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Research Article
Study of Orchid Resistance Induction Using Rhizoctonia Against ORSV Infection Based on Anatomical Characters of Roots and Leaves

Mahfut, Mauli Maro Hidayat and Sahira Josy Arifannisa

Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Lampung 35141, Indonesia

Abstract

Background and Objective: *Phalaenopsis amabilis* and *Dendrobium discolor* are orchids that are widely cultivated in Indonesia. Orchid cultivation has several problems, one of which is infection with *Odontoglossum ringspot virus* (ORSV). Rhizoctonia can be used in the induction of orchid resistance by triggering lignification in roots and leaves. However, there is little information about the induction of mycorrhizal resistance. It is necessary to study to determine of orchids resistance to virus through analysis of anatomical character on root and leave. **Materials and Methods:** Factorial block random design. The factor I: Orchids types: *P. amabilis* (A1) dan *D. discolor* (A2). Factor II: Inoculation treatment: Control (K), Mycorrhiza (M), Virus (V) and Mycorrhiza-Virus (MV). Parameters observed were root anatomical structure includes lignification and the presence of peloton and leaf anatomical structure includes leaf damage and thickness. **Results:** All types of experimental plants experienced root and leaf tissue damage due to virus inoculation and mycorrhiza. The anatomy of the treated roots had differences in the thickness of the epidermal lignin and the thickness of the carrier bundle lignin. Meanwhile, changes in the anatomical character of leaves as a result of virus inoculation showed damage to the epidermis and stomata tissue. **Conclusion:** Based on the anatomical observations of roots and leaves, *D. discolor* was more resistant to ORSV infection than *P. amabilis*. The results of this study become a recommendation for the types of orchids cultivated in ORSV endemic area.

Key words: Induced resistance, *Phalaenopsis amabilis*, *Dendrobium discolor*, rhizoctonia, ORSV, root anatomy, leaf anatomy

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Corresponding Author: Mahfut, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Lampung 35141, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Orchids are commodities that are not difficult to cultivate. The type of orchid that is most in demand by consumers and dominates the market is *Dendrobium*, followed by *Phalaenopsis*[1]. This species is widely enjoyed because of its uniqueness in the form of durable and beautiful flowers, with varied shapes and colors[2]. Until now, infectious diseases are still the main obstacle in orchid cultivation[3,4], including *Odontoglossum ringspot virus* (ORSV)[1,5].

Efforts to protect against viral infections that interfere with orchid productivity can be done by utilizing orchid mycorrhiza fungi (OMF)[6]. Mycorrhizal interactions with plants will form a mutualistic symbiotic structure. Mahfut[7] explained that the association of mycorrhizae in roots can help orchids to be more resistant to disease. The decrease in the severity of viral infections is due to OMF being able to activate jasmonic acid and methyl jasmonic which play a role in activating plant resistance signals.

There are few studies related to OMF induction in controlling viral infection in orchids. Several previous studies only reported the effectiveness of OMF in increasing height growth, increasing the number of roots and leaves[8], increasing leaf thickness[9], decreasing infection symptoms and disease intensity in leaves caused by ORSV[10], but there is no information about the effectiveness of this OMF on the anatomical structure of orchid roots and leaves.

This study was conducted to determine the resistance induction of orchids using Rhizoctonia against ORSV infection based on the anatomical characteristics of roots and leaves. The results of this study were expected to be a reference in efforts to protect orchids against infectious diseases.

MATERIALS AND METHODS

Study area and sample collection: This study was carried out in the experimental garden of the Laboratory of Botany, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, from October, 2020 to March, 2021. The materials used in this study were *P. amabilis*, *D. discolor*, mortar, pestel, object glass, cover glass, microscope, ocular micrometer lens, optilab lens, petri dish, tweezers, erlenmeyer, measuring cup, Potato Dextrose Agar (PDA) powder, carborundum, phosphate buffer, water, alcohol, phloroglucin, methylene blue and HCl solution.

Methodology: The study used a factorial block random design, factor I was a type of orchid consisting of *P. amabilis* and *D. discolor*. Factor II was the inoculation treatment consisting of control (K), mycorrhiza (M), virus (V) and mycorrhiza-virus (MV).

This study used 3-4 month old orchid plantlets. The sterile plantlets were removed from the bottle and then planted in a 2.5 cm diameter plastic pot containing moss media. Plantlets were grown well (acclimatization) for 3 months before treatment. Watering is done every day using a sprayer. The PDA media preparation using a total of 39 g of PDA media was dissolved with 1000 mL of distilled water in a beaker glass. Furthermore, the media is heated with a hotplate stirrer until it boils. Then the media was sterilized in an autoclave at a pressure of 1 atm at 121°C for 15 min. After that, the media was poured into a Petri dish and allowed to solidify[5,10]. Rhizoctonia subculture was grown on PDA media which had added chloramphenicol antibacterial. The isolate in the cup was taken approximately 0.5 cm and then placed on PDA media with three points. Then incubated at room temperature for 5-7 days. Rhizoctonia isolates were rejuvenated in 8-10 cups[5]. The OMF induction method was carried out using the method of Mahfut *et al.*[11]. The orchids were removed from the moss media and then placed in a petri dish containing Rhizoctonia isolates for 3×24 hrs. The orchids are then re-grown in moss growing media.

The ORSV inoculation on orchids was carried out using an inoculum of tobacco leaf samples that had been infected with ORSV. Tobacco leaves were ground by adding phosphate buffer at a ratio of 1:10 (m/v), modified. Phosphate buffers play a role in destroying cells so that viruses are released from cells. Before being inoculated, the surface of the orchid leaves was sprinkled with carborundum until evenly distributed[1]. Inoculation is done gently in the direction of the leaf bone with your fingers or a cotton bud. The orchids were then reared in sterile moss growing media and observed for infection symptoms including necrosis, chlorosis, streak yellowing, mosaic, leaf malformations and leaf curling during the incubation period until these symptoms appeared[1,10-13].

