Biosaintifika 18 (2) (2026): 263-272 p-ISSN 2085-191X | e-ISSN 2338-7610
Journal of Biology & Biology Education https://journal.unnes.ac.id/nju/index.php/biosaintifika

Orchid Mycorrhizal Fungi Combination to Overcome Water Stress in Dendrobium Spectabile Orchids

R. Soelistijono[1]*, Prisma Aditya Rianto[2], Achmad Fatchul Aziez[2], Cecep Hidayat[3], Herry Utomo[4]

[1]Agricultural Faculty, Universitas Pembangunan Nasional "Veteran" Yogyakarta (55283) Indonesia
[2]Agricultural Faculty, UniversitasTunas Pembangunan Surakarta(57139) Indonesia
[3]Faculty of Science and Technology, UIN Sunan Gunung Djati Bandung (40614) Indonesia
[4]LSU AgCenter H.Rouse Caffey Rice Research Station Crowley LA (70578) USA

*Correspondence author: soelistijono@upnyk.ac.id

Submitted: 2026-03-23. Revised: 2026-05-28. Accepted: 2026-07-29.

Abstract. One strategy to mitigate drought stress is using orchid mycorrhizal fungi (OMF) of the *Rhizoctonia* type. This study aims to determine the use of a combination of *Rhizoctonia* mycorrhiza isolated from the roots of two *Dendrobium* orchid species and watering intervals on *Dendrobium spectabile* seedlings. The research method used was a randomized block design (RBD) with 2 factors and 5 replications: the first factor was inoculation of OMF isolated from the roots of *Dendrobium fimbriatum* (D1), *Dendrobium phalaenopsis* (D2), and a combination of OMF D1 + D2 (D3). The second factor included watering intervals every 2-days (W1), 4 days (W2), and every 6-days (W3). The results showed that OMF inoculation factors on *D. spectabile* gave significantly different results on plant height, leaf length, root length, plant fresh weight, and plant dry weight, with the best results using the OMF combination (D3). The watering interval factor gave significantly different results on plant height and number of roots, with the best results on watering every 4-days (W2). The highest chloroplast content was found in D3W3 (0.89), while proline accumulation reached its peak at D2W3 (0.135). Microscopic observations confirmed the formation of pelotons in the root cortex. Overall, the combined OMF inoculum applied with a 4-day watering interval (D3W2) is a promising approach to improve drought tolerance in *D. spectabile* seedlings. This research supports SDG 15 (Life on Land Ecosystems), as a solution to overcome water stress, the main cause of desertification and land degradation that affects the growth of orchid species in nature

Keywords: Orchid Mycorrhizal Fungi (OMF); *Rhizoctonia* mycorrhiza; *Dendrobium spectabile*; chloroplast; proline.

How to Cite: Soelistijono, R., Rianto, P. A., Aziez, A. F., Hidayat, C., & Utomo, H. (2026). Orchid Mycorrhizal Fungi Combination to Overcome Water Stress in Dendrobium Spectabile Orchids. *Biosaintifika: Journal of Biology & Biology Education, 18(2)*, 263-272.

DOI: http://dx.doi.org/10.15294/biosaintifika.v18i2.42840

INTRODUCTION

Orchids have high economic value as a commodity for the domestic market and for export (Dodson, 2025). Dendrobium orchids are one of the orchids that are in demand by the public because these orchids have flowers that vary in the diversity of shapes and colors of their flowers. One of the orchids that has the potential to be developed is *Dendrobium spectabile, having* ornamental plants with high economic value and relatively more durable color beauty. Currently, several orchid species, including *D. spectabile*, are rarely found in the wild. This is due to abiotic factors, including prolonged drought stress in Indonesia and the long-term removal of many from their natural habitats (Alderton-moss, 2022; Soelistijono et al., 2024). Chemical treatments are often used to promote growth, but these methods are not environmentally friendly and provide only short-term benefits because they do not penetrate plant tissues and are not systemic (Ario & Setiawan, 2020). An alternative approach is the inoculation of orchids with orchid mycorrhizal fungi (OMF).

There are several OMF groups in nature that are able to associate with orchids, including *Tunasnela*, *Sebacina*, *Ceratobacidium*, and *Rhizoctonia* (Attri, 2023; Gao, 2021; Tian et al., 2022). Of the several OMFs in orchids, *Rhizoctonia* is the most common group found in orchid species. Each orchid in nature has a different relationship with its *Rhizoctonia* mycorrhiza as endophytic fungi**.** When associated with orchids, *Rhizoctonia* mycorrhiza will form a peloton structure on the orchid root cortex, which functions to supply several nutrients needed by orchids from their environment when there is a lack of nutrients (Salazar et al., 2020). In nature, *D. spectabile* orchids have begun to decline due to environmental factors that are not supportive, one of which is drought stress; therefore, new techniques are needed to overcome this (Ario & Setiawan, 2020).

Several previous studies have been conducted on the use of *Rhizoctonia* mycorrhiza to overcome drought stress in hybrid orchids propagated in vitro (Soelistijono et al., 2018; Li et al., 2021). From this study, the ability of *Rhizoctonia* mycorrhiza association with *Dendrobium macrohyllum* can be seen from the formation of a peloton structure in the roots of *D. macrohyllum*. However, this study does not explain the extent of the effect of *Rhizoctonia* mycorrhiza inoculation on plant growth. Although each orchid seed has a specific relationship with each *Rhizoctonia* mycorrhiza, conducted by Chen et al., (2012) proved that *Rhizoctonia* mycorrhiza isolated from *Dendrobium lasiantera* was able to associate with *Dendrobium aggregatum*. From this study, it can be seen that *Rhizoctonia* mycorrhiza inoculation has a significant effect on seedling height, leaf length, number of roots, and fresh weight of seedlings. This shows that several isolates of *Rhizoctonia* mycorrhiza isolated from different orchids will affect the vegetative growth of plants. Rhizoctonia mycorrhiza has two benefits: it is ecologically benign and systemic, as shown by the development of pelotons in the root cortex of *D. aggregatum*.

