Hashemite Kingdom of Jordan

Jordan Journal of Biological Sciences

An International Peer-Reviewed Scientific Journal

Financed by the Scientific Research and Innovation Support Fund

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DAFTAR ISI

2.

Halaman Pengesahan

HALAMAN PENGESAHAN

Judul	: Physiological Analysis of Orchid Chlorophyll Against *Odontoglossum ringspot virus* Infection
Penulis	: **Mahfut**, Irni Yuni Minarni, Sri Wahyuningsih, Tundjung Tripeni Handayani
NIP	: 198109092014041001
Instansi	: Jurusan Biologi, Fakultas MIPA, Universitas Lampung
Publikasi	: Jordan Journal of Biological Sciences, Vol. 14, No. 5, Pp. 1025 – 1029, 2021
Alamat Web (Link)	: https://doi.org/10.54319/jjbs/140522 http://repository.lppm.unila.ac.id/id/eprint/43097
Penerbit	: Hashemite University
ISSN	: 1995-6673
Indeks	: Scopus Q2, SJR 0.29 (2021)
Jenis Publikasi	: Jurnal Internasional Bereputasi

Bandar Lampung, 14 Juli 2022

Mengetahui,
Dekan Fakultas MIPA

Dr. Eng. Suripto Dwi Yuwono, M.T.
NIP. 197407052000031001

Penulis

Dr. Mahfut, M.Sc.
NIP. 198109092014041001

Menyetujui,
Ketua LPPM Universitas Lampung

Dr. Ir. Lusmeilia Afriani, D.E.A
NIP. 196505101993032008

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Artikel Final Yang Sudah Dipublikasi

المجلة الأردنية للعلوم الحياتية
Jordan Journal of Biological Sciences (JJBS)
http://jjbs.hu.edu.jo

Jordan Journal of Biological Sciences (JJBS) (ISSN: 1995–6673 (Print); 2307-7166 (Online)): An International Peer- Reviewed Open Access Research Journal financed by the Scientific Research and Innovation Support Fund, Ministry of Higher Education and Scientific Research, Jordan and published quarterly by the Deanship of Scientific Research , The Hashemite University, Jordan.

Submission Address
Professor Atoum, Manar F
The Hashemite Universit y
P.O. Box 330127, Zarqa, 13115, Jordan
Phone: +962-5-3903333 ext.4147
E-Mail: jjbs@hu.edu.jo

المجلة الاردنية للعلوم الحياتية
Jordan Journal of Biological Sciences (JJBS)
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JJBS

Volume 14, Number 5,December 2021
ISSN 1995-6673

Jordan Journal of Biological Sciences

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Original Articles

JJBS

Volume 14, Number 5,December 2021
ISSN 1995-6673
Pages 1025 – 1029
https://doi.org/10.54319/jjbs/140522

Jordan Journal of Biological Sciences

Physiological Analysis of Orchid Chlorophyll against *Odontoglossum ringspot virus* Infection

Mahfut[1,*], Irni Yuni Minarni[2], Sri Wahyuningsih[1], Tundjung Tripeni Handayani[1]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia;* [2]*Undergraduate Student, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia*

Received: July 21, 2021; Revised: October 23, 2021; Accepted: November 6, 2021

Abstract

Orchids (Orchidaceae) are one of the most popular ornamental plants having diverse flower shapes and colors. They are vastly applied as cut flowers, potted plants, and garden elements. Infectious diseases are still a major obstacle in the cultivation of orchids in Indonesia. *Odontoglossum ringspot virus* (ORSV) is one of the most widely reported viruses that infect orchids worldwide, including Indonesia. This research was done by mechanically injecting the virus on *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy. This study aimed to determine the symptoms of the disease, plant resistance, and chlorophyll content. The results showed that each orchid had severe disease symptoms, the incidence of both orchids was 80%, and *Phalaenopsis amabilis* reaction was more susceptible than *Dendrobium* Salaya Fancy that showed tolerant response to ORSV. Physiological response analysis also showed that the content of chlorophyll A,B, and total *Dendrobium* Salaya Fancy was higher (0.35 ± 0.02; 0.29 ± 0.05; 0.63 ± 0.05) than *Phalaenopsis amabilis* (0, 18 ± 0.0; 0.31 ± 0.06; 0.48 ± 0.04) respectively, after viral infection. This proves that *Phalaenopsis* is the most susceptible type of orchid virus compared to *Dendrobium*.

Keywords: selection of resistance; analysis of chlorophyll; orchid; ORSV

1. Introduction

Orchidaceae are ornamental plants that have a high aesthetic value (Mose et al., 2020), because they have a variety of colors and flower shapes. For that, high demands of markets on orchids have been raised in the form of cut flowers and potted plants (Mahfut et al., 2016). One of the obstacles in cultivating orchids is infectious diseases that affect flower quality. Orchids can be infected with 50 types of viruses (He et al., 2019), including *Odontoglossum ringspot virus* (ORSV) (Pai et al., 2019). The virus is an important type that attacks orchids and is popular in the world.

