Pak. J. Phytopathol., Vol. 37 (01) 2025. 15-26 DOI: 10.33866/phytopathol.037.01.1138

Official publication of Pakistan Phytopathological Society

Pakistan Journal of Phytopathology

ISSN: 1019-763X (Print), 2305-0284 (Online)

https://pjp.pakps.com

RESEARCH ARTICLE

Study of Orchid Mycorrhiza Fungi (Omf) on Phalaenopsis Violacea Drought Strees

[a]Raden Soelistijono*, [a]Daryanti, [a]Hadad M. N. Kholiq, [b]Cecep Hidayat, [c]Achmadi Priyatmojo

[a] *Department of Agrotechnology, Faculty of Agricultural, Tunas Pembangunan University, Jl. Balekambang No.1, Manahan Surakarta 57139, Indonesia.*

[b] *Department of Agrotechnology, Faculty of Science and Technology, UIN Sunan Gunung Djati Bandung, Jl. A.H. Nasution No.105, Bandung 40614, Indonesia.*

[c] *Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada, Jl. Flora No. 1, Bulaksumur, Sleman 55281, Yogyakarta, Indonesia.*

Corresponding Author:
Raden Soelistijono, Email: sulistyo.utp@gmail.com

Article History:
Submitted: June 13, 2024; Revised: November19, 2024; Accepted for Publication: May 30, 2025.

A B S T R A C T

Water stress is a plant disease that is abiotic in nature and affects the growth of orchid species in nature. Economically, it is very detrimental because it can cause losses in orchid production. One of the countermeasures is the use of Orchid Mycorrhizal Fungi (OMF) which form symbiotic associations with orchids, including the mycorrhizal *Rhizoctonial* group. This study aims to examine the effectiveness of *Rhizoctonia* isolated from the roots of *Dendrobium macrophyllum* and *Dendrobium secundum* orchids and watering intervals on *Phalaenopsis violacea* seedlings. The research method used a Complete Randomized Block Design (CRBD) with 2 factors and 3 replications.The first factor was the application of mycorrhizal *Rhizoctonia:* (R0) control (no mycorrhiza), (R1) mycorrhiza isolated from *D. macrophyllum*, (R2) mycorrhiza isolated from *D. secundum*, and (R3) a combination of R1 and R2. The second factor was the watering intervals: twice, four times, and six times a day. The results of the study on the first factor showed that Seedling *Phalaenopsis violacea* gave significantly increased growth with the R3 treatment, as indicated by increases in plant height (20.7 cm), leaf length (19.2 cm), root number (14 pieces), root length (21.3 cm), fresh weight (17.5 g), and dry weight (2.7 g). For the second factor, the growth increased significantly when watering was done once every four days (P2), with plant height reaching 20.3 cm and leaf length 18.8 cm. The interaction between *Rhizoctonia* mycorrhiza application and the best watering interval was in the M3P2 combination treatment, with the highest plant height (8.0 cm) and leaf length (7.5 cm). The proline content was M2P3 (0.135) and the highest number of chloroplasts was in the M3P3 treatment (0.89), while all those applied with mycorrhizal *Rhizoctonia* formed peloton structures in the root cortex. Thus, the combination of mycorrhizal *Rhizoctonia* and a four-day watering interval represents an effective strategy in overcoming drought stress in *P. violacea. This approach* has the potential to improve orchid cultivation practices. It is hoped that mycorrhizal *Rhizoctonia* can be used to induce drought resistance in orchids in the future.

Keywords: Drought stress, OMF, *Phalaenopsis violacea*, mycorrhizal *Rhizoctonia*, Watering interval.

INTRODUCTION

Orchids (Orchidaceae) are non-food horticultural plants admired for their unrivaled beauty and elegance. Among the most popular groups of orchids is the moon orchid, valued for its aesthetic appeal and high economic value due to its attractive shape, vibrant color and pattern, making it the main choice as an ornamental plant both indoors and outdoors. One of the most favoured moon orchid varieties in Indonesia is *Phalaenopsis violacea*, renowned for its beautiful petal shape and the flower coloration. Like other orchids, moon orchids can also be attacked by plant diseases during cultivation. These can be caused by biotic factors such as pathogenic microorganisms fungi, bacteria, viruses (Li *et al.,* 2021) and abiotic stressors including nutrition deficiencies and environmental stress (He *et al.,* 2018).

One of the main challenges in orchid cultivation in Indonesia is weather factor. Long-term observations by the Meteorology, Climatology, and Geophysics Agency (BMKG) show that due to the unpredictable weather anomalies, can lead to long dry seasons and drought stress/low rainfall. Of the various effects of environmental stress, drought stress has the greatest effect on orchid growth. This type of stress is unstable and is influenced by unpredictable climate changes. Drought stress reduce water availability which is the main factor influencing orchid growth, because it affects the metabolic level of plants which really need water in the absorption or distribution of nutrients throughout the tissue, making it an important key in determining the quality of orchid plant growth (Sabatini *et al.,* 2022). Due to drought stress, many orchid productions experience growth constraints, leading to substantial economic losses in production (De and Medhi, 2014).

However, in nature, several other groups of orchid plants have demonstrated the ability to adapt to drought stress, namely the *Dendrobium* sp. (Salazar *et al.,* 2022). *Dendrobium* sp. generally grows well at an altitude of around 1,000 meters above sea level. Interestingly, when moved from the highlands to lowland environments with reduced rainfall, they are still able to adapt. This adaptability Is believed to result from a drought resistance mechanism facilitated by their association with an endophytic fungus known as Orchid Mycorrhizal Fungi (OMF). These fungi colonize the root cortex and form a specialized type of mycorrhiza in orchid plants, thereby enabling them to tolerate different levels of drought (He *et al.,* 2018; Khan *et al.,* 2021; Shah *et al.,* 2022). OMF associated with orchid roots include members of the mycorrhizal *Rhizoctonia*, *Tunasnela*, *Sebacina*, and *Ceratobasidium* groups (Ahu *et al.,* 2008; Shakoor *et al.,* 2015). Among these, the mycorrhizal *Rhizoctonia*. are the most frequently found in orchid roots (Jacquemyn *et al.,* 2017). Research conducted by Lucky Kumar Attri (2023) stated that the Rhizoctonia group of species classified as mycorrhizae contributes to the water supply to support the growth process. The *Rhizoctonia* group that is able to associate with orchids is grouped into mycorrhizal *Rhizoctonia* and has the characteristic of having 2 nuclei/binucleate (Sneh *et al.,* 1992; Soelistijono *et al.,* 2011; Tian *et al.,* 2021). Like other OMF fungi, (Salazard *et al.,* 2020), mycorrhizal *Rhizoctonia* is also classified as an endophytic fungus because it is located in the root cortex (Shah *et al.,* 2020). Therefore, to get this mycorrhizal *Rhizoctonia*, we have to isolate it from healthy orchid roots and then transfer it to a fungal growth medium (Soelistijono *et al.,* 2018). Mycorrhizal *Rhizoctonia* itself has been tried in preventing the fungal pathogen *Fusarium* sp. (Soelistijono *et al.,* 2024).

