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Diversity of Arbuscular Mycorrhizal Fungi in the Rhizosphere of *Xanthostemon petiolatus* (Valeton) Peter G. Wilson at the Halu Oleo University Botanical Garden, Kendari

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ABSTRACT. Arbuscular mycorrhizal fungi (AMF) are one type of mycorrhizal association with plant roots. AMF have a fungal structure in the form of arbuscules, vesicles, and hyphae, which are found in roots and spores in the soil. The research aimed to determine the diversity of arbuscular mycorrhizal fungi on the rhizosphere of *Xanthostemon petiolatus* in the Botanical Gardens of Universitas Halu Oleo (UHO), Kendari. Studies that have been conducted show that Nona wood has a symbiotic relationship with AMF. The soil and plant root sampling method was carried out at the Botanical Gardens UHO Kendari, randomly (non-proportionally) based on the distribution of plants. Isolated plant roots and identification of AMF were carried out at the Plastic House of the Indonesian Mycorrhiza Association (AMI) Kendari Branch, Southeast Sulawesi Province, which took place during May-June 2024. The results of the research showed that *X. petiolatus* roots were colonized by AMF, as evidenced by the discovery of internal hyphae and external hyphae. A total of 6 species of AMF from the genus Glomus (5 species) and the genus Acaulospora (1 species) were found. The dominant type is Glomus. Diversity is included in the low category (H ' = 0.235). This type of AMF has the potential to be developed as a biofertilizer.

Keywords : *Glomus,* Secondary forest, Myrtaceae, Wallaceae

INTRODUCTION

Botanical Garden of Universitas Halu Oleo (BG UHO) is the first botanical garden in Indonesia managed by a university with an area of 22.8 hectares (Rahayu et al., 2018). The main function of the botanical garden is as a conservation, research, education, tourism, and environmental service area (Purnomo et al., 2010). Most of the BG UHO area is a secondary forest that has a variety of vegetation. Kandari et al. (2020) reported that there are 112 species in the BG UHO, and one of the tree species is nona wood.

Nona wood (*Xanthostemon petiolatus*) is spread in Sulawesi, Maluku to Papua New Guinea (Whitmore et al., 1989). One of its characteristics is the gray or blackish wood color with dark black lines and a smooth texture and intersecting grain direction. The wood is very hard, so it is often used for building wood, floors, sleepers, shipbuilding wood, telephone poles, and bridges. This tree can reach a height of 40 meters (Erwin et al., 2020). The existence and growth of *X. petiolatus* in the BG UHO is thought to be supported by the presence of arbuscular mycorrhizal fungi.

Arbuscular mycorrhizal fungi (AMF) are one type of mycorrhizal association with plant roots (Smith and Read, 2008). AMF have a mutualistic symbiotic relationship between fungi and the roots of higher plants (Husna et al., 2018). AMF have a fungal structure in the form of arbuscules, vesicles, and hyphae found in roots and spores in the soil (Smith and Read, 2008). In Indonesia, 26 AMF species have been reported, consisting of 4 families, namely Acaulosporaceae, Endogonaceae, Gigasporaceae, and Glomeraceae (Husna et al., 2021). The most commonly found type of AMF is Glomus sp. AMF is known to be symbiotic with several

types of local plants in Southeast Sulawesi, including *Pericopsis mooniana* (Thw. Thw) (Husna et al., 2014), *Pterocarpus indicus* Willd (Husna et al., 2018), and *Kalappia celebica* (Husna et al., 2022).

A previous study by Puspita (2018) showed that X. petiolatus plants are symbiotic with AMF in the post-nickel mining land of PT Wijaya Inti Nusantara in South Konawe Regency. The most common AMF structure found is internal hyphae. 8 types of AMF spores were found, consisting of 3 genera, namely Gigaspora (1 type), Acaulospora (2 types), and Glomus (5 types). However, information related to AMF symbiosis in *X. petiolatus* in the UHO Botanical Garden is not yet known. The diversity and species of AMF populations are greatly determined by differences in location, rhizosphere, and environment (Hartoyo et al., 2011). Therefore, this study was conducted in order to reveal the types of AMF that are symbiotic with nona wood in the UHO Botanical Garden.

MATERIALS AND METHODS

Soil and Root Sampling in the Field

Soil and root samples were taken randomly (Nusantara et al., 2012). Soil samples were taken from 12 *X.petiolatus* trees at the Botanical Gardens UHO Kendari at a soil depth of approximately 30 cm. Soil samples were taken from four points in the cardinal directions, each weighing 250 grams, for a total of 1 kg of soil per tree. The soil samples were placed in plastic bags and labeled. (Husna, 2015).

