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Identification of root-associated arbuscular mycorrhizal fungi in cassava (*Manihot esculenta* Crantz) in Manokwari Regency, West Papua, Indonesia

Identifikasi fungi mikoriza arbuskula pada akar ubi kayu (*Manihot esculenta* Crantz) di Kabupaten Manokwari, Papua Barat, Indonesia

Antonius Suparno*[1], Darmawanta Tarigan[1], Yohanis A. Mustamu[1], Linda E. Lindongi[1], Wiwik Hardaningsih[2]

[1]Department of Agrotechnology, Faculty of Agriculture, Universitas Papua, West Papua, Indonesia
[2]Department of Food Crop Production, Payakumbuh State Polytechnic of Agriculture, West Sumatra, Indonesia

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*Corresponding author
E-mail: anton.sprn@gmail.com

ABSTRACT
Arbuscular mycorrhizal fungi (AMF) are crucial for plants growing on marginal soils, especially those with a very high dependence on AMF; one of them is plants with few roots, such as cassava. This study aimed to identify the AMF associated with cassava in six locations in Manokwari Regency, West Papua, Indonesia. The research was carried out using an observation method and purposive sampling at 6 locations. Soil samples were taken from the rhizosphere of 8-month-old cassava plants, with 2 kg of soil at each site. Furthermore, using the host plant *Sorghum bicolor* L, AMF was trapped in rhizosphere soil samples for three months. The spore morphology identification showed that cassava in North Manokwari District was associated with 3 genera 7 species, West Manokwari District-Manggoapi obtained 3 genera 7 species, Warmare District obtained 2 genera 7 species, Prafi SP-3 District obtained 3 genera 6 species, Masni SP-5 District obtained 3 genera 7 species, and Masni SP-8 District obtained 2 genera 7 species. The highest AMF colonization rate (94%) was found in the roots of the host plant *Sorghum bicolor* (L.) originating from Masni SP-5 District with the AMF composition consisting of *Acaulospora cf. rehmi*, *Acaulospora cf. spinosa*, *Acaulospora cf. gerdemanii*, *Glomus cf. clarum*, *Gigaspora cf. margarita*, *Gigaspora cf. rosea*, and *Gigaspora cf. gigantea*.

ABSTRAK
Keberadaan fungi mikoriza arbuskula (FMA) sangat penting pada tanaman yang tumbuh di tanah marginal terutama tanaman yang memiliki ketergantungan sangat tinggi terhadap FMA yaitu tanaman dengan perakaran sedikit seperti ubi kayu. Penelitian ini bertujuan untuk mengidentifikasi spesies FMA yang berasosiasi dengan ubi kayu di enam lokasi di Kabupaten Manokwari, Papua Barat, Indonesia. Penelitian dilaksanakan dengan metode observasi dan pengambilan contoh secara purposif pada 6 lokasi. Sampel tanah diambil dari rizosfer tanaman singkong berumur 8 bulan, sebanyak 2 kg tanah tiap lokasi. Selanjutnya dilakukan trapping AMF dari sampel tanah rhizosphere selama 3 bulan menggunakan tanaman inang *Sorghum bicolor* L. Hasil penelitian identifikasi morfologi spora menunjukkan bahwa ubi kayu di Distrik Manokwari Utara berasosiasi dengan 3 genus 7 spesies, Distrik Manokwari Barat-Manggoapi diperoleh 3 genus 7 spesies, Distrik Warmare diperoleh 2 genus 7 spesies, Distrik Prafi SP-3 diperoleh 3 genus 6 spesies, Distrik Masni SP-5 diperoleh 3 genus 7 spesies dan Distrik Masni SP-8 diperoleh 2 genus 7 spesies. Tingkat kolonisasi FMA tertinggi (94%) ditemukan pada akar uji tanaman inang *Sorghum bicolor* (L.) berasal dari Distrik Masni SP5 dengan komposisi FMA adalah *Acaulospora cf. rehmi*, *Acaulospora cf. spinosa*, *Acaulospora cf. gerdemanii*, *Glomus cf. clarum*, *Gigaspora cf. margarita*, *Gigaspora cf. rosea*, dan *Gigaspora cf. gigantea*. .