Previous research by Sabatini *et al.* (2022) attemted to overcome the drought stress in orchids by using chemical agents such as Poly Ethylene Glycol (PEG). However, this method has several limitations. Not only is it non-permanent because it must be carried out in vitro continuously, there is also a relatively low survival percentage among treated plants. Research by Soelistijono *et al.* (2023) tries to overcome the problem of drought biologically by applying mycorrhizal *Rhizoctonia* to *Dendrobium aggregatum* orchid seeds. This new method is more environmentally friendly because it does not use chemicals and is permanent because mycorrhizal *Rhizoctonia* is associated in the orchid root cortex (endophytic). Thus, it is hoped that orchids that have been inoculated with mycorrhizal *Rhizoctonia* can adapt to lowland environments with lower water availability. The successful association of endophytic fungi with orchids will be aimed at forming a peloton structure in the roots (Soelistijono *et al.,* 2018). The peloton structure in the roots contains carbohydrate components which will be formed into nutrients needed by orchids when there is no supply of nutrients from the environment. The peloton structure store carbohydrate components that can be converted into essential nutrients, supporting plant survival during periods when environmental nutrient availability is low. Interestingly, when external nutrients become sufficiently available, the peloton structures undergo lysis (Soelistijono *et al.,* 2023).

Drought stress or limited water availability, typically triggers physiological, biochemical and molecular responses in plants as survival mechanisms (Tyagi *et al*, 2022). These anatomical and chemical changes play an important role in providing plants with nutritional needs from the environment. One of these responses is the activation of the formation of secondary (chemical) metabolites such as proline (Cheour *et al.,* 2014; Chun *et al.,* 2018). Proline is an amino acid produced by plants as an indicator of plant resistance to water stress. The presence of proline is greatly influenced by plants that are able to associate with mycorrhiza. Proline is synthesized via the glutamate pathway and the ornithine pathway by the enzyme pyrroline-5-carboxylate synthase which is important in plant metabolism. The presence of proline is needed in plants that lack carbohydrates as an effect of inhibiting the rate of photosynthesis in the leaves (Wang *et al.,* 2022). Proline is produced by cells immediately after the cells experience stress and will function to protect the plasma membrane and cell proteins.

Drought stress can also affect structural changes (anatomy) such as chlorophyll (Luigi *et al.,* 2018; Putri *et al.,* 2019; Bashir *et al.,* 2021). Chlorophyll is a green pigment that is important in the process of photosynthesis, which allows plants to convert solar energy into usable chemical energy. When plants experience drought, they can undergo changes in their chlorophyll structure as part of an adaptation to survive in water-poor conditions. For example, plants can change the ratio between chlorophyll an and chlorophyll b, or can even produce chlorophyll variants with slightly different structures to increase their water use efficiency. Research by Sasongko *et al.* (2016) shows that drought stress led to differences in the amount of chlorophyll in *Grammatophyllum scriptum* plantlets cultured in the laboratory under various different nutrient treatments. Apart from changes in chlorophyll structure, drought stress also triggers the formation of proline compounds as a source of carbohydrates which can be induced by microbes that are able to interact with plants. This mechanism is known as induction of resistance in plants (Soelistijono *et al.,* 2020).

The novelty of this study compared to previous studies lies in two main aspects. First, it involves (1) the utilization of a combination of two Orchid Mycorrhizal Fungi (OMF), specifically two mycorrhizal *Rhizoctonia* isolated from two different *Dendrobium* sp. species known for their drought stress. This combination is expected to have a synergistic effect in enhancing the drought tolerance of *P. violaceae* under varying watering intervals. Second, the resistance properties obtained are systemic because the consortium isolates of two mycorrhizal *Rhizoctonia* that form endophytic fungi are already associated with the root cortex tissue. This endophytic relationship not only provides long-term resistance but also has the potential to be inherited by subsequent generations, paving the way for the development of new drought-resistant orchid varieties.

MATERIALS AND METHODS

The research was carried out at the Tissue Culture and Greenhouse Laboratory, Faculty of Agriculture, Tunas Pembangunan University (UTP), Surakarta, located at an altitude of 105 meters above sea level. The study took place from July 2023 to March 2024. The plant material used consisted of 8 *Phalaenopsis violacea* orchid seedlings aged eight months, resulting from acclimatized tissue culture (juvenile phase) in the greenhouse according to the method of Soelistijono *et al.* (2011). Additional plant sources included *D. macrophyllum* and *D. secundum* orchids from the orchid collection in the UTP greenhouse., Other materials included Potato Dextrose Agar (PDA), Potato Dextrose (PD), dry moss (moss), alcohol, safranin, and distilled water. The tools and equipment used in this research include Petridish, Erlenmeyer, measuring cup, razor, ose needle, tweezers, pipette, Bunsen, aluminum soil, lighter, cotton, stirrer, tissue, ruler, plastic pot, hand sprayer, plastic wrap, microscope, autoclave, laminar air flow, electric stove, optilab, laptop, label paper, documentation tools and writing tools.

