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Research Article
Variation of Resistance and Physiological Response of Orchid from Induction *Trichoderma* to Infection *Odontoglossum ringspot virus* (ORSV)

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Abstract

Background and Objective: The OMF *Trichoderma* is a biocontrol capable of increasing plant resistance to *Odontoglossum ringspot virus* (ORSV) infection. The objective of this study was to determine the resistance and physiological responses of orchids induced by OMF and infected with ORSV. **Materials and Methods:** The study was arranged in a factorial block randomized design. Orchid type (factor 1): *Phalaenopsis amabilis* (A1) and *Dendrobium discolor* (A2). Inoculation treatment (factor 2): Control (K), mycorrhiza (M), virus (V) and mycorrhizal-virus (MV). The parameter observation was a response to plant resistance through symptoms of infection, the intensity of disease infection and level of plant resistance, as well as knowing the physiological response of plants through analysis of chlorophyll content. **Results:** The OMF *Trichoderma* is proven to be able to prevent the appearance of symptoms of infection and disease development. The results of mycorrhizal and virus inoculations on orchids cause more severe symptoms of infection than those inoculated with viruses only. The OMF *Trichoderma* also has not been able to increase plant resistance in both orchids. The results of mycorrhizal and viral inoculations on average showed a very susceptible response compared to treatments that were only inoculated with viruses. Total chlorophyll (a, b and total) was induced by OMF and ORSV inoculation on *P. amabilis* and *D. discolor* was the same. **Conclusion:** The results of the analysis of observational data showed the resistance of *P. amabilis* and *D. discolor* to infection of ORSV as a result of OMF *Trichoderma* induced through resistance and physiological responses, there was no difference in resistance between the two. It can be the basis for efforts to protect orchids against disease infections through OMF induction.

Key words: Induced resistance, *Phalaenopsis amabilis*, *Dendrobium discolor*, *Trichoderma*, *Odontoglossum ringspot virus*, resistance response, physiological response

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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 very famous for their beautiful and long-lasting flowers and variety among the most diverse family of flowering plants in the world. Orchid is one of the plants which is in great demand as an ornamental plant by the general public because it has a variety of colors and patterns as well as high economic value. The most popular types of orchids are *Phalaenopsis* and *Dendrobium*[1]. In the effort to develop orchid cultivation, there are still serious disease infection problems[2], including viruses[3,4]. One type of plant virus that is reported to infect orchids the most and has a very wide distribution in the world including those that have arrived in Indonesia is *Odontoglossum ringspot virus* (ORSV)[3-5]. Symptoms of this virus infection are generally in the form of ring spots on leaves and color breaking on flowers. Furthermore, the symptoms of infection cause a decrease in the rate of photosynthesis and stunting.

Orchids that have been infected with a virus can be protected by inducing orchid mycorrhiza fungi (OMF), namely *Trichoderma* sp.[6-8]. The association of mycorrhizae is able to increase plant resistance to viral infections by changing the activity of secondary metabolites, namely phenol and peroxidase enzymes. Both of these compounds cause lignification in the thickening of plant cell walls making it difficult for pathogens to penetrate[3].

Several studies regarding the benefits of OMF as a biocontrol agent in controlling viral infections in orchids have been reported[9-12]. Mahfut[13] showed changes in the anatomical character of the root and leaves of orchids resulting in the induction of OMF resistance to viral infections. Changes in the anatomical character of *P. amabilis* and *D. discolor* roots induced by OMF and ORSV inoculated have differences in epidermal lignin thickness and carrier bundle lignin thickness. 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 OMF 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*.

Until now there has been no research related to the effectiveness of induction of *Trichoderma* can be done through analysis of resistance response and plant physiological response. The objective of this research was to determine the response of plant resistance is known by observing symptoms, the intensity of disease infection and the level of plant resistance, while the physiological response of plants is through analysis of chlorophyll content. This study complements the results of previous research on orchids resulting in the induction of OMF resistance to viral infections.

MATERIALS AND METHODS

Research area and collection of sample: This research was carried out at the greenhouse and Laboratory of Botany, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, from January to May, 2023.

Research materials include plantlets of orchids (*P. amabilis* and *D. discolor*) 12 months old from Anggrek Bandung (West Java, Indonesia), potato dextrose agar (PDA) powder (Merck), carborundum, phosphate buffer, alcohol, aquadest, petri dish, mortar, pestle, tweezers, measuring glass, erlenmeyer, micropipette, filter paper, erlenmeyer, test tube, balance, magnetic stirrer, hot plate, volumetric flask, vortex mixer (Sigma Aldrich, USA), centrifuge (Hettich, Germany), UV-Vis spectrophotometer (Hitachi, Japan), autoclave (Memmert, Germany) and freezer (Modena, Italia).

Methodology: The study was arranged for 2 treatment factors and 4 replications with a factorial block randomized design. Orchid type (factor 1): *P. amabilis* (A1) and *D. discolor* (A2). Inoculation treatment (factor 2): Control (K), mycorrhiza (M), virus (V) and mycorrhizal-virus (MV). After 8 months of age, the orchid plantlets were acclimatized. Orchid care routinely in the greenhouse until 3-4 leaves appear. The OMF was grown on a PDA medium in a Petri dish. The OMF isolates were subcultured on media and incubated for 7 days at room temperature. The orchids were then placed in a Petri dish containing OMF and incubated for 72 hrs. Furthermore, the orchid was replanted in a sterile moss growing medium.

