BIODIVERSITAS
Volume 23, Number 10, October 2022
Pages: 5290-5297

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d231038

Diversity of Arbuscular Mycorrhizal Fungi of *Kalappia celebica*: An endemic and endangered plant species in Sulawesi, Indonesia

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Manuscript received: 19 August 2022. Revision accepted: 13 October 2022.

Abstract. *Husna, Tuheteru FD, Albasri, Arif A, Basrudin, Nurdin WR, Arman E, Agustin DI, Saribadu J, Rahmat, Dermawansyah A, Daliana, Lody LP, Deri AS, Safitri I. 2022. Diversity of Arbuscular Mycorrhizal Fungi of* Kalappia celebica*.): An endemic and endangered plant species in Sulawesi, Indonesia. Biodiversitas 23: 5290-5297.* Arbuscular Mycorrhizal Fungi (AMF) are a group of fungi belonging to Phylum Glomeromycota. AMF has a symbiotic relationship with higher plants, including endangered species in various climatic regions and land types. Research on AMF diversity related to endemic and endangered tree species in the tropics is still limited. This study aimed to examine the diversity of AMF in the natural habitat of *Kalappia celebica* Kosterm. (*kalapi*) in Southeast Sulawesi Province, Indonesia. Samples of soil and roots of *K. celebica* were collected from six natural habitat locations, i.e., Konawe District (Anggatoa and Abuki Villages), East Kolaka District (Lalingato and Anggaloosi Villages) and Kolaka District (Lalonaha and Lapao Pao Villages). The AMF spores were isolated by using the pouring method, followed by wet filtration. AMF identification was carried out by observing the morphology of AMF spores. A total of eight AMF species belonging to three genera Viz. *Glomus*, *Funneliformis* and *Rhizophagus* were isolated. Among the species identified, five species belong to genus *Glomus*, two species belong to the genus *Funneliformis* and one species belongs to the genus *Rhizophagus*. *Glomus* sp.1 and 2 and *F. mosseae* had the highest spore density, relative density and frequency, and importance index values. The AMF highest spore density was obtained in Anggaloosi Village of East Kolaka District. Soil properties such as organic C, total N and clay were positively correlated with spore density and negatively correlated with AMF colonization. The Simpson's Index ranged from 0.42 ± 0.117 to 0.86 ± 0.068. The range of Shannon-Wiener Index was 0.12 ± 0.208 to 0.99 ± 0.198, while the Evenness Index ranged from 0.17 ± 0.189 to 0.75 ± 0.106. These findings indicated that *K. celebica* species are rich in AMF diversity. These symbiont fungi are key components of the ecosystem. Further research is needed to develop their use as promoters of plant establishment in conservation and restoration of those sites.

Keywords: Glomeraceae, *Kalappia celebica*, spore density, Sulawesi

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are a group of fungi from the phylum Glomeromycota that are symbiotic with the root system of higher plants (Smith and Read 2008) in various climatic zones and continents (Stürmer et al. 2018), global biomes (Brundrett and Tedersoo 2018) and land use types (Tuheteru et al. 2020; Ceola et al. 2021). AMF include beneficial soil microbes (Ishaq 2018; Powell and Rillig 2018; Diagne et al. 2020; Riaz et al. 2021; Mitra et al. 2021) and can be developed as biological fertilizers to support the conservation of endangered tree species (Suharno et al. 2013; Husna et al. 2018; Evelin et al. 2019; Manjula et al. 2022). AMF are broadly classified into three classes, 11 families, 25 genera and nearly 250 species (Schüßler et al. 2001; Spatafora et al. 2016; Tedersoo et al. 2018). As of early 2021, there have been 75 species of AMF from 23 genera and 11 families in Indonesia identified (Husna et al. 2021).

There is ample scientific evidence showing that AMF significantly increases plants' succession in species conservation programs. AMF are reported to be effective in the improvement and cultivation of endangered species and can significantly accelerate the succession and survival of species in conservation and restoration programs (Sharma et al. 2008; Zubek et al. 2009; Bothe et al. 2010; Husna et al. 2018). In Indonesia, the AMF application studies for the conservation of endangered species have been reported in *Aquilaria malaccensis* and *A. crassna* (Turjaman et al. 2006a), *A. filaria* (Turjaman et al. 2006b), *Kalappia celebica* (Husna et al. 2019a; 2021a; Arif et al. 2021), *Pericopsis mooniana* (Husna et al. 2016; 2019b; 2021b), and *Pterocarpus indicus* (Husna et al. 2021b).

