

Mycorrhizal status of *Diospyros celebica* Bakh. (Ebenaceae), an endangered endemic species from Sulawesi Island, Indonesia

Yusran Yusran^{1*}, Wardah Wardah¹, Annadira Annadira², Husain Umar¹, and Rukmi Rukmi¹

¹Department of Forestry, Faculty of Forestry, Tadulako University, Jl. Soekarno-Hatta Km.9, Palu, Central Sulawesi, 94118, Indonesia.

²Master of Agricultural Sciences Study Program, Postgraduate Program, Tadulako University, Jl. Soekarno-Hatta Km.9, Palu, Central Sulawesi, 94118, Indonesia.

*E-mail: yyusranyusran610@gmail.com

Received: 07 June 2022 / Accepted: 22 October 2022 / Available online: 07 November 2022

Abstract

Arbuscular mycorrhizal fungi (AMF) play an important role in the composition and growth of plants in forest ecosystems. Research on mycorrhizal status in endemic plant species is still lacking. This study aims to determine the AMF status of ebony (*Diospyros celebica* Bakh.), which is one of the endemic plant species that are threatened with extinction on the island of Sulawesi, Indonesia. The collection of rhizosphere soil samples and ebony roots was carried out in three ebony stands on Sulawesi Island, namely Maleali village and Tovalo village, Parigi Motong district, Central Sulawesi province and in Ako village, Pasangkayu district, West Sulawesi province, Indonesia. Identification of AMF species was carried out at the Forest Biotechnology Laboratory, Bogor Agricultural Institute. We found 19 AMF species from two genera (*Glomus* and *Acaulospora*) associated with ebony, with the distribution of 7 AMF species in Maleali village, 5 and 7 AMF species in Tovalo village and Ako village, respectively. Furthermore, AMF colonisation on ebony roots was in the low category, between 0.33 % and 2.47 %, with a spore density of 38–162 per 50 g of soil. The results of this study indicate that *Diospyros celebica* is associated with AMF. This information can be used as a guide in the use of AMF to optimize the cultivation, conservation and restoration programs of this species in the future.

Key words: *Acaulospora*, colonisation, ebony, *Glomus*, spore density.

Introduction

Currently, there are very few data regarding the status of mycorrhizae in endangered plants in the world (Evelin et al. 2019). Bothe et al. (2010) reported that based on the International Union for Conservation of Nature (IUCN 2022a) database, only 139 rare plant species belong-

ing to 44 families have been screened for mycorrhizal status (Wang and Oiu 2006). The 54 plant species are classified as endangered, 13 species as Near Threatened and 72 are included in the Least Concern category. And among the 54 species that are endangered, 11 plant species are categorised as critically endangered, 6 endangered and 30 vulnerable.

Tree species are threatened with extinction in Indonesia, which have been studied in symbiosis with arbuscular mycorrhizal fungi, among others; Kuku wood (*Pericopsis mooniana* Thw.) (Husna et al. 2015), Kalapi (*Kalappia celebica* Kosterm.) (Asrianti et al. 2016), Bisbul (*Diospyros blancoi* A.DC.) (Ningsih et al. 2013) and Jenang (*Daemonorops draco* Blume) (Purwati et al. 2019). Meanwhile, there are still many other forest plant species that are threatened with extinction and it is not yet known whether they are in symbiosis with mycorrhizae or not. One example is *Diospyros celebica* Bakh. which by the people in Sulawesi island is known as ebony. This species has been categorised as an endangered (vulnerable A1cd) since 1998 in the Red List of IUCN (2022b).

Ebony (*D. celebica*) is a genus of Ebenaceae family, one of the endemic plant species on the island of Sulawesi, where natural populations can only be found in Central Sulawesi (the districts of Poso, Parigi Moutong and Donggala), South Sulawesi (the district of Gowa, Barru, Maros, Sidrap and Mamuju) and Gorontalo (Hendromono 2008). Ebony produces luxury wood that has high economic value and has been ratified as the mascot of the province of Central Sulawesi through the Decree of the Governor of Central Sulawesi No. 660/78/1995 dated 27 February 1995 (Wulandari et al. 2017). Ebony has a very beautiful wood pattern arranged in black and brownish red strips, because of its distinctive wood pattern, very strong and durable. This species has a high commercial value and is one of the trade timbers classified as a luxurious type of wood that is in great demand by the public, both in the domestic and international markets and is one of the causes of its limited existence in nature.

Ebony is a type of wood that grows

very slowly, with a life cycle of about 100 years and even up to 200 years. The slow growth will be an obstacle in its development, besides there is no much data about its growth criteria (Rahman and Abdullah 2002). Many replanting efforts have been carried out in logged-over areas, but the success rate for planting is very low, presumably due to a lack of knowledge about the ecology of where ebony species grow (Allo 2002).

Arbuscular mycorrhizal fungi (AMF) have the ability to colonise various types of plants found in various habitats in the tropics (Zhi-Wei et al. 2001). They are found abundantly in almost all natural terrestrial communities and are in an obligate symbiosis with the roots of about 80 % of terrestrial plants. This form of symbiosis is the oldest, the origins of which can be traced back to 400 million years ago (Harley and Smith 1983, Smith and Read 2008). Arbuscular mycorrhizal fungi including Glomeromycota are beneficial soil microorganisms that have a key function in a complex network of biotic interactions below and above the soil (Turrini and Giovannetti 2012). However, the effectiveness of AMF in infecting host plants varies, because AMF has certain specifications for selecting host plants. Host plants and conditions of the surrounding environment will indicate the level of presence of AMF (Goltapeh et al. 2008).

One of the efforts to increase the growth of ebony seedlings in the context of their conservation efforts is the use of AMF. Utilisation of natural AMF from local plants will be more effective than the use of isolates from outside where the plants are grown. This is because AMF is a living microorganism with adaptability to a specific host and environment. Differences in location and rhizosphere will result in differences in the diversity of species and

populations of AMF (Widiastuti and Kramadibrata 1992). Therefore, a study was conducted on the status of AMF in ebony rhizosphere which is the first step in the exploration and utilisation of local superior AMF to increase the growth of ebony plants and before this species becomes extinct on the island of Sulawesi.

The objectives of this research were to study the colonisation and density of AMF spores in *Diospyros celebica* stands in Sulawesi Island, Indonesia.

Material and Methods

Research sites

This research was carried out for 8 months starting from April to December

2020. The locations of soil and root sampling of ebony plants were carried out in three locations (Fig. 1 and Table 1). Identification of the type, colonisation and spore density of AMF was carried out at the Forest Biotechnology Laboratory, Bogor Agricultural University (IPB), Bogor, West Java. Analysis of soil properties was carried out at the Soil Science Laboratory, Faculty of Agriculture, Tadulako University, Indonesia.

Diospyros celebica is a medium to large tree with a straight trunk that can reach a height of 40 m with a trunk diameter up to 120 cm and often large buttresses. The outer bark (Pepagan) is grooved and peeled off into small pieces, brown-black in colour. Single leaf alternating, oblong-elongated, measuring 10–35 cm × 2–7 cm, the underside is downy and gray-

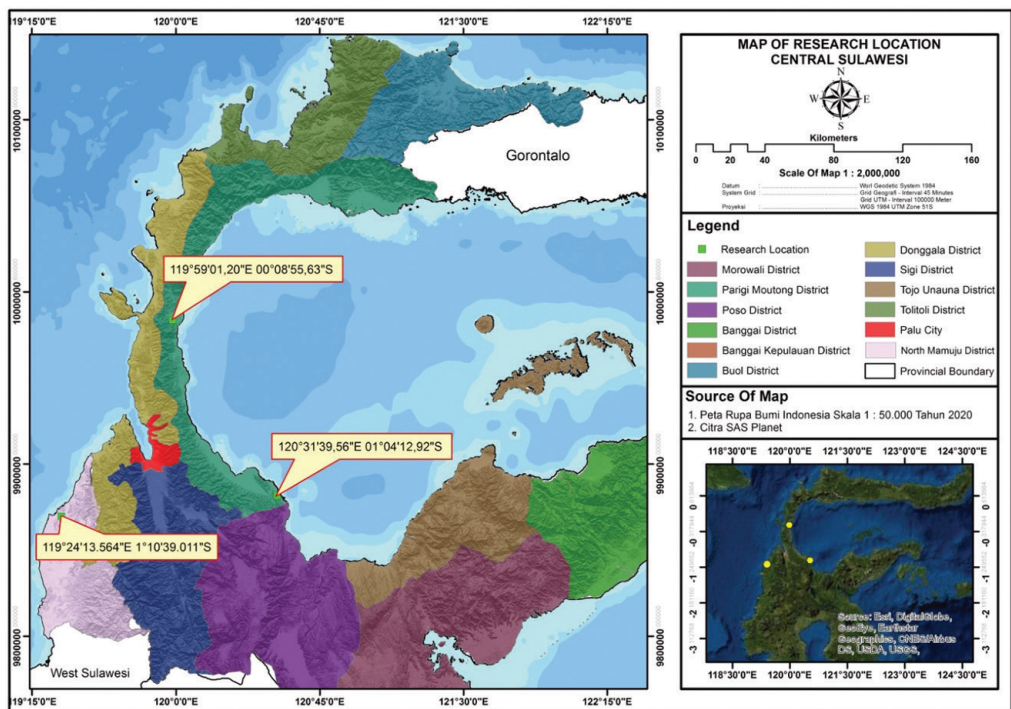


Fig. 1. Map of soil and root sampling locations in three ebony stands as a source of ebony seeds on the island of Sulawesi.

