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SHORT COMMUNICATION

THE USES OF *Rhizoctonia* MYCORRHIZAE AS A DRY RESISTANCE INDUCTION OF *Dendrobium aggregatum* SEEDLINGS

Orchids are cultivated by tissue culture because their seeds do not have food reserves (*endosperm*) for their growth. All nutrient requirements are obtained from tissue culture media. Propagation by tissue culture causes them to have no association with *Rhizoctonia* mycorrhizae, which are needed in their growth so that they can interfere with orchid growth both vegetatively and generatively. *Rhizoctonia* mycorrhizae are a group of *Rhizoctonia* sp. which form a mutual symbiotic association with orchids. Each orchid has a different association with *Rhizoctonia* mycorrhizae. At the time of association with orchids, *Rhizoctonia* mycorrhizae will form a *peloton* structure in the orchid root cortex, which supplies several nutrients needed by orchids from their environment during drought. *Rhizoctonia* mycorrhizae inoculation has been carried out on orchid *seedlings* of the same species orchids *invitro*. However, *Rhizoctonia* mycorrhizae inoculation has never been carried out on seedlings of different species orchid using a simpler spraying method. The success of simpler *Rhizoctonia* mycorrhizae inoculation on *Dendrobium aggregatum* seedlings might provide an understanding of the increased resistance of orchids to water stress. The materials used were isolates of *Rhizoctonia* mycorrhizae isolated from the roots of *Dendrobium lasiantera* and seedlings of *Dendrobium aggregatum* aged 6 months. The seedlings consisted of two groups. The former included seedlings exposed to *Rhizoctonia* mycorrhizae (M1) and watering with the intervals of 1, 2, 3 days and those treated without *Rhizoctonia* mycorrhizae (M0) but with watering at the same intervals. The experiment used RCBD with 14 replications. Meanwhile, the control group was not exposed to both *Rhizoctonia* mycorrhizae and watering. The results showed that *Rhizoctonia* mycorrhizae inoculation had a significant effect on seedling height, leaf length, number of roots, and fresh weight of seedlings. Meanwhile, the watering interval did not give a real effect.

Key words: *Dendrobium Aggregatum* seedling, *Dendrobium lasiantera, Rhizoctonia* mycorrhizae, water stress,

Dendrobium sp. is very popular because it is easy to care for, has fragrant flowers, is long lasting, and has medicinal properties. However, according to the Central Bureau of Statistics (BPS) in 2019, the production and quality of orchids in Indonesia decreased compared to the previous years. They were 20,727,891 stalks in 2016, 20,277,672 stalks in 2017, and 19,739, 627 stalks in in 2018 respectively (Indonesia, 2019). The decline in production and quality of orchids is caused by many factors, and one of which is the lack of water (water stress). Orchids need water for their growth, especially epiphytic orchids such as *Dendrobium* because this type of orchid that attaches itself to trees is unable to absorb water and nutrients from the soil like ground orchids (Soelistijono et al., 2011). To minimize and even prevent this from happening at the nursery level (seedlings), *Rhizoctonia* mycorrhizae fungi inoculation is carried out. This is due to the seedling of *Dendrobium* sp. from tissue culture which does not have food reserves (*endosperm*) so that it requires *Rhizoctonia* mycorrhizae to provide additional nutrients for orchids seed growth.

Rhizoctonia sp. consists of 3 groups based on morphological characteristics, namely uninucleate, binucleate which is mycorrhizal, and multinucleate which is pathogenic that can last long time in the soil such as *Rhizoctonia solani* (Webb et al., 2011); (Ajayi-Oyetunde & Bradley, 2018). Binucleate *Rhizoctonia* isolate if inoculated on orchid seedlings will occur symbiotic association between the fungus and the root thereby causing the ability of the orchids to fulfill their own nutrients. *Rhizoctonia* mycorrhizae benefit from a friendly environment and a stable supply of sugar (carbohydrates) from the roots, which is contributed by orchid seedlings. In return, *Rhizoctonia* mycorrhizae will provide nutrients and other minerals in the form of peloton for orchid seedlings. *Rhizoctonia* mycorrhizae secrete growth factors that stimulate root growth and development in tropical soils (Cardoso et al., 2017).