Before OMF isolation, the laboratory room was sterilized overnight. Similarly, all equipment was sterilized using 90% alcohol. Meanwhile, the Potato Dextrose Agar (PDA) media was sterilized in an autoclave and left for three days to monitor for potential contamination (Atiq *et al.,* 2024; Usman *et al.,* 2025). Only PDA plates that remained uncontaminated were used for OMF isolation from orchid roots. To avoid contamination from the material, the orchid roots used as explants were surface-sterilized with 90% alcohol inside a laminar air flow (LAF) cabinet before being placed onto sterile PDA in Petridishes (Ahmad *et al.,* 2024). The OMF in this study were isolated from the roots of *D. macrophyllum* (isolate M1) and *D. secundum* (isolate M2.). A third isolate (M3) was prepared as a combination of M1 and M2 (M1+M2) according to the method of Soelistijono *et al.* (2011). Healthy orchid roots are cut at the ends 1 cm long, sterilized with 70% alcohol, then placed on PDA media. Fungal growth was monitored over 3 to 6 days, after which emerging fungal isolates are then identified as mycorrhizal *Rhizoctonia* (Soelistijono *et al.,* 2011). Isolates M1 and M2 are isolates that must have the same group anastomosis. Therefore, these isolates must also be tested first by placing them together in 1 petri dish. If the two isolates (M1 and M2) hyphae that meet are identical or do not cancel each other out (antagonistic), then it can be suspected that they are the same group of group anastomosis (Misawa and Kurose, 2019; Olutoyosi and Bradley, 2020). The three isolates (M1, M2, and M3) were cultured on PDA media for 7 days, then macroscopic and microscopic observations were carried out to identify the colony shape and hyphal structure according to Zhu *et al.* (2008). The mycorrhizal Rhizoctonia group has 2-nucleated hyphae (binucleate), with right-angled branches, and hyphae thickness ranging (Soelistijono *et al.,* 2011). Identification of the number of nuclei is important because the Rhizoctonia group also has more than 2 nuclei which are known as multinucleate and are pathogenic, for example *Rhizoctonia solani* (Oyetunde and Bradley, 2017). After being identified as the mycorrhizal *Rhizoctonia* group, each M1, M2, and M3 isolate was placed into PD liquid media with a ratio of 1 g isolate (M1, M2, M3) in 100 ml PD and incubated for 7 days. After incubation for 7 days, 10 ml of each M1, M2, and M3 isolate was applied to the roots of 8-month old *P. violacea* seedlings which were placed in pots filled with moss, and acclimatized for 2 months. The growth of *P. violacea* seedlings was observed weekly from 8 to 10 months of age.

The experimental design used a Complete Randomized Block Design (CRBD) with two treatment factors. The first factor was the application of mycorrhizal *Rhizoctonia* consisting of: no application (M0), application of mycorrhizal *Rhizoctonia* isolated from *D. macrophyllum* roots (1), application of mycorrhizal *Rhizoctonia* from *D. secundum* roots (M2), and combined application of M1 andM2 (M3). There were 12 treatment combinations with three blocks and 3 replications, so there were a total of 118 experimental units. Data were analyzed using Analysis of Variance (ANOVA) with a significance level of 5% and 1%, as well as the Duncan Multiple Range Test (DMRT) with a significance level of 5%. The parameters observed included the shape of the mycorrhizal *Rhizoctonia* colony, mycorrhizal *Rhizoctonia* hyphae, peloton structure, plant height, leaf length, number of leaves, root length, number of roots, plant fresh weight, plant dry weight, proline content, and amount of chlorophyll. Proline measurements were carried out using the photoacoustic spectrometer method (Chun *et al.,* 2018), while chlorophyll measurements were carried out using the method by Suminar *et al.* (2020).

RESULTS AND DISCUSSION

After subculturing various MFO isolates (M1, M2, and M3) onto new PDA media, within 7 days' white hyphae colonies were visible forming a circle in the middle of the Petridish. The hyphae colony grew quickly and almost covered the entire PDA medium, without any contamination in the Petridish medium. Each colony showed typical characteristic white mycorrhizal *Rhizoctonia* hyphae, with whorls reaching to the periphery (Figure 1).

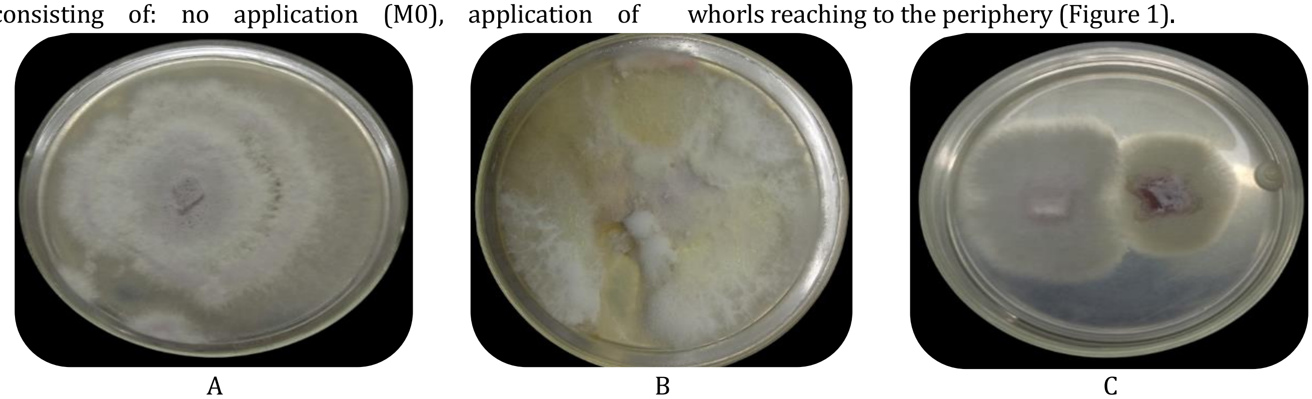
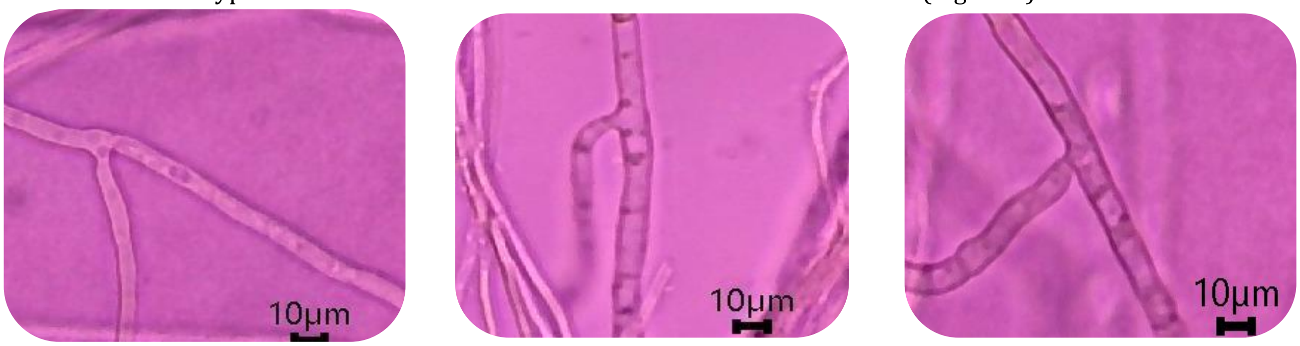