Table 1. Characteristics of ebony stands at the study site.

Location	Coordinates	Altitude, m a.s.l.	Stand age, years
Tovalo village, Kasimbar District, Parigi Moutong	00°08'55.63" S 119°59'01.20" E	56	30
Maleali village, Sausu District, Parigi Moutong Regency	01°04'12.92" S 120°31'39.56" E	36	40
Ako village, Pasangkayu District, Pasangkayu	1°10'39,011" S 119°24'13,564" E	73	25

green. The upper surface of the leaves is glossy and dark green. The flowers are clustered in the axils of the leaves, white. The fruit is ovate, hairy, young fruit is green and ripe fruit is yellow to brown (Gunawan et al. 2019, Fig. 2).

Soil and root sampling

Soil samples were carried out from the surface to a depth of 20 cm. They were taken randomly from ebony stands, where 10 trees with relatively the same growth

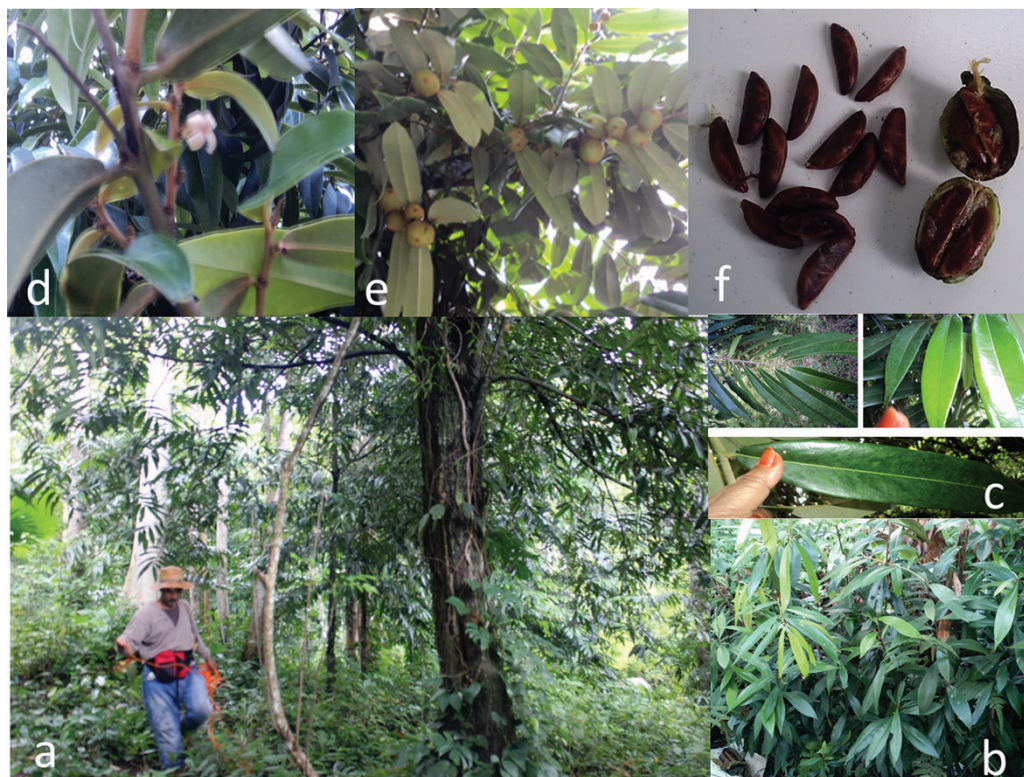


Fig. 2. Morphological features of *Diospyros celebica*: a) trees, b) seedlings, c) leaves, d) flowers, e) fruits, e) seeds (photos: Rukmi Rukmi).

vigor were selected at each study site. Furthermore, for each ebony tree, 4 soil sampling points were set with a distance of 1–2 m from the tree trunk, namely on the west, east, north and south sides. At each point 250 g of soil samples were taken and then composited, so that a total of 1000 g was obtained from each tree. Each soil sample was put in a plastic bag and labeled accordingly. All soil samples were air-dried in the laboratory for the purpose of isolation and identification of arbuscular mycorrhizal fungi spores and for analysis of soil properties.

Fine root samples from ebony trees were also collected at random from the tree from which the rhizosphere soil was taken. These roots were ensured to be taken from at least three lateral roots towards the smooth roots of the ebony tree. After harvesting, these were put in a plastic box, labeled according to the research location and taken to the laboratory. Root samples were rinsed under running tap water to remove soil and adhering dirt. After cleaning, they were cut into 1 cm size and stored in 70% alcohol solution until further analysis.

Isolation, identification of AMF and analysis of physico-chemical properties of soil

The technique used for the isolation of AMF spores was the wet pour-filter technique (Pacioni 1992). The spore extraction was then followed by a centrifugation technique (Brundrett et al. 1996). The spore preparations used Melzer's dye and polyvinyl lacto glycerol (PVLG) preservative placed separately on a glass slide. AMF spores obtained from the extraction after counting were placed in Melzer's and PVLG solutions and their types present in these two solutions were

the same. Then the spores were carefully broken down by pressing the cover slip. Healthy spores were selected and stored on a slide that had been given a solution of PVLG and Melzer then covered with a cover glass. The colour change of spores in Melzer's solution is one of the indicators to determine the type of spores. The identification of was carried out by observing the morphological colour, shape, size, hyphae, spore ornamentation, and the reaction to the fusing solution (Schenck and Pérez 1988, Schüßler and Walker 2010). After that, the AMF spores were identified manually based on the description of the current AMF species (International Culture Collection of Vesicular-Arbuscular Endomycorrhizal Fungi in INVAM 2020). The spores were observed under a stereo microscope and were identified using an Axio Imager microscope with a magnification of 200 $\times$.

The spore density (per 50 g of soil) was calculated by directly counting their number present in each sample. AMF species richness was defined as the number of AMF species present per soil sample (Koske 1987).

The physico-chemical properties of the selected soil were analysed using a standard protocol. Organic C was quantified by Walkley-Black method (Walkley and Black 1934). Total N was analysed using Kjeldahl method (Kjeldahl 1883). P availability was analysed using the method by Bray and Kurtz (1945). Soil pH was determined using a digital pH meter and soil texture using a pipette method.

Arbuscular mycorrhizal fungi colonisation

The roots were put into a 10% KOH solution and stored for 2 weeks in the room. After that the KOH solution was discard-

ed and the root samples were washed with clean water. Furthermore, they were soaked in alkaline H_2O_2 and then heated for 45–60 min at a 90 °C. Next, the roots were cleaned and soaked in 1% HCL solution for 24 h, then were immersed in a dye solution (Trypan blue 0.05% + Glycerin 70% + 30% aquadest) for 24 h. The roots were then washed again and put into a 50% glycerin solution.

AMF colonisation counts used the infected root length method (Giovannetti and Mosse 1980). From the roots that have been treated with trypan blue, root samples with a length of ± 1 cm were taken and arranged in the glass microscopy slides, and were carefully observed, the field of view showing colonisation was marked with (+), and if there was no colonisation it was marked with (-). In general, colonisation was characterised by the presence of AMF structures within the root such as extraradical and intraradical hyphae, vesicles, arbuscles or spores (Brundrett et al. 1984). To calculate the percentage of AMF infection in roots, formula (1) (Brundrett et al. 1996) was used:

$$\frac{A}{B} \cdot 100, \% \quad (1)$$

where: *A* is the number of field of view colonised, *B* is total observed field of view.

