

Research Article

New Record of Arbuscular Mycorrhizal Fungi (AMF) Association with Kebar Grass (*Biophytum petersianum* Klotzsch.) in the Grassland Area of Kebar, Tambrauw Regency, West Papua, Indonesia

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are an important form of symbiosis between fungi and plants in an ecosystem. One of the medicinal plants used by the people in West Papua is kebar grass (*Biophytum petersianum* Klotzsch.). This study aims to determine the AMF association in the rhizosphere of *B. petersianum* in grasslands. Survey method was used in this study. The presence of AMF was observed by examining root colonization and spore diversity. The results showed that the percentage of AMF colonization in roots was between 46.7–90.0% with an average of 71.66%. Meanwhile, the number of spores found in the plant rhizosphere averaged 119.8 spores per 10 grams of soil sample. There were 18 species of AMF dominated by the genus *Glomus* (7 species), *Acaulospora* (3 species), while the genus *Claroideoglossum*, *Entrophospora*, *Gigaspora*, and *Scutellospora* were dominated each with 2 species. This finding is the first record on the presence of AMF on *B. petersianum* in West Papua.

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INTRODUCTION

Indonesia has a great wealth of biological resources (Kartikasari et al. 2012; von Rintelen et al. 2017) and a diverse forest ecosystem that influences the presence of other organisms (Kartikasari et al. 2012), including fungi (Suharno et al. 2014). In Papua, the condition of the forest and its content are even better than in other areas with a fairly large variety of fungi (Suharno et al. 2014). Mycorrhizae are a form of mutual symbiosis (mutual benefit) between fungi and plants.

Arbuscular mycorrhizal fungi (AMF) is one of the mycorrhizal groups classified as large in membership and symbiotic in most groups of vascular plants (Souza 2015; Suharno et al. 2020). This association involves the root system of plants with fungi belonging to the phylum Glomeromycota (Smith & Read 2008; Souza 2015). Furthermore, this symbiosis plays an important role in nutrient cycling and uptake by plant roots (Zhang et al. 2021). Moreo-

ver, mycorrhizae can associate around 80–90% of plant species (agriculture, forestry, and plantations), including natural plants (Giovannetti et al. 2006; Smith & Read 2008; Suharno et al. 2020) such as halophytic, hydrophytic, and xerophytic plants (Souza 2015) and also helps in increasing the efficiency of nutrient uptake (especially phosphorus) on some marginal lands (Souza 2015; Tuheteru et al. 2019; Suharno et al. 2021).

In addition to the development program of medicinal ingredients (including traditional medicine), the role of fungi in increasing plant growth is very important. The potential of indigenous fungi is very useful and has the potential to be developed. Subsequently, the most important thing is to develop the function of fungi and medicinal plants as superior products (Suharno et al. 2018). However, supporting factors are necessary for optimal production, and not just only the physical and chemical factors of the soil, but the most important is also the involvement of microorganisms that supports plant growth. AMF is one of the multifunctional microorganisms in symbiosis with plants.

AMF plays a role in the growth of medicinal plants. In China, several types were found in symbiosis with the roots of medicinal plants such as ginseng (*Panax* spp.), *Datura stramonium*, *Atractylodes macrocephaly*, and other types of medicinal plants (Wang & Shi 2008). Furthermore, it is also found to be associated with traditional medicinal plants in India (Kumar et al. 2021). Under the salt stress condition, some AMF are also able to rise proline dan phenol (Duc et al. 2021). AMF plays important role in facilitating secondary metabolite synthesis as a protection from several pathogen (Sun et al. 2021). While in Indonesia, research on AMF in medicinal plant is still limited. Inoculation of AMF on *Centela asiatica* was increased asiaticoside (0.1–0.6%) (Trisilawati et al. 2019). In Papua, the medicinal plant Wati (*Piper methysticum*) is found to be associated with AMF (Suharno et al. 2018).

Kebar grass (*Biophytum petersianum* Klotzsch.) is widely known in Indonesia as a traditional medicinal plant for women's fertility (Unitly & Inara 2011; Sembiring & Darwati 2013). It is also used for the immune system, inflammation, fever, and wound healing (Inngjerdingen et al. 2006; Inngjerdingen et al. 2008; Kayadoe et al. 2012). Besides that, *B. petersianum* is also used for the treatment of malaria (Giovannetti et al. 2010; Kayadoe et al. 2012), hypertension (Mouzou et al. 2009), thrush medicine, an antidote to snake bites, and laxative for children (Unitly & Inara 2011).

The distribution of this herbaceous plant is quite wide in tropical regions such as Africa, Madagascar, and Asia (Sambodo et al. 2018), including Papua, New Guinea. In Indonesia, it is found in several areas such as Java and Papua (Tambrau and Manokwari) (Sembiring & Darwati 2013). It belongs to the family of Oxalidaceae (Inngjerdingen et al. 2006; Sambodo et al. 2018) and has a synonymous name *B. sensitivum* (L.) DC (Inngjerdingen et al. 2006). Although it is not included in the grass family (Poaceae), its stature looks like grass and is often found among weeds (*Imperata cylindrica*), creates a perception for local people to call it “grass” kebar. In Papua, *B. petersianum* is

commonly found in Kebar District, Tamberau Regency, especially in grassland areas. The relationship between AMF and plants in absorbing nutrients to improve plant growth is very important. Therefore, it is necessary to study the association of *B. petersianum* with AMF in grassland areas.

MATERIALS AND METHODS

Study Area

This study was conducted in 2 sub-districts, Kebar and East Kebar in Tamberau Regency, West Papua. The selection of the location was based on information about the existence of kebar grass from the local community. The Tamberau regency is located in the bird's head area of Papua (Figure 1). Furthermore, the total area is 11,529,179 km² and consists of 29 sub-districts. Moreover, Kebar and East Kebar are savanna grassland areas in Tamberau Regency, West Papua.

