

Review Article

Important Role of Mycorrhiza for Seed Germination and Growth of *Dendrobium* Orchids

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ABSTRACT

Indonesia is a tropical country that has natural forests and is suitable for orchid species habitat, leading to more than 5,000 species of orchids grow. The tropical area is the main distribution centre for epiphytic orchids, one of which is Dendrobium, which grows more than 1,000 species throughout the world. Orchid seeds are very small and do not have an endosperm, making germination difficult in their natural habitat. Mycorrhizal association with orchids plays a role in the survival of orchids in nature through seed germination and growth. This study aims to provide a deeper understanding about the important role of mycorrhiza in seed germination and growth of Dendrobium. The mechanism of mycorrhizal association with orchids begins with the initial contact of the fungus with the orchid, hyphae enter the cortex cells to form peloton, peloton lysis, and exchange of nutrients occurs. Orchid mycorrhiza that mostly found groups in Dendrobium are Rhizoctonia (Epulorhiza, Tulasnella, Rhizoctonia). Mycorrhiza plays a role in increased secretion of phytohormone and enzyme activity which supports seed germination and growth of orchids. Specific mycorrhizal data on orchids can be used as an effort for in-situ and ex-situ conservation of Indonesian orchids, including Dendrobium.

Keywords: conservation, Dendrobium, orchid mycorrhiza, seed germination

INTRODUCTION

Orchidaceae is one of the largest family of flowering plants including more than 28,000 species in 880 genera (Fochiet al. 2017; Xing et al. 2017). Orchids are distributed worldwide both in tropical and subtropical areas with different habitats. *Dendrobium* is one of the epiphytic orchids commonly found in Indonesia and there are more than 275 species (Xing et al. 2013; Juswara et al. 2016).

Several species of *Dendrobium* in Indonesia are *Dendrobium lineale* (from Papua), *Dendrobium phalaenopsis* (from Larat Island, Southeast West Maluku), and *Dendrobium nobile* which widely distributed in Indonesia. The flowers are beautiful and can withstand flowering for a long time, thus have high economic value and often taken freely from nature causing population decrease of orchid species, even *D. lineale* categorized as endangered orchid species (Chadburn & Schuiteman 2018). The diversity of *Dendrobium* orchid continued to decrease in nature as a result of overexploitation and was not balanced with conservation effort (Kusmana & Hikmat 2015).

The unique trait of orchid is the seeds are very small (microscopic) so inside one fruit there are millions of seeds. Although one fruit contains a large number of seeds, only 0,2-0,3% could be germinated in nature, and the rest died. Besides that, orchid seeds usually do not have an endosperm and only contain a small amount of complex carbohydrates as nutrient sources for seed germination. Thus, germination is difficult to occur in their natural habitat. Orchid seeds require symbiosis with mycorrhiza in order to change complex carbohydrates into small molecules that initiate germination and growth (Chen et al. 2014; Yeh et al. 2019; Kaur 2020).

All orchid species are myco-heterotrophic at certain stages during their life cycle. Several specific mycorrhizas were symbiosis with orchid roots or seeds. Mycorrhizal associations were formed during the entire life cycle of orchids, provide nutrients, sugar, and minerals, but some orchids species are also known to form mycorrhizal associations only when plants under stress (Kaur 2020). Furthermore, the study about mycorrhizal identification is important in order to obtain specific mycorrhizal species in orchids that increase germination and growth either in the natural habitat or in vitro culture, as conservation effort for Indonesian orchid species including *Dendrobium*.

Epiphytic orchids are the largest group of class in Orchidaceae, mainly *Dendrobium*, however, there are not many kinds of researches focused on orchid mycorrhizal association (Dearnaley et al. 2012). This review aims to provide a deeper understanding of the mechanism of mycorrhizal association with *Dendrobium* orchids, identify mycorrhiza on *Dendrobium*, and the role of mycorrhiza in seed germination and growth of *Dendrobium*.

