became more evident from 45 DAT onward compared with the

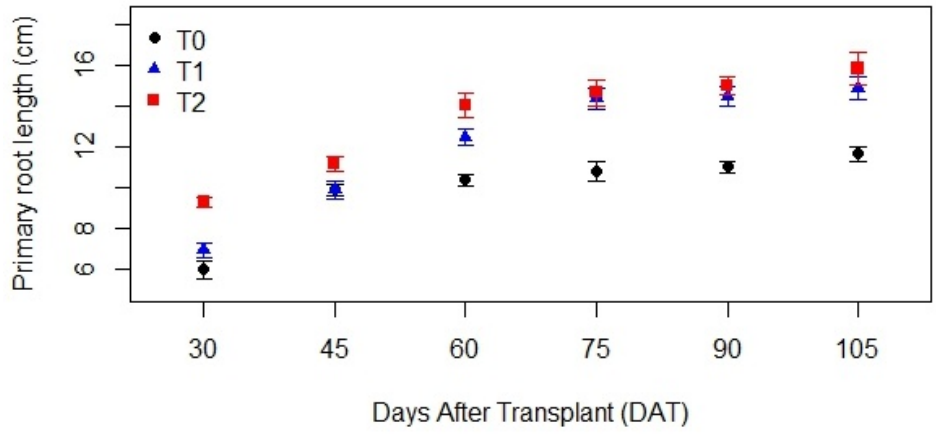

Fig. 2. Primary root length of coffee seedlings (Cenicafé 1) inoculated with arbuscular mycorrhizal fungi (*G.* sp.) at doses of 5 cm³ (T1), 10 cm³ (T2), with a non-inoculated control (T0), evaluated at different days after transplanting (DAT). Vertical bars indicate standard deviation.

control. Increases of 35.9% (15.86 cm) for T2 and 27.4% (15.25 cm) for T1 were observed relative to T0 (12.12 cm). These results are comparable to those reported by León-Burgos et al. (2022), Domínguez and Chávez (2023), and Castro and Memenza (2024), who recorded primary root lengths of 21.0 cm at 100 DAT, 22.19 cm at 93 DAT, and 21.05 cm at 90 DAT, respectively, under applications of 15 and 20 g plant^{-1}, and without mycorrhizal application. These discrepancies may be attributed to differences in growing conditions, plant material, substrate type, container volume, as well as the concentration and type of mycorrhizal inoculum used. Although these studies provide useful benchmarks for seedling growth under nursery conditions, direct comparisons should be interpreted with caution because they differ in the mycorrhizal species or inoculum employed, inoculation strategy, substrate characteristics, and environmental conditions. Therefore, the comparisons presented here are intended to place the magnitude of the observed responses within the range reported for coffee seedlings rather than to establish direct equivalence between experimental systems.

Another important factor is the number of secondary roots comprising the root system. Initially, NSR results showed no significant differences ($p > 0.05$) among treatments at 30 DAT; only after the third sampling (60 DAT) were graphical and statistically significant differences observed among treatments (Fig. 3).

A parallel increase in NSR was observed between 30 and 45 DAT, followed by a progressive rise among treatments from 75 to 105 DAT. This increase coincided with the application of DAP fertilizer at 60 DAT. Previous studies have reported that arbuscular mycorrhizal fungi may enhance phosphorus uptake (Hernández-Acosta et al., 2020); however, because root colonization was not quantified in the present study, this mechanism cannot be confirmed for the observed responses. At 105 DAT, the mean NSR values for each treatment were 90.0 (T2), 79.4 (T1), and 62.3 (T0) roots per plant, representing statistically significant increases of 44.4% for T2 and 27.4% for T1 compared with the control (T0).

