

The Various ORSV Infection Symptoms on Ceratorhiza Induction in Orchid Plants

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ABSTRACT

Several types of orchids that are in great demand and cultivated by various groups are *Phalaenopsis* sp. and *Dendrobium* sp. Mycorrhizae are needed by orchid plants to complete their life cycle. This is because mycorrhizae are capable of symbiosis with the root system of the host plant so that the plant's capacity to absorb elements will increase. This study aims to determine the extent to which Ceratorhiza induction on *Phalaenopsis amabilis* and *Dendrobium discolor* orchids can suppress the symptoms of Odontoglossum ringspot virus (ORSV) infection. The method used in this study was a factorial completely randomized design (CRD) with 2 factors and 4 replications. The first factor is the type of orchid used, namely *Phalaenopsis amabilis* (A1) and *Dendrobium discolor* (A2), while the second factor is the type of administration of mycorrhizae, viruses, and mycorrhizal viruses. Observation of disease symptoms is done by looking at the symptoms that appear on leaves that have been infected with ORSV. The results showed that the symptoms that appeared in *Phalaenopsis amabilis* were in the form of mosaic, necrosis, leaf malformation, while in *Dendrobium discolor* were mosaic and necrotic. Ceratorhiza was not able to suppress the symptoms of disease infection because based on the results obtained; the treatment on orchids inoculated with mycorrhizae and viruses showed more severe symptoms compared to the treatment only inoculated with viruses increased response and *Dendrobium discolor* showed a decreased response in leaf length, width, and number. Meanwhile, in the root organ, *Dendrobium discolor* showed an increased response and *Phalaenopsis amabilis* showed a decreased response in root length and number of roots.

ARTICLE INFO

Received: 26 June 2021

Accepted: 17 November 2021

Published: 19 November 2021

KEYWORDS

Phalaenopsis amabilis, *Dendrobium discolor*, ORSV, Ceratorhiza disease, disease, resistance response

INTRODUCTION

Indonesia is a country that is rich in various types of orchids and the largest in the world considering that Indonesia has 6000 species of orchids (Heriswanto, 2009). Due to the attractiveness of flowers and their wide distribution, Orchids are included in the second largest family of flowering plants. Several types of orchids that are in great demand and cultivated by various groups are *Phalaenopsis* sp. and *Dendrobium* sp. (Mahfut et al., 2019).

Mycorrhizal fungi are needed by orchid plants to complete their life cycle. This is because mycorrhizae are able to symbiotically with the root system of the host plant which can increase the plant's capacity to absorb elements (Syahierah, 2010). Rungkat (2009) reported that mycorrhizae can produce growth regulating substances such as vitamins and several hormones that trigger faster and optimal growth.

Odontoglossum ringspot virus (ORSV) is one of the viruses that infect orchids with a fairly wide spread. ORSV is the dominant virus infecting orchids in the world. Virus infection in orchids causes a decrease in flower quality (Mahfut et al., 2017).

LITERATURE REVIEW

Dendrobium is one of the largest orchid genera in the family *Orchidaceae*, and includes more than 2,000 species (Uesato, 1996). *Dendrobium* is one of the natural resources owned by Indonesia where the number is estimated to reach 275 species (Sastrahidayat, 2011). The genus *Dendrobium* has a very large diversity which includes habitat, size, pseudobulb shape, leaves and flower color. Based on their way of life, most *Dendrobium* is epiphytic, but some live as lithophytes (Bechtel et al., 1992). The growth pattern of *Dendrobium* is sympodial or has limited pseudobulb growth. *Dendrobium* is liked by the public because it diligently blooms with varied and attractive flower colors and shapes.

One of the popular types of orchids is the *Phalaenopsis* genus or better known as the moon orchid. The peculiarity of *Phalaenopsis* is the shape of its larger flowers with varied colors and longer flower blooms than other types of orchids (Jenny et al., 2009). The specialty of orchids is the large size of the flowers and their elegant appearance, both of which are the main attraction. Another feature is that the color of the orchid flowers is varied, the flower crown is not easy to fall off and lasts one to two months (Yuswanti et al., 2015). *Phalaenopsis* flowers can be larger than the *Dendrobium* type. Flower stalks emerge from the base of the stem or the growing point of the plant and flowers can last for 24-29 days. The intensity of flowering is twice in one year.



