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Original Article

Evaluation of arbuscular mycorrhizal fungi isolated from the rhizosphere of two wild plants inoculated in *Zea mays* and *Phaseolus vulgaris*, crops of economic importance

Evaluación de hongos micorrízicos arbusculares aislados en la rizosfera de dos plantas silvestres inoculados en *Zea mays* y *Phaseolus vulgaris*, cultivos de importancia económica

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Abstract

Arbuscular mycorrhizal fungi [AMF] establish mutualistic symbioses with plant roots, enhancing nutrient and water uptake in exchange for carbohydrates, which boosts plant growth, stress resistance, and ecosystem health. This study focused on developing a biological inoculant from native AMF of a dry forest, utilizing *Rhynchosia minima* and *Cenchrus echinatus* as trap plants for their collection. The methodology involved the identification of arbuscular mycorrhizae from the rhizospheric soil of each plant species, where root and soil samples were collected, followed by the preparation of the inoculum with antibiotics (gentamicin and streptomycin). Inoculation was performed by direct application of the inoculum to the roots of maize (*Zea mays*) and bean (*Phaseolus vulgaris*) plants in a randomized block design with three treatments (T1 = control without mycorrhizal inoculum; T2 = mycorrhizae + antibiotic; T3 = mycorrhizal inoculum only) and six replicates; the sterilized substrate consisted of a 1:1 ratio of peat and sand. Plant growth and mycorrhizal colonization were evaluated and compared using Student's t-test. The results revealed a mycorrhizal colonization exceeding 50% and a spore abundance (101–132 spores g^{-1} of soil) in the trap plants, with a predominance of the genera *Acaulospora*, *Glomus*, *Gigaspora*, and *Scutellospora*. Inoculation with these native AMF increased colonization in maize (32%) and beans (37%) compared to the non-inoculated controls of each species, demonstrating the effectiveness of the developed inoculant. In conclusion, the use of native arbuscular mycorrhizal fungi represents an effective strategy to improve the growth of important crops such as maize and beans, contributing to more sustainable agricultural practices.

Keywords: biofertilizer, bioprospecting, mycorrhizal colonization, spores, symbiosis.

Resumen

Los hongos micorrízicos arbusculares [HMA] establecen simbiosis mutualistas con las raíces de las plantas, mejorando la absorción de nutrientes y agua a cambio de carbohidratos, lo que potencia el crecimiento vegetal, la resistencia al estrés y la salud del ecosistema. Este estudio se enfocó en desarrollar un inoculante biológico a partir de HMA nativos de un bosque seco, utilizando *Rhynchosia minima* y *Cenchrus echinatus* como plantas trampa para su obtención. La metodología implicó la identificación de las micorrizas arbusculares de suelo rizosférico de cada especie vegetal, donde se recolectó muestras de raíz y suelo, para luego realizar preparación del inóculo con antibióticos (gentamicina y estrepto-

micina). La inoculación se realizó mediante la aplicación directa del inóculo a las raíces de plantas de maíz (*Zea mays*) y fréjol (*Phaseolus vulgaris*) en un diseño de bloques aleatorizados con tres tratamientos (T1 = control sin inóculo de micorrizas; T2 = micorrizas + antibiótico; T3 = solo con inóculo micorrizas) y seis réplicas, el sustrato fue esterilizado estaba constituido por una proporción 1:1 de turba y arena. El crecimiento vegetal y la colonización micorrízica se evaluaron y compararon mediante la prueba t de Student. Los resultados revelaron una colonización micorrízica superior al 50% y una abundancia de esporas (101-132 esporas g^{-1} de suelo) en las plantas trampa, con predominio de los géneros *Acaulospora*, *Glomus*, *Gigaspora* y *Scutellospora*. La inoculación con estos HMA nativos incrementó la colonización en maíz (32%) y fréjol (37%) en comparación con los controles de cada especie no inoculados, demostrando la efectividad del inoculante desarrollado. En conclusión, el uso de hongos micorrízicos arbusculares nativos representa una estrategia eficaz para mejorar el crecimiento de cultivos importantes como el maíz y el fréjol, contribuyendo a prácticas agrícolas más sostenibles.

Palabras clave: biofertilizante, bioprospección, colonización micorrízica, esporas, simbiosis.