Root Colonization Observation

The root colonization procedure used the basic method developed by Brundrett et al. (1996) with several modifications. Fine roots from the nona tree roots were selected, then washed thoroughly, and placed in a 10% KOH solution for 1 hour at 90°C. The KOH solution was then discarded, and the root samples were washed with running water until clean. The roots were then immersed in a 2% HCl solution for 30 minutes. The HCl solution was then discarded. The root samples were then immersed in a staining solution (trypan blue 0.05% glycerol 70% + 30% distilled water) for 24 hours. After that, the staining solution was discarded, and the roots were placed in a 50% glycerol solution. After the staining was complete, the root samples could be observed. Root observation was carried out by cutting 10 roots approximately 1 cm long, then arranging the roots on the preparation, pressing them, and covering them with a cover glass. Once the preparation was ready, they were observed under a microscope.

Isolation and Identification of AMF from soil

The AMF spore isolation technique uses the pour-filter technique from Pacioni (1992) and is continued with the centrifugation technique from Brundrett et al. (1996). Identification of AMF spores is carried out by observing the morphology (shape, size, color, hyphae, carrier, spore ornamentation, spore mother cells, and 'bulbous suspensor ') (Schüßler and Walker, 2010).

Research Variables

The research variables for AMF diversity at the Botanical Gardens UHO Kendari are presented in Table 1.

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Table 1. Research Variable

Research Variable	Formula
Relative Frequency (RF)	[number of soil samples where the type or genus of AMF was found/total samples] x 100% *
Relative Abundance (RA)	Percentage of the number of spores of a type or genus
Important Values (IV)	(RF + RA)/2. NP ≥ 20, including the dominant type or genus
Spore density	Number of spores per 100 g of soil
Species richness	Number of types of each soil sample
Diversity index	Shannon-Weiner indeks -H' = -Σpi In Pi* H' < 1: Low diversity 1< H' < 3: Medium diversity,H'> High uniformity
Uniformity index	Evenness-E' = H'/H' maxE' < 0.4 : Low uniformity, 0.4 < E' < 0.6 : Medium uniformity E' > 0.6 High uniformity
Dominance index	Simpon's -D = H' = -Σ[ni(ni-1)/N(N-1)]*0.00 < D ≤ 0.05 : Low dominance, 0.50 < D ≤ 0.75 : Medium dominance, 0.75 < D ≤ 1.00 : High dominance
AMF colonization	[Σ mycorrhizal field of view / Σ total observed field of view] x 100%**

Description: Pi = ni /N, where ni is the number of spores per type and N is the total number of spores identified. H 'max = In S is the total number of types identified (Husna, 2015).

Data Analysis

All data were analyzed using descriptive analysis of all observed variables. The results are presented in tables and figures.

RESULTS AND DISCUSSION

AMF colonization

The average AMF colonization was 8.06%, with the AMF structures found being internal hyphae (81.20%) and external hyphae (18.80%).

Jenis FMA

Based on observations of the roots of *X.petiolatus* at the Botanical Gardens UHO Kendari, five types of AMF from the genus Glomus and one type from the genus Acaulospora were identified. A description of the AMF characteristics is presented in Table 2.

Table 2. Characteristics of AMF that are symbiotic with *X.petiolatus* in the Botanical Gardens UHO Kendari

AMF species	General Characteristics				
	Arrangement	Colour	Shape	Size (µm)	Trees
Glomus sp1.	Single	Brown	Globose	67.72	1-12
Glomus sp2.	Single	Black	Globose	122.23	1-12
Glomus sp. 3.	Single	Reddish brown	Oval	66.11	2,7,9
Glomus sp. 4.	Single	Yellow-white	Oval	57.72	3,4
Glomus sp. 5.	Single	Yellow	Globose	38.70	3,4
Acaulospora sp	Single	Brown	Oval	145.45	2-12

Spore count data per 100 g of soil are presented in Table 3. Table 3 shows that Glomus sp. 1 had the highest spore count, at 21.14 spores/100 g of soil, while Glomus sp. 4 had the lowest spore count, at 1.0 spore/100 g of soil.

AMF ecological parameters, including relative frequency (RF), relative abundance (RA), and importance value (IV) at the Botanical Gardens UHO Kendari, are presented in Table 3. Table 3 shows that *Glomus* sp. 1 had the highest RF, RA, and IV, at 28.57%, 45.32%, and 36.94%, respectively. Meanwhile, *Glomus* sp. 4 had the lowest RF, RA, and IV, at 4.76%, 2.14%, and 3.45%, respectively.