Overall, the greater primary root length and secondary root production observed in the inoculated treatments indicate that mycorrhizal inoculation was consistently associated with enhanced root system development throughout the nursery stage. Beyond increasing root size, the earlier differentiation of secondary roots observed from 60 DAT suggests a progressive modification of root architecture, which may increase the volume of soil explored by the seedling and improve the potential for water and nutrient acquisition (Cuervo, 2017; Guo et al., 2024; Nie et al., 2024). Similar coordinated responses between root architectural development and subsequent plant growth have been reported in mycorrhiza-inoculated coffee seedlings under controlled conditions (Silveira et al., 2023; Chialva et al., 2023). Nevertheless, because mycorrhizal root colonization was not quantified in the present study, these morphological responses should be interpreted as being associated with the inoculation treatment rather than as direct evidence of successful fungal establishment or functional symbiosis.

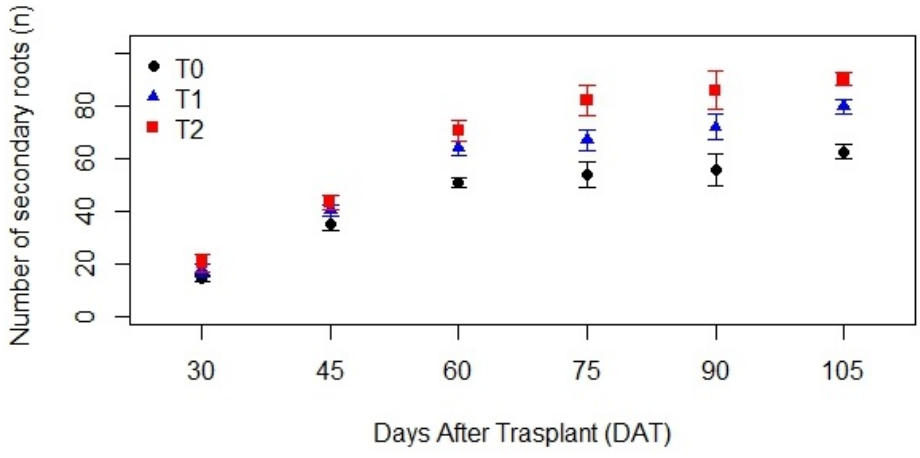

Fig. 3. Number of secondary roots in coffee seedlings (Cenicafé 1) inoculated with arbuscular mycorrhizal fungi (*G.* sp.) at doses of 5 cm³ (T1), 10 cm³ (T2), with a non-inoculated col (T0), evaluated at different days after transplanting (DAT). Vertical bars indicate standard deviation.

Stem development

Stem length (SL) of coffee seedlings showed a progressive increase throughout the evaluation period in all treatments, indicating continuous growth as DAT progressed (Fig. 4). However, clear differences were observed among treatments, with the most pronounced increase occurring in seedlings inoculated with mycorrhizal fungi.

Treatment T2 consistently exhibited the highest SL values throughout the evaluation period, with an average GR of 0.062 cm day^{-1}, followed by T1 (0.052 cm day^{-1}), while the control treatment (T0) showed the lowest values (0.049 cm day^{-1}). This trend suggests a positive effect of mycorrhizal inoculation on longitudinal stem growth, particularly at the higher dose evaluated.

The regression models fitted for each treatment showed a strong linear relationship between stem length (SL) and time, with high coefficients of determination ($R^2 \geq 0.97$), indicating a good fit of the data to the models and a consistent stem growth response over time. At the end of the study (105 DAT), T2 exhibited the highest slope, with a statistically significant difference of 21.33% (SL = 11.89 cm), followed by T1 with an 8.67% difference (SL = 10.65 cm) compared with the control treatment (SL = 9.8 cm).

These results are consistent with those of Silveira et al. (2023), who reported stem growth of 34.1 cm at 200 DAT with the application of *G.* spp.; Moreira et al. (2019) observed growth of 24.5 cm at 150 DAT with the application of *Dentiscutata heterogama*; and León-Burgos et al. (2022), who recorded 12 cm of growth at 100 DAT in the absence of mycorrhizal inoculation.