Inoculation of ORSV was carried out using inoculum resulting from virus multiplication on ORSV-infected tobacco plants[8,13]. Tobacco leaves are weighed 1 g and then crushed in cold conditions with a mortar and pestle that has been sterilized using 70% alcohol. The mashed leaves are then added with phosphate buffer on a ratio of 1:10 (m/v). This ORSV culture was then poured onto the leaves with carborundum on the top surface[1]. Inoculation was done slowly in the direction of the leaf reinforcement using a cotton bud and waiting until it dries. Furthermore, the inoculated leaves were washed in running water to remove carborundum on the leaf surface. Observations are carried out periodically until the plants show symptoms including yellowing, chlorosis, necrosis, mosaic, streak, curling and leaf malformations[3,5,8,11,13].

Table 1: Leaf disease severity categories

Categories	Symptom
Healthy plant	No symptoms or infection
Very mild	Leaves infected between >0-10%
Mild	Infected leaves between >10-20%
Moderate	Infected leaves between >20-40%
Severe	Infected leaves between >40-60%
Very severe	Leaves infected between >60%

Table 2: Plant resistance level to ORSV infection

	Host plant reaction	
Plant resistance level	Symptom	Incident
Very resistant	Symptomless	-
Resistant	Clorotic	+
Mild resistant	Mild Mosaic	+
Tolerant	Severe mosaic	++
Vulnerable	Wilting leaf	++
Very vulnerable	Nekrotic, curling leaf	++

-: No incidence of disease, +: Incidence of disease 0%<×≤40%, ++: Incidence of disease 40≤×≤100%

Analysis of disease symptom: This analysis was carried out by observing variations in symptoms of infection and incubation time on leaves that had been inoculated with the virus and OMF. The results of subsequent observations were carried out by matching the documentation of research results with reported literature[8,11-13].

Analysis of disease progression: The stages of this research were carried out by observing the incidence of the disease and the severity of the disease to determine the progress of the disease. Disease incidence was obtained by calculating the number of infected plant organs from all plant samples. While the severity of the disease is the proportion of infected plants to the total surface area of the plants observed. The intensity of the disease was obtained by calculating the damage scale (%) of the disease that appeared on the host plant. Observation of the severity of the disease is done visually. Calculation of the percentage of infected plants is carried out if the disease-causing infection occurs systematically so that the internal symptoms may have spread to all parts of the leaf even though the external symptoms are only slightly visible. The calculation of disease severity score categories was shown in Table 1[12,13].

Analysis of the level of resistance of orchids to disease infection was carried out according to the method of Mahfut *et al.*[8] as shown in Table 2.

Analysis of chlorophyll: The orchid leaves that had been taken from the midrib as a result of the inoculation were weighed to a maximum of 0.01 g, added to 10 mL of ethanol and then ground with a mortar. Whatman No. 1 paper was used to filter the solution before it was added to the flacon. The 1 mL of the sample and 1 mL of the standard solution were added to several cuvettes. With three repeats, absorption measurements were taken with a UV spectrophotometer at wavelengths of 648 and 664 nm. Chlorophyll content was calculated using UV-Vis spectrophotometer (Hitachi, Japan) with the following procedure[8]:

Chlorophyll total = 17, 3 λ648+7, 18 λ664 mg L^{-1}
Chlorophyll a = 12, 21 λ664-2, 81 λ648 mg L^{-1}
Chlorophyll b = 20, 13 λ648-5, 03 λ664 mg L^{-1}

Statistical analysis: Data analysis adopted the statistical method of Mahfut *et al.*[8,13] using Analysis of Variance (ANOVA) with a significance level of 5%. The data was previously homogenized using the Levene Test. Then it was analyzed using ANOVA and continued with the Tukey's Test at the 5% level.

RESULTS

Symptoms of disease: The results of mycorrhizal induction and ORSV inoculation on *P. amabilis* showed a variety of infection symptoms and different incubation times. Some of the symptoms were mild mosaic and necrotic in the treatment of orchids inoculated with viruses and mycorrhizae in replicate 1 (MA1VU1) and replicate 4 (MA1VU4) on 10th day after treatment. In addition, there was clear mosaic in the treatment MA1VU3, as well as mild mosaic and necrotic in MA1VU4 at 13th day, respectively. Other symptoms were clear mosaic in MA1VU3, as well as clear and necrotic mosaic on MA1VU2 at 18th day, respectively. Furthermore, the symptoms of MA1VU1 developed into necrosis on 15th day.

Whereas, in *D. discolor*, the results showed mosaic in the MA2VU1 and MA2VU3 treatments on the 10th day, clear mosaic on MA2VU2 on the 13th days, mosaic on MA2VU3 and MA2VU4 on the 18th day. In MA2VU2 there is a mixture of symptoms, namely mild mosaic and necrotic. In addition, the MA2VU1 treatment was symptomless until the end of the observation. Variations of symptoms of infection and incubation period resulting from mycorrhizal induction and ORSV inoculation in *P. amabilis* and *D. discolor* were shown in Table 3.

