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Arbuscular Mycorrhizal Fungi Associated with Wati (*Piper methysticum*), a Medicinal Plant from Merauke Lowland, Papua, Indonesia

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Abstract

Indonesia is rich of natural resources, including the diversity of agricultural crops and other valuable plants. Many plant species used by local people for traditional herbal medicine and some other species used by drug industries. Among these, Wati (*Piper methysticum*, Piperaceae) is one of the medicinal plant found in Merauke lowland, Papua. It has been cultivated by local people because of it high value as medicinal and cultural uses. Wati plant is used to treat anti-stress, rheumatism, respiratory tract infections, tuberculosis, gonorrhea, headache etc. The habitat, including the microorganism in the soil plays an important role in the growth of this plant. Therefore, this study was conducted to identify the arbuscular mycorrhizal fungi (AMF) associated with the rhizosphere of Wati from Merauke lowland. Soil and root samples were collected from different locations and the colonization percentage on the root sample were determined. Our results showed that the number of the spores in the soil samples was 45-89 spores/50 g soil, while the colonization percentage on the root was 38.46-83.3%. Among 13 AMF morphospecies that found on the soil samples, 10 were identified to genus level such as *Glomus, Clariodeoglomus, Acaulospora* and *Scutellospora*, while the other were unidentified. Further work will be needed such as trap-plant culture method to get more information on the diversity of AMF associated with Wati.

How to Cite

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INTRODUCTION

Piper methysticum is one of the plant species of the Piperaceae family and known as a medicinal plant to cure rheumatism, respiratory infections, tuberculosis, gonorrhea, and headaches. The leaves and stems contain several compounds that can be used as antidepressant, analgesic, and psychoactive drug (Suharno *et al.*, 2016), antibacteria (Zhao *et al.*, 2010) and anticancer (Tabudravu & Jaspars, 2005). Known as "kava", this species has been widely explored in the South Pacific region, for example in Fiji, Tonga, Vanuatu, Samoa and some Micronesia regions, because it is important for community tradition events (Davis & Brown, 1999). In the lowlands of Merauke, New Guinea part of Indonesia, this species is called "Wati" and used by local communities as medicinal plants (Tanjung *et al.*, 2014; Suharno *et al.*, 2016). In addition, the plant is of high cultural and economic value therefore it gave impacts on the income of local communities.

Local people in Merauke have been trying to cultivate it in small scale using conventional methods (Suharno *et al.*, 2016). Some people failed to cultivate and they believe in the existence of "superstitius powers" that only certain people can cultivate (Tanjung *et al.*, 2014; Suharno *et al.*, 2016). Therefore, it need a touch of technology in cultivating "wati" (Suharno *et al.*, 2016). One is the involvement of soil microorganisms, including arbuscular mycorrhizal fungi (AMF) (Turk *et al.*, 2006; Souza, 2015; Pagano *et al.*, 2016).

AMF forms a symbiotic mutualism with the root system of plants. More than 80% of vascular plants form symbiotic mutualism with AMF (Turk *et al.*, 2006; Smith & Read, 2008, Souza, 2015). Plants derive nutrients from the absorption carried out by the fungus, while the fungi derive energy sources from the photosynthesis of plants (Smith & Read, 2008; Souza, 2015). The role of AMF (Phylum *Glomeromycota*) in the growth of medicinal plants in various countries is significant (Sundar *et al.*, 2011; Raei & Weisany, 2013; Chandra *et al.*, 2014), including in China (Wang & Zhao, 2008). Several types of AMF are found to be symbiotic with the roots of medicinal plants such as ginseng (*Panax ginseng*, *P. notoginseng,* and *P. quiquefolium*), *Datura stramonium, Atractylodes macrochepala*, and several other medicinal plants (Wang & Zhao, 2008). It can even improve the composition of essential oils and antioxidant activity (da Luz *et al.*, 2015).