Figure 1. *Phalaenopsis amabilis* orchid (Rukmana, 2018)

Endophytic mycorrhizae is a symbiosis between fungi and plant roots during a certain time in the orchid life cycle that forms colonies on plant tissues without harming the host (Mahfut et al., 2019). The working principle of this mycorrhizal is to infect the root system of the host plant, producing an intensive network of hyphae so that plants containing mycorrhizae are able to increase the absorption capacity of elements needed by plant growth (Verena, 2010). The role of mycorrhizae on host plants as a result of the symbiotic process is quite large, even mycorrhizae play a role in the success of germination and the development of sprouts to become individuals that grow normally with adequate nutrition in orchid plants (Verena, 2010).

Odontoglossum ringspot virus (ORSV) or also called Tobacco mosaic virus orchid strain (TMV-O) is one of the important viruses that infect orchids. ORSV belongs to the genus Tobamovirus and the family Virgaviridae (Mahfut et al., 2016). ORSV infection will cause stunted growth and plant resistance, as well as reduce the aesthetic value and marketability of plants on a regular basis. Orchids infected with ORSV will show symptoms in the form of necrotic, mosaic, chlorotic, and curling leaves (Mahfut et al., 2016). Necrotic is characterized by physical damage or death of cells or tissues. Some of the symptoms belonging to the necrotic type are necrose, rot, die back, and cancer (dead bark dries up with vivid boundaries) (Rungkat, 2009).



Figure 2. Symptoms of ORSV Infection in Natural Orchids (Mahfut et al., 2017)

METHODS

This research was conducted at the Botanical Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences in October–March 2021. The orchids used in this study were *Phalaenopsis amabilis* and *Dendrobium discolor*. The steps taken were putting the orchid into a culture bottle which was then acclimatized. The plantlets were placed in a petri dish containing Ceratorhiza for 24 hours, 48 hours, and so on. The plantlets were then regrown in sterile moss growing media. Observations were made by observing changes in the roots of orchids that had been incubated for 1 week with ceratorhiza. Furthermore, ORSV was inoculated on the leaves of *Phalaenopsis amabilis* and *Dendrobium discolor* orchids. Subsequent observations were made for one month. If during the observation there are no signs of infected orchids, virus reinoculation will be carried out.

The research design used was a Factorial Completely Randomized Design (CRD) with 2 treatment factors and 4 replications. The first factor was the type of orchid used, namely *Phalaenopsis amabilis* and *Dendrobium discolor* (A1 and A2). Meanwhile, the second factor is the type of administration of Mycorrhizal

(M), Virus (V), and Mycorrhizal Virus (MV). In this study, 6 (2x3) treatment combinations were obtained as presented in Table 1.

Table 1. Combination of treatment

F2 F1	M	V	MV
A1	A1M	A1V	A1MV
A2	A2M	A2V	A2MV

Description:

A1 : *Phalaenopsis*

amabilis A2 :

Dendrobium discolor M

: *Ceratorhiza* sp.

V: *Odontoglossum ringspot virus* (ORSV)

This study does not use statistical tests to describe the results of the study, but this is done descriptively.

RESULTS

In this study, observations of disease symptoms on the leaves were carried out. Observations were made regarding the development of symptoms of the disease that appeared. Based on observations, the symptoms of disease infection due to ORSV infection in *Phalaenopsis amabilis* orchids found can be classified based on the variation of symptoms and different incubation times. Based on observations, the variation of symptoms and incubation time related to symptoms of disease infection due to ORSV infection in *Phalaenopsis amabilis* orchids is presented in Table 2. The differences in infection symptoms in each type of orchid plant affect the level of plant response to the virus.