Environmental Condition

The environmental condition such as altitude, temperature, and humidity of the study site were measured. Soil properties of the *B. petersianum* rhizosphere such as pH, organic C, N total, C/N ratio, available P, kalium, Ca, Na, Mg, Cation Exchange Capacity (CEC), and soil texture were tested. Soil samples were only taken in three locations, i.e.: Kebar (DK1), Kebar (DK4), and East Kebar (DKT2), because of the similar condition. The analysis of the soil samples was carried out at the Seameo-Biotrop Bogor laboratory.

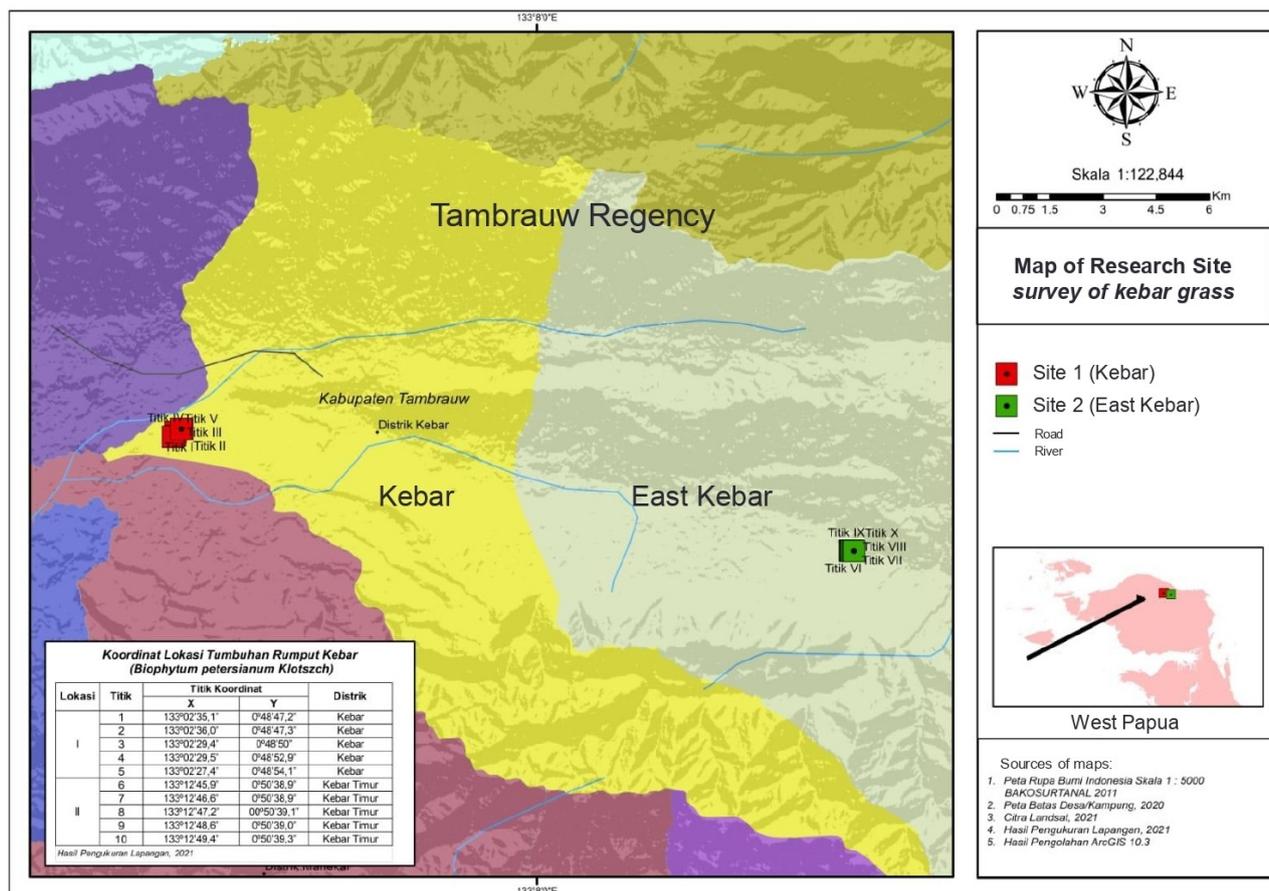


Figure 1. Sampling location in Tamberau regency, West Papua.

Survey of *B. petersianum* Presence

A survey of kebar grass was carried out in 10 locations in the districts of Kebar and East Kebar. These locations were chosen because they are kebar grass producer areas according to the local community. The initial survey showed that *B. petersianum* was not found in the primary and secondary forest areas, while it was abundant in the grasslands. Therefore, the grassland area was the main focus of the observation. Furthermore, five observation sites were located in each district, then 10 plots (1 x 1 m²) were randomly distributed with 20 meters distance between plots to have totally 100 plots. The presence of kebar grass was observed and the number of individuals in each plot was counted.

Survey of AMF Presence

The survey of AMF existence in the rhizosphere of *B. petersianum* was carried out by observing the AMF spores; the method used was the extraction of spores by wet sieving. Then, 1 kg of soil samples was taken and the AMF was isolated using the wet sieving method with *sucrose centrifugation technique* (Vierheilig et al. 2005). Meanwhile, the soil samples (100 g) was filtered using a stratified sieve with a mesh size of 250, 100, and 30 µm. Furthermore, the solution containing the filtered spores was centrifuged (2,500 rpm, 10 minutes) with the addition of 50% sucrose. The separated spores were washed on filter paper and then observed and counted under a stereo microscope (30x).

AMF Association in *B. petersianum*

The AMF association with *B. petersianum* was determined by observing the infection and colonization of AMF in the plant root system. The method used in this observation is root staining (Brundrett et al. 1996). Plant roots was taken randomly at two district locations, each at five different locations (Table 1). To determine fungal colonization, the slide method was used (Brundrett et al. 1996; Vierheilig et al. 2005) by observing 30 of 1 cm root slices, while staining was carried out with trypan blue (Kormanik & Mc.Graw 1984; Vierheilig et al. 2005; Sun & Tang 2012). The status of AMF is known through symbiotic characteristics, such as the presence of intraradical and extraradical hyphae, vesicles, arbuscules, as well as the possible presence of intraradical spores in root tissue.

