

Research Article

Arbuscular Mycorrhizal Fungi Associated with *Myrciaria dubia* in the Amazonia Region, Peru

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Myrciaria dubia (Kunth) McVaugh (camu-camu) is a shrub native to the Amazon region that produces fruits with a high content of vitamin C and various bioactive compounds, making it a functional food with antioxidant, anti-inflammatory, and antimicrobial properties. However, it is unknown which microorganisms are associated with its root system and can influence its growth and productivity. Arbuscular mycorrhizal fungi (AMF) are associated with most plants and are essential for their establishment, survival, and productivity since they facilitate their nutrition, increase water absorption, and improve soil structure. Although the AMF association is already known in some species of *Myrciaria*, no report is available on its association in *M. dubia*. This study presents, for the first time, the symbiotic association between AMF and *M. dubia* from the INIA San Roque experimental station located in the Amazon region, Peru. For the morphological and molecular analyses of the AMF, samples of rhizospheric soil and roots from two native accessions of the National Germplasm Bank of *M. dubia* were collected. Eighteen AMF morphospecies were identified in rhizospheric soil, belonging to nine genera *Acaulospora*, *Ambispora*, *Entrophospora*, *Diversispora*, *Gigaspora*, *Glomus*, *Paraglomus*, *Funnelformis*, and *Sclerocystis*, being the first one the most frequent. The roots of *M. dubia* showed high colonization by AMF (mean = 91%), and characteristic structures of arbuscular mycorrhizae, such as vesicles, hyphae, and arbuscules, could be observed. Likewise, the molecular analysis detected the presence of genetic material (rDNA) corresponding to AMF in the roots of both accessions. Our results evidenced the symbiotic association between AMF and *M. dubia*, which encourages further investigation of the functional potential of these microorganisms in this economically crucial agricultural plant in Peru.

1. Introduction

Myrciaria dubia (Kunt) McVaugh, popularly known as camu camu, is native to the Amazon region of Peru, Brazil, Venezuela, and Colombia [1], and its most prominent natural population is located in the eastern Peruvian Amazon [2]. It is a shrub species

found naturally in flooded environments near streams or rivers in the Amazon region [3–5]. However, it is cultivated in agricultural areas (nonflooded lands) in association with other crops [6], demonstrating good adaptive capacity [7].

The pulp of the *M. dubia* fruit has a tremendous nutritional potential that lies in the high concentration of ascorbic acid (vitamin C), higher than other fruits such as acerola, lemon, and orange [8]. This fruit is consumed worldwide as a beverage, frozen pulp, or extract [1, 9]. Likewise, it presents antioxidant [10], anti-inflammatory [1], and photoprotective [11] properties. Consequently, almost all research on this species has focused on its nutraceutical, pharmaceutical, and cosmetological properties, with few studies on the diversity of microbial communities present in the rhizosphere of this plant.

Among the microbial communities, the arbuscular mycorrhizal fungi (AMF) stand out [12], which are associated with approximately 80% of plant species in almost all terrestrial ecosystems [13, 14]. This association is defined as an obligatory mutualistic symbiotic relationship that inhabits the root cells of the host plant [15], improving the uptake of P, N, S, K, and various microelements (Fe, Cu, Zn, and many other minerals) [16]. Likewise, it provides resistance against pathogens and unfavorable environmental conditions and improves soil quality [17].

Root colonization by AMF is characterized by presenting fungal structures, such as mycelium, auxiliary cells, arbuscules, vesicles, and spores [18, 19], being this vast structure it is most important for its correct identification using the morphological approach [18–22]. Currently, in the Peruvian Amazon, some studies have recorded a great diversity of AMF [23–27]. New species were identified in different crops [23, 28–38] using morphological and molecular tools [39–41], and molecular analyses of the SSU-ITS-LSU region of rDNA [42].

In this context, to know the symbiotic association between AMF and *M. dubia*, we evaluated: (i) the presence of intraradical fungal structures typical of AMF symbiosis: vesicles, hyphae, and arbuscules; (ii) the presence of AMF rDNA inside the roots of *M. dubia*; and (iii) diversity of AMF species present in *M. dubia* rhizospheric soil in the Amazonas region, Peru.