Observation of root lignification and peloton: Observation of plantlet root anatomy using manual incision method (freehand sections). The roots were cut transversely using a razor blade, then placed on an object of glass. Observation of lignification in roots using phloroglucin-HCl staining which shows a purple-red color if lignification occurs[11]. Then observed using a microscope at 40× and 100× magnification.

Leaf anatomy observations: Observation and identification of leaf surface anatomical characters were carried out using the replica method[9]. The leaves that have been taken are cleaned on the top and bottom surfaces with a tissue to remove dust and dirt. Brush the top and bottom surfaces of the leaves with nail polish and let them stand for 10 min to dry. The smear that has dried is pasted with insulation and leveled. The insulation is slowly removed, then stick on the object glass. Flatten the insulation, then label the left side of the glass object with a description of the type of plant and replication. Observe the slides under a microscope at 400× magnification.

Parameters: The parameters observed in the anatomical character of the roots were epidermal lignification, lignification of the carrier bundle and peloton. While the anatomical characteristics of the leaves are the type of stomata, neighboring cells, the shape of the epidermal cells, the length and width of the epidermis and the stomata index. The stomata index was calculated using the formula according to Tohari *et al*.[8] namely:

$$\frac{\text{Number of stomata}}{\text{Number of epidermis} + \text{Number of stomata}} \times 100\%$$

Statistical analysis: The data obtained were homogenized using Levene's Test and then analyzed by ANOVA and Tukey's follow-up test at 5% level, adopted statistical method of Tohari *et al*.[8].

RESULTS

Root lignification: Hasil Observation of lignin thickness in root cross-sections of *P. amabilis* and *D. discolor* were presented in Table 1. The table showed *P. amabilis* control had a range of lignin thickness of epidermal cells and root carrier bundles measuring 12.38 and 28.43 m. Meanwhile, the control *D. discolor* had a thickness range of lignin in epidermal cells and root bundles measuring 19.48 m and 20.78 m, respectively. The thickness of the epidermal lignin thickness of the control roots of *D. discolor* had a size that was not much different from the thickness of the root bundle lignin.

Based on Table 1, it can be seen that the range of lignin thickness of root epidermal cells of *P. amabilis* is thinner than that of *D. discolor*. In contrast, the thickness range of root bundle lignin of *P. amabilis* was thicker than that of *D. discolor*. The results of the MV treatment on both orchids had the thickest range of epidermal cell lignin thickness among all treatments. Meanwhile, the results of the M treatment on both orchids had the thickest range of carrier bundle lignin thickness among all treatments. The results of the observation of lignification in the transverse incision of the orchid roots showed that the epidermis and transport bundles were pinkish-purple in color, indicating the presence of thickening of lignin in the cell wall (Fig. 1 and 2a-d). This lignification occurs in the epidermis and carrier bundle which results in different thicknesses in each treatment.

Peloton: The results showed that the presence of peloton in the transverse section of the roots of *P. amabilis* treated by mycorrhizal and mycorrhizal viruses was located in the cortex. However, the transverse section of the roots of *P. amabilis* treated by virus and control showed no presence in the epidermis, cortex and transport bundle. In addition, a cross-section of the roots of *D. discolor* treated with mycorrhizae showed the presence of peloton only in the cortex. Meanwhile, the cross-section of the roots of *D. discolor* in the mycorrhizal virus treatment did not show the presence of peloton either in the epidermis, cortex or in the carrier bundle. The results of peloton observations on the transverse section of the roots of *P. amabilis* and *D. discolor* were presented in Table 2.

Table 1: Root lignification of *Phalaenopsis amabilis* and *Dendrobium discolor*

	Lignin thickness	
Treatment	Epidermis (µm)	Vascular bundle (µm)
KA1	12.38	28.43
KA2	19.48	20.78
MA1	10.30	35.60
MA2	18.73	26.10
VA1	11.94	31.52
VA2	19.76	13.05
MVA1	14.03	34.55
MVA2	21.27	11.82

K: Control, A1: *P. amabilis*, A2: *D. discolor*, M: Mycorrhiza. and V: ORSV

Table 2: Peloton on the roots of *Phalaenopsis amabilis* and *Dendrobium discolor*

		Presence of peloton		
Treatment	Peloton	Exoderm	Cortex	Vascular bundle
KA1	-	-	-	-
KA2	-	-	-	-
MA1	+	-	+	-
MA2	+	-	+	-
VA1	-	-	-	-
VA2	-	-	-	-
MVA1	+	-	+	-
MVA2	-	-	-	-