MYCORRHIZAL ASSOCIATION MECHANISM WITH ORCHIDS

The symbiosis of mycorrhiza and orchids has a mutual relationship. Mycorrhiza provides minerals, such as nitrogen, phosphor, and potassium for plants, and plants provide carbon (photosynthetic products) to mycorrhiza (Garcia & Zimmermann 2014; Hijri & Bâ 2018). Availability of more mineral sources will increase growth. In general, fungi also play a role in increasing tolerance to abiotic stresses, such as inundation, salinity, temperature, and biotic stresses, including pathogens and insects (Yeh et al. 2019).

Orchid seeds are very small and do not have an endosperm (Fochi et al. 2017; Xing et al. 2017), hence seed germination carries out a symbiosis process with mycorrhizal fungi (Xing et al. 2013, 2017; Qin et al. 2020; Wu et al. 2020). Orchids germination requires mycorrhiza to get nutrients and organic carbon (Fochi et al. 2017). Orchid seedlings that have not yet carried out the photosynthetic process get a supply of carbohydrates and nutrients from mycorrhiza.

Association between *Dendrobium* seeds and their symbionts begins with fungal invasion in the first week during the imbibition stage of water throughout the testa (tissue of death cell layer which encapsulated embryo). Hyphae enter through the posterior end of the embryo suspensor cell then invaded cortex cells and form the peloton. Peloton is formed in phases 1-3. Peloton is intracellular hyphae coiled inside cortex cells that contain accumulated organic materials such as protein, glycogen, and lipid because of nutrient absorption from the soil. The plant will absorb the organic materials and peloton degraded. Hyphae degenerate after protocorm formation, usually in phases 4-5, leaving the empty hyphae (Chen et al. 2014; Soelistijono et al. 2020).

Mycorrhizas enter embryo cells through the posterior end and then invaded several layers of basal cells during stage 1. Not all embryo cells can be colonized by fungi especially the meristem area. Along with embryo



Figure 1. Mycorrhizal association during seed germination of *D. officinale.* a. Stage 1, fungal invasion at embryo basal cell. b. Stage 2, peloton formation. c. Stage 3, peloton degeneration along with protocorm formation. d. Stage 4, hyphae remaining forms clumps and peloton fully degraded. Bar: 20 µm (Chen et al. 2014).



Figure 2. The *D. phalaenopsis* orchid (Hoeve 2020) and transverse slices of orchid roots. a. Fungal invasion in the parenchyma cells of the cortex. b. The peloton is connected to each other with the peloton in another cell. c. Lysed peloton (Kaur 2020).

development, fungi spreads from cell to cell until the basal the area is fully colonized. Fungal hyphae form pelotons in the outer cortical cell on entrance into the embryo cell (stage 2). Pelotons have degenerated in the inner cortical cell (stage 3). Mostly digested pelotons form clumps in stage 4 as shown in Figure 1 (Chen et al. 2014).

Initial contact of fungi mycorrhiza in adult orchid plants occurs during penetration through root hairs. Fungi mycorrhiza enters parenchymal cortex cells and hyphae coiled forms peloton. Intact peloton activity provided nutrients and mediated the transport between the orchid and mycorrhiza. The peloton is connected to each other with the peloton in another cell. Peloton then lysis and nutrient released (Figure 2) (Kaur 2020).

The mycorrhizal invasion caused biochemical and cytological changes in cells. The newly colonized cells by mycorrhiza have a large amount of starch in contrast, cells with degraded peloton only have a small amount. Peloton degradation caused changes in structural and functional cells. Nucleus cells with degraded peloton observed have differentiation into bigger size with increase of DNA content inside (Kaur 2020).

Mycorrhizal association in orchid can be distinguished into two types of the host cell as digestive cells and host cells. Digestive cells play a role in forming intact peloton then continued to degrade and again reinvasion. Host cells have hyphae that active in nutrient exchange and peloton not yet degraded or were not ready to degrade yet. This model of nutrient exchanges is called phytophagy. This model involves fungal lysis inside the cells. The fungal part that degraded is the tip area (growing end), then released the cell amount into interspace area between plant cells and hyphal membrane which later would be used by plant (Kaur 2020).