2. Materials and Methods

2.1. Soil Collection and Conservation. The sampling was carried out in two elite accessions (which exhibited the best agronomic qualities, such as percent pulp, fruit weight, ascorbic acid content, and yield. These accessions gave rise to the first variety of *M. dubia* “INIA 395-Vitahuayo,” <https://www.gob.pe/institucion/inia/noticias/349846>) of the National Germplasm bank of *M. dubia* of the Agrarian Experimental Station “San Roque” Iquitos, National Institute of Agrarian Innovation (INIA) (Figure 1). This station is located at 25 km of the Iquitos–Nauta highway (03°57'17" S, 73°24'55" W, 112 m of elevation) and has a sandy loam texture soil, pH = 4.10, M.O = 1.63%, and P = 3.01 ppm (Supplementary 1). The *M. dubia* accessions were introduced from plant material collected from native populations located in the Loreto region (Table 1). The biological samples were composed of 1.5 kg of rhizospheric soil and 10 g of root tissue, which were extracted from three random plants for each accession. From each plant, subsamples were collected from three equidistant points around the main stem from 0 to 20 cm deep, which were mixed and stored in

polyethylene bags in a cooler at 4°C and then transported to the biology and molecular genetics laboratories at the National University of San Martín (Tarapoto, Peru). In the laboratory, the roots and soil were separated. The roots were pooled in a sample composed of each accession, washed, dried with paper, cut into 1–2 cm pieces, and homogenized in water. Two 200 mg aliquots were frozen at –80°C for molecular analysis. The remaining roots were kept in ethanol at 70° to determine mycorrhizal colonization. Soil samples were also mixed for each accession, dried at room temperature for 48 hr, and sieved through a 5-mm mesh to remove root debris and stones. Finally, they were stored in airtight bags and kept at 4°C until use.

2.2. Morphological Analyses. The isolation and specimen preparation were performed according to Corazon-Guivin et al. [34]. For the morphological identification of AMF, the classification proposed by Oehl et al. [39] was followed, and the taxonomic organization of orders, families, and genera suggested by Blaszkowski et al. [40] and Wijayawardene et al. [41] was followed.

2.3. Fungal Intraradical Colonization. Approximately 5 g from the roots of each accession were stained, according to Vierheilig et al. [43]. Once stained, the roots were cut into 1 cm segments, mounted on slides, and examined in a compound microscope (20x) by intersection method [44].

2.4. Molecular Analysis. DNA was extracted from 100 mg fine roots (a mix of the three plants for each accession) using the cetyltrimethylammonium bromide (CTAB) protocol. Subsequently, a two-step PCR (using gDNA) was conducted to amplify the ribosomal fragment of AMF consisting of partial SSU, ITS1, 5.8S, ITS2, and partial LSU rDNA using the primers SSUmAf/LSUmAr and SSUmCf/LSUmBr, consecutively [42]. The PCR was carried out according to Corazon-Guivin et al. [23, 28–34]. PCR products from the second round of amplifications (~1500 bp) were separated by electrophoresis on 1.2% agarose gel, stained with Diamond™ Nucleic Acid Dye (Promega), and revealed by UV illumination.

2.5. Comparative Analysis. Sørensen index was used to assess the similarity of AMF species among accessions. The Venn diagram was constructed using the calculate and draw custom Venn diagrams tool available online (<http://bioinformatics.psb.ugent.be/webtools/Venn/>).