ORSV, also known as *Tobacco mosaic virus* orchid strain (TMV-O), belongs to the genus *Tobamovirus* and the family Virgaviridae (Forterre et al., 2017). There is very little information about ORSV infection in Indonesia. The virus is reported to be able to infect *Phalaenopsis* and *Dendrobium* orchids in West Java, Central Java, East Java, Banten, Yogyakarta, and Bali (Mahfut et al., 2016). ORSV infection causes damage to chlorophyll and affects the growth and development of orchids.

Efforts to protect orchids against viral infections need to be carried out to reduce the spread and preserve orchids in Indonesia. The initial stage of protection is considered through observing symptoms to determine the type and nature of a disease (He et al., 2017; Ko et al., 2020). This data is then used in determining plant resistance. In addition, chlorophyll analysis was also carried out, and so the physiological response of plants due to viral infection was collected.

This study was conducted to distinguish plant responses in the form of disease symptoms, plant resistance, and chlorophyll content between *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy against ORSV infection. The results of this study are expected to provide information about the response and level of resistance of orchids to ORSV infection, and it could be used as a reference for the right type of orchid to be cultivated in disease endemic areas or there has been a history of previous ORSV infection.

2. Materials and Methods

2.1. Plantlet Acclimatization

This study used two orchid species, *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy on six replicates. Plantlets were immersed in a fungicide Benlate solution, with active ingredient Benomyl (2 grams/l water) for 20 minutes and then planted in plastic pots containing sterile of moss media (Mahfut et al., 2021). Orchids were well cared for before treatment in a green house.

2.2. Virus Inoculation

The inoculum used was prepared from inoculation of the Magelang isolate virus on tobacco plants that had been previously analyzed (Mahfut et al., 2016). The inoculum was then mechanically inoculated. The initial stage of inoculation is to weigh 1 gram of viral inoculum, then

* Corresponding author e-mail: mahfut.mipa@fmipa.unila.ac.id.

grind in a sterile mortar by adding 10 ml of 0.01 M phosphate buffer solution (pH 7) (w : v = 1 : 10). Before being inoculated, 100 mess of carborundum powder was sprinkled on the upper surface of the leaves, then the virus was applied to the two youngest leaf surfaces that were fully opened. After the virus sap dries, the carborundum that remains attached to the leaf surface of the test plant was cleaned by spraying sterile water (Mahfut et al., 2016).

2.3. Observation of Infection Symptoms

The results of the inoculation test on plants were noted for variations in symptoms and incubation time. Observations were made every three days for one month to determine the response among host plants that were more quickly infected with symptoms of the disease. ORSV inoculation on each host plant was carried out at different times depending on the fast or slow growth of the plant and indicated whether or not the number of leaves was sufficient.

2.4. Plant Resistance

Determination of disease resistance criteria for various types of plants against ORSV infection was based on several factors, including symptoms of viral infection and the percentage of disease incidence. Analysis of plant resistance was used to determine the development of the observed disease, namely disease incidence. The incidence of disease is carried out by calculating the scale of damage (%) of the disease that appears on the host plant. Plant resistance was grouped into very resistant, resistant, moderately resistant, tolerant, susceptible, and very susceptible, following the method of Dwipa et al. (2018).

2.5. Chlorophyll Content Test

This test was carried out following Sedjati et al. (2020) using a spectrophotometer. For measuring chlorophyll content, the sample was applied an orchid leaf that had been identified as infected with ORSV. In the first step, 1 gram of treated orchid leaves were weighed, the leaves had been removed, then crushed with a mortar and added 10 ml of ethanol. The solution was filtered with Whatman paper no. 1 and put into a flakon, then tightly closed. Sample solution and standard solution (ethanol) 1 ml were put into different cuvettes. Furthermore, absorption readings were carried out with a UV spectrophotometer at wavelengths (λ) 648 nm and 664 nm, the measurements were carried out three times for sample replication.

3. Results

3.1. Observation of Infection Symptoms

The response of plants after virus inoculation showed that ORSV could infect all types of host plants with variations in symptoms and different incubation times. The results showed that in general the response began to appear about 2-3 weeks after inoculation. The symptoms showed necrotic *Phalaenopsis amabilis*, while *Dendrobium* Salaya Fancy showed necrotic and mosaic symptoms. Variations in response to symptoms of viral infection in both host plants are shown on (Figure 1).