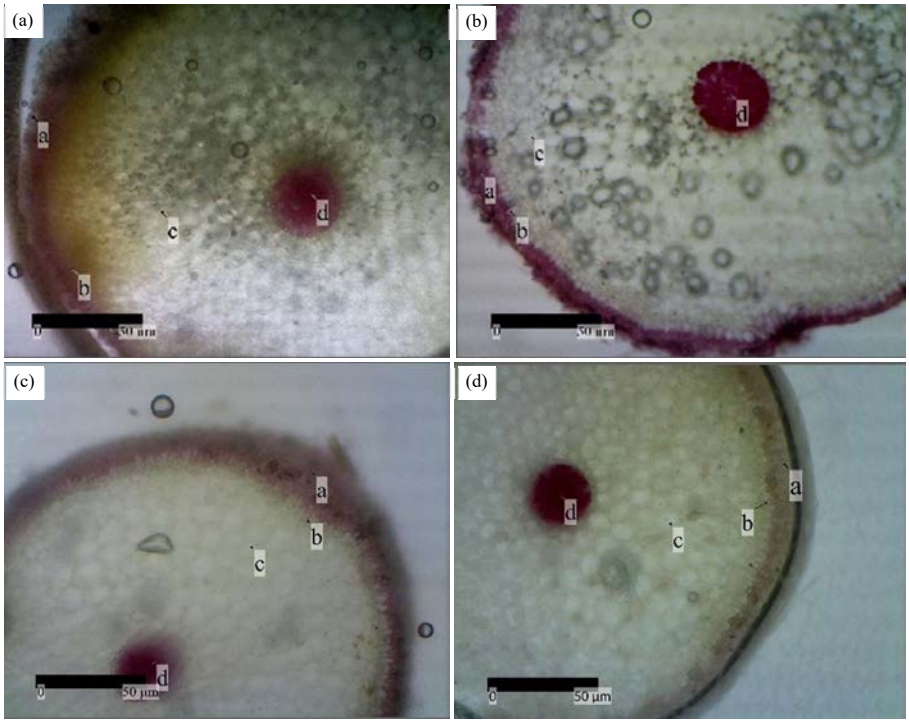

Fig. 1: Cross-section of *Phalaenopsis amabilis* root, (a) KA1, (b) MA1, (c) VA1 and (d) MVA1
a: Velamen, b: Exoderm, c: Cortex, d: Vascular bundle, Magnification 40× and Bar 50 µm

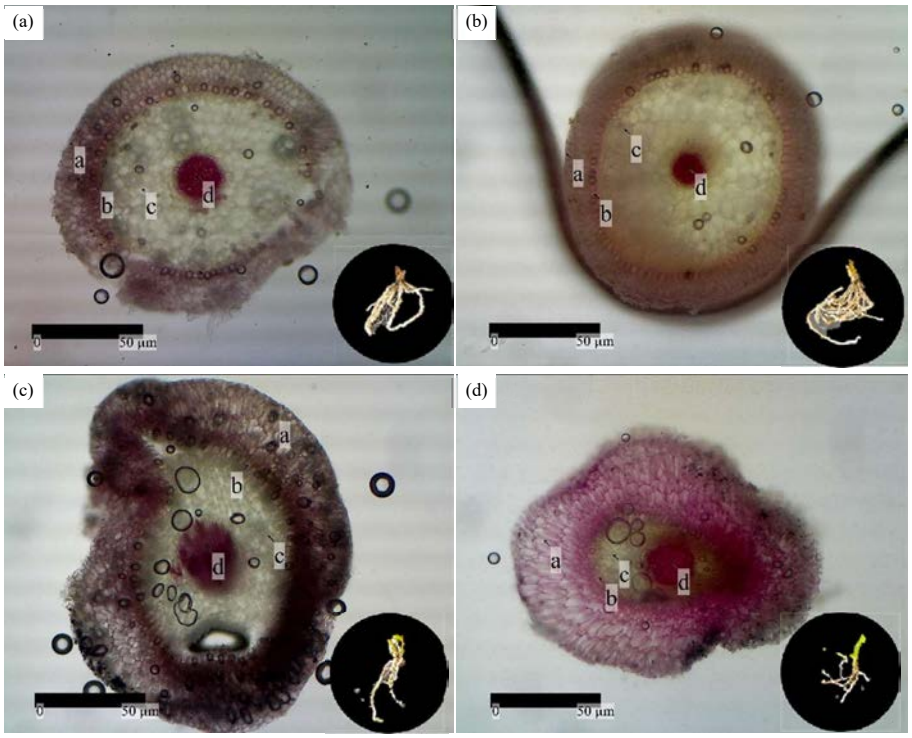

Fig. 2: Cross-section of the roots of *Dendrobium discolor*, (a) KA2, (b) MA2, (c) VA2 and (d) MVA2
a: Velamen, b: Exoderm, c: Cortex, d: Vascular bundle, Magnification 40× and Bar 50 µm

