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Submission date: 24-Sep-2020 08:38PM (UTC+0700)

Submission ID: 1395756213

File name: 30._AKIB2121-222020PCBMB7490.pdf (213.91K)

Word count: 3418

Character count: 19150

Plant Cell Biotechnology and Molecular Biology 21(21&22):123-129; 2020 ISSN: 0972-2025

INDIGENOUS ARBUSCULAR MYCORRHIZAL FROM JOMPIE BOTANICAL GARDEN OF PAREPARE WHICH ISOLATED AT *Pterospermum diversifolium*

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Article Information

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Reviewers:
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Received: 24 May 2020
Accepted: 01 August 2020
Published: 19 August 2020

Original Research Article

ABSTRACT

Jompie Botanical Garden of Parepare is a regional botanical garden managed by the Center for Plant Conservation of the Botanic Gardens-Indonesian Institute of Sciences, the Ministry of Public Works and Public Housing, and the Government of the City of Parepare, South Sulawesi Province. Jompie Botanical Garden of Parepare has implemented five botanical garden functions, namely conservation, research, education, tourism, and environmental services. The master plan vegetation shows that found 90 species of plants that grow naturally and are planted by the government and the people of the city of Parepare, one of the native plants found are *Pterospermuni diversivolium*. In addition to various types of plants, there are also various kinds of microorganisms that have not been identified, one of which is a fungus that is capable of symbiosis with plant roots known as arbuscular mycorrhizae. The aim of the research was to determine the abundance and identify arbuscular mycorrhizal spores found in *Pterospermuni diversivolium* rhizosphere, using a sieve and wet techniques and staining method, which were carried out in Microbiology Laboratory, Research, and Development Center for Environment and Forestry in Makassar, Indonesia. The results showed that abundance of Glomus mycorrhizal spores, on average, 25 spores per 100 g of rhizosphere samples with morphological shapes of small spores, colored of brown to black, thin cell walls, visible hollow interior, have hyphae, smooth surface and spore diameter 136.8 - 198.5 µm. While the genus Acaulospora, has an average abundance of 21 spores per 100 g of rhizorfer samples with morphological forms of the small clear round, clear-colored, thick cell walls, surfaces appear nodules and spore diameters of 143 - 159 µm.

Keywords: Jompie botanical; indigenous species; arbuscular; *Pterospermum diversifolium*.

INTRODUCTION

Jompie Botanical Garden of Parepare has an area of 13.5 ha and constitutes the part of the Alitta forest. The term "Alitta" taken from the name of the one Parepare heroes that is Andi Pangeran Pettarani. While the name of "Jompie" quoted from the ancient bugines language, which means water comes out from land naturally or called springs. This spring greatly contributes to fulfilling the clean water needs in Parepare city [1,2].

Pterospermuni diversivolium (*P. diversivolium*) is one of the native trees of Sulawesi that grows naturally in the Jompie Botanical Garden of Parepare. *Pterospermum* sp. or redwood, often used as material for mixing palm sugar by the Sulawesi community [2]. Research to determine the pharmacognostic of *Pterospermum* sp and the screening of chemical components by chromatography reported that the leaves, bark, and stems of *Pterospermum* sp contain tannin, catechin, phenol and steroid compounds [3].

Spores are one of the self-propagation tools of some microorganisms, one of which is arbuscular mycorrhizal, which is formed from extraradical hyphae that have stumps or colony (sporocarps) [4]. Spores contain polysaccharides, lipids, proteins, and chitin [5]. Besides that, spores also have organs such as mitochondria, endoplasmic reticulum, and vacuoles [6]. In the process of propagation, spores first experience germination to produce hyphae [7]. Hyphae formed from spore germination. This is what plays a role in absorbing nutrients and water from the outside into the roots and then used in the process of growth and development of host plants [8].

An arbuscular mycorrhiza is a group of fungi that live in the soil, which belongs to the group endomikoriza [9], which has a hyphal structure called arbuscular. Arbuscular acts as a place of contact and transfer of mineral nutrients between fungi and host plants in the root cortex tissue [10].

Arbuscular mycorrhizae associated with plant roots can act as biological agents [11], there are five benefits of mycorrhiza for host plant growth, namely: improving soil health [12], increasing absorption of nutrients from the soil [13], as a biological barrier to infection of root pathogens [14], increases host resistance to drought [15], increases growth-promoting hormones [16], and ensures implementation of the biogeochemical cycle [4]. Therefore, it is necessary to conduct a study to identify the type and number of indigenous arbuscular mycorrhizal spores on the rhizosphere of *P. diversivolium* that can be used as biological agents for preserving the Jompie Botanical Garden of Parepare.

