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MORPHOLOGICAL AND ANATOMICAL IDENTIFICATION OF Rhizoctonia MYCORRHAZA FROM FOUR SPECIES OF Phalaenopsis sp.

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ABSTRACT

Rhizoctonia mycorrhiza is an endophytic fungus that plays an important role in orchid growth and can increase plant resistance to environmental stress and pathogens. The purpose of this study was to examine the morphology and anatomy of Rhizoctonia mycorrhiza isolated from four Phalaenopsis orchid species. The study used a quantitative descriptive method through direct observation and analysis of the percentage of hyphal cell nuclei. The results showed that the four Rhizoctonia mycorrhiza isolates had different colony morphological characteristics. Isolates from Phalaenopsis venosa had brownish-white colonies with rapid mycelial growth, isolates from Phalaenopsis amabilis showed grayish-white colonies with thinner growth and Phalaenopsis celebensis, colonies were white-brown and began to form dark masses suspected to be sclerotia. Meanwhile, isolates from Phalaenopsis gigantea showed white-dark green colonies with a circular growth pattern and rapid spread.

Anatomical observations showed that all isolates had the typical characteristics of Rhizoctonia, namely hyphal branching at a 90° angle, septate hyphae, and the presence of more than one cell nucleus. Based on the count of 120 hyphal cells, isolates from P. venosa (63.3%), P. amabilis (66.6%), and P. gigantea (60%) were dominated by binucleate cells, while isolates from P. celebensis (70%) were dominated by multinucleate cells. This indicates the physiological diversity of Rhizoctonia mycorrhiza among orchid species. Thus, this study confirms that the roots of four species of Phalaenopsis orchid species contain Rhizoctonia mycorrhiza with morphological and anatomical variations that can be used as an inoculum source for the development of orchid cultivation as well as further research related to its physiological function and application as a biological agent.

KEYWORD

Rhizoctonia Mycorrhiza, Phalaenopsis sp., Binucleate

INFORMATION

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1. INTRODUCTION

Orchids (Orchidaceae) are one of the most popular ornamental plants and are loved by many for their beautiful charm. The importance of orchids lies in the beauty of their flowers, and this beauty can be achieved if the orchid is cultivated correctly from a young age (Husodo et al., 2023). The most common orchid genera cultivated as ornamental plants are the Cattleya, Dendrobium, Oncidium, Phalaenopsis and Vanda genera (Syahputra & Wibowo, 2020). The Phalaenopsis genus requires relatively low levels of sunlight, as this orchid species thrives best in shady, humid environments with good air circulation. Therefore, understanding the differences in characteristics between orchid genera is crucial. By recognizing the specific needs of each genus, growers can provide more appropriate care, ensuring optimal orchid growth, tailored to each plant's unique characteristics (Syahputra & Wibowo, 2020).

Orchid cultivation often faces various challenges, such as disease attacks and drought stress. Diseases in orchids generally arise from infections by microorganisms, including fungi, bacteria, and viruses. One frequently problematic pathogen is Fusarium oxysporum, a fungus that causes wilt disease that can hinder successful orchid cultivation. F. oxysporum infection is usually characterized by yellowing of the leaves and stems, followed by decay, which ultimately leads to plant death (Monawati et al., 2021). One way to overcome these obstacles is by using a biological agent, namely Rhizoctonia mycorrhiza. Rhizoctonia mycorrhiza can also be a control agent for pathogenic fungi (Soelistijono et al., 2024). Rhizoctonia not only transfers nutrient ions to plants but also increases plant resistance to disease and unfavorable environments. Regarding plant resistance, not only mycorrhizal-forming Rhizoctonia play a role, but also avirulent to low-virulence (hypovirulent) Rhizoctonia can also increase plant resistance to drought stress (Suryantini & Soelistijono, 2021). Rhizoctonia mycorrhiza inoculation was able to maintain plant growth rates for at least 6 days of drought. Significant results were found in leaf length, number, and area, which were closely related to the amount of chlorophyll and CO2, which increased photosynthesis rates, and were also associated with tuber growth and the formation of new shoots in orchid tubers (Soelistijono et al., 2025). Therefore, research on Rhizoctonia mycorrhiza from various types of orchids is important to be carried out so that it can be used in orchid cultivation because in fact orchids cannot grow without mycorrhiza due to the absence of food reserves (endosperm) in the seeds (Attri, 2023)

In research by Soelistijono, et al., (2020) entitled "Characterization of Rhizoctonia-Like Mycorrhizae Associated With Five Dendrobium Species in Java, Indonesia". The results of observations of fungal colonies on PDA showed that the fungus has a white color. Rhizoctonia mycorrhiza colonies on orchids are generally white. Macroscopic observations showed that fungal colonies growing on PDA almost covered the surface of the petri dish. Color differences in the same isolate can occur due to differences in sporangium maturity. Sporangium maturity affects the color of the sporangium from white, brown, and dark.