The percentage level of AMF colonisation was classified according to the coloni-

sation standard of O'Connor et al. (2001), namely: a) Not colonised: 0 % colonisation value; b) Low: colonisation value <10 %; c) Medium: colonisation value 10–30 %; d) High: colonisation value >30 %.

Data analysis

Levels for the treatment were calculated by analysis of variance. The mean density and number of spores and the percentage of colonisation of arbuscular mycorrhizal fungi were compared using the test Tukey's Honestly Significant (LSD 0.05% confidence). Significant (ANOVA) using the SPSS software package (IBM SPSS Statistics 25, Armonk, NY, USA).

Results and Discussion

Arbuscular mycorrhizal fungi associated with ebony plants

The results showed that there were 7 types of AMF associated with ebony plants in Maleali village, Sausu district, Parigi Moutong Regency, Central Sulawesi, 5 species associated in Tovalo village, Kasimbar district, Central Sulawesi. Parigi Moutong, Central Sulawesi and 7 in Ako village, Pasangkayu District, Pasangkayu Regency, West Sulawesi. The description of each type can be seen in Table 2.

Table 2. Description of Arbuscular mycorrhizal fungi spores associated with ebony plants.

Species	Description		Location
	Shape	Size	
<i>Glomus</i> sp. 1	globalus	100×90 µm	brownish yellow
<i>Glomus</i> sp. 2	globalus	100×90 µm	yellow
<i>Glomus</i> sp. 3	globalus	130×120 µm	blackish brown

Species	Description				Location
	Shape	Size	Colour	Other ornament	
<i>Glomus</i> sp. 4	globalus	110×110 µm	blackish brown	one spore wall, does not have a germinal wall, there are non-figmental hyphae	A
<i>Glomus</i> sp. 5	globalus	180×180 µm	yellowish	hyphae and spore walls	A
<i>Glomus</i> sp. 6	ellipsoid	100×100 µm	yellow	one spore wall, do not have a germinal wall, have hyphae	B
<i>Glomus</i> sp. 7	globalus	90×90 µm	yellow	spore walls and primidiums	B
<i>Glomus</i> sp. 8	globalus	120×130 µm	brown	spore walls and primidiums	B
<i>Glomus</i> sp. 9	ovoid	90×90 µm	yellow	spore walls and non-figmental hyphae	B
<i>Glomus</i> sp. 10	globalus	150×150 µm	blackish brown	non-figmental hyphae	C
<i>Glomus</i> sp. 11	globalus	120×120 µm	reddish brown	spore walls, do not have a germinal wall	C
<i>Glomus</i> sp. 12	ovoid	110×110 µm	yellowish	hyphae, spore walls, do not have germinal walls	C
<i>Glomus</i> sp. 13	globalus	120×120 µm	brownish black	clear hyphae and a spore wall	C
<i>Glomus</i> sp. 14	globolus	110×110 µm	yellowish	non-figmental hyphae and a spore wall	C
<i>Acaulospora</i> sp. 1	globalus	250×350 µm	brown	spore wall and germinal wall, also non-figmental hyphae	A
<i>Acaulospora</i> sp. 2	globalus	290×320 µm	brown	one spore wall and germinal wall, there are cicatrix and saccule	A
<i>Acaulospora</i> sp. 3	globalus	180×180 µm	yellowish brown	a germinal wall, there are cicatrix	B
<i>Acaulospora</i> sp. 4	globalus	360×310 µm	brownish	one spore wall, germinal wall, there are cicatrix	C
<i>Acaulospora</i> sp. 5	globalus	270×270 µm	brownish	one spore wall and germinal wall	C

Note: A – Maleali village, Sausu District, Parigi Moutong Regency; B – Tovalo village, Kasimbar District, Parigi Moutong Regency; C – Ako village, Pasangkayu District, Pasangkayu Regency.

The types of each AMF associated with ebony at the three research sites can be seen in Figure 3.

Based on the results of spore identification from the three research locations, it was found that only 2 AMF genera as-

sociated with ebony plants, namely *Glomus* and *Acaulospora*, where both genera had yellow to brownish black colour on average and were globalus shaped, but could be distinguished based on the characteristics and structure of each genus.

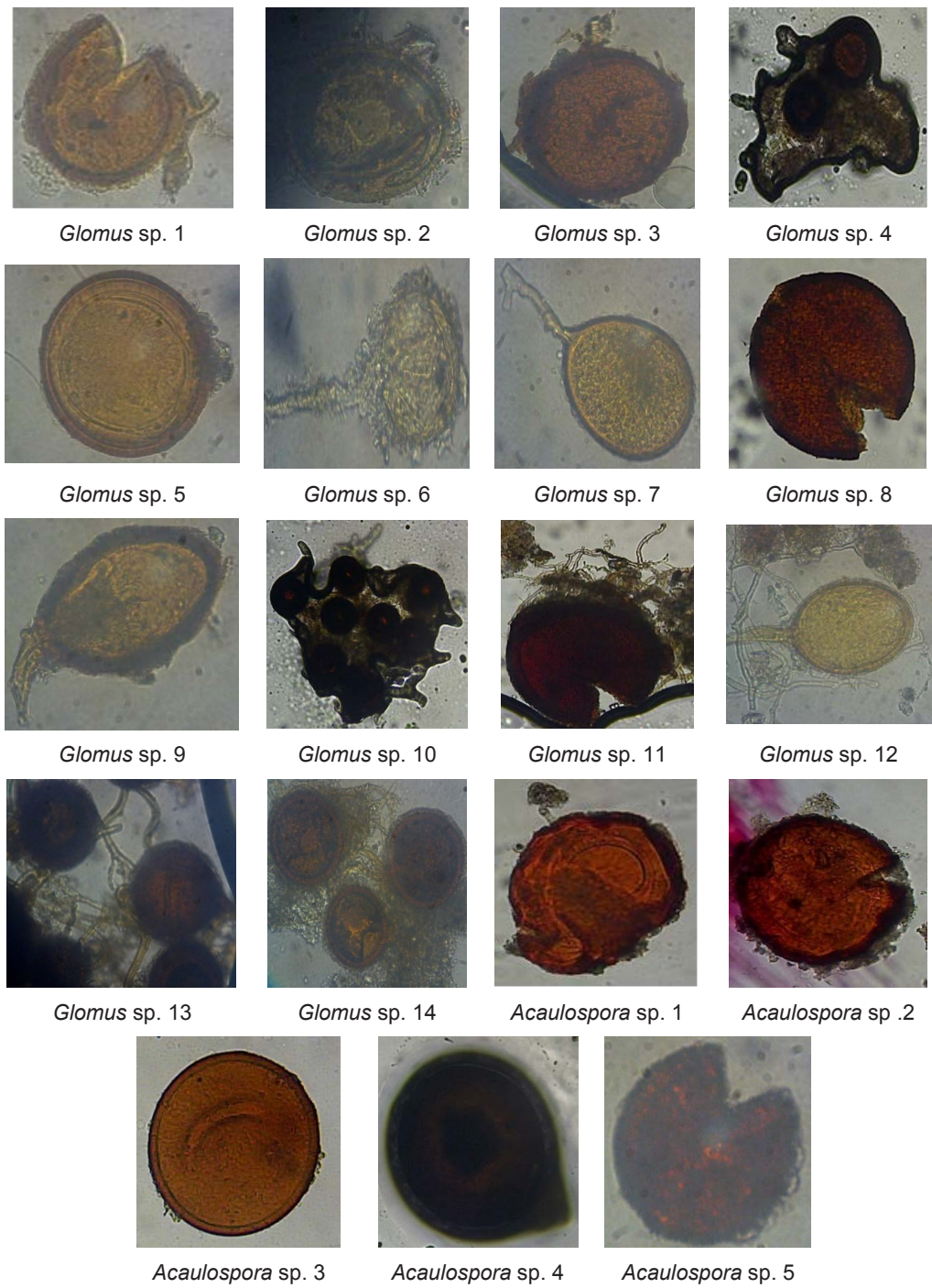


Fig. 3. Micromorphology of AMF associated with ebony in three locations.