In Papua, the occurence of AMF has been reported in corn, *Setaria italica*, weeds, and *Brachiaria precumbens* (Suharno *et al.*, 2006; Suharno *et al.*, 2015; Suharno *et al.*, 2016; Suharno *et al.*, 2017). However, little is known about the presence of AMF on medicinal plant in Papua. It was reported that in addition to endophytic fungi, in the root of *P. methysticum* indicated the presence of AMF (Tanjung *et al.*, 2014). This preliminary information becomes important to reveal the presence of AMF in medicinal plants. Therefore, this study was conducted to determine the presence AMF in the rhizosphere of wati from the lowlands of Merauke, Papua. The results of this study are important in the utilization of the types of AMF found for the purpose of endurance and enhancement of plant growth. It is only on *P. methysticum* plants, but also in other crops such as agriculture, plantation and forestry in general.

METHODS

Study sites

The survey was conducted in the area of *P. methysticum* plantation in lowland area of Merauke, Papua Province. Three sites namely Wasur, Pinje and Sota were selected to sampling (Figure 1).

Soil sampling

Rhizosphere soil samples (1 kg) and roots from each individual plant were collected. Soil samples were mixed to form a composite sample for analysis of soil properties and extraction of AMF spores.

AMF colonization

The roots were separated from the stem and cleaned with running water, then were soaked in a FAA fixative solution (formalin-acetic acid-5: 5: 90) for 1 hour. Fixed roots were cleared in 10% KOH solution for 24 hours and acidified with 1% HCl for 24 hours. After washing with aqueous solution, the roots were stained with trypane blue (0.05% in lactoglycerol) for 24 hours (Vierheilig *et al.*, 2005). Then they were examined with compound microscope for AMF structures (intraradical hyphae, extraradical hyphae, vesicles, arbuscules and intraradical spores). The estimation of root colonization percentage was done by the slide method (Kormanik & McGraw, 1984; Brundrett *et al.*, 1996; Sun & Tang, 2012).

Extraction and identification of AMF spores

Extraction of AMF spores were followed wet sieving method with sucrose centrifugation technique (Vierheilig *et al.*, 2005). 50 g and 100 g soil samples were filtered using a multilevel filter of 250 μm, 100 μm, and 30 μm. Subsequently, the

solution containing the spores was centrifuged (2500 rpm, 10 min) by the addition of 50% sucrose. The separated spores were observed under a microscope. Morphological identification were based on spore characteristics (spore shape, spore color, glare stalk attachment, spore wall and spore reaction with Melzer's solution) according to Brundrett *et al*. (1996) and Schenck & Perez (1990).

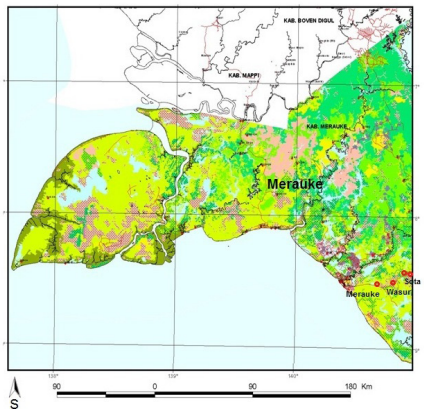

Figure 1. Study sites in lowland area of Merauke, Papua Province.

Data analysis

Soil analysis was conducted at Soil Laboratory SEAMEO-Biotrop, Bogor. The observational data were analyzed qualitatively. The data is shown in the form of pictures or tables.

RESULT AND DISCUSSION

Soil properties

The texture of the soil in this field was categorized as loam to clay loam. The pH of the soil was acidic (5.1-5.7). The macro and micronutrient content in Sota was relatively higher than in other sites, except for available P which was the lowest compared to others (Table 1).