Fungi enter through suspensors or rhizoids, depending on the type of orchid and associated mycorrhizal (Yeh et al. 2019). Hyphae enter through





the posterior end of the embryo in relation to the structure of the suspensor cell which consists of elongation of cells and dead cells, thereby reducing colonization resistance (Chen et al. 2014). The incoming hyphae will form a peloton. The plant-fungus symbiosis occurs where the fungus provides a source of minerals, and the plant provides a carbon source for the fungus. The orchid-fungus symbiosis is unique since the nutrient transfer does not occur bidirectionally at the early stage of growth and in some adult plant species. Most of the orchids have photosynthetic abilities, whereas more than 100 species are myco-heterotrophic, which do not have chlorophyll during their life cycle. Partial mycohetero trophy has two growth stages, namely chlorophyllous and achlorophyllous, thus it can survive in a shaded environment where the ability of photosynthesis is reduced (Yeh et al. 2019).

Nutrient exchange on photosynthetic orchid is different from the nonphotosynthetic one (Figure 3). In photosynthetic orchid, the plant gives carbon to mycorrhiza and in exchange, the plant receives phosphor from the symbionts. Amino acid transport from mycorrhiza to plant stopped because plants need only inorganic material source. This action is regulated by fungal amino acid transporters/permeases (TcAAT1, TcAAT2, TcAAT6). Mycorrhiza lysis releases nitrogen (N), phosphor (P), carbon (C) which absorb by plants however in non-photosynthetic orchid, a small amount of ammonium (NH₄⁺) transported from plant to fungal symbionts. NH₄⁺ produce to attract mycorrhiza so that colonized orchid seed and initiated germination. A study on protocorm *Serapias vomeracea* shows orchid received nitrogen from fungi because fungal arginase and urease genes stimulated amino acid breakdown (Dearnaley & Cameron 2017; Yeh et al. 2019).

Orchids receive N, P, C through membrane. N and C are amino acids from fungal symbionts. Mycorrhizae provide inorganic and organic material for orchids because of their inability to doing photosynthesis. Mineral N, P, C released from peloton lysis also would be absorbed by the plant (Yeh et al. 2019). Thus, explain that symbiosis mutualism of mycorrhizal association with orchid occurs in all growth either photosynthetic or non-photosynthetic stage (Dearnaley & Cameron 2017).

MYCORRHIZA ON Dendrobium

The association of orchids with mycorrhiza is interesting to study. Since the embryo, orchids need the help of mycorrhiza to obtain nutrients during

germination in nature (Chen et al. 2014). The mycorrhizal identification studies obtained various types of specific mycorrhiza associated with orchids as described in Table 1.

Orchidaceae considered to interact largely with group *Rhizoctonia*. The form of genus *Rhizoctonia* is a polyphyletic group of filamentous fungi which have many similarities in their anamorph phase (asexual). *Rhizoctonia-like* disparate based on the number of nucleus in hyphae, anastomosis ability (hyphae fusion), and morphological character including colony colour, concentric pattern, sclerotia (joined to the mycelium), and hyphae size. Based on the number of nucleus in hyphae, *Rhizoctonia* disparate into uninucleate (one nucleus) such as *Ceratobasidium bicorne* strain, binucleate (number of nucleus 1-3) such as *Rhizoctonia* sp. (Dearnaley et al. 2012; Suryantini et al. 2015).

Mycorrhizas that are commonly found in *Dendrobium* are *Epulorhiza* (11 species), *Tulasnella* (8 species), *Rhizoctonia* (6 species), and *Mycena* (5 species) (Table 1). *Epulorhiza* is the asexual form of genus *Tulasnella* which belongs to *Rhizoctonia* group (Dearnaley et al. 2012) so that the most found mycorrhiza

Table 1. Identified Mycorrhizal orchids in Dendrobium sp.