The dominant genus *Glomus* was found at three locations (71.3–80 %) compared to the genus *Acaulospora* (Fig. 4). Some of the characteristics of genus *Glomus* were a distinctive hyphal attachment that was not found in other genera. It was globose-sub globose, ovoid and obovoid in shape, yellow, brownish red, brown, and black in colour, wall layers 1–2. This genus can thrive at a pH of less than 5 to neutral. Furthermore, the genus *Acaulospora* had several characteristics, including globulus to elliptical, clear, yellow, or yellowish red in colour, 2–3 spore walls, there were substending hyphae (INVAM 2020). This genus was also more adapted to acidic soil conditions with a pH of less than 5 to neutral. The characteristic found was that the spores develop on the edge of the sporiferous saccule, so that in adult spores there would be one saccule which if released will leave a hole which is also

called cycatric.

In this study, it was found that Glomeraceae family was abundant, where only two dominant genera were found, namely *Glomus* and *Acaulospora* which were found in the rhizosphere soil of ebony plants. Previous studies have also reported that they were also dominant in the forest which may be complementary to their host plant functions (Zhao et al. 2003, Jamiolkowska et al. 2018, Wang et al. 2019, Furrazola et al. 2020). The dominance of *Glomus* and *Acaulospora* in plantations may be related to the smaller spore size which allows them to reproduce faster in a shorter time (Wang et al. 2019). Confirmed by Husband et al. (2002), Li et al. (2007), Jefwa et al. (2012) and Pereira et al. (2014) is that *Glomus* and *Acaulospora* are the most common and most common arbuscular mycorrhizal fungi genera and species found infecting terrestrial plants

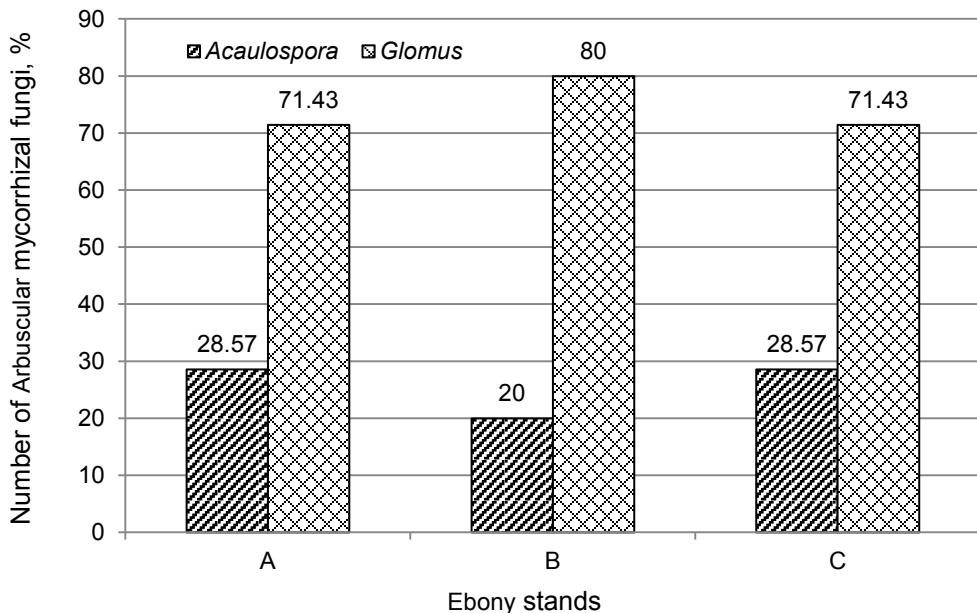


Fig. 4. Percentage of the number of arbuscular mycorrhizal fungi.

Note: A – Maleali village, Sausu District, Parigi Moutong Regency; B – Tovalo village, Kasimbar District, Parigi Moutong Regency; C – Ako village, Pasangkayu District, Pasangkayu Regency.

throughout the world, both in natural environments and those that have been modified by humans. Both genera were able to adapt to a very wide range of environmental conditions, including the different physico-chemical soil conditions in the three research sites (Table 3). The absence of other AMF genera in this study maybe due to the size of their spores. As explained by Hepper (1984) that large spores of AMF species require a longer period to develop compared to AMF species that have small spore sizes, and also because rhizosphere soil sampling is only done once.

The different soil physico-chemical conditions between the three research sites (Table 3), was one of the factors that greatly affected the level of spore density and the diversity of AMF species found. Based on observations, the shape and number of spores, type and genus of AMF found in each research location varied. This situation shows the diversity of AMF found in each research location. The diversity of species/genus and the number of spores found in this study can be

caused by differences in rhizosphere conditions and research locations (Widiastuti and Kramadibrata 1993, Diop et al. 2021, Alrajhei et al. 2022).

The number of AMF species found in this study was less than the results of research by Nobre et al. (2018), where they found 16 AMF species associated with Babassu palm (*Attalea speciosa* Mart.) in the eastern suburbs, Amazonia, Brazil, with the dominance of *Acaulospora* (6 species) followed by *Glomus* (3 species). More results were also found by Songachan et al. (2015), where they found 20 types of AMF in the rhizosphere of *Clerodendrum* species in Meghalaya, India.

According to Nurhalimah et al. (2014) the distribution of AMF is influenced by many factors, including soil type and structure, N and P nutrients in the soil, water, pH and soil temperature. Environmental factors affect the level of mycorrhizal infection in plants. Each AMF genus has a different character. *Glomus* with a wide distribution pattern is a genus that is able to survive and tolerate extreme soils and also has symbiotic ability and high adaptability to local conditions (Sianturi et al. 2005). According to Clark (1997), *Glomus* has the shortest dormancy period, has fairly good germination and the fastest germination time among other mycorrhizal genera (± 6 weeks), while the narrowest distribution of AMF genus is *Acaulospora*.

Spore density and arbuscular mycorrhizal fungi colonisation

The spore density of arbuscular mycorrhizal fungi (AMF) per 50 g of soil, the average number of spores, the percentage of colonisation of arbuscular mycorrhizal fungi and their

Table 3. Soil characteristics.

Parameters	Soil Sampling Location		
	A	B	C
C-organic, %	1.87 $\pm$ 0.01	2.41 $\pm$ 0.02	1.72 $\pm$ 0.01
N-total, %	0.18 $\pm$ 0.02	0.25 $\pm$ 0.02	0.18 $\pm$ 0.02
pH (H ₂ O)	5.43 $\pm$ 0.01	5.56 $\pm$ 0.04	5.39 $\pm$ 0.02
P ₂ O ₅ Bray, ppm	6.18 $\pm$ 0.03	7.80 $\pm$ 0.17	5.96 $\pm$ 0.02
Soil texture	Clay	Clay	Clay
Sand, %	39.02 $\pm$ 0.03	42.74 $\pm$ 0.02	5.52 $\pm$ 0.04
Clay, %	43.34 $\pm$ 0.65	40.90 $\pm$ 0.57	70.84 $\pm$ 0.60
Silt, %	17.64 $\pm$ 0.35	16.36 $\pm$ 0.13	23.94 $\pm$ 0.10

Note: A – Maleali village, Sausu District, Parigi Moutong Regency; B – Tovalo village, Kasimbar District, Parigi Moutong Regency; C – Ako village, Pasangkayu District, Pasangkayu Regency. Each value is mean of three replications, $\pm$ Standard Deviation.

categories at three locations of ebony stands can be seen in Table 4.

Table 4. Density, average number of spores and colonisation.

Location study	Spore density per 50 g of soil	Colonisation, %	Category	Forms of colonisation		
				Mycelium	Vesicle	Arbuscular
A	72 $\pm$ 5 b	0.73 $\pm$ 0.15 b	Low	+	+	-
B	38 $\pm$ 3.05 b	2.47 $\pm$ 0.44 a	Low	+	+	-
C	162 $\pm$ 12 a	0.33 $\pm$ 0.26 b	Low	+	+	-

Note: A – Maleali village, Sausu District, Parigi Moutong Regency; B – Tovalo village, Kasimbar District, Parigi Moutong Regency; C – Ako village, Pasangkayu District, Pasangkayu Regency. Each value is the mean of three replications, $\pm$ Standard Deviation, + present, - absent. Means followed by same letter within a column are not significant at $p \leq 0.05$ level (Tukey's Honestly Significant test).