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INTRODUCTION

Arbuscular mycorrhiza fungi (AMF) is a soil microorganism that shows a significant function in the relationship between plants and their growth sites (Begoude et al., 2016; Fall et al., 2022), one of them in cassava plants that have very minimal roots (Miyakasa & Habte, 2001). AMF is the most frequently encountered symbiosis on earth, which is used to increase the productivity of living and non-biological natural resources (Gianinazzi et al., 2010; Prasad, 2020). This fungus is essential because it is involved in various biogeochemical cycles of carbon and nutrient elements to ensure the fitness of plants and the stability of terrestrial ecosystems. Arbuscular mycorrhizal fungi survive as propagules, i.e., spores, extraradical hyphae, soil, and colonized roots. AMF is the oldest symbiont, predicted to be as old as 400 million to 1 billion years old, and it is older than monocotyl and dicotyl plants as well as other symbionts (Smith & Read, 2008; Selosse et al., 2015). Four orders of AMF, namely, Glomerales, Archaeosporales, Paraglomerales, and Diversisporales, have been identified in this sub-phylum, including 25 genera (Redecker et al., 2013).

The AMF association is formed by the obligate fungi associated with approximately 80 – 90% of terrestrial plants, including flowering plants, bryophytes, and ferns (Ahanger et al., 2014) or 73% of higher plant ordo (Brundrett, 2009). Each plant has functional compatibility that differs from AMF inoculant due to the plant's preference towards the specific AMF. The plant's small rooting system, which has short root hair, is indicated with highly dependent on mycorrhiza fungi. Cassava (*Manihot esculenta* Crantz) has primarily been planted on dry land with a wet and/or dry climate, like inceptisol soil. This soil is spread widely in Indonesia, with approximately 40.8 million ha (Hanudin et al., 2021). However, cassava is a kind of plant with very little in its rooting system, so it needs very fertile land conditions and especially other organisms' roles to help its growth and production. In Manokwari, West Papua, cassava is vital as a local food crop, especially for indigenous Papuans. Based on the research result in Cameron (Begoude et al., 2016), there are three genera of AMF associated with cassava: Gigaspora, Glomus, and Acaulospora. Soedarjo (2019) stated that in addition to the increased nutrition absorption, AMF also increases cassava's tolerance to drought and soil salinity. Ceballos et al. (2013;2019) & Widiatma et al. (2015) asserted that AMF was commonly found in the rhizosphere of cassava plants because, during the vegetative stage, cassava requires a high enough supply of phosphorous (P) for the tuber growth process to obtain the optimal tuber yield. AMF was reported to increase the growth and production of Bangka cassava accession (Lestari et al., 2018).

According to Miyasaka & Habte (2007), plant dependency on AMF is classified into four categories: not dependent, somewhat dependent, highly dependent, and highly dependent on AMF. The fact shows that the response of cassava to AMF depends on the variety of intraspecific AMF (Ceballos et al., 2013). However, the diversity of AMF types associated with cassava in Manokwari, West Papua, is still unknown. Therefore, this research was conducted to identify the diversity of AMF based on morphological features or phenotypic spores associated with cassava in Manokwari.

MATERIALS AND METHODS

The research was conducted in Manokwari, West Papua, which comprised six districts or locations where cassava was planted as one of a local Papuan staple food, i.e., North Manokwari (-0°43'37"S; 133°51'1"E), West Manokwari (-0°50'15"S; 134°3'58"E), Warmare (-0°59'17"S; 133°50'39"E), Masni SP-5 (-0°51'0"S; 133°46'29"E), Masni SP-8 (-0°46'51"S; 133°42'13"E), Prafi SP-3 (-0°52'58"S; 133°49'4"E). The trapping process and morphological observation of AMF were performed in the Agroclimate Laboratory, Faculty of Agriculture, Papua University, Manokwari (see Figure 1).