AMF Type Diversity

The AMF diversity was identified based on the morphological characteristics of the spores. The morphological identification was based on spore characteristics (spore shape, color, hyphae stalk attachment, wall, and reaction of spore contents with Melzer's solution). Meanwhile, identification of genus to species used several literatures from Schenck and Perez (1990), Brundrett et al. (1996), and Suharno et al. (2020).

Analysis Data

Observational data are displayed in the form of tables and figures.

RESULTS AND DISCUSSION

Environmental conditions

Observations showed that the study site is at an altitude of 572–619 m above sea level (Table 1) which was quite high during the day, with a humidity of 28 to 68%. Moreover, soil characteristics in this area vary, ranging from dusty clay to loamy sand (Table 2).

Table 1. Location, coordinate, and environmental condition of the study site in Kebar Sub-district, Tambrau, West Papua.

No	Location (code)	Coordinate	Altitude (m asl.)	Temperature (°C)	Humidity (%)
1	East Kebar (DKT2)	S : 00°50'38,9" E : 133°12'45,9"	619	38	31
2	East Kebar (DKT7)	S : 00°50'38,9" E : 133°12'46,6"	604	36	30
3	East Kebar (DKT9_1)	S : 00°50'39,1" E : 133°12'47,2"	603	32	28
4	East Kebar (DKT9_2)	S : 00°50'39,0" E : 133°12'48,6"	606	33	29
5	East Kebar (DKT10)	S : 00°50'39,3" E : 133°12'49,4"	604	32	31
6	Kebar (DK1)	S : 00°48'47,2" E : 133°02'35,1"	574	22	68
7	Kebar (DK3)	S : 00°48'47,3" E : 133°02'36,0"	575	28	42
8	Kebar (DK4)	S : 00°48'50" E : 133°02'29,4"	573	27	46
9	Kebar (DK5)	S : 00°48'52,9" E : 133°02'29,5"	573	33	38
10	Kebar (DK6)	S : 00°48'54,1" E : 133°02'27,4"	572	32	32

The analysis of the soil sample shows that the soil quality in the Tambrau area was acidic, with a soil pH value of 4.8 to 6.4 (pH analysis (H₂O) classified as acidic. Meanwhile, the organic C content is between 1.56-4.01 % (low–high), with an average of 2.65% including the medium category. The total N content ranged from 0.19-0.49% with an average of 0.31% including the medium category. Then, the C/N ratio between 8.00-9.00 with an average of 8.33 (low) (Table 2). According to Sorensen (1993) and Warren et al. (2017), the high and low C content are associated with the carbon stock value in an area. This result shows that under low acidity soil and low to high organic carbon condition, AMF still could be found with the presence of 18 species of AMF on the *B. petersianum* rhizosphere. AMF could grow at pH 2.7 – 9.2 (Aguilera et al. 2015). *Acaulospora* is one of AMF that could well adapted to the acid soil condition.

The available P content (available P₂O₅) ranged from 10.7–16.2 ppm, with an average of 14.0 ppm (high). Meanwhile, the K content ranged from 0.19–0.79 cmol.kg⁻¹ with an average of 0.38 cmol.kg⁻¹ (low), Ca averaged 2.42 cmol.kg⁻¹ (low), and Mg 0.97 cmol.kg⁻¹ (low). Subsequently, the cation exchange capacity (CEC) of soil is between 9.55–21.22 cmol.kg⁻¹ with an average of 14.55 (low), with a base saturation (BS) level of around 16.82–45.65 %, with an average of 29.79% (low). Its category of soil properties were based on [Eviati and Sulaeman \(2009\)](#). Furthermore, the soil analysis shows the area overgrown with Kebar grass has a loamy sand texture to dusty clay. [Carrenho et al. \(2007\)](#) reported that host plant species and soil texture were influenced to the level of AMF colonization. Host plant species is associated with the compatibility of plant-fungus symbiosis. Some AMF only have association with specific host plant. Colonization of AMF were also influenced by the nutrient content and its availability which related to the soil texture. In some condition, soil texture also influenced the availability of water which would have impact on the spore production in the rhizosphere.

The soil characteristics in several locations in Sub-district of Kebar are quite suitable for agricultural land, which has long been known as a producer of peanuts and corn. Although some investors seem to be developing corn cultivation in grasslands, this land is located in a plain area lower than the topography of the higher plains, hills, and surrounding mountains.

Table 2. Soil physicochemical characteristics in the Kebar grass area in West Papua.

Soil physicochemical characteristics	Location and code			Average
	Kebar (DK1)	Kebar (DK4)	East Kebar (DKT9_1)	
pH (H ₂ O)	6.4	5.3	4.8	5.5
pH (CaCl ₂)	4.9	4.8	4.1	4.6
Organic C (%)	2.37	1.56	4.01	2.65
N total (%)	0.26	0.19	0.49	0.31
C/N Ratio	9.00	8.00	8.00	8.33
P ₂ O ₅ available (ppm)	15.1	10.7	16.2	14.00
K-dd (cmol.kg ⁻¹)	0.20	0.19	0.76	0.38
Ca-dd (cmol.kg ⁻¹)	4.24	1.58	1.45	2.42
Na-dd (cmol.kg ⁻¹)	0.22	0.21	0.25	0.27
Mg-dd (cmol.kg ⁻¹)	1.21	0.59	1.11	0.97
CEC (cmol.kg ⁻¹)	12.88	9.55	21.22	14.55
Base saturation (%)	45.65	26.91	16.82	29.79
<i>Al-H_{dd}KCl 1N:</i>				
Al-dd (me.100g ⁻¹)	0.00	0.00	2.56	0.85
H-dd (me.100g ⁻¹)	0.10	0.35	0.65	0.37
Soil texture				
Sand (%)	89.4	84.4	2.9	58.90
Dust (%)	5.3	9.4	45.4	20.03
Clay (%)	5.3	6.2	51.7	21.07
	Loamy sand	Loamy sand	Dusty clay	Sandy loam

Note: Soil analysis was carried out at Seameo-Biotrop soil laboratory, Bogor.