The results showed that the spore density varied between the three locations. The density found in the ebony rhizosphere soil in Ako village was higher (162 spores per 50 g soil) and was significantly different from the ebony stands in Maleali village and Tovalo village, which were only 72 and 38 spores per 50 g soil, respectively. This is lower than that of *C. crinite* rhizosphere soil, which is an average of 756 spores per 100 g of soil (Furazola et al. 2020). Much higher results were reported that the AMF spore density associated with tree species in Eastern China plantations was between 4.38 to 76.38 spores per gram of soil (Wang et al. 2019) and 100 to 302 spores per gram of soil in the rhizosphere of *Attalea speciosa* (Nobre et al. 2018, Wang et al. 2019).

Liu et al. (2021) reported that soil pH is a major factor and negatively affects the mycorrhizal status of the rhizosphere and endosphere. The effect of soil pH on the results of this study also confirmed by Guo and Gong (2014) who observed that soil pH is the main factor in the formation of AMF composition in ecosystems that are stressed by salt levels, because soil pH affects spore germination and AMF hyphae growth (Gai et al. 2004). Soil pH at three locations of ebony stands tends to

be acidic, namely 5.39–5.56. Low or very acidic soil pH has a direct relationship with an increase in the number and presence of AMF spores in the soil (Saputra et al. 2015). Generally, mycorrhizae are resistant to changes in soil pH so that in alkaline or very acidic soils AMF can still be found. However, the number of AMF depends on the adaptability of each type to be able to develop properly (Samsi et al. 2017). Soti et al. (2014) reported that the AMF colonisation rate was high in soils with low acidity (5.5–6.0) compared to soils with high acidity (4.0–4.5). Differences in season, temperature and humidity can also affect AMF sporulation (Koske 1987), and this causes the highest number of spores found in the rhizosphere of ebony plants in Ako village, Pasangkayu district, West Sulawesi, compared to the other two locations.

According to Suharno et al. (2016) the duration of the interaction between fungi and hosts is influenced by variations in the dependence of mycorrhizae on the host plant and the influence of land conditions on the ability of mycorrhizae to grow. The diversity of AMF is highly dependent on natural conditions. Differences in the location of growth and rhizosphere cause differences in the diversity of species

and populations of AMF (Cui et al. 2018, Velázquez et al. 2020, Husein et al. 2022, Islam et al. 2022). The diversity of AMF spores is due to, among other things, differences in soil properties (Alimi et al. 2021), management practice and elevation level (Marizal et al. 2016), organic matter content in the soils (Bedini et al. 2013, Rini et al. 2017), plant nutrition, light intensity and temperature change (Sun et al. 2013). The next environmental factor that affects the number of spores is C-organic (Samsi et al. 2017).

C-organic in ebony rhizosphere soil samples in Maleali village, Tovalo village and Ako village was 1.87 %, 2.41 %, 1.72 % respectively. The stand with high organic C content (Tovalo village) had fewer spores than the other rhizosphere soil samples. This is in line with the results of Sianturi et al. (2005) who also reported that a small number of AMF spores were found in soils with the highest organic C content. However, the highest number was found in soils with a very low C-organic content compared to soil samples with a high content (Samsi et al. 2017).

The results in Table 4 show that the samples of ebony roots from Tovalo village, Kasimbar District had the highest percentage of colonisation by AMF (2.47 %) which was significantly different from the percentage of colonisation in ebony root samples from Maleali village, Sausu District (0.73 %) and the lowest was in the sample of ebony roots from Ako village, Pasangkayu District (0.33 %). Ebony root colonisation was categorised as low. The same results were obtained by Turjaman et al. (2019), where they found a low percentage of AMF colonisation on several plant roots in the peat swamp forest of Jambi, Sumatra, Indonesia, namely *Alostonia scholaris* (L.) R.Br. (7 %) and *Macaranga pruinosa* (Miq.) Müll.Arg. (9 %).

The low AMF colonisation in ebony roots may be influenced by many factors, for example growth conditions, interactions with host plants, light conditions, temperature, competition of AMF with other soil microbes (Turjaman et al. 2019). The age factor of the old ebony plants (25–40 years) (Table 1) has relatively hard roots so that it has difficulty in the process of infection. AMF infection is more common in young roots (Hermawan et al. 2015). Edaphic and climatic factors, compatibility between fungi and their hosts, root properties and the presence of other soil microorganisms can affect AMF sporulation and its symbiosis with certain trees (Ferdousee et al. 2012).

Furthermore, soil P is also an important factor affecting the intensity of colonisation and growth of AMF spores. When it is abundant, plants will obtain a lot of P nutrients through their roots so they can inhibit growth, extension rate and activity of AMF extraradical hyphae (Zhang et al. 2014). This condition causes a reduction in the level of root colonisation, resulting in reduced P uptake and plant growth (Kiers et al. 2011, Li et al. 2013). Similar to available P and organic C, in this study, the highest total soil N was also obtained from ebony stands in Tovalo village, 0.25 %, followed by ebony stands in Maleali village and Ako village 0.18 %. N is a limiting nutrient in many terrestrial ecosystems (Vitousek and Howarth 1991), including influencing plant associations with AMF. Their development, both in terms of hyphal proliferation and spore formation is influenced and stimulated by addition of organic matter containing high N (Gryndler et al. 2003, Bukovská et al. 2016).

Based on the data obtained, the high density and average number of spores in Ako village was not followed by a high percentage of root colonisation by AMF. In

accordance with the explanation by Smith and Read (1997) it is not certain that plants with a high percentage of AMF colonisation will have high spore densities. This is due to spore formation factor which is influenced by many factors including edhaptic ones and seasonal variation (Sivakumar 2013), the host plant (Sun et al. 2022), growth medium, fertilizer, temperature (Kilpeläinen et al. 2020) and light (Menge 1984). Zhang et al. (2014) stated that the level of root colonisation by AMF and spore density were closely related to the soil factor.

In addition, soil texture is also closely related to the development of plant roots, and indirectly affects the percentage of AMF in plant rhizosphere (Saputra et al. 2015). According to Foth (1994) soil that contains a lot of dust is stronger at storing water because it has small pores and has water absorption slowly so that water is stored in the soil for a long time. In this condition, the available water content is more so that mycorrhizal colonisation on the roots is very low. The results of observations of AMF infection in ebony roots can be seen in Figure 5.

Based on the results of observations, the percentage of AMF infection was characterised by the presence of structures such as AMF vesicles and hyphae

in the ebony root tissue, whereas in roots that did not show any infection, these structures were not found. In the observation of root colonisation, the presence of arbuscular was not found in all the observed root samples. According to Smith and Read (1997) the existence of the arbuscular in the root is relatively short, ranging from 1–3 days. However, with the presence of one or more AMF structures, it can be said that there has been an association by AMF to its host plant.

Conclusions

Arbuscular mycorrhizal fungi associated with ebony plants from the three research sites consisted of 19 species belonging to two genera, namely the genus *Glomus* and *Acaulospora*. The spore density found in Ako village was higher (162 spores per 50 g soil). AMF colonization on ebony roots also varied the highest percentage of AMF infection was in ebony roots in Tovalo village (2.47 %). The results of this study provide basic information about the association of AMF with ebony plants under natural conditions. From the research results, it is recommended that further research is needed, especially the identification of AMF species in natural ebony for-

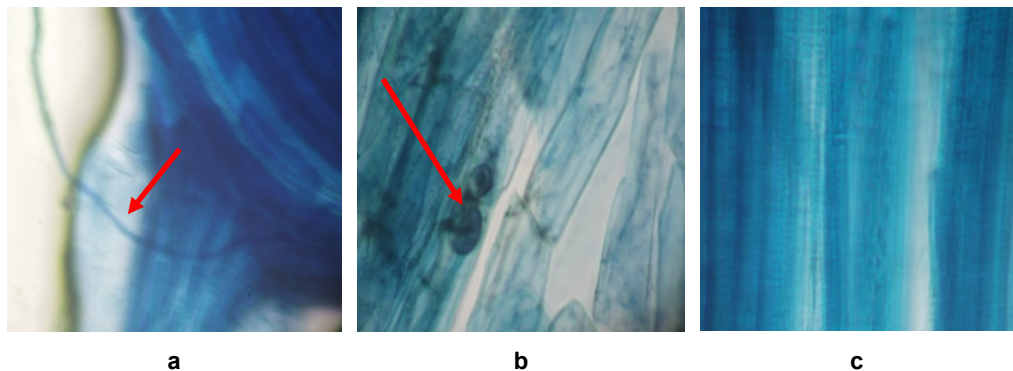


Fig. 5. Ebony roots infected by AMF: a – hyphae, b – vesicles, c – uninfected roots.

ests in various distribution areas, so that data on the diversity of AMF species associated with ebony is more complete and can be used as a reference in future conservation efforts, especially the application of these species. AMF has been found to support the cultivation, conservation and restoration of this species in the future.