Two OMF isolates from *Dendrobium fimbriatum* and *Dendrobium phalaenopsis* were employed in this work, and *D. spectabile* seeds from tissue culture will be used for testing. The advantage of inoculation with the double OMF isolate can help each other in increasing the ability to absorb complementary nutrients from the environment, which causes increased photosynthesis in *D. spectabile* and becomes a solution in overcoming drought stress during the dry season. Therefore, the purpose of this study is to explore the effect of two OMF isolates in controlling drought stress occurring in nature in *D. spectabile* orchids, which are very important for conservation and propagation because they are increasingly threatened by drought and habitat loss. The benefits that can be obtained include supporting science on the use of microbes/germplasm from nature (in the concept of sustainable agriculture) to overcome water stress in *D. spectabile* orchids in nature, while the benefits for orchid farmers are that they can increase the cultivation of *D. spectabile* orchids as one of the sources of germplasm for orchid species in nature.

METHODS

Isolation of Orchid Mycorrhizal Fungi (OMF)

Orchid mycorrhiza fungi (OMF) were obtained from OMF isolation on *D. fimbriatum* roots (D1); D2 isolates were obtained from OMF isolation on *D. phalaenopsis* roots, while D3 isolates were a combination of D1 and D2 isolates (D1 + D2) according to the method of (Tian et al., 2022). The three OMF isolates (D1, D2, and D3) were cultured on potato dextrose agar (PDA) media for 7 days, then macroscopic observations were carried out to identify the colony shape and hyphal structure of OMF which is a group of *Rhizoctonia* mycorrhiza (Suryantini et al., 2015) and microscopic observations and anatomical observations using safranin O (Bandoni R.J., 2018). After being identified as a *Rhizoctonia* mycorrhiza group, each isolate D1, D2, and D3 was placed in potato dextrose (PD) liquid media with a ratio of 1 g of isolate D1, D2, and D3 in 100 ml PD and incubated for 7 days.

OMF Inoculation and Watering Interval Treatments

D. spectabile seedlings aged 8 months were placed in pots containing moss and applied with 10 ml of PD liquid media on the roots according to each treatment (D1, D2, D3) and without OMF (D0), and acclimatized for 2 months. The growth of *D. spectabile* seedlings was observed every week from 8 to 10 months of age. Successful inoculation was evidenced by the growth ratio and formation of a peloton structure in cross-sections of *D. spectabile* seedling roots stained with safranin O in the 9th month according to Soelistijono et al., (2023).

Research Design

The experimental design used a Complete Randomized Block Design (CRBD) with two treatment factors. The first treatment consisted of: without OMF application (D0), with OMF application from *D. fimbriatum* roots (D1), with OMF application from *D. phalaenopsis* roots (D2), and with OMF application combination D1+D2 (D3). The second treatment was a watering interval of every 2 (W1), 4 (W2), and 6 (W3) days. There were 12 treatment combinations with three blocks and 3 replications, resulting in a total of 118 experimental units. The parameters observed included the formation of the peloton structure, plant height, leaf length, number of leaves, root length, number of roots, plant fresh weight, plant dry weight, chlorophyll amount, and proline content. Data were analyzed using Analysis of Variance (ANOVA) with a significance level of 5% and 1%, as well as Duncan's Multiple Range Test (DMRT) with a significance level of 5%.

RESULTS AND DISCUSSION

Observations showed that the OMF isolate from *D. fimbriatum* formed a small, irregularly developed circular colony, whereas the isolate from *D. phalaenopsis* produced a larger, uniformly circular colony with evenly distributed hyphae along the edges. The *D. phalaenopsis* isolate also exhibited faster hyphal colony expansion compared to the *D. fimbriatum* isolate (Table 1). The results of OMF isolation from *D. fimbriatum* and *D. phalaenopsis* on PDA media showed different colony shapes (Figure 1).

Both isolates were suspected to belong to the *Rhizoctonia* mycorrhiza group because their colonies displayed the characteristic evenly circular morphology, despite differences in colony color. This was consistent with Mahfut et al., (2020), who reported that *Rhizoctonia* colonies typically had a uniformly circular surface pattern. The faster growth of the *D. phalaenopsis* isolate might be related to morphological traits such as larger or more rapidly extending hyphae, indicating the need for detailed observations of hyphal shape and size. However, anatomical examination was still required to confirm whether these isolates were indeed *Rhizoctonia* mycorrhiza by examining the number of cells and the shape of the hyphal branches (Figure 2).