Similarly, SD in coffee seedlings showed a progressive increase throughout the evaluation period, with an average GR of 0.019 mm day^{-1} for T2, followed by T1 with 0.017 mm day^{-1} and T0 with 0.016 mm day^{-1} (Fig. 5).

Additionally, statistically significant differences were observed between treatments at specific time points. At 45 DAT, treatments T2 and T1 showed increases of 30.38% (SD = 2.65 mm) and 20.69% (SD = 2.45 mm), respectively, compared with T0 (SD = 2.03 mm). Similarly, at 60 DAT, increases of 21.59% (SD = 3.02 mm) in T2 and 14.11% (SD = 2.83 mm) in T1 were recorded relative to T0 (SD = 2.48 mm). These results are consistent with those reported by León-Burgos et al. (2022) and Silveira et al. (2023), who recorded SD values of 4.0 and 4.87 mm, respectively, at 200 DAT, both with and without mycorrhizal inoculation (*G.* spp.).

Overall, the greater stem elongation observed in inoculated seedlings indicates that the treatment was consistently associated with enhanced shoot development during the nursery stage. The stronger response observed for stem length than for stem diameter suggests that, under the experimental conditions, biomass allocation preferentially favored longitudinal growth during the early vegetative phase, when rapid canopy establishment is essential for subsequent leaf expansion and light interception. Similar patterns have been reported in coffee seedlings inoculated with arbuscular mycorrhizal fungi, where improvements in shoot growth occur concurrently with enhanced root development and increased vegetative vigor (Kalamulla et al.,

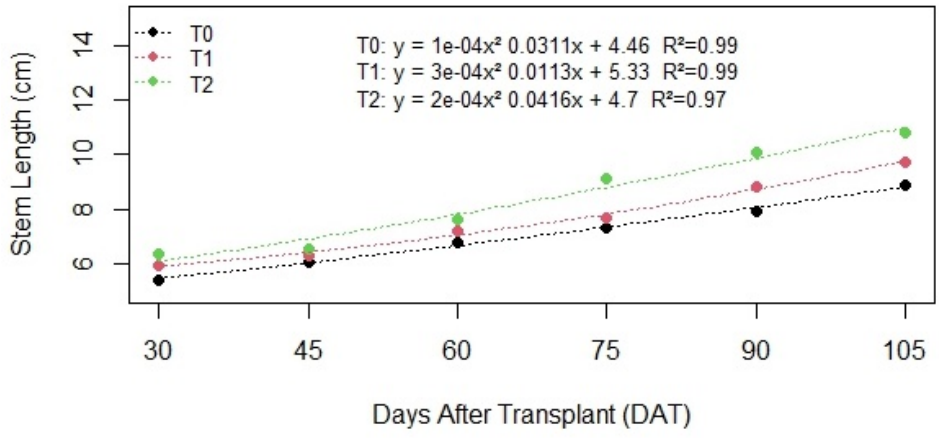

Fig. 4. Stem growth curve in coffee seedlings (Cenicafé 1) inoculated with arbuscular mycorrhizal fungi (*G.* sp.) at doses of 5 cm³ (T1), 10 cm³ (T2), with a non-inoculated control (T0), evaluated at different days after transplanting (DAT).