Figure1. Growth and development of Orchid Mycorrhizal Fungus (OMF) on PDA media.
Description: a. OMF from *Dendrobium macrophyllum* root (M1). b. OMF from *Dendrobium secundum* root (M2). c. OMF from the combination *D. macrophyllum* dan *D. secundum* roots (M3)

In Figure 1 above, it can be seen that the OMF isolates from the roots of *D. macrophyllum* (M1), *D. secundum* (M2), and a combination of both (M3), each one week old, exhibit distinct colony morphologies. The OMF colonies from *D. macrophyllum* (Figure 1a) had white hyphal clumps, thinner than the OMF colonies from *D. secundum* (Figure 1b) and the two OMF isolates had different colors and shapes (Figure 1c). The brownish white color of the three OMF groups (M1, M2, M3) is one of the characteristics of mycorrhizal *Rhizoctonia*. This is in accordance with the opinion of Sneh *et al.,* (2004) that mycorrhizal *Rhizoctonia* has color variations such as white, light brown and brown. However, the growth rate of mycorrhizal *Rhizoctonia* hyphae in forming colonies vary depending on each species. The fast hyphal growth rate of mycorrhizal *Rhizoctonia* is expected to accelerate the formation of mycorrhizal associations with orchid seeds and the formation of peloton structures in the root cortex. In Figure 1c, the absence of zoning where the M1 and M2 isolates meet indicates that M3 is a compatible combination (a fusion) of both isolates. the to determine the structure of the hyphae and nuclei of the three isolates which were mycorrhizal *Rhizoctonia*, microscopic observations of the hyphae were carried out.

To ensure that the OMF isolate belongs to the mycorrhizal *Rhizoctonia* group, it is necessary to make microscopic observations of the shape of the hyphal branches and the number of cell nuclei (Figure 2).

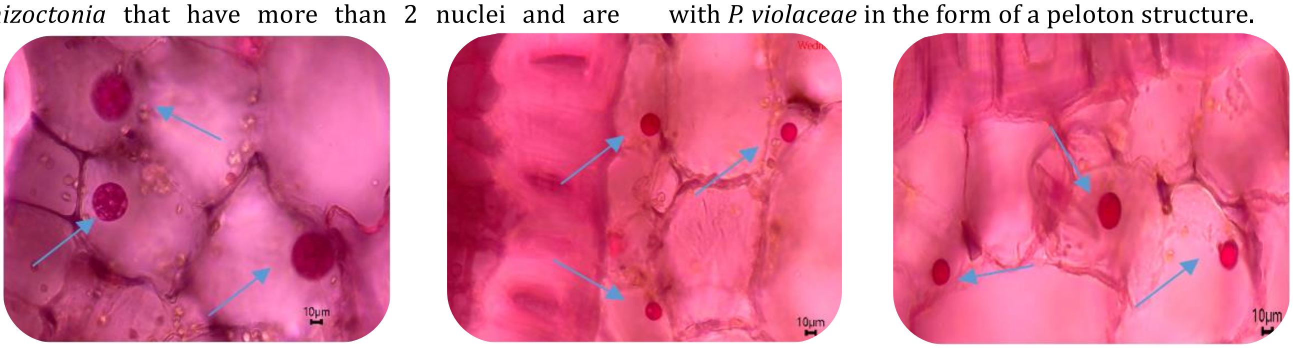


A B C

Figure 2. Structure of mycorrhizal *Rhizoctonia* hyphae

Description: a. Mycorrhizal *Rhizoctonia* from *Dendrobium macrophyllum* roots (M1) b. Mycorrhizal Rhizoctonia from *Dendrobium secundum* roots (M2). c. *Rhizoctonia* mycorriza from combination of *Dendrobium macrophyllum* & *Dendrobium secundum* (M3)

In Figure 2, mycorrhizal *Rhizoctonia* hyphae isolated from the roots of *D. macrophyllum*, *D. secundum*, and the combination of the two produces branches at a 90° angle and has partitions near the branches, which is one of the main characteristics of mycorrhizal *Rhizoctonia*. This is in accordance with the opinion of Sneh *et al.,* (2004); Hossain, (2019), stated that *Rhizoctonia* hyphae form branches at right angles (90°) and there are partitions near these branches. Both Figures 2a, 2b, show *Rhizoctonia* hyphae with 2 cell nuclei, indicating that this isolate belongs to the mycorrhizal binucleate *Rhizoctonia* (BNR) group and is known as mycorrhizal *Rhizoctonia* (Fauziah *et al.,* 2014). Identification of the number of nuclei is important because there are also *Rhizoctonia* that have more than 2 nuclei and are pathogenic (Oyetunde & Bradley 2017). In Figure 2c, the combination of M1 and M2 in the middle of the hyphaeshows that fusion is occurring. According to Olutoyosi & Bradley (2020), the presence of fusion shows that the two isolates are compatible / there is an anastomotic relationship so that the hyphae that meet are identical or do not cancel each other out (antagonistic). Isolate M3, which is a fusion of M1 and M2, is expected to be more optimal in helping the growth of *P. violaceae* seedlings against drought stress because it is a combination of 2 mycorrhizal *Rhizoctonia* isolates. Therefore, it is necessary to make anatomical observations on the roots of *P. violaceae* to see the association of mycorrhizal *Rhizoctonia* (M1, M2, and M3) with *P. violaceae* in the form of a peloton structure.