In general, endemic and endangered plants are reported to be in symbiosis with AMF. In a review by Wang and Qiu (2006), it was reported that there were 139 species of endangered plants out of 2,469 species which were in symbiosis with AMF. Husna et al. (2015) reported that 15 AMF species were found (predominantly Glomeraceae) and grouped into 5 families and 9 genera from the rhizosphere of *Pericopsis mooniana* in Southeast Sulawesi Province, Indonesia. AMF was found to be in symbiosis

with three endangered medicinal plant species in Rajasthan, India (Panwar and Tarafdar 2006). Root colonization of *Vitis vinifera* subsp. *sylvestris* was found along the Neretva River in southwestern Bosnia and Herzegovina (Radić et al. 2018). Sixteen AMF species were found to be in symbiosis with *Coccothrinax crinita* (Arecaceae) in Cuba (Furrazola et al. 2020). Several native AMF were associated with *Artemisia umbelliformis* Lam, in Southern French Alps (Binet et al. 2011), *Astragalus applegatei* Peck in Oregon State, USA (Barroetavena et al. 1998), *Carissa edulis* in Kenya (Ogoma et al. 2021), endemic plants from ultramafic soils of New Caledonia (Crossay et al. 2018), *Ulmus chenmoui* Cheng in China (Song et al. 2019), *Picconia azorica* in two Azorean islands, Portugal (Melo et al. 2019) and *Ferula sinkiangensis* (Luo et al. 2020).

Kalappia celebica Kosterm. (Fabaceae), locally named "*kalapi*", is an endangered species endemic in the Lowland Forest in Sulawesi, Indonesia (Trethowan 2019; Tuheteru et al. 2022). *Kalappia celebica* is an endemic species in Indonesia which is on conservation priority and listed as vulnerable (VU B1 ab, ii, iii, iv, v) in the IUCN Red List 2019 (Trethowan 2019). *Kalappia celebica* populations and habitats are threatened by habitat degradation for large-scale agricultural and plantation expansion, over-exploitation, and low natural regeneration. Knowledge of seed handling (Arif et al. 2018), vegetative propagation (Arif et al. 2022) as well as nursery-scale AMF testing (Husna et al. 2019a; 2021a) and field-scale (Arif et al. 2021) are known. AMF symbiosis related to the endangered *K. celebica* tree species has been reported by Arif et al. (2016). Research on the AMF diversity on the *K. celebica* rhizosphere is still very limited in its natural distribution in Kolaka. AMF species identification is still limited to up to the genus level (Arif et al. 2016). Exploration, isolation, identification and propagation of AMF from various *K. celebica* natural habitats in Sulawesi need to be carried out to reveal the diversity of *K. celebica* AMF in the Wallacea Region. AMF collections can be an alternative technology for *K. celebica* conservation in Indonesia.

MATERIALS AND METHODS

Site description

The study was conducted in six villages in Southeast Sulawesi of Indonesia from January to June 2022 (Table 1).

Soil and roots sampling

Soil and root samples were taken from the *K. celebica* rhizosphere at each location using a soil drill to a depth of 20 cm (Husna et al. 2015). At each location, 250 g of soil sample was taken from 4 sampling points, so that 1 kg of composite soil samples were obtained from each location (Husna et al. 2015). Soil samples were stored in labeled plastic bags. Root samples were preserved with 70% alcohol and then poured into labeled film tubes. Subsequently, the soil and root samples were taken to the laboratory. The 1 kg composite soil from each location (three replicates each) was delivered to the SEAMEO BIOTROP Soil and Plant Laboratory in Bogor.