Symptoms in *P. amabilis* indicate the development of infection, namely MA1VU1, the color of the leaves changes from green to brown and necrosis appears on the edges of leaves A (V1) and leaves B (V2). In MAVU2 there is necrotic on leaf C (V1), also necrotic and mosaic on leaf D (V2). In MA1VU3 there is a mosaic from the middle to the tip of the E (V1) leaf, while in the F (V2) leaf, there is a mosaic on the entire leaf

Table 3: Variation of symptoms of infection and incubation period resulting from mycorrhizal induction and ORSV inoculation on *Phalaenopsis amabilis* and *Dendrobium discolor*

Orchid	Treatment	Leaves	Variation of symptom	Incubation period
Phalaenopsis amabilis	M1VU1	L1	N, MR	10
		L2	N, MJ	15
	M1VU2	L1	N	18
		L2	N, MJ	18
	M1VU3	L1	MJ	13
		L2	MJ	15
	M1VU4	L1	N, MR	10
		L2	N, MR	13
Dendrobium discolor	M1VU1	L1	MR	10
		L2	TG	18
	M1VU2	L1	N, MJ	13
		L2	N, MJ	18
	M1VU3	L1	M	10
		L2	MJ	18
	M1VU4	L1	MR	13
		L2	MJ	18

MR: Mild mosaic, MJ: Severe mosaic, N: Necrotic and TG: Symptomless

Table 4: Disease incidence on *P. amabilis* and *D. discolor* induced of mycorrhiza and inoculation of ORSV

	Disease incidence (%)			
Repetition	VA1	MVA1	VA2	MVA2
1	50	40	50	50
2	50	66	66	66
3	50	66	50	66
4	66	50	66	50
Sum	216	222	232	232
Average (%)	54	55.5	58	58

A1: *Phalaenopsis amabilis*, A2: *Dendrobium discolor*, V: Virus and MV: Mycorrhiza virus

blade. On MA1VU4 there was a mild mosaic on the entire surface of the leaf and necrotic in the middle of the G (V1) leaf, while on the H (V2) leaf there were symptoms of a mild and necrotic mosaic.

Whereas, in *D. discolor*, MA2V1 there was also a change in symptoms to a mosaic in the middle of the leaf on leaf A (V1) (Fig. 1a), while on leaf B (V2) it showed symptomlessly (Fig. 1b). In MAVU2 there is a mosaic from the base to the middle of the leaf and there are brown necrotic spots on the leaf edges on leaves C (V1) and D (V2) Fig. 1(c-d). In MA2VU3 there were mild mosaic changes on the entire surface of leaves E (V1) and F (V2) Fig. 1(e-f). Whereas MA2VU4 has vein banding on G leaves (V1) Fig. 1(g) and vein clearing on H leaves (V2) Fig. 1(h). Various disease symptoms of *P. amabilis* and *D. discolor* induced mycorrhiza and inoculation of ORSV are shown in Fig. 1.

Disease progression: The results of this study are based on an analysis of disease incidence and disease intensity. The results of calculating the incidence of disease in *P. amabilis* revealed that the mycorrhizal induced and virus inoculated (MVA1) had a greater disease incidence than the virus inoculated only (VA1). In VA1 it has an average percentage of disease incidence of 54%, whereas in MVA1 it has an average percentage of disease incidence of 55.5%. Whereas in *D. discolor*, the treatment of virus inoculation (VA2) and the treatment of mycorrhizal induction and virus inoculation (MVA2) had the same average percentage of disease incidence, namely 58%. Calculation of the disease incidence on *P. amabilis* and *D. discolor* induced mycorrhiza and inoculation of ORSV was shown in Table 4.

The results of the analysis of disease intensity in *P. amabilis* showed that the virus inoculation treatment (VA1) was 26.75%, while the mycorrhizal induction and virus inoculation treatment was 32.5%. Whereas in *D. discolor*, the disease intensity in the virus inoculation treatment (VA2) was higher, namely 33.32%, while the mycorrhizal induction and virus inoculation treatments were smaller, namely 24.57%.

Resistance level of plants: The results of the resistance level analysis of plants on *P. amabilis* showed that the virus inoculation treatment (A1V) in 3 replicates was semi moderate, susceptible, very susceptible and tolerant. Meanwhile, in the mycorrhizal induction and virus inoculation (MVA1) treatment, the resistance level was very susceptible and tolerant. Overall, the results of the study showed