A B C

Figure 4. Peloton observations

Description: a. Peloton mycorrhizal *Rhizoctonia* from the roots of *Dendrobium macrophyllum.* b. Peloton mycorrhizal *Rhizoctonia* from the roots of *Dendrobium secundum.* c. Peloton mycorrhizal *Rhizoctonia* combimation from the roots of *Dendrobium macrophyllum* and *Dendrobium secundum*

In Figure 4a, a cross section of *P. violacea* roots inoculated with mycorrhizal *Rhizoctonia* (M1) reveals the presence of round structures within the cortical cells. Round lumps are observed in the middle cell and at the edge of the cell which is the peloton structure. This finding is consistent with research conducted by Zhu *et al.* (2008), which states that peloton is an intracellular hyphal structure that forms clumps in the middle tissue of the root cortex, functions as a carbohydrate reserve and is generally only present for a certain period of time before being decomposed and absorbed by the plant. Peloton infection and decomposition occurs repeatedly in the same cells and tissues for as long as the orchid requires. The formation of this structure is characteristic of mycorrhizal *Rhizoctonia* in orchid plants. However, *P. violacea* roots that had been applied with mycorrhizal *Rhizoctonia* (M2) showed a different peloton structure, namely the peloton clumps were flatter and not round (Figure 4b). This shows that the peloton structure of mycorrhizal *Rhizoctonia* in the root cortex has different shapes. This is in accordance with the opinion of Chen *et al.* (2012) that the peloton structure of each mycorrhizal *Rhizoctonia* isolate has a different shape. In Figure 4c, it can be seen that the mycorrhizal *Rhizoctonia* peloton from both *D. macrophyllum* and *D. secundum* roots is in the *P. violaceae* root cortex, which proves that the two M1 and M2 isolates are not antagonistic, so it is hoped that the two peloton structures formed can function as a source of carbohydrates when *P. violaceae* experiences drought stress.

To increase the capacity of nutrient uptake by orchids in plants, mycorrhizal *Rhizoctonia* first infects the roots and forms a network of hyphae (peloton) in the root cortex. Mycorrhiza in orchids initiates a flow of nutrients where the fungus receives carbon directly from the plant instead of phosphorus or as a substitute for nitrogen for the plant. However, according to Sugiyarto *et al.* (2016), the flow of carbon from fungi to plants or from plants to fungi alternately often occurs, where this flow involves nitrogen and phosphorus nutrients from fungi moving towards plants. Although there are approximately 400 species of orchids that have little carbon nutrient flow from plants, fungi can provide nutrients to orchids, confirming that mycorrhizal *Rhizoctonia* can reduce stress caused by water shortages.

Observations on plant morphology were carried out to provide a visual description of the research results, facilitate understanding, and an explanation of the visualized results based on the mycorrhizal *Rhizoctonia* application factor with watering every 2, 4, and 6 days can be seen in Figure 3.

A,B,C,D E,F,G,H I,J,K,L

Figure3.Morphological appearance of orchid plants based on watering factors every 2, 4, and 6 days.
Description:
a. M0P1: No. application mycorrhizal *Rhizoctonia* & watering once every 2 days
b. M1P1: Application mycorrhizal *Rhizoctonia* M1 & watering once every 2 days
c. M2P3: Application mycorrhizal *Rhizoctonia* M2 & watering once every 2 days
d. M3P1: Application mycorrhizal *Rhizoctonia* M3 & watering once every 2 days
e. M0P2: No. application mycorrhizal *Rhizoctonia* & watering once every 4 days
f. M1P2: Application mycorrhizal *Rhizoctonia* M1 & watering once every 4 days
g. M3P2: Application mycorrhizal *Rhizoctonia* M2 & watering once every 4 days
h. M2P2: Application mycorrhizal *Rhizoctonia* M3 & watering once every 4 days
i. M0P3: No. application mycorrhizal *Rhizoctonia* & watering once every 6 days
j. M1P3: Application mycorrhizal *Rhizoctonia* M1 & watered once every 6 days
k. M2P3: Application mycorrhizal *Rhizoctonia* M2 & watered once every 6 days
l. M2P3: Application mycorrhizal *Rhizoctonia* M3 & watered once every 6 days

From the results of the analysis in Figure 3, it can be seen that the application of mycorrhizal *Rhizoctonia* to the watering interval factor has a different effect on plant height, leaf length, number of roots and root length. Each treatment in Figure 3 is a combination of specific treatments of mycorrhizal *Rhizoctonia* (M0–M3) and watering intervals (P1–P3). Plant morphology in the 12 treatments showed that the plants in the M3P1 treatment were the best with longer and straighter leaves compared to the other treatments. This shows that the combination of mycorrhizal *Rhizoctonia* (M3) from the roots of the orchid *D. macrophyllum* (M1) and the roots of the orchid *D. secundum* (M2) provides a mutually supportive influence on the absorption of nutrients in *P. violacea* seedlings. The effect of mycorrhizal *Rhizoctonia* application and watering interval on plant morphology is quantitatively shown in Table 1.

Table 1. Application of mycorrhizal *Rhizoctonia* and watering intervals on the growth of *Phalaenopsis violacea* seedlings

Treatment	Parameters						
	Tall Plant (cm)	Number Of leaves (cm)	Leaf length (sheet)	Number Of roots (sheet)	Root Length (cm)	Fresh weight of the plant (g)	Plant dry weight (g)
Mycorrhizal *Rhizoctonia* applications (M)							
M_0	15.6 a	9.0	14.1 a	10.0 a	18.3 a	13.7 a	2.5 a
M_1	19.8 bc	11.3	18.3 bc	12.3 b	22.3 bc	15.3 b	2.8 b
M_2	19.3 b	10.3	17.8 b	11.7 b	22.0 bc	16.8 c	3.4 c
M_3	20.7 c	11.7	19.2 c	14.0 c	21.3 b	17.5 c	3.5 c
Time interval for giving water (P)							
P_1	18.5 ab	11.0	17.0 ab	11.5	21.5	16.0	3.1
P_2	20.3 c	11.0	18.8 c	12.3	21.9	16.7	3.3
P_3	17.9 a	9.8	16.4 a	12.3	19.6	14.7	2.7

Note: In each treatment, numbers followed by the same letter are not significantly different in Duncan's Multiple Range Test at the 5% level.

As shown in Table 1, the application of mycorrhizal *Rhizoctonia* had a significant impact on root development, leaf length, plant height, and plant weight. These results confirm that the application of mycorrhizal *Rhizoctonia* showed more optimal growth compared to not applying mycorrhizal *Rhizoctonia*. Application of mycorrhizal *Rhizoctonia* not only accelerates growth, but also forms an effective symbiosis with the root tissue of orchid plants, increasing nutrient absorption, as explained by Oyetunde *et al.* (2020).

