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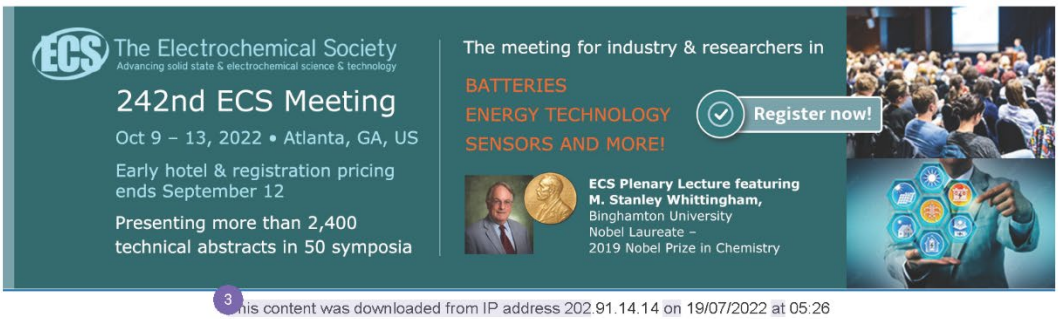

Isolation of Mycorrhizal *Rhizoctonia* as resistance inducer of *Dendrobium macrophyllum* to drought

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Abstract. One of the obstacles encountered in the cultivation of orchids *Dendrobium macrophyllum* is difficult to cultivate in areas with high drought due to the slow absorption of nutrients. Based on previous research, the mycorrhizal binucleate *Rhizoctonia* (BNR) has the ability to increase the resistance of vanilla (*Vanilla planifolia* Andrews) to drought, but it has never been tried on orchid *Dendrobium macrophyllum*. The objectives of this study was to isolate resistance inducer organisms by induced resistance techniques on orchids against drought. It is expected that the administration of mycorrhizal *Rhizoctonia* can increase the absorption of nutrients in *D. macrophyllum* which is exposed to high water stress. Each treatment consisted of 3 replications of 3 potted plants. The characterization of mycorrhizal *Rhizoctonia* isolate from *D. macrophyllum* root from Surakarta, Kopeng, Magelang, and Yogyakarta did not different morphologically. Character equations are in colony color, cell length and number of cores, while character differences are present in cell width and all isolates are capable of forming a *peloton* structure.

1. Introduction

Drought is a major factor in orchid cultivation. Long-term observation results indicated that the long dry season due to the phenomenon of La Nina global climate anomalies in general occur periodically every 5 years. Orchid *D. macrophyllum* is a group of *Dendrobium* sp. which is resistant to drought stress. *D. macrophyllum* is an endemic orchid at the foot of Merbabu Mountain. However, in recent years, this orchid is very hard to find in its habitat. This is due to environmental conditions that are less supportive (drought stress factor). By the Department of Commerce, the orchid *D. macrophyllum* is categorized into Appendix 2 plants, which are rare and non-tradable crops except cultivation.

D. macrophyllum is rare because it is not able to live in environment with drought stress, this because in the flowering phase requires enough water, thus, inhibit the flowering phase (generative phase) in the dry season (Figure 1).

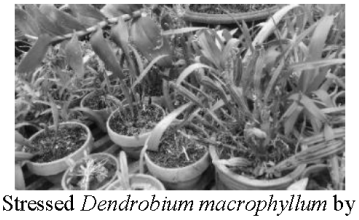

Figure 1. Stressed *Dendrobium macrophyllum* by low water

Lack of nutrients absorbed by the root of epiphytic orchids caused by high water stress factors affect the productivity of flowering *D. macrophyllum*. This because the structure of epiphytic orchids do not intersect with the soil, thereby can not directly absorb nutrients from the soil and rely solely on water uptake in the epiphytic root that causes the lack of nutrients. Water stress factor is a major factor in the cultivation of orchids and influences the flowering phase of the plant. If the minimum water content within the plant roots is fulfilled, then the flowering process will occur quickly. Increased plant resistance to water stress can be done by inoculation using various biological agents due to the increase of several compounds that stimulate the nutrient uptake process and this is one of the effects of induced mechanisms *in vitro*.

The mycorrhizal *Rhizoctonia* is thought to have the ability to induce orchid plants [4]. The resistance mechanism in *D. macrophyllum* inoculated mycorrhizal *Rhizoctonia* presumably due to the formation of *peloton* structures [9], which indicates the existence of intermediate associations between mycorrhizal *Rhizoctonia* and *D. macrophyllum.* Until now there has been no report that definitely mentions the use of mycorrhizal *Rhizoctonia* in improving the resilience of orchid plants in high water stress conditions. It is therefore necessary to conduct research on the role of mycorrhizal *Rhizoctonia* in increasing flowering productivity in *D. macrophyllum* on high water stress by isolating it from the wild.