3. Results and Discussion

According to the morphological and molecular analyses, the mutualistic symbiotic relationship between AMF and *M. dubia* was evidenced for the first time. Likewise, we report the taxonomic diversity of AMF associated with two accessions from the National Germplasm Bank of *M. dubia*, Peru. Previous studies showed that species of the genus *Myrciaria* establish symbiosis with AMF. For example, a study evaluated *M. cauliflora* plants in the field and reported 40% root colonization [45]. In the same way, it was observed that *M. glomerata* seedlings, under controlled conditions, showed similar results [46]. In our study, AMF root colonization reached a mean value of 91%. We were able to observe the presence of different typical AMF

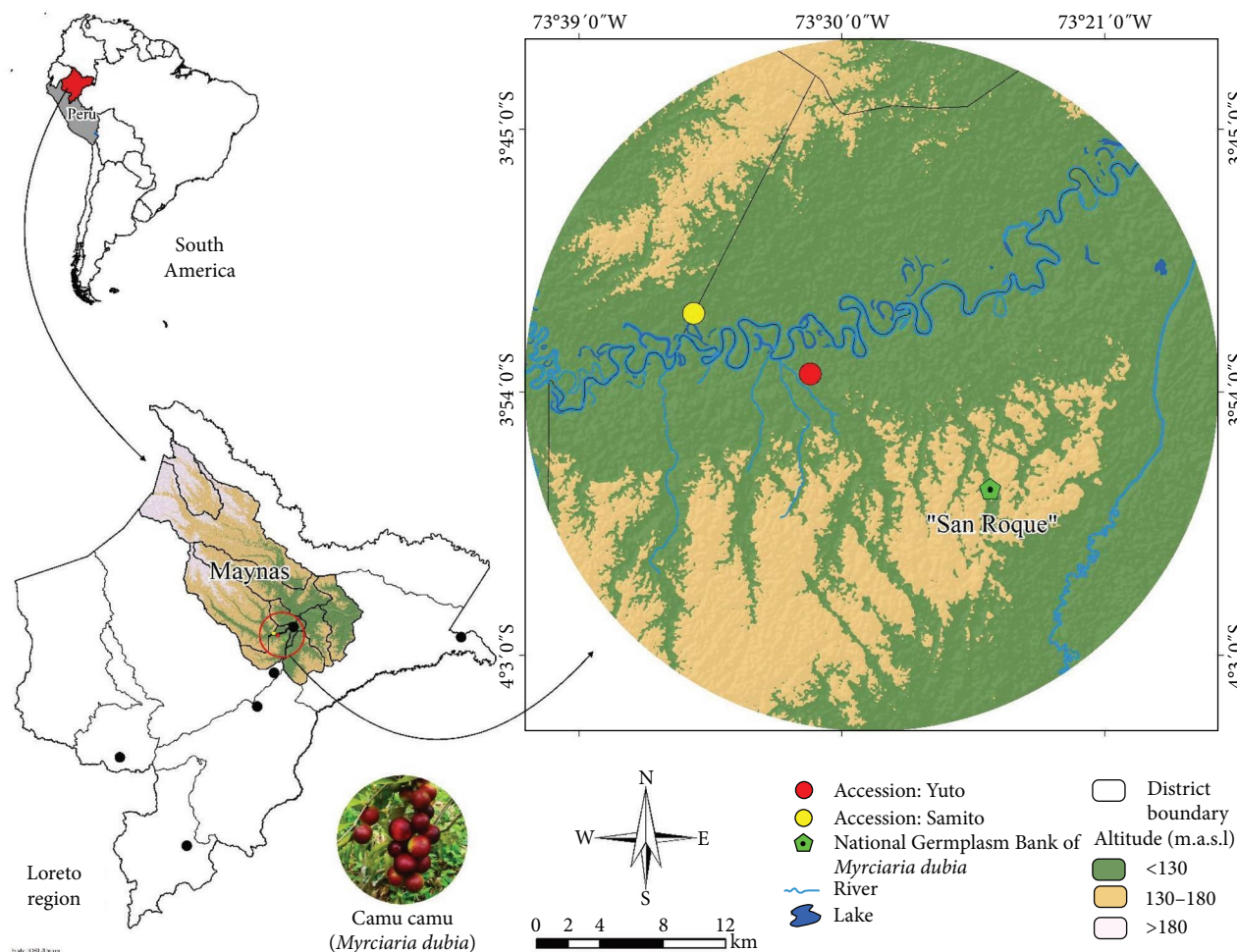


FIGURE 1: Spatial location of the National Germplasm Bank of *M. dubia* (Kunth) McVaugh at the Agrarian Experimental Station “San Roque” in Loreto Region, National Institute of Agrarian Innovation (INIA).