On the 10th day of the virus incubation period, mild mosaic and necrotic symptoms appeared on the orchids inoculated with the virus and mycorrhizal in replication 1 (MA1VU1) and 4 (MA1VU4). On the 12th day, 2 symptoms were found, namely vivid and necrotic mosaics in the treatment of orchids inoculated with virus in the replication 3 (A1VU3). On the 13th day, the symptoms found were mild mosaic symptoms in the treatment of orchids inoculated with virus in replication 1 (A1VU1), vivid mosaic symptoms were seen in the treatment of orchids inoculated with mycorrhizal virus in the replication 3 (MA1VU3), and 2 symptoms, namely mild mosaicism and necrotic on virus in replication 4 (MA1VU4). On the 15th day, vivid mosaic symptoms appeared in the treatment of orchids inoculated with virus and mycorrhizae in replication 3 (MA1VU3), while in replication 1 (MA1VU1), the symptoms found were vivid and necrotic mosaics. On day 18th mild mosaic, leaf malformation, and necrotic symptoms were found in the treatment of orchids inoculated with virus in replication 1 (A1VU1), vivid necrotic and mosaic symptoms in replication 2 (A1VU2), mild mosaic in replication 3 (A1VU3), necrotic on both orchid leaves in replication 4 (A1VU4), vivid mosaics and necrotic

were also found in the treatment of mycorrhizal and viral inoculated orchids in replication 2 (MA1VU2). Variations in symptoms of ORSV infection and complete incubation period in *Phalaenopsis amabilis* are presented in Table 2.

Table 2. Virus incubation period in *Phalaenopsis amabilis*

Treatment	Leaf	Variation of ORSV	Incubation
		Infection Symptom	Final Period (Days to)
A1VU1	V1	MR	13
	V2	MR, MD, N	18
A1VU2	V1	MJ	18
	V2	N	18
A1VU3	V1	MJ, N	12

In replication 1 (A1VU1) which had been inoculated with the virus, symptoms of a change of color to yellow were found in the middle of the leaf (mosaic) on leaf A (V1), while on leaf B (V2), symptoms of leaf change were almost yellow (mosaic) and shrunken leaves which are usually called leaf malformations and black (necrotic) spots in the middle of the leaf surface. In replication 2 (A1VU2), there were symptoms of a change in leaf color from green to yellow (vivid mosaic) on leaf C (V1) while on leaf D there were 2 symptoms, namely a change in leaf color from green to yellow (vivid mosaic) and black spots at the base leaf. In replication 3 (A1VU3), there were symptoms of changes in leaf color (vivid mosaic) and black spots (necrotic) on leaves E (V1). In leaf F (V2), there was a change in leaf color from green to yellow (mosaic). In replication 4 (A1VU4), there were symptoms in the form of white spots in the middle of the leaves (necrotic) on both leaves. Variations in disease symptoms in virus inoculated *Phalaenopsis amabilis* (A1V) are shown in Figure 3.

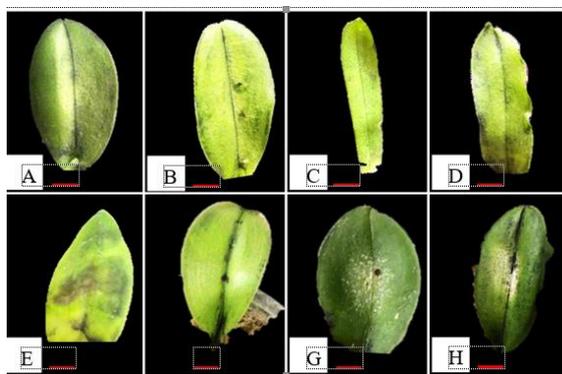


Figure 3. Symptoms of infection in *Phalaenopsis amabilis* Orchid inoculated with Virus (A1V); (A) Vivid Mosaic, (B) Vivid Mosaic, Leaf Malformation and Necrotic, (C) Vivid Mosaic, (D) Vivid Mosaic and Necrotic, (E) Vivid Mosaic and Necrotic, (F) Mild Mosaic, (G) Necrotic, (H) Necrotic.