According to research Harieni et al., (2021) entitled "The Effect of Rhizoctonia Mycorrhiza Application and Watering Interval on the Growth of Dendrobium Stockelbuschii Schettler Orchid Seedlings". The results of the observations showed that microscopic observations of the brownish-white Rhizoctonia mycorrhiza hyphae showed the presence of hyphal branches at right angles and the presence of two cell nuclei. These characteristics, including the color of the colonies formed and the number of cell nuclei in the hyphae, are important indicators in identifying the characteristics of Rhizoctonia mycorrhiza.

2. METHODS

This research was conducted in the tissue culture laboratory and greenhouse of the Faculty of Agriculture, Tunas Pembangunan University, Surakarta and the research time was carried out from April 2025 to October 2025. The materials used in this study were moon orchids with species, P. venosa, P. amabilis, P. celebensis and P. gigantea, Potato Dextrose Agar (PDA) media, distilled water, alcohol, and safranin. While the tools used were 1000 ml Erlenmeyer, magnetic stirrer, digital scales, 250 ml measuring cup, autoclave, Petridish, flushing tool, deck glass, cover glass, microscope, tissue paper, dissection needle, knife/cutter, scissors, cotton, stick stick, aluminum foil, spray bottle, cork bur, label, Bunsen flame, stationery, and iPhone 11 brand cellphone camera with 12 mp.

The collection of Rhizoctonia mycorrhiza isolates from the roots of the P. venosa, P. amabilis, P. celebensis and P. gigantea was carried out aseptically according to the method Michael, et al. (2023). Observations of colony shape, Rhizoctonia mycorrhiza angle, and number of cell nuclei were carried out according to Sneh et al., (2004). Observation parameters include: colony shape, colony color, hyphae shape, and number of cell nuclei. The observation data were analyzed descriptively quantitatively through percentage calculations and data distribution to compare variations in the number of hyphal cell nuclei between mycorrhizal isolates from 4 orchid species P. venosa, P. amabilis, P. celebensis and P. gigantea.

3. RESULTS AND DISCUSSION

From the identification carried out at the Tissue Culture Laboratory of the Faculty of Agriculture, UTP Surakarta, the results of Rhizoctonia mycorrhiza fungus isolates obtained from the roots of the orchid plants P. venosa, P. amabilis, P. celebensis and P. gigantea on PDA media were as follows:

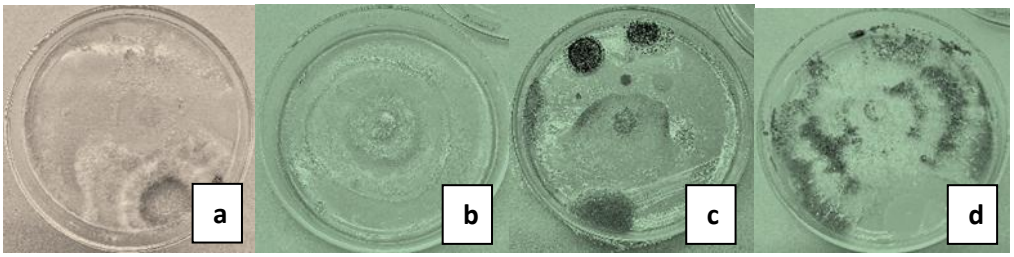

Figure 1. Development and growth of Rhizoctonia mycorrhiza fungal colonies on PDA media isolated from the roots of Phalaenopsis sp.

Information: (a) Phalaenopsis venosa, (b) Phalaenopsis amabilis, (c) Phalaenopsis celebensis, (d) Phalaenopsis gigantea.

The four isolates of Rhizoctonia mycorrhiza fungi from the roots of the orchid plants P. venosa, P. amabilis, P. celebensis, and P. gigantea showed several different shapes and colors of fungal colonies. From the several fungal colonies that were visible, the isolates could not be identified as Rhizoctonia mycorrhiza, although the isolate obtained from the roots of P. amabilis had a shape similar to the definition of Sneh et al., (2004), namely white and having circular rings on its colonies. However, these isolates as a whole can be classified as mycorrhizal fungi in the Rhizoctonia-like mycorrhiza group (Soelistijono et al., 2020). However, it is necessary to observe the structure of the hyphae to determine the form of a single isolate of Rhizoctonia mycorrhiza as seen in Figure 2.

