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Identification of Arbuscular Mycorrhizal Fungi associated with Arabica coffee root (*Coffea arabica*) in the Arfak Mountains region of West Papua, Indonesia

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Abstract. Suparno A, Prabawardani S, Nisa DK, Ruimassa RR. 2023. Identification of Arbuscular Mycorrhizal Fungi associated with Arabica coffee root (*Coffea arabica*) in the Arfak Mountains region of West Papua, Indonesia. *Biodiversitas* 24: 3207-3213. Coffee is a global commodity widely consumed as a beverage globally and has a high economy. Currently, the trend of drinking coffee is no longer dominated by older people but has become part of the millennial and celebrity lifestyle; therefore, worldwide demand for coffee continues to increase. Efforts to develop area expansion and productivity continue to be pursued. The Arfak Mountains Region of West Papua, located 800 - 2,500 m above sea level (masl), is a potential area for coffee plantations. The local farmers in this area have grown coffee independently and with gradual government support for the last years. Given the benefits of Arbuscular Mycorrhizal Fungi (AMF), which can increase plant growth and productivity, this study aims to identify the types of AMF that are associated with coffee plants in 4 districts, Mokwam, Anggi Giji, Anggi Gida, and Membey in Arfak Mountains region, West Papua. Identification of AMF types was observed based on the morphology of mycorrhizal spores. The research was conducted using the observation method with a purposive sampling technique from November 2022 to March 2023. Based on the observation, the coffee plants in 4 districts were associated with AMF. The types of AMF associated with coffee in Mokwam were more numerous than in other locations, namely the Acaulosporaceae Genus with five species, Glomeaceae Genus with five species, one species of Simiglomus sensu stricto, one species of Funneliformis sensu stricto, and one species of Septoglomus sensu stricto. AMF in Anggi Giji District consisted of Acaulosporaceae Genus with two species, Glomeaceae Genus with four species, while in Anggi Gida District consisted of Acaulosporaceae Genus with one species, Glomeaceae Genus with three species, one species Septoglomus sensu stricto. AMF species in the coffee plants of the Membey District are the Acaulosporaceae Genus with three species, the Glomeaceae Genus with two species, and one species of Septoglomus sensu stricto. From the soil analysis results, the soil fertility level is low; conversely, the mycorrhizal presence level is higher because of the more infertile soil, the more active mycorrhiza.

Keywords: Coffee arabica, identification, mycorrhiza, symbiosis, West Papua

INTRODUCTION

Arbuscular Mycorrhizal Fungi (AMF) are one of the most important symbiont groups for plants, universally forming relationships with plant roots of more than 80 % of terrestrial plants, acting as bioprotection, biodegrade, increasing roots surface absorption area, biological fertilizer, and play a significant role in efficient absorption of nutrients, particularly N, P, K (Smith and Read 2008; Nell et al. 2010; Khaliq et al. 2022). The role of AMF in plant ecosystems is like a bridge that connects plants with the conditions of their growth media, namely their influence on root length, root diameter, root surface area, and root hair density (Smith et al. 2011; Liu et al. 2018). The response of plants to AMF symbiosis is very significant, especially for plants that grow under abiotic stress conditions, such as marginal soils, acid soils, soils contaminated with heavy metals and oil, saline soils, and dry land (Mojib et al. 2018; de la Hoz et al. 2021). The presence of AMF can increase the range or exploration of roots in the soil, increase plant fitness, increase plant tolerance to drought stress, salinity stress, and high

temperatures (Khaliq et al. 2022), facilitating the absorption of P, increasing soil P availability by producing acid phosphatase enzymes, and increasing soil aggregation through the production of glomalin by AMF (Meng et al. 2021; Liu et al. 2021b).

The ability of AMF to help its host plants is to overcome environmental stress in crop cultivation systems increase growth and yield. AMF is an obligate fungus; therefore, it requires an association with plant roots to complete its life cycle (Khaliq et al. 2022). In addition, AMF can increase plant resistance due to abiotic and biotic stress, including invasion of soil-borne pathogens, which will damage the plant roots' function (Powell and Rillig 2018; de la Hoz et al. 2021; Bell et al. 2022).