Spores and Colonization of AMF in *P. methysticum*

The results showed that AMF was found in the roots of *P. methysticum* and rhizosphere soil (Figure 1; Figure 2). The percentage of AMF colonization in all study sites was varied (38.46 - 83.3%, average 56.65%) (Table 2). The number of spores found in the rhizosphere soil of *P. methysticum* varies widely, ranging from 45 to 89 spores

Table 1. Physical and chemical properties of *P. methysticum* rhizosphere soil in Merauke, Papua.

Physicochemical parameters Sota (P-52)		Study site (Samples code)			
		Wasur 1 (W-11)	Pinje (P-21)	Wasur 2 (W-21)	
pH (H_2O) (1:1)		5.1	5.7	5.3	5.4
pH ($CaCl_2$)(1:1)		4.4	4.5	4.6	4.6
Organic C	(%)	5.35	0.58	1.75	0.67
N total	(%)	0.54	0.08	0.12	0.08
C/N ratio		44.6	7.3	14.6	8.4
Available P (P_2O_5)	(ppm)	3.4	8.2	14.0	5.6
K	($cmol.kg^{-1}$)	0.78	0.23	0.51	0.43
Ca	($cmol.kg^{-1}$)	2.06	1.98	3.66	4.44
Na	($cmol.kg^{-1}$)	2.55	2.45	1.33	1.18
Mg	($cmol.kg^{-1}$)	1.48	1.28	1.05	1.56
Cation exchange capacity	($cmol.kg^{-1}$)	26.69	6.95	9.94	9.68
KB	(%)	25.74	85.47	65.90	78.62
Al-H_{dd}KCl 1N:					
Al^{3+}	($me.100g^{-1}$)	0.30	0.00	0.00	0.00
H^+	($me.100g^{-1}$)	1.63	0.10	0.10	0.10
Soil texture					
Sand	(%)	2.3	14.8	39.3	26.0
Clay	(%)	67.0	45.6	18.6	28.8
Loam	(%)	30.7	39.6	42.1	45.2
		Clay loam	Clay loam	Sandy loam	Sandy clay loam

with an average of 66,4 spores per 50 g of soil samples and 93 to 162 spores with an average of 122,2 spores per 100 g of soil samples (Table 3).

Diversity of AMF

Based on the spore morphology, 10 species of AMF that belong to 4 genera were identified but 3 species were remain unknown (Figure 2). From the known genera, the dominant genus was *Glomus* (4 types; *Glomus* sp1., *Glomus* sp2., *Glomus* sp3., *Glomus* sp4), followed by *Acaulospora* (3 types; *Acaulospora foveata, Acaulospora* sp1., *Acaulospora* sp2.), *Gigaspora* (1 types; *Gigaspora* sp.) and *Scutellospora* (2 types; *Scutellospora* sp1; *Scutellospora* sp2).

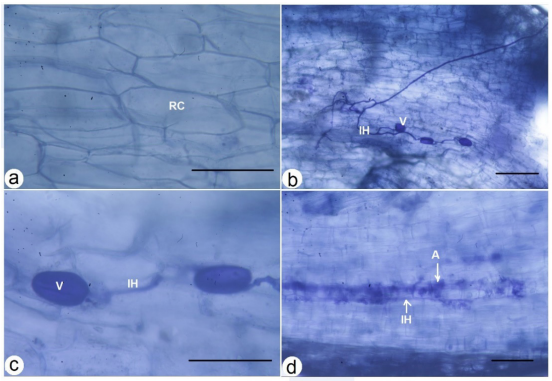

Figure 2. Colonization of AMF in *P. methysticum* roots. a. Uncolonized root, b - d. Infected roots. RC: root cells, IH: intraradical hyphae, V: vesicles, A: arbuscules (Scale bar: 100 µm).