The land in the form of grassland is a suitable location for livestock food. According to public information, this area was once a center for cattle breeding and development. However, it continues even after the cattle population has decreased. These are provided by the government in form of assistance to the community capital to develop a cattle farming business that often consumes kebar grass. According to Xu et al. (2017), land use change will have impact on the diversity of AMF. AMF diversity was significantly higher in grassland than in forest or arable land. Significant differences in AMF community composition were found among different land use types.

The population of *B. petersianum*

The results show that the kebar grass distribution is not evenly distributed throughout the Kebar and East Kebar Districts. The average population of *B. petersianum* is 11.2 per m². However, Kebar grass was not found in site 3 (East Kebar) site 6, dan 7 (Kebar) (Figure 2). Moreover, when the survey was conducted, Kebar grass was not found in primary and secondary forest areas. This is because the habitat and other plant associations do not allow it to grow and to develop. Furthermore, forests with higher plant species can cause land cover, and kebar grass which is less than six inches in height cannot compete for light for growth.

The small kebar grass habitus (< 15 cm) grows in association with other plants in the grassland dominated by plant species from the *Poaceae* family. Meanwhile, the one grassland in Kebar (DK4) is different, but weeds (*Imperata cylindrica*) are widely distributed throughout the savanna area. Kebar grass is known to be most commonly found in association with weeds (*Imperata cylindrica*) in Kebar Sub-district area. Moreover, it is also associated with “road” grass (*Themeda triandra*), melastoma (*Melastoma malabathricum*), tekian (*Cyperus* spp.), *Heteropogon* spp., and other types of grass in East Kebar Sub-district.

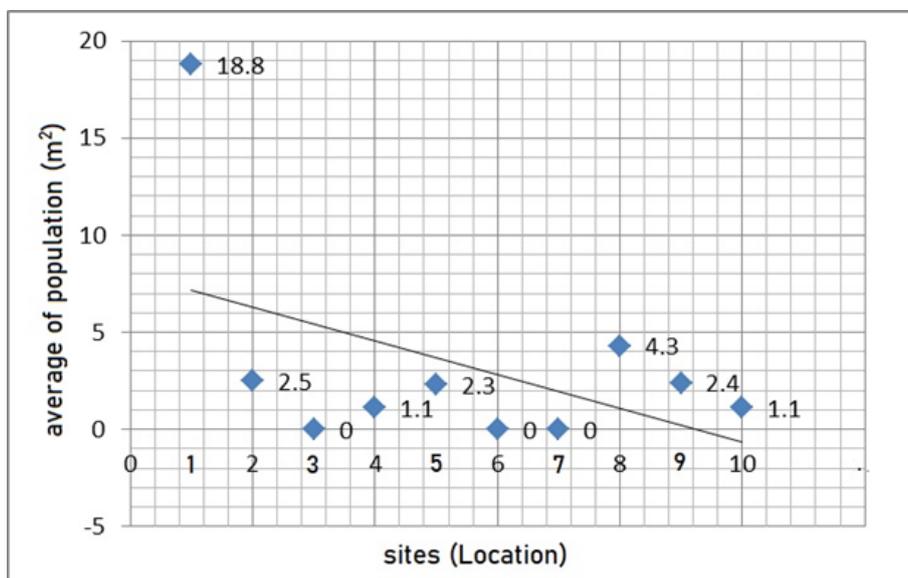


Figure 2. Distribution and trend of the kebar grass population in Tambrauw Regency, West Papua. Observation locations 1 – 5 in East Kebar, and 6 – 10 in Kebar.

Presence and Colonization of AMF

The presence of AMF in this area is known from spores found in the rhizosphere of kebar grass (Table 3). The results show the average number of spores found is 119.8 spores per 10 g (1,198 spores per 100 g) of soil sample. Furthermore, Tao et al. (2004) found an average of 1,530 spores per 100 g in a savanna field in southwest China. The number of spores found depends on the depth of the soil. In general, it is very high to a depth of 20 cm, but it decreases with the depth of the soil and depends on the type and composition of the plant (Cuenca & Lovera 2010).

Table 3. The number of spores and the colonization of AMF in the rhizosphere of *B. petersianum* in Tambrauw Regency, West Papua.

No	Sample code	Location (sub-district)	Number of spores (10 g soil sample)	AMF colonization (%)
1.	DK1	Kebar	124.5	86.7
2.	DK3	Kebar	160	90
3.	DK4	Kebar	100	90
4.	DK5	Kebar	113	70
5.	DK6	Kebar	132	66.7
	Average		125.9	
6.	DKT2	East Kebar	116	76.7
7.	DKT7	East Kebar	120.5	46.7
8.	DKT9_1	East Kebar	108	60
9.	DKT9_2	East Kebar	113	63.3
10.	DKT10	East Kebar	111	66.7
	Average		133.7	
	Total average		119.8	

The results show that there is an AMF colonization in the root system of *B. petersianum* (Figure 3). Meanwhile, the AMF colonization rate is in the range of 46.7–90.0%, with an average of 80.0% (high category) (Table 3). Furthermore, Suharno et al. (2018) found the level of AMF colonization in the medicinal plant wati (*Piper methysticum*) originating from the lowlands of Merauke between 38.46–83.3%. According to Suharno et al. (2020), the percentage of AMF colonization is included in the large category. Moreover, Casazza et al. (2017) and Zhang et al. (2021) suggested that the intensity and diversity of AMF colonization levels varied for each plant species and habitat differences. Several factors influence the content of the soil water (Cuenca & Lovera 2010), pH (Coughlan et al. 2000), temperature, and altitude (Zhang et al. 2021). However, AMF colonization rates are lower at high altitudes in different mountainous environments (Zhang et al. 2021).

The level of AMF colonization is very important in the symbiotic process with plants (Smith & Read 2008; Suharno et al. 2021). The intraradical mycelium will influence the ecosystem processes indirectly through the nutrition and performance of host plants, while the extraradical mycelium is directly related to the ecosystem function (Barceló et al. 2020). Therefore, the infection and colonization of AMF in the ecosystem are very important.