Plants with shallow and few roots are generally highly dependent on symbiosis with AMF. Most terrestrial plants show their dependence on AMF, including adjusting the architecture of the root system to enhance plant growth and development (Chen et al. 2021). Marginally dependent plant groups include *Ananas comosus* (L.) Merr., *Glycine max* (L.) Merr., *Lycopersicon esculentum* Mill., *Zea mays* L., Moderately dependent include *Acacia mangium* Willd.,

Colocasia esculenta (L.) Schott, *Sesbania grandiflora* (L.) Poir, Highly dependent includes *Allium cepa* L., *Carica papaya* 5 *Coffea arabica* L. Very highly dependent includes *Leucaena leucocephala* (Lam.) de Wit, *Manihot esculenta* Crantz, and *Sophora chrysophylla* (Salisb.) See 14

Indonesia is the world's fourth-largest Arabica coffee-producing country after Brazil, Vietnam, and Columbia (ICO 2021). In 2019, the area of coffee plantations in Indonesia was around 1,245 million ha, with an average production of 794 kg/ha (BPS 2020), lower than coffee productivity in Vietnam which reached 2.78 tons/ha in the same year (USDA 2019). It has high economic value and 3 globally important (Krishnan 2017). Indonesia generates a large percentage of coffee export revenue besides Mexico, Peru, Brazil, Indonesia, Ecuador, Costa Rica, Ghana, Uganda, Cameroon, Ivory Coast, Rwanda, Burundi, Kenya, and DR Congo (Munyuli 2014).

Arabica coffee is widely developed on land with over 800 masl. These lands are generally located in the mountains/hills with cool-cold air conditions, low soil fertility, acidic soil, and low available P nutrient content. In Arfak Mountains District, West Papua Province, coffee development has been started since 2010 in villages/districts at an altitude of 800-2500 masl. Planting is done with an insertion system between food crops and upland vegetables, not in a monoculture system because the development area is conservation. In addition, plants bear fruit from about two years of age.

Coffee growth and production highly depend on the type of AMF (De Beenhouwer et al. 2015). Therefore, coffee growth will be suppressed if it is not symbiosis with AMF. Coffee plants depend heavily on AMF for optimal nutrition, especially in eroded soils and low 15 fertility levels common in the tropic land (Cogo et al. 2017 15 The presence of AMF in the coffee plantation has been associated with the roots of the Arabica coffee plant due to the nature of the

11 Arabica coffee plant, which has a high dependence on AMF. The higher colonization rate indicates that the plant has a better association with AMF. However, the type of AMF present in the coffee plantation of 127 Arfak Mountain Region is not yet known. Therefore, this study was conducted to identify the types and levels of AMF colonization on Arabica coffee roots in this area, as identification of AMF in plant roots is important because the colonization level indicates the fungi activity.

31 MATERIALS AND METHODS

Study area

The research was conducted by observation and purposive sampling technique (Taherdoost 2016) in four districts of the Arfak Mountains region, which comprised nine locations ordinate where Arabica coffees are planted. In Mokwam District, 10-year-old coffee plants were taken as a sample on the ordinate (S -01°06'02"; E 133°54'31"); in Anggi Giji District, 4-year-old coffee plants with two samples on the ordinate (S -01°20 '690"; E 133°54'713" and S -01°20'52"; E 133°55'1"), in Anggi Gida District coffee plants aged five years with three samples on the ordinate (S -01°22'043"; E 133°57'592", S -01°22'094"; E 133°57'560" and S -01°22'046"; E 133°57'594"), and Membey District, 8 years old coffee plants with three samples on the ordinate (S 01°18'441"; E 134°01'403", S 01°18'702"; E 134° 01' 011" and S 01°20'509"; E 134°02'146") (Figure 1). The number of samples at each study location was not the same, depending on the area, where the wider the area, the greater the number of samples.

The trapping process for observing AMF morphology was carried out at the Agroclimatology Laboratory, Faculty of Agriculture, Papua University, Manokwari, West Papua, Indonesia, from December 2022 to March 2023.