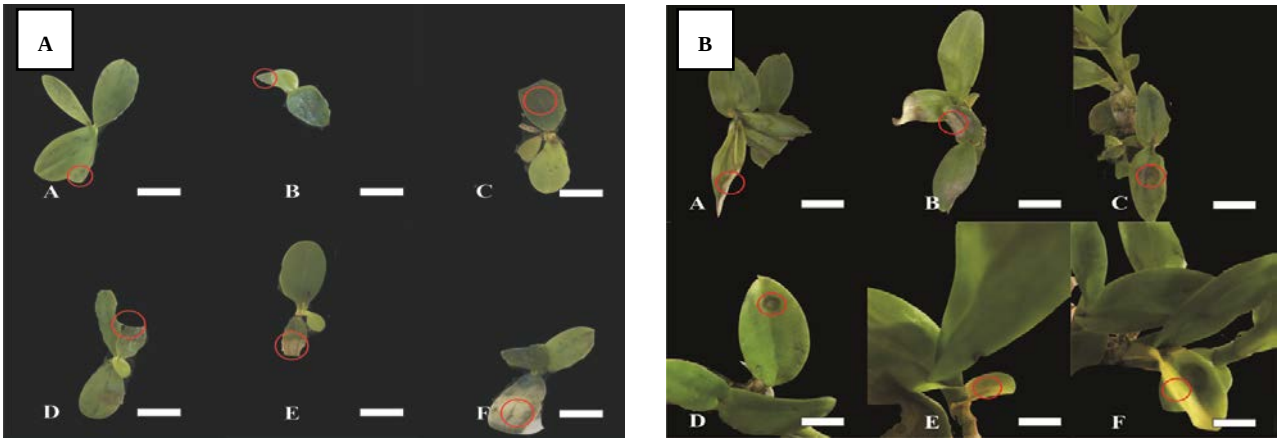

Figure 1. Symptoms of virus infection on (A) *Phalaenopsis amabilis* and (B) *Dendrobium* Salaya Fancy. Bar= 1cm

3.2. Plant Resistance Analysis

The results of the analysis of plant resistance to ORSV infection were based on variations in symptoms, incubation times, and disease incidence. The results of the analysis showed that the level of resistance is very susceptible to symptoms of very severe infection. Meanwhile, *Dendrobium* Salaya Fancy showed a level of resistance that was tolerant to a fairly severe variety of symptoms, but not as severe as the symptoms of infection in *Phalaenopsis amabilis*.

3.3. Chlorophyll Content Test

The results of the physiological response analysis showed that the chlorophyll A, chlorophyll B, and chlorophyll total content of *Dendrobium* Salaya Fancy was higher than *Phalaenopsis amabilis* after being infected with the virus. The complete test results for the content of chlorophyll on both types of host plants for 30 days after ORSV inoculation are shown in Table 1.

Table 1. Tukey's test of chlorophyll A, B, total content of two types of host plants 30 days after inoculation

Treatment	Type of Chlorophyll	Species of Host Plant	
		Phalaenopsis amabilis	*Dendrobium* Salaya Fancy
Control	Chlorophyll A	0,17 ± 0	0,37 ± 0
	Chlorophyll B	0,24 ± 0,01	0,28 ± 0
	Chlorophyll Total	0,41 ± 0,01	13,9 ± 13,27
Virus Inoculated	Chlorophyll A	0,18 ± 0,01	0,35 ± 0,02
	Chlorophyll B	0,31 ± 0,06	0,29 ± 0,05
	Chlorophyll Total	0,48 ± 0,04	0,63 ± 0,05
Total of Average	Chlorophyll A	$0,18^{a}$ ± 0,05	$0,36^{b}$ ± 0,01
	Chlorophyll B	0,28 ± 0,04	0,29 ± 0,03
	Chlorophyll Total	0,45 ± 0,025	7,27 ± 6,66

Note: The values followed by the same letter are not significantly different at the 5% level. Chlorophyll A: HSD Cell [.05] = 0.05. HSD Columns [.05]= 0.02. Chlorophyll B: Values followed by the same letter are not significantly different at the 5% level. HSD Cell [0.05] = 0.12. HSD Columns [.05]= 0.06. Total Chlorophyll: HSD Cell [0.05] = 26.86. HSD Columns [.05]= 14.5

The homogeneity of Levene's test variance at 5% significance level showed that the variance of the samples of the two host plants was homogeneous (P=0.201>0.05). Analysis of variance at the 5% level of significance showed that the virus treatment had no significant effect on the chlorophyll a content of the host plant (P=1>0.05), but the type of plant had a significant effect on the chlorophyll a content (P<0.0001). Thus, the interaction between virus inoculation and plant species did not significantly affect the chlorophyll a content (P=1>0.05). The content of chlorophyll A after virus inoculation with two types of host plants is shown in (Figure 2).