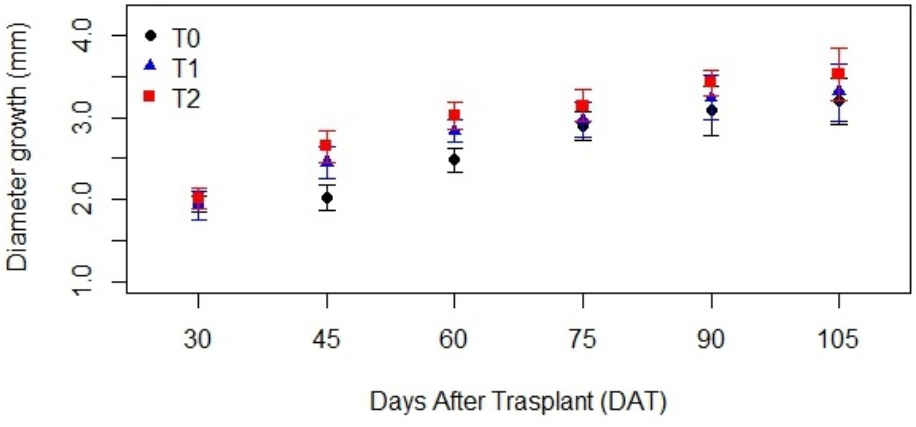

Fig. 5. Stem diameter of coffee seedlings (Cenicafé 1) inoculated with arbuscular mycorrhizal fungi (*G.* sp.) at doses of 5 cm³ (T1), 10 cm³ (T2), with a non-inoculated control (T0), evaluated at different days after transplanting (DAT).

2022; Silveira et al., 2023; Chialva et al., 2023). In contrast, stem diameter exhibited smaller treatment differences, indicating that radial growth was less responsive than stem elongation during the evaluation period. Nevertheless, because mycorrhizal root colonization was not quantified, these responses should be interpreted as associated with the inoculation treatment rather than as direct evidence of successful fungal establishment or functional symbiosis.

Leaf development

Fig. 6 shows the evolution of TLA (cm²) of coffee seedlings over DAT, where all treatments exhibited accelerated growth, as indicated by the quadratic nature of the fitted functions. Furthermore, the coefficients of determination (R^2) were high (0.96 for T0 and T1, and 1.0 for T2), indicating that the fitted models adequately describe the observed leaf growth dynamics and fall within the range of values reported by Urriago-Muñoz et al. (2015) and León-Burgos et al. (2022) for the same evaluation model.

From 60 DAT onward, treatment effects became increasingly evident, with T2 exhibiting the highest leaf development (141.05 cm²), followed by T1 (95.36 cm²). At the end of the evaluation period, these treatments increased leaf area by 84.5% and 24.7%, respectively, compared with T0, which exhibited the lowest value (76.45 cm²).

These findings are consistent with those

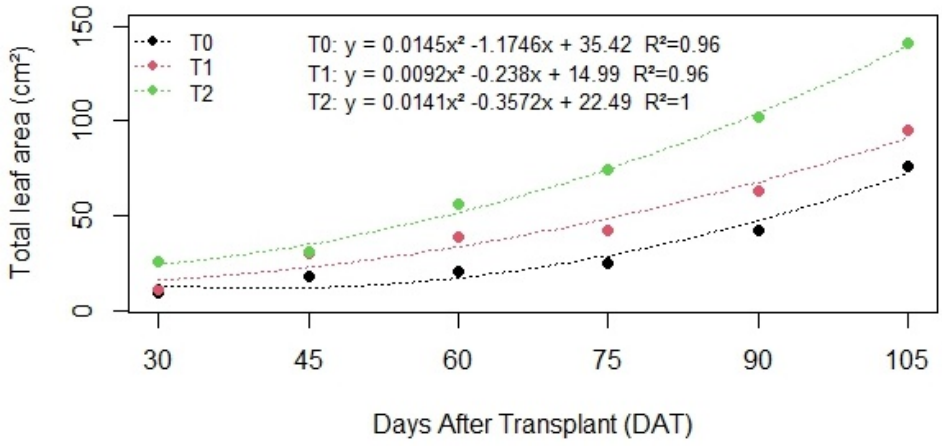

Fig. 6. Leaf area of coffee seedlings (Cenicafé 1) inoculated with arbuscular mycorrhizal fungi (*G.* sp.) at doses of 5 cm³ (T1), 10 cm³ (T2), with a non-inoculated control (T0), evaluated at different days after transplanting (DAT).