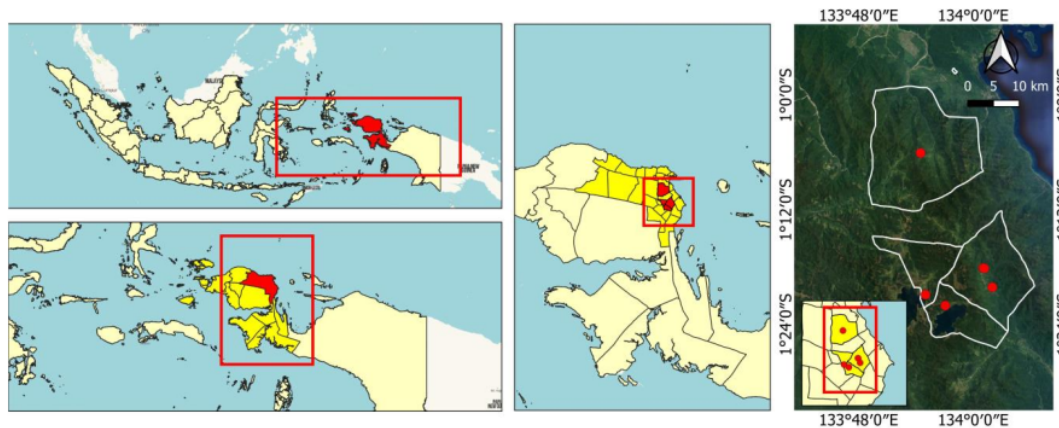


Figure 1. Research locations in Arfak Mountains, West Papua, Indonesia

Research materials

Materials utilized in this research were samples of the ready-to-harvest rhizosphere coffee soil, sterile zeolite sand as an AMF trapping medium, *Sorghum bicolor* L. as a trapping host for AMF, glucose 60 % (derived from Gulaku: 100% cane sugar, PT Sweet Indolampung-Indonesia) for wet sieving of AMF spores, polyvinyl lactoglycerol-PVLG (Lactic acid 88%, Carlson Kent-Ohio USA; Glycerol 85%, Merck Darmstadt - Germany) and Melzer's reagent (Biodiversity Laboratory, IPB University Indonesia) for AMF identification, KOH (Merck, Darmstadt - Germany), HCl (HCl 37%, Merck, Darmstadt-Germany), trypan blue (Merck, Darmstadt-Germany) for staining mycorrhizal roots. If the root stain was too dark, the root sample was immersed in a staining solution (lactic acid 88%: Glycerol 85%: 1 distilled water) with a ratio of 2:2:1.

Isolation and trapping of AMF

Coffee-plant rhizosphere soil samples were taken at each research location, namely the District of Mokwam, Anggi Giji, Anggi Gida, and Membey, consisting of 1, 2, 3, and 3 samples, respectively. The soil sample of 2 kg per plant was taken at a 10 cm depth of the Arabica coffee plant rhizosphere for isolation and AMF trapping. The rhizosphere soil samples were taken from two plants in each ordinate. Soil sampling was taken using a shovel by scraping the soil attached to the Arabica coffee roots, then put in sterile plastic bags. The soil samples in each ordinate were composited and air-dried in the laboratory. Half of the soil sample was used to observe early AMF existence, and the other half was used for trapping culture.

A trapping culture was conducted to increase the number of AMF spores in coffee rhizosphere soil. The trapping process used plastic cups (size 7.5 × 9 × 4 cm / 220 mL) with the media of rhizosphere soil mix and sterilized zeolite sand with *Sorghum bicolor* L. for about three months (Auli and Kasiamdari 2019; Husein et al. 2021). In the last two weeks of the trapping process, water stressing was conducted to stimulate AMF spore production.

