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Symbiotic associations between arbuscular mycorrhizal fungi and *Cinchona officinalis*, the Quina tree, in the Andean Amazon of Peru

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Summary

Most studies on *Cinchona officinalis*, the 'quina tree', have focused on its pharmaceutical properties, while agro-ecological aspects have received little attention to date. Studies about its association with arbuscular mycorrhizal fungi (AMF), which represent an important clade of beneficial soil fungi, are still scarce. Especially, the AMF partners of *C. officinalis* and their diversity has never been determined. These microorganisms play a crucial role in plant establishment across diverse ecosystems by enhancing water and nutrient uptake from the soil, suppressing root pathogens and pests, and contributing to overall plant health and resilience. The current study aimed at analyzing the arbuscular mycorrhizal root structures, at visualizing this mutualistic relationship between *C. officinalis* and AMF, and to identify the AMF species in the rhizosphere of the 'quina tree'. Rhizosphere soil and roots were collected from a native population of *C. officinalis* in an Andean tropical forest in the Amazonas region, in Peru. Seventeen AMF species were isolated and morphologically identified. They belonged to eight genera (*Acaulospora*, *Glomus*, *Funneliformis*, *Rhizoglomus*, *Septoglomus*, *Scutellospora*, *Sclerocystis* and *Ambispora*) with highest diversity on species level among the AMF detected in *Acaulospora*. Roots of *C. officinalis* showed high AMF colonization (mean = 89%) in the form of different symbiotic structures, such as, vesicles, arbuscules and hyphae. By means of molecular analysis, we also detected rDNA of AMF in *C. officinalis* roots. Our results confirm the AMF association in *C. officinalis* with multiple species and a very high AMF root colonization under natural conditions, which opens opportunities for future research to screen the potential of this symbiosis to increase the sustainable productivity on this representative Peruvian tree.

Key words: Symbiosis; Mycorrhizae; Colonization; Biodiversity; *Cinchona officinalis*.

Introduction

Cinchona officinalis, also known as 'quina tree' represents the national tree of Peru. This plant species is naturally distributed in the montane tropical forests of the Andean Mountains in South America, particularly within the Peruvian Amazon, between 1500 and 3000 m a.s.l. (Anderson, 1998; Aymard, 2019; Chaves et al., 2020). The bark of *C. officinalis* contains high proportions of natural alkaloids like quinine, quinidine, cinchonine, and cinchonidine (Bharadwaj et al., 2018) proved to be effective against malaria (Achan et al., 2018; Álvarez, 2013) and having antifungal effects (Solari et al., 2000; Baroni et al., 2007). Due to these reasons, it is considered a high valued species with ample uses in industry (Minam, 2014). Despite its inherent interest, few studies have been carried out to describe the microbiome associated with the root system of *C. officinalis*.

Among the microbial communities, arbuscular mycorrhizal fungi (AMF) are of key importance (Yang et al., 2018; Brundrett and Tedersoo, 2019). This fungal group, belonging to the phylum Glomeromycota, forms mutualistic symbioses with between 70 and 90% of terrestrial plant species (Smith and Read, 2008; Taylor et al., 2017). These fungi serve as the interface between plant roots and the soil (Huang and Zhang, 2020) and the hyphal network developed in the soil can gather different nutrients such as P, N, S, K and various microelements (Bukovská et al., 2018; Jansa et al., 2019; Datta et al., 2020). Within plant roots, AMF develop different types of structures (arbuscules, vesicles, and hyphae) able to penetrate plant cells to exchange soil nutrients by photosynthetic products of the plant (Smith and Read, 2008).

For *C. officinalis* there were no reports about the occurrence of natural symbiosis with AMF. However, commercial Myco Grow® product which contain the complex of AMF *Glomus intraradices*, *Glomus mosseae*, and *Glomus aggregatum* as inoculum was used in the germination and development of seedlings of *C. officinalis* to improve its growth and development (Fernandez-Zarate et al., 2024; Fernandez-Zarate et al., 2022). Based on this data gap, we aimed to explore the establishment of the AM symbiosis with *C. officinalis* to particularly identify: i) the presence of intraradical fungal structures typical of the AM symbiosis with hyphae, vesicles, and arbuscules; ii) the presence of AMF rDNA within roots of *C. officinalis* and iii) the diversity of AMF species, detected by morphological spore analysis, in soils associated to *C. officinalis* in the Amazonas region of Peru.