In this study, the *P. methysticum* were clarified to the mycorrhizal by the presence of AMF stuctures such as hyphae, vesicles, arbuscules and spores in the root cortical cells. However, the arbuscules were only observed in 5 of the 10 roots *P. methysticum*. This result was in confirmation with Maffia *et al*. (1993) which was found only 2 of the 8 species of Piperaceae indicated the presence of vesicles and arbuscules of AMF. The presence of colonization and development of hyphae in the root system rhizosphere affects plant growth (Torrecillas *et al.*, 2010; Koul *et al.*, 2012; Seema & Garampalli, 2015). This is because hyphae plays a role in helping root function in nutrient absorption (Moreira *et al.*, 2006; Turk *et al.*, 2006).

The number of spores in the rhizosphere soil of *P. methysticum* are varied. The highest number of spores was found in Pinje 4 (89 spores per 50 g soil, 162 spores per 100 g soil), while the lowest was found in Wasur 5 (45 spores per 50 g soil, 97 spores per 100 g soil). Soil condition in Pinje was sandy loam and in Wasur was sandy clay loam. The texture of soil the soil influenced the presence of AMF. The spore number and the infection of AMF was higher in loamy and sandy loam soil (Rachel *et al.*, 1993; Sundar *et al.*, 2011). The average number of spore was 122.2 per 100 grams of soil samples indicates the production of spores in this area was quite high. This high number was due to soil sampling is taken during the dry season. According to Cuenca & Lovera (2010) AMF in the dry season tends to produce spores than to form both intraradical and extraradical hyphae.

In this study, 4 generations have been registered namely *Glomus, Acaulospora, Scutellospora* and *Gigaspora*. However, *Glomus* was the dominant genus in in the rhizosphere soil of *P. methysticum*. This result was supported by Maffia *et al*. (1993) that *Glomus, Acaulospora*, and *Gigaspora*

Table 2. Percentage root colonization of AMF in *P. methysticum* from Merauke, Papua.

Study Sites	Samples Code	∑ of root segment	AMF Structures					∑ of colonized root	AMF colonization %
			IH	EH	V	A	S		
Pinje	P1-1	16	10	2	13	0	0	10	62.50
Pinje	P2-2	30	17	0	13	1	1	17	56.67
Pinje	P3-1	30	16	2	16	0	1	16	53.33
Pinje	P4-1	30	25	5	22	1	1	25	83.33
Pinje	P5-1	30	16	1	14	0	0	16	53.33
Sota	S5-2	14	7	0	3	0	0	7	50.00
Wasur	W1-2	30	15	0	14	2	0	15	50.00
Wasur	W2-2	30	21	2	18	0	0	21	70.00
Wasur	W3-2	13	5	1	0	1	0	5	38.46
Wasur	W5-2	30	20	2	15	1	1	20	66.67
	Average								56.65

were observed in the Piperaceae rhizosphere, the genus *Scutellospora* cannot be found in this study.

Table 3. AMF spores number in the rhizosphere soil of *P. methysticum* from Merauke, Papua.

Sudy sites	Samples code	∑ spores	
		50 g	(100 g $^{-1}$ soil)
Pinje 1	P1-1	53	112
Pinje 2	P2-2	64	141
Pinje 3	P3-1	62	93
Pinje 4	P4-1	89	162
Pinje 5	P5-1	65	104
Sota	P5-2	73	125
Wasur 1	W1-2	60	123
Wasur 2	W2-2	81	152
Wasur 3	W3-2	72	113
Wasur 5	W5-2	45	97
Average		66.4	122.2