Identification of AMF spore

A wet-sieving technique collected spores of AMF with 250 μm, 125 μm, 63 μm, and 45 μm tiered strainer (Pacioni 1992; Satomura 2007). The filtered spores were put in a 15 ml test tube, added 60% glucose, and centrifuged for 3 mins at 3,000 rpm, and the spores obtained were freed of glucose. Furthermore, the obtained AMF spores were identified on the spore's morphological characteristics, including spore size, spore color, spore wall layer, spore ornamentation, the presence and dimensions of sub-standing hypha, and their reaction to Melzer's solution. The genus and species of AMF were determined by comparing the spore properties with various references. The spore slide was made using PVLG and Melzer's, and the observation was conducted using a stereo microscope and a compound microscope up to 400 times magnification. In addition, identification was carried out on spore morphology and its reaction to Melzer's (Vizzini et al. 2020).

Root colonization by AMF

The calculation of root colonization by AMF was done using the root coloring technique (Koske and Gemma 1989; Kiheri et al. 2016). First, ten pieces of root 1 cm long were soaked in 10% KOH for 4-5 days until the roots looked clear. Then, the root pieces were washed and soaked in 2% HCl for 24 hours. After that, the roots were soaked in Trypan Blue dye solution for 24 hours. Each root piece was arranged on a slide for observation; the level of colonization was based on the microscope's field of view. The sign of AMF infection in the roots was calculated based on the infection mark of AMF found in the root (spore, hypha, vesicular, arbuscular) in the frame size. The observation of root infection by AMF was conducted using a compound microscope.

The observation variable comprised the variety of AMF types based on the morphological spore and the level of root colonization resulting from trapping. The root colonization was calculated based on the following formula (Deguchi et al. 2017):

$$\text{Level of root colonization (\%)} = \frac{\text{Number of infected frame size}}{\text{Total of frame size}} \times 100 \%$$

Soil analysis

Soil samples from each location were taken as much as 1 kg at 20-40 cm depth, packed in a labeled plastic container, and then taken to the Soil Laboratory of the Faculty of Agriculture, Papua University, Manokwari. Observation of soil physical properties was carried out in the field, while soil chemical analysis was carried out at the Soil and Land Resources Laboratory of IPB University, Bogor. Soil chemical analysis consisted of pH, organic C, soil organic matter, CEC, base saturation, total N, available P, K, salinity, and Al saturation (Table 2).

RESULTS AND DISCUSSION

The results of wet sieving of coffee rhizosphere soil samples showed that at each soil sampling location, there were AMF spores whose number ranged from 328 to 828. At the same time, the analysis of the degree of root colonization by AMF showed variations between 50.6% to 93.2% with a correlation of $r = 0.79$ (Figure 2). The degree of root colonization depends not only on AMF activity; but also on the AMF species that infect the roots of the host plant, following the study result by Turrini et al. (2018). The presence of internal hyphae in cortical cells by AMF belonging to the Gigaspora and Scutellospora tends to be less than in other AMF genera, which do not form vesicles in cortical cells. Still, if the development of external hypha is more numerous, this can result in low colonization rates (Alkobaisy 2022). On the other hand, more extraradical hypha development will be more effective and wider in boosting root exploration for plant nutrients in the rhizosphere (Posada et al. 2018; Rojas et al. 2019).

A positive correlation exists between spore count, colonization rate, and soil fertility. The high number of spores and the high level of AMF colonization on coffee

roots indicates that Arabica coffee plants developed in the Arfak Mountains region have a good association with AMF. The soil fertility level in the Arfak mountainous areas is relatively low due to the frequent leaching of nutrients caused by high rainfall. The low level of soil fertility will encourage AMF to the existence of AMF to be more active in supplying plant nutrients, especially the P element increase the growth and yield of host plants (Posada et al. 2018; Rojas et al. 2019; Kha et al. 2022).

The level of AMF colonization on the roots of the host plant is one of the indications that there has been an association of AMF with the host. Besides being influenced by AMF species, the colonization rate is also affected by plant age. The age of the coffee plants in Mokwam (10 years) is older than the coffee plants in Anggi Gida (5 years), Anggi Giji (4 years), while the coffee plants in Konei, Membei, and Menti are each 8 years old. The level of root colonization and AMF diversity will increase with the increasing age of the Arabica coffee plant (Aguila et al. 2022). A decrease in AMF colonization on old soils shows a positive correlation between soil pH and root colonization by AMF (Liu et al. 2021a). AMF colonization shows a positive association with $Al^{+3} + H^+$ and a negative association with soil pH (Aguila et al. 2022). There is a positive correlation between silt (dust) and AMF colonization because of the role of silt (dust) in pore

formation and soil retention, and air circulation so that it comes a suitable place for AMF development (Banstola et al. 2021). The high level of AMF colonization can indicate the abundance of AMF in soil (Barceló et al. 2020).