Materials and methods

Site description and sample collection

The sampling was carried in July 2021, during the wet season. Samples of rhizosphere soil and roots were collected from five native individuals of *C. officinalis*, growing in a tropical Andean forest (Fig. 1), located in the Province of Luya, San Jerónimo District at Amazonas region, Peru (6º 0' 19.344" S, 78º 1' 22.889" W, 2,783 m a.s.l.) and has a sandy loam texture soil, pH = 5.7, Organic C (%) = 4.24 and P = 16.97 ppm (Tab. 1). A permit for scientific research on wild flora (RDG N° D000330-2021-MIDAGRI-SERFOR-DGGSPFFS,

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Fig. 1: Native trees of *Cinchona officinalis* in San Jerónimo. (A) Native tree, (B) Rhizosphere soil collection.

Tab. 1: Physical-chemical soil properties around the native trees of *C. officinalis* in San Jerónimo Distric.

Soil properties	
pH	5.7
EC (dS/m)	0.24
Organic C (%)	4.24
P (ppm)	16.97
K (ppm)	207.9
N (%)	0.37
Texture	
Sand (%)	80.0
Silt (%)	8.0
Clay (%)	12.0
CEC	12.0
Changeable cations	
Ca^{+2}	6.88
Mg^{+2}	1.52
K^{+2}	0.35
Na^{+}	0.65
$Al^{+3} + H^{+}$	0.00

Electrical conductivity (EC), Organic C (%), phosphorus (P), potassium (K), cation exchange capacity (CEC), calcium cation (Ca^{+2}), magnesium cation (Mg^{+2}), potassium cation (K^{+}), sodium cation (Na^{+}), aluminum cation (Al^{+3}+H^{+}).

with authorization code N° AUT-IFL-2021-035) was provided by the Servicio Nacional Forestal y de Fauna Silvestre (SERFOR). Mean annual temperatures in the area range between 10 and 20 °C, varying between 5 and 29 °C throughout the year. Mean annual precipitation is approximately 1500 mm.

The five sampled individuals were separated by at least 10 m. Plant positions were georeferenced and three subsamples per plant containing approximately 10 g of roots and 1.5 kg of soil were carefully collected between 0 and 15 cm depth from three equidistant points, each at 0.5 m from the main stem of each plant. Roots were excavated to trace them back to the stem of the quina tree to ensure they belong to the targeted plant. Subsamples were pooled, mixed and placed into polyethylene bags inside a cooler at 4 °C and transported to the laboratory on the same day of collection. In the laboratory, the samples were divided into root and soil components. The roots underwent a thorough washing process, followed by drying with paper towels, cutting into 1-2 cm segments, and subsequent homogenization. One 100 mg aliquots were preserved by freezing at -30 °C for molecular analysis. The remaining root portions were stored in ethanol (70°) for later assessment of AMF intraradical colonization. Soil samples underwent a 72-hour drying period at room temperature and were sieved through a 5-mm mesh to eliminate any residual roots and stones. Once fully dried, the soil samples were securely sealed in airtight bags and stored at 4 °C until further use.

Extraction, quantification, and morphological characterization of spores

The isolation of AMF spores was performed using 50 g of soil (0-20 cm) per extraction by wet sieving through two meshes sizes of 250 and 38 µm [18] followed by sucrose gradient centrifugation (Gerdemann and Nicolson, 1963) as also described previously in Corazon-Guivin et al. (2022). The identification of the morphological features and the spores was based on observations of specimens mounted in lactic acid, polyvinyl alcohol and glycerol (PVLG), and a mixture of PVLG and Melzer's reagent (1:1) (Koske and Tessier, 1983; Błaszkowski, 2012). An average of 10 spores per morphotype was used for the morphological identification of AMF. AMF species were identified according to the manuals of Schenck and Pérez (1990) and Błaszkowski (2012), considering also all original and emended AMF genus and species descriptions available. Glomeromycota classification was based on Wijayawardene et al. (2020) and Hyde et al. (2024), considering all updates on the higher taxa to species level since then (e.g. Goto et al., 2025). Notably, the number of AMF genera increased since 2000 from 5, and since 2011 from 29 to currently 64 genera following Oehl et al. (2026).