In replication 1 (MA1VU1) which had been inoculated with mycorrhizae and virus, symptoms appeared in the form of a change in leaf color from green to brown and black spots on the leaf margins on leaf A (V1) and leaf B (V2). In replication 2 (MAVU2), there were symptoms of black spots in the middle of the leaf on leaf C (V1), while on leaf D (V2), symptoms were not only necrotic but also leaf color changes from green to yellow on

the entire leaf surface. (mosaic). In replication 3 (MA1VU3), symptoms of a yellow color change (mosaic) appeared from the middle of the leaf to the tip of the leaf on leaf E (V1) while on leaf F (V2), the symptoms were green leaves mixed with yellow color (mosaic). In replication 4 (MA1VU4), there was a change in leaf color from green to yellow (light mosaic) on the entire leaf surface and black (necrotic) spots in the middle of the leaf on leaf G (V1), while on leaf H (V2), there were symptoms of green mixed with yellow (light mosaic) on the leaf and black spots (necrotic) in the middle of the leaf. The full variation of disease symptoms in virus-inoculated *Phalaenopsis amabilis* (A1V) is shown in Figure 4.



Figure 4. Symptoms of Infection in *Phalaenopsis amabilis* Orchid Inoculated with Virus (A1V); (A) Vivid Mosaic, (B) Vivid Mosaic, Leaf Malformation and Necrotic, (C) Vivid Mosaic, (D) Vivid Necrotic and Mosaic, (E) Vivid Necrotic and Mosaic, (F) Mild Mosaic, (G) Necrotic, (H) Necrotic.

In replication 1 (A2V1) which had been inoculated with the virus, symptoms of a color change from green to yellow (vivid mosaic) were found in the middle to the tip of the leaf on leaf A (V1), while on leaf D (V2) no symptoms were found. In replication 2 (A2VU2), there were symptoms of a color change from green to yellow (mosaic), leaf B (V1) showed vivid mosaic symptoms, while on leaf C (V2), mild mosaic symptoms appeared. In replication 3 (A2VU3), the leaves turned yellow (mosaic) on D (V1) leaves, while on H (V2) leaves, there were no symptoms. In replication 4 (A2VU4), black spots were found at the base of the leaves on both leaves (E and F). However, the leaves of E (V1) in this case experienced symptoms of a color change from green to yellow (mosaic). Variations in disease symptoms in virus inoculated *Phalaenopsis amabilis* (A1V) are more fully shown in Figure 5.

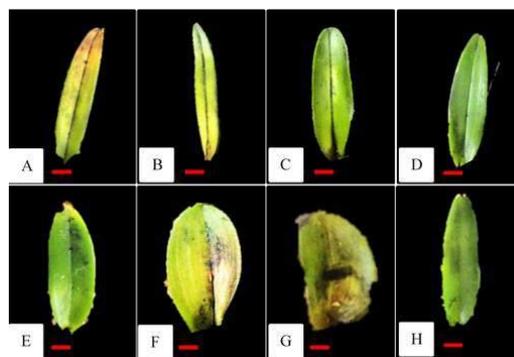


Figure 5. Symptoms of Infection in Virus Inoculated *Dendrobium discolor* (A2V): (A) Vivid Mosaic, (B) Vivid Mosaic, (C) Mild Mosaic, (D) No Symptoms, (E) Mild Mosaic, (F) Vivid Mosaic and Necrotic, (G) Necrotic, (H) Asymptomatic

Green to yellow (mosaic) was found in the middle of the leaf on leaf A (V1), while on leaf B (V2) there were no symptoms of disease. In replication 2 (MAVU2), there were symptoms of a change in color from green to yellow (mosaic) from the base to the middle of the leaf. In replication 2, brown spots appeared on the leaf margins on leaves C (V1) and D (V2). In replication 3 (MA2VU3), symptoms of leaf changes from green to yellow (vivid mosaic) appeared on the entire surface of E (V1) and F (V2) leaves. In replication 4 (MA2VU4), there were symptoms of color change at the base of the G (V1) leaf and color changes at the base to the middle of the H leaf (V2).