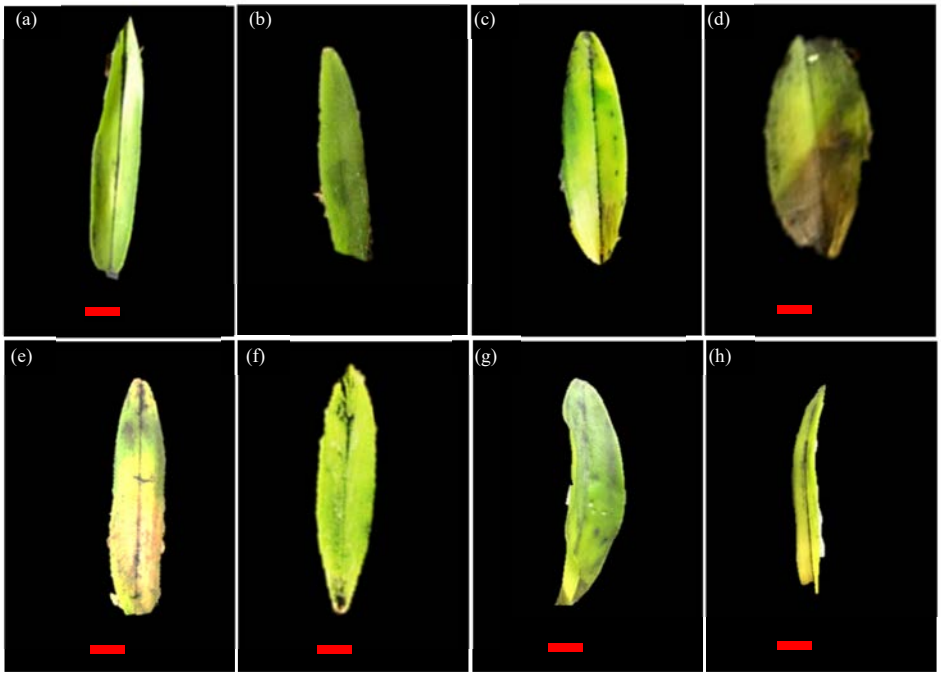

Fig. 1(a-h): Symptoms of *P. amabilis* and *D. discolor* induced of mycorrhiza and inoculation of ORSV: (a) Mild mosaic, (b) Symptomless (c) Severe mosaic and necrotic, (d) Severe mosaic and necrotic, (e) Severe mosaic, (f) Severe mosaic, (g) Mild mosaic and (h) Severe mosaic
Bar 1 cm

Table 5: Resistance Level of plants on *P. amabilis* induced of mycorrhiza and inoculation of ORSV

Treatment	Leaves	Resistance level	Disease incidence
A1VU1	L1	Semi moderate	+
	L2	Very susceptible	++
A1VU2	L1	Susceptible	++
	L2	Very susceptible	++
A1VU3	L1	Very susceptible	++
	L2	Tolerant	++
A1VU4	L1	Very susceptible	++
	L2	Very susceptible	++
MVA1U1	L1	Very susceptible	++
	L2	Very susceptible	++
MVA1U2	L1	Very susceptible	++
	L2	Very susceptible	++
MVA1U3	L1	Tolerant	++
	L2	Tolerant	++
MVA1U4	L1	Very susceptible	++
	L2	Very susceptible	++

A1: *Phalaenopsis amabilis*, V: Virus, MV: Mycorrhiza virus, +: Disease incidence 0%<×≤40%, ++: Diseases incidence 41≤×<100%

that there were 11 very susceptible leaves, 1 susceptible leaf, 3 tolerant leaves, 1 semi moderate leaf. The resistance level of plants on *P. amabilis* induced by mycorrhiza and inoculation of ORSV showed in Table 5.

In contrast, the results of the resistance level analysis of plants on *D. discolor* showed that the treatment of virus inoculation (A2V) in 3 replicates was very moderate, very susceptible and tolerant. Meanwhile, in the mycorrhizal induction and virus inoculation (MVA2) treatment, the resistance level was semi moderate, very moderate, susceptible, very susceptible and tolerant. Overall, the results showed that 3 leaves were very susceptible, 1 susceptible leaf, 7 tolerant leaves, 2 semi-moderate leaves and 3 very moderate leaves. The resistance level of plants on *D. discolor* induced by mycorrhiza and inoculation of ORSV showed in Table 6.

Table 6: Resistance level of plants on *D. discolor* Induced by Mycorrhiza and Inoculation of ORSV

Treatment	Leaves	Resistance level	Disease incidence
A2VU1	L1	Tolerant	+
	L2	Very moderate	-
A2VU2	L1	Tolerant	++
	L2	Tolerant	++
A2VU3	L1	Tolerant	++
	L2	Very moderate	-
A2VU4	L1	Very susceptible	++
	L2	Very susceptible	++
MVA2U1	L1	Semi moderate	+
	L2	Very moderate	-
MVA2U2	L1	Very susceptible	++
	L2	Susceptible	++
MVA2U3	L1	Tolerant	++
	L2	Tolerant	++
MVA2U4	L1	Semi moderate	+
	L2	Tolerant	++

A2: *Dendrobium discolor*, V: Virus, MV: Mycorrhiza virus, -: No disease incidence, +: Disease incidence 0<x≤40%, ++: Diseases incidence 41%≤x<100%

Table 7: Comparison of chlorophyll (a, b, total) content in *P. amabilis* and *D. discolor* induced by mycorrhiza (M), inoculation by virus (V) and mycorrhizal virus (MV) with chlorophyll a content in controls 4 weeks after treatment