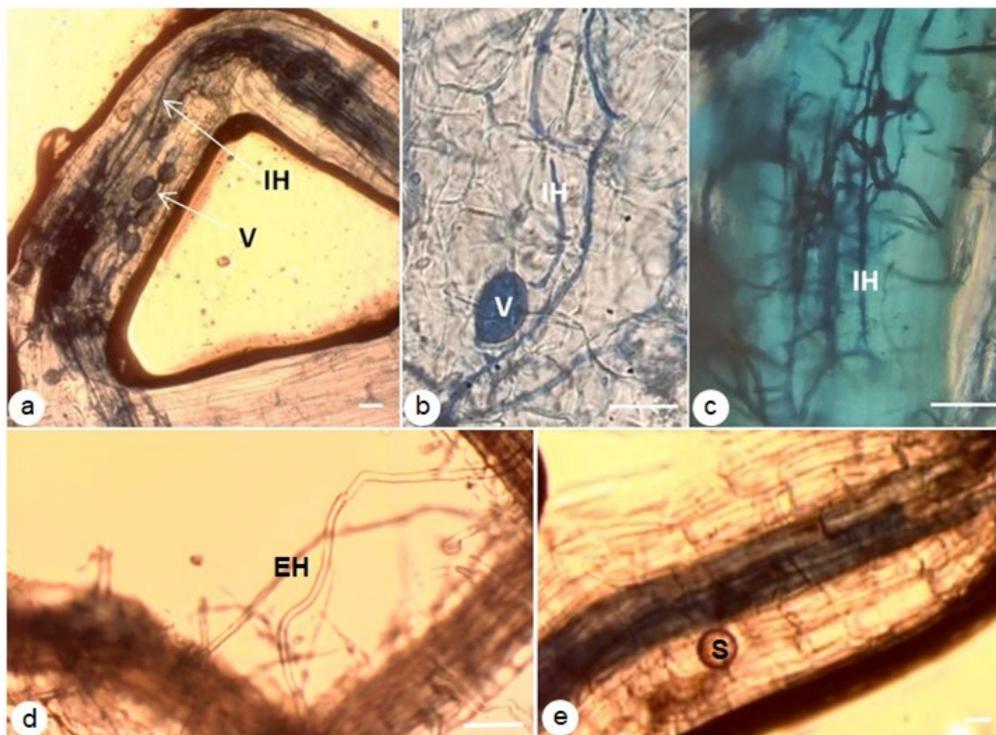


Figure 3. The presence of AMF on the root system of kebar grass (*B. petersianum*). a. intraradical hyphae and vesicles in the root, b. vesicles c. intraradical hyphae, d. extraradical hyphae on the root hairs, e. intraradical spore. (v: vesicula, IH: intraradical hyphae, EH: extraradical hyphae, S: intraradical spores, scale bar: 100 μ m).

Moreover, AMF hyphae proliferation and spore production can also contribute to mycorrhizal activity and function related to ecosystems and environmental variables (Furrazola et al. 2015). In this study, on low pH soil (Table 2) AMF colonization was high and may be maximal if pH would be increased. Rohyadi et al. (2004) reported that under acid condition, AMF colonization also low, but it will improve in line with the increasing of pH. The increment of AMF colonization has positive correlation with the host plant growth.

AMF Diversity

Based on the characteristics of the spore, there are 18 species of AMF (Table 4; Figure 4). These species belong to six genera, including *Glomus* (7 species of AMF), *Acaulospora* (3 species), and the genus *Claroideoglossum*, *Entrophospora*, *Gigaspora*, *Scutellospora* with two species each (Table 4; Figure 4). Some spore could not be identified yet since the identification process was only based on morphological characteristics.

In Papua, a study on AMF diversity based on the morphological characteristics of the medicinal plant Wati (*Piper methysticum*) found around 10 species (Suharno et al. 2018), while nine species were found in the pokem plant (*Setaria italica*) nine types (Suharno et al. 2015). *G. etunicatum*, *A. foveata*, *Glomus* sp. DK1-1., and *Scutellospora* sp. DK4-2., were widely distributed, while *Gigaspora* sp. DKT9-2, *Gigaspora* sp. DK9, *Claroideoglossum lamellosum*, and *Acaulospora* sp. DK4. have limited distribution. Suharno et al. (2018), reported that *G. etunicatum* and *A. foveata* are AMF which have broad distribution in

Table 4. The diversity of AMF types found in the rhizosphere of *B. petersianum* in Kebar, Tambrau Regency, West Papua.

No	Species of AMF	Location (sub-district)									
		Kebar					East Kebar				
		1	2	3	4	5	6	7	8	9	10
1	<i>Acaulospora</i> sp. DK4	-	-	-	+	-	-	-	-	-	-
2	<i>Acaulospora</i> sp. DK1-3	+	-	+	-	-	+	-	-	-	-
3	<i>Acaulospora foveata</i> DK2-3	+	+	-	+	-	+	-	+	-	-
4	<i>Clariodeoglossum etunicatum</i> DK8	-	+	-	+	-	-	+	-	+	+
5	<i>Clariodeoglossum lamellosum</i> DKT10	+	+	-	-	+	-	-	-	-	+
6	<i>Entrophospora</i> sp. DKT6-2	-	-	-	-	-	+	+	+	-	-
7	<i>Entrophospora</i> sp. DKT9-3	-	-	-	-	-	-	-	-	+	+
8	<i>Gigaspora</i> sp. DK9	-	-	-	-	+	-	-	-	-	-
9	<i>Gigaspora</i> sp. DKT9-2	-	-	-	-	-	-	-	-	+	-
10	<i>Glomus agregatum</i> DKT2	-	-	+	-	-	-	+	-	-	-
11	<i>Glomus</i> sp. DK1-1	+	-	+	-	+	+	-	+	+	+
12	<i>Glomus</i> sp. DK2-4	+	+	-	-	-	-	-	-	-	+
13	<i>Glomus</i> sp. DK3-2	-	+	-	+	-	-	-	-	-	-
14	<i>Glomus</i> sp. DKT6-1	-	-	-	+	-	+	+	-	-	-
15	<i>Glomus</i> sp. DKT6-4	-	-	-	+	-	-	+	-	-	-
16	<i>Glomus</i> sp. DKT8-1	-	-	-	-	-	-	+	+	+	-
17	<i>Scutellospora</i> sp. DK2-2	+	-	+	-	-	+	-	-	-	-
18	<i>Scutellospora</i> sp. DK4-2	-	+	+	+	-	+	-	+	-	+