This study is different from previous studies because *Rhizoctonia* mycorrhizae isolates used as resistance inducers were derived from root *Dendrobium lasiantera* and inoculated to *Dendrobium aggregatum* seedlings to be induced. *Rhizoctonia* mycorrhizae isolates were isolated from *D. lasiantera* roots in nature while the seedlings used were *D. aggregatum* from tissue culture. From the previous research, *Rhizoctonia* mycorrhizae were isolated from various orchids of *Dendrobium* sp. in Java. *Rhizoctonia* sp., whose isolates obtained from *D. lasiantera* were better than the others (Soelistijono et al., 2020). We expected that there would be a symbiotic mutualism between *Rhizoctonia* mycorrhizae and *Dendrobium* orchid seedlings of different species.

The research was conducted from August 2019 to July 2020. *Rhizoctonia* mycorrhizae isolates were obtained from the roots of *D. lasiantera* orchids in nature according to the modified Sneh B. method (Zumri et al., 2017). The 100 seedlings of 6-month-old *D. aggregatum* to be inoculated were obtained from the tissue culture laboratory of Tunas Pembangunan University. The experiment was laid out in five replicated Randomized Complete Block Design (RCBD). *Rhizoctonia* mycorrhizae isolates were grown on Potato Dextrose Agar (PDA) and incubated for 9 days, and identification of the colony form and hyphal structure was carried out (Suryantini et al., 2011). After 9 days, 5 grams of *Rhizoctonia* mycorrhizal culture were mixed with 100 ml of sterile water. Seedling of *D. aggregatum* aged 6 months was placed in the pot containing the moss. Each *D. aggregatum* seedling was sprayed with 1 ml of *Rhizoctonia* mycorrhizae inoculum and was acclimatized in the greenhouse for 2 months. After 8 months the roots of *D. aggregatum* were cut and examined under a microscope to see *Rhizoctonia* mycorrhizae associations in the form of peloton structure. Seedlings were 8 months old, and watering was carried out according to 3 treatments, namely once a day, every 2 days, and every 3 days for 2 months. The growth of *D. aggregatum* seedlings was observed every week from 8 to 10 months of age, both those in the treatment group inoculated with *Rhizoctonia* mycorrhizae (M1) and those without *Rhizoctonia* mycorrhizae (M0) as well as those in control group. After 10 months, the root length measurements were done according to the method of Pesci and Beffagna (Ábrahám et al., 2010) to determine the level of stress against drought.

Rhizoctonia mycorrhizae colonies were isolated from the roots of *D. lasiantera*. It was seen that the colonies were white with a brown circle in the middle almost covering part of the Petridish. This is in accordance with the results of research from Soelistijono et al., (2020) that *Rhizoctonia* mycorrhizae isolates were isolated from 5 *Dendrobium* sp. in Java, which most have a different colony color from the original culture. *Rhizoctonia* mycorrhizae are fungi that are facultative and easy to grow on PDA. *Rhizoctonia* mycorrhizae isolates have the following characteristics: they do not form spores (only white mycelia), mycelia colonies grow very fast so that at the age of 9 days after culturing they have filled the Petridish. Beside forming mycelia, it also forms a *sclerotium* structure with thick and hard walls (Soelistijono et al., 2011). According to (Kumar & Chaurasia, 2016) and (Li'atul Mufidah et al., 2017), the growth rate of *Rhizoctonia* mycorrhizae hyphae in forming colonies will vary depending on each species (Soelistijono et al., 2020). The rapid growth rate of *Rhizoctonia* mycorrhizae is expected to accelerate the formation of mycorrhizal associations with orchid seedling and the formation of peloton structures in the root cortex.

Microscopic observations show that the hyphae form right angles at the branches and form hyphal septa. According to (Muzhinji et al., 2015) the branching of the *Rhizoctonia* mycorrhizae hyphae forms right-angles branches and the pigment of the hyphae is brownish. The *Rhizoctonia* mycorrhizae isolates obtained have two cell nuclei (Figure 1), so that in accordance with the opinion of (Ajayi-Oyetunde & Bradley, 2018), they can be grouped in the *Rhizoctonia* mycorrhizal group (Binucleate *Rhizoctonia*).

Rhizoctonia mycorrhizae inoculation on the *D. aggregatum* seedlings cause the formation of peloton structures in the root cortex (Figure 2). The presence of hyphae penetrating the root cell wall indicated of tolypophagy infection (Suryantini et al., 2015). The presence of peloton structures in the root cortex proves that there is an association of *Rhizoctonia* mycorrhizae with orchid roots. The existence of this *peloton* is very important because it will supply nutrients that are needed by *D. aggregatum* in times of water shortage. If the environmental conditions are sufficient for the elements of the nutrients, the peloton will lysis (Soelistijono, 2015).