Acknowledgement

The authors would like to thank the Head of the Forest Biotechnology Laboratory, Bogor Agricultural University (IPB), Bogor, West Java who has provided laboratory facilities, especially in the identification of AMF species. The author also expresses his gratitude to the International Publication and Collaborative Center (IPPC) and Rector of Tadulako University, for all the help and services until this article was published.

References

- ALIMI A.A., ADELEKE R., MOTEETE A. 2021. Soil environmental factors shape the rhizosphere arbuscular mycorrhizal fungal communities in South African indigenous legumes (Fabaceae). *Biodiversitas* 22(5): 2466–2476. doi: 10.13057/biodiv/d220503
- ALRAJHEI K., SALEH I., ABU-DIEYEH M.H. 2022. Biodiversity of arbuscular mycorrhizal fungi in plant roots and rhizosphere soil from different arid land environment of Qatar. *Plant Direct* 6(1), e369. doi: 10.1002/pld3.369
- ALLO M.K. 2002. Ebony dan Habitatnya, Balai Penelitian Kehutanan, Ujung Pandang. *Berita Biologi*. 6(2): 259–266. doi: 10.1088/1755-1315/522/1/012018
- ASRIANTI A., TUHETERU F.D., HUSNA KANDARI A.M., MEKUO MASNUN I.S. 2016. Status and Culture of Arbuscular Mycorrhizal Fungi isolated from rhizosphere of Endemic and Endangered Species of Kalapi (*Kalappia celebica* Kosterm). *European Journal of Sustainable Development* 5(4): 395–402. doi: 10.14207/ejsd.2016.v5n4p395
- BEDINI S., AVIO L., SBRANA C., TURRINI A., MIGLIORINI P., VAZZANA C., GIOVANNETTI M. 2013. Mycorrhizal activity and diversity in a long-term organic Mediterranean agroecosystem. *Biology and Fertility of Soils* 49(7): 781–790. doi: 10.1007/s00374-012-0770-6
- BOTHE H., TURNAU K., REGVAR M. 2010. The potential role of arbuscular mycorrhizal fungi in protecting endangered plants and habitats. *Mycorrhiza* 20(7): 445–457. doi: 10.1007/s00572-010-0332-4
- BRAY R.H., KURTZ L.T. 1945. Determination of Total Organic and Available Forms of Phosphorus in Soils. *Soil Science* 59(1): 39–45. <http://dx.doi.org/10.1097/00010694-194501000-00006>
- BRUNDRETT M.C., PICHE Y., PETERSON R.L. 1984. A new method for observing the morphology of vesicular arbuscular mycorrhizae. *Canadian Journal of Botany* 62(10): 2128–2134. doi: 10.1139/b84-290
- BRUNDRETT M., BOUGHER N., DELL B., GROVE T., MALAJCZUK N. 1996. Working With Mycorrhizas in Forestry and Agriculture. Monograph 32. Australian Centre For International Agriculture Research, Canberra, Australia. 374 p.
- BUKOVSKÁ P., GRYNDLER M., GRYNDLEROVÁ H., PÜSCHEL D., JANSÁ J. 2016. Organic Nitrogen-Driven Stimulation of Arbuscular Mycorrhizal Fungal Hyphae Correlates with Abundance of Ammonia Oxidizers. *Frontiers in Microbiology* 7, 711. <https://doi.org/10.3389/fmicb.2016.00711>
- CLARK R.B. 1997. Arbuscular Mycorrhizal Adaptation, Spore Germination, Root Colonization and Host Plant Growth and Mineral Acquisition at Low pH. *Plant and Soil* 192: 15–22. <https://doi.org/10.1023/A:1004218915413>
- CUI J., BAI L., LIU X., JIE W., CAI B. 2018. Arbuscular mycorrhizal fungal communities in the rhizosphere of a continuous cropping soybean system at the seedling stage. *Brazilian Journal of Microbiology* 49(2): 240–247. <https://doi.org/10.1016/j.bjm.2017.03.017>
- DIOP I., NDOYE F., DIEDHIU A.G., KRASOVA-WADE T., DO REGO F.A., NOBA K., AMBROSI J.P.,

- KANE A. 2021. Diversity and spore density of arbuscular mycorrhizal fungi in the rhizosphere of Cowpea (*Vigna unguiculata* [L.] Walp.) cultivated in different soils in Senegal. *Journal of Animal and Plant Sciences* 48(1): 8552–8565. <https://doi.org/10.35759/JANmPISci.v48-1.1>
- EVELIN H., SHARMA E., KAPOOR R. 2019. Arbuscular Mycorrhizal Fungi: Potential Role in Conservation of Endangered Plants. *Plant Reproductive Biology*: 314–326.
- FERDOUSEE N., MISBAHUZZAMAN K., HOQUE A.T.M.R. 2012. Arbuscular mycorrhizal colonization in five tropical forest tree legumes of Chittagong University Campus in Bangladesh. *Journal of Basic & Applied Sciences* 8(2): 353–361. <https://doi.org/10.6000/1927-5129.2012.08.02.17>
- FOTH H.D. 1994. *Dasar-dasar Ilmu Tanah*. Erlangga. Jakarta. 374 p. (in Indonesian).
- FURRAZOLA E., SÁNCHEZ-RENDÓN J.A.S., GUADARRAMA P., PERNÚS M., TORRES-ARIAS Y. 2020. Mycorrhizal status of *Coccothrinax crinita* (Arecaceae), an endangered endemic species from Western Cuba. *Revista Mexicana de Biodiversidad* 91, e913048. 10 p. <https://doi.org/10.22201/ib.20078706e.2020.91.3048>
- GAI J.P., FENG G., LI X.L. 2004. Diversity of arbuscular mycorrhizal fungi in field soils from North China. *Biodiversity Science* 12(4): 435–440. doi: 10.17520/biods.2004053
- Giovannetti M., Mosse B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytologist* 84: 489–500. <https://doi.org/10.1111/j.1469-8137.1980.tb04556.x>
- GOLTAPEH E.M., DANESH Y.R., PRASAD R., VARMA A. 2008. Mycorrhizal Fungi: What We Know and What Should We Know? In: Varma A. (Ed.), *Mycorrhiza*. 3rd ed. Springer, Berlin, Heidelberg: 3–27. https://doi.org/10.1007/978-3-540-78826-3_1
- GRYNDLER M., JANSÁ J., HRŠELOVÁ H., CHVÁTALOVÁ I., VOSÁTKA M. 2003. Chitin stimulates development and sporulation of arbuscular mycorrhizal fungi. *Applied Soil Ecology* 22(3): 283–287. [https://doi.org/10.1016/s0929-1393\(02\)00154-3](https://doi.org/10.1016/s0929-1393(02)00154-3)
- GUNAWAN H., SUGIARTI, WARDANI M., MINDAWATI N. 2019. 100 Spesies Pohon Nusantara Target Konservasi *Ex Situ* Taman Keanekaragaman Hayati. Bogor, IPB Press. 237 p.
- GUO X., GONG J. 2014. Differential effects of abiotic factors and host plant traits on diversity and community composition of root colonizing arbuscular mycorrhizal fungi in a salt-stressed ecosystem. *Mycorrhiza* 24(2): 79–94. doi: 10.1007/s00572-013-0516-9
- HARLEY J.L., SMITH S.E. 1983. *Mycorrhizal Symbiosis*. Academic Press, London. 483 p.
- HENDROMONO 2008. Teknik penanaman ebony (*Diospyros celebica* Bakh.) di daerah agak kering. *Jurnal Hutan Tanaman* 5(1): 53–61 (in Indonesian).
- HERMAWAN H., MUIN A., WULANDARI R.S. 2015. Abundance of Arbuscular Mycorrhizal Fungi (AMF) on Plants Eucalyptus (*Eucalyptus pellita*) Based on Level Depth In Peatland [Kelimpahan Fungi Mikoriza Arbuskula (FMA) Pada Tegakan Ekaliptus (*Eucalyptus pellita*) Berdasarkan Tingkat Kedalaman Di Lahan Gambut]. *Jurnal Hutan Lestari* 3(1): 124–132 (in Indonesian).
- HEPPER C.M. 1984. Isolation and culture of VA mycorrhizal (VAM) fungi. In: Powell C.L., Bagyaraj D.J. (Eds), *VA Mycorrhizae*. CRC Press, Florida, USA: 95–112.
- HUSEIN M., UMAMI N., PERTIWININGRUM A., RAHMAN M.M., ANANTA D. 2022. The Role of Arbuscular Mycorrhizal Fungi Density and Diversity on the Growth and Biomass of Corn and Sorghum Forage in Trapping Culture. *Tropical Animal Sciences Journal* 45(1): 37–43. <https://doi.org/10.5398/tasj.2022.45.1.37>
- HUSBAND R., HERRE E.A., TURNER S.L., GALLERY R., YOUNG J.P.W. 2002. Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest, *Molecular Ecology* 11(12): 2669–2678. doi: 10.1046/j.1365-294x.2002.01647.x
- HUSNA, BUDI S.W., MANSUR I., KUSMANA D.C. 2015. Diversity of Arbuscular Mycorrhizal Fungi in the Growth Habitat of Kayu Kuku (*Pericopsis mooniana* Thw.) in Southeast Sulawesi. *Pakistan Journal of Biological Sciences* 18(1): 1–10. doi: 10.3923/pjbs.2015.1.10
- INVAM 2020. Davis College of Agriculture, Nat-