Isolation and identification of AMF from soil

Soil samples (100 g) were collected from the field were examined for spore density analysis. Spores were extracted from the soil by the wet filter pour method (Gerdemann and Nicolson 1963) followed by centrifugation of the supernatant with 50% added sugar solution (Brundrett et al. 1996). The extracted AMF spores were observed and counted under a dissecting microscope with 35x magnification. Identification of AMF spores was carried out by observing morphological characteristics (composition, color, shape, size) (Schenck and Perez 1988). The nomenclature of AMF spores follows the latest nomenclature according to Schüßler and Walker (2010) and Redecker et al. (2013).

Root colonization

Root colonization was observed by the root staining technique of Brundrett et al. (1996). A total of 20 pieces of fresh roots (1 cm long) were taken from plant roots at random. Fresh roots were immersed in 10% KOH for 2 days, followed by immersion in H_2O_2 for 10-20 minutes, then rinsed thoroughly. The roots were then given 0.2% HCl treatment for 20 minutes, followed by 0.05% Trypan Blue. The 10 root samples from each plant were placed on a glass slide, covered with a cover glass, and then observed under a microscope with 200x magnification. Calculation of root colonization was conducted using the formula: [Σ mycorrhizal field of view/ total field of view observed] x 100% (Brundrett et al. 1996).

Table 1. Description of research site

District/village	Coordinate	Elevation (m)	Dominant vegetation
Konawe District			
Abuki	121° 55' 20,78" BT - 3° 44' 14,84" LS	81-91	*Garcinia tertandra*
Anggotoa	122° 6' 26,76" BT - 3° 49' 7,36" LS	96-105	*Syzygium* sp.
Kolaka Timur District			
Anggaloosi	121° 51' 34,49" BT - 4° 6' 56,35" LS	321-322	Clove (*Syzygium aromatica*)
Lalingato	121° 50' 44,70" BT - 3° 59' 56,14" LS	237-259	Eha (*Castanopsis buruana*)
Kolaka District			
Lalonaha	121° 15' 28,87" BT - 3° 49' 8,51" LS	76-117	Eha (*Castanopsis buruana*)
Lapao-pao	121° 20' 30,30" BT - 3° 52' 39,91" LS	397-425	Clove (*Syzygium aromatica*)

Tabel 2. Parameters of AMF diversity and calculation methods

Parameter	Formula
Frequency of Isolation	[the amount of soil samples where AMF presence/total sample] x 100%
Relative abundance	Percentage of spores amount from a species or a genus
Importance Value (IV)	(Isolation frequency + Relative abundance)/2. Importance Index ≥ 20 means dominant species or genus
Spore densities	Spores amount per 100 g soil
Species richness	Species in each soil sample
Shannon-Wiener Index	$H' = -\Sigma pi \ln pi$
Evenness Index	$E = H'/H'max$
Simpson's Index	$D = \Sigma[n_i(n_i-1)/N(N-1)]$
AMF colonization	[Σ mycorrhizal field of view/total field of view observed] x 100%

Soil properties

Analysis of soil properties at each location was also carried out SEAMEO BIOTROP Soil and Plant Laboratory, Bogor. Soil pH was measured using the method described in SNI 03-6787-2002. Soil organic C was assessed by the method of SNI 13-4720-1998 (Walkey-Black). The total N was measured by micro-Kjeldahl (SNI 13-4721-1998), while the P_2O_5 was measured by Bray method I/II (SL-MU-TT-05). Texture analyses of 3 fractions of sand, dust, clay were conducted by using the SL-MU-TT-10 (Hydrometer) method.

Data

The diversity data observed in this study were isolation frequency, relative abundance, importance value, spore density, species richness, spore diversity (Shannon-Wiener, Evenness and Simpson's Indices) and root colonization as presented in Table 2.

Data analysis

The data were analyzed by using analysis of variance (F test). Parameters analyzed included soil chemical properties, spore density, AMF colonization, species richness, Shannon-Wiener, Evenness and Simpson Indices. If the test results showed a significant effect, a different significance test were carried out at the 95% level, with SAS 9.1.3 Portable. The correlation analyses between soil chemical properties with spore density and colonization were carried out using Pearson's correlation.