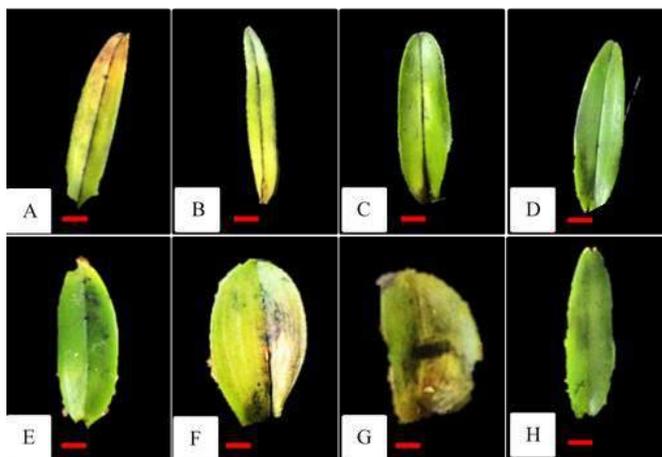


Figure 6. Symptoms of Infection in Mycorrhizal and Virus (MA2V) Inoculated *Dendrobium discolor* : (A) Mild Mosaic, (B) Asymptomatic (C) Vivid Necrotic and Mosaic, (D) Vivid necrotic and mosaic, (E) Vivid Mosaic, (F) Vivid Mosaic, (G) Mild Mosaic.

Variations in disease symptoms in virus-inoculated *Dendrobium discolor* (A2V) are shown in Figure 6.

DISCUSSION

Based on the observation of symptoms of infection due to ORSV in *Phalaenopsis amabilis* and *Dendrobium discolor*, different variations of symptoms and incubation period were found. The difference in symptoms of infection in each type of orchid affects the level of plant response to viral infection (Lakani et al., 2015). In Table 2. the symptoms that appear in *Phalaenopsis amabilis* with different incubation period treatments are shown. Symptoms that often appear in *Phalaenopsis amabilis* include necrotic, mosaic, and leaf malformations. (Mahfut et al., 2017) reported that the most frequently observed symptoms in *Phalaenopsis* due to ORSV infection were mosaic, necrotic and chlorotic. In addition, some symptoms appear in the form of curling leaf and wilting leaf. In a previous study, *Phalaenopsis* sp. It was also found to show necrotic symptoms in the form of small concave dark brown circles that cause the leaves to turn yellow (Fery et al., 2018).

In *Dendrobium discolor*, the average symptoms of infection appear on day 18. (Syahierah, 2010) reported that the average ORSV symptoms in *Dendrobium discolor* orchids appeared on the 11th day after virus inoculation. Table 3. Presents that the symptoms that often appear are mosaic symptoms. In *Dendrobium discolor*, the type of mosaic symptom seen is bright green and yellow molting on the leaves of the orchids *D. nindii*, *D. lasiantera*, *D. Burana Jadex D. nindii*, and *D. Burana Mainil Wrap x D. Stip*, chlorotic spotting rings on *D. Woxin* and *D. stratiotes* and concentric spots on *D. schulleri* orchids. (Syahierah, 2010).

In this *Dendrobium discolor* treatment, there were 3 leaves that did not experience symptoms. In a previous study, it was also reported that no disease symptoms appeared until the end of the observation in several observed plants, one of which was *Dendrobium stratiotes* (Syahierah, 2010).

CONCLUSION

Ceratorhiza was concluded to be unable to overcome the symptoms of disease infection and disease progression. This is because based on the results obtained in the treatment of orchids inoculated with mycorrhizae and viruses, it was found that symptoms were more severe than those that were only inoculated with viruses.

ACKNOWLEDGEMENTS

The first author would like to thank Dr. Mahfut, S.Si., M.Sc., Dra. Tundjung T. Handayani, M.S., and Dr. Sri Wahyuningsih, M.Sc. who have helped a lot in providing input in this research

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