1. Introduction

Arbuscular mycorrhizal fungi [AMF] are essential symbiotic microorganisms for sustainable agriculture, acting as biostimulants by promoting plant growth and as bioprotectors by enhancing plant tolerance to environmental stress (Shi et al., 2023). Their ecological importance extends beyond these direct benefits to plants. Extensive mycorrhizal networks have been shown to contribute to carbon sequestration, with plants transferring approximately 13 gigatons of CO_2 per year to these fungi (Hawkins et al., 2023). This process highlights the crucial role of mycorrhizae in ecosystem functioning, providing services that go far beyond simple plant nutrition. Therefore, understanding and harnessing these complex symbioses is essential for developing more sustainable agricultural and forestry practices capable of mitigating plant stress, optimizing nutrient acquisition, and contributing to planetary health (Martin et al., 2024).

To unravel the complex framework of the ecophysiology and ecosystem services provided by the different types of arbuscular mycorrhizae, it is essential to conduct large-scale trials complemented by detailed analyses under controlled conditions (Tedersoo & Bahram, 2019). The composition of fungal communities colonizing plant roots is not random but appears to result from a dual influence: fungal selection by the plant and the pressures exerted by the environment (Noguchi & Toju, 2024). This intricate relationship, particularly evident in ectomycorrhizal fungi, shows sensitivity to environmental changes in nutrient-rich environments (Zhang et al., 2024), with significant consequences for the dynamics of plant communities. The presence of mycorrhizal fungi can, in fact, profoundly alter their composition and structure (Tedersoo et al., 2020). The specialization of soil microbes in association with specific wild plants emerges as a key factor for the successful establishment of new seedlings, acting as a mechanism that shapes the composition of plant communities over time (Eck et al., 2019).

AMF inoculation induces beneficial early physiological responses in plants subjected to saline-alkaline stress. Fifteen days after inoculation, improved root development and increased production of secondary metabolites have been observed (Fan et al., 2024). Similarly, recent studies have demonstrated that inoculation with *Funneliformis mosseae* stimulates metabolic pathways enriched in fructose and mannose, as well as the biosynthesis of fatty acids and flavonoids (Shao et al., 2023). This early metabolic activation may be associated with greater phosphorus [P] acquisition efficiency during the initial stages of plant development, particularly in the presence of established connectivity between communities of *Rhizophagus* sp. and *Streptomyces* sp. (Jin et al., 2024). However, fertilization practices in conventional agricultural systems may negatively affect AMF diversity, resulting in a reduction in the number of species (Ma et al., 2021).

Whereas chemical fertilization tends to reduce plant dependence on their association with AMF, organic fertilization appears to strengthen this symbiosis in agricultural systems (Liu et al., 2020). In fact, organic production systems promote complex interactions between bacteria and fungi, fostering a more dynamic soil microbial community (Matteoli et al., 2024). It is important to note that the process of root colonization by AMF involves the recruitment of a specific microbial assemblage, suggesting the existence of a distinctive microbial signature associated with the AMF of each plant (Zhou et al., 2020). The objective of this study was to develop a biological inoculant from native arbuscular mycorrhizal fungi collected from a remnant of tropical dry forest, using two wild plant species as trap hosts, to improve the growth of two crops of major agricultural importance: maize and common bean.

2. Materials and Methods

2.1. Study area

The exploration of arbuscular mycorrhizae in wild plant species was conducted at the Guayaquil campus of the Universidad Politécnica Salesiana, within the forest remnant located on the María Auxiliadora Campus at kilometer 19 of Vía a la Costa (Delgado-Bohórquez et al., 2024). A census of the most representative plant species in the study area was carried out at coordinates 2°11′55″S, 80°02′31″W. From the geographic sampling point, three circular plots with a diameter of five meters were established to identify two plant species, one belonging to the Fabaceae family and the other to the Poaceae family. Plant species were identified in the field using the PlantNet application developed by the Tecnológico de Costa Rica, Ecuadorian databases such as BioWeb (https://bioweb.bio/), and the *Catalogue of the Vascular Plants of Ecuador* from the Missouri Botanical Garden (https://www.mobot.org/mobot/research/ecuador/welcomesp.shtml) (Naranjo-Moran et al., 2024). Based on the mean values obtained from the circular plots, the Margalef and Shannon plant diversity indices were calculated.