RESULTS AND DISCUSSION

Soil properties

The chemical and physical properties of the soil are presented in Table 3, showing that the soil pH at the Lalingato location was the highest and significantly different from other locations. Lapao Pao Village had higher organic C and total N which were significantly different from other locations. The highest available P were shown in Abuki and Lapao Pao Villages where values were significantly different from that in other locations. In general, soil texture in the growing habitat of *K. celebica* is clay. At the Anggatoa and Lalingato locations, the sand fraction was dominant.

AMF colonization and spore density

Variations in *K. celebica* growing sites had a significant effect on spore density and no significant effect on AMF colonization (Figure 1). The highest number of spores per 100 g of soil sample was shown in Anggaloosi Village (447 spores). Organic C, total N and clay were positively correlated with spore density and negatively correlated with soil pH and sand fraction (Table 4 and Figure 2). Roots of *K. celebica* in six natural habitats in Southeast Sulawesi were colonized by AMF. The percentage range of AMF colonization was 20-49% (Figure 1). The commonly found structures of AMF were internal and external hyphae. AMF colonization was not affected by habitat differences. Soil properties such as organic C, total N and clay fraction were significantly having negative correlation with AMF colonization (Table 4).

Species diversity

Morphological characterization of AMF spores revealed a total of eight AMF species belonging to three genera Viz. *Glomus*, *Funneliformis* and *Rhizophagus* (Table 5). Five species belongs to genus *Glomus*, two species belongs to genus *Funneliformis* and one species belongs to genus *Rhizophagus* (Table 5). *Glomus* sp.1, and *Glomus* sp.2 had 35.5 percent species occurrence. The relative abundance of AMF species in the rhizosphere of *K. celebica* varied significantly. *Glomus* sp.1 was the most dominant species isolated from all the *K. celebica* followed by *Funneliformis* cf. *mosseae* (37.9 %) and *Glomus* sp. 2 (21.2). *Glomus* sp.1, *Funneliformis* cf. *mosseae* and *Glomus* sp. 2 had the highest Importance and Shannon-Wiener Diversity Indices. The richness and diversity of AMF species differed among locations (Figure 3). Anggatoa Village had the highest species richness although not significantly different from Abuki and Anggaloosi Villages. The species richness found was between 1-5 species per soil sample with an average of 3.6 species in Anggatoa Village and 1.33 species in Lalingato and Lapao Pao Villages (Figure 3).

Diversity indices

The Simpson's index of diversity (Ds) ranged from 0.42 ± 0.117 to 0.86 ± 0.068, Shannon-Wiener diversity index (Hs) ranged from 0.12 ± 0.208 to 0.99 ± 0.198, and Evenness index (J) ranged from 0.17 ± 0.189 to 0.75 ± 0.106 (Figure 3). The diversity index varied significantly except Simpson's index of diversity and evenness index in

the natural habitat of *K. celebica*. There was no significant difference observed in the species evenness and Simpson's index of diversity in the natural habitat of *K. celebica*. Anggatoa Village had the highest species diversity (H'-Shannon-Wiener index) (0.99) and was different from other locations (Figure 3). Lalingato Village had a low uniformity index but a high Simpson's dominance index (D). Lapao pao Village had a low uniformity index and dominance index.

Table 3. Soil chemical and physical properties at various *Kalappia celebica* locations in Southeast Sulawesi, Indonesia

Site	pH	C-organic (%)	N total (%)	P_2O_5 (ppm)	Texture		
					Sand (%)	Silt (%)	Clay (%)
Anggotoa	4.66±0.03 b	0.91±0.01 e	0.11±0.00 f	5.15±0.39 cd	52.50±0.85 a	32.90±0.10 c	14.60±0.90 c
Abuki	4.40±0.00 c	2.18±0.01 b	0.25±0.03 b	7.41±0.21 a	9.16±0.43 e	48.16±0.59 a	42.66±0.80 a
Lalonaha	4.33±0.03 cd	1.57±0.02 d	0.19±0.03 d	5.88±0.26 bc	28.73±0.72 c	42.83±0.34 b	28.43±1.04 b
Lapao-pao	3.80±0.00 e	2.37±0.04 a	0.28±0.00 a	6.48±0.35 ab	28.63±0.74 c	41.933±2.24 b	29.43±1.99 b
Lalingato	5.06±0.03 a	0.92±0.01 e	0.14±0.01 e	4.57±0.36 d	43.66±3.25 a	45.53±3.04 ab	10.80±2.48 c
Anggaloosi	4.30±0.00 d	1.95±0.01 c	0.24±0.00 c	6.24±0.21 b	21.50±0.75 d	35.06±1.30 c	43.43±1.70 a
Mean	4.43±0.09	1.65±0.14	0.20±0.02	5.59±0.25	30.70±3.47	41.07±1.43	28.23±3.07
Pr>f	<.0001	<.0001	<.0001	0.0004	<.0001	0.0002	<.0001