Inoculation of *Rhizoctonia* mycorrhizae on *D. aggregatum* seedling showed better vegetative growth (plants height, leaf length, number of roots, and plant fresh weights) than seedlings that were not inoculated with *Rhizoctonia* mycorrhizae (Table 1). *Rhizoctonia* mycorrhizae had a very significant effect on the seedling heights (Figure 3). This shows that *Rhizoctonia* mycorrhizae inoculation actively plays a role in stimulating the growth of seedlings height. The results of this study are consistent with (Wu J. et al., 2010) who state that the application of *Rhizoctonia* sp. on *Cymbidium georingii* orchids showed a significant difference in plant height. These young orchid seedlings are vulnerable to individual planting. Intensive fertilization is required until the plantlets are ready to be acclimatized in the greenhouse.

Morphological observations were carried out at the end of the study by looking at the color of the leaves and roots of the *D. aggregatum* orchids, which show that, in seedlings with *Rhizoctonia* mycorrhizae (M1) inoculation, leaf length was higher compared to that in plants without *Rhizoctonia* mycorrhizae (M0). This is due to the inoculation of *Rhizoctonia* mycorrhizae (M1), *Dendrobium* seedlings obtain nutrients from the peloton structures (Soelistijono et al., 2020). Peloton contains nutrients needed by orchid seedlings until their growth reaches the plantlet phase. The observations on the roots showed that all plant roots were white for mature roots, brown for old roots, and greenish for young roots.

In the observation of leaf length, it was seen that seedlings with *Rhizoctonia* mycorrhizae (M1) had a very significant effect compared to those without *Rhizoctonia* mycorrhizae (M0) (Table 1). *Rhizoctonia* mycorrhizae which are applied to *D. aggregatum* and associated with the root of the orchid play a role in providing nutrients for plant growth. Leaf length and leaf area are closely related to more effective light and CO^2 capture so that the rate of photosynthesis increases and is also associated with bulb growth, the formation of new shoots on the bulb, and the number of leaves in orchids.

In calculating the number of roots, inoculation of *Rhizoctonia* mycorrhizae (M1) had an effect compared to that without *Rhizoctonia* mycorrhizae (M0). The role of plant roots is as a channel to supply nutrients and water from the planting medium to plants. *Rhizoctonia* mycorrhizae play a role in increasing plant survival against extreme conditions such as drought and disease and increasing orchid growth by increasing the ability of roots to absorb the nutrients needed (Ningsih & Ambardini, 2014). The greater the number of roots in the orchid seedlings, the more nutrients will be absorbed. This is because the *Rhizoctonia* mycorrhizae hyphae that have infected plant roots can help the roots absorb nutrients and water in areas that are not reached by plant roots. The direct role of mycorrhizae is to help roots increase water absorption because fungal hyphae are still able to absorb water from soil pores when plant roots have difficulty in absorbing water. This is because the main *Rhizoctonia* mycorrhizae hyphae outside the roots (hyphosphere) form smaller hyphae and fine of the root hair with a diameter of approximately 2 µm (Cardoso et al., 2017).

The administration of *Rhizoctonia* mycorrhizae (M1) to the plants had a very significant effect on the plant fresh weight if compared to that without *Rhizoctonia* mycorrhizae (M0) (Table 1). The highest value in the M1 treatment was 4.307 g while the lowest value in the M0 treatment was 3.053 g., indicating that *Rhizoctonia* mycorrhizae inoculation had a significant effect on fresh weight in orchids. In the watering interval treatment (1, 2, and 3 days for 2 months), it did not significantly affect the parameters of leaf length, plant height, number of leaves, number of roots, and plant fresh weight. In the future, more researches with watering intervals every 2 days, 4 days, and 6 days might give a significant result. The moss (dry moss) media with its excellent binding and water retention power might have caused shorter watering interval treatment not to have a significant effect.

Rhizoctonia mycorrhizae inoculation on *D. aggregatum* seedlings also had a significant effect on the proline content in leaves, compared to those without inoculation with *Rhizoctonia* mycorrhizae (Table 2). Proline is a compound that is widely synthesized and accumulated in cytosol and plastids when plants experience drought stress (Verbruggen & Hermans, 2008). Plants that accumulate proline generally have good cell wall osmotic tension, and protein structure which was damaged due to lack of water can be repaired and have a higher survival rate than those that do not (Basu et al., 2016).