TABLE 1: Origin sites of the elite accessions from the National Germplasm Bank of *M. dubia* (Kunth) McVaugh at the Agrarian Experimental Station “San Roque” in Iquitos, National Institute of Agrarian Innovation (INIA), Peru.

| Department | Province | District | Basin | Location | Codnac | Codentban | Coordinates |
|------------|----------|-------------------|-------|----------|------------|-----------|------------------------------|
| Loreto | Maynas | San Juan Bautista | Nanay | Samito | PER1000394 | MD-014 | 3°51'18.3" S 73°35'4.5" W |
| | | | | Yuto | PER1000395 | MD-015 | 3°53'22.9" S 73°31'5.0" W |

structures, i.e. hyphae, vesicles, and intraradical arbuscules (Figure 2). Also, we provide molecular evidence on the presence of AMF in roots of *M. dubia* (Figure 3). This analysis was carried out using the primers SSUmAf/LSUmAr and SSUmCf/LSUmBr [38], which amplify a much more informative region of rDNA than is typically used for root-level molecular analysis [47].

We found a total of 18 AMF species belonging to nine genera and seven families in the rhizospheric soil of two *M. dubia* accessions (Supplementary 2). *Acaulospora* is the most dominant genus, with five species: *A. mellea*, *A. morrowiae*, *A. undulata*, and two unidentified species. *Diversispora* followed it with four

species: *D. aurantia*, *D. eburnea*, *D. invermanium*, and *D. tortuosa*, and *Ambispora* with three species: *A. appendicula*, *A. reticulata*, and an unidentified one. Furthermore, *Funnelformis geosporus*, *Glomus macrocarpum*, *Sclerocystis sinuosum*, *Entrophospora etunicata*, *Gigaspora margarita*, and *Paraglomus* sp. were found (Figure 4). The presence of possible unidentified new species could be related to the fact of an area not yet characterized and a poorly studied plant species. Thus, our research group recently reported 11 new AMF species in the Peruvian Amazon such as *Funnelliglomus sanmartinensis*, *Microkamiensia peruviana*, *Acaulospora*

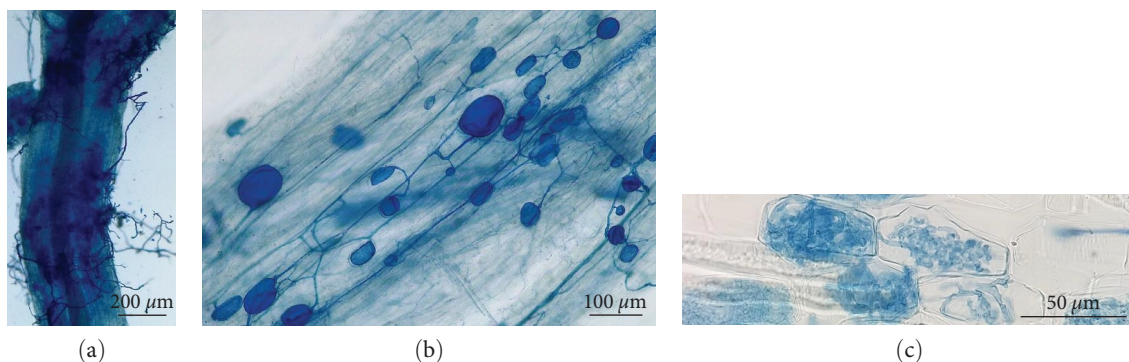


FIGURE 2: Arbuscular mycorrhizal colonization in *M. dubia* (Kunth) McVaugh: (a) extraradical hyphae of arbuscular mycorrhizal; (b) vesicles in roots; and (c) branched arbuscles.