	Phalaenopsis amabilis			*Dendrobium discolor*		
Plants	M	V	MV	M	V	MV
Chlorophyll a	2.60	2.17	2.65	2.70	1.87	2.48
Control		2.01			2.07	
Difference	0.59	0.16	0.64	0.63	-0.20	0.41
Chlorophyll b	2.14	1.85	2.15	2.11	1.90	2.26
Control		1.74			1.87	
Difference	0.40	0.11	0.41	0.24	0.03	0.48
Chlorophyll total	4.82	4.02	4.81	4.79	3.87	4.97
Control		3.96			3.56	
Difference	0.86	0.06	0.85	1.23	0.31	1.41

Chlorophyll (a, b and total): The results of the tukey test for chlorophyll content. In combination with *P. amabilis* (2.27 b) and *D. discolor* (1.87 b) treatments with virus induction (V) produced the same chlorophyll a (not significantly different), but the results were lower and significantly different when compared to the combination treatment between *Phalaenopsis amabilis* which was induced by mycorrhiza (M) 2.60 a and mycorrhizal virus (MV) 2.65 a similarly when compared with the combination treatment between *Dendrobium discolor* which was induced by mycorrhiza (M) 2.70 a and mycorrhizal virus (MV) 2.48 a. The results of the Tukey's Test for chlorophyll (b, total) content in *P. amabilis* and *D. discolor* with mycorrhizal (M), virus (V) and mycorrhizal virus (MV) treatments, it was shown that *Phalaenopsis amabilis* and *Dendrobium discolor* had the same effect. Comparison results of chlorophyll (a, b, total) content in *P. amabilis* and *D. discolor* induced by mycorrhiza (M), inoculation by virus (V) and mycorrhizal virus (MV) with chlorophyll b content in control are shown in Table 7.

DISCUSSION

The OMF induction in controlling viral infection in orchids in decreasing infection symptoms and disease intensity in leaves caused by ORSV[8,13]. Observation of these two orchid showed OMF *Trichoderma* has not been proven to be able to reduce the symptoms of infection and disease progression. Observations of symptoms of ORSV infection showed differences in symptoms of infection and incubation period in *Phalaenopsis amabilis* and *Dendrobium discolor*. *Phalaenopsis amabilis* showed a variety of ORSV symptoms in the form of mosaics and necrosis on days 13 and 18 after inoculation. This was in accordance with what was reported by Mahfut *et al*.[1] that the most common ORSV symptoms in *Phalaenopsis* were mosaic, necrotic, mixed necrotic and chlorotic, leaf curl and wilted leaves. In previous studies, *Phalaenopsis* also showed necrotic in the form of small blackish-brown circles that were concave and over time caused yellowing of the leaves[2,3,14].

In contrast to *Dendrobium discolor*, the average infection symptoms appear on the 18th day. Minarni *et al.*[13] reported that ORSV symptoms appeared on the 11th day after inoculation in the form of leaf discoloration. Symptoms that often appear are mosaics. Several research reports on ORSV symptoms in *Dendrobium*, namely *D. discolor* showing mosaic symptoms, *D. lasiantera*, *D. nindii*, *D. burana* Jade × *D. nindii* and *D. burana* Mainil Wrap × *D. stip* in the form of a mosaic with mixed bright green and yellow spots, *D. woxin* and *D. stratioties* in the form of ring-shaped chlorotic spots and *D. schulleri* in the form of concentric spots. In this study, all treatments showed 3 leaves of *D. discolor* that did not show ORSV symptoms. Research also reported that there were several orchids that show symptomless until the end of the observation, including *D. stratiotes*[8,13]. The difference in the symptoms of infection in these two orchids is influenced by the level of plant response to viral infection[1,3,5,8,12].

Disease development was obtained by calculating the incidence of each plant and the disease intensity of each plant. Based on the results showed variations in the incidence of the disease in each different replication. The average incidence of disease in *P. amabilis* is 54%, while in *D. discolor*, the average is 58%. *Phalaenopsis amabilis* has an average of 4 total leaves and 2 infected leaves, a disease incidence of 54% is obtained, while *D. discolor* has an average of 3 and 4 total leaves and 2 infected leaves so an average of 58 %. This means that the development of ORSV in *D. discolor* infects more than in *P. amabilis*, this is because the total number of leaves analyzed in *P. amabilis* is more, namely 4 leaves compared to 2 leaves in *D. discolor*. In this study it was shown that the incidence of disease in *D. discolor* was greater than in *P. amabilis*. The presence of mycorrhiza does not provide an effective role in reducing disease infection. In a previous study, Minarni *et al.*[12] reported that there was no interaction between the application of different types of planting media and doses of mycorrhizal biofertilizers on the growth and flowering of *Dendrobium*, this was made possible because *Dendrobium* sp., unable to respond well to both treatments, so that each treatment has a separate effect.