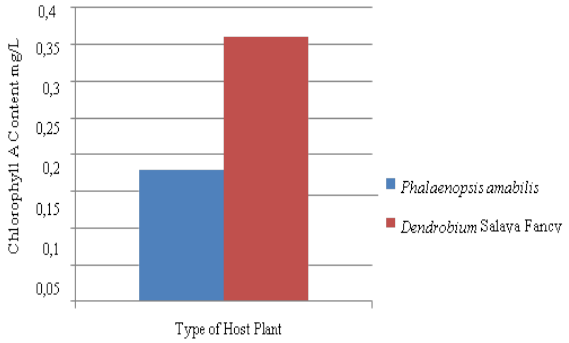

Figure 2. Curves of chlorophyll A content after virus inoculation in two types of host plants

Chlorophyll B is one of the parameters that affect plant metabolism through photosynthesis. The homogeneity of Levene's test variance at 5% significance level showed that the variance of the samples of the two host plants was homogeneous (P= 0.076>0.05). Analysis of variance at 5% significance level showed that the virus treatment (P=0.15>0.05) and plant species (P>0.05) had no significant effect on chlorophyll B levels, respectively. Likewise, the interaction between virus inoculation and plant species did not significantly affect the chlorophyll B content (P>0.05). Virus inoculation and plant species did not significantly affect chlorophyll B. ORSV inoculation on chlorophyll B content on *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy is shown in (Figure 3).

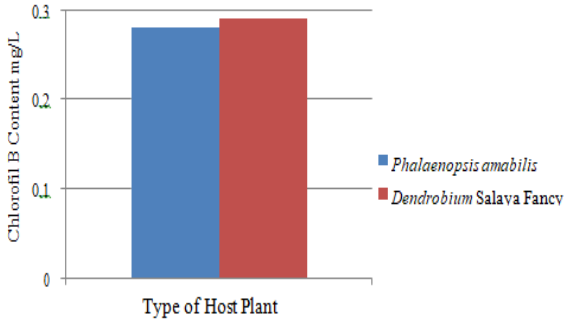

Figure 3. Curves of chlorophyll B content after virus inoculation in two types of host plants

The results of the total chlorophyll content test in both host plant samples also showed homogeneity of the Levene test variance at the level of significance 5 (P=0.224). The results showed that the virus treatment had no significant effect (P=0.34) and the type of plant also had no significant effect on the total chlorophyll content (P=0.32) in the analysis of variance at 5% significance level.

Likewise, the interaction between virus inoculation and plant species did not significantly affect the total chlorophyll content (P=0.33). ORSV virus inoculation, plant species and interactions on host plants did not significantly affect total chlorophyll. ORSV virus inoculation on total chlorophyll content in *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy is presented in (Figure 4).

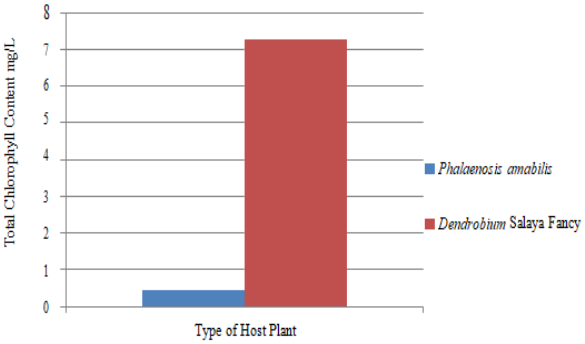

Figure 4. Curves of total chlorophyll content after virus inoculation in two types of host plants

4. Discussion

The symptomatic response of ORSV in host plants shows a wide range of symptoms on the examined host plants *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy. Based on the results of the virus inoculation on the two orchids did not show symptoms until the 30th day, so reinoculation was necessary. Then, reinoculation is performed and observed again until symptoms can be seen. *Phalaenopsis amabilis* showed necrotic symptoms on the 18th day. Observations continued until day 30 and showed worsening necrotic symptoms. In a previous study of Mahfut et al. (2020[a]), it was also known that ORSV infection in *Phalaenopsis* sp. showed necrotic symptoms on 23rd day.

Dendrobium Salaya Fancy also showed necrotic symptoms on the 15th day. Necrotic symptoms in *Dendrobium* were shown more rapidly than *Phalaenopsis*. Based on the observation of symptoms up to day 25, necrotic symptoms turned into a mosaic indicating that

ORSV infection in *Dendrobium* was getting worse. In previous studies, it was known that ORSV infection in *Dendrobium* sp. also appeared necrotic and mosaic symptoms on 15th and 23rd day (Mahfut, 2020; Mahfut et al., 2020[a]; Mahfut et al., 2020[b]; Mahfut et al., 2021).

The results of disease incidence analysis showed that each host plant had the same response that the disease incidence was > 40% and disease infection was found. This proves that the inoculation of ORSV on the whole host plant was successful. In previous research (Mahfut, 2020), it is also known that ORSV inoculation in *Phalaenopsis* sp. and *Dendrobium* sp. showed an incidence of disease > 40% and found the presence of disease infection.

Based on the results of the study, it is known that the host plant *Phalaenopsis amabilis* which shows a very susceptible level of resistance with very severe symptoms of infection. Meanwhile, *Dendrobium* Salaya Fancy orchid showed a tolerant level of resistance with a fairly severe variation of symptoms, but not as severe as the symptoms of infection in *Phalaenopsis amabilis*. This means that *Phalaenopsis amabilis* orchids are more susceptible to ORSV than *Dendrobium* Salaya Fancy.