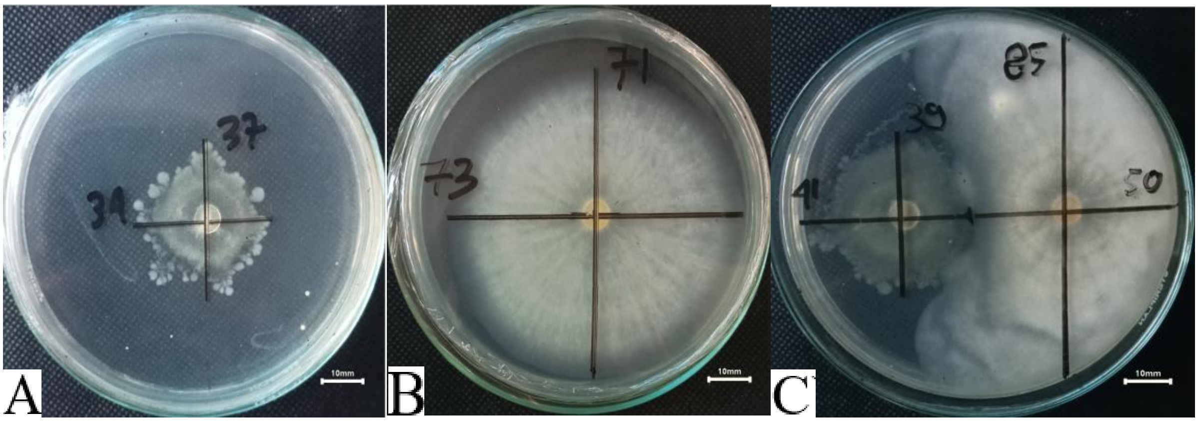

Figure 1. (A) OMF isolate colony of *Dendrobium fimbriatum* aged 7 days, (B) OMF isolate colony of *Dendrobium phalaenopsis,* aged 7 days, (C) Colonies of OMF isolates from *Dendrobium fimbriatum* and *Dendrobium phalaenopsis* were 7 days old

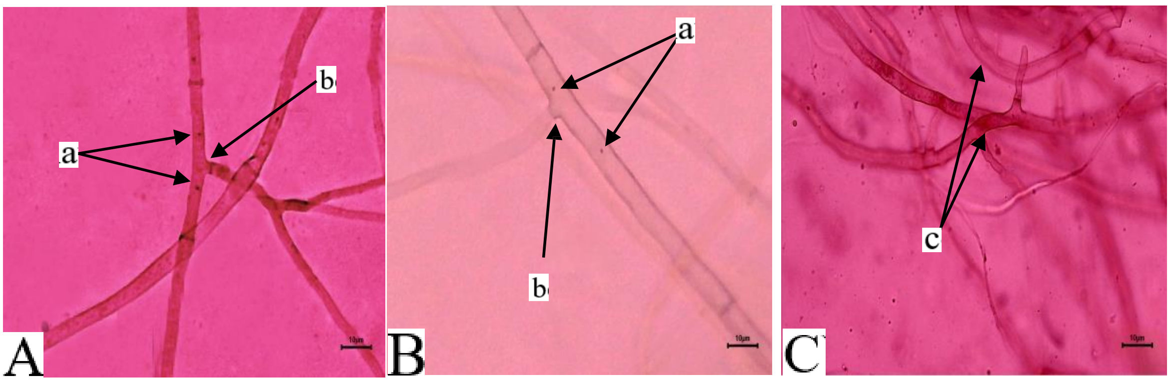

Figure 2. (A) Hyphae OMF isolate from *Dendrobium fimbiratum*, (B) Hyphae OMF isolate from *Dendrobium phalaenopsis*, (C) Hyphae OMF isolates from *Dendrobium fimbiratum* and *Dendrobium phalaenopsis*
(a) 2 nuclei/binucleate; (b) Right-angled hyphae branching; (c) Two kinds of hyphae

Figure 2 shows that the OMF hyphae isolated from *D. fimbriatum* had a thin hyphae diameter of about 3 µm, thinner than those isolated from *D. phalaenopsis* roots, which had a thickness of about 5 µm. The difference in hyphae size was likely a factor in the faster growth rate of OMF isolated from *D. phalaenopsis* compared to the growth of OMF isolated from *D. fimbriatum* (Figure 1). This was because, with the larger hyphae size, OMF isolated from *D. phalaenopsi*s absorbed more nutrients from the PDA.

Anatomical identification from Figure 2 showed that the OMF (D1, D2, and D3) carried out showed the presence of brown mycelium with branching hyphae that form right angles (90^0 angles), were septate, and each cell had 2 nuclei (binucleate). The observation results in Figure 2 showed that all OMF (D1, D2, and D3) included the characteristics of the *Rhizoctonia* mycorrhiza group according to Sneh et al., (2004) namely hyphae forming right-angled branches and having 2 nuclei. This finding was in line with the research of Soelistijono et al., (2020) and Gao, (2021) which showed that OMF isolated from orchids had irregularly shaped brown hyphae connected by brown mycelial threads forming right angles and having 2 nuclei and were classified as *Rhizoctonia* mycorrhiza, although some *Rhizoctonia* mycorrhiza were also 3-nucleated (Moliszewska et al., 2023). Figure 2C showed that the two OMF hyphae did not meet each other (conjugation) thus proving that the two OMF were from different groups based on their anastomosis groups (AGs). This was in accordance with the opinion Sneh et al., (2004) that only the same species (group) of *Rhizoctonia* sp. was capable of fusion in its hyphae. To see the effect of providing OMF isolate on the growth of *D. spectabile* seedlings inoculated with OMF both D1, D2, D3 and those not inoculated with OMF (D0) and combined with watering intervals of 2 (W1), 4 (W2), and 6 (W3) days can be seen in Figure 3.