The Margalef Index [DMg] focuses on species richness, that is, the number of species present in the sample, and is adjusted according to the total sample size (equation [1]). Where *S* is the total number of species present in the sample, *N* is the total number of individuals of all species in the sample, and *ln(N)* is the natural logarithm of the total number of individuals.

$$DMg = \frac{S-1}{\ln(N)} \quad [1]$$

The Shannon Index [H] considers both the number of species and the evenness of their abundance distribution. A community with many species and similar relative abundances will have a high *H* value (equation [2]).

$$H = -\sum_{i=1}^{S}(\rho_i \times \ln(\rho_i)) \quad [2]$$

Where *S* is the total number of species present in the sample, *pi* is the proportion of individuals of species *i* relative to the total number of individuals (*pi* = *ni* / *N*, where *ni* is the number of individuals of species *i* and *N* is the total number of individuals of all species), *ln*(*pi*) is the natural logarithm of the proportion of species *i*, and ∑ indicates the summation over all species from *i* = 1 to *S*.

2.2. Analysis of the mycorrhizal symbiosis

A small inventory of the Fabaceae (legume) and Poaceae (grass) species was conducted in the study area to identify the dominant and abundant species in the tropical dry forest remnant where the inoculum was to be obtained. This involved identifying the species that constituted a significant proportion of the plant biomass within an area of 500 square meters. Different habitat types were considered, including grasslands, roadsides, disturbed areas, and native vegetation. Plant floristic compositions may be abundant in specific niches, with the Poaceae family frequently dominating herbaceous shrubland communities (Bonifaz, 2023).

Once one trap species from each plant family had been selected, root (60 g) and soil (600 g) samples were collected in triplicate. The mycorrhizal symbiosis was verified by staining colonized roots with trypan blue. Fine roots were collected and thoroughly washed with tap water using a 250 µm sieve. After washing, the roots were placed in a 10 mL amber container, and a 10% KOH solution was added, followed by autoclaving for 30 minutes. The solution was then discarded, and the roots were treated with 9% hydrogen peroxide for 30 minutes. After another thorough washing, the roots were immersed in 1% HCl for 30 minutes. The excess solution was removed, and finally, a 0.05% trypan blue solution in lactoglycerol was added (Ho-Plágaro et al., 2020), followed by autoclaving for 30 minutes. The stained roots were then mounted on microscope slides with coverslips and examined under a compound microscope at 40× magnification. The percentage of root colonization for each sample was determined using the formula proposed by Sieverding (1983) (equation [3]).

$$Colonization\ (\%) = \frac{No\ of\ infected\ plots}{No\ of\ observed\ pltos} \times 100 \quad [3]$$

To determine the number of spores in the rhizosphere, 100 g of soil surrounding the roots of the trap plants was used. The soil was placed in a 1 L beaker, 500 mL of water was added, the suspension was stirred, and the volume was adjusted to 1 L. The suspension was then poured three times through a series of sieves with mesh sizes of 250, 106, and 45 µm. The sediment retained in the final sieve was collected into a 50 mL Falcon tube. A sucrose and Tween 20 solution was prepared, and each tube was filled with 10 mL of the sample and 10 mL of the sucrose solution before being centrifuged at 500 rpm for 5 minutes using a Thermo Scientific™ centrifuge (Sieverding et al., 1991). For taxonomic characterization, the most distinctive spores were mounted on microscope slides and iden-

tified to the genus level using the *International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi* [INVAM], hosted by the University of Kansas, United States (https://invam.ku.edu/), and the website of Professor Janusz Błaszkowski (http://www.zor.zut.edu.pl/Glomeromycota_2/Blaszkowski%20Janusz.html), affiliated with the West Pomeranian University of Technology, Szczecin, Poland. The identification of mycorrhizal fungal spores was based on microscopic observation of their morphological characteristics, including spore shape, size, and wall color. Particular attention was given to the wall structure, including its layers and ornamentation, as well as the morphology of the subtending hypha and the presence of accessory structures. Finally, the reaction to Melzer's reagent complemented the analysis, allowing the differentiation of AMF genera and species.