Previous research (Mahfut, 2020) reported that *Phalaenopsis* is a highly susceptible host orchid plant and most susceptible to ORSV. In other research (Mahfut et al., 2020[a]; Mahfut et al., 2020[b]; Mahfut et al., 2021), it was also known that the plants *Phalaenopsis amabilis, P. small* Red White Lips x, *D. nindii*, *D. kyosimori*, *D. liniae*, *D. schulerii* had a response, i.e. are susceptible to ORSV.

Chlorophyll content analysis aims to determine the chlorophyll content in the host plant (Jaelani et al., 2016; Alananbeh et al., 2018; Saeed, 2019). Result of Tukey's test at the 5% significance level showed that two host plants, namely *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy, had a significant effect only on plant species but had no significant influence on viral treatment on chlorophyll A. The content of chlorophyll B in the leaves of *Dendrobium* Salaya Fancy was not much different from the content of chlorophyll B on *Phalaenopsis amabilis* leaves. This shows that the plants *Dendrobium* Salaya Fancy and *Phalaenopsis amabilis* have the same level of resistance, which is very susceptible to diseases not only caused by ORSV virus but can be caused by other factors such as fungi, bacteria, nutrients found in the media.

Total chlorophyll content in *Dendrobium* Salaya Fancy orchid leaves is relatively higher than the total chlorophyll content in *Phalaenopsis amabilis* orchid leaves. This indicates that the *Dendrobium* Salaya Fancy orchid is more resistant than the *Phalaenopsis amabilis* orchid to disease. Chlorophyll is a green pigment found in chloroplastide. In general, chlorophyll is found in leaf mesophyll cell chloroplasts, i.e. in palisade parenchyma cells and parenchyma sponge cells. In chloroplasts, chlorophyll is present in the gamma thylakoid membrane. In higher plants, the types of chlorophyll are chlorophyll A and chlorophyll B. Under normal circumstances, the proportion of chlorophyll A is much greater than that of chlorophyll B (Sedjati et al., 2020).

According to the chlorophyll data in this study, it was found that the chlorophyll data on *Dendrobium* Salaya Fancy plants are more than *Phalaenopsis amabilis* plants because seen morphologically, *Phalaenopsis amabilis* orchid plants have more severe symptoms than *Dendrobium* Salaya Fancy. It is possible that the leaf mesophyll tissue in *Dendrobium* Salaya Fancy is not damaged by the virus, and can produce more chlorophyll for photosynthesis. Therefore, it can be concluded that *Dendrobium* Salaya Fancy is more resistant to ORSV virus or other viruses than *Phalaenopsis amabilis*. Data on chlorophyll B and total chlorophyll in both host crops are stated to be statistically similar because usually the amount of chlorophyll B is less than that of chlorophyll A (Jaelani et al., 2016; Sedjati et al., 2020). Virus treatment and plant species interactions did not significantly affect the two host plants, so further testing was not performed. Therefore, it can be concluded that *Dendrobium* Salaya Fancy have a higher level of resistance than *Phalaenopsis amabilis*.

5. Conclusion

The results showed that the indicator crops and the host crops had quite severe disease with various symptoms. Each crop had the same response, the indicator crop had a disease incidence of > 40% i.e. 75% while the host crop with a disease incidence of > 40% i.e. 80%. Indicator and host plant responses to ORSV are highly susceptible, except that *Dendrobium* Salaya Fancy orchid host plants have a tolerant response to ORSV. The results of physiological response analysis showed that the content of chlorophyll A, B, and total *Dendrobium* Salaya Fancy were higher (0.35 ± 0.02; 0.29 ± 0.05; 0.63 ± 0.05) than *Phalaenopsis amabilis* (0.18) ± 0.0; 0.31 ± 0.06; 0.48 ± 0.04) respectively, after being infected with the virus. This proves that *Phalaenopsis* is the most susceptible type of orchid virus compared to *Dendrobium*.

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4.

Akreditasi Jurnal

Source details

Jordan Journal of Biological Sciences

Scopus coverage years: from 2014 to Present

Publisher: Hashemite University

ISSN: 1995-6673 E-ISSN: 2307-7166

Subject area: Agricultural and Biological Sciences: General Agricultural and Biological Sciences

Biochemistry, Genetics and Molecular Biology: General Biochemistry, Genetics and Molecular Biology

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H-INDEX

15

PUBLICATION TYPE

Journals

ISSN

19956673, 23077166

COVERAGE

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INFORMATION

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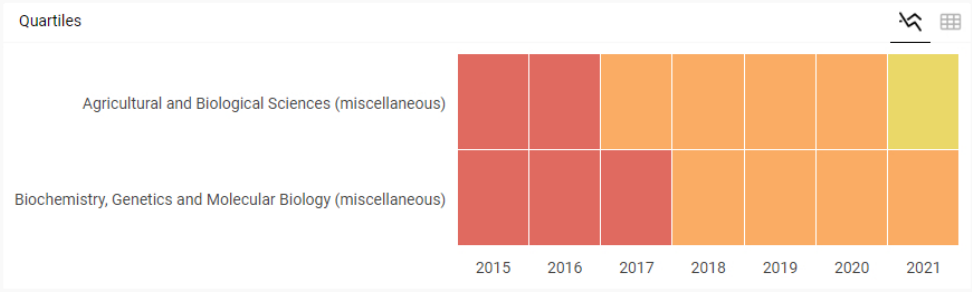