AMF colonization

Roots collected from the five individual plants were pooled and approximately 5 g were stained according to the methodology proposed by Vierheilig et al. (1998). Subsequently, the stained root segments (1 cm) were mounted on slides, and examined in a compound microscope (20X). To calculate the percentage of AMF colonization was used the intersection method (Brundrett et al., 1996).

Molecular analysis

DNA was extracted from an approximately 100 mg sample of fine roots, which consisted of a combination of roots from five plants. A two-step PCR procedure was utilized to amplify a ribosomal DNA fragment of AMF using the primers specified by Krüger et al. (2009). The subsequent steps were carried out as described by Pineda-Lázaro et al. (2024).

Results

Symbiotic associations between AMF and *Cinchona officinalis*

To our knowledge, this is the first report describing the establishment of the AMF symbiosis in *C. officinalis* and the species diversity of AMF associated to this tree in natural areas of the tropical Andean forests, Amazonas of Peru. In the current study, the root colonization reached a value of 88% of root length (Tab. 2), and we could reveal the existence of the multiple characteristic structures of the AM symbiosis, i.e. intraradical hyphae, vesicles and arbuscules (Fig. 2). We have also provided molecular evidence about the presence of AMF in *C. officinalis* roots (Fig. 3). The analysis was conducted using the SSUmAf/LSUmAr and SSUmCf/LSUmBr primers.

Tab. 2: Spore abundance, species richness and percentage of root colonization of arbuscular mycorrhizal fungi in the rhizospheric soil of a native population of *C. officinalis*.

Species AMF	N° spores
Glomeromycetes	
Acaulosporales	
Acaulosporaceae	
Acaulospora laevis	18
Acaulospora longula	12
Acaulospora sieverdingii	28
Acaulospora sp. 1 resembling *A. baetica*	20
Acaulospora sp. 2 resembling *A. punctata*	2
Acaulospora sp. 3	80
Acaulospora sp. 4	36
Acaulospora sp. 5	8
Gigasporales	
Scutellosporaceae	
Scutellospora calospora	56
Scutellospora sp. 1	36
Scutellospora sp. 2	4
Glomerales	
Glomeraceae	
Glomus macrocarpum	270
Rhizoglomus clarum	220
Funneliformis geosporus	70
Septoglomus constrictum	10
Sclerocystis sp.	30
Archaeosporomycetes	
Archaeosporales	
Ambisporaceae	
Ambispora sp.	92
AMF spores/100g soil	992
AMF colonization (%)	88

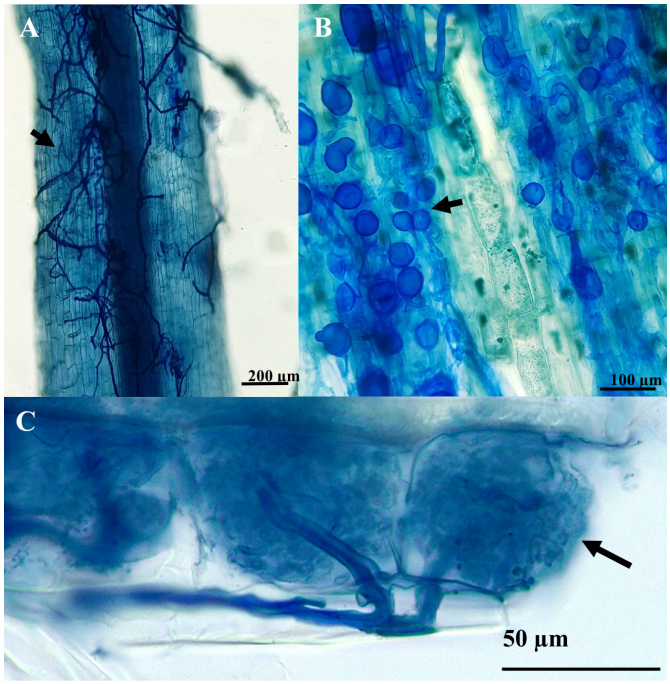

Fig. 2: Arbuscular mycorrhizal colonization in *C. officinalis* roots: (A) intrradical hyphae, (B) vesicles, (C) arbuscules.