2.3. Production of the mycorrhizal inoculum

The native mycorrhizal inoculum from Poaceae and Fabaceae was obtained using the wet-sieving method described by Sieverding (1991). The AMF inoculum consortium from each trap species (*Rhynchosia minima* and *Cenchrus echinatus*) exhibited a cosmopolitan distribution, consisting of genera with a high capacity to adapt to diverse edaphoclimatic conditions. Among these, the genus *Glomus* stands out for its ubiquity and efficiency in colonizing a wide range of plant species. The cosmopolitan inocula obtained also contained the genera *Claroideoglomus* and *Funneliformis*, although in lower abundance. These genera are recognized for their ability to improve plant nutrition and enhance stress tolerance in different agricultural and natural ecosystems (Bilgili, 2025). The consortium tends to be multifaceted, as exemplified by *Gigaspora margarita*, which exerts a cascade-like effect on the symbiosis with the host plant it colonizes (Bonfante, 2022). The selection of these genera was based on their proven effectiveness and their capacity to establish functional symbioses with a wide diversity of plant species worldwide. Once the spore inocula had been extracted from each plant species, they were stored in a 50 mL Falcon tube, to which 20 mL of an antibiotic solution containing 280 mg of gentamicin and 1 g of streptomycin was added (Pérez et al., 2011). The spores were subjected to this antibiotic treatment to reduce the bacterial load and were left to stand for 2 hours. Each inoculum contained an average of 546 spores suspended in 25 mL of distilled water. Finally, the inocula were stored in a refrigerator at 10 °C until they were used for bare-root inoculation of maize and common bean seedlings.

2.4. Experimental design

To evaluate the effectiveness of this inoculant, an experimental trial was conducted with two randomized blocks and three treatments: T1 (control without mycorrhizae), T2 (mycorrhizae + antibiotic), and T3 (mycorrhizae only), with six replicates for each treatment. Individual seeds of hard yellow hybrid maize variety "Hércules" and "Rojo moteado" common bean were used, which were obtained from a local agricultural supply store. Maize and bean seeds were germinated in sphagnum peat A2 substrate, Novarbo brand, under shade-house conditions. Two rigid black plastic seedbeds with 120 cells and dimensions of 30 × 50 cm were used. The environmental conditions inside the shade house included temperatures ranging from 25–32 °C and high relative humidity between 70–90%. Although light intensity was high due to the equatorial location, it was reduced by 55–65% using shade nets. Rainfall varied seasonally, and the experiment was conducted during January and February 2024. Seedlings with one true leaf were transplanted into terracotta-colored plastic pots, No. 15, with dimensions of 15 × 14 cm and a volume of 1.35 L. Subsequently, sterilized substrate containing peat and river sand in a 1:1 ratio was added.

At the time of transplantation, a double inoculation was applied: the first consisted of 25 mL of mycorrhizal inoculum placed in the pothole before planting, and the second involved immersing the seedling in 250 mL of each inoculum solution before transplanting into the pot, in order to ensure greater connection with the bare root system. For treatment T2, the inoculum was mixed with a solution prepared with gentamicin (280 mg) and streptomycin (1 g) to reduce the microbial load. In other words, the inoculation of this treatment aimed to reduce the presence of contaminating microorganisms that could compete with AMF establishment or negatively affect plant growth. This treatment sought to ensure more efficient and pure colonization by the desired mycorrhizae. To maintain plant nutrition, 70 mL of complete fertilizer Fertisol® Inicio (10 N; 40 P; 10 K) from Walco S.A. was applied weekly at 10:00 a.m., at a dose of 1 gram per liter of water. Applications were carried out after routine irrigation and after seedling establishment. Growth evaluations, such as plant height and number of leaves, were performed weekly, along with chlorophyll measurements using the TYS-4N equipment, nitrogen content (mg g^{-1}), and the percentage of mycorrhizal colonization assessed at the end of the experiment, which lasted four weeks (Rodríguez-Larramendi et al., 2022; Chávez-Hernández et al., 2021).

2.5. Statistical analysis

Data analysis was performed using analysis of variance (ANOVA) followed by Tukey's test ($p < 0.05$) (Balzarini et al., 2015; Correa-Álvarez et al., 2019) in InfoStat Statistical Software (2017).