The observation results in Figure 3 showed that the provision of OMF and watering intervals had different effects on plant height, leaf length, number of roots, and root length. Plant morphology in 12 treatments showed that plants with the D3W2 treatment were better than other treatments, with longer, straighter leaves and more roots. It was suspected that inoculation with the OMF combination isolated from the roots of *D. fimbriatum* and *D. spectabile* accelerates the ability of *D. spectabile* seedlings to absorb nutrients. Therefore, it was necessary to conduct anatomical observations on the roots of *D. spectabile* that had been inoculated to determine the presence or absence of OMF in *D. spectabile* seedlings.

Figure 3. Effect of inoculation of various OMF on *Dendrobium spectabile* seedlings with different watering factors.

(A) D0W1: Without OMF and watering once every 2 days
(B) D1W1: Inoculated with OMF isolation from *D. fimbriatum* roots and watered every 2 days
(C) D2W1: Inoculated with OMF isolation from *D. phalaenopsis* roots and watered every 2 days
(D) D3W1: Inoculated with OMF isolation from *D. fimbriatum* + *D. spectabile* roots and watered every 2 days
(E) D0W2: Without OMF and watered once every 4 days
(F) D1W2: Inoculated with OMF isolation from *D. fimbriatum* roots and watered every 4 days
(G) D2W2: Inoculated with OMF isolation from *D. phalaenopsis* roots and watered every 4 days
(H) D3W2: Inoculated with OMF isolation from *D. fimbriatum* + *D. spectabile* roots and watered every 4 days
(I) D0W3: Without OMF and watered once every 6 days
(J) D1W3: Inoculated with OMF isolation from *D. fimbriatum* roots and watered every 6 days
(K) D2W3: Inoculated with OMF isolation from *D. phalaenopsis* roots and watered every 6 days
(L) D3W3: Inoculated with OMF isolation from *D. fimbriatum* + *D. spectabile* roots and watered every 6 days

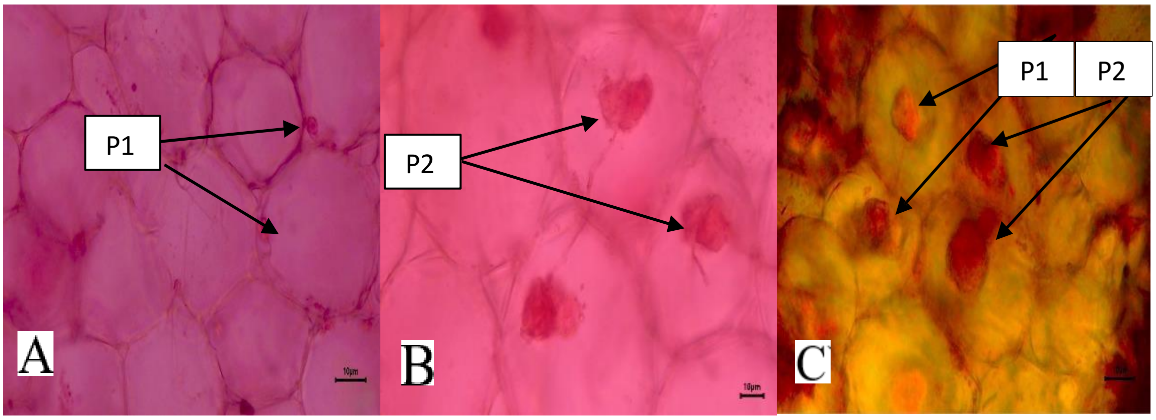

Figure 4. (A) Peloton structure in the root cortex of *Dendrobium spectabile* inoculated with OMF isolate from *Dendrobium fimbriatum* (D1), (B) Peloton structure in the root cortex of *Dendrobium spectabile* inoculated with OMF isolate from *Dendrobium phalaenopsis* (D2), (C) Peloton structure in the root cortex of *Dendrobium spectabile* inoculated with OMF combination isolates from *Dendrobium fimbriatum* and *Dendrobium phalaenopsis* (D3)

The penetration of OMF hyphae into the orchid roots formed a peloton structure. The presence of a peloton structure and two nuclei (Figure 4) within the hyphae indicated that the OMF was *Rhizoctonia* mycorrhiza (Sneh et al., 2004). The peloton structure indicated the occurrence of a symbiosis between orchid roots and *Rhizoctonia* mycorrhiza(Smith & Read, 2008; Soelistijono et al., 2020; Michael et al., 2023) and this symbiosis was mutually beneficial. The peloton was a collection of intracellular hyphae found in the root cortex and was only found during certain periods when needed, such as when the orchid experiences drought stress before finally lysing (Soelistijono et al,.2018). The peloton served as a site for nutrient exchange between the fungus and the orchid. The orchid obtained carbohydrates, glutamine, glutamic acid, aspartic acid, and nicotinic acid (Sugiarto et al., 2016). The penetration of OMF hyphae, D1, D2, and D3, into the epidermal tissue of *D. spectabile* roots and ultimately reaching the cortex, forming a peloton structure, as seen in Figure 4.

The size of the peloton (P1) in the root cortex of *D. spectabile* induced with OMF isolate from *D. fimbriatum* (D1) had a smaller clump diameter and was somewhat spaced compared to the peloton (P2) induced with OMF isolate from *D. phalaenopsis* (D2). This was thought to be because the development of the D1 fungus was slower when compared to the D2 fungus, because the hyphae size of the OMF isolate from *D. phalaenopsis* had a larger hypha diameter so it was easier to absorb nutrients from the environment (Figure 2B).When D. spectabile was infected with OMF combination isolates (D3) from *D. fimbriatum* and *D. phalaenopsis*, pelotons in the root cortex were seen,, its appearance was denser, and there were 2 forms of large and small pelotons (P1 and P2) because this consortium (D3) was a combination of 2 different OMF isolates (D1 and D2).