reported by León-Burgos et al. (2022), who observed an average TLA of 130 cm² at 90 DAT, as well as with Araújo et al. (2020), who reported significant increases in leaf area in coffee seedlings inoculated with Rhizophagus clarus. Similarly, França et al. (2014) reported leaf area values between 100 and 150 cm² at 105 DAT, with the highest values occurring in inoculated plants (G. clarum). From a physiological perspective, leaf expansion represents one of the main determinants of the photosynthetically active surface available for biomass production during the nursery stage. Therefore, the greater TLA observed in T2 from 60 DAT onwards suggests that inoculated seedlings developed a larger assimilatory surface capable of supporting subsequent plant growth. This temporal pattern is consistent with recent studies in coffee demonstrating coordinated responses between root development, shoot growth, and leaf expansion following mycorrhizal inoculation (Silveira et al., 2023; Chialva et al., 2023). Nevertheless, because mycorrhizal root colonization was not quantified in the present study, these responses should be interpreted as being associated with the inoculation treatment rather than as direct evidence of enhanced physiological performance mediated by an established mycorrhizal symbiosis.

Dry mass

The results for root dry mass (RDM), shoot dry mass (SDM), and TDM showed significant differences ($p \leq 0.05$) among the evaluated treatments (Table 1). In RDM, treatment T2 exhibited the highest mean value (0.82 ± 0.014 g), differing significantly by 8.08% from T0 (0.76 ± 0.010 g), while T1 (0.79 ± 0.015 g) displayed intermediate values, not differing statistically from either T2 or T0.

Similarly, SDM was highest in T2 (0.75 ± 0.010 g), showing a significant difference of 3.47% compared with T0 (0.72 ± 0.005 g), while T1 (0.74 ± 0.004 g) had intermediate values. The greater accumulation of root, shoot and total dry mass observed in T2 indicates that the inoculation treatment was associated with a more efficient conversion of plant resources into structural biomass during the nursery stage. Rather than representing isolated increases in individual organs, the simultaneous improvement in root and shoot dry mass suggests a coordinated growth response consistent with previous reports in coffee seedlings inoculated with arbuscular mycorrhizal fungi (González-Osorio et al., 2022; Kalamulla et al., 2022; Silveira et al., 2023; Guo et al., 2024; Nie et al., 2024; Gomes et al., 2025). Similar responses have also been reported across a wide range of plant species, where a meta-analysis demonstrated that AMF inoculation significantly increases root, shoot and total dry biomass through improvements in root morphology and nutrient acquisition, supporting biomass accumulation under contrasting environmental conditions (Chandrasekaran, 2022). Furthermore, greater biomass production has been associated with integrated improvements in root development, leaf expansion and nutrient acquisition, allowing plants to sustain greater carbon assimilation and vegetative growth (Chialva et al., 2023). Nevertheless, because mycorrhizal root colonization was not quantified in the present study, these responses should be interpreted as

Table 1. Dry mass of coffee seedlings (Cenicafé 1) inoculated with arbuscular mycorrhizal fungi (*G.* sp.) at doses of 5 cm³ (T1), 10 cm³ (T2), with a non-inoculated control (T0), evaluated at 105 days after transplanting (DAT).

Parameter	Treatmen	Mean
Root Dry Mass [Adim]	T2	0.82 ± 0.014 a
	T1	0.79 ± 0.015 ab
	T0	0.76 ± 0.010 b
Shoot Dry Mass [Adim]	T2	0.75 ± 0.010 a
	T1	0.74 ± 0.004 ab
	T0	0.72 ± 0.005 b
Total Dry Mass [Adim]	T2	1.57 ± 0.017 a
	T1	1.53 ± 0.018 b
	T0	1.48 ± 0.013 c

Different superscript letters within the same column indicate significant differences ($p < 0.05$).

being associated with the inoculation treatment under the experimental conditions rather than as direct evidence of functional mycorrhizal establishment.