Based on the identification of AMF spore morphology which was associated with Arabica coffee plants in the Arfak Mountains, there were 14 AMF species (Figure 3), namely 4 Acaulospora species, 7 Glomus species, and Simiglomus, Funneliformis, Septoglomus consisted of 1 species each (Table 1).

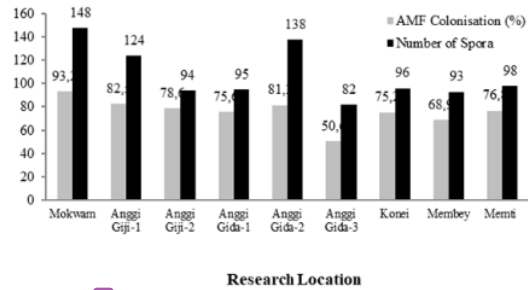


Figure 2. The number of spores and colonization rate of AMF in coffee plants

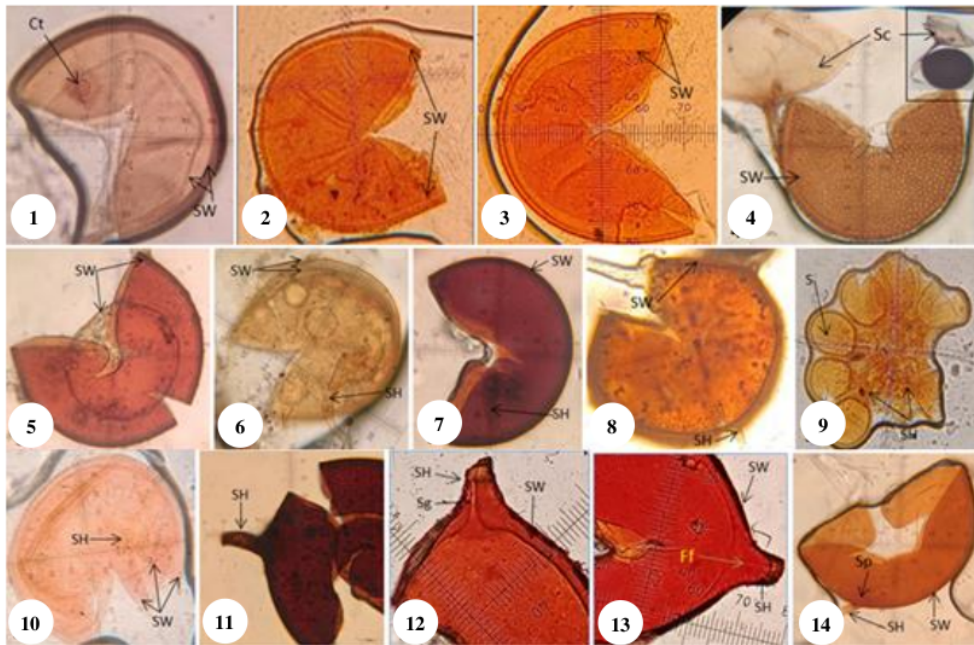


Figure 3. AMF spores associated with Arabica coffee roots (400 x signification; correction factor 2,5). (Note: 1. *Acaulospora cf. foveata*, 2. *Acaulospora cf. tuberculata*, 3. *Acaulospora cf. lacunosa*, 4. *Acaulospora denticulata*, 5. *Glomus manihotis*, 6. *Glomus coronatum*, 7. *Glomus cf. etunicatum*, 8. *Glomus cf. clarum*, 9. *Glomus aggregatum*, 10. *Glomus cf. caledonium*, 11. *Glomus cf. ambisporum*, 12. *Simiglomus sensu stricto*, 13. *Funneliformis sensu stricto*, 14. *Septoglomus sensu stricto*. [Ct: Citrix, SW: Spore Wall, Sc: Sacculle, SH: Substanding Hypha, S: Spore, Sg: Simiglomus, Ff: Funneliformis, Sp: Septoglomus, cf: Confirm] *Acaulospora cf. foveata* and *Glomus cf. ambisporum* were AMF species found at each soil sampling location, while 6 species *A. cf. lacunosa*, *G. cf. clarum*, *G. cf. caledonium*, *G. aggregatum*, *Simiglomus sensu stricto*, and *Funneliformis sensu stricto* were only found in Mokwam)