Previous research has shown that there is an increase in nutrient absorption capacity in orchids after OMF from *Rhizoctonia* mycorrhiza infection occurs first in the roots and forms a hyphal network in the root cortex (Soelistijono et al., 2020). Orchid mycorrhiza initiates a nutrient flow in which the fungus receives carbon directly from the plant, either as a substitute for phosphorus or as a substitute for nitrogen in the plant. However, according to Sugiarto et al., (2016), carbon flow from fungus to plant or from plant to fungus often occurs alternately, and this flow transfers nitrogen and phosphorus nutrients from fungus to plant. Therefore, it can be confirmed that OMF can reduce stress due to water shortages. The vegetative growth of *D. spectabile* that has been inoculated with isolates D1, D2, and D3 showed significant differences in several growth parameters as can be seen in Table 1.

Table 1. Growth of *Dendrobium spectabile* orchids in OMF induction treatment and watering interval treatment

Treatment	Parameter						
	Plant Height (cm)	**Number of Leaves (Sheets)**	**Leaf Length (cm)**	**Fresh Plant Stove Weight (g)**	**Dry Plant Slab Weight (g)**	**Root length (cm)**	**Number of Roots**
Orchid Mycorrhiza Fungi (D)							
D0	6.33 a	2.10	3.29 a	4.88 a	1.13 a	3.10 a	4.78 a
D1	6.89 ab	2.33	3.47 ab	5.60 ab	1.26 ab	3.51 ab	7.11 ab
D2	7.2 bc	2.41	3.62 ab	6.47 ab	1.58 ab	4.17 ab	7.56 b
D3	8.86 d	2.69	4.13 c	8.67 c	2.28 c	5.56 b	10.33 c
Watering Interval (W)							
W1	7.2 ab	2.25	3.69	6.49	1.46	3.85	7.17 ab
W2	7.97 b	2.36	3.80	7.01	1.60	4.97	9.08 b
W3	6.79 a	2.25	3.38	5.70	1.62	3.48	6.08 a

Description: In each treatment, numbers followed by the same letter indicate no significant difference in Duncan's Multiple Range Test at 5% level.

Based on the results of the 5% DMRT test in Table 1, the application of OMF showed a very significant effect on the parameters of plant height, leaf length, number of roots, fresh weight of plants and dry weight of plants, but did not have a significant effect on the parameter of number of leaves. This indicates that the application of OMF plays an active role in stimulating the growth of orchid plants for each growth parameter with the best results in the D3 treatment (combination). The results of this study are in line with the opinion of Soelistijono et al., (2018) namely that the administration of OMF mycorrhiza to orchid plants has shown promising effects in increasing their resistance to drought. OMF from *Rhizoctonia* mycorrhiza groups applied to plants (D1, D2, and D3) and associated with orchids in the form of peloton (P1, P2, and P3) plays a role in providing nutrients for plant growth, especially in terms of leaf length which is closely related to more effective light and CO2 capture so that the rate of photosynthesis increases and is also related to the growth of orchid tubers, the formation of new shoots on the tubers. The combination of OMF (D3) increases the number of *D. spectabile* roots. The more roots, the more nutrients are absorbed. This is because OMF hyphae that have infected plant roots can help roots in absorbing nutrients and water in areas that are inaccessible to plant roots (Ajayi-oyetunde et al., 2017). This causes an increase in the weight of fresh orchid stover so that it is expected to be able to withstand drought stress.

The watering-interval treatment significantly influenced plant height and root number, but had no significant effect on leaf number, leaf length, root length, fresh weight, or dry weight. These results suggest that watering frequency plays an important role in stimulating the growth of *D. spectabile*, with the most favorable response observed under watering every 2 days (W1). This aligns with Sabatini et al., (2022), who reported that watering intervals have a substantial impact on orchid growth and development.

In the interaction between the administration of OMF and the watering interval, the study found a significant impact on plant height, leaf length, number of roots, root length, and fresh weight of the plant, but there was no significant impact on other variables such as the number of leaves and dry weight of the plant. These results indicate that the combination of these two factors can provide a significant increase in the growth and development of orchid plants, especially in terms of plant height and number of roots, with the treatment factor of OMF administration playing a greater role (D1, D2, and D3). Previous research by Soelistijono et al., (2018) also supports these findings by showing that plant growth responses often occur through interactions between two factors working together and that OMF from *Rhizoctonia* mycorrhiza groups plays a more significant role.

Table 2. Effect of combination of OMF application and watering interval on the growth of *Dendrobium spectabile* seedlings