Orchid species	Isolated part	Mycorrhiza	Reference
Dendrobium sp.	root	Tulasnella pinicola	Sathiyadash et al. 2020
D. aphyllum	seed	<i>Tulasnella</i> sp.	Zi et al. 2014
		Rhizoctonia sp.	Soelistijono et al. 2020
D. candidum	root	Mycena dendrobii	Kaur 2020
D. crepidatum	seed	<i>Epulorhiza</i> sp.	Swangmaneecharern et al. 2012
D. chrysanthum	protocorm	Sebacinales	Chen et al. 2012
c .	root	Epulorhiza sp., Mycena	Kaur 2020
D. chrystallinum	root	Tulasnella calospora	Sathiyadash et al. 2020
c .	seed	Epulorhiza sp.	Swangmaneecharern et al. 2012
D. crumenatum	root	Epulorhiza sp.	Sathiyadash et al. 2020
D. dicuphum	root	Tulasnella irregularis	Sathiyadash et al. 2020
		Tulasnella deliquescens	
D. fimbriatum	root	Epulorhiza sp.	Xing et al. 2013
-		Rhizoctonia sp.	Mala et al. 2017
		Tulasnella calospora	Soelistijono et al. 2020
		Tulasnella deliquescens	
D. findlayanum	seed	Tulasnella deliquescens	Mala et al. 2017
		Epulorhiza sp.	
D. friedericksianum	root	Epulorhiza sp.	Sathiyadash et al. 2020
-		Tulasnella calospora	
D. hancockii	protocorm	Epulorhiza anaticula	Liu et al. 2010
D. lasianthera	root	Rhizoctonia sp.	Soelistijono et al. 2020
D. lindenii	root	<i>Tulasnella</i> sp.	Soelistijono et al. 2020
D. lineale	root	Rhizoctonia sp.	Soelistijono et al. 2020
D. nobile	protocorm	<i>Epulorhiza</i> sp.	Chen et al. 2012
	root	Epulorhiza sp., Mycena sp.,	Zhang et al. 2012; Xing et al. 2013;
		Rhizoctonia sp.,	Kaur 2020
		Mycena orchidicola	
D. officinale	root	Mycena dendrobii	Liu et al. 2010; Zhang et al. 2012;
		<i>Mycena</i> sp.	Sathiyadash et al. 2020
		<i>Epulorhiza</i> sp.	
D. sinense	root	Mycena orchidicola	Xing et al. 2013
D. speciosum	root	<i>Tulasnella</i> sp.	Sathiyadash et al. 2020
D. phalaenopsis	root	Rhizoctonia sp.	Soelistijono et al. 2020
D. pulchellum	seed	Epulorhiza sp.	Swangmaneecharern et al. 2012

belongs to genus Rhizoctonia.

Based on anastomosis ability, *Rhizoctonia* multinucleate diverse into 14 anastomosis groups (AG), *Waitea circinata, Rhizoctonia globulis,* and *Tulasnella* sp. (orchid mycorrhiza). *Rhizoctonia-like* binucleat dispersate into AG 1- AG 13, *W. circinata* var. *zeae* and var. *circinata,* member of AG A – AG except for AG BI, *Ceratobasidium cerealis, C. ramicola, Epulorhiza repens,* and *E. calendulina* (Dearnaley et al. 2012; Suryantini et al. 2015).

Rhizoctonia-like associated with orchids are from taxa Sebacinales, Ceratobasidiaceae, and Tulasnellaceae (class Agaricomycetes). Fungi can distinguish in anamorph or teleomorph form. Anamorph phase is an asexual form that appeals when fungi formed spores. Teleomorph phase is a sexual form that appeals when fungi using nuclei fusion to reproduce. Species anamorph *Epulorhiza* sp. (also known as *Rhizoctonia repens*) have teleomorph form *Tulasnella* sp. (Dearnaley et al. 2012; Suryantini et al. 2015).

MYCORRHIZA ROLES ON SEED GERMINATION AND GROWTH OF *Dendrobium*

Many studies observed the influence of mycorrhiza in seed germination and growth of orchids. Mycorrhiza that identified from *Dendrobium* orchid known as their role to initiate seed germination and growth of orchid including *Rhizoctonia*, *Tulasnella*, *Epulorhiza*, and *Mycena*.

Rhizoctonia produced niacin that increased germination and growth (Soelistijono et al. 2020). *Rhizoctonia* also increased seed germination up to 44-91% and advanced protocorm growth (Jiang et al. 2015). *Tulasnella* increased seed germination, protocorm formation, and orchid seedling development (Zi et al. 2014; Zhang et al. 2020; Freitas et al. 2020). *Tulasnella* has a role to stimulated growth and metabolism in *Dendrobium* (Wu et al. 2020). Roots with the addition of *Mycena* inoculum show change in morphology and has a bigger size. Mycorrhiza was isolated back and showed the same traits as the original inoculated strain (Zhang et al. 2012). *Epulorhiza* also increased the growth of *D. aphyllum* seedling. This shows that mycorrhiza and orchid have a specific relation at different growth phases.