The thing that affects the calculation of disease incidence is the large number of plants infected with the virus so that the ratio of infected leaves to the total number of leaves is obtained. The genetic composition of host plants and viruses, plant age and environmental conditions before and after infection are factors that can influence the development and severity of disease symptoms. The intensity of the disease obtained shows that there are variations in the intensity of the disease. The results of this study showed that the average intensity of the disease in *P. amabilis* was more than 40%, i.e., there were 10 leaves showing necrotic symptoms, the rest were mild mosaic and severe mosaic. Meanwhile, the average disease intensity on *D. discolor* was 30%, i.e. there were 11 leaves showing mosaic symptoms. This indicated that the disease of *P. amabilis* was more severe than *D. discolor*, so the severity of each leaf increased. In a previous study, Mahfut *et al.*[4,5] found black-brown necrotic symptoms on the leaves of *Cattleya* with disease intensity between 20-80%.

The level of resistance in this study was obtained at different plant levels, namely very resistant, resistant, tolerant, susceptible and very susceptible. On average, *D. discolor* shows a tolerant response. In *D. discolor* there are also 3 leaves showing a very resistant response. Previous research by Mahfut *et al.*[4,5] reported that *D. stratiotes* and *P. violacea* orchids were tolerant to ORSV infection. In *P. amabilis,* the average resistance level is very susceptible. Several types of orchids are known to have different levels of resistance to ORSV infection. *D. burana* mainil wrap × *D. strip*, *D. burana* jade × *D. nindii*, *D. woxin*, *O. golden* shower and *V. violacea* showed a resistant response, while *D. nindii*, *D. schulerii, D. kyosimori, D. liniae*, *P. amabilis*, *P. tiny* white red lip × white red lip, *G. scriptum* and *C. black* lucky man × *C. black* lijinan pearl showed a susceptible response. *Dendrobium nindii*, *D. discolor*, *D. lasiantera*, *D. kyosimori*, *D. schulerii*, *P. amabilis*, *O. golden* shower and *C. pandurata* showed a tolerant response (*P. violacea* and *D. stratiotes*) and showed a mild response resistant (*D. burana* mainil wrap × *D. strip*, *D. burana* jade × *D. nindii*, *D. woxin* and *G. scriptum*) showed a susceptible response.

This study showed that the resistance level of *D. discolor* is more resistant than *P. amabilis*. In the *D. discolor* treatment, the virus inoculated (A1V) was more resistant than those inoculated with mycorrhizae and viruses. A comparison was also obtained of the level of resistance in the treatment (A1V), which was very susceptible (5): Susceptible (1): Tolerant (1): Moderately resistant (1) whereas, in the treatment inoculated with mycorrhiza and virus (MA1V) very susceptible (6): Tolerant (2). The treatment (A2V) had a comparison of plant resistance levels, namely very susceptible (2): Tolerant (4): Very resistant (2). In the treatment (MA2V2) obtained a comparison of the level of resistance very susceptible (1): Susceptible (1): Tolerant (3): Somewhat resistant (1): Very resistant (1). From the results obtained from the two treatments on both orchids it can be concluded that the presence of mycorrhizae does not play an effective role in plant resistance, because pathogenic viruses can also increase peroxidase activity as shown by Mahfut *et al.*[8,13] which stated that pathogen infection will increase peroxidase activity. The higher the peroxidase activity, the higher the level of plant resistance against pathogenic infections. Judging from the results obtained, more orchids were inoculated with viral mycorrhizae which showed a very susceptible response.

The results of the analysis of the content of chlorophyll a in *P. amabilis* and *D. discolor* which were inoculated with virus (V) showed a low amount. This is thought to be due to the absence of mycorrhizal roles in the formation of chlorophyll where the formation of chlorophyll requires nutrients in the form of carbon, phosphorus, nitrogen, magnesium and water which can be used in the process of forming chlorophyll a. However, although there is no Ceratorhiza, the presence of ORSV in *Phalaenopsis amabilis* and *Dendrobium discolor*, in addition to being pathogenic, is thought to induce the formation of phenols which can act as an anti-virus so that the presence of ORSV in *P. amabilis* and *D. discolor* are suspected not to damage the leaf mesophyll tissue. So it doesn't damage chlorophyll a. This conjecture was consistent with the theory put forward by Mahfut *et al*.[8,13] declare that ORSV enters cells and replicates so that orchid plants will activate a resistance response by forming secondary metabolites in the form of phenols. Mahfut *et al*.[3,11] stated that the formation of phenol can function as an antiviral. The conjectures and theories mentioned above caused the chlorophyll content in the combined treatment of both orchids with mycorrhiza (M), inoculation of viruses (V) and mycorrhizal viruses (MV) induced to be the same (not significantly different). However, when compared to the controls found in *P. amabilis* and *D. discolor* chlorophyll content was higher on both orchids induced by mycorrhiza (M), inoculation of viruses (V) and mycorrhiza viruses (MV). So it can be concluded that there is a role for Ceratorhiza induction and ORSV inoculation in *P. amabilis* and *D. discolor*. Although it does not produce resistance that is different from the chlorophyll content produced. Based on the results of the Tukey's Test for chlorophyll b content on both orchids with mycorrhizal (M), virus (V) and mycorrhizal virus (MV) treatments was shown that both orchids had the same effect. The similarity of chlorophyll b content which has been induced by mycorrhizae (M), viruses (V) and mycorrhizal viruses (MV) is thought to be due to the formation of phenols on both orchids.