Materials that been utilized in this research were samples of the ready-to-harvest rhizosphere cassava soil, sterile zeolite sand as an AMF trapping medium, *Sorghum bicolor* L. as a trapping host for AMF, glucose 60 % (Gulaku: 100% cane sugar, PT Sweet Indolampung-Indonesia) for wet sieving of AMF spores, polyvinyl lacto-glycerol (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.

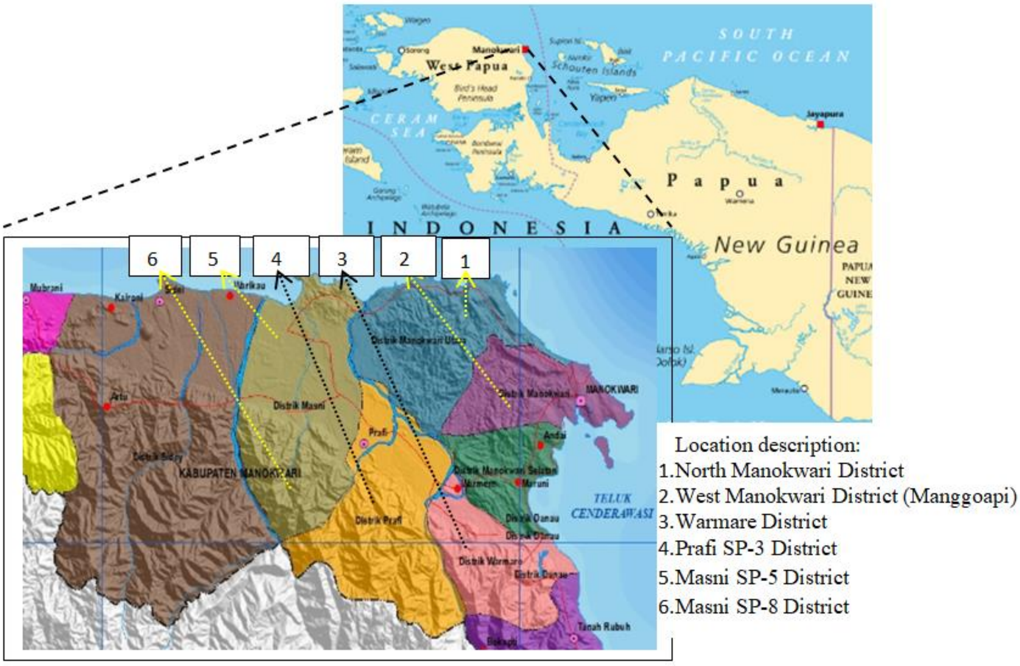

Figure 1. Research location of the diversity of AMF in Manokwari, West Papua

Isolation and trapping of AMF

The research was conducted by observation and purposive sampling techniques (Priyono, 2016). The sample of cassava rhizosphere soil was taken in ready-to-harvest (8 months) of 5 cassava plants in each location from a total of six research locations: North Manokwari, West Manokwari, Warmare, Masni SP-5, Masni SP-8, and Prafi SP-3 where cassava was planted as a portion of local Papuan food. The soil sample was taken at a 10-cm-depth from 8 months of cassava plant rhizosphere for 2 kg/plant. Rhizosphere soil samples were taken from three plants in each location. Soil sampling was carried out using a knife by scraping the soil attached to the cassava tubers and roots and then put in a sterile plastic bag. The soil sample in each location was composited and air-dried in the laboratory. Half of the soil sample was used for observing early AMF existence, and the other half for trapping culture.