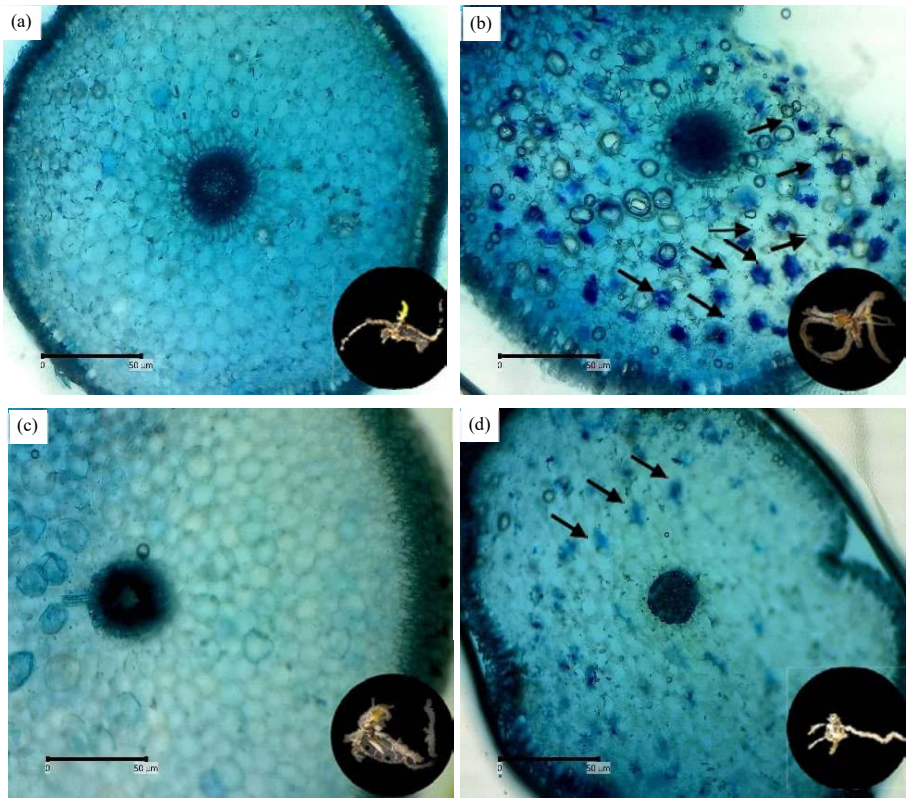

Fig. 3: Observation of a cross-section of the roots of *Phalaenopsis amabilis*, (a) KA1, (b) MA1, (c) VA1 and (d) MVA1
Arrow indicates the peloton, Magnification 40x and Bar 50 µm

The presence of peloton was not seen in all parts of the roots of *D. discolor* in both control and virus treatment. The cross-section of the orchid roots shows the presence of peloton which was presented in Fig. 3 and 4a-d. Observations of this peloton use methylene blue staining, so the peloton will show a dark blue color. The results of the transverse incisions on the two orchids were peloton visible on the root cortex and not on the endodermis and on the carrier bundle.

Based on Fig. 3 and 4, these two orchids have different observations. Observation of the transverse section of *P. amabilis* roots in the treatment of MI and MV showed the presence of peloton in the cortex and did not show the presence of peloton in the exodermis and transport bundle. The number of peloton roots of *P. amabilis* in treatment M seemed more than in the MV treatment. Transverse sections of *P. amabilis* roots in treatment V and control did not show the presence of peloton (Fig. 3).

The results of the observation of transverse incisions of *D. discolor* roots were different from those of *P. amabilis* roots because in the treatment of MV, *D. discolor* roots did not show peloton in the cortex, exodermis and carrier bundle. Meanwhile, the roots of *D. discolor* as a result of mycorrhizal treatment showed small blue dots on some cortical cells. These small blue dots were thought to be degraded peloton so that the results of a cross-section of the root show a cortex that has peloton but is not completely filled with the peloton (Fig. 4).

Variation of leaf surface anatomical characteristics: The anatomical characteristics of the leaf surface were seen in the stomata, neighboring cells and the epidermis. Epidermal observations were made to determine the average number of epidermis, the average size of epidermal cells and the shape of the epidermis. Stomata observations were carried out to determine the average number of stomata, the average stomata size, stomata index and to determine the type of stomata. The results of the observations can be seen in Table 3 and 4.

Leaf surface anatomical characters observed through paradermal cross-section of *P. amabilis* and *D. discolor* orchids at 400× magnification were stomata and epidermal cells. The stomata characters are the number of stomata, stomata type, stomata size and stomata index, while the epidermal cell characteristics are the number of epidermis, the shape of the epidermis and the size of the epidermis.

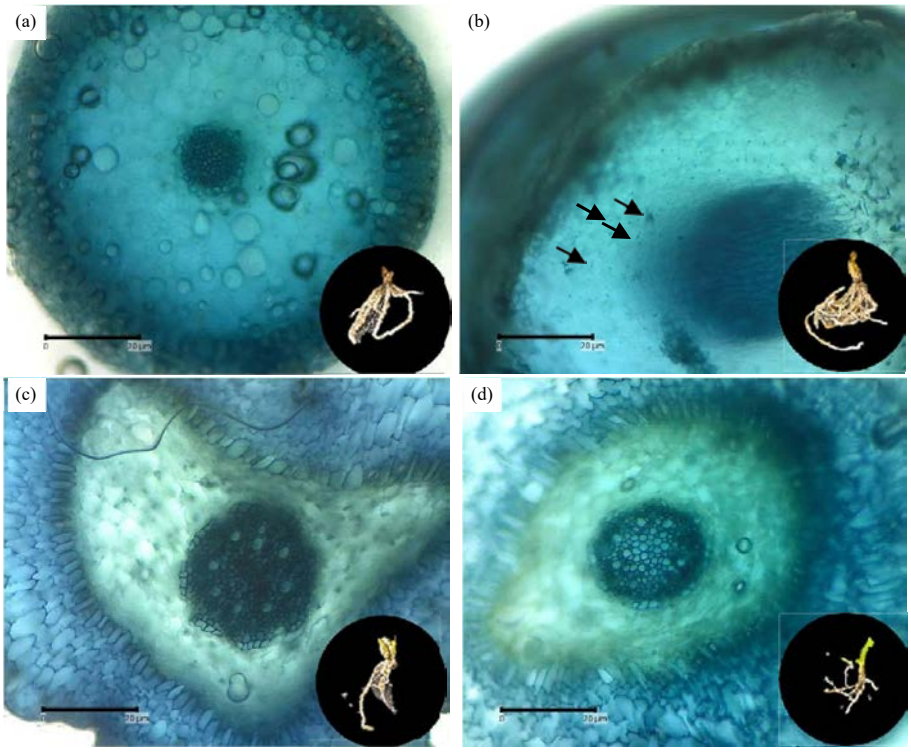

Fig. 4(a-d): Observation of a cross-section of the roots of *D. discolor*, (a) KA2, (b) MA2, (c) VA2 and (d) MVA2
Arrow indicates the peloton, Magnification100× and Bar 20 µm

Table 3: Anatomical comparison of epidermal tissue and stomata on the upper surface of the leaves of *Phalaenopsis amabilis* and *Dendrobium discolor*