Table 4. Analysis of the correlation between environmental factors and plant symbiotic AMF on *Kalappia celebica*

Parameter	pH	C	N	P	Sand	Silt	Clay
Spore density	-0.33	0.46	0.51	0.28	-0.46	-0.14	0.59
	(0.11)	(0.21)*	(0.21)*	(0.07)	(0.21)*	(0.20)	(0.35)**
AMF colonization	0.41	-0.55	-0.60	-0.39	0.48	-0.029	-0.53
	(0.17)	(0.29)*	(0.36)**	(0.15)	(0.23)*	(0.001)	(0.28)*

Note: ** very significant at $P < 0.01$

Table 5. Spore density, relative frequency, relative density, importance and diversity indices of AMF species in the natural habitat of *Kalappia celebica*

AMF species	SD	FI	RA	IV	H	Location
Rhizophagus aggregatus	4	3.9	0.2	2.1	0.013	Anggaloosi
Funneliformis geosporus	10	5.9	0.6	3.3	0.032	Anggatoa
Glomus multicaulis	1	2.0	0.1	1.0	0.005	Anggaloosi
Funneliformis cf. *mosseae*	604	9,8	37.9	23.8	0.368	Lalonaha, Lalingato, Abuki
Glomus sp.1	631	35.3	39.6	37.4	0.367	Anggatoa, Abuki, Lalonaha, Anggaloosi, Lapao Pao
Glomus sp. 2	339	35.3	21.2	28.3	0.329	Anggatoa, Lalingato, Abuki, Anggaloosi
Glomus sp. 3	6	5.9	0.4	3.1	0.020	Anggatoa
Glomus sp. 4	1	2.0	0.1	1.0	0.005	Anggaloosi

Notes: SD: Spore density, FI: relative frequency, RA: Relative abundance, IV: Importance value, and H: diversity

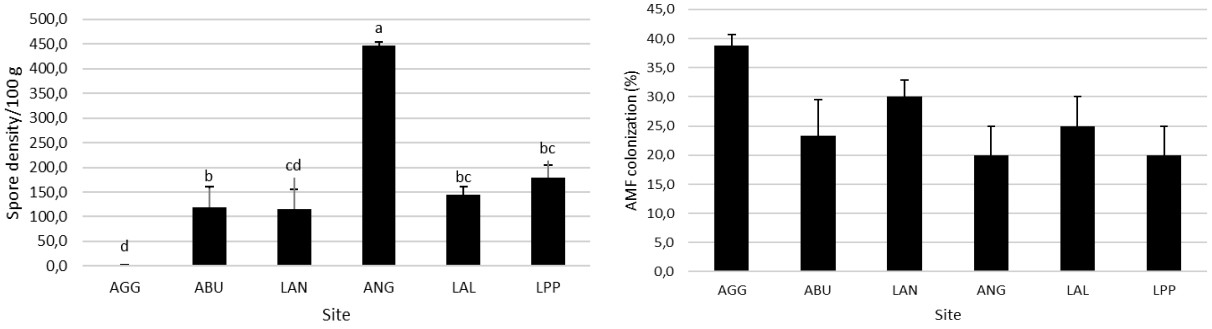

Figure 1. AMF colonization and spore density. Notes: AGG: Anggotoa, ABU: Abuki, LAN: Lalingato, ANG: Anggaloosi, LAL: Lalonaha, LPP: Lapao Pao