Consequently, TDM followed the same pattern, with T2 reaching the highest mean value (1.57 ± 0.017), followed by T1 (1.53 ± 0.018) and T0 (1.48 ± 0.013). Statistical differences confirmed that treatment T2 resulted in a 5.83% increase in total biomass accumulation compared with the control, while T1 showed an intermediate effect, with a 2.88% increase.

Overall, the results indicate that treatment T2 was the most effective in enhancing dry biomass production, both in individual organs and in the whole plant. This behavior suggests that this treatment optimizes growth conditions, favoring improved distribution and accumulation of dry matter (Kalamulla et al., 2022; Wu et al., 2024; Xu et al., 2024).

Technical and economic analysis

The technical and economic analysis assessed the production costs of 400 coffee seedlings, Cenicafé 1 variety in a temporary nursery. This analysis included labor and material costs associated with nursery establishment, which had sufficient capacity to accommodate up to twice the number of seedlings produced. Materials accounted for 56.07% of the total investment, while labor costs reached a value of 35.22 USD (Table 2).

According to the sale prices in the northern zone of the Huila Department, the average cost of Cenicafé 1 coffee seedlings for planting ranges between 500 and 600 Colombian Pesos (COP) in 2023 (Table 3). In the present study, costs increased by 46.62% (USD 51,02, primarily due to labor associated with loading, transportation, and unloading the seedlings at the crop establishment site. This additional cost would apply if the coffee seedlings were purchased from the nursery closest to the study site.

Purchasing coffee seedlings from nurseries located near the planting site is 36.34% (USD 29.14) more expensive than producing seedlings using conventional methods. In addition to the higher cost, the potential for seedling damage during loading, transport, and unloading should be considered (Lee et al., 2012; Afsharnia and Moosavi, 2021; Zeeshan et al., 2024; Carlin et al., 2025).

CONCLUSIONS

The use of arbuscular mycorrhizal fungi in coffee seedlings of the Cenicafé 1 variety during the nursery stage resulted in a significant increase in primary root length (PRL) of 35.9% for T2 and 27.4% for T1. Consistently, an increase in the number of secondary roots (NSR) was also observed, reaching 44.4% in T2 and 27.4% in T1, when applying doses of 10 and 5 cm³ L^{-1} of mycorrhizae, respectively.

The mycorrhizal inoculation treatment was associated with significant increases in stem length (SL) and total leaf area (TLA), reaching 21.33 and 84.5% for T2, and 8.67 and 24.7% for T1, respectively, compared with the non-inoculated control.

Additionally, increasing the mycorrhizal dose promoted a 2.95% increase in total dry mass (TDM) of coffee seedlings in the nursery stage under treatment T2 compared with T1.

Table 2. Production costs associated with establishing the temporary nursery at the "El Santuario" farm, Hobo, Huila.

Materials	Unit	Quantity	Unit Price	Total Price
Guadua 3'x6 m	Unit	3	$7,000.00	$21,000.00
60% Shade Net	m^2	4	$5,000.00	$20,000.00
Coffee Seedling Bags 17 × 23 cm, 100 Unit	Package	4	$9,700.00	$38,800.00
Cenicafé 1 Variety Seeds	kg	1	$48,000.00	$48,000.00
Biofermex®	L	1	$46,080.00	$46,080.00
Twine Cone	Unit	1	$5,000.00	$5,000.00
			Materials Cost	$178,880.00
Labor Cost	**Unit**	**Quantity**	**Unit Price**	**Total Price**
Germinator Construction	h	2	$4,833.33	$9,666.67
Land Preparation	h	4	$4,833.33	$19,333.33
Nursery Construction	h	6	$4,833.33	$29,000.00
Substrate Preparation	h	4	$4,833.33	$19,333.33
Filling of Bags	h	5	$4,833.33	$24,166.67
Transplanting of Seedlings	h	4	$4,833.33	$19,333.33
Fertilization	h	1	$4,833.33	$4,833.33
Nursery Cleaning	h	3	$4,833.33	$14,500.00
			Labor Cost	$140,136.67
Prices as of July 26, 2023			**Total Value in COP**	$319,046.67
USD Exchange Rate: $3,978.83 COP			**Total Value in USD**	USD 80.19
Daily Labor Rate (8 h): $38,666.67 COP				

Table 3. Costs of purchasing Cenicafé 1 coffee seedlings from commercial nurseries.