	Phalaenopsis amabilis (A_1)				*Dendrobium discolor* (A_2)			
Character	MA_1	VA_1	MVA_1	KA_1	MA_2	VA_2	MVA_2	KA_2
Average number of stomata	1	0.75	0.5	1.25	1	0.5	1	1
Average length of stomata (µm)	1.83	1.31	0.96	2.17	1.29	2.01	0.80	2.96
Average width of stomata (µm)	1.38	1.11	0.75	1.64	1.10	1.45	0.60	2.09
Average number of neighbor cells	2.75	2.5	1.75	4.5	2	2.25	1	4.75
Average number of epiderm	25.5	16.5	10.75	35	8	6	3	17
Average length of epiderm (µm)	4.74	3.44	2.36	3.70	3	2.87	1.35	5.22
Average width of epiderm (µm)	2.67	2.07	1.47	2.41	1.72	1.80	0.83	2.80
Stomata index	3.95	3.26	2.23	3.82	2.95	3.84	1.92	5.77
Stomata type	Tetracytic	Tetracytic	Tetracytic	Tetracytic	Tetracytic	Tetracytic	Tetracytic	Pentacytic
Epidermis shape	Poligonal	Poligonal	Poligonal	Poligonal	Poligonal	Poligonal	Poligonal	Poligonal

Magnification: 400×

Table 4: Anatomical comparison of epidermal tissue and stomata on the lower surface of the leaves of *Phalaenopsis amabilis* and *Dendrobium discolor*

	Phalaenopsis amabilis (A_1)				*Dendrobium discolor* (A_2)			
Character	MA_1	VA_1	MVA_1	KA_1	MA_2	VA_2	MVA_2	KA_2
Average number of stomata	1	1	1	1	1.75	1.25	1.25	2
Average length of stomata (µm)	1.91	2.21	0.60	1.99	3.13	1.55	2.13	2.65
Average width of stomata (µm)	1.58	1.62	0.36	1.40	1.98	0.99	1.72	1.72
Average number of neighbor cells	4.25	4	1	4	7.25	5	5	8
Average number of epiderm	19	20.25	5	24.25	18	9.25	11.5	23.25
Average length of epiderm (µm)	5.33	5.01	1.19	4.89	4.59	2.19	3.32	4.24
Average width of epiderm (µm)	2.97	3.04	0.77	2.99	2.71	1.31	2.26	2.79
Stomata index	5.08	4.83	1.19	4.27	8.75	5.90	7.20	8.30
Stomata type	Tetracytic	Tetracytic	Tetracytic	Tetracytic	Tetracytic	Tetracytic	Tetracytic	Tetracytic
Epidermis shape	Poligonal	Poligonal	Poligonal	Poligonal	Poligonal	Poligonal	Poligonal	Poligonal

Magnification: 400×

Number of stomata: On the anatomical observation of the upper surface of the leaves with 400× magnification, there were differences in the average number of stomata in each treatment and control. *Phalaenopsis amabilis* in the M inoculation treatment showed an average number of stomata 1, the V inoculation treatment was 0.75 and the MV treatment was 0.5. Based on the observations, the highest number of stomata was found in the M treatment and the least in the MV treatment, with K having an average number of stomata of 1.25. In contrast to observations on the upper surface of *D. discolor*, 2 of the 3 treatments, namely M and MV, had an average number of 1 stomata, while in treatment V was 0.5, with K having an average number of stomata of 1. While observations on the lower surface *P. amabilis* leaves showed in all treatments and K had an average number of stomata of 1, respectively. There are no variations or differences between them. Meanwhile, in *D. discolor*, 2 of 3 treatments, namely V and MV, had the same average number of stomata, which was 1.25, while in treatment M 1.75 and K 2. More number of stomata than *P. amabilis*.

Stomata type: On the upper surface of the leaves of *P. amabilis* and *D. discolor*, the stomata types were not diverse and tended to be almost the same. The type of stomata on the upper surface of the leaves of each orchid is tetracytic. But the K in *D. discolor* is pentacytic. While on the lower surface of the leaves, both types of orchids in each treatment had the same type of stomata and there was no difference, namely the tetracytic type.

Stomata size: The results of observations on the upper surface of *P. amabilis* leaves showed that the average stomata length in treatment M was 1.83 m and width 1.38 m. In treatment, V has an average length of 1.31 m and a width of 1.11 m. While the MV treatment was 0.96 and 0.75 m. In the three treatments, treatment M had the largest average stomata size with K having an average stomata length of 2.17 m and an average width of 1.64 m. Meanwhile, on the upper surface of *D. discolor* leaves, the average size of stomata in each treatment also varied. In treatment M of 1.29 and 1.10 m, treatment V of 2.01 m and 1.45 m, while treatment M and V of 0.80 and 0.60 m with K of 2.96 and 2.09 m. The results of observations of stomata size on the lower surface of the leaves of both types of orchids also showed a different average stomata size in each treatment and control. The results showed that *D. discolor* had a larger stomata size than *P. amabilis*.

Stomata index: The calculation of the stomata index shows the diversity of the number of stomata and epidermis. Based on the results of observations, on the upper surface of the leaves of *P. amabilis*, the largest stomata index was obtained, namely in the M treatment of 3.95%. While on the upper surface of *D. discolor* leaves, the largest stomata index value was found in treatment V and the lowest was in treatment MV, with K having a stomata index greater than that in treatment M, which was 5.77%. Furthermore, on the lower surface of the leaves, each *P. amabilis* treatment had a different stomata index value. The greatest stomata index was found in the M treatment and the lowest in the MV treatment. The K has a stomatal index of 4.27% which is not greater than the M treatment. Meanwhile, the observation of the lower surface of the leaves of *D. discolor* also obtained differences in the stomata index in each treatment and control. The largest stomata index in treatment M was 8.75%, while the lowest in treatment V. The K had a stomata index of 8.30%. Based on observations, the overall comparison showed that *D. discolor* had a higher stomata index than *P. amabilis*, especially on the underside of the leaves.

