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# MICROSCOPIC IDENTIFICATION OF VESICULAR ARBUSCULAR MYCORRHIZAE (VAM) ASSOCIATED WITH ORCHID *Cymbidium* sp.

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## ABSTRACT

*Cymbidium* sp. is one of the most popular orchid desired in public, it means high productivity is needed in its fulfillment. One of the obstacle of *Cymbidium* sp. orchid in order to fullfilment of productivities are the stress and orchid's seed size which is very small. Vesicular Arbuscular Mycorrhizae is a symbiont fungus that can help absorption of nutrients in stress phase. VAM help *Cymbidium* sp. to maintenance and increase the productivity. This research aimed to determined VAM species and colonization in rhizospher of *Cymbidium* sp. orchid. This research has been conducted from November 2020 untill April 2021. Spore isolation was conducted by using wet shievings method, meanwhile VAM colonization was conducted by using roots staining techniques. The result showed there were 4 genus consisted by 6 different type of spores which has been found. There was *Glomus* (2 types), *Dentiscutata* (2 types), *Acaulospora* (1 type), and *Gigaspora* (1 type). Colonization of VAM in sample plant has been known by presence of VAM special structure, there were vesicular and internal hyphae.

*Keywords : colonization, Cymbidium* sp*, spore, Vesicular Arbuscular Mycorrhizae*

## INTRODUCTION

Orchid (Orchidaceae) are plants that have high diversity and are popular among the public. Based on data from Badan Pusat Statistik ("Outlook Komoditas Pertanian Subsektor Hortikultura Anggrek," 2015) orchid production have inclined to ±16,53 million unit per year. This fact is evidence that market demand for orchid is directly proportional to fulfillment. Therefore, it is important to maintain the stability and maximality of orchid production. One of the obstacle factors to maintain and maximize orchid production is at germination process, orchid's seed does not have endosperm which makes natural germination mechanism hampered. According to Andersen and Rasmussen (1995), the existence of mycelium mycorrhizae that are compatible can help the process of initiation and inclined orchid germination significantly. The capability of orchid's root that is limited in absorbing nutrition in soil that is not available for the plant also make orchid productivity tend to be slow or

limited. According to Setiawati (2008), orchid need mycorrhizae infection to fulfill their life cycle. Versicular Arbuscular Mycorrhizae (VAM) have a role in organic and inorganic nutrition such as phosphor, carbon, water, and nitrogen provision can increase the efficiency of groundwater usage by plants so that it can help to increase the vegetative growth rate of orchid plant (Miransari, 2014).

Research about VAM is reported to have been done in several plant's rhizospheres such as cocoa, sweet potato, cassava, and cogon grass with a diversity of VAM including the genera Gigaspora, Acoulospora, and Glomus. However, there is no one has reported any research on Vesicular Arbuscular Mychorrizal (VAM) associated with *Cymbidium* sp. *Cymbidium* sp. that have been found in Indonesia has a diversity of approximately 60 species spread from Sumatra to Sulawesi, with the level of species diversity is directly proportional to the diversity of types of VAM. Based on the description above, It's necessary to conduct research on Microscopic Identification of Vesicular Arbuscular Mycorrhizae (VAM) Associated with Orchid *Cymbidium* sp.

## MATERIALS AND METHODS

This research was conducted from November 2020-April 2021. Plant sample was collected from perumahan Karanglo Indah Blok FF No. 12-14 Singosari Subdistrict, Malang Regency. The research sample identification was conducted in the Laboratory of Genetic Resource and Molecular Biology of Udayana University.

This research used soil samples were taken from the rhizosphere of *Cymbidium* sp. The procedure of taking soil samples used diagonal point sampling method. Isolation and identification of Mycchorizae fungi used wet sieving method (Gerdemann & Nicolson, 1963) with modification. Soils were taken from rhizosphere of *Cymbidium* sp. weighed for 100 g, and put into 1000 ml beaker glass, and then pour 1 liter of water. Afterwards, stir until homogenous for ±2 minutes, and soil aggregates are broken by hand makes the spores are free from the soil. Leave it for ± 5 minutes. Afterwards, the supernatant liquids were poured into a multilevel sieve: 650 μm, 425 μm, 250 μm, 0,065 mm, 60 μm (procedure was repeated 2-3 times). After that, the residues of each sieve is rinsed with water. The residues which had been rinsed by water were poured into a pretridish and can be observed under stereo microscope. Identification was done manually based on INVAM (www.INVAM.wvu.edu.com) and another source of VAM Identification.