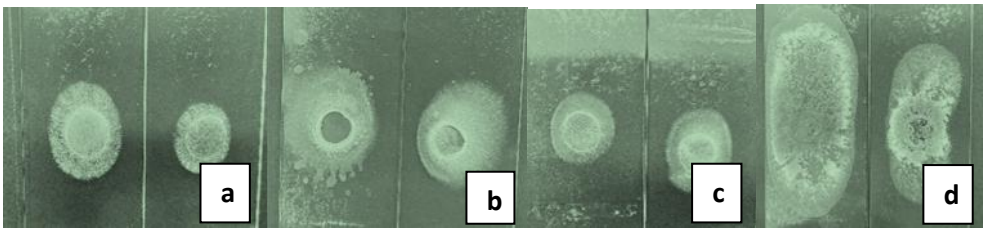

Figure 2. Colony development of Rhizoctonia mycorrhizae isolates from the roots of Phalaenopsis venosa (a), Phalaenopsis amabilis (b), Phalaenopsis celebensis (c), and Phalaenopsis gigantea (d).

From Figure 2 shows the different growth patterns of each Rhizoctonia mycorrhiza isolates. The Rhizoctonia mycorrhiza isolate from P. venosa exhibits colony growth with white mycelium at the edges and brownish in the center. This color change indicates a difference in growth stage, with young mycelium being white and older mycelium pigmented to brown. Binucleate Rhizoctonia (BNR) is characterized by white colonies and brown hyphae (Soelistijono, et al. 2020). In P. amabilis, colony growth is evenly distributed with a grayish-white color. Colonies appear smooth and thin, with a circular growth pattern from the inoculation point towards the edge of the medium. Colonies of Rhizoctonia mycorrhiza from P. celebensis roots have a deep dark green color with clearly visible circular layers from the center to the edge of the colony. The color variation from white to dark green in the colony indicates a physiological development gradient, where young mycelium (white) actively grows and expands the network, while the center is dark green. This pattern reflects the shift in gene expression from vegetative growth to the formation of reproductive structures, which is usually controlled by complex genetic regulation and the influence of the surrounding environment of the culture medium (Chen et al., 2021). Rhizoctonia mycorrhiza obtained from P. gigantea showed a yellowish-white center of the colony, indicating the initial inoculation area where the mycelium begins to grow. Meanwhile, the edges of the colony were lighter green, indicating young mycelium that was still actively growing. The surface texture of the colony appeared dense and in the form of a fine powder. Colony growth occurred at a high rate, as evidenced by the large area covered in a relatively short time. The deep green color of the colony indicates high metabolic activity and the production of secondary pigments. Overall, the growth characteristics of this colony indicate that the isolate has high growth potential, active reproductive ability, and good morphological stability. The circular growth pattern, dark green color, and powdery texture indicate that the fungus is in the final stage of its growth cycle (maturation phase) with the potential for spore formation or defense structures. To confirm that the isolate obtained is Rhizoctonia mycorrhiza, it is necessary to observe the hyphae to determine the number of nuclei and the presence or absence of right-angled branches, as can be seen in Figure 3.

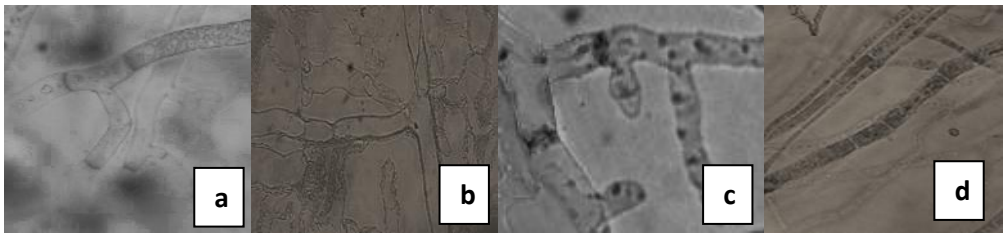

Figure 3. Anatomy of Rhizoctonia mycorrhiza isolate colonies from the roots of Phalaenopsis venosa (a), Phalaenopsis amabilis (b), Phalaenopsis celebensis (c), and Phalaenopsis gigantea (d).

From the observations in Figure 3, it can be seen that the Rhizoctonia mycorrhiza isolate obtained from P. venosa roots has septate hyphae that branch at an angle of almost 90°. This branching shape is a characteristic of Rhizoctonia fungi (Hossain, 2022), which can help distinguish it from other types of mycorrhiza fungi. The hyphae appear clear to slightly brownish in color, and in some sections, each hyphal cell appears to have two nuclei (binucleate). These two nuclei also indicate that the fungus is in a period of active growth, where the cells are actively dividing and developing. Furthermore, septa are visible separating each cell in the hyphae. These septa function to regulate food flow and maintain cell stability as the fungus grows. The hyphal branching pattern, which forms a 90° angle, also indicates anastomosis, the process of connecting between hyphae. This process is important for the formation of strong fungal tissue and helps the connection between the fungus and the orchid roots. Other characteristics of Rhizoctonia mycorrhiza include 90° hyphal branching, narrowing of the hyphae at the branching point, and the presence of sclerotia (Nafisa et al., 2021).