Total epidermis: Based on observations, the average number of epidermis in each treatment and K of each orchid is different. On the upper surface of the leaves of *P. amabilis*, the highest average number of the epidermis was in treatment M and the lowest was in the MV treatment, with K having a greater value than treatment M. On the upper surface of the *D. discolor* leaves, the highest average number of the epidermis was obtained in treatment M and the lowest was in treatment V. The K had a greater value than treatment M. Furthermore, on the lower surface of the leaves of *P. amabilis*, the average number of epidermis was highest in treatment V while the lowest was in treatment MV. The K was not higher than the K treatment. On the lower surface of *D. discolor*, the highest average number of the epidermis was found in the M treatment and the lowest in the V and MV treatments, which had the same amount. The K was higher than that of orchids treated with mycorrhizae. Based on observations, it can be compared overall that *P. amabilis* has more epidermal cells than *D. discolor*.

Epidermis shape: The results showed that there were no differences between the epidermis in the treatment of *P. amabilis* and *D. discolor*. Overall the shape of the epidermis is polygonal.

Epidermis size: The results of the observation of the size of the epidermal cells which include the average length and the average width of the epidermal cells, it can be seen that the length and width of each treatment and K in *P. amabilis* and

D. discolor varied. On the upper surface of the leaves, *P. amabilis* had the largest epidermal size, namely in the M treatment with an average length of 4.74 m and a width of 2.67 m, while the smallest in the MV treatment was 2.36 and 1.47 m. The K has an epidermal size that is not larger than the M treatment, namely 3.70 and 12.41 m. On the upper surface of the leaves of *D. discolor*, the largest epidermis size was in treatment M of 3 m and 1.72 m, while the smallest were MV of 1.35 and l of 0.83 m. The K has a larger epidermis size than M, namely 5.22 and 2.80 m. Furthermore, the results of observations on the lower surface of *P. amabilis* leaves showed that the largest epidermis size was found in the M treatment and the smallest was found in the MV. While on the lower surface of the leaves of *D. discolor*, the largest epidermis size was obtained in treatment M and the smallest in treatment V. When compared, the size of the epidermis in *P. amabilis* was larger than in *D. discolor*.

DISCUSSION

Transverse sections of the roots of *P. amabilis* and *D. discolor* showed a reddish-purple color in the epidermal tissue and transport bundles. The purple-red color on phloroglucin-HCl staining indicated the presence of lignification[7]. Lignification occurs when there is a pathogenic infection. This study used ORSV as a pathogen to trigger lignification in orchid cells resulting from V and MV treatments. In the M treatment, a reddish-purple color appeared in each epidermal cell and carrier bundle indicating the presence of lignin thickness. This was in accordance with the research of Mahfut *et al*.[11] that OMF is able to induce orchids to trigger lignification in the epidermis of orchid roots.

Observation of the transverse incision on the control of these two orchid roots showed a reddish-purple color on the epidermis and carrier bundle. This illustrates the presence of lignification in epidermal cells and transport bundles in orchids that were not induced by mycorrhizae or were not infected with pathogens. Orchids that were not induced by mycorrhizae or that were not infected with the pathogen were suspected to be still lignified but had a thinner lignin thickness than those that were induced by mycorrhizae or those infected with the pathogen or those with both. Orchids that are not induced by mycorrhizae or that are not infected with pathogens still undergo lignification, presumably because apart from orchids needing lignin to defend themselves before being infected with pathogens, orchids need lignin for their survival. This assumption was reinforced by the research of Tohari *et al*.[8] which stated that in the epidermis of orchid roots there were velamen cells that have suberin and lignin which function to reduce water evaporation in the roots. Meanwhile, in the carrier bundle, there is also lignin which functions to protect the evaporation of water during the transportation of nutrients and photosynthetic products. Then the thickness of lignin in the two orchids treated M, V and MV will be compared with the thickness of lignin in K of each orchid.

The roots of *P. amabilis* in the MV treatment had thicker lignin thickness of the epidermal cells and carrier bundles compared to K, treatment M and treatment V. This was presumably because the combination of mycorrhizal induction and viral infection was able to trigger thicker lignification in the cells. Epidermal cells and transport bundles so that the orchid is able to make stronger self-defense. This assumption was reinforced by the statement of Izzati *et al*.[10], namely the presence of pathogenic infections triggers orchids to form peroxidase enzymes in order to protect them from pathogenic infections. Meanwhile, the results of the study Arifannisa *et al*.[9] showed that orchids induced by Rhizoctonia were able to increase peroxidase activity and stimulate lignification in the roots of the Spathoglottis plicata orchid. Therefore, in this study, the induction of orchid resistance by Rhizoctonia and ORSV inoculation triggered the orchid to increase the activity of the peroxidase enzyme, which functions to maintain the life of the orchid.

The results of the observation of transverse section lignification of the roots of *D. discolor* in the MV treatment were different from the observations of the transverse section lignification of the roots of *P. amabilis* in the MV treatment. The difference was seen in the root epidermal cells of *D. discolor* which had a thickness of lignin that was not much different from that of the epidermal cells of K. Meanwhile, the epidermal cells of *P. amabilis* roots had thicker lignin thickness than K. The root carriers of *D. discolor* were thinner than those of K. In addition, the *D. discolor* root carriers in the MV treatment had a size that was not much different from the results of the V treatment.