AMF diversity associated with *Cinchona officinalis*

After confirmation of the presence of AMF in the *C. officinalis* rhizosphere, we morphologically identified the AMF spores present in the collected samples. A total of 17 AMF species were found belonging to eight genera and four families in the rhizosphere soil of *C. officinalis* (Tab. 2) belonging two two classes. Of the Glomeromycetes, *Acaulospora* was the most diverse genus, having eight species: *A. laevis*, *A. longula*, *A. sieverdingii*, *Acaulospora* sp. 1 resembling *A. baetica*, *Acaulospora* sp. 2 resembling *A. punctata*, and three unknown *Acaulospora* species (named as: *Acaulospora* sp. 3, *Acaulospora* sp. 4, *Acaulospora* sp. 5). *Scutellospora* had three species from which only one had been previously described (*S. calospora*) and two so far undescribed species (named here as: *Scutellospora* sp. 1 and *Scutellospora* sp. 2). Finally, Glomerales: *Glomus macrocarpum*, *Funneliformis geosporus*, *Rhizoglomus clarum*, *Septoglomus constrictum*, and *Sclerocystis* sp., were identified. Of the class Archaeosporomycetes, there was only one undescribed *Ambispora* sp. detected, clearly attributable to this genus due to its diagnostic spore morphology on the genus level.

Discussion

In this study, the arbuscular mycorrhizal root structures were analyzed and illustrated for the first time in *Cinchona officinalis*, the 'quin tree'.

Additionally, molecular evidence was provided about the presence of AMF in *C. officinalis* roots (Fig. 3). In the rhizospheric soils of 'quina tree', seventeen AMF species were separated by morphological spore identification, belonging to eight genera, four families and orders, and two AM fungal classes. This might be a surprisingly high number. However, the arbuscular mycorrhizal symbiosis generally is of very low specificity, so that it can be concluded from several studies that a arbuscular mycorrhizal plant might accept the large majority if not all AMF species living in its rhizosphere, independent if it is a tree, a shrub, a bush, a grass, or a herb species (Oehl et al., 2011a; Oehl and Körner, 2014; Oehl and Koch, 2018).

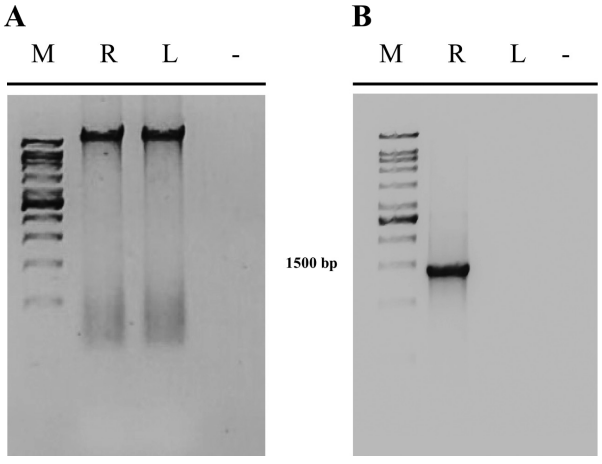

Fig. 3: Gel electrophoresis: (A) Lane 1: (M) 1 Kb molecular marker (Invitrogen, USA), Lane 2 (R) Genomic DNA from root (San Jerónimo samples), Lane 3 (L) Genomic DNA from leaf (San Jerónimo samples), Lane 4: (-) negative control, no DNA was added; (B) PCR reaction, Lane 1: (M) 10 Kb molecular marker (Invitrogen, USA), Lane 2-3: (+) 1500 bp fragments of mycorrhizal DNA (San Jeronimo Samples), Lane 4: (-) negative control reaction, no DNA was added.