Materials	Unit	Quantity	Unit Price	Total Price
Coffee seedling at 105 DPT	Unit	400	$ 550.00	$ 232,000.00
Loading seedlings at the nursery	Unit	400	$ 50.00	$ 20,000.00
Transportation	Unit	1	$ 175,000.00	$ 175,000.00
Unloading seedlings at the farm	Unit	400	$ 50.00	$ 20,000.00
Prices as of July 26, 2023			**Total Value in COP**	$ 435,000.00
USD Exchange Rate: $3,978.83 COP			**Total Value in USD**	US 109.33

Finally, beyond demonstrating the positive growth responses observed under the experimental conditions, this study provides experimental validation of arbuscular mycorrhizal inoculation under commercial temporary nursery conditions in southern Colombia. The incorporation of a technical and economic assessment further extends the contribution of the study by demonstrating the practical feasibility of this management strategy, showing that local seedling production with appropriate mycorrhizal management can reduce production costs by USD 29.14 compared with purchasing seedlings from external nurseries, thereby supporting its adoption in regional coffee production systems.

One limitation of this study is that mycorrhizal root colonization was not quantified. Therefore, although significant improvements in root development, shoot growth, leaf area, and biomass were observed in inoculated seedlings, the successful establishment of the symbiosis could not be directly verified through colonization indicators. Consequently, the beneficial responses reported here should be interpreted as being associated with the mycorrhizal inoculation treatment rather than as direct evidence of root colonization. Additionally, all seedlings were established in a germination substrate disinfected with Tricho-D®, a Trichoderma-

based commercial product reported to promote root development. Because this treatment was uniformly applied to all experimental units before mycorrhizal inoculation, it does not affect treatment comparisons but may have contributed to the overall growth of the seedlings and should therefore be considered when interpreting the absolute magnitude of the observed responses.

Because the nursery substrate consisted of non-sterilized field soil, indigenous soil microorganisms, including native arbuscular mycorrhizal fungi and other microbial communities, may have been present in all treatments. However, since the same substrate was uniformly used across all experimental units, this condition did not affect treatment comparisons, although it may have influenced the overall biological response observed during seedling development.

Future studies should incorporate quantitative assessments of mycorrhizal colonization to strengthen the biological interpretation of these responses. In addition, future experiments should compare different commercial inoculants and native arbuscular mycorrhizal communities under contrasting nursery substrates and environmental conditions to determine whether the responses observed in the present study can be generalized across production systems.

Declaration on the use of generative Ai

Generative AI tool: ChatGPT. Version: GPT-5.5. Sections of the manuscript: Results and Discussion, Conclusions, and Response to Reviewers. Specific purpose: The generative AI tool was used to improve the scientific writing, language clarity and grammatical quality of the manuscript, and to improve the overall consistency of the revised version. All scientific interpretations, revisions, and final decisions were critically reviewed and approved by the authors.

Author contributions

Carlos Alberto Garzón-Pajoy: Investigation, Data curation, Formal analysis, Writing – original draft. Jordan Alexis Castillo-Coronado: Formal analysis, Visualization, Writing – review & editing. Juan Gonzalo Ardila-Marín: Conceptualization, Methodology, Supervision, Project administration, Writing – review & editing. Diana Carolina Polanía-Montiel: Conceptualization, Methodology, Supervision, Formal analysis, Writing – review & editing. Jean Carlos Acosta-Vargas: Data curation, Formal analysis, Writing – review & editing. All authors reviewed and approved the final version of the manuscript.

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