METHODOLOGY

A descriptive study consisting of three phases was conducted in the cities of Parepare and Makassar, South Sulawesi, Indonesia, in five months: *P. diversifolium* rhizosphere sampling in the Jompie Botanical Garden of Parepare using the diagonal method as the first stage. Rhizozfer samples were entered into samples envelope to be sent to the Microbiology Laboratory, Research, and Development Center for Environment and Forestry in Makassar, Indonesia.

The second stage, the filtering of arbuscular mycorrhizal spores using method modification of the wet sieve and glucose centrifugation [17]. Stages of the wet sieving method, i.e. (1) mix 100 g of rhizosphere soil sample and 100 ml of water, then stirred evenly for eight minutes and allowed to stand for 4 four minutes so that large particles settle. (2) Pour the solution into a stratified sieve (consecutive 40 µm, 50 µm, and 125 µm) from top to bottom. (3) Flush using flowing water to speed up the filtering process. (4) Filtering results are centrifuged for five minutes with a speed of 2 500 rpm to separate the spores with dirt. (5) Results of first centrifugation, added 500 ml glucose then centrifuged at speed 1200 rpm for two minutes with the intention of binding spores. (6) Results of second centrifugation poured on a 125 mm sieve and doused with running water to separate spores with glucose. (7) Results of filtering poured to a petri dish for a counted number of spores based on morphotypes using a light microscope. Mycorrhiza arbuscular spore density of calculated based on a formula [18]:

$$Spore\ density = (Number\ of\ spores) / (Weight\ rhizosphere\ soil\ sample)$$

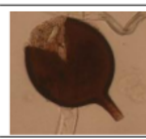
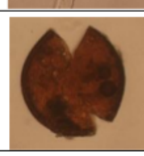
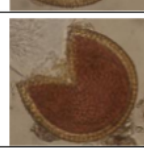
The third step, morphotype and morphology identification using the method of staining [19] with Polyvinyl alcohol-lacto-glycerol (PVLG) solution and Melzer's reagent. Spore identification based on the similarity of spore morphological characteristics, including color and shape of spores. Color of spores determined using a color chart to INVAM website (http://www. invam.caf.wvu.edu). Colors of spore consist of hyaline yellow, greenish-yellow, brown, reddish-brown to dark brown, while the form of spores consists of globes, subglobose, ovals, and oblong. Identification of spores using an electron microscope.

RESULTS AND DISCUSSION

Results of isolation and identification of arbuscular mycorrhizal spores (MA) in *P. diversifolium* soil rhizosphere taken from Jompie Botanical Garden of Parepare based on morphotype and morphology of spore showed that fourth morphotypes MA spores wich identification have different diameters and spore abundances and including in genus Glomus and Acaulospora (Table 1).

Morphological identification result at mycorrhizal spore of genus Glomus showed that spore diameter reached 136.8-198.5 µm, whereas according to the INVAM website (http://www. invam.caf.wvu.edu) the spore size for Acaulospora sp could be 15-167.5 µm in diameter. Spores of Glomus have the characteristics of round and oval, yellowish black, reddish-yellow, brownish yellow, yellowish-brown, light brown, dark brownish-black, and Black. The spore wall surface appears smooth and has a thin spore wall. Glomus spores found have hyphae. Hyphae on spores are found directly fused with the spore wall with a color that is almost the same as the spore wall [20]. The process of Glomus spore development starts from the tip of the hypha, which enlarges to the maximum size and formed spores [21]. Because the spores come from the development of hyphae, they are called chlamydospores, sometimes hyphae are branched, and each branch forms chlamydospores and forms

Table 1. Characteristics and Abundance of MA Spores Isolated from Rhizosphere Soil *of P. diversifolium*