The water interval treatment also had a significant impact on plant height and leaf length with the highest value of plant height at P2 (20.3 cm) and the highest leaf length at P2 (18.8 cm). This difference is caused by the interval of once every 4 days being higher than every 2 days which is more or less influenced by mycorrhizal *Rhizoctonia* in increasing the availability of nutrients and water for plants. These findings are consistent with previous research by (Xiaolu W. *Et al.,* 2016), which showed a significant impact of providing water on the growth of *Dendrobium moniliforme*. Collaboration between plants and mycorrhizal *Rhizoctonia* in root associations provides great benefits for plant growth. Overall, the interaction between mycorrhizal *Rhizoctonia* application and watering interval can be seen in Table 2.

In the interaction between the application of mycorrhizal *Rhizoctonia* and the interval of water application, there was a significant impact on plant height and leaf length, but there was no significant impact on other variables such as number of leaves, number of roots, root length, plant fresh weight, and plant dry weight. The highest results were obtained in treatment with a combination of 2 mycorrhizal *Rhizoctonia* (M3) for all watering factors (2, 4, and once every 6 days). This shows that the combination of 2 mycorrhizal *Rhizoctonia* factors plays a greater role in increasing plant vegetative growth when compared to just being applied separately (M1 and M2). This shows a mutually supportive symbiosis between the 2 mycorrhizal *Rhizoctonia* combined resulting in a significant increase in the growth and development of orchid plants, especially in terms of plant height and leaf length. Previous research by Soelistijono *et al.,* (2024). also supports these findings by showing that the growth response of orchid plants often occurs through interactions between two factors working together.

Table 2. Effect of mycorrhizal *Rhizoctonia* application and watering interval on the growth of *Phalaenopsis violacea* seedlings

Treatment	Parameters						
	Tall Plant (cm)	Number Of leaves (cm)	Leaf length (sheet)	Number Of roots (sheet)	Root Length (cm)	Fresh weight of the plant (g)	Plant dry weight (g)
M_0P_1	6.0 bc	3.3	5.5 b	3.3	6.6	4.8	0.9
M_0P_2	5.6 b	3.0	5.1 b	3.3	6.0	5.0	1.0
M_0P_3	4.1 a	2.7	3.5 a	3.3	5.7	3.9	0.6
M_1P_1	6.3 bc	3.7	5.8 bc	3.7	7.2	5.4	1.0
M_1P_2	6.9 cde	3.7	6.4 cd	4.7	7.9	5.1	0.9
M_1P_3	6.6 bcd	4.0	6.1 bc	4.0	7.3	4.8	0.8
M_2P_1	6.2 bc	3.7	5.7 bc	3.7	7.7	5.5	1.1
M_2P_2	6.6 bcd	3.7	6.1 bc	3.7	7.1	6.0	1.3
M_2P_3	6.5 bc	3.0	6.0 bc	4.3	7.2	5.3	1.1
M_3P_1	6.1 bc	4.0	5.6 b	4.7	7.2	5.6	1.2
M_3P_2	8.0 cde	4.3	7.5 d	4.7	8.2	6.2	1.2
M_3P_3	6.7 bcd	3.3	6.2 bcd	4.7	5.9	5.6	1.1

Note: In each treatment, numbers followed by the same letter are not significantly different in Duncan's Multiple Range Test at the 5% level.

Factors that have the potential to increase the number of leaves on plants, such as providing sufficient water and the availability of adequate nutrients, are important factors in plant growth. This is in accordance with the opinion of Hans *et al.* (2017) that the peloton formed in the roots contains carbohydrate components that will be formed into nutrients needed by orchids when there is no supply of nutrients from the environment. Orchids, which are plants with slow growth (at least 2 years to flower), have the potential to be stimulated in growth through the application of OMF (mycorrhizal *Rhizoctonia*). This idea is in line with the view of (Zettler & Corey, 2018), which states that mycorrhiza can increase the ability of plants to absorb water and nutrients from the soil, while plants provide organic material to the fungi. Apart from that, the role of moss planting media is also important in providing a place to grow and store nutrients and water for orchid growth (Soelistijono *et al.,* 2023). Therefore, it is recommended to use moss-based planting media for *P. violacea* seedlings cultivated in areas with minimal water availability or experiencing drought stress. The application factors of mycorrhizal *Rhizoctonia* and water watering intervals also influence the increase in proline and chlorophyll ratios in *P. violacea* leaves. This can be seen in table 3 below.

Table 3. Proline content and chlorophyll ratio comparison in *Phalaenopsis violacea* seedlings

Treatment	Prolin (µmol/g)	Chlorophyll A (mg/g)	Chlorophyll B (mg/g)	Total Chlorophyll (mg/g)
M0P1	0.021	0.04	0.05	0.09
M0P2	0.071	0.13	0.16	0.29
M0P3	0.142	0.10	0.25	0.35
M1P1	0.019	0.05	0.06	0.10
M1P2	0.010	0.20	0.25	0.45
M1P3	0.015	0.48	0.17	0.56
M2P1	0.014	0.33	0.20	0.53
M2P2	0.021	0.20	0.25	0.45
M2P3	0.027	0.17	0.09	0.26
M3P1	0.019	0.31	0.18	0.50
M3P2	0.029	0.35	0.23	0.58
M3P3	0.135	0.57	0.32	0.89

From Table 3 above, it can be seen that the lowest proline results were in the M2P1 treatment, which shows that the application of mycorrhizal *Rhizoctonia* and watering every 2 days (M2P1) shows the ability to control plant stress caused by drought stress. Meanwhile, the highest proline yield was in the M0P3 treatment, which shows that without the application of mycorrhizal *Rhizoctonia* (M0) and watering every 6 days it would cause high stress in the plants. This is in accordance with the opinion of Chun *et al.* (2018); Wang *et al.* (2022) that an increased amount of proline is considered an indication of tolerance to drought stress because proline functions as a nitrogen storage compound and osmoregulator and/or as a protector of certain enzymes. Proline accumulation is higher in drought tolerant plants than in sensitive types, this is due to the higher water holding capacity of the tolerant types. From Table 3 it can also be seen that the application of mycorrhizal *Rhizoctonia* M2 has more influence on resistance to stress than M1, even though the two mycorrhizal *Rhizoctonia* are combined (M3). The proline content in plants correlated with the chlorophyll levels, further indicating that mycorrhizal *Rhizoctonia* treatment enhance drought tolerance by improving both nutrient uptake and stress response mechanisms.