2. Methods

Isolation of mycorrhizal *Rhizoctonia* was performed from healthy *D. macrophyllum* root from various sites according to Otero *et al*. [7]. Identification of mycorrhizal Rhizoctonia by schlerotium and mycorrhizal identified by Barnett & Hunter [3] included: (1) sclerotium form, (2) hyphae branching forms, and (3) the number of nucleotide.

D. macrophyllum development stage *in vitro* by [8] conducted in the tissue culture laboratory of Agricultural Faculty of UTP included: Stage 1: Observation of embryo development (0-3 weeks after seed was sown); Stage 2: Protocorm observation (3-10 weeks after seed was sown); Stage 3: Observing the growth of seedling (10-20 weeks after the seed is sown).

Observation of *peloton* on transverse slices of orchid root of *D. macrophyllum for* each treatment were done by floroglucinol painting using method of Kutscha [6]. *Peloton* observations were performed 24, 48, and 96 hours after induction of mycorrhizal *Rhizoctonia*.

3. Results and discussion

Figure 2 shows the identification of mycorrhizal according to Barnett & Hunter [3] includes the color of the colony, the shape of the hypha branch, and the number of nuclei seen. Isolation of mycorrhizal *Rhizoctonia* from *D. macrophyllum* root in various places was obtained by several isolates of mycorrhizal *Rhizoctonia*, from Surakarta obtained isolate R1, from Magelang obtained isolate R2, from Kopeng obtained isolate, and from Yogyakarta obtained R4.

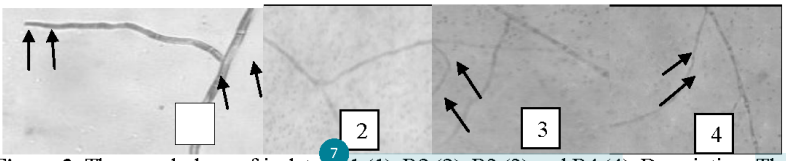

Figure 2. The morphology of isolates R1 (1), R2 (2), R3 (3) and R4 (4). Description: The arrow pointed the cell nucleus.

Figure 3 shows the propagation of culture in vitro obtained exsplant *D. macrophyllum* to be inoculated mycorrhizal *Rhizoctonia* to see the *peloton* in the orchid cortex. From figure 3, we can see the exsplant *D. macrophyllum* to able inoculated mycorrhizal *Rhizoctonia* is 8 weeks after seed planting (WASP), because the root of explant has occurred. Mycorrhizal *Rhizoctonia* is able to

symbiotize with orchid root tissue and form a hyphae coagulant that clumps on the cortical tissue of the roots. The coagulated hifa coil structure is called a *peloton* [9].

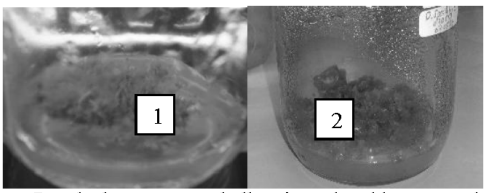

Figure 3. Explant *Dendrobium macrophyllum* inoculated by mycorrhizal *Rhizoctonia*.
Note: 1) on 4 weeks after seed planting (WASP): 2) on 8 WASP

Figure 4 shows the *pelotons* are intracellular hyphae in the cortex network of roots and usually only exist in a certain period before lysis [11] which can be seen the assosiation betwen mychorrhizal *Rhizoctonia* with exsplant *D. macrophyllum*.

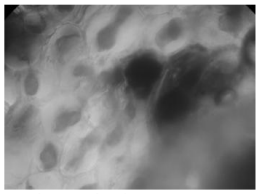

Figure 4. The structure of the *pelotons* in the root cortex *Dendrobium macrophyllum.*

Table 1 shows characteristics of various isolates of mycorrhizal *Rhizoctonia* isolated from Surakarta, Kopeng, Magelang, and Yogyakarta having differences in cell width and length. The isolations were done with 40 sample from each location.