3. Results and Discussion

3.1. Selection of ruderal plants as trap species for arbuscular mycorrhizae

As a result of the circular census conducted at the sampling point, 23 plant species belonging to eight botanical families were identified, as shown in Table 1. The most abundant species in the studied area within the Fabaceae and Poaceae families were *Rhynchosia minima* L., with 80 individuals, and *Cenchrus echinatus* L., with 149 individuals on average in the circular plots. The plant *R. minima* was selected because it establishes symbiotic relationships with rhizobia through root hairs in meristematic, vascular, and parenchymatous tissues for the formation of nodules that contribute to nitrogen fixation (Bianco, 2020). However, the selected species is also a host of the virus known as golden yellow mosaic virus (EU021216); this *RhYMV* virus can infect other cultivated plants of the Fabaceae family (Akram et al., 2025). It may also serve as a trap species for the bioprospecting of arbuscular mycorrhizal fungi. The second selected species was *C. echinatus* due to its potential for heavy metal mycoremediation (Andrade et al., 2015).

The Margalef index result, with a value of 2.83, indicates that there is a relatively moderate species richness for the sample size, since a higher value would suggest a greater number of species in relation

Table 1. Plant species identified in a tropical dry forest remnant.

Botanical Family	Scientific Name	Individuals
Acanthaceae	*Ruellia blechum* L.	145
	Ruellia floribunda Hook.	84
Asteraceae	*Bidens pilosa* L.	65
	Tridax procumbens L.	118
	Lagascea mollis Cav.	23
Convolvulaceae	*Ipomoea coccinea* L.	35
Fabaceae	*Desmanthus virgatus* (L.) Willd.	42
	Rhynchosia minima L.*	80
	Mimosa pigra L.	29
	Mimosa albida Humb. & Bonpl. ex Willd.	47
	Prosopis juliflora (Sw.) DC.	7
	Senna occidentalis (L.) Irwin	8
Malvaceae	*Sida rombifolia* L.	148
	Guazuma ulmifolia Lam.	34
Poaceae	*Dichantium annulatum* (Forssk.) Stapf.	9
	Bouteloua gracilis H.B.K (Lag.).	14
	Panicum maximum Jacq.	124
	Urochloa decumbens (Stapf.) R.D. Webster	98
	Brachiaria brizantha (A.Rich.) Stapf	87
	Cynodon dactylon (L.) Pers.	101
	**Cenchrus echinatus* L.	149
Scrophulariaceae	*Stemodia durantifolia* (L.) Sw.	26
Verbenaceae	*Phyla nodiflora* (L.) Greene	78

* Selected ruderal species for the study of arbuscular mycorrhizae.

to the total number of individuals. For the Shannon index, a value of $H = 2.67$ was obtained, indicating moderately high diversity. This demonstrates that the analyzed plant community presents moderate species richness, but overall diversity is relatively high due to a fairly even distribution in the abundance of the different species. This suggests a ruderal community with a good mixture of species, without an overwhelming dominance of a few species. A similar study reported that the diversity of herbaceous species growing under forest canopy is affected by both soil characteristics and the spatial arrangement of trees (Sheng et al., 2024). Therefore, it is recommended to develop further spatiotemporal biodiversity studies in this area to investigate seasonal dynamics.

The results of the present study showed mycorrhizal colonization greater than 50% in both plant species inoculated with a density of 100 spores per gram of soil (Table 2). These findings are consistent with recent studies in other Fabaceae species, such as *Dipteryx alata*, which reported a colonization rate of 72.87% (Souza et al., 2023). On the other hand, research conducted on *Leymus chinensis*, belonging to the Poaceae family and subjected to drought and salinity conditions, showed a mycorrhizal colonization percentage of 46.67% in its roots (Wang et al., 2022). The number of spores required for effective colonization can vary considerably, influenced by complex biotic interactions, soil nutrient availability, and the specific compatibility between host plants and mycorrhizal fungi (Tenzin et al., 2022).

The structures of mycorrhizal colonization, which are a source of native inoculum, can be observed in Figure 1. Despite stability in the number of AMF species over time, the specific composition is influenced by temporal changes in abiotic factors such as temperature, pH, organic matter, and nitrogen availability (Peralta-Valencia et al., 2023).

As a result of the morphological analysis of the spores, four representative genera were identified from both inocula (*R. minima* and *C. echinatus*), namely *Acaulospora*, *Glomus*, *Gigaspora*, and *Scutellospora* (Figure 2). *Acaulospora* spores have an irregular shape and are dark brown in color, although some lighter areas are present. Under microscopic observation, black spores, spore-forming sacs, unusually

Table 2. Mycorrhizal colonization and spore count for selected species of the Fabaceae and Poaceae families.