In the inoculation of virus (V), phenol functions as an anti-virus. Viruses that enter orchid leaves trigger phenol to terminate virus synthesis so that the virus cannot damage the mesophyll tissue of the leaves but the virus is still able to damage the surface of the epidermis, then shows symptoms of infection in the form of chlorotic, mosaic and necrotic. Prevention of virus entry by phenol in the leaf mesophyll keeps the leaf chloroplasts from being damaged and disturbed so that chlorophyll can be formed. This was in accordance with the theory of Mahfut *et al*.[8,13] which states that plants that experience pathogen infection will develop plant resistance. Pathogens can cause activation of plant defense responses in the form of hypersensitive responses. This response will induce PR-Protein so that it can stimulate lignin formation and slow down the spread of pathogens.

In the induction of mycorrhiza (M), *P. amabilis* and *D. discolor* form induced systemic resistance (ISR). The ISR itself is an increase in plant resistance that is formed due to the presence of non-pathogenic induced agents. Factors causing the formation of ISR as a result of changes in plant physiology that stimulate plant defense against pathogens through the formation of chemical compounds. This was in accordance with Mahfut *et al*.[8,13], who explained that the physiological changes caused by ISR can be in the form of structural modifications to the cell wall or changes in biochemical reactions in plants. Some of the changes that occur are the strengthening of the epidermis and the cortex of the cell walls as well as the formation of a barrier around the site of infection in the form of phenolic compounds to prevent the entry of viruses and prevent damage to chloroplast tissue due to viral infections.

In the induction of mycorrhizal virus (MV), *P. amabilis* and *D. discolor* formed induced systemic resistance (ISR) in the administration of mycorrhiza and formed system acquired resistance (SAR) in the administration of viruses. In this treatment, it was found that chlorophyll b did not decrease. This is because in plants that were previously infected by mycorrhiza (M) and induced resistance when orchids are induced by virus (V) the plants will be able to defend themselves by producing a hypersensitive reaction (HR). HR is a reaction that increases respiration, accumulation and phenol so that there will be anti-pathogenic and non-pathogenic which results in increased plant resistance so that plants will still be able to produce chlorophyll properly due to preventing damage to the chloroplast tissue.

The conjectures and theories mentioned above caused the chlorophyll b content in the combined treatment on both orchids with induced mycorrhiza (M), inoculation of virus (V) and mycorrhizal virus (MV) to be the same (not significantly different). However, when compared to the controls found on both orchids the chlorophyll b content was higher on both orchids induced by mycorrhiza (M), inoculation of viruses (V) and mycorrhiza viruses (MV). that's it can be concluded that there is a role for *Trichoderma* induction and ORSV inoculation in both orchids. Although it does not produce resistance that is different from the chlorophyll content produced.

The results of the analysis of total chlorophyll content on both orchids induced by OMF on virus infection showed the same effect. Similar results obtained in mycorrhizal (M) inoculation, virus induction (V) and mycorrhizal virus (MV) are produced from the presence of phenol which causes viruses

that enter orchid leaves to trigger phenol to terminate viral synthesis so that viruses cannot damage tissues. leaf mesophyll but the virus can still damage the surface of the leaf epidermis and show symptoms in the form of mosaic, chlorotic or necrotic. Prevention of virus entry by phenol in the leaf mesophyll keeps the leaf chloroplasts from being damaged and disturbed so that chlorophyll can be formed. At the time of mycorrhiza (M) induction, the formation of induced resistance occurs which results in several changes occurring. These changes take the form of strengthening the epidermis and cortex of the cell walls as well as the formation of a barrier around the site of infection in the form of callus, lignin and phenolic compounds so as to prevent the entry of the virus and prevent damage to the chloroplast tissue due to viral infection. Therefore, plants will still be able to produce chlorophyll properly due to preventing damage to the chloroplast tissue[1,5,8-10,13,14].

The conjectures and theories mentioned above caused the total chlorophyll content in the combined treatment on both orchids with mycorrhiza (M) induced, virus (V) inoculation and mycorrhizal virus (MV) were the same (not significantly different). However, when compared to the controls found on both orchids the chlorophyll b content was higher on both orchids induced by mycorrhiza (M), inoculation of viruses (V) and mycorrhiza viruses (MV). So that it can be concluded that there is a role for Ceratorhiza induction and ORSV inoculation on both orchids Although it does not produce resistance that is different from the chlorophyll content produced[1,8,13].

According to research, there is no evidence that the induction of OMF *Trichoderma* increases orchid resistance to viral infections or slows the spread of disease. However, the findings of this research may provide recommendations for the kinds of orchids to be planted in the endemic regions of ORSV. This study's drawbacks include the fact that it was conducted under *in vivo* circumstances under various environmental variables, which prevented it from producing the intended results. *In vitro* research should be done more in the future to ensure that the findings support the theory. To determine the secondary metabolites produced by OMF *Trichoderma* in the indication of plant disease resistance, additional research was also carried out.