Notes: + = present, - absence

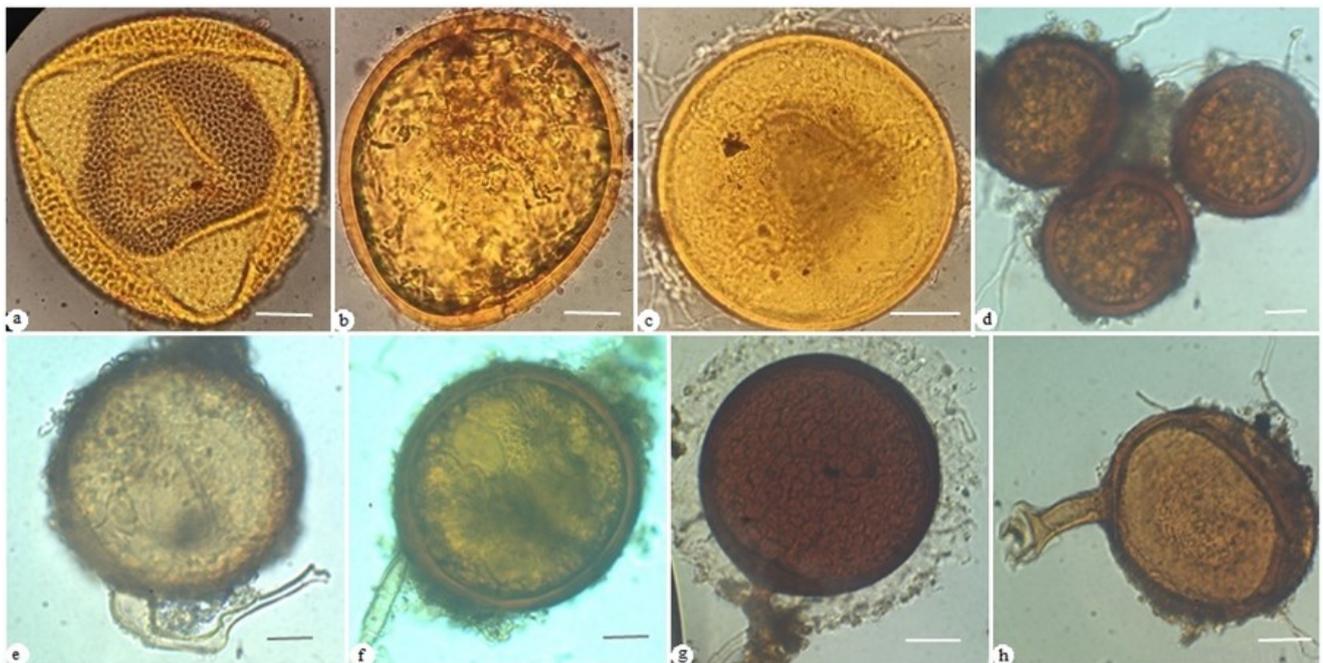


Figure 4. Diversity of AMF spores found in the rhizosphere of the kebar grass plant, in Tambrau, West Papua. a. *Acaulospora foveata*, b. *Glomus* sp. DK2-4, c. *Glomus* sp. DK3-2, d. *Glomus agregatum*, e. *Gigaspora* sp. DK9, f. *Clariodeoglossum etunicatum*, g. *C. lamellosum*, h. *Entrophospora* sp. DKT9-3 (scale bar: 50 μm).

various types of habitat, while *C. lamellosum* often found in sandy soil habitat (Suharno et al. 2016). The physicochemical characteristics of the soil condition in the study site were similar, such as acidic soil, low-high C organic, medium N, low K, therefore the AMF distributed widely in that grassland.

C. etunicatum is capable of symbiosis with medicinal plants such as *Aloe vera*, *Artemisia nilagrica*, and *Withania somnifera*. *G. aggregatum* has symbiosis with *Coleus aromaticus*, and *Acaulospora foveata* associated with *Paris polyphylla* (Sun et al. 2021). However, *C. lamellosum* which has symbiosis with Kebar grass is not known to have symbiosis with other medicinal plants.

The results showed that the *Glomus* (38.89%) and *Acaulospora* (16.67%) were the most dominant genus found in the area. Among the other genera, the genus *Glomus* has the most species (Schübler & Walker 2010; Souza 2015). There are approximately 76 types (56.72%) of AMF from the genus *Glomus*, and 37 types (27.61%) from the genus *Acaulospora*, which have been identified based on morphological and molecular characteristics. A total of approximately 134 AMF types have been identified (Schübler & Walker 2010).

G. aggregatum, *A. foveata*, *C. etunicatum*, *C. lamellosum* and some other species were found to be associated with *B. petersianum* in the study site at 572–619 m asl, temperature of 22–38 °C, and 30–68% humidity. Moreover, the distribution of AMF is derived from the distribution of individual plant biomes and climatic factors. However, dispersal limitations, local environmental conditions, and interactions between AMF taxa also determine local diversity and global distribution (Kivlin et al. 2011).

CONCLUSIONS

Kebar grass is in symbiosis with the AMF in a grassland area, Tambrau Regency. The high number of AMF spores and the level of its colonization indicate that the kebar grass has a very close symbiotic form. Several species such as *G. aggregatum*, *A. foveata*, *C. etunicatum*, *C. lamellosum* and others found to be associated with kebar grass. The information of this study show new record on the AMF association with *B. petersianum*. Therefore, there is a need to conduct a compatibility test on the AMF diversity, including the possibility of obtaining the species that plays the most effective role in the growth of various other plants.