	Parameter						
Treatment	**Plant Height (cm)**	**Number of Leaves (Sheets)**	**Leaf Length (cm)**	**Number of Roots**	**Root length (cm)**	**Fresh Plant Stove Weight (g)**	**Dry Plant Slab Weight (g)**
D0W1	6.57^{a}	2.14	3.13^{a}	4.00^{a}	2.56^{a}	5.50a	1.24
D0W2	6.70^{a}	2.14	3.23^{a}	5.33^{a}	3.40^{a}	5.00^{a}	1.12
D0W3	5.73^{b}	2.00	3.50^{a}	5.00^{a}	3.33^{a}	4.13^{a}	1.01
D1W1	6.70^{a}	2.40	3.70^{a}	7.00^{b}	2.93^{a}	5.80^{a}	1.31
D1W2	7.50^{b}	2.33	3.66^{a}	9.66^{c}	4.40^{b}	5.66^{a}	1.27
D1W3	6.47^{a}	2.25	3.03^{a}	4.66^{a}	3.20^{a}	5.33^{a}	1.20
D2W1	7.10^{b}	2.81	3.63^{a}	7.66^{b}	3.80^{a}	6.33^{a}	1.42
D2W2	7.87^{c}	2.22	3.83^{b}	9.66^{c}	5.00^{b}	6.76^{a}	1.54
D2W3	6.63^{a}	2.18	3.40^{a}	5.33^{a}	3.70^{a}	6.30^{a}	1.78
D3W1	8.43^{c}	2.74	4.30^{b}	1.00^{c}	6.10^{b}	8.33^{b}	1.88
D3W2	9.80^{c}	2.74	4.50^{b}	11.66^{c}	7.10^{b}	10.63^{b}	2.46
D3W3	8.33^{c}	2.59	3.60^{a}	9.33^{b}	3.70^{a}	7.05^{b}	2.50

Description: In each treatment, numbers followed by the same letter indicate no significant difference in Duncan's Multiple Range Test at the 5% level.

Factors that have the potential to increase leaf number in plants, such as adequate water supply and nutrient availability, are important factors in plant growth. Orchids, which are slow-growing plants, have the potential to stimulate their growth through the application of OMF. This idea aligns with the view (Tsulsiyah et al., 2021), which states that mycorrhizae can increase a plant's ability to absorb water and nutrients from the soil, while the plant provides organic matter to the fungus. Furthermore, the role of moss as a growing medium is also important in providing a place for plants to grow and storing nutrients and water for orchids. Therefore, it is recommended to use moss-based growing media for *D. spectabile* seedlings cultivated in areas with minimal water availability or experiencing drought stress. Plants experiencing drought stress form proline. Proline is a compound (with the chemical formula C5H9NO2C5H9NO2) that is an amino acid that functions as an important component in protein synthesis (Xi et al., 2020). Although often considered an amino acid, proline has a unique structure because the nitrogen in the amino group is bound to two carbons, making it a secondary amine (Suminar et al., 2020; Wang et al., 2022).

Table 3 showed that the lowest proline yield was found in the D2W1 treatment, which indicated that the application of OMF isolated from *D. Phalaenopsis* and watering every 2 days (D2W1) showed the ability to control plant stress caused by drought. Meanwhile, the highest proline yield was found in the D0W3 treatment, indicating that without the application of OMF (D0) and watering every 6 days, high stress was caused to the plants. This is in accordance with the opinion of Chun et al., (2018) and Wang et al., (2022) that increased proline levels are 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 was lower in drought-tolerant plants compared to sensitive types, this was due to the higher water retention capacity of the tolerant type (Wang et al., 2022). Table 3 also showed that the administration of OMF D1 had a greater effect on stress resistance compared to D2, even though both OMFs are combined (D3). This indicates that the watering interval factor plays a greater role than the OMF factor (D1, D2, D3) in drought stress.

Table 3. Proline test results from leaves subjected to various treatments.

Treatment	Proline (µmol/G)
D0W1	0.033
D0W2	0.107
D0W3	0.139
D1W1	0.011
D1W2	0.010
D1W3	0.014
D2W1	0.011
D2W2	0.015
D2W3	0.131
D3W1	0.020
D3W2	0.020
D3W3	0.026

Drought stress in orchids also affects chlorophyll content, causing a decrease in leaf chlorophyll levels (Bashir et al., 2022). This occurs because water deficiency inhibits chlorophyll synthesis, which plays a vital role in photosynthesis. When plants experience stress due to water shortages, chlorophyll production decreases, causing leaves to turn yellow or pale (Livia . et al., 2025) (Table 4).

Table 4. Chlorophyll test results from leaves subjected to various treatments.

Treatment	Chlorophyll A (Mg/G)	Chlorophyll B (Mg/G)	Total Chlorophyll (Mg/G)
D0W1	0.04	0.04	0.08
D0W2	0.03	0.04	0.07
D0W3	0.03	0.03	0.06
D1W1	0.05	0.05	0.10
D1W2	0.20	0.23	0.43
D1W3	0.09	0.19	0.28
D2W1	0.30	0.21	0.51
D2W2	0.21	0.24	0.45
D2W3	0.16	0.10	0.26
D3W1	0.29	0.19	0.48
D3W2	0.34	0.23	0.57
D3W3	0.57	0.32	0.89

Table 4 shows that the highest total chlorophyll content was found in the D3P3 treatment, which indicated that the combination of OMF (D3) application and watering every 6 days showed the ability to control plant stress due to drought, even though it was only watered once every 6 days. Meanwhile, the lowest results were found in the D0W1 treatment, which was without OMF application (control) and watering every 2 days. This is in accordance with the opinion of Nurcahyani et al., (2020) that chlorophyll content in plants can be an indicator of drought tolerance. Drought stress treatment significantly affected the content of chlorophyll a, b, and total plants. The same opinion was also expressed by Guidi et al., (2019) that plants can adapt to reduce the impact of water stress, as seen in sunflower and corn varieties with different drought tolerances by increasing or decreasing the rate of photosynthesis which is greatly influenced by the amount of chlorophyll in the leaves (Livia T. et al., 2025).

The novelty of this research is based on previous research that only used one OMF isolate in controlling drought stress in *D. aggregatum* (Soelistijono et al., 2023), while this study used 2 OMF isolates isolated from *D. fimbriatum* and *D. phalaenopsis* and wastested on *D. spectabile* seedlings obtained from tissue culture. The advantage of inoculation with double OMF isolates is that they can help each other in increasing the ability to absorb complementary nutrients from the environment causing increased photosynthesis in *D. spectabile* and being a solution in overcoming drought stress during the dry season.