Internal structure of VAM were observed using 10 roots. The roots were taken around ± 3-5 cm. Washed the root in

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water, subsequently immersed in KOH 10%, heated at 110°C for 15-20 minutes, washed in the water and subsequently immersed in $H_2O_2$ 3% heated at 110°C for 60 minutes. Washed in the water, subsequently immersed in HCl 1% for 30 minutes. Afterwards immersed in 0,05% *tryphan blue* for 24 hour. Thereafter remove 0,05% *tryphan blue* then add *lactoglycerol* heated at 250°C for 5-10 minutes. Afterwards, the colored roots arranged in preparation glass and were analyzed under compound microscope. VAM Colonization percentage used Giovannetti & Mosse method (1980) with modification. Colonization can be known by the structure of VAM in roots. The VAM Colonization percentage was calculated by following formula :

$$\text{Colonization Percentage}\,(\%) = \frac{\Sigma \text{ Colonized root piece}}{\Sigma \text{ Colonized root observed}}$$

## RESULTS AND DISCUSSION

### Characteristic of Spores

The result of spore characterization which has been isolated from the rhizosphere of *Cymbidium* sp. obtained four genera with six different types of VAM spore. The genera include *Glomus, Dentiscutata, Gigaspora* and *Acaulospora.* In this study, identification was conducted up to the genera level with the characterization of spores which include: color, shape, diameter, spore wall, spore surface, and subtending hyphae. The result of spore characterization was dominated by Glomus, Dentiscutata, and followed by Acaulospora and Gigaspora. Picture of VAM spores can be seen in Fig 1 and characteristic of VAM spores in rhizosphere of *Cymbidium* sp. can be seen in Table 1.

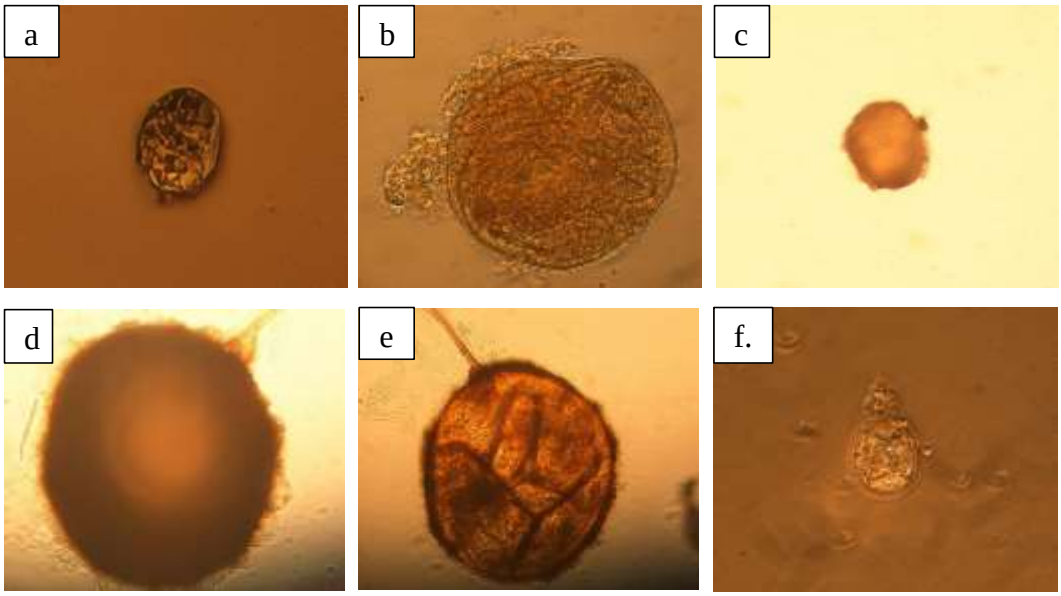


**Fig 1.** VAM Spores. a. Dentiscutata type 1 (magnification 200 times), b. Acaulospora (magnification 1000 times), c. Dentiscutata type 2 (magnification 400 times), d. Glomus type 1, e. Glomus type 2 (magnification 200 times), f. Gigaspora (magnification 400 times)