The results of observations of *P. amabilis* root bundle lignification in the M, V and MV treatments showed a difference in the size of the carrier bundle lignin which was thicker than the control. Meanwhile, the root carrier bundle of *D. discolor* in treatment M had the thickest lignin thickness compared to control, treatment V and MV. The thicker lignification of the carrier bundle was thought to be because the carrier bundle must be more protected during pathogen infection because the carrier bundle is a place for transporting nutrients and photosynthetic products which are more important for plants.

This was in accordance with the research of Mahfut[7] which states that the lignin in the orchid transporting bundle functions to protect the evaporation of water during the transportation of nutrients and photosynthesis products so that the lignin is thicker. In this context, the above statement was supported by the research of Arifannisa *et al*.[9] which stated that the lignin contained in the xylem of tomato plantlets induced by salicylic acid showed a greater thickness than K.

The anatomical characters of the roots of *P. amabilis* and *D. discolor* induced by mycorrhizae, inoculated with virus and their combination has differences. The range of lignin thickness of the root epidermal cells of *P. amabilis* was thinner than that of *D. discolor*. In contrast, the thickness range of root bundle lignin of *P. amabilis* was thicker than that of *D. discolor*. The results of the MV treatment on both orchids had the thickest lignin in epidermal cells among all treatments. Meanwhile, the results of the M treatment on both orchids had the thickest range of carrier bundle lignin thickness among all treatments.

In this study, it was seen that the epidermal lignin in the roots of *D. discolor* did look thicker than the control *P. amabilis*. The thicker thickness of *D. discolor* root epidermal lignin is thought to be because naturally *D. discolor* has better resistance than *P. amabilis*. This assumption was supported by the results of study Izzati *et al*.[10] which showed that the disease index of *P. amabilis*, whether induced by Rhizoctonia or not induced by Rhizoctonia, had a higher severity of disease index caused by ORSV than *D. discolor*.

This assumption is further strengthened by the research of Minarni *et al*.[13] which states that *Dendrobium* has a higher level of resistance to ORSV compared to *P. amabilis* based on data on the amount of chlorophyll. Minarni's research did not use mycorrhizal induction. This statement reinforces the notion that *D. discolor* has higher natural resistance than *P. amabilis* so that from the start *D. discolor* has thicker lignin than *P. amabilis*. In this regard, it is suspected that Rhizoctonia is less able to induce *D. discolor* because *D. discolor* has thicker lignin before being induced by Rhizoctonia so Rhizoctonia hyphae have little difficulty in penetrating root epidermal cell defenses.

Peloton is a dense coil in the form of coils of mycorrhizal hyphae that induce orchids found in orchid root cells. Peloton observations on cross-sections of *P. amabilis* and *D. discolor* roots used methylene blue staining to show peloton that was marked dark blue while those that were not stained would show light blue. The presence of peloton in orchid root cells indicates the success of Rhizoctonia induction in this study.

The results of peloton observations on transverse sections of the roots of *P. amabilis* and *D. discolor* against all treatments did not indicate the presence of peloton in endodermal cells and transport bundles. This was in accordance with the research of Mahfut[6] that peloton was found in cortical cells. Although some peloton is found in the cortex. the inner part that is near the endodermis and the transport bundle but the peloton is not found in the cells of the endodermis and the transport bundle.

The cross-section of the roots of the two types of orchids had different observations. The cross-section of the roots of *P. amabilis* treated with MV showed the presence of peloton in the cortex, while the cross-section of the roots of *D. discolor* from the treatment of MV did not show the presence of peloton in all parts of the root tissue, either in the cortex, endodermis or transport bundle. However, the results of peloton observations on root transverse incisions of the two types of orchids have similarities to the M treatment, namely the presence of peloton in some cortical cells. Due to this difference, initially, the induction of Rhizoctonia in *D. discolor* was thought to have not been successful. However, the assumption was wrong when a cross-section of the roots of *D. discolor* treated with Mycorrhizae found small dots on some cortical cells. Although the shape is different from the peloton found in *P. amabilis* roots, the small dots on some cells of the *D. discolor* root cortex are thought to be the degraded peloton.

This assumption is reinforced by the research of Mahfut *et al*.[11] that the results of the transverse incision of the roots of the *Thrixspermum subulatum* orchid showed that peloton was degraded in some cortical cells with peloton not completely filling the cortical cells. This assumption was reinforced by the results of research by Arifannisa *et al*.[9] who found two types of peloton in the dendrobium root cortex, namely peloton that filled cortical cells completely and did not completely fill cortical cells. A peloton that does not completely fill cortical cells is declared a degraded peloton.

The presence of peloton observed in the cross-section indicates the success of Rhizoctonia induction in orchids. Observation of the cross-section of the roots of *D. discolor* had different results in the treatment of M and MV. The results of the observation of the cross-section of the roots of *D. discolor* in the M treatment still found the presence of peloton even though it had degenerated, while in the MV treatment, no peloton was found. Accordingly, the results of observations of root lignification of *D. discolor* in the treatment of M and MV were different. The thickness of the root bundle lignin of *D. discolor* in the M treatment was

thicker than that of the control *D. discolor* root. Meanwhile, the thickness of the root bundle lignin of *D. discolor* in the MV treatment was thinner than the *D. discolor* control. This difference in lignin thickness proves that the presence of peloton indicates the success of Rhizoctonia induction which triggers peroxidase activity so that lignification occurs in the carrier beam of *D. discolor* root mycorrhizal treatment. However, it is not certain whether the induction of Rhizoctonia in *D. discolor* as a result of the MV treatment has not been successful because the induction of Rhizoctonia in *D. discolor* was carried out simultaneously.