The AMF diversity components, which include the diversity index (H'), uniformity index (E'), and dominance index (D'), at the Botanical Garden UHO Kendari are presented in Table 3. Table 3 shows that the diversity index has a total value of 0.235, the uniformity index has a total value of 0.131, and the dominance index is 0.042.

Table 3 Ecological Parameters of AMF in Botanical Gardens UHO Kendari

Species	Spore number/100 g	RF (%)	RA (%)	IF (%)	H'	E'	D'
Glomus sp.1	21,14	28,57	45,32	36,94	0,359	0,200	0,138
Glomus sp.2	13,00	28,57	27,87	28,22	0,356	0,199	0,085
Glomus sp.3	3,33	7,14	7,14	7,14	0,188	0,105	0,004
Glomus sp.4	1,00	4,76	2,14	3,45	0,082	0,046	0,000
Glomus sp.5	5,00	4,76	10,72	7,74	0,239	0,134	0,003
Acaulospora sp	3,18	26,19	6,82	16,50	0,183	0,102	0,019
Total	7,78	100,0	100,0	100,0	1,4	0,8	0,3

Description: RF=Relative frequency, RA=Relative abundance and IV=Importance value, H' = Diversity index, E' = Evenness index and D' = Dominance index

Observations indicate that the roots of nona wood are colonized by Arbuscular Mycorrhizal Fungi (AMF). Colonization is characterized by the presence of AMF structures, including internal hyphae (81.20%) and external hyphae (18.80%). This finding aligns with research conducted by Puspita (2018) that showed AMF colonized nona wood plants in the Post-Nickel Mining Area of South Konawe Regency. The AMF structure commonly found in the Botanical Gardens UHO Kendari is internal hyphae (81.20%). According to Souza (2015), internal hyphae originate from cellular symbiosis and have limited growth. Internal hyphae function as a means of translocating nutrients from fungi to plants and vice versa.

The results of this study indicate that the average colonization rate in nona wood roots is 8.06%, which is considered low. This is in accordance with the root colonization percentage according to O'Connor et al. (2001), where if the colonization value is <10%, then the category is low. The low percentage of root colonization is thought to be caused by soil fertility, resulting in vegetation being unable to grow optimally and impacting low root exudates in the form of carbohydrates, which are essential for spores to grow and develop (Wisnubroto et al., 2023).

The research results found 5 types of AMF from the genus Glomus and 1 type from the genus Acaulospora (Table 2), while the results of research conducted by (Puspita, 2018) were more numerous, namely the discovery of three genera of AMF spores symbiotically in nona wood plants in the Post-Nickel Mining Land of South Konawe Regency, namely Glomus (5 types), Acaulospora (2 types), and Gigaspora (1 type). Differences in the number of spores in the rhizosphere of a plant are likely influenced by soil acidity, high soil temperature, and the amount of vegetation. Glomus is the most commonly found AMF genus. This is in accordance with research conducted by Husna et al. (2015) showing that Glomus is the dominant genus found. Husna (2015) stated that the dominance of Glomus is related to several factors, including Glomus having small spores and the ability to sporulate (produce spores) in various environmental conditions. This is suspected because Glomus has the ability to produce inoculum

in the rhizosphere of various types of plants. The components of the ecological approach used in this study are Relative Frequency (RF), Relative Abundance (RA), and Importance Value (IV). The highest importance value is possessed by the *Glomus* sp.1 (36.94%) and *Glomus* sp.2 (28.22%) species, which are included in the dominant category. This is in accordance with the importance value criteria according to Husna (2015), where if the NP value is >20, then the species is the dominant species. This species has the highest importance value because it is influenced by relative frequency and relative abundance, which have the highest value because *Glomus* sp.1 and *Glomus* sp.2 have a wider ability to adapt to various extreme environmental conditions (Yanti et al., 2023).

The AMF diversity components in this study were the diversity index (H'), the uniformity index (E'), and the dominance index (D'). The results showed that the diversity index (H') was low with a total value of 0.235, the uniformity index (E') was low with a total value of 0.131, and the dominance index (D') was low with a value of 0.042. The high and low levels of the diversity index, uniformity index, and dominance index are not influenced by a single factor, but are influenced by the mycorrhizae themselves, host plant varieties, and environmental conditions (Alayya and Prasetya, 2022)

CONCLUSION

This study concludes that the roots of nona wood in the UHO Kendari Botanical Gardens are colonized by AMF, with 6 types of AMF found from the genus Glomus (5 species) and Acaulospora (1 species). The dominant genus is Glomus, and AMF diversity is in the low category.

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