AUTHORS CONTRIBUTION

S. designed the research and supervised all the process, I.R. collected and analyzed the data and wrote the manuscript, R.H.R.T and S.S. supervised this manuscript.

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CONFLICT OF INTEREST

We confirm that there is no conflict of interest associated with this publication.

REFERENCES

- Aguilera, P. et al., 2015. Diversity of arbuscular mycorrhizal fungi in acidic soils and their contribution to aluminum phytotoxicity alleviation. *In*: S.K. Panda, F. Baluska (eds.), Aluminum Stress Adaptation in Plants, Signaling and Communication in Plants 24, pp.203–228. doi: 10.1007/978-3-319-19968-9_11.
- Barceló, M. et al., 2020. The abundance of arbuscular mycorrhiza in soils is linked to the total length of roots colonized at ecosystem level. *PLoS ONE*, 15(9), e0237256. doi: 10.1371/journal.pone.0237256.
- Brundrett, M. et al., 1996. Working with mycorrhizas in forestry and agriculture. ACIAR. Canberra. Australia. doi: 10.13140/2.1.4880.5444.
- Carrenho, R. et al., 2007. The effect of different soil properties on arbuscular mycorrhizal colonization of peanuts, sorghum and maize. *Acta Bot. Bras.*, 21(3), pp.723–730. doi: 10.1590/S0102-33062007000300018.
- Casazza, G. et al., 2017. The abundance and diversity of arbuscular mycorrhizal fungi are linked to the soil chemistry of screes and to slope in the Alpic paleo-endemic *Berardia subacaulis*. *PLoS ONE*, 12(2), e0171866. doi: 10.1371/journal.pone.0171866.
- Coughlan, A. P. et al., 2000. Soil pH-Induced changes in root colonization, diversity, and reproduction of symbiotic arbuscular mycorrhizal fungi from healthy and declining maple forests. *Canadian Journal of Forest Research*, 30, p.10. doi: 10.1139/x00-090.
- Cuenca, G. & Lovera, M., 2010. Seasonal variation and distribution at different soil depths of arbuscular mycorrhizal fungi spores in a tropical sclerophyllous shrubland. *Botany*, 88, pp.54–64. doi: 10.1139/B09-100.
- Duc, N. H. et al., 2021. Arbuscular mycorrhizal fungi improve tolerance of the medicinal plant *Eclipta prostrata* (L.) and induce major changes in polyphenol profiles under salt stresses. *Front. Plant Sci.*, 11, 612299. doi: 10.3389/fpls.2020.612299.
- Eviati & Sulaeman, 2009. Chemical analysis of soil, plants, water and fertilizer. Balai Penelitian Tanah. Bogor.
- Furrazola, E. et al., 2015. Functionality of arbuscular mycorrhizal fungi in three plant communities in the Managed Floristic Reserve San Ubaldo-Sabanalamar, Cuba. *Int. J. Trop. Biol*, 63(2), pp.341–356.
- Giovannetti, M. et al., 2006. At the root of the wood wide web; self recognition and non-self incompatibility in mycorrhizal networks. *Plant Signal. Behav.*, 1(1), pp.1–5. doi: 10.4161/psb.1.1.2277.

- Giovannetti, M. et al., 2010. Fungal spore germination and pre-symbiotic mycelial growth—physiological and genetic aspects. In *Mycorrhizas : Physiology and Function*. London: Springer Science+Business Media B.V. pp.3–32. doi: 10.1007/978-90-481-9489-6_1.
- Inngjerdingen, K.T. et al., 2006. A complement fixing polysaccharide from *Biophytum petersianum* Klotzsch, a medicinal plant from Mali, West Africa. *Biomacromolecules*, 7(1), pp.48–53. doi: 10.1021/bm050330h.
- Inngjerdingen, M. et al., 2008. Pectic polysaccharides from *Biophytum petersianum* Klotzsch, and their activation of macrophages and dendritic cells. *Glycobiology*, 18(12), pp.1074–1084. doi: 10.1093/glycob/cwn090.
- Kartikasari, S. N. et al., 2012. Ecology of Papua, Indonesian ecology series. Vol VI. Second Printing. Yayasan Obor, Jakarta. [Indonesian].
- Kayadoe, M. et al., 2012. Pengaruh penggunaan rumput kebar (*Biophytum petersianum* Klotzsch) dalam konsentrasi berdasarkan kandungan protein kasar 19% terhadap penampilan kelinci. *Sains Peternakan*, 10(2), pp.64–68. doi: 10.20961/sainspet.v10i2.4850.
- Kivlin, S. N. et al., 2011. Global diversity and distribution of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry*, 43(11), pp.2294–2303. doi: 10.1016/j.soilbio.2011.07.012.
- Kormanik, P. P. & Mc.Graw, A.–C., 1984. Quantification of vesicular–arbuscular mycorrhizae in plant roots. In *Methods and principles of mycorrhizal research* (N.C. Schenck, Ed). Minnesota: The American Phytopathological Society. pp. 37–45.
- Kumar, A. et al., 2021. Ethno-medicinal and AMF diversity conservation aspects of some weeds of Himachal Pradesh, India. *Journal of Research in Weed Science*, 4(1), pp. 43–56. doi: 10.26655/JRWEEDSCI.2021.1.4.
- Mouzou, A. P. et al., 2009. Effects du decocte de *Biophytum petersianum* Klotzsh (Oxalidaceae), sur le calcium libre intracellulaire dans les cellules musculaires. *Int. J. Biol. Chem. Sci.*, 3(4), pp.834–839. doi: 10.4314/ijbcs.v3i4.47190.
- Rohyadi, A. et al., 2004. Effects of pH on mycorrhizal colonization and nutrient uptake in cowpea under conditions that minimise confounding effects of elevated available aluminium. *Plant Soil*, 260, pp.283–290. doi: 10.1023/B:PLSO.0000030183.87228.0b.
- Sambodo, P. et al., 2018. Subchronic toxicity test of kebar grass extract of nHexane (*Biophytum petersianum* Klotzsch) on weight, ADG, and vital organ of Rat (*Rattus norvegicus*). *J. Fooof Pharm. Sci.*, 6, pp. 14–16. doi: 10.14499/jfpps.
- Schenck, N. C. & Perez, Y., 1990. Manual for the identification of VA mycorrhizal fungi. Gainesville, USA: Synergitis Publication.
- Schüßler, A. & Walker, C., 2010. The Glomeromycota. A species list with new families and new genera. With correction on July 2011. [online]. The Royal Botanic Garden Kew, Botanische Staatssammlung Munich, and Oregon State University.