Presence of VAM spore is affected by several factors, namely environmental factors, host plant factors, and factors of VAM fungus itself. Environmental factors that affect to presence of VAM spores are organic matter and soil type. Organic matter is able to affect the presence of spores in soil, due to it is able to increase the nutrient content in the soil (Yusnaini, 2009). According to Johns (2017) that the addition of organic matter is able to increase the density of VAM spores. The type of soil sampling is a type of alluvial soil that contains low organic matter. However, sample plants are cultivated plants that get fertilization which contains organic matter, and allegedly the organic matter has given, has not undergone a perfect decomposition process. Besides that, Soil type affects the presence of VAM spores. Soil type that suits the most with particular genera of spore will support the sporulation of VAM. Soil with sand fraction suitable for the development

either *Gigaspora* and *Acaulospora* (Puspita si *et al*., 2012). Type of soil with silt loam suitable for development of Glomus genera, meanwhile type of soil gravelly - clayey loam suitable for development of *Dentiscutata* genera (Khade, 2010). The soil sampling included to type of alluvial soil, which has sandy texture to loamy sand, dominated by sand fraction (Subardja *et al*., 2016). This type of soil is allegedly suitable for development of VAM spores that have been found in rhizosphere of *Cymbidium* sp.

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Table 1. Characteristic of VAM spores in rhizosphere of *Cymbidium* sp.

| Genus | Characteristic of Spores | | | | | |
|---|---|---|---|---|---|---|
| | **Color** | **Shape** | **Diameter** | **Spore Wall** | **Spore surface** | **Subtending hyphae/ bulbeous suspensor** |
| *Dentiscutata* Type 1 | Brownish dark orange | Sub globose | 595,5 µm | Layered | Subtly without ornament | *bulbeous suspensor* is dark orange colored |
| *Acaulospora* | Bright to dark orange | Sub globose | 1945,4 µm | Layered | Subtly without ornament | *Cicatrix* doesn't visible |
| *Dentiscutata* Type 2 | Bright orange slightly brownish | Sub globose | 1047,5 µm | Non - layered | Subtly without ornament | *bulbeous suspensor* is bright orange slightly brownish colored |
| *Glomus* Type 1 | Dark brown with orange hint | Globose | 6050,2 µm | Layered | Subtly without ornament | Cylindrical, Flared to funnel shaped |
| *Glomus* Type 2 | Palle yellow to orange | Globose | 4453,3 µm | Layered | Subtly without ornament | Cylindrical, Flared to funnel shaped |
| *Giga spora* | Brownish Bright orange | Globose | 1211,8 µm | Layered | Subtly without ornament | *bulbeous suspensor* is brownish bright orange colored |

Besides of environmental factors. Host plants also affect spore presence. Sample are cultivated plant which generally gets the application of fungicides and fertilizers intensively, meanwhile, mycorrhizae can develop well in a particular condition such like in critical land and minimum fertilization. Cultivated plants that generally get the application of fungicides and fertilizers intensively cause the ability of mycorrhizae decreases. According to Dewi *et al*. (2014) mycorrhizae ability in order to assist plant roots to absorb, the nutrient and minerals through their hyphae will active when roots can not absorb the nutrient well.

VAM fungus factor is an ability of fungus to adapt. According to Janos (1992) that the distinction of VAM spores presence which have been found, is caused by adaption ability of VAM spores in an ecosystem. According to (Saputra *et al*.,

2015) *Glomus* and *Gigaspora* have a great adaptation. Meanwhile *Acaulospora* is able to adapt only in a particular condition.

The identification result showed that the diversity of spore VAM has relatively variated. The diversity of spore obtained from rhizosphere of *Cymbidium* sp. due to environmental factor such as type of soil. Each type of soil have different characteristic of pH and organic content, thereby there is a possibility to find variated spores of VAM (Kurnia *et al.*, 2019). The type of soil sampling is a type of soil that contains a lot of sand and clay and also rough profile, with the problem of lack of organic matter (Yulianah, 2018). Alluvial soil has sand fraction to sandy loam (Subardja *et al*., 2016), which means this kind type of soil suitable for development of *Gigaspora* and *Acaulospora*, big soil pores in this type of soil able to give space for development of *Gigaspora and Acaulospora*. *Glomus* develop well in silt loam soil, as well as its high adaptability to the environment makes *Glomus* be able to develop well in different types of soil. Meanwhile, *Dentiscutata* has known to be able to develop well in gravelly-clayey loam type of soil, specifically in tropical climates areas (Khade, 2010).