A trapping culture was conducted to increase the number of AMF spores found in cassava rhizosphere soil. The trapping process used plastic cups (size 7.5 × 9 × 4 cm / 220 mL) with the media of rhizosphere soil mix and (sterile) zeolite sand with *Sorghum bicolor* L. for about three months (Auli & 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 (Nusantara et al., 2012). The filtered spores were put in a 15 ml test tube, added 60% glucose, and centrifuged for 3 mins at 3000 rpm, and the spores obtained were cleaned of glucose. Furthermore, the obtained AMF spores were identified on the spore's morphological characteristic that covered spore size, spore color, spore wall layer, spore ornamentation, 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. 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 & 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 pieces of root were washed and soaked in 2% HCl for 24 hours. Afterward, the roots were soaked in Trypan Blue dye solution for 24 hours; each root piece was arranged on a slide and observed. The level of colonization based on the microscope's field of view to observe signs 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 level of root colonization resulting from trapping. The root colonization was calculated based on the following formula (Deguchi et al., 2017):

$$\text{Level of root colonization (\%)} = \frac{\text{Number of infected frame size}}{\text{Total frame size}} \times 100\% \qquad (1)$$

RESULTS AND DISCUSSIONS

There were three families, i.e., Glomaceae, Acaulosporaceae, and Gigasporaceae, and four genera, Glomus with 11 species, Acaulospora with seven species, Gigaspora with three species, and Scutelospora with one species (Table 1). Some photos of AMF spores are presented in Figure 2.

Table 1. Diversity of AMF associated with cassava (*Manihot esculenta* Crantz) in Manokwari West Papua

Location	Genus	Species
North Manokwari District	Acaulospora	*Acaulospora cf. foveate*
		Acaulospora cf. rehmi
		Acaulospora cf. spinosa
	Glomus	*Glomus cf. etunicatum*
		Glomus cf. callosum
	Gigaspora	*Gigaspora cf. margarita*
Total	*3 genera*	*6 spesies*
West Manokwari District (Manggoapi)	Acaulospora	*Acaulospora cf. foveata*
	Glomus	*Glomus cf. mosseae*
		Glomus cf. monosporum
		Glomus cf. callosum
	Gigaspora	*Gigaspora cf. gigantea*
Total	*3 genera*	*5 spesies*
Warmare District	Acaulospora	*Acaulospora cf. scorbiculata*
		Acaulospora cf. tuberculata
		Acaulospora cf. spinosa
	Glomus	*Glomus cf. etunicatum*
		Glomus cf. mosseae
		Glomus cf. monosporum
		Glomus cf. aggregatum
Total	*2 genera*	*7 spesies*
Prafi SP-3 District	Acaulospora	*Acaulospora cf. laevis*
	Glomus	*Glomus cf. clarum*
		Glomus cf. maculosum
		Glomus cf. deserticola
		Glomus cf. albidum
	Scutellospora	*Scutellospora cf. fulgida*
Total	*3 genera*	*6 spesies*
Masni	Acaulospora	*Acaulospora cf. rehmi*

SP-5 District		*Acaulospora cf. spinosa*
		Acaulospora cf. gerdemanii
	Glomus	*Glomus cf. clarum*
	Gigaspora	*Gigaspora cf. margarita*
		Gigaspora cf. rosea
		Gigaspora cf. gigantea
Total	*3 genera*	*7 spesies*
Masni	Acaulospora	*Acaulospora cf. laevis*
SP-8 District		*Acaulospora cf. tuberculata*
		Acaulospora cf. rehmi
		Acaulospora cf. gerdemanii
	Glomus	*Glomus cf. monosporum*
		Glomus cf. gerdemanii
		Glomus cf. callosum
Total	*2 genera*	*7 species*

Note: *cf.* = *confirm* (resemblant)