The results of the cross-section of the roots of *P. amabilis* in the M treatment showed more peloton than the cross-section of the roots of *P. amabilis* in the MV treatment. In this regard, the results of the cross-section of the roots of *D. discolor* in the M treatment showed more peloton than those in the MV treatment. The results of the cross-section of the roots of *D. discolor* in the MV treatment did not even show peloton either in the cortex, exodermis or transport bundle. These comparisons led to the suggestion that ORSV infection could suppress Rhizoctonia induction in orchid roots. Based on this statement, Rhizoctonia that induces *P. amabilis* and *D. discolor* is thought to be less effective in defending orchids against ORSV infection based on peloton observations on root anatomy.

One of the observed stomata characters is the number of stomata. The number of stomata of *P. amabilis* and *D. discolor* on the lower surface of the leaf (abaxial) was more than on the upper surface of the leaf (adaxial). This was in accordance with the research of Tohari *et al.*[8] which states that the number of stomata in the abaxial part is more than the adaxial part because in the adaxial part, there is a thick cuticle layer that covers the stomata so that it prevents the transpiration process.

The type of stomata observed was based on the number of neighboring cells (Table 3 and 4). The stomata types in *P. amabilis* and *D. discolor* were almost entirely tetracytic type, which was determined based on the number of 4 neighboring cells. However, the control *D. discolor* had pentacytic type stomata because the number of neighboring cells was 5. Mahfut *et al.*[11] explained that the character of stomata based on the number and location of neighboring cells can be taxonomically useful.

The diversity of the number of stomata and epidermis can be seen through the stomata index. In the data obtained, the largest stomata index between *P. amabilis* and *D. discolor* was found in *D. discolor* mycorrhizal treatment. Previous research of Mahfut *et al.*[11] showed that the greater the value of the stomata index, the greater the number of stomata on the leaf surface.

The shape of the epidermal cells from each treatment of the two orchids observed was polygonal (Fig. 1 and 2). In addition, data on the average length and width of the epidermis are also shown in Table 3 and 4. The largest epidermal cell size between the two types of orchids was found in *P. amabilis* with M treatment. The size and shape of the epidermal cells vary greatly in each species[2,7,11]. Measurements of epidemic cells have been attempted to distinguish closely related species, but differences in epidermal size make them unreliable for distinguishing between species. The difference in size can only be used as a data comparison[11,13].

In the observation of leaf anatomy, *P. amabilis* inoculated with M and V had the lowest resistance even compared to leaves inoculated with the virus alone. This was presumably because Rhizoctonia works less effectively and plays a less role in fighting viral infections. The effectiveness of mycorrhizae is highly dependent on the suitability of plant factors and housing media. The type of plant has an effect on the difference in the level of dependence on mycorrhizae because there are certain plants that really need the presence of mycorrhizae and some do not[7,11].

The induction of Rhizoctonia OMF on *P. amabilis* in this study showed no significant effect on several research parameters such as leaf thickness and the number of stomata as well as the number and size of the epidermis. This was presumably due to the relatively short research time so that mycorrhizae have not been fully induced into the plant through the roots as in the study of Mahfut *et al.*[11]. Meanwhile, in *D. discolor*, Rhizoctonia had a significant effect in fighting viral infections, such as leaf thickness and leaf characteristics. *Phalaenopsis amabilis* leaves inoculated with V showed symptoms of the necrotic virus, as well as leaves treated with MV. The symptoms on leaves treated with MV were more severe than on leaves that were only inoculated with V. In leaves of *D. discolor* inoculated with the virus, the symptoms were mosaic and on leaves treated with MV had necrotic symptoms starting from chlorotic. Previous research[1,9-13] reported that the most common symptoms of viral infection were mosaic, necrotic, chlorotic, curling leaf, streak, wilting and ringspot which are typical symptoms of this ORSV.

CONCLUSION

Changes in the anatomical character of *P. amabilis* and *D. discolor* roots induced by OMF Rhizoctonia and ORSV inoculated have differences in epidermal lignin thickness and carrier bundle lignin thickness. While the presence of peloton, Rhizoctonia was less effective in inducing resistance of *P. amabilis* and *D. discolor* to ORSV infection. Changes in the

anatomical character of the leaves of the two orchids inoculated with ORSV showed similarities, namely damage to epidermal cells, decreased number of stomata, leaf thickening in mycorrhizal-induced leaves and leaf thinning in ORSV inoculation. Meanwhile, the anatomical differences of the leaves of the two orchids induced by Rhizoctonia and ORSV inoculation, namely the leaves of *P. amabilis* suffered severe damage when compared to *D. discolor*, which was characterized by more severe damage to the epidermis and stomata tissue. Likewise, the thickness of the leaves of *P. amabilis* inoculated with ORSV was also thinner due to a lot of damaged tissue compared to the leaves of *D. discolor*. The results showed that *D. discolor* was more resistant to ORSV infection than *P. amabilis*.

SIGNIFICANCE STATEMENT

The purpose of this research to determine orchid resistance induction using OMF Rhizoctonia to virus infection through analysis of anatomical structure on root and leave. The results showed that all orchids root and leaf tissue damage due to virus infection, even though mycorrhizal induction has been carried out. The anatomy of the treated roots had differences in the thickness of the epidermal lignin and the thickness of the carrier bundle lignin. Meanwhile, changes in the anatomical character of leaves as a result of virus inoculation showed damage to the epidermis and stomata tissue. This results are important to determine the ability of Rhizoctonia as a biocontrol agent and the level of resistance of orchids to viral infections.

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