The hyphae branch of P. amabilis at almost a 90° right angle. These septa help the fungus regulate food flow and maintain cell stability, especially during active growth. The presence of two nuclei indicates that this fungus is a binucleate Rhizoctonia, a type of fungus that often lives in symbiosis with orchid roots (Soelistijono et al., 2020). Three morphological characteristics of Rhizoctonia mycorrhiza are white colonies, perpendicular branches and two nuclei (Soelistijono et al., 2023) indicates that this fungus is most likely an orchid mycorrhizal fungus from the Rhizoctonia group.

P. celebensis has branches at angles approaching 90°. This branching pattern is one of the main characteristics often used to identify the Rhizoctonia fungus that lives symbiotically with orchids (Hossain, 2022). The nucleus is binucleate, but there are also several or more nuclei in each hyphal cell. Although there are different nuclei (multinucleate), it does not affect the properties of the isolate because the roots of P. celebensis can grow well. The presence of these two nuclei is a characteristic marker of the binucleate Rhizoctonia (BNR) group, known as mycorrhizal fungi that live symbiotically with orchid roots (Moliszewska et al., 2023).

P. gigantea has branched hyphae at 90° right angles, a characteristic of Rhizoctonia, and this distinguishes it from other types of mycorrhizal fungi. There are septa that function to separate the hyphal cells so that they are orderly and help regulate the flow of food between fungal cells and play a crucial role in the union between hyphae (anastomosis), which is the process in which hyphae connect to each other to form a strong network in the orchid roots (Pujasatria et al., 2024).

Rhizoctonia mycorrhiza isolates from the roots of P. amabilis, P. venosa, P. celebensis, and P. gigantea according to research Soelistijono et al., (2011) that isolates can be grouped (isolates grouping) based on their anastomosis ability (Anastomosis Groups) can be seen in Table 1.

Table 1. Number of cell nuclei in Rhizoctonia mycorrhiza hyphae (120 hyphal cells)

Rhizoctonia mycorrhiza	The number of 30 hyphae cells observed in each isolates			Highest percentage (%)	Information
	1 inti	2 inti	>2 inti		
P. venosa	2	19	9	63,3%	binucleate
P. amabilis	2	20	8	66,6%	binucleate
P. celebensis	0	9	21	70%	multinucleate
P. gigantea	2	18	10	60%	binucleate

Source: research data

Based on the results of observations of 120 hyphal cells from Rhizoctonia mycorrhiza isolates obtained from four Phalaenopsis orchid species, it is known that each isolate has a tendency for a different number of hyphal cell nuclei. This grouping is carried out based on the dominant number of cell nuclei (1 nucleus, 2 nuclei, or >2 nuclei) in 30 cells observed from each isolate. Isolates from P. venosa showed that most hyphal cells had two nuclei, namely 63.3%. This indicates that the isolate is included in the binucleate group. The same pattern was also found in the isolate from P. amabilis, with a percentage of binucleated hyphal cells of 66.6%, so this isolate is also classified as a binucleate group. The isolate from P. gigantea showed results in line with the two previous isolates. The percentage of binucleated hyphal cells in this isolate was 60%, so it was categorized as a binucleate isolate. In contrast to the three species, the isolate from P. celebensis showed a different pattern of cell nucleus number. In this isolate, the largest dominance was found in hyphal cells with more than two nuclei, namely 70%. This indicates that the isolate from P. celebensis has a multinucleate character, so it is included in the multinucleate grouping. Overall, the results of this study indicate that three isolates (P. amabilis, P. venosa, and P. gigantea) have binucleate hyphal characters, while one isolate, namely P. celebensis, is a multinucleate isolate.

4. CONCLUSION

The morphological characteristics of Rhizoctonia mycorrhiza isolates from the four Phalaenopsis sp. species were brownish white, grayish white, and dark green with different spreading speeds. Anatomically, the four isolates had septate hyphae, right-angled hyphal branching (90°), and an average of 2 cell nuclei each hypha, indicating different physiological diversity variations between isolates.

5. SUGGESTIONS

1. Molecular identification (DNA/ITS rDNA) is necessary to determine the grouping of isolates into anastomosis groups.
2. Some isolates have formed sclerotia, which can be used as culture collections. It is highly recommended that these isolates be used in future orchid cultivation research and applications.

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