Previous studies revealed the potential of genera and species closely related to *Cinchona officinalis* to establish the AMF symbiosis (e.g. Laksono et al., 2016). At the morphological level, the AMF species richness was higher in *C. officinalis* than that found in C.*pubescens* (Herrera et al., 2024). For example, Herrera et al. (2024) determined AMF communities associated with *C.pubescens* trees located in Santa Cruz and two sites in the province of Loja from Ecuador and they found 36 AMF Operational Taxonomic Units (OTUs) associated with *C.pubescens* in the root system, most of them belonging to the genus *Glomus*. The use of molecular tools to identify specific AMF in *C. officinalis* opens the possibility that in the near future, to link the taxonomic description of these spore communities to their presence in roots, as, during the last two decades, most of the described AMF species had been sequenced by using the SSUmAf/LSUmAr and SSUmCf/LSUmBr primers sets (e.g. Aguila et al., 2022; Corazon-Guivin et al., 2019a; Corazon-Guivin et al., 2019b; Corazon-Guivin et al., 2019c; Corazon-Guivin et al., 2020; Corazon-Guivin et al., 2021; Corazon-Guivin et al., 2022; Oehl et al., 2019a; Oehl et al., 2019b; Turrini et al., 2018).

AMF communities living in high mountains of South America have been barely studied (Lugo et al., 2008; Velázquez et al., 2016; Soteras et al., 2019). The species richness detected (17) seems to be low compared to a previous study in Peru by Senés-Guerrero and Schüssler (2016), who reported 41 AMF species in the Peruvian Andes. However, the different sampling coverage could be behind this difference since in that study the authors collected in four different locations in Peru in comparison with the single one of our study. The AMF symbiosis constitutes a key strategy to assist plants to face unfavorable conditions (Smith and Read, 2008) as occurs in high altitude ecosystems (e.g. mountain forests), in which the genus *Acaulospora* has often been found to be the most abundant (Gai et al., 2012; Oehl et al., 2017; Senès-Guerrero and Schüssler, 2016; Velázquez et al., 2013; Shi et al., 2014). In our study, the diversity and frequency of occurrence of the AMF detected could be explained by the high altitude of the study site. Several species of this genus have been identified in mountainous and alpine areas in Europe and the Chilean Andes (Oehl et al., 2011b; Oehl et al., 2012; Palenzuela et al., 2015). Senés-Guerrero and Schüssler (2016) also reported *Acaulospora* as the most diverse genus associated to potato (*Solanum tuberosum*) in a study carried out between 2,658 and 4,075 m.a.s.l. Gai et al. (2012) and Shi et al. (2014), studying altitudinal gradients in China, reported *Acaulospora* as the most frequent genus, too. The Acaulosporaceae family has also been described as dominant in well conserved forests (Melo et al., 2019), a fact that could be attributed to high soil organic matter contents (Melo et al., 2020). In agreement, the soils of the targeted *C. officinalis* in this study have an average of 7.3% of soil organic matter content. Indeed, species of *Acaulospora* have also been widely found in nature reserves and protected areas (Velázquez et al., 2016; Velázquez et al., 2010; Turrini et al., 2012).

Conclusions

Our study represents the first report of the symbiotic association between the *C. officinalis* root system and AMF, with a high species richness of native arbuscular mycorrhizal fungi in the 'quina tree' rhizosphere, displaying typical structures of vesicular-arbuscular mycorrhizal fungi, essentially hyphae, vesicles, and arbuscules. The presence of the AMF in the roots was also confirmed on molecular level by DNA analyses. From the eight genera identified, highest diversity of AMF was found for *Acaulospora* species in the rhizosphere soil of this plant species. Future studies should encompass a comprehensive exploration of the functional roles played by the identified AMF taxa in *C. officinalis*. Additionally, investigations concerning the ecological implications and potential agricultural applications of these native AMF species could provide valuable insights for sustainable cultivation practices.

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Conflict of interest

No potential conflict of interest was reported by the authors.

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