CONCLUSION

The OMF *Trichoderma* has not been proven to be able to reduce the symptoms of infection and disease progression. Based on the results obtained in the mycorrhizal induction treatment and virus inoculation showed a more severe disease infection compared to those only inoculated with the virus. The OMF could not increase resistance in *P. amabilis* and *D. discolor*, this was due to the mycorrhizal induction treatment and virus inoculation showed an average response that was very susceptible compared to the treatment that was only inoculated with the virus. The content of chlorophyll (a, b, total) mycorrhizal induction and virus inoculation treatment on both orchids was the same. In addition, there was also no difference in the level of resistance of both orchids to induction of mycorrhizal (M), inoculation of virus (V) and mycorrhizal virus (MV). This research can be used as a recommendation to determine the types of orchids cultivated in ORSV endemic areas based on the best resistance response.

SIGNIFICANCE STATEMENT

This research aims to determine the response of plant resistance through symptoms of infection, intensity of disease infection and level of plant resistance, as well as knowing the physiological response of plants through analysis of chlorophyll and carbohydrate content. The result showed OMF *Trichoderma* is proven to be able to prevent the appearance of symptoms of infection and disease development. The results of mycorrhizal and virus inoculations on orchids cause more severe symptoms of infection than those inoculated with viruses only. The OMF *Trichoderma* also has not been able to increase plant resistance in both orchids on resistance and physiological response.

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REFERENCES

1. Mahfut, M., T.T. Handayani, S. Wahyuningsih and S. Sukimin, 2021. Identification of *Dendrobium* (orchidaceae) in Liwa botanical garden based on leaf morphological characters. J. Trop. Biodivers. Biotechnol., Vol. 6. 10.22146/jtbb.59423.
2. Mahfut, A. Anggreiny, S. Wahyuningsih, T.T. Handayani and Sukimin, 2020. Identification of disease and efforts to protect native orchid plants against bacteria infection in Liwa botanical garden J. Phys. Conf. Ser., Vol. 1641. 10.1088/1742-6596/1641/1/012098.
3. Mahfut, T. Joko and B.S. Daryono, 2016. Molecular characterization of *Odontoglossum ringspot virus* (ORSV) in Java and Bali, Indonesia. Asian J. Plant Pathol., 10: 9-14.

4. Mahfut, H. Shafira, S. Wahyuningsih, T.T. Handayani and Sukimin, 2021. Identification of virus infection on native orchids in Liwa botanical garden. J. Phys. Conf. Ser., Vol. 1751. 10.1088/1742-6596/1751/1/012063.
5. Mahfut, 2020. Identification and detection *Odontoglossum ringspot virus* on native orchids collection of nurserys in Java, Indonesia. J. Phys.: Conf. Ser., Vol. 1641. 10.1088/1742-6596/1641/1/012075.
6. Mahfut, A. Indrianto, S. Somowiyarjo and B.S. Daryono, 2020. Molecular phylogeny of orchids mycorrhiza isolated from native tropical orchids in Indonesia. Malays. J. Microbiol., 16: 68-72.
7. Mahfut, 2021. Morphological identification of mycorrhizal fungi isolated from native orchid in Indonesia. Jordan J. Biol. Sci., 14: 1031-1034.
8. Mahfut, I.Y. Minarni, S. Wahyuningsih and T.T. Handayani, 2022. Physiological analysis of orchid chlorophyll against *Odontoglossum ringspot virus* infection. Jordan J. Biol. Sci., 14: 1025-1029.
9. Tohari, E.N., M. Mahfut, T.T. Handayani and S. Wahyuningsih, 2021. Induction of *Rhizoctonia* resistance to *Odontoglossum ringspot virus* (ORSV) infection on orchid leaf organ viability. J. Sustainability Sci. Technol. 1: 52-63.
10. Arifannisa, S.J., S. Wahyuningsih, T.T. Handayani and Mahfut, 2021. The effect of Rhizoctonia induction on leaf thickness of orchids infected by *Odontoglossum ringspot virus* (ORSV) [Indonesian]. Biota: J. Ilmiah Ilmu-Ilmu Hayati, 6: 186-191.
11. Izzati, F.N., M. Mahfut, E. Ernawiati and S. Wahyuningsih, 2021. Variation of resistance response of orchids induced by Rhizoctonia against the infection of *Odontoglossum ringspot virus* (ORSV) based on percentage of disease development. J. Sustainability Sci. Technol., 1: 23-34.
12. Minarni, I.Y., Mahfut, S. Wahyuningsih and T.T. Handayani, 2021. Orchid resistance selection against *Odontoglossum ringspot virus* (ORSV) [Indonesian]. Teknosains, 15: 228-233.
13. Mahfut, M. Hidayat and S. Arifannisa, 2023. Study of orchid resistance induction using rhizoctonia against ORSV infection based on anatomical characters of roots and leaves. Asian J. Plant Sci., 22: 239-249.
14. Windiyani, I.P., M. Mahfut, P. Purnomo and B.S. Daryono, 2022. Morphological variations of superior sugarcane cultivars (*Saccharum officinarum*) from Lampung, Indonesia. Biodiversitas, 23: 4109-4116.