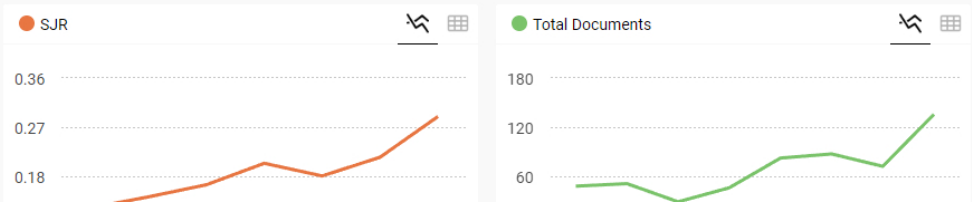


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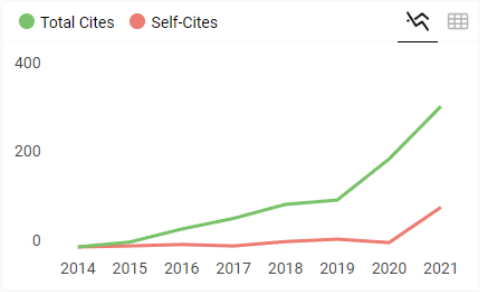
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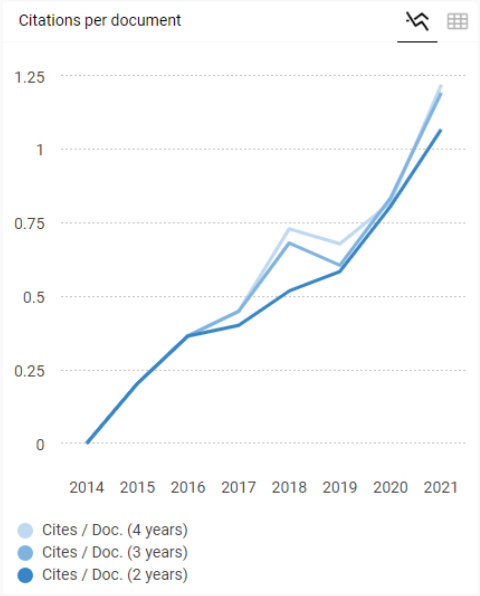
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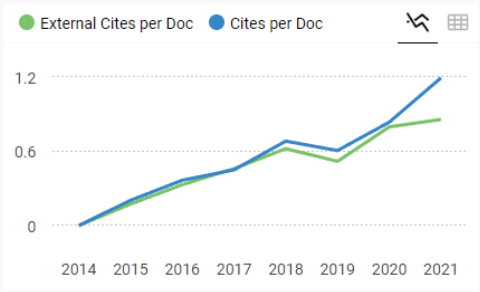
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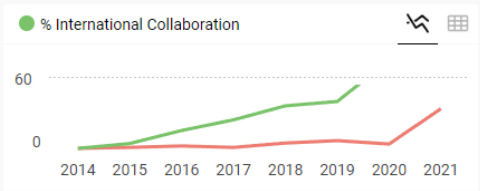
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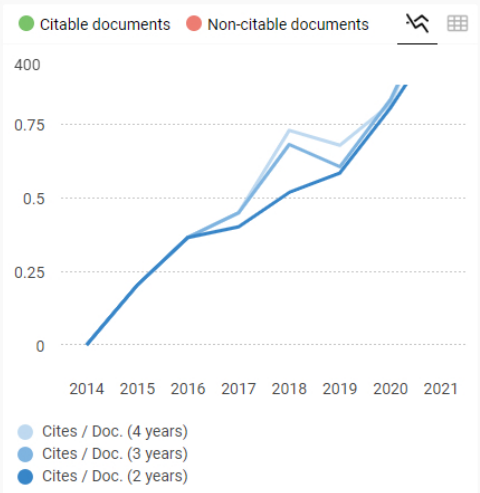
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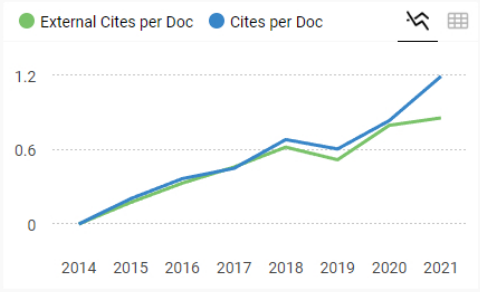
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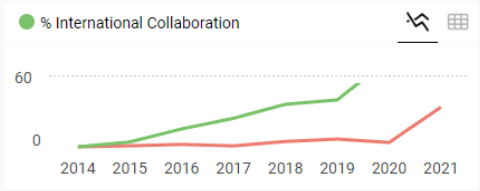
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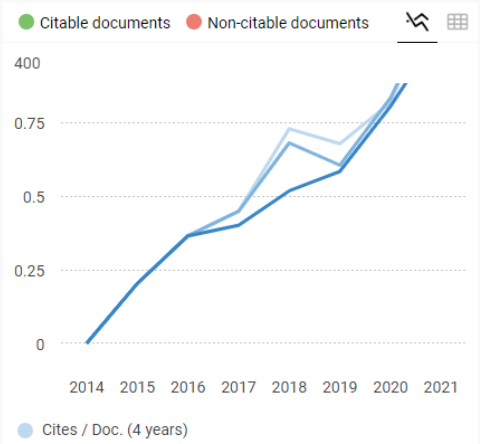
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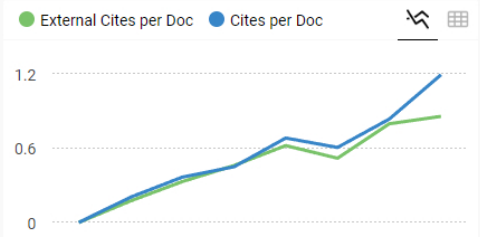
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Review 1