Trap Plants	% MIC*	E 100 g^{-1} of soil
Rhynchosia minima	57.51 ± 2.10	101 ± 1.11
Cenchrus echinatus	53.62 ± 2.12	132 ± 0.97

* % MIC = mycorrhization rate; E = spores; ± = standard deviation.

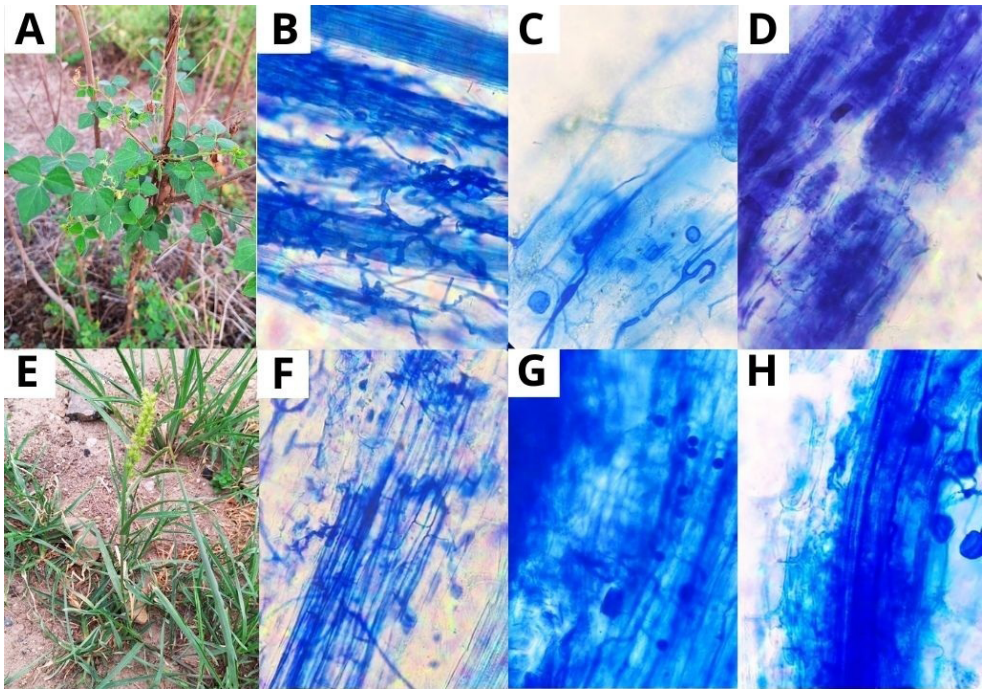

Figure 1. Selected ruderal species for bioprospecting of arbuscular mycorrhizae; A. *Rhynchosia minima* plant; *B. Hyphae*; C. Vesicles and hyphae; D. Arbuscules; E. *Cenchrus echinatus* plant; F. Hyphae; G-H. Vesicles and hyphae. Photographs taken under a microscope at 40X magnification (B-D y F-H).