D. aggregatum seedlings without *Rhizoctonia* mycorrhizal inoculation will accumulate greater proline. When experiencing water stress, the proline concentration will increase to 80% of normal capacity. This is to maintain of cell turgor, hydration, accumulation abscisic acid, synthesis protein, and the rate of photosynthesis (Lisar S.Y., et al., 2012). However, the high proline accumulation in *D aggregatum seedlings* will affect the rate of plant vegetative growth and can be seen in the parameters of plant height, leaf length, number of roots, and plant fresh weigh in its growth rate which are lower than that of *Rhizoctonia* mycorrhizae inoculations.

CONCLUSION

Rhizoctonia mycorrhizae isolated from different orchid species and applied by spraying had a very significant effect on plant height, leaf length, number of roots, and fresh weight on *D. agregatum* seedling. As daily watering interval did not have a significant effect, future research will compare longer watering interval.

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CONFLICT OF INTEREST

This article is original and has not been submitted or published in any other journal.

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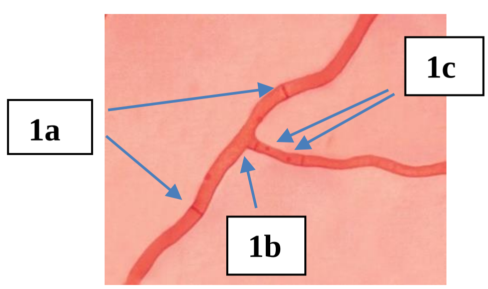

Figure 1. Identification of *Rhizoctonia* mycorrhizae isolates from *Dendrobium lasiantera.*
Description: (1a) septa or hyphal septum (1b) hyphal branching, (1c) number of cell nuclei.

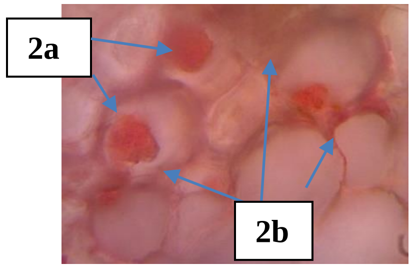

Figure 2. Structure of the peloton in the root cortex of *Dendrobium aggregatum*.
Description: (2a) The structure of peloton (2b) Root cortical cell wall.

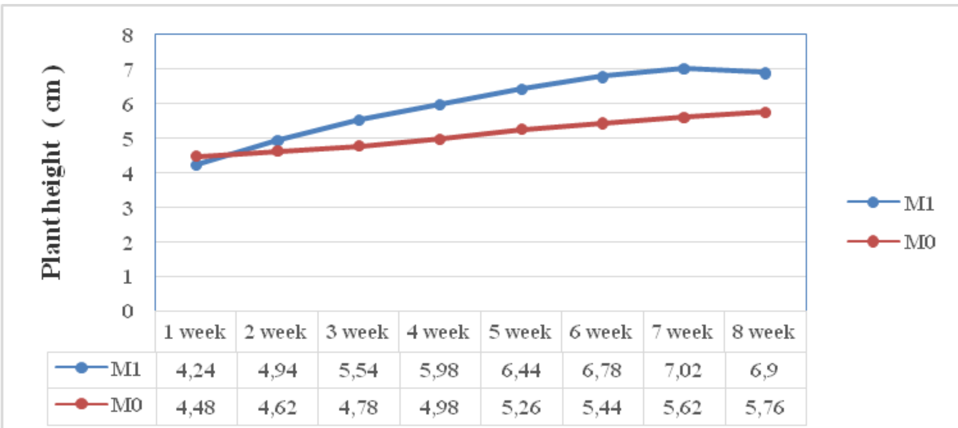

Figure 3. Relationship between orchid plant height due to the influence of *Rhizoctonia* mycorrhizae (M1) and without *Rhizoctonia* mycorrhizae application (M0).