No	Genus	Morphotypes	Picture	Morphology		Diameter (µm)	Density (spora.100g^{-1})
				PVLG	MELTZER		
1	Glomus sp1	Round, Black, Small.		Small round shape, brown color, thin cell wall, visible hollow inside, and have a smooth surface.	Not react to Meltzer solution	136.8	40
2	Glomus sp2	Round, Chocolate, Small.		Small round shape, colored of Black to brown colored, thin cell walls, have hyphae, appear to be surrounded by fluid.	Not react to Meltzer solution	198.5	11
3	Acaulospora sp1	Round, Clear, Small.		Small clear round shape, the color of clear, thick cell walls, and liquid comes out when broken down	React to Meltzer solution	159	26
4	Acaulospora sp2	Round, Yellow, Small.		Small round shape, the color of yellow to brown, thick cell walls, the surface looks like a pimple.	React to Meltzer solution	144.3	17

sporocarp [22]. As adults mature the spores are separated from the hyphae, spore walls consisting of more than one layer and spore shaped of globos, sub globos, ovoid or obovoid [9,23], According to Peraza [24], the genus spores of Glomus can be found in the form of single or loose aggregates, sporocarp unlike in Sclerocystis, and sporocarp consists of spores with lateral walls that are attached to each other, Glomus spores can be produced singly or in groups forming aggregates [8].

The morphological identification results of the Acaulospora genus indicate that the spore size found at rhisozfer of P. diversifolium has a diameter of 144-159 µm. Whereas according to the INVAM website (http://www. invam.caf.wvu.edu), the spore size for Acaulospora sp can reach an average diameter of 74-289 µm. The process of development of Acaulospora spores starts from the tip of the hypha (subtending hyphae) that enlarges like a spore called hyphal terminus. Between hyphal terminus and subtending hyphae, a small sphere will appear, which is getting bigger, and spores will form. In its development, the hyphae terminus will be damaged, and its contents will enter the spores. Damage to the terminus hyphae will leave the former small holes called Cycatric [25,26]. According to Guzman [20], Acaulospora spores are single spores in the sporocarp. Spores attached laterally to the hyphae whose ends are bulging with a size almost the same as the spores, spore forms globos, subglobose, ellipse or fusiform widened. Acaulospora spores were found to have an oval shape, have relatively thick spore walls, and reddish-orange colored [27].

The morphotype, morphological, and sprora diameter differences are thought to be strongly influenced by environmental factors. Jompie Botanical Garden of Parepare, located at an altitude of 5 - 55 m asl at coordinates 3°59'51.168 S, and 119°38'24.336 E. According to Adlyansah et al. [28] the average rainfall range is 150.08 mm^3 per month, the total rainy day is 114 days, the daily average air temperature around 28.53°C and soil pH 6-7.

The development of arbuscular mycorrhizal fungal spores is strongly influenced by soil temperature and pH [29]. Glomus, usually in alkaline soils, can germinate well at pH 6 to 7 [30]. Whereas, Kumar [31] states that spore density, diversity, and arbuscular mycorrhizal infections negatively correlated with soil pH. The degree of acidity directly influences the enzyme activity that plays a role in spore germination [32]. The optimum acidity (pH) for spore germination does not only depend on fungal species but nutrient content in the soil. There is a tendency to increase the number of spores by reducing the amount of rainfall, fluctuations in soil moisture can also affect the formation of spores or sporulation, although it is not yet concluded that dry conditions will always produce more spores [33]. Drought does not inhibit mycorrhizal growth but increases lateral root development, and after re-wetting, the rate of root elongation and the number of mycorrhizae increase rapidly. Zhou [34] added that in the dry season, VAM is active for sporulating to form spores, whereas, in the rainy season, conditions occur otherwise. The number of spores found in P. diversifolium stands is presented in Table 1.

According to Tahat [33] and Holste [35], the number of spores is not only influenced by one factor but is influenced by the accumulation of several factors, including mycorrhiza itself, host plant varieties and environmental conditions, such as light and temperature, because sunlight plays a role in the formation of carbohydrates through carbon assimilation then VAM will use the carbon as an energy source for its growth. Hasid et al. [36] states that it is uncertain that plants with a high percentage of root infections will produce a high number of spores in the rhizosphere or vice versa.

CONCLUSION

Morphotype, morphology, diameter, and abundance of Acaulospora and Glomus mycorrhizal spores are found in shapes and sizes of spores which different. The genus Glomus and Acaulospora are indigenous microorganisms owned by Jompie Botanical Garden of Parepare, which need to be exposed and introduced to the community, to be used as environmentally friendly biological agents in the development of Jompie Botanical Gardens of Parepare.

ACKNOWLEDGEMENTS

The author thanks the Republic of Indonesia's Ministry of Research, Technology, and Higher Education for providing support through a multi-year basic research competition (2019-2021) and to Dr. Muhammad Akhsan Akib and the team, which involved us in basic research as a final project at the undergraduate level.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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