This research contributes to supporting SDG 15, namely Life on Land Ecosystems, which focuses on sustainable land management by reducing desertification and stopping land degradation, where water stress is one of the main causes of desertification and land degradation. Benefits that can be obtained include supporting science on the use of fungal microbes/germplasm from nature (in the concept of sustainable agriculture) in overcoming water stress in *D. spectabile* orchids, which are very important for conservation and ex situ propagation because they are increasingly threatened by drought and loss in their natural habitat. Meanwhile, the benefit for orchid farming communities is maintaining the survival of *D. spectabile* orchids as germplasm for orchid species in nature.

CONCLUSIONS

The application of Orchid Mycorrhizal Fungi (OMF) belonging to the *Rhizoctonia* group enhances the drought resistance of young *D. spectabile* plants. The combined OMF treatment (D3) produced the strongest response, significantly increasing plant height, leaf number, root length, proline accumulation, and chlorophyll content. It is recommended for further research to map the resistance genes to water stress in OMF isolated from *D. fimbriatum* (D1) and *D. phalaenopsis* (D2) using PCR, and later these resistance genes can be compared with the DNA mapping of *D. spectabile* seedlings that have been inoculated with the OMF consortium (D3).

ACKNOWLEDGEMENT

The authors would like to thank the Directorate of Research, Technology, and Community Service, Higher Education, Ministry of Education and Culture, for the financial support for this research grant for the 2024 fiscal year, based on Decree Number 0459/E5/PG.02.00/2024 dated May 30, 2024.

CONTRIBUTION OF AUTHORS

RS Conducting and coordinating each stage of research, especially identification and

determining the combination of *Rhizoctonia* mycorrhiza (M3 isolates). PAR Care and maintenance of *D. spectabile* seedlings in the greenhouse, watering interval in green house and growth parameter measurement. AFA Propagating *D. spectabile* using tissue culture techniques in a plant breeding laboratory. CH The selection provides orchid species *D. fimbriatum* and *D. phalaenopsis* from Gede mountain. HU English Grammarly correction article.

CONFLICT OF INTEREST STATEMENT

This section states that each author has no personal interests or conflicts of interest. For example: The authors declare that there is no conflict of interest regarding the publication of this paper

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

REFERENCES

Ajayi-oyetunde, O. O., Bradley, C. A., & Sciences, C. (2017). *Identification and Characterization of Rhizoctonia Species Associated with Soybean Seedling Disease*. *101*(4), 520–533.

Alderton-moss, J. J. (2022). *Exploring the orchid mycorrhizal symbiosis in the context of conservation : identity , environmental availability , and ontogenesis By*.

Ario & SetiawanArio & Setiawan (2020). *The Effect of Benzyl Amino Purine (BAP) Concentration on the Growth Amount of the Explant of Dendrobium spectabile Orchid by*. *3*(2), 33–38.

Attri, L. K. (2023). Studies on Mycorrhizal Associations in an Orchid. *Asian Journal of Biological and Life Sciences*, *12*(1), 179–186. https://doi.org/10.5530/ajbls.2023.12.24

Bandoni R.J. (2018). Safranin O as a Rapid Nuclear Stain for Fungi. *Mycologia.* Taylor & Francis Online. P: 873-874. https://doi.org/ 10.1080/00275514.1979.12021088

Bashir, S. S., Hussain, A., Hussain, S. J., Ali, O., Nabi, S. Z., Dar, N. A., & Baloch, F. S. (2022). Plant drought stress tolerance: understanding its physiological, biochemical and molecular mechanisms. *Biotechnology & Biotechnological Equipment*, *35*(1), 1912–1925. https://doi.org/10.1080/13102818.2021. 2020161

Chen, J., Wang, H., & Guo, S. (2012). *Isolation and identification of endophytic and mycorrhizal fungi from seeds and roots of Dendrobium (Orchidaceae)*. 297–307. https://doi.org/10. 1007/s00572-011-0404-0

Chun, S. C., Paramasivan, M., & Chandrasekaran, M. (2018). *Proline Accumulation Influenced by Osmotic Stress in Arbuscular Mycorrhizal Symbiotic Plants*. *9*(October), 1–13. https://doi.org/10.3389/fmicb.2018.02525

Dodson C.H., (2025). Orchid plants. *Encyclopedia Britanica. https://www. britannica.com/plant/orchid/Characteristic-morphological-features*.

Gao, J. (2021). *Progress and Prospects of Mycorrhizal Fungal Diversity in Orchids*. 12(May). https://doi.org/10.3389/fpls.2021. 646325

Guidi, L., Piccolo, E. Lo, Landi, M., & Esteban, R. (2019). *Chlorophyll Fluorescence , Photoinhibition and Abiotic Stress : Does it Make Any Difference the Fact to Be a C3 or C4 Species ? 10*(February), 1–11. https://doi.org/ 10.3389/fpls.2019.00174

Livia T., Nurcahyani, E., Qudus, H. I., & Irawan, B. (2025). *Characterization of Drought Stress Responses in Phalaenopsis amabilis Based on Survival Percentage and Stomatal Density*.