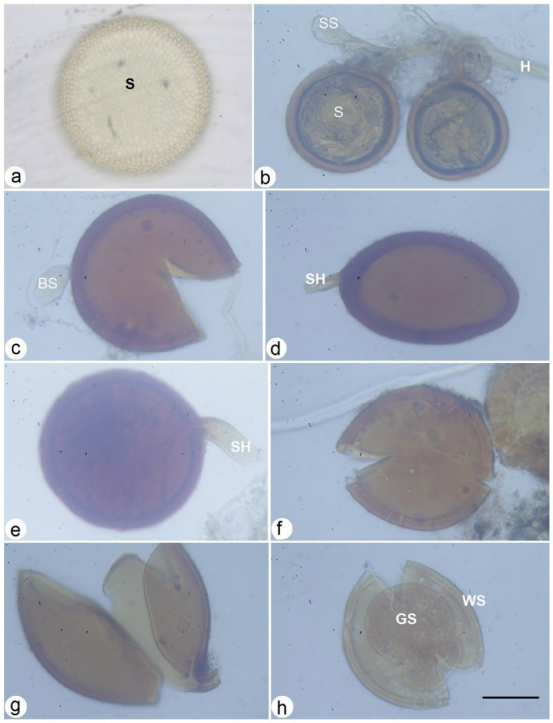

Figure 3. Photomicrograph of AMF in the rhizosphere soil of *P. methysticum* from Merauke, Papua. a. *Acaulospora foveata* b. *Acaulospora* sp., c. *Gigaspora* sp., d-g. *Glomus* spp., h. *Scutellospora* sp. (S: spore, SS: soporiferous saccule, H: hyphae, BS: bulbous suspensor, SH: subtanding hyphae, GS: germinating shield, WS: wall spore. Scale bar: 50 µm).

AMF can be utilized in increasing plant growth, including medicinal plants (Thapa *et al.*, 2015; Chandra *et al.*, 2014). According to Gogoi & Sigh (2011), AMF has an effect on the growth of *Piper longum* (Gogoi & Sigh, 2011; Seema & Garampalli, 2015) and *P. nigrum* (da Luz *et al.*, 2015). Significantly, AMF increases plant growth compared to uncolonized one. Several AMF species such as *Glomus fasciculatum*, *G. versiforme*, *G. clarum*, *Glomus* sp.2, *G. mosseae* and *G. etunicatum* play an important role in the growth of medicinal plants (Gogoi & Sigh, 2011). Furthermore, AMF was able to increase the composition of essential oils and antioxidant activity in *Piper nigrum* (da Luz *et al.*, 2015).

The results of this study indicated that AMF were found in the lowlands of Merauke at very low pH conditions. Previously, there was not research, which has been done on AMF in rhizosphere of wati plant (*P. methysticum*) and in lowland area of Merauke, which having low pH. The plant species of *P. methysticum* are known to contain a fairly high metabolite (Tanjung *et al.*, 2014), but AMF can be well-associated (Chandra *et al.*, 2014). In fact, besides AMF, various plant species are also able to associate with endophytes (Cape *et al*, 2014; Hasanah *et al.*, 2017).

The present study clarify the presence of AMF in the *P. methysticum* rhizosphere in Merauke, Papua. Further work need to be conducted such as reinoculation of the native spores to this medicinal plant in pot to test their influence in the improvement of the plant growth. In the future time, those native AMF spores may have potency to be developed as an inoculum for application as biofertilizer in cultivation of medicinal plant. These types of AMF can be utilized in the processes of land rehabilitation (Suharno *et al.*, 2017; Setyaningsih *et al.*, 2018), and improvement the performance of plant growth, both agricultural crops, plantations and forestry (Pagano *et al.*, 2016).

CONCLUSION

P. methysticum which is known as traditional medicinal herb has association with AMF. The percentage of AMF colonization in all study sites was varied with average 56.65%. The number of spores found in the rhizosphere soil of *P. methysticum* varies widely, ranging from 93 to 162 spores with an average of 122.2 spores per 100 g of soil samples. Based on the spore morphology, there are 10 species of AMF that belong to 4 genera. The dominant genus was *Glomus* (4 types; *Glomus* sp1., *Glomus* sp2., *Glomus* sp3., *Glomus* sp4), followed by *Acaulospora* (3 types; *Acaulospora foveata, Acaulospora* sp1., *Acaulospora* sp2.), *Gi-*

gaspora (1 types; *Gigaspora* sp.) and *Scutellospora* (2 types; *Scutellospora* sp1; *Scutellospora* sp2)

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