**Colonization of Vesicular Arbuscular Mychorrizal in Plant Root Tissue**

The result of VAM Colonization with staining technique shows the existence of VAM structures in the form of vesicular, internal hyphae, and external hyphae. Besides vesicular and hyphae structure, arbuscular is one of the most important structure of VAM fungus. However, there is no arbuscular structure was found in this study. It's because of arbuscular characteristic is unstable and easily degraded by plant cytoplasm (Widiatma *et al*., 2016). In line with research by Widiatma *et al*. (2016) Vesicular Arbuscular Mycorrhizae (VAM) Identification of Sweet Potato (*Ipomoea batatas* L.) and Cassava (*Manihot esculenta Crantz*) Rhizosphere, there is arbuscular structure was found. Allegedly, it's because of arbuscular characteristic. Picture of VAM Structure in root tissue of *Cymbidium* sp. can be seen in Fig 2.

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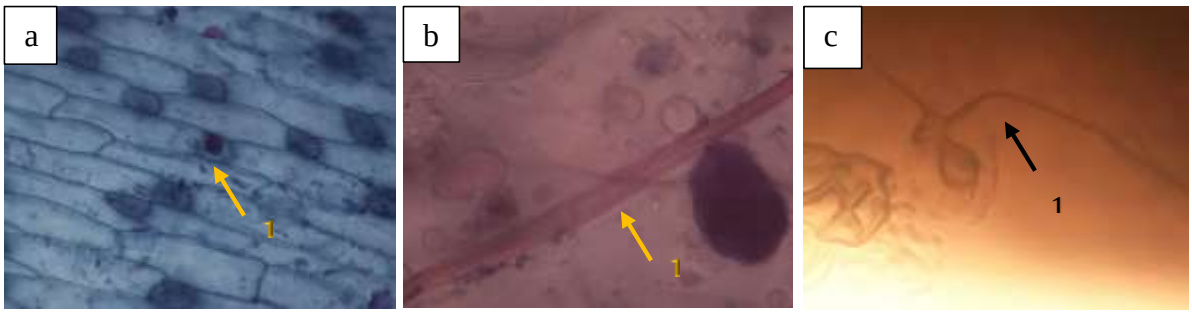


**Fig 2.** VAM Structure in root tissue of *Cymbidium* sp. (a.1. vesicular, magnification 200 times, b.1 internal hyphae, magnification 1000 times, c.1 eksternal hyphae, magnification 200 times)

The ability of VAM fungus in colonizing a plant can be known by calculating the intensity level of the colony. The result of the calculation of colony intensity indicates the intensity of of the colony is at 30%. According to Rajapakse & Miller (1992) regarding the intensity level of the colony, ability of VAM colonization can be categorized in medium category. Percentage Classification of VAM Colonization can be seen in Table 2.

Table 2. Percentage Classification of VAM Colonization

| Classification | VAM Infection | Category |
|---|---|---|
| 1 | 0 – 5% | Very low |
| 2 | 6 – 25% | Low |
| 3 | 26 – 50% | Medium |
| 4 | 51 – 75% | High |
| 5 | 76 – 100 % | Very High |

Source : Methods for Studying Vesicular-arbuscular Mycorrhizae Root Colonization and Related Root Physical Properties. Methods in Microbiology (Rajapakse & Miller, 1992).

VAM Colonization can be affected by several factors such as sensitivity of the host plant, climate, soil condition, also the presence of VAM propagules/spores in soil. The type of plant used in the multiplication of VAM inoculum is a plant that is able to

adapt to the production site of the inoculum and is able to associate with the fungus. Meanwhile, sample plant is a cultivated plant that intensively obtains fertilizers and fungicides that cause mycorrhiza ability to decrease (Dewi *et al*., 2014). Another factor that affects colonization of VAM is the presence of VAM spores/propagules. The presence of VAM spores/propagules itself is affected by organic matter in soil. Organic matter affects the presence of VAM spores/propagules caused by type of soil. The type of soil sampling in Singosari Subdistrict Malang Regency is an alluvial soil that has the problem of lack of organic matter (Yulianah, 2018). It causes the percentage of VAM colonization in *Cymbidium* sp. categorized in third grade that is medium.

## CONCLUSIONS

Based on this study it can be concluded that VAM spores were successfully identified in the rhizosphere of *Cymbidium* sp. are consisted of 4 different genera. Glomus, Acaulospora, Dentiscutata, and Gigaspora. Glomus and Dentiscutata are two different types of spores , meanwhile, Acaulospora and Gigaspora are each one type of spores.

The colony of VAM found in orchid plant's rhizosphere by the structure of VAM, vesicular and internal hyphae in root's tissue of *Cymbidium* sp. by colonization's precentage 30%, which categorized in the medium level of colonization.

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