Li T., Yang W., Wu S., Selosse M.A. , Gao, J. (2021). *Progress and Prospects of Mycorrhizal Fungal Diversity in Orchids*. 12(May). https://doi.org/10.3389/fpls.2021.646325

Mahfut, Izzati F.N., Ernawiati E., Wahyuningsih S. (2022). *View of* Variasi Respon Anggrek Hasil Induksi Rhizoctonia Terhadap Infeksi Odontoglossum ringspot virus (ORSV*).BIOTA*.

Michael, M., Sukarno, N., Mursidawati, S., Sandra, E., & Rahayu, N. D. (2023). Isolasi dan Identifikasi Cendawan Endofit Akar Anggrek Epifit dan Hemiepifit. *Jurnal Sumberdaya Hayati*, *9*(4), 152–163. https://doi.org/10. 29244/jsdh.9.4.152-163

Moliszewska, E., Maculewicz, D., & Stępniewska, H. (2023). Characterization of three-nucleate Rhizoctonia AG-E based on their morphology and phylogeny. *Scientific Reports*, *13*(1), 1–12. https://doi.org/10.1038/s41598-023-44448-1

Nurcahyani E., Sholekhah, Sumardi, Qudus. (2020). Analysis of Total Carbohydrate and Chlorophyll Content of The Orchid Plantlet [

Phalaenopsis amabilis (L .) Bl .] Resistant *Fusarium* Wilt Disease. *Journal of Physics: Conference Series*. https://doi.org/10.1088/1742-6596/1751/1/012061

Sabatini, A. P., Nurcahyani, E., & Agustrina, R. 2022. *Respon Planlet Anggrek Cattleya sp . Hasil Seleksi In Vitro Terhadap Cekaman Kekeringan dengan Polietilenglikol (PEG) 6000*. *6*(2), 61–67.

Salazar J., Pomavila M., Pollard A.T., Chica E.J., Pena Denise F. (2020). Endophytic Fungi Associated With Roots Of Epiphytic Orchids In Two Andean Forests In Southern Ecuador And Their Role In Germination. *Lankesteriana*. Vol. 20 (January), 37–47.

Smith, S.E. and D. J. Read. 2008. *Mycorrhiza Symbiosis*, 3rd Edition. Academic Press. New York. 805 p.

Sneh, B., Yamoah, E., & Stewart, A. (2004). Hypovirulent Rhizoctonia SPP. Isolates From New Zealand Soils Protect Radish Seedlings Against Damping-off Caused By R. solani. *Bioprotection* Vol.58, 54–58.

Soelistijono R., Daryanti and M.T. Handayani. (2018). *Isolation of Mycorrhizal Rhizoctonia as resistance inducer of Dendrobium macrophyllum to drought Isolation of Mycorrhizal Rhizoctonia as resistance inducer of Dendrobium macrophyllum to drought*. IOP Conference.

Soelistijono, R., Utami, D. S., Daryanti, Faizin, M., & Dian, R. (2020). Short Communication_ Characterization of Rhizoctonia-like mycorrhizae associated with five Dendrobium species in Java, Indonesia.*Biodiversitas.pdf*. (n.d.).

Soelistijono, R., Jakti, A. W., Budiyono, A., Suprapti, E., & Daryanti. (2023). The Uses of Rhizoctonia Mycorrhizae As a Dry Resistance Induction of Dendrobium aggregatum Seedlings.Malaysian Applied Biology.

Soelistijono, R., Daryanti, Rakhmawati, D., Rianto, P. A., & Utomo, H. (2024). Utilization of Rhizoctonia Mycorrhizae for Orchid Late Blight Control in Sustainable Agricultural. *Biosaintifika*, *16*(1), 1–9. https://doi.org/10.15294/biosaintifika.v15i1.3498

Sugiarto L., Umniyatie S., Henuhili V. (2016). The Diversity Of Orchid Mycorrhiza Existence In Turgo Village Pakem, Sleman Yogyakarta. *J. Sains Dasar* Vol. 5 (2) p:71 - 80.

Suminar, E., Lestari, F. W., Nuraini, A., Mubarok, S., & Ezura, H. (2020). *Kandungan Prolin , Klorofil , dan Hasil Tanaman Tomat Mutan IAA9 pada Kondisi Cekaman Suhu Tinggi*. *31*(3), p:280–287.

Suryantini, R., Wulandari, R. S., & Kasiamdari, R. S. R. I. (2015). *Orchid Mycorrhizal Fungi: Identification of*. *9*(4), 157–162. https://doi.org/10.5454/mi.9.4.3

Tian, F., Liao, X., Wang, L., Bai, X., & Yang, Y. (2022). Isolation and identification of beneficial orchid mycorrhizal fungi in Paphiopedilum barbigerum (Orchidaceae). *Plant Signaling & Behavior*, *17*(1). https://doi.org/10.1080/15592324.2021.2005882

Tsulsiyah, B., Farida, T., Sutra, C. L., & Semiarti, E. (2021). Important role of mycorrhiza for seed germination and growth of dendrobium orchids. *Journal of Tropical Biodiversity and Biotechnology* (Vol. 6, Number 2). https://doi.org/10.22146/JTBB.60805

Wang, Z., Yang, Y., Yadav, V., Zhao, W., & He, Y. (2022). *Drought-induced proline is mainly synthesized in leaves and transported to roots in watermelon under water de fi cit*. *8*(September).

Xi, G., Shi, J., Li, J., & Han, Z. (2020). Isolation and identification of beneficial orchid mycorrhizal fungi in Bletilla striata (Thunb .) Rchb . f . (Orchidaceae). *Plant Signaling & Behavior*, *15*(12). https://doi.org/10.1080/15592324.2020.1816644