Table 1. Distribution of AMF species in study locations

Spesies AMF	M	AG-1	AG-2	AGD-1	AGD-2	AGD-3	K	MB	M
<i>Acaulospora cf. foveata</i> (Janos & Trappe)	v	v	-	v	v	-	-	v	-
<i>Acaulospora cf. tuberculata</i> (Janos & Trappe)	v	-	v	-	v	v	v	v	v
<i>Acaulospora cf. lacunosa</i> (Morton)	v	-	-	-	-	-	v	-	-
<i>Acaulospora cf. denticulata</i> (Sieverd. & Toro)	-	v	v	-	v	-	-	v	v
<i>Glomus cf. etunicatum</i> (Becker & Gerd)	v	v	-	v	-	-	-	-	-
<i>Glomus cf. clarum</i> (Nicolson & Schenck)	v	-	-	-	-	-	-	-	-
<i>Glomus cf. aggregatum</i> (Schenck & Smith)	v	-	-	-	-	-	-	-	-
<i>Glomus cf. caledonium</i> (Morton)	v	-	-	-	-	-	-	-	-
<i>Glomus cf. ambisporum</i> (Smith & Schenck)	v	v	v	v	v	v	v	v	v
<i>Glomus manihotis</i> (Becker & Gerd)	-	v	-	-	-	-	-	-	-
<i>Glomus coronatum</i> (Walker & Schöbler)	-	v	v	v	v	v	v	v	v
<i>Simiglomus sensu stricto</i> (Berch & Trappe)	v	-	-	-	-	-	-	-	-
<i>Funneliformis sensu stricto</i> (Walker & Schöbler)	v	-	-	-	-	-	-	-	-
<i>Septoglomus sensu stricto</i> (Oehl)	v	-	-	v	v	v	-	v	v
Amount	11	6	4	5	6	4	4	6	5

Note: M: Mokwam, AG: Anggi Giji, AGD: Anggi Gida, K: Konei, MB: Membei, M: Memti

Table 2. The result of analysis of soil physical and chemical properties in Mokwam, Anggi Gida, Anggi Giji, Membey, Arfak Mountains Region, West Papua, Indonesia

Parameter	Research site			
	Mokwam	Anggi Gida	Anggi Giji	Membey
s Slope (%)	6 - 45%	8-45%	8-45%	25-45%
r Soil physical property				
- Effective soil depth (cm)	60 - 90	60-80	50 -70	60-100
- Soil texture	Dusty sandy loam	Dusty loam	Dusty loam	Sandy loam
- Surface stone (%)	10- 15	10-15	10 - 15	5-15
d Waterlogged				
Drainage class	Good	Good	Good	Good
n Soil chemical property (0-30 cm)				
- pH	6.10 (slightly acid)	5.57 (acid)	6.15 (slightly acid)	5.04 (slightly acid)
- Organik C (%)	0.43	4.37	4.34	5.05
- OM (%)	5.91 (high)	6.04 (high)	5.96 (high)	6.98 (high)
- CEC (me/100 g)	10.77 (medium)	10.87 (low)	11.03 (low)	19.33 (medium)
- BS (%)	32.37 (low)	41.49 (low)	51.91 (low)	100.00 (very high)
- N (total (%))	0.16 (low)	0.27 (medium)	0.31 (medium)	0.33 (medium)
- Available P (ppm)	10.82 (medium)	11.58 (low)	12.31 (low)	36.43 (very high)
- K (me/100 grams)	163.67 (very high)	270.67 (very high)	186.97 (very high)	1297.28 (very high)
x Toxicity				
- Salinity (mm hos/cm)	0.19 (very low)	0.07 (very low)	0.12 (very low)	0.17 (very low)
- Al saturated (%)	4.19 (low)	4.60 (low)	4.13 (low)	3.87 (low)