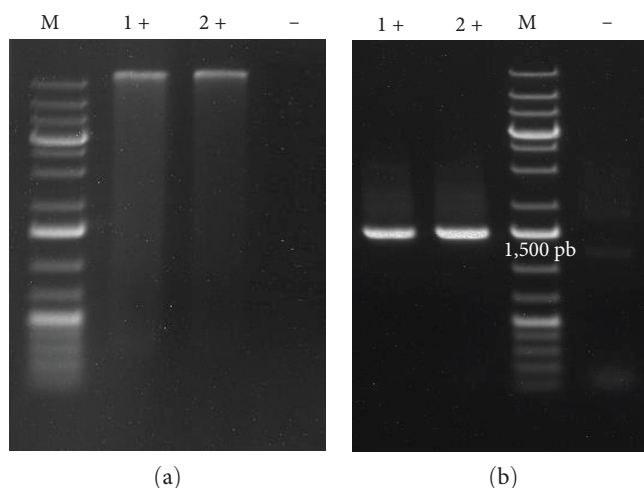


FIGURE 3: Gel electrophoresis. (a) Carril 1: (M) 1 kb molecular marker (Invitrogen, USA), Carril 2–3: (1+, 2+) AMF genomic DNA, Carril 4: (–) negative control, no DNA was added; (b) PCR reaction, Carril 1–2: (1+, 2+) 1,500 bp fragment of AMF DNA, Carril 3: (M) 1 kb molecular marker (Invitrogen, USA), Carril 4: (–) negative control reaction, no DNA was added.

aspera, *Nanoglomus plukenetiae*, *Rhizoglomus variabile*, *Paraglomus occidentale*, *Acaulospora flava*, *Paraglomus peruvianum*, *Acaulospora flavopapillosa*, *Rhizoglomus cacao*, and *Diversispora alba* [28–38]. A study conducted in three agroecosystems associated with *Theobroma cacao* in the Peruvian Amazon rainforest reported 46 AMF species [48]. Meanwhile, another study carried out in the Colombian Amazon rainforest reported 18 AMF species, similar to the species richness found in our study [49].

Approximately, 65% of the soils of the Amazon lowlands in Peru are classified as ultisols, characterized by being extraordinarily acidic and having low availability of phosphorus [50]. The pH can exert a fundamental influence on the composition and diversity of bacteria and fungi living in the soil, owing to its direct impact on the regulation and mobilization of several essential nutrients [23, 51]. In this context, AM symbiosis constitutes a key strategy to help plants efficiently take up phosphorus from the soil [52]. In this type of ecosystem, it has been reported that the genus *Acaulospora* is abundant [48], similar to our study, where the

sampling area is classified as ultisol. In this regard, Veresoglou et al. [53] and Coutinho et al. [54] mentioned that the genus *Acaulospora* is adapted to an acid soil pH (<5.0). Likewise, the absence of species from the *Rhizoglomus* genus in our study could be influenced by pH, as certain limitations in the growth of some species of this genus in slightly acidic soil have been reported [55].

Among the 18 AMF species identified, two were found exclusively in the rhizospheric soil of Samito, six species in the rhizospheric soil of Yuto, and 12 AMF species were shared between both accessions (Figure 5). The high percentage of similarity (67%) of AMF species can be attributed to the proximity of both accessions within the Germplasm bank, exhibiting similar edaphoclimatic properties. In their study, Vieira et al. [56] demonstrated a higher percentage of similarity of AMF species in two ecosystems that shared similar soil properties. Furthermore, the presence of certain AMF species exclusively in the rhizospheric soil of one accession and not the other could indicate that the genotype of each accession might have an influence. In this regard,