From Table 3, it can be seen that the total chlorophyll content was highest in the M3P3 treatment, which shows that the combined application of mycorrhizal *Rhizoctonia* (M3) and watering every 6 days shows the ability to control plant stress caused by drought stress. Meanwhile, the lowest results in the M0P1 treatment were without application of mycorrhizal *Rhizoctonia* (control) and watering every 2 days. This is in accordance with the opinion of Putri *et al.* (2022) that the chlorophyll content in plants can be an indicator of the level of tolerance to drought. Drought stress treatment significantly affected the contents of chlorophyll a, b, and total in plants. The same opinion was also expressed by Lucia *et al.* (2018) that plants can adapt to reduce the impact of water stress, as seen in sunflower and corn varieties with different drought tolerance by increasing or decreasing the rate of photosynthesis which is strongly influenced by the amount of chlorophyll inside the leaf (Ding *et al.,* 2018). Overall, the combined application of mycorrhizal *Rhizoctonia* (M3) resulted in the most significant increase in plant height, number of leaves, root length, proline content, and chlorophyll levels compared to other treatments.

CONCLUSIONS

The application of orchid mycorrhizal fungi (especially *Rhizoctonia*) significantly enhances the drought resistance of *P. violacea* seedlings. The combined application of mycorrhizal *Rhizoctonia* (M3) resulted in the most significant improvements in plant height, leaf number, root length, proline content, and chlorophyll levels compared to other treatments.

FUTURE RESEARCH

In the future, research can be developed in applying OMF isolates to several other orchids besides *Phalaenopsis* sp. such as *Vanda* sp. *Cattleya* sp. *Paphiopedillum* sp. and so on. So that later it can be used as the best OMF comparison in increasing resistance to drought stress in orchids that are vulnerable in Indonesia.

ACKNOWLEDGMENT

The authors would like to thank the Directorate of Research, Technology, and Community Service, Higher Education, Ministry of Education and Cultural, for the financial support of this research grant for the 2023 fiscal year contract number: 185/E5/PG.02.00.PL/2023

REFERENCES

Ahmad, H., N.A. Rajput, M. Atiq, G.A. Kachelo, M. Usman, H. Tariq and M. Wahab. 2024. Detection of *Phytophthora nicotiana* induced citrus gummosis by the loop mediated isothermal amplification. Pakistan Journal of Botany, 56(5): 1-11.

Ajayi-Oyetunde, O.O. and C.A. Bradley. 2017. *Rhizoctonia solani*: Taxonomy, population biology and management of Rhizoctonia seedling disease of soybean. Plant Pathology, 67: 3–17.

Ajayi-Oyetunde, O.O. and C.A. Bradley. 2020. Identification and characterization of *Rhizoctonia* species. A Lab & Experiment, DOI: 10.1094/PDIS-06-16-0810-RE.

Atiq, M., L. Malik, N.A. Rajput, M. Shabbir, M. Ashiq, M.J. Matloob, A. Waseem, A. Jabbar, A. Ullah, R. Zawar and A. Majeed. 2024. Evaluating the efficacy of plant-mediated copper-silver nanoparticles for controlling Cercospora leaf spot in mung beans. Plant Protection, 08 (04): 661-670.

Attri, L.K. 2023. Studies on mycorrhizal associations in orchids. Asian Journal of Biological and Life Sciences, 2(1): 179–186.

Bashir, S.S., A. Hussain, S.J. Hussain, O.A. Wani, S. Zahid Nabi, N.A. Dar and S. Mansoor. 2021. Plant drought stress tolerance: Understanding its physiological, biochemical and molecular mechanisms. Biotechnology & Biotechnological Equipment, 35(1): 1912–1925.

Chen, J., H. Wang and S.X. Guo. 2012. Isolation and identification of endophytic and mycorrhizal fungi from seeds and roots of *Dendrobium* (Orchidaceae). Mycorrhiza, 22: 297–307.

Chéour, F., I. Kaddachi, D. Achouri, S. Bannour and L.

Zorgui. 2014. Effects of water stress on relative water, chlorophylls and proline contents in barley (*Hordeum vulgare* L.) leaves. IOSR Journal of Agriculture and Veterinary Science, 7: 13–16.

Chun, C.S., M. Paramasivan and M. Chandrasekaran. 2018. Proline accumulation influenced by osmotic stress in arbuscular mycorrhizal symbiotic plants. Frontiers in Microbiology, 9: 2525.

De, L.C. and R.P. Medhi. 2014. Climate change and its impact on orchid productivity. International Journal of Scientific Research, 3(10): 500–504.

Ding, L., Z. Li, G. Limun, S. Guo and Q. Shen. 2018. Is nitrogen a key determinant of water transport and photosynthesis in higher plants upon drought stress? Frontiers in Plant Science, 9: 1143.

He, M., C.Q. He and N.Z. Ding. 2018. Abiotic stresses: General defenses of land plants and chances for engineering multistress tolerance. Frontiers in Plant Science, 9: 1771.

Hossain, M.M. 2019. Morpho-molecular characterization of *Ceratobasidium* sp.: A mycorrhizal fungi isolated from a rare epiphytic orchid *Gastrochilus calceolaris* (J. E. Sm.) D. Don. Bangladesh Journal of Plant Taxonomy, 26(2): 249–257.

Jacquemyn, J., M. Waud, R. Brys, F. Lallemand, P.E. Courty, A. Robionek and M.A. Selosse. 2017. Mycorrhizal associations and trophic modes in coexisting orchids: An ecological continuum between auto- and mixotrophy. Frontiers in Plant Science, 8: 1497.

Khan, Y., A. Sohail, T. Yaseen, K.U. Rehman, M. Noor and K. Akbar. 2021. *Arbuscular mycorrhizal* fungi improved the growth and yield productivity of Lens esculenta under the influence of poultry litter. Pakistan Journal of Phytopathology, 33(1): 14-25.

Lucia, G., E.L. Piccolo and M. Landi. 2018. Chlorophyll fluorescence, photoinhibition and abiotic stress: Does it make any difference the fact to be a C3 or C4 species? Frontiers in Plant Science, 10: 174.

Misawa, T. and D. Kurose. 2019. Anastomosis group and subgroup identification of *Rhizoctonia solani* strains deposited in NARO Genebank, Japan. Journal of General Plant Pathology, 85(4): 282–294.