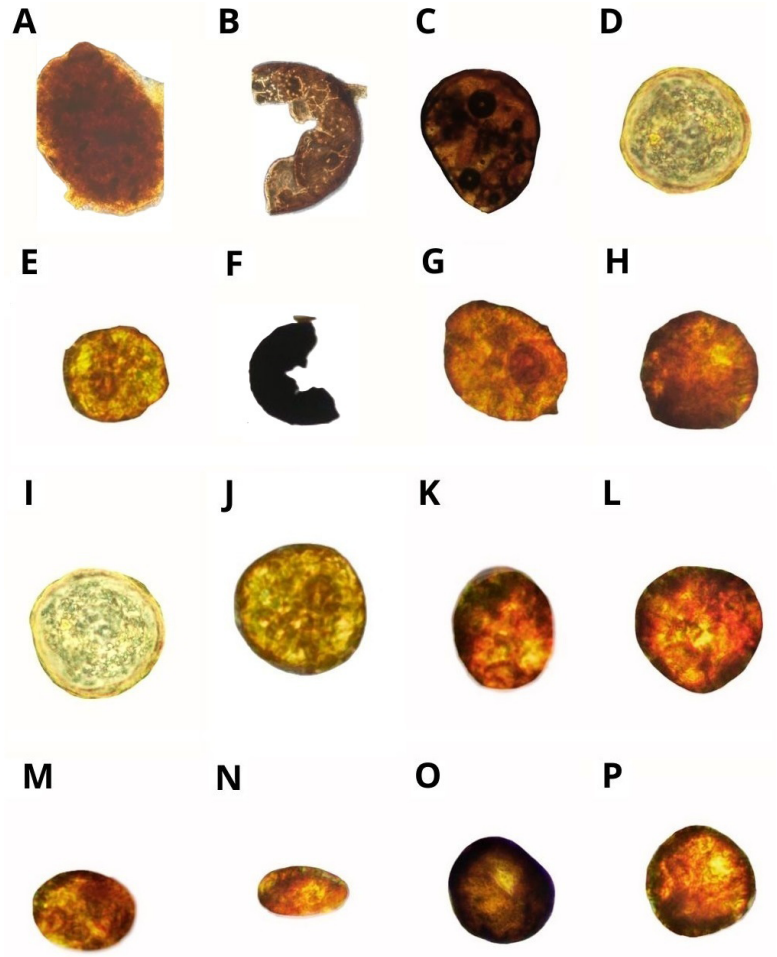

Figure 2. Arbuscular mycorrhizal spore genera from *Rhynchosia minima* (A-H) and *Cenchrus echinatus* (I-P); A. *Scutellospora*; B-C. *Acaulospora*; D-E. *Gigaspora*; F. *Glomus*; G-H-I-J-K-L. *Gigaspora*; M. *Scutellospora*; N-O. *Glomus*; P. *Gigaspora*. Photographs were taken with an inverted phase-contrast microscope (Köhler infinite field, 40x–600x).

shaped hyphae, and remnants of cells can be observed (da Silva et al., 2022). Spores of the genus *Glomus* have an almost spherical shape or irregular lobes, are soft, and possess a white outer layer (Guillén et al., 2020). Internally, the spore content is dark brown and has an ovoid shape. Spores of the genus *Gigaspora* exhibit a simple soil morphology, characterized by a large size, yellowish coloration, smooth surface, and a distinctively sized cell wall (Bonfante, 2022). Finally, *Scutellospora* spores are distinguished by their thick laminated wall, prominent auxiliary cells, and complex apical structure, forming unique structures in the soil (de Souza et al., 2005).

Recent studies, conducted over three years and across different seasons, have detected the consistent presence of arbuscular mycorrhizal fungal species such as *Acaulospora* and *Funneliformis*. These findings suggest a close relationship between the composition of these fungal communities and key environmental variables such as temperature and soil organic matter content. The remarkable ability of these species to adapt to diverse environmental conditions positions them as promising candidates for the restoration of degraded ecosystems (Peralta-Valencia et al., 2023). Furthermore, other arbuscular mycorrhizal fungal species, such as *Gigaspora albida* and *Gigaspora margarita*, have been shown to significantly contribute to plant growth (Souza et al., 2023).

In the experiment, bean plants inoculated with arbuscular mycorrhizae from *R. minima* showed a higher average height (32.8 cm) compared to the control without mycorrhizae (32.6 cm), suggesting a possible positive effect of inoculation. At the end of the study, these plants presented an average of five leaves. Regarding chlorophyll content, treatments 1 and 2 exhibited 39 SPAD units, slightly lower than the 41 SPAD units recorded in the control. Nitrogen content remained constant at approximately 15 mg g^{-1} across all treatments, and the mycorrhizal colonization percentage reached approximately 30% by the fourth week, indicating a developing symbiosis. It is important to note that the number of leaves is not detailed in Table 3, since all plants had the same number of leaves during the symbiotic stage of both species. According to the literature, this process could continue beyond the four weeks evaluated, with higher colonization levels

Table 3. Growth assay results using native arbuscular mycorrhizal spore inocula.