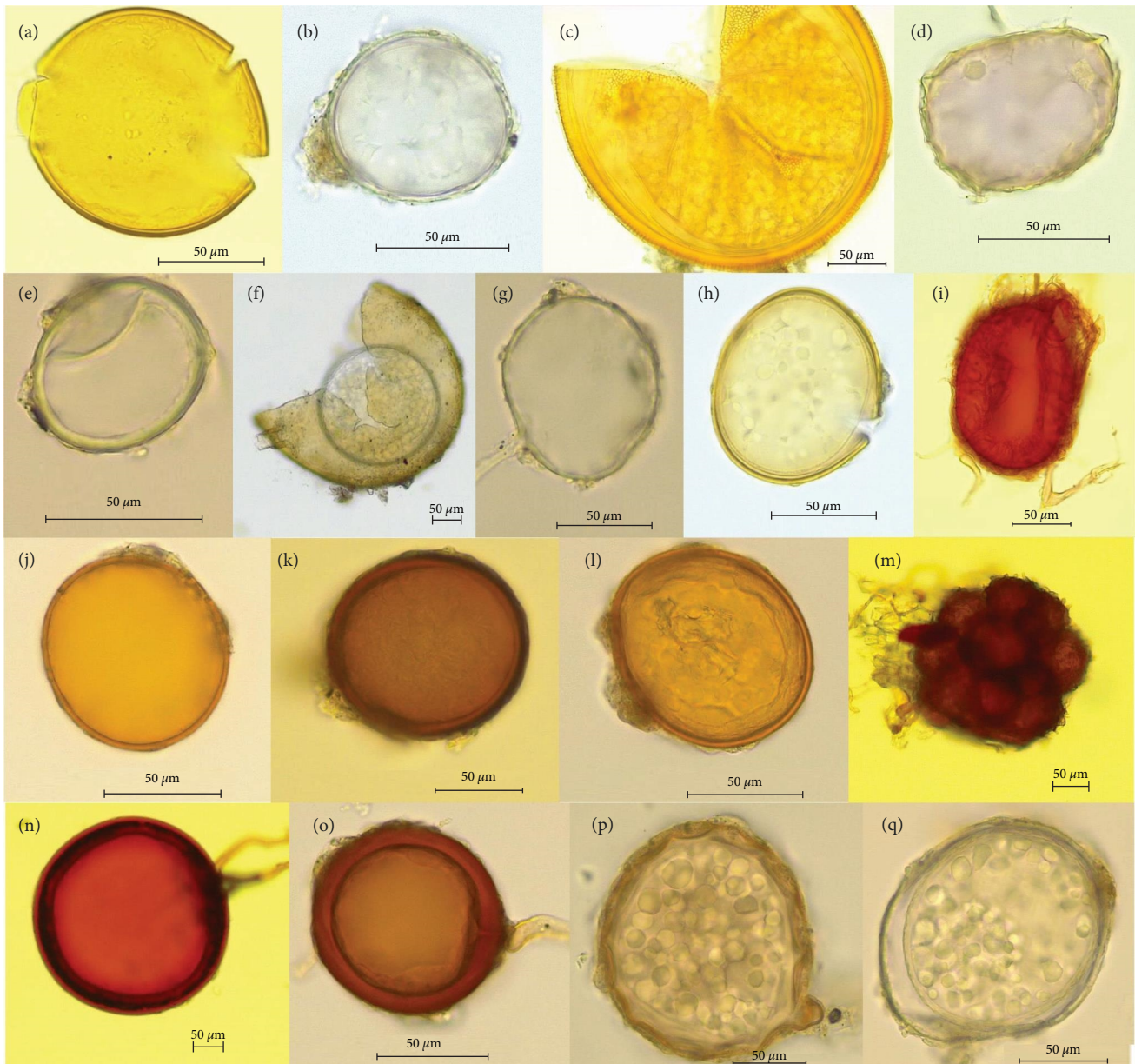


FIGURE 4: AMF species in *M. dubia* (Kunth) McVaugh: (a) *Entrophospora etunicata*; (b) *Acaulospora morrowiae* (c) *Acaulospora* sp1.*; (d) *Acaulospora* sp2.*; (e) *Paraglomus* sp.; (f) *Ambispora appendicula*; (g) *Diversispora eburnea*; (h) *Acaulospora mellea*, (i) *Diversispora tortuosa*; (j) *Diversispora aurantia*; (k) *Diversispora invermanium*; (l) *Funneliformis geosporum*; (m) *Sclerocystis sinuosum*; (n) *Gigaspora margarit*; (o) *Glomus macrocarpum*; (p) *Ambispora* sp.*; (q) *Ambispora reticulata*. (*) still unidentified.