Variations in the distribution of AMF were affected by pH and nutrition. According to Shukla et al. (2013; Tedersoo et al. 2020), besides pH and nutrient content, soil texture, and chemistry, soil depth influences the distribution of AMF. The soil acidity, moisture, temperature, structure, and nutrient content level are important as they can restrict the spread of AMF (Bunn et al. 2009; Alkobaisy 2022; Janowski et al. 2022). Plants with AMF can grow at a higher pH than those without AMF (Peat and Fitter 1993; Gosling et al. 2006). Soil pH is a key factor affecting the composition of AMF (Stürmer et al. 2018). It is related to complex matters because it is related directly to the availability of several plant nutrients

(Cruz-Pareda et al. 2021). Soil type and depth, season, and vegetation affect the distribution of AMF (Becerra et al. 2014; Neffar et al. 2015; Delvian and Rambey 2021). The AMF population appears to be able to adjust to the gradual changes in the environment, especially to the level of colonization (Vieira et al. 2019). However, if more extreme or rapid environmental changes occur, such as mining or erosion, this can significantly reduce the presence of mycorrhiza. More aggressive agricultural practices promote less mutualism of AM fungal traits, such as fewer quantities of extraradical mycelium or arbuscular colonization (Chagnon et al. 2022). Continuous monoculture farming systems reduce soil microbial

diversity (Mo et al. 18) 6), whereas a rotational cropping system can increase soil microbial diversity (Venter et al. 2016). Posada et al. (2018) stated that in older and well-maintained coffee plants, more AMF species were found. The greater number of AMF species on coffee plants in Mokwam was because the coffee plants were 10 years old, which is older than the ages of the coffee plants in Anggi Giji, Anggi Gida, and Membey, which were 4, 5, and 8 years respectively.

Glomus, known to be the most common genus with 7 species, and the *Acaulospora* genus, with 4 species, is especially abundant in Mokwam. At the same time, the genera *Simiglomus*, *Funneliformis*, and *Septoglomus* each have 1 species (Table 1). At the Membey location, the genus *Acaulospora* was more common. *Acaulosporaceae* can be found on acid soils, while *Glomeraceae* prefers alkaline or neutral soils (Melo et al. 2019). Al-Arequi et al. (2013) in his research found that 13 species were obtained from coffee plants in Yemen; *Glomus proliferum*, *G. etunicatum*, *Acaulospora sporocarpia*, *Acaulospora sp. 1*, *Archeospora sp. 1*, *Scutellospora nigra* and the genus *Glomus* were the most dominant AMF. The large number of AMF species in Mokwam is due to the lower C-organic, organic matter, CEC, base saturation (KB) N-total, available P, and K compared to other locations (Table 2). In general, silt (dust) can form pores, increasing soil retention and air circulation to become a suitable place for the development of AMF (Instola et al. 2021). The diversity of fungal species is related to physical factors, soil chemistry, soil aggregate stability, and agricultural land management practices.

In conclusion, Arabica coffee plants in Mokwam, Anggi Giji, Anggi Gida, and Membey are associated with AMF. Arabica coffee plants in Mokwam District have been associated with more AMF species, namely 11 species, while Anggi Giji, Anggi Gida, and Membey, respectively, have 6, 5, and 6 species. Overall, AMF species that have been associated with Arabica coffee plants in The Arfak Mountains Region are *Acaulospora cf. foveata*, *Acaulospora cf. tuberculata*, *Acaulospora cf. lacunosa*, *Acaulospora denticulata*, *Glomus cf. etunicatum*, *Glomus cf. clarum*, *Glomus agregatum*, *Glomus cf. caledonium*, *Glomus cf. ambisporum*, *Glomus manihotis*, *Glomus coronatum*, *Simiglomus sensu stricto*, *Funneliformis sensu stricto*, and *Septoglomus sensu stricto*.

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