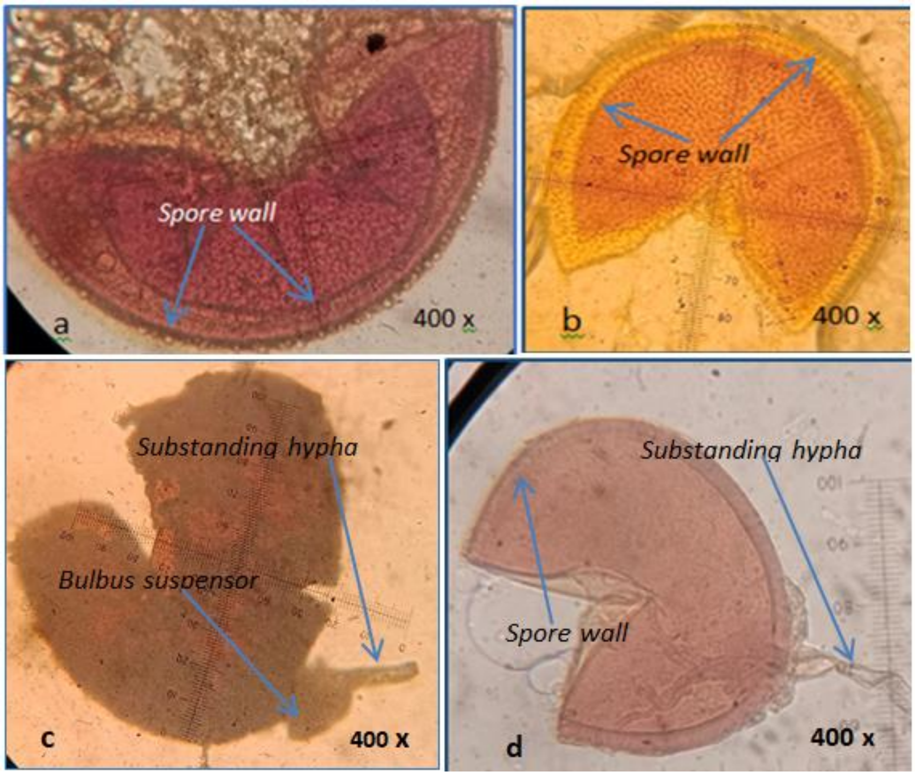

Figure 2. Spores morphology of a: *Acaulospora cf. foveata*; b. *Acaulospora tuberculata*; c. *Scutellospora cf. fulgida*; d. *Glomus cf. etunicatum* (400 times magnification).

The main characteristic of AMF spores in the Acaulospora genus was that the spores react positively with Melzer's solution, which was marked by a change in the color of the spores to brown. In contrast, the spores of other AMF genera were negative. The following identification was to observe the special features on the surface of the spore, the number of layers of the spore wall, and the characteristics of other spore accessories, such as the substending hypha and bulbous suspensor. These special characteristics determined the genus and species of AMF (Figure 2).

The highest level of AMF colonization (%) of cassava from Masni SP-5 District in the *Sorghum bicolor* L. as the host plant was 94% (Figure 3, Table 2).