- ural Resources and Design. West Virginia University. Web site. <http://fungi.invam.wvu.edu/the-fungi/species-descriptions.html>
- ISLAM M., AL-HASHIMI A., AYSHASIDDEKA M., ALI H., EL ENSHASY H.A., DAILIN D.J., SAYYED R.Z., YEASMIN T. 2022. Prevalence of mycorrhizae in host plants and rhizosphere soil: A biodiversity aspect. *PLoS ONE* 17(3), e0266403. <https://doi.org/10.1371/journal.pone.0266403>
- IUCN 2022a. The IUCN Red List of Threatened Species. Version 2021-3. Web site. <https://www.iucnredlist.org/>
- IUCN 2022b. *Diospyros celebica*, Indonesian Ebony. The IUCN Red List of Threatened Species. World Conservation Monitoring Centre. e.T33203A9765120. <https://www.iucnredlist.org/species/33203/9765120>
- JAMIOŁKOWSKA A., KSIEŹNIAK A., GALĄZKA A., HETMAN B., KOPACKI M., SKWARYŁO-BEDNARZ B. 2018. Impact of abiotic factors on development of the community of arbuscular mycorrhizal fungi in the soil: A Review. *International Agrophysics* 32: 133–140. doi: 10.1515/intag-2016-0090
- JEFWA J.M., OKOTH S., WACHIRA P., KARANJA N., KAHINDI J., NJUGUINI S., ICHAMI S., MUNGU'ATU J., OKOTH P., HUISING J. 2012. Impact of land use types and farming practices on occurrence of arbuscular mycorrhizal fungi (AMF) Taita-Taveta district in Kenya. *Agriculture, Ecosystems and Environment* 157: 32–39. <https://doi.org/10.1016/j.agee.2012.04.009>
- KIERS E.T., DUHAMEL M., BEESETTY Y., MENSAH J.A., FRANKEN O., VERBRUGGEN E., FELLBAUM C.R., KOWALCHUK G.A., HART M.M., BAGO A., PALMER T.M., WEST S.A., VANDENKOORNHUYSE P., JANS A., BÜCKING H. 2011. Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science* 333(6044): 880–882. doi: 10.1126/science.12084
- KILPELÄINEN J., APHALO P.J., LEHTO T. 2020. Temperature affected the formation of arbuscular mycorrhizas and ectomycorrhizas in *Populus angustifolia* seedlings more than a mild drought. *Soil Biology and Biochemistry* 146, 107798. <https://doi.org/10.1016/j.soilbio.2020.107798>
- KJELDAHL J. 1883. A New Method for the Determination of Nitrogen in Organic Matter. *Zeitschrift für Analytische Chemie* 22: 366–382. <http://dx.doi.org/10.1007/BF01338151>
- KOSKE R.E. 1987. Distribution of VA mycorrhizal fungi along a latitudinal temperature gradient. *Mycologia* 79(1): 55–68. <https://doi.org/10.1080/00275514.1987.12025370>
- LI Y.F., JU L.H., ZHANG L.Y., XU G.H. 2013. Effects of AM fungi on plant growth and nitrogen and phosphorus utilization in rice/mungbean intercropping under different phosphorus application levels. *Jiangsu Journal of Agricultural Sciences* 41: 51–55.
- LI L.F., ZHANG Y., ZHAO Z.W. 2007. Arbuscular mycorrhizal colonization and spore density across different land-use types in a hot and arid ecosystem, Southwest China. *Journal of Plant Nutrition and Soil Science* 170(3): 419–425. <https://doi.org/10.1002/jpln.200625034>
- LIU R.-C., XIAO Z.-Y., HASHEM A., ABD ALLAH E.F., WU Q.S. 2021. Mycorrhizal Fungal Diversity and Its Relationship with Soil Properties in *Camellia oleifera*. *Agriculture* 11(6), 470. <https://doi.org/10.3390/agriculture11060470>
- MARIZAL S., MUZAKIR., SYARIYAH A. 2016. The Diversity of Arbuscular Mycorrhiza Fungus (AMF) Indigenous in Peanuts (*Arachis hypogaea* L.) Rhizosphere under Different Elevation. *Journal of Tropical Soils* 21(2): 109–114. doi: 10.5400/jts.2016.21.2.109
- MENGE J.A. 1984. Inoculum Production. Powel C.L., dan Bagyaraj D.J. (Eds), Dalam VA Mycorrhiza. CRC Press. Inc. 243 p.
- NINGSIH D.R., KRAMADIBRATA K., GUNAWAN A.W. 2013. Arbuscular Mycorrhizal Fungi in Bisbul Trees (*Diospyros blanco*) in Bogor. *Biotropia* 20(2): 112–121. <https://doi.org/10.11598/btb.2013.20.2.381>
- NOBRE C.P., DA COSTA M.G., GOTO B.T., GEHRING C. 2018. Arbuscular mycorrhizal fungi associated with the babassu palm (*Attalea speciosa*) in the eastern periphery of Amazonia, Brazil. *Acta Amazonia* 48(4): 321–329. <https://doi.org/10.1590/1809-4392201800092>
- NURHALIMAH S., NURHATIKA S., MUHIUBDIN A. 2014. Eksplorasi Mikoriza Vesikular Ar-