Source of inoculum HMA	Treatments	Commercial species	Height (cm)	Clorophyll (SPAD)	Nitrogen (mg/g)	% MIC
Rhynchosia minima	T1	Bean	32,63±1,6 [a]	41	16	31,33±0,47 [a]
	T2		32,89±0,45 [a]	39	15	32,45±0,37 [b]
	T3		32,9±1,2 [a]	39	15	32,21±0,36 [b]
Cenchrus echinatus	T1	Maize	20,83±1,3 [a]	34	13	36,43±0,12 [a]
	T2		21,89±3,1 [b]	40	15	37,17±0,23 [b]
	T3		21,9±2,5 [b]	38	15	37,65±0,16 [b]

*T1 = control without mycorrhizal inoculum; T2 = mycorrhizae + antibiotic; T3 = mycorrhizal inoculum only. Different letters in the same column indicate statistically significant differences according to Tukey test ($p < 0.05$). ± = standard deviation; n = 6; % MIC = mycorrhization rate.

expected near the flowering stage.

For maize, inoculation with *C. echinatus* in treatment 3 was also associated with a slightly higher height (21.54 cm) compared to the control (20.83 cm). At the fourth week, maize plants developed an average of three true leaves. Treatment 2 showed the highest chlorophyll concentration, with 40 SPAD units. Like common bean, nitrogen levels remained around 15 mg g^{-1}, and the mycorrhizal colonization percentage was close to 30% at the fourth week, suggesting an ongoing symbiotic process. As with common bean, the complete dynamics of the symbiosis in maize could extend beyond the study period, with effective colonization occurring during later growth stages.

Inoculation with *Acaulospora scrobiculata* and *Rhizoglomus clarum* has resulted in greater biomass development in sorghum and maize crops (Barros et al., 2024). Species of the genus *Glomus* have been predominant among the AMF identified. The abundance of these fungi is affected by edaphic factors such as organic matter and total nitrogen (Tenzin et al., 2022).

Although arbuscular mycorrhizal symbiosis is often described as a highly specific mutualistic association, this concept has been challenged by several studies. Research conducted in Cuba over the past 20 years suggests that soil conditions, rather than specific selection by the host, play a determining role in the efficiency of the symbiosis and do not show significant differences according to vegetation formations. This indicates that the criteria for selecting native inoculum strains in solid substrates do not vary (Mujica-Pérez, 2020). Our own results support this perspective. The morphological analysis of spores isolated from the rhizosphere of the two evaluated plant species (*R. minima* and *C. echinatus*) revealed the presence of four representative AMF genera: *Acaulospora*, *Glomus*, *Gigaspora*, and *Scutellospora* (Figure 2). This finding indicates that, under the conditions of our study, there is no high specificity between AMF species and plant families, since the same fungal communities were associated with both hosts. Therefore, it is important to study related species within the same botanical family. Given the convergent evolution of arbuscular mycorrhizal symbiosis, future studies focused on species such as *R. minima* could be crucial for unraveling the specific mechanisms by which different hosts detect and reward the most beneficial fungal partners (Shi et al., 2023). Further studies on these interactions, both individually and collectively, are essential to understand the complex dynamics of arbuscular mycorrhizae in tropical dry forest remnants.

4. Conclusions

This study demonstrates the potential of native arbuscular mycorrhizal fungi from a dry forest remnant to improve the growth of agriculturally important crops such as maize and common bean. Inoculation with AMF isolated from *Rhynchosia minima* and *Cenchrus echinatus* increased colonization in both crops, suggesting the viability of the obtained inoculum. These results support the idea that the use of mycorrhizal symbiosis can contribute to more sustainable agricultural practices by reducing dependence on chemical inputs and promoting ecosystem health. However, it is important to recognize that the complete dynamics of the symbiosis, including the timing of maximum colonization and the long-term effects on crop growth and yield, require further extensive studies covering longer evaluation periods, particularly analyses during the different phenological stages of economically important plants.

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Contributor roles

- Jaime Naranjo-Moran: conceptualization, investigation, methodology, resources.
- Nicolle Vargas-Huacon: research, software, writing – original draft.
- Allison Morales-Núñez: validation, visualization, writing – review & editing.

Data Availability

The data will be available on request.

Use of Artificial Intelligence

The authors declare that no artificial intelligence has been used in the preparation of the manuscript.

Ethical Implications

The authors declare that there are no ethical implications.

Conflict of Interest

The authors declare that they have no affiliation with any organization with a direct or indirect financial interest that could have appeared to influence the work reported.

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