Carrascosa et al. [57] also demonstrated that purslane genotypes from different geographical areas can significantly influence the composition of microbial communities in their rhizospheres.

4. Conclusions

Our study reports and describes, for the first time, the interaction between AMF and *M. dubia* through the observation of structures such as hyphae, vesicles, and arbuscules in the

root system of this plant. Likewise, molecular techniques allowed the confirmation of the presence of AMF in *M. dubia* roots, at the time of sampling. On the other hand, in soil samples, it was possible to identify a high diversity of AMF associated with the rhizosphere of *M. dubia*, the *Acaulospora* genus being the richest in morphospecies. Our results indicate that AMF may play a very important role in the establishment, nutrition, and productivity of *M. dubia* cultures, which is why it is important to carry out research to elucidate the functional role of these fungi in the flow of nutrients

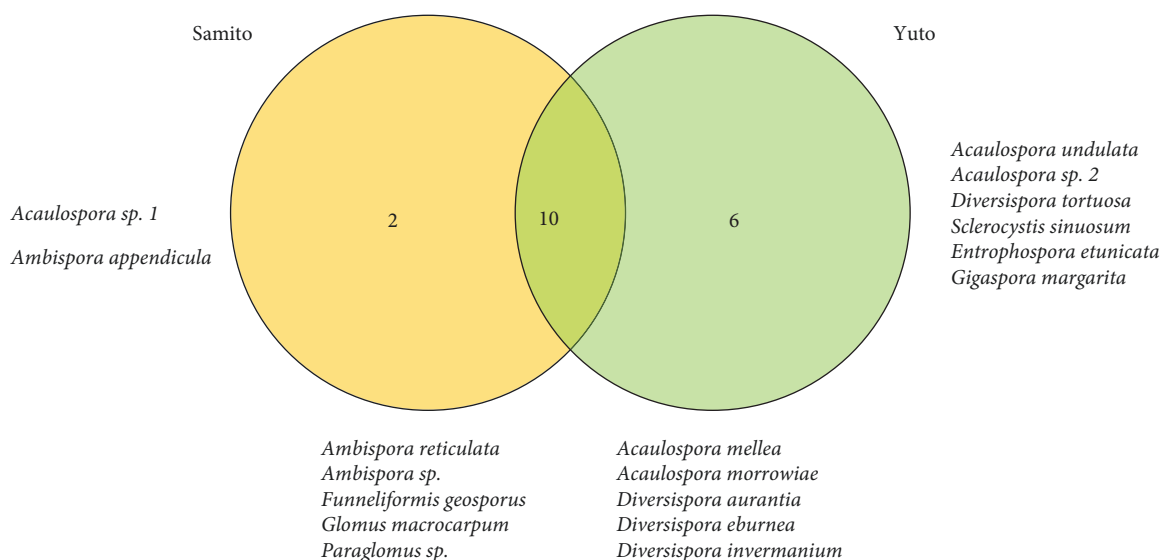


FIGURE 5: Venn diagram showing the AMF species richness (exclusive and shared) in the rhizospheric soil of the Samito and Yuto accessions of *M. dubia* (Kunth) McVaugh.

between the soil and plant. This will help to propose better strategies for the use, management, and formulation of inoculants that promote sustainable production of *M. dubia* crops in Peru.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The author and co-authors declare that there are no conflicts of interest regarding the publication of this paper.

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Supplementary Materials

Supplementary 1. Table 1: physical–chemical soil properties of the National Germplasm Bank of *M. dubia* (Kunth) McVaugh at the Agrarian Experimental Station “San Roque” in Loreto Region, National Institute of Agrarian Innovation (INIA).

Supplementary 2. Table 2: abundance, richness of species, and colonization of arbuscular mycorrhizal fungi in the rhizospheric soil of two elite accessions from the National Germplasm Bank of *M. dubia* (Kunth) McVaugh of the Agrarian Experimental Station “San Roque” Iquitos, of the National Institute of Agrarian Innovation (INIA).

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