Jordan Journal of Biological Sciences (JJBS)
ISSN 1995- 6673 (Print), 2307- 7166 (Online)
http://jjbs.hu.edu.jo

Dear Dr. Surapog Chatpun **October 16, 2021**

A Pilot Study: **Selection of Resistance and Physiological Analysis of Chlorophyll Orchid Against Infection of Odontoglossum ringspot virus**

Manuscript Number: JJBS 69/21/A19

Thank you for submitting the above mentioned manuscript to Jordan Journal of Biological Sciences (JJBS). It has been considered by an Editor, a Member of the Editorial Board and three independent expert referees. They agree that the paper presents an interesting piece of work, however, before your paper can be accepted for publication, several changes are required; you should also respond satisfactorily to some questions of methodology and interpretation which have been raised by the referees (See attached and Comments Below). **The current decision does not guarantee an automatic accept decision following revision and the handling editor may still reject your manuscript. Please make sure that all References are according to JJBS Format as well as you send me the consent form and the results of plagiarism software (I authenticate or turnitin) with similarity results less than 15%.**

If you decide to submit a revised version of your paper within two weeks, please send to me one copy of the revised MS and your response to each Referee at separate sheet. Please be sure to include detailed Response to Reviewers' comments on separate file.

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Sincerely yours,

Professor Manar Atoum

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Jordan Journal of Biological Sciences (JJBS)
ISSN 1995- 6673 (Print), 2307- 7166 (Online)
http://jjbs.hu.edu.jo

Informed Consent

Manuscript Number: JJBS 69/21/A19
Title: Selection of Resistance and Physiological Analysis of Chlorophyll Orchid Against Infection of *Odontoglossum ringspot virus*

Authorships [Mahfut, Irni Yuni Minarni, Sri Wahyuningsih, Tundjung Tripeni Handayani] and Affiliation:
1- Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia
2- Graduate Student, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia
3- Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia
4- Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia

Conflict of interest/sponsorship (if any):

Ethical committee approval\ Human Research Protections \ or Institutional Review Board (IRB): If Applicable, Please provide a copy of the Approval.

We affirm that the submission represents work that has not been published previously and is not currently being considered by another journal. Also, I confirm that each author has seen and approved the contents of the submitted manuscript.

Signature (on behalf of all co-authors (if any))

Corresponding author
Name: Mahfut
Affiliation: Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia
Tel.: +62 721 702673
Fax: +62 721 702767

E-mail address: mahfut.mipa@fmipa.unila.ac.id
Submission date: July 21, 2020

Auhor,

Mahfut

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Review 2

Jordan Journal of Biological Sciences (JJBS)
ISSN 1995- 6673 (Print), 2307- 7166 (Online)
http://jjbs.hu.edu.jo

Dear Dr. Surapog Chatpun **October 16, 2021**

A Pilot Study: **Selection of Resistance and Physiological Analysis of Chlorophyll Orchid Against Infection of Odontoglossum ringspot virus**

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If you decide to submit a revised version of your paper within two weeks, please send to me one copy of the revised MS and your response to each Referee at separate sheet. Please be sure to include detailed Response to Reviewers' comments on separate file.