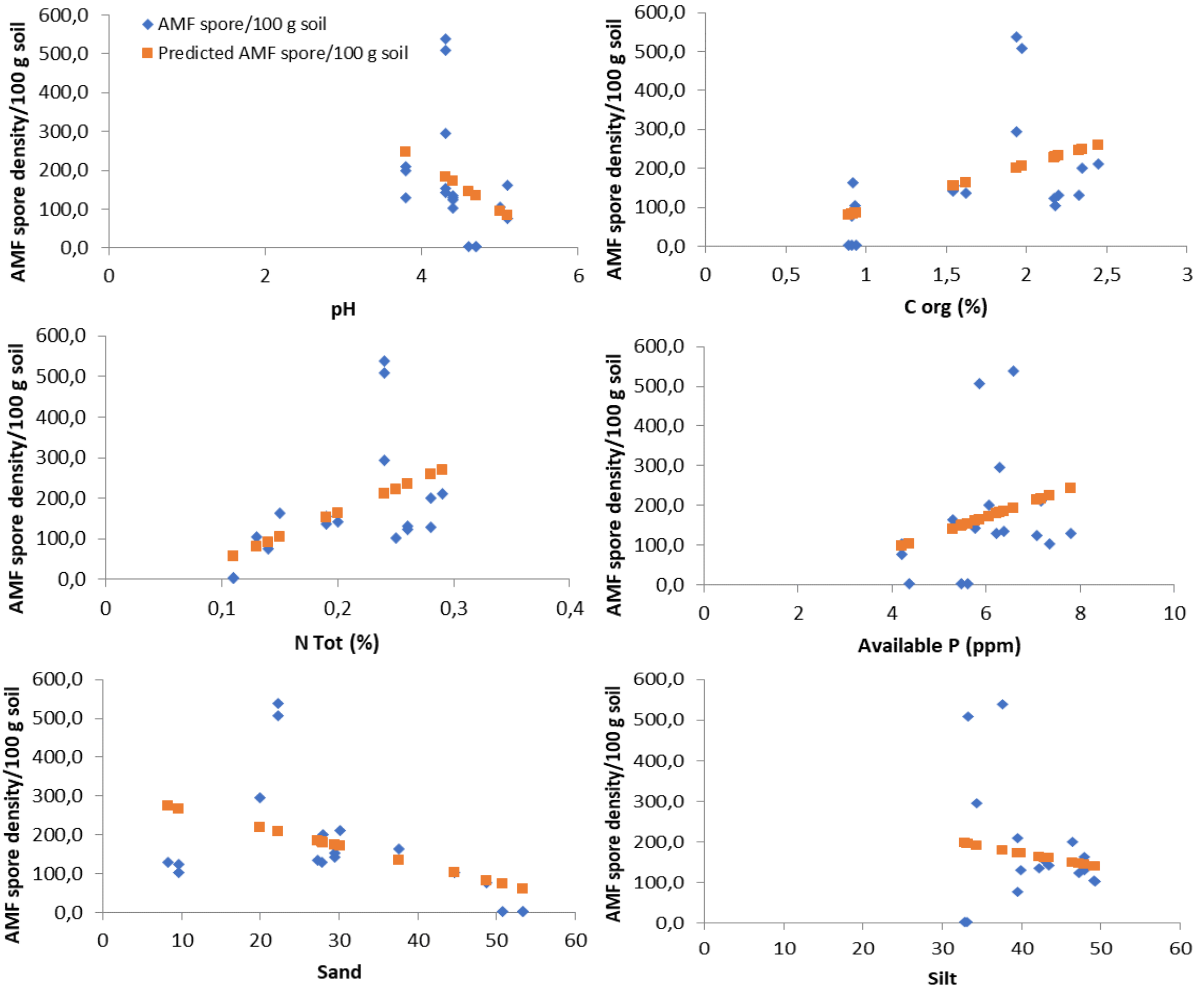

Figure 2. Relationship between soil properties and AMF spore density

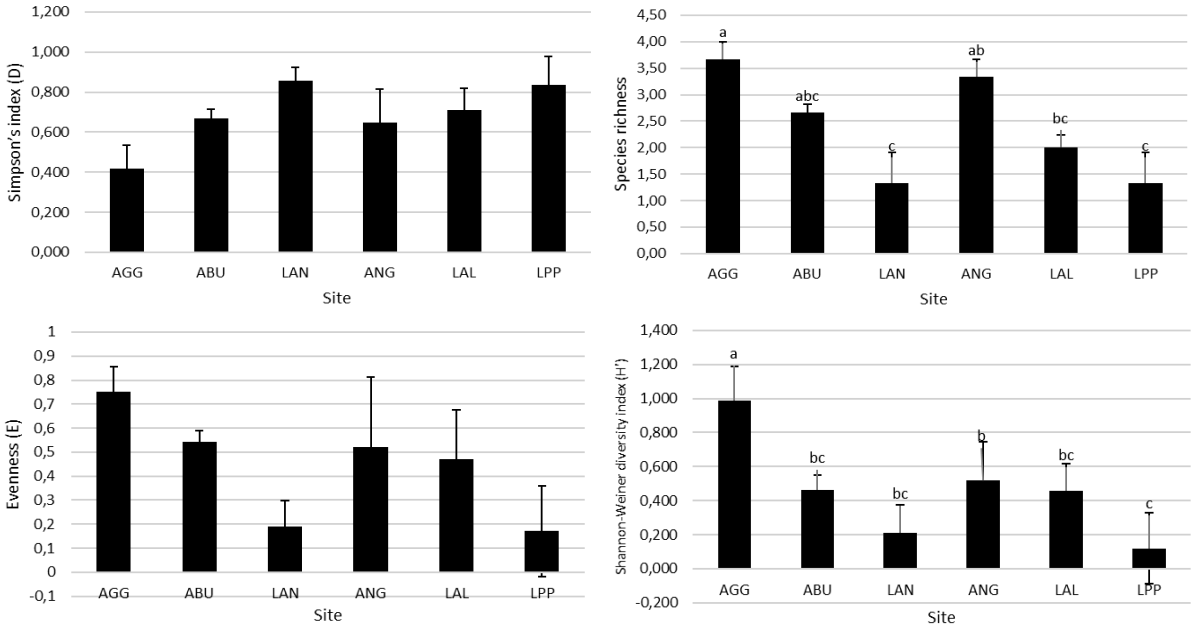

Figure 3. Diversity indices of AMF species in the natural habitat of *Kalappia celebica*, Southeast Sulawesi Province, Indonesia. Note: AGG: Anggotoa, ABU: Abuki, LAN: Lalingato, ANG: Anggaloosi, LAL: Lalonaha, LPP: Lapao Pao

Discussion

AMF is in symbiosis with *K. celebica* in its 6 natural habitat locations in Southeast Sulawesi Province. The average spore density ranged from 3-447 spores per 100 g of soil. The percentage of mycorrhizal colonization was low to moderate and ranged from 20 to 49%. In this study, AMF colonization was negatively correlated with organic C ($r = -0.55$, $p<0.05$), total N ($r = -0.60$, $p <0.01$) and clay fraction ($r = -0.53$, $p < 0.05$). However, the colonization was positively correlated with the sand fraction ($r = 0.48$, $p<0.05$). Soil pH and available P did not correlate with AMF colonization and spore density. The uncorrelated available P is in line with research done by Ruotsalainen et al. (2002), Becerra et al. (2007) and Songachan and Kayang (2012).

However, several studies have reported that the concentration of available P affects spore density (Mirzaei and Moradi 2017). High concentration of available P decreases the diversity of AMF (Husna et al. 2015; Abdedaiem et al. 2020). Chaudhary et al. (2018) identified the same biotic and abiotic factors driving AMF species composition in tropical and temperate regions. Several factors have been identified that may influence AMF distributions, including abiotic (e.g., soil physicochemical properties, latitude, climate) (Melo et al. 2019; Zhu et al. 2020) and biotic (e.g., host plant) factors (Songachan and Kayang 2012; Thanni et al. 2022), and intrinsic properties of species (e.g., dispersal ability). Organic C, total N and pH affect the diversity of AMF species (Luo et al. 2020). The relative contribution of environmental factors showed that factors such as elevation, relative air humidity, soil pH, and soil available P, K, and Mg influenced AMF spore production and root colonization of *Picconia azorica* (Melo et al. 2019). Organic C, N, P and pH were positively correlated with species richness (Zhu et al. 2020).