- buskular (MVA) Indigenous Pada Tanah Regosol di Pamekasan Madura. *Jurnal Sains dan Seni Pomits* 3(1): 1–5.
- O'CONNOR P.J., SMITH S.E., SMITH F.A. 2001. Arbuscular mycorrhizal association in the Southern Simpson desert. *Australian Journal of Botany* 49(4): 493–499. doi: 10.1071/BT00014
- PACIONI G. 1992. Wet Sieving and Decanting Technique for the Extraction of Spores of Vesicular- Arbuscular Fungi. In: *Methods in Microbiology*, Norris J.R., Read D.J., Varma A.K. (Eds). Chapter 16, Academic Press, San Diego, CA, USA: 317–322.
- PEREIRA C.M.R., SILVA D.K.A., FERREIRA A.C.A., GOTO B.T., MAIA L.C. 2014. Diversity of arbuscular mycorrhizal fungi in Atlantic forest areas under different land uses. *Agriculture, Ecosystems and Environment* 185: 245–252. <https://doi.org/10.1016/j.agee.2014.01.005>
- PURWATI B., BUDI S.W., WASIS B. 2019. Status fungi mikoriza arbuskular (FMA) pada rizosfer jernang (*Daemonorops draco* Blume) di Jambi. *Media Konservasi* 24(3): 261–268. doi: 10.29244/medkon.24.3.261-268
- RAHMAN W., ABDULLAH M.N. 2002. Effect of shade and tiller origin on growth of ebony (*Diospyros celebica* Bakh.) [Efek naungan dan asal anakan terhadap pertumbuhan eboni (*Diospyros celebica* Bakh.)]. *Berita Biologi* 6(2): 297–301 (in Indonesian).
- REDECKER D., SCHÜSSLER A., STOCKINGER H., STÜRMER S.L., MORTON J.B., WALKER C. 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi (Glomeromycota). *Mycorrhiza* 23(7): 515–531. doi: 10.1007/s00572-013-0486-y
- RINI M.V., SITIO S.N.S., HIDAYAT K.F. 2017. Population and Diversity of Arbuscular Mycorrhizal Fungi in the Rhizosphere of Kasetasrt Cassava Clone Grown on two Different Locations. *Journal of Tropical Soils* 22(3): 183–189. <http://dx.doi.org/10.5400/jts.2017.v22i3.183-189>
- SAMSI N., PATA'DUNGAN Y.S., THAHA A.R. 2017. Isolation and Morphology Identification of Spores of Arbuscular Mycorrhiza Fungi in The Rhizosfer Area of Some Horticulture-Plants in Farming Land of The Sidera Village [Isolasi dan Identifikasi Morfologi Spora Fungi Mikoriza Arbuskula Pada Daerah Perakaran Beberapa Tanaman Hortikultura di Lahan Pertanian Desa Sidera]. *Fakultas Pertanian. Universitas Tadulako. Palu. Jurnal Agrotekbis* 5(2): 204–211 (in Indonesian).
- SAPUTRA B., LINDA R., LOVADI I. 2015. Jamur Miko-riza Vesikular Arbuskular (MVA) Pada Tiga Jenis Tanah Rhizosfer Tanaman Pisang Niah (*Musa paradisiaca* L Var. nipah) di Kabupaten Pontianak. *Universitas Tanjungpura. Jurnal Protobiont* 4(1): 160–169.
- SCHENCK N.C., PÉREZ Y. 1988. *Manual for the Identification of VA Mycorrhizal Fungi*. 2nd Edn., University of Florida, Gainesville, Florida. 241 p.
- SCHÜSSLER A., WALKER C. 2010. *The Glomeromycota: A Species List with New Families and New Genera*. Schüssler A., Walker C., Gloucester, published in libraries at Royal Botanic Garden Edinburgh, Kew, Botanische Staatssammlung Munich, and Oregon State University. 56 p.
- SIVAKUMAR N. 2013. Effect of edaphic factors and seasonal variation on spore density and root colonization of arbuscular mycorrhizal fungi in sugarcane fields. *Annals of Microbiology* 63: 151–160. doi: 10.1007/s13213-012-0455-2
- SMITH S.E., READ D.J. 1997. Vesicular arbuscular mycorrhizas: Growth and carbon economy of VA mycorrhizal plants. In *Mycorrhizal Symbiosis*. 2nd New York (US), Acad. Press. 605 p.
- SMITH S.E., READ D.J. 2008. *Mycorrhizal symbiosis*. Elsevier Limited, Amsterdam. 800 p.
- SIANTURI F., LINDA R., KHOTIMAH S. 2005. Kepadatan Spora Jamur Vesikular Arbuskular Pada Tiga Tingkat Kematangan Gambu Di Kawasan Hutan Lindung Gunung Ambawang Kabupaten Kubu Raya. *Universitas Tanjungpura. Jurnal Protobiont* 4(2): 96–102.
- SONGACHAN L.S., KAYANG H., MOINAO P. 2015. Diversity and species composition of arbuscular mycorrhizal fungi in *Clerodendrum species*. *Mycosphere* 6(2): 150–158. doi: 10.5943/mycosphere/6/2/5
- SOTI P.G., JAYACHANDRAN K., PURCELL M., VOLIN

- J.C, KITAJIMA K. 2014. Mycorrhizal symbiosis and *Lygodium microphyllum* invasion in south Florida – a biogeographic comparison. *Symbiosis* 62(2): 81–90. doi: 10.1007/s13199-014-0272-4
- SUHARNO, KASIAMDARI R.S., SOETARTO E.S., SANCAYANINGSIH R.P. 2016. Presence of Arbuscular Mycorrhizal Fungi on Fern from Tailoring Deposition Area of Gold Mine in Timika. Indonesia. *International Journal of Environmental Bioremediation and Biodegradation* 4(1): 1–7. doi: 10.12691/ijebb-4-1-1
- SUN X.F., SU Y.Y., ZHANG Y., WU M.Y., ZHANG Z., PEI K.Q., SUN L.F., WAN S.Q., LIANG Y. 2013. Diversity of arbuscular mycorrhizal fungal spore communities and its relations to plants under increased temperature and precipitation in a natural grassland. *Chinese Science Bulletin* 58: 4109–4119. <https://doi.org/10.1007/s11434-013-5961-5>.
- SUN X., FENG J., SHI J. 2022. Stimulation of Hyphal Ramification and Sporulation in *Funneliformis mosseae* by Root Extracts Is Host Phosphorous Status-Dependent. *Journal of Fungi* 8, 181. <https://doi.org/10.3390/jof8020181>
- TURJAMAN M., HERDYANTARA B., FAULINA S.A., AGUSTINI L., IRIANTO R.S.B., HIDAYAT A., WAHNO I., MURDANI., TJAHYONO B., INDRAYADI H. 2019. Mycorrhizal colonization of indigenous tropical tree species grown in peat swamp forest of Sumatera, Indonesia. *IOP Conference Series: Earth and Environmental Science* 308, 012049. 6 p. doi: 10.1088/1755-1315/308/1/012049
- TURRINI A., GIOVANNETTI M. 2012. Arbuscular mycorrhizal fungi in national parks, nature reserves and protected areas worldwide: a strategic perspective for their in situ conservation. *Mycorrhiza* 22(2): 81–97. doi: 10.1007/s00572-011-0419-6
- VELÁZQUEZ M.S., FABISIK J.C., BARRERA M. ALLEGUCCI N., VALDÉS F.E., ABARCA C.L., CABELLO M. 2020. Diversity and abundance of arbuscular mycorrhizal fungi (Glomeromycota) associated with *Ilex paraguensis* in Northeastern Argentina. *Revista de Biología Tropical* 68(4): 1231–1240. <http://dx.doi.org/10.15517/rbt.v68i4.41543>
- VITOUSEK P.M., HOWARTH R.W. 1991. Nitrogen limitation on land and in the sea; how can it occur? *Biogeochemistry* 13: 87–115. <https://doi.org/10.1007/BF00002772>
- WANG J., WANG G.G., ZHANG B., YUAN Z., FU Z., YUAN Y., ZHU L., MA S., ZHANG J. 2019. Arbuscular mycorrhizal fungi associated with tree species in a planted forest of Eastern China. *Forests* 10(5), 424. <https://doi.org/10.3390/f10050424>
- WANG B., QIU Y.L. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza* 16: 299–363 doi: 10.1007/s00572-005-0033-6
- WALKLEY A.J., BLACK I.A. 1934. Estimation of soil organic carbon by the chromic acid titration method. *Soil Science* 37: 29–38.
- WIDIASTUTI H., KRAMADIBRATA K. 1992. Jamur mikoriza bervesikula-arbuskula di beberapa tanah masam dari Jawa Barat. *Menara Perkebunan* 60(1): 9–19 (in Indonesian).
- WIDIASTUTI H., KRAMADIBRATA K. 1993. Identifikasi jamur mikoriza ber vesikula arbuskula di beberapa kebun kelapa sawit di Jawa Barat. *Menara Perkebunan* 61(1): 13–19 (in Indonesian).
- WULANDARI R., KUSTIAWAN W., SUKARTININGSIH, SIMARANGKIR B.D.A.S. 2017. Genetik Diversity and Kinship of Ebony Population (*Diospyros celebica* Bakh.) in its Natural Population in Central Sulawesi. *Journal of Biodiversity and Environmental Sciences* 10(6): 50–58. <https://europub.co.uk/articles/-A-39147>
- ZHANG Q., SUN Q., KOIDE R.T., PENG Z., ZHOU J., GU Z., GAO W., YU M. 2014. Arbuscular Mycorrhizal Fungal Mediation of Plant-Plant Interactions in a Marshland Plant Community. *The Scientific World Journal* 14, 923610. 10 p. doi: 10.1155/2014/923610
- ZHAO Z.W., WANG G.H., YANG L. 2003. Biodiversity of arbuscular mycorrhizal fungi in a tropical rainforest of Xishuangbanna, Southwest China. *Fungal Diversity* 13: 233–242.
- ZHI-WEI Z, YONG-MEI X, XIN-ZHENG Q, XI-WU L, LI-ZHONG C, TAO S, GUO-HUA W. 2001. Arbuscular mycorrhizal status of plants and the spore density of arbuscular mycorrhizal fungi the tropical rain forest of Xishuangbanna, Southwest China. *Mycorrhiza* 11(3): 159–162. doi: 10.1007/s005720100117