Table 1. Summary of research results

No	**Parameters**	**Mycorrhizae Applications (M)**	**Watering Interval (P)**	**Factor Interaction (M x P)**	**Value**	
					Highest	**Lowest**
1.	Plant height (cm)	**	ns	ns	9,2 (M_1P_2)	4,4 (M_0P_1)
2.	Leaf length (cm)	**	ns	ns	6,8 (M_1P_2)	2,9 (M_0P_3)
3.	Number of leaves (sheet)	ns	ns	ns	10 (M_1P_1)	2 (M_0P_2)
4.	Number of roots (sheet)	**	ns	ns	16 (M_1P_3)	4 (M_0P_3)
5.	Plant fresh weight (g)	**	ns	ns	6,7 (M_1P_3)	2,1 (M_0P_3)

Footnote: ns: not significant*: significant**: very significant

Table 2. Content of proline in *Dendrobium aggregatum* leaves

No.	Code	520 nm	(µ mol proline/g)
1	M0A1	0.97	0.328
2	M0A2	0.115	0.730
3	M0A3	0.281	4.437
4	M1A1	0.093	0.238
5	M1A2	0.086	0.082
6	M1A3	0.124	0.931

Footnote:M0A1: Without *Rhizoctonia* mycorrhizae with watering every 1 days
M0A2: Without *Rhizoctonia* mycorrhizae with watering every 2 days
M0A3: Without *Rhizoctonia* mycorrhizae with watering every 3 days

M1A1: With *Rhizoctonia* mycorrhizae with watering every 1 days
M1A1: With *Rhizoctonia* mycorrhizae with watering every 2 days
M1A1: With *Rhizoctonia* mycorrhizae with watering every 3 days

Supplementary materials.

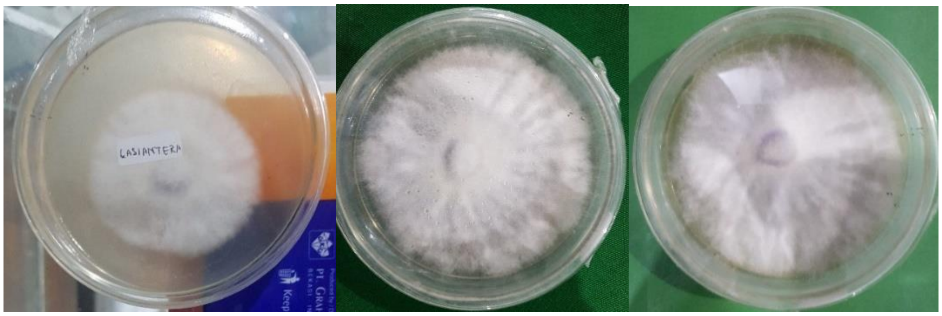

Figure 4. Growth and development of *Rhizoctonia* mycorrhizae colonies (isolated from *Dendrobium lasiantera*) on PDA.
Description: Observation of the development of *Rhizoctonia* mycorrhizae colonies on the third day (1a), (1b) sixth day, and ninth day (1c).

Figure 5. Plant nursery on greenhouse.

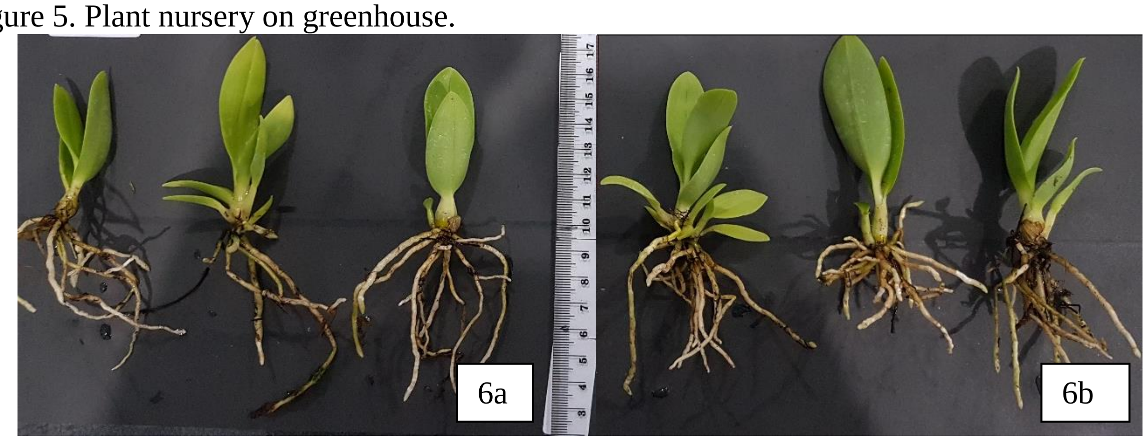

Figure 6. Comparison of *Dendrobium aggregatum* seedlings morphological appearance.
Description: (6a) Without *Rhizoctonia* mycorrhizae (M0)
(6b) With *Rhizoctonia* mycorrhizae (M1)

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