In our present investigation, *Glomus* sp. (Glomeraceae) was the most dominant AMF species. The dominance of *Glomeraceae* in the habitat of *K. celebica* has also been reported by many researchers. Endemic and endangered species such as *Artemisia umbelliformis* (Binet 2011), *Pericopsis mooniana* (Husna et al. 2015), *Amygdalus scoparia* (Mirzaei and Moradi 2017), *Ulmus chenmoui* (Song et al. 2018), *Coccothrinax crinita* (Furrazola et al. 2020), *Ferula sinkiangensis* (Luo et al. 2020), *Carissa edulis* (Ogoma et al. 2021), and several other species are threatened with extinction in the Himalayas (Jishtu et al. 2019). *Glomus* is also dominant on disturbed land such as ex-mining tailing (Suting and Devi 2021), semi-arid and arid (Abdedaiem et al. 2020). According to Strumer et al. (2018), the Glomeraceae family has a very wide distribution, found in 4 climatic zones, 7 continents, 17 biomes and many countries. In addition, *Glomus* is a genus with a large number of AMF species (Schüßler and Walker 2010).

AMF species with Importance Index of more than 20 in the 6 habitats of *K. celebica* were *Funneliformis mosseae*, *Glomus* sp.1 and *Glomus* sp. These three species are thought to have adaptations to various habitat conditions. Mycorrhizae consist of a number of complex species and have tolerance and adaptation to various environmental conditions and habitats. The dominance of the two Glomus species is thought to be due to several factors, including 1) have small spore sizes, 2) have the ability to sporulate (spore production) in various environmental conditions, 3) have the ability to adapt to various soil and climatic conditions and 4) have the ability to produce spores. inoculum (propagules) in nail wood rhizosphere (Kivlin et al. 2011; Shukla et al. 2013; Husna et al. 2015). *F. mosseae* has been observed in various geographic locations under very different environmental conditions (Chaudhary et al. 2008). *F. mosseae* is also found predominantly in limestone mine spoils (Suting and Devi 2021), on several endangered plant species in the Himalayas (Jishtu et al. 2019). Anggatoa Village had the highest AMF species richness (3.67 species) compared to other locations except for the villages of Abuki and Anggaloosi. The highest AMF diversity (H') was found in Anggatoa Village ($H' = 0.99$) (Fiqure 3). The value of H' in this study was lower than that of *Solanum* sp. (Songachan and Kayang 2012). Simpson's dominance index showed no differences among the six habitats of *K. celebica*, with a range of 0.42 - 0.86. The lower index of dominance for AMF in Anggatoa Village as compared to Lalingato and Lapao Pao villages indicated higher number of shared dominances of AMF species. The value of E was higher in Anggatoa Village. The value of E tended to be the same for the villages of Abuki (0.54) and Anggaloosi (0.52) and Lalingato (0.19) and Lapao pao (0.17). The same value of E indicates that the distribution of AMF types is uniform at both locations. The AnggatoaVillage (Konawe District) had high species richness. The Shannon-Wiener, Evenness and Simpson indices in this village were high, except for the variable number of spores. The high Diversity Index in Anggatoa Village was forgotten due to soil conditions, climate, and support for the distribution and population of AMF spores. In general, the distribution of AMF is strongly influenced by many environmental factors, including soil texture, land degradation, humidity and temperature as well as nutrient availability (Kivlin et al. 2011). The number of AMF species per host species at a given location may differ between regions and habitat types (Stürmer et al. 2018; Husna et al. 2015). Species richness and AMF diversity may be related to host type, life cycle and site-specific conditions (Öpik et al. 2006).

In conclusion, this study provides information on the status of AMF colonization and diversity of *K. celebica* species. Mycorrhizal status and selection of appropriate microbial strains to inoculate plants could be of particular value to improve the quality and quantity of plant material to conservation program.

ACKNOWLEDGEMENTS

The authors wish to thank the Directorate General of Higher education, Research and Technology, Ministry of Education, Culture, Research and Technology of the Republic of Indonesia and general director of LPDP for funding support via Program Riset Keilmuan Tahun 2021 (No. 025/E4.1/AK.04.RA/2021).

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