Table 1. The characteristics mycorrhizal *Rhizoctonia* of *D. macrophyllum*

Mychorrhizal *Rhizoctonia*	Cell width	Cell length	Colony color	Sclerotium formation
Rl	5,3-9,1	43,0-128,0	Brownish white	+
R2	5,1-9,0	51,0-131,0	Light brown	+
R3	7,2-9,0	40,0-137,3	Light brown	+
R4	5,9-11,5	42,5-171,5	Light brown	+

Note: R1: Mycorrhizal *Rhizoctonia* isolated from Surakarta; R3: mycorrhizal *Rhizoctonia* isolated from Kopeng; R2: Mycorrhizal *Rhizoctonia* isolated from Magelang, and R4: mycorrhizal *Rhizoctonia* isolated from Yogyakarta.
+ = Sclerotium is formed.

Table 2 shows the isolate mycorrhizal *Rhizoctonia* isolated from Surakarta, Kopeng, Magelang, and Yogyakarta also had differences in the number of nucleotide within each cell.

Table 2. The nuclei number of hyphae cell of mycorrhizal *Rhizoctonia*

Rhizoctonia sp. (4 isolate)	The number cells based on the number of nucleotide					*Range*	Information
	1	2	3	4	5		
R1	3	15	7	5	0	2	binucleate
R3	3	16	4	7	0	2	binucleate
R2	7	14	5	5	0	2	binucleate
R4	4	15	8	3	0	2	binucleate

Note: R1: Mycorrhizal *Rhizoctonia* isolated from Surakarta; R3: mycorrhizal *Rhizoctonia* isolated from Kopeng; R2: Mycorrhizal *Rhizoctonia* isolated from Magelang, and R4: mycorrhizal *Rhizoctonia* isolated from Yogyakarta.

Colonies of mycorrhizal *Rhizoctonia* are different depending on their group (isolates grouping). The mycorrhizal *Rhizoctonia* isolates (R2, R3, and R4) obtained, were mostly brownish-brown, while the colonies of *Rhizoctonia* isolate R1 were white colored. [1] found that 9 isolates of mycorrhizal *Rhizoctonia* isolated from *Spathoglottis plicata* from various places in Thailand showed white colonies. Meanwhile Agustini *et al.* [2] found different things in the botanical garden of Cycloops Jayapura, that were 10 orchids. The exsplants of *D. macrophyllum* inoculated by mycorrhizal *Rhizoctonia* were 8 weeks after seed planting (WASP), because the root of explant has occurred. The *pelotons* structure is already formed in the root cortex part of *D. macrophyllum*. It is expected that the *peloton* structure will help to absorb the nutrients needed by *D. macrophyllum* from the surrounding environment.

Mycorrhizal *Rhizoctonia* has cell size ranges from 5.1µ to 11.5µ. Table 1 showed that each of the mycorrhizal *Rhizoctonia* isolates obtained from various places in Surakarta, Kopeng, Magelang, and Yogyakarta have a more uniform cell size. This is in accordance with research on mycorrhizal *Rhizoctonia* in orchids such as *Ceratobasidium*, *Thanatephorus* (Ceratobasidiales), *Sebacina* (Exidiales) and *Tulasnella* (Tulasnellales), in which the width of mycorrhizal *Rhizoctonia* cells can be more than 10 µm and grouped in *Tulasnella* [5].

Isolates Rl, R3, R2, and R4 are mostly hypha cells with average nuclei ranging (range) 2. According to Sneh *et al.* [9], isolates with a nucleotide number of 1-3 cells were binucleic groups, whereas isolates with more than 3 nuclei cells were multinucleated. From the opinion of Sneh *et al.* [9] isolates Rl, R3, R2, and R4 are binucleate. This is in accordance with previous research in orchid plants [1], [5], [10], [12], which concluded most of the isolates of *Rhizoctonia* sp. are mycorrhizal and belong to the binucleate group. Mycorrhizal isolates obtained colony color varied from white to black. Therefore, based on morphological observation of colloidal color of the mycorrhizal *Rhizoctonia* isolate from *D. macrophyllum* orchids, it can be said that colony color can not be used as a differentiator between each isolate.

4. Conclusion

The characterization mycorrhizal *Rhizoctonia* isolates of *D. macrophyllum* root from Kopeng, Magelang, Surakarta, and Yogyakarta did not different morphologically. Character similarity is in colony color, cell length and number of cores, while character differences are in cell width. The association results of *D. macrophyllum* with mycorrhizal *Rhizoctonia*, formed a *peloton* structure in the root cor

Acknowledgments

On this occasion, we are grateful to the Directorate General of Higher Education, whom funded the study in accordance with the Contract of Research Execution Number: 007/K6/KM/BASERISET/ 2016 dated May 4, 2016.

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