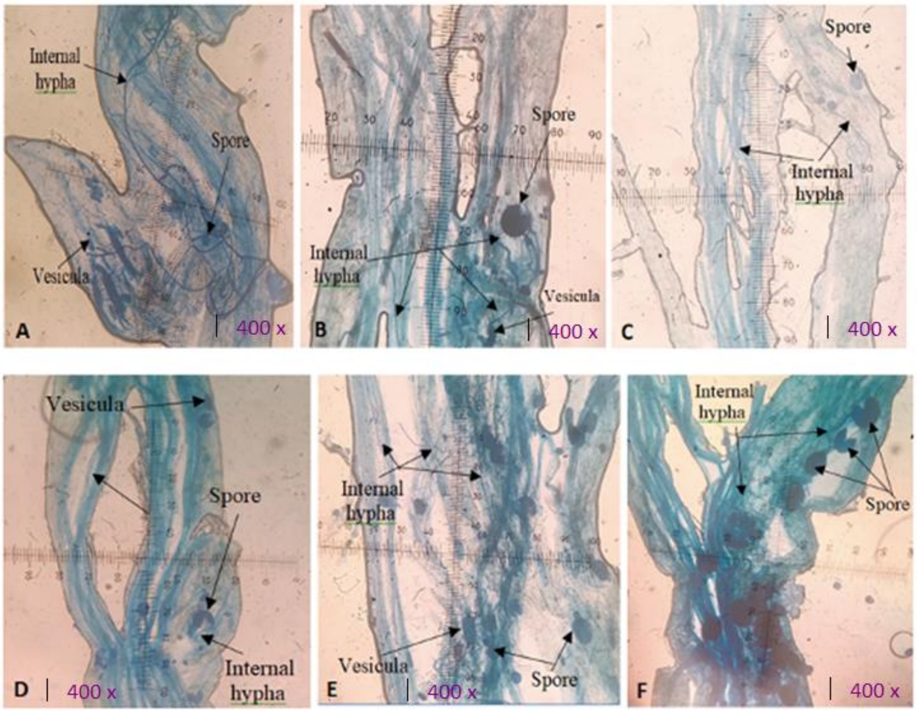

Figure 3. Cassava AMF infection on roots of *Sorghum bicolor* L. as a host plant in trapping (400 times magnification with trypan blue stain); A. North Manokwari District; B. West Manokwari District (Manggoapi); C. Warmare District; D. Prafi SP3 District; E. Masni SP5 District; F. Masni SP8 District

Table 2. Level of cassava AMF colonization in white sorghum (*Sorghum bicolor* L.) host plant and soil pH

Location	Colonization level (%)	pH (H_2O)
North Manokwari District	61	5.8
West Manokwari District (Manggoapi)	49	6.6
Warmare District	55	5.8
Prafi SP-3 District	86	5.9
Masni SP-5 District	94	4.7
Masni SP-8 District	80	5.7

Description: Colonization level (Anggraeny et al., 2017)

>75% : high level of colonization

51-74% : medium level of colonization

<50% : low level of colonization

The high level of cassava AMF colonization from Masni SP-5 District in the host plant's root showed that the species was more effective in infecting and forming colonization with their host plant (see Figure 3). The specialty of AMF from the cassava rhizosphere that came from Masni SP-5 District, which grew in lower pH soil, was the existence of the Gigaspora genus comprised of 3 species (Table 1). Soil condition in Masni SP-5 District was acid (Suharno et al., 2019; Taberima et al., 2020). The results of research on AMF diversity in Cassava in East and South Cameron showed that Andom soil, which had less available P, had higher AMF diversity and contained 10 site-specific species, compared to Bityili soil, which only found two site-specific species (Sarr et al., 2019). According to Fitriani et al. (2008), AMF's acid phosphate enzyme activity would be higher if it was located in P-deficient soil because AMF was more active. Increasing organic fertilizer (AMF, Microbe, Phosphate Solvent) increased the activity of acid phosphatase enzyme to increase P solubility. In acid soil, the P availability for plants is limited because it is fixed by Al and Fe (Supriyanto, 2019).

CONCLUSIONS

Based on spore morphological character and AMF spore accessories associated with cassava (*Manihot esculenta* Crantz) in six locations, it was comprised of 4 genera, i.e., Acaulospora, Glomus, Gigaspora, and Scutellospora. The highest AMF diversity of species in the genus of Aculospora was found in Masni SP-8 District (4 species), the most diverse species in the genus Glomus were found in Warmare District and Prafi SP-3 District (4 species each), the most diverse species in the genus Gigaspora were found in Masni SP-5 District (4 species). Scutellospora was only located in Prafi SP-3 District (1 species). AMF associated with cassava in Masni SP-5 District produced the highest root colonization level (94%) in *Sorghum bicolor* L. as the host plant and the lowest (49%) AMF associated with cassava plant in West Manokwari – Manggoapi District. The diversity of AMF associated with cassava in Manokwari consisted of 11 species from the genus Glomus, seven species from the genus Acaulospora, three species from the genus Gigaspora, and only one species from the genus Scutellospora.

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