Orchid mycorrhiza colonization increased both vegetative and generative growth in orchid, increased plant defense, initiate early flowering, increased flower quality, and reduced seed abnormality. Colonization of *Tulasnella repens* increased plant biomass. Colonization of *Epulorhiza* sp., *M. dendrobii*, and *M. anoectochila* on *D. nobile* increased height and plant biomass. Colonization of *D. officinale* roots by *Mycena* sp. increased plant height, biomass, and the number of shoots (Sathiyadash et al. 2020). *Epulorhiza* sp., *Mycena* sp., Tulasnellales, Sebacinales, Cantharellales increased nutrient intake in plants and increased seed germination on *D. nobile* and *D. chrysanthum*. Colonization of *M. dendrobii* on *D. candidum* helps growth with phytohormone secretion (Kaur 2020).

Mycorrhiza as symbionts has a role in widening surface area and increased intake ability of water and minerals in epiphytic orchid which has limited source access (Dearnaley et al. 2012). Mechanism of symbiont in seed germination and plant growth are provided nitrogen, phosphor, potassium in orchid also with secreting phytohormone indole-acetic acid (IAA), indole-3acetonitrile, gibberellic acid (GA), zeatin, zeatin riboside and naphthalene acetic acid (NAA) and increased enzyme activity such as chitinase, β -1,3glucase, phenylalanine ammonia-lyase, and polyphenol oxidase (Liu et al. 2010; Sathiyadash et al. 2020; Kaur 2020).

Seed germination of orchids needs mycorrhizal symbiosis. Orchid seeds are very small and do not have endosperm, thus germination is difficult to occur in their natural habitat. Orchid seeds required symbiosis with mycorrhiza to initiate germination (Chen et al. 2014; Yeh et al. 2019). The nutrients from the peloton helped the embryo development process since orchid seeds did not have an endosperm. Embryonic development would form sprouts and produced new plants (Yeh et al. 2019). The symbiosis of mycorrhiza and orchids for seed germination was key for seed-based orchid conservation. It is very important to conserve over-taken orchids, for example, many of *Dendrobium* species (Wu et al. 2020).

Dendrobium is the largest epiphytic orchid genus in the world and Indonesia. Mycorrhiza is important for the survival of orchids in nature for seed germination and growth. Specific mycorrhizal data on orchids can be used as a basis for increasing seed germination and growth of *Dendrobium* in their natural habitat as well as in vitro culture. It can be an effort to conserve Indonesian orchids and alternative information for the public regarding the handling of problems in orchid cultivation, especially *Dendrobium* in order to encourage the progress of orchid species cultivation quickly with good quality.

CONCLUSION

Orchidaceae naturally depend on mycorrhizae during their lifecycle. Fungi and orchid form symbiosis in several developmental stages including both non-photosynthetic and photosynthetic phases. The mycorrhizal association plays a role in orchids survival through seed germination since its seed lacks endosperm and improves plant development. The initiative begins with contact between fungi and orchid followed by hyphae entering cortex cells. Hyphae coils and form peloton in cells thus initiate the exchange of nutrients between fungi and plant cells. Mycorrhiza provides nutrients, sugar, and minerals for plants, and as exchange photosynthetic orchids would give carbon, however, non-photosynthetic orchids transported small amounts of NH4⁺ to fungal symbionts due to their inability in photosynthesis. Peloton lysis and other hyphae would enter cells for reinvasion. Largely interact with group Rhizoctonia, mycorrhizal colonization influences seed germination, plant height, plant biomass by widening surface area and increased ability of water and minerals intake, also secreting phytohormone and increased enzyme activity.

AUTHORS CONTRIBUTION

B.T., T.F., and C.L.S. searched the literatures and wrote manuscript, E.S. supervised all the process.

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CONFLICT OF INTEREST

There is no conflict interest regarding this research and research funding.

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