Putri, A.E., E. Ernawiati, Priyambodo, R. Agustrina and L. Chrisnawati. 2022. Chlorophyll as indicator of drought tolerance level in upland rice germination of Lampung local variety, Lumbung Sewu Cantik. Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati, 7(2): 142–150.

Putri, F.S., E. Nurcahyani, Yulianty and B. Irawan. 2019. Effect of drought-stress conditions in chlorophyll content of *Dendrobium* sp. planlets. Jurnal Ilmiah Biologi Eksperimen dan Keanekaragaman Hayati, 6(1): 20–26.

Sabatini, A.P., E. Nurcahyani, Yulianty and R. Agustrina. 2022. Response of *Cattleya* sp. orchid planlet in vitro selection results against drought stress with polyethylene glycol (PEG) 6000. Indonesian Journal of Biotechnology and Biodiversity, 6(2): 61–67.

Salazar, J., M. Pomavila, A.T. Pollard, E.J. Chica and D.F. Pena. 2020. Endophytic fungi associated with roots of epiphytic orchids in two Andean forests in southern Ecuador and their role in germination. Lankesteriana: International Journal on Orchidology, 20(1): 1–12.

Sasongko, A.B., F. Asruwaidah and I. Ari. 2016. The growth improvement of *Grammatophyllum scriptum* (Lindl.) Bl. in vitro plantlet using photoautotrophic micropropagation system. Indonesian Journal of Biotechnology, 21(2): 109–116.

Shah, S., S. Biva, R. Singh, B. Rekawad, Y.S. Shouce, J. Sharma and B. Pant. 2022. Colonization with non-mycorrhizal culturable endophytic fungi enhances orchid growth and indole acetic acid production. BMC Microbiology, 22: 101.

Shakoor, S., M. Inam-ul-Haq, S. Bibi and R. Ahmed. 2015. Influence of root inoculations with Vasicular arbuscular mycorrhizae and rhizomyx for the management of root rot of chickpea. Pakistan Journal of Phytopathology, 27(2): 1-15.

Sneh, B., E. Yamoah and A. Stewart. 2004. Hypovirulent *Rhizoctonia* spp. isolates from New Zealand soils protect radish seedlings against damping-off caused by *R. solani*. New Zealand Plant Protection, 57: 54–58.

Sneh, B., L. Burpee and A. Ogoshi. 1992. Identification of *Rhizoctonia* species. CABI, 133 pp.

Soelistijono, A.P., S. Endang and S. Christanti. 2011. Characterization of pathogenic *Rhizoctonia* sp. and mycorrhizal *Rhizoctonia* isolates on terrestrial orchid plant *Spathoglottis plicata*. Biota, 16(2): 121–130.

Soelistijono, R., A.W. Jakti, A. Budiyono, E. Suprapti and Daryanti. 2023. The uses of *Rhizoctonia* mycorrhizae as a dry resistance induction of *Dendrobium aggregatum* seedlings. Malaysian Applied Biology, 52(2): 147–151.

Soelistijono, R., Daryanti and M.T. Handayani. 2018. Isolation of mycorrhizal *Rhizoctonia* as resistance inducer of *Dendrobium macrophyllum* to drought. IOP Conference Series: Earth and Environmental Science, 142(1): 012013.

Soelistijono, R., E. Puspitasari, H. Haryuni, S. Priyadi, A.F. Aziez and D. Rakhmawati. 2024. Utilization of *Rhizoctonia* mycorrhiza in the management of *Fusarium* sp. seedling orchid *Dendrobium nindii*. AIP Conference Proceedings, 2957(1): 012021.

Sugiyarto, L., S. Umniyatie and V. Henuhili. 2016. Diversity of natural orchids and the presence of orchid mycorrhiza in Turgo Pakem Hamlet, Sleman Yogyakarta.. Jurnal Sains Dasar, 5(2): 71–78.

Suminar, E., S. Mubarok, A. Nuraini, H. Ezura and F.W. Fitriatin. 2020. Proline, chlorophyll, and yield content of *IAA9* mutant tomato plants under high temperature stress conditions. Jurnal Agrikultura, 31(3): 280–287.

Tian, F., X.F. Liao, L.H. Wang, Y.B. Yang, Z.Q. Luo and F.X. Yan. 2021. Isolation and identification of beneficial orchid mycorrhizal fungi in *Paphiopedilum barbigerum* (Orchidaceae). Plant Signaling & Behavior, 17(1): 2005882.

Tyagi, E., A. Singh, A. Gupta, M. Prasad and R. Ranjan. 2022. Mechanism and function of salicylate in plant toward biotic stress tolerance. Emerging Plant Growth Regulators in Agriculture, Chapter 4: 131–164.

Usman, M., M. Atiq, N.A. Rajput, M. Sagheer, Y. Xia. 2025. Computational and experimental approaches to explore defense related enzymes conferring resistance in *Fusarium* infected chilli plants by regulating plant metabolism through nutritional products. PLoS One. 20(1): e0309738

Wang, Z., Y. Yang, V. Yadav, W. Zhao, Y. He, X. Zhang and C. Wei. 2022. Drought-induced proline is mainly synthesized in leaves and transported to roots in watermelon under water deficit. Horticultural Plant Journal, 8(5): 615–626.

Xiaolu, W., J. Yuan, A. Luo, Y. Chen and Y. Fan. 2016. Drought stress and re-watering increase secondary metabolites and enzyme activity in *Dendrobium moniliforme*. Industrial Crops and Products, 94: 385–393.

Zettler, L.W. and L.L. Corey. 2018. Orchid mycorrhizal fungi: Isolation and identification techniques. Springer Protocols Handbooks, 27–59.

Zhu, G.S., Z.N. Yu, Y. Gui and Z.Y. Liu. 2008. A novel technique for isolating orchid mycorrhizal fungi. Fungal Diversity, 33: 123–137.

Contribution of Authors:

Raden Soelistijono	:	Conducting and coordinating each stage of research, especially identification and determining the combination of mycorrhizal *Rhizoctonia* (M3 isolates)
Daryanti	:	Care and maintenance of *Phalaenopsis violaceae* seedlings in green house
Hadad M. N. Kholiq	:	Watering interval in green house and growth parameter measurement
Cecep Hidayat	:	The selection provides orchids species *D.* macrophdyllum and *D. secundum* from Gede mountain
Achmadi Priyatmojo	:	Identification of mycorrhizal *Rhizoctonia* based on their anastomosis group