- Sembiring, B. & Darwati, I., 2013. Kebar grass (*Biophytum petersianum*) as a fertility enhancer. *Warta Penelitian dan Pengembangan Tanaman Industri*, 19 (2), pp. 15–18. (In Indonesian).
- Smith, S. E. & Read, D., 2008. *Mycorrhizal symbiosis*. Third Edition. New York: Academic Press, Elsevier.
- Sorensen, K. W., 1993. Indonesian peat swamp forests and their role as a carbon sink. *Chemosphere*, 27(6), pp. 1065-1082. doi: 10.1016/0045-6535 (93)90068-G.
- Souza, T., 2015. *Handbook of arbuscular mycorrhizal fungi*. Switzerland: Springer International Publishing.
- Suharno, et al., 2014. Keragaman makrofungi di Distrik Warmare Kabupaten Manokwari, Papua Barat. *Jurnal Biologi Papua*, 6(1), pp.136–144. (in Indonesian).
- Suharno, et al., 2015. Keragaman fungi mikoriza arbuskula pada tumbuhan pokem [*Setaria italica* (L.) Beauv.] dengan metode trapping. *Jurnal Biologi Papua*, 7(2). pp.68–77.
- Suharno, et al., 2016. Presence of arbuscular mycorrhizal fungi on fern from tailing deposition area of gold mine in Timika, Indonesia. *International Journal of Environmental Bioremediation & Biodegradation*, 4, pp.1–7. doi: 10.12691/ijebb-4-1-1.
- Suharno, et al., 2018. Arbuscular mycorrhizal fungi associated with Wati (*Piper methysticum*), a medicinal plant from Merauke Lowland, Papua, Indonesia. *Biosaintifika: Journal of Biology & Biology Education*, 10(2), pp.260–266. doi: 10.15294/biosaintifika.v10i2.14303.
- Suharno, et al., 2020. Fungi mikoriza arbuskula mempercepat rehabilitasi lahan tambang. Yogyakarta: UGM Press.
- Suharno, et al., 2021. The growth response of pokem (*Setaria italica* L.) inoculated with arbuscular mycorrhizal fungi (AMF) from tailings area. *Journal of Degraded and Mining Lands Management*, 8(4), pp.2873-2880. doi: 10.15243/jdmlm.2021.084.2873.
- Sun, R.-T., et al. 2021. A review of the interaction of medicinal plants and arbuscular mycorrhizal fungi in the rhizosphere. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 49(3), 12454. doi: 10.15835/NBHA49312454.
- Sun, X.-G. & Tang, M., 2012. Comparison of four routinely used methods for assessing root colonization by arbuscular mycorrhizal fungi. *Botany*, 90(11), pp.1073–1083. doi: 10.1139/b2012-084.
- Tao, L. et al., 2004. Arbuscular mycorrhizas in a valley-type savanna in southwest China. *Mycorrhiza*, 14, pp.323–327. doi: 10.1007/s00572-003-0277-y.
- Trisilawati, O. et al., 2019. Application of AMF (Arbuscular Mycorrhizal Fungi) and organic fertilizer to increase the growth, biomass and bioactive content of *Centella*. *IOP Conf. Series: Earth and Environmental Science*, 292, pp.012067. doi: 10.1088/1755-1315/292/1/012067.

- Tuheteru, F. D. et al., 2019. Arbuscular mycorrhizal fungi associated with adaptive plants in gold mines tailing. *Biodiversitas*, 20, pp.3398-3404. doi: 10.13057/biodiv/d201137.
- Unity, A. J. A. & Inara, C., 2011. The potential of kebar grass (*Biophytum petersianum* Klotzch) in improving reproductive performance. *Prosiding Seminar Nasional Pengembangan Pulau-Pulau Kecil Tahun 2011*, pp. 329–333 (in Indonesian).
- Vierheilig, H. et al., 2005. An overview of methods for the detection and observation of arbuscular mycorrhizal fungi in roots. *Physiol. Plant*, 125, pp.393–404. doi: 10.1111/j.1399-3054.2005.00564.x.
- von Rintelen, K. et al., 2017. A review of biodiversity-related issues and challenges in megadiverse Indonesia and other Southeast Asian Countries. *Research Ideas and Outcomes*, 3, e20860. doi: 10.3897/rio.3.e20860.
- Wang, F. Y. & Shi, Z. Y., 2008. Biodiversity of arbuscular mycorrhizal fungi in China : a Review. *Adv. Environ. Biol*, 2(1), pp.31–39.
- Warren, M. et al., 2017. An appraisal of Indonesia's immense peat carbon stock using national peatland maps: uncertainties and potential losses from conversion. *Carbon Balance Manag*, 12, pp.12. doi: 10.1186/s13021-017-0080-2.
- Xu, M. et al., 2017. Land use alters arbuscular mycorrhizal fungal communities and their potential role in carbon sequestration on the Tibetan Plateau. *Nature: Scientific Report*, 7, pp.3067. doi: 10.1038/s41598-017-03248-0.
- Zhang, M. et al., 2021. Molecular diversity and distribution of arbuscular mycorrhizal fungi at different elevations in Mt. Taibai of Qinling Mountain. *Front. Microbiol*, 12, pp.609386. doi: 10.3389/fmicb.2021.609386.