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Manuscript Evaluation Report- Referee 1

Manuscript ID: JJBS 69/21/A19 **Due date: October 2, 2021**

MS Title: Selection of Resistance and Physiological Analysis of Chlorophyll Orchid Against Infection of Odontoglossum ringspot virus

Type of Article: ☐ Review Article ☐ Research Paper ☐ Case Report

PART A:

On a scale of 1 – 5 (1 being lowest and 5 being highest), rate the manuscript based on the following criteria;

NO.	Criteria	Score
1	**Is the topic of the manuscript within the scope of the journal?**	4
2	**Does the title clearly and sufficiently reflect its content?**	3
3	**Are the keywords and abstract sufficient and informative?**	4
4	**What is the scholarly quality of the manuscript?**	2
5	**Is this a new and/ or original contribution?**	3
6	**Is the research methodology utilized appropriate and properly administered?**	3
7	**Are the methods of data analysis acceptable?**	3
8	**Are the results and conclusions clear, adequately presented and organized in relation to rest of manuscript?**	3
9	**Are the illustrations and tables necessary and in acceptable format?**	3
10	**Are the interpretations/ conclusions sound and justified by the data?**	3
11	**Are the References in a proper format according to JJBS author Instruction?**	5
12	**Is the MS written in correct and satisfactory English?**	2

Please rate the priority for publication of this article (10 is the highest priority, 1 is the lowest priority)

6

PART B: Comments per Section of Manuscript:

Abstract	It's been informative so far just needs to be improved in writing style
Introduction	Almost in fair level.
Methodology	This part in fair level and some details need more explanation like in 2.3 and 2.4.
Results	Repetitive style has been noticed in presenting the results especially in chlorophyll contents part. My big concern is related to resistance outcomes, what proved here are more likely screening the two species against the virus and showing symptom intensity level. So resistance is not clearly covered in this work.
Discussion and Conclusion	I have pointed out some problems in this division like poor English level and weak styles so huge job needs to be achieved.
References	So far match journal criteria.

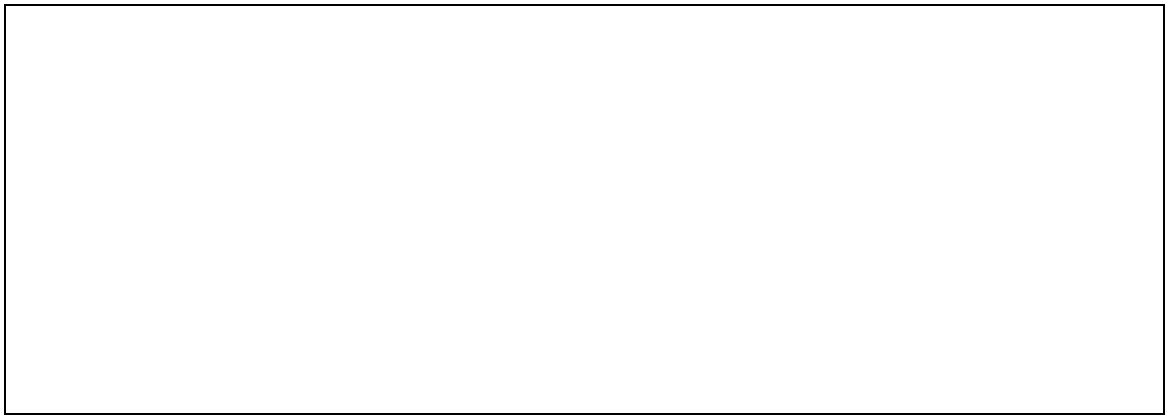
PART C: Recommendation (Kindly Mark With An ✓)

Acceptable in its Present Form	
Acceptable with Minor Revision	
Reconsidered after Major Revision	✓
Reject on Ground of (Please be Specific)	

PART D: Additional Comments:

Please add any other additional comments or specific suggestions on the enclosed comments sheet:

The title needs to exclude resistance from and focus on the achieved study and I suggest it as ((Physiological Analysis of Chlorophyll Orchid Against Infection of *dontoglossum ringspot virus*)).



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Manuscript Evaluation Report- Referee 2

Manuscript ID: JJBS 69/21 **Due date: October 22, 2021**

MS Title: Selection of Resistance and Physiological Analysis of Chlorophyll Orchid Against Infection of Odontoglossum ringspot virus

Type of Article: ☐ Review Article ☐ **Research Paper** ☐ Case Report

PART A:

On a scale of 1 – 5 (1 being lowest and 5 being highest), rate the manuscript based on the following criteria;

NO.	Criteria	Score
1	**Is the topic of the manuscript within the scope of the journal?**	5
2	**Does the title clearly and sufficiently reflect its content?**	4
3	**Are the keywords and abstract sufficient and informative?**	4
4	**What is the scholarly quality of the manuscript?**	4
5	**Is this a new and/ or original contribution?**	4
6	**Is the research methodology utilized appropriate and properly administered?**	4
7	**Are the methods of data analysis acceptable?**	4
8	**Are the results and conclusions clear, adequately presented and organized in relation to rest of manuscript?**	3.5
9	**Are the illustrations and tables necessary and in acceptable format?**	3.5
10	**Are the interpretations/ conclusions sound and justified by the data?**	4
11	**Are the References in a proper format according to JJBS author Instruction?**	3
12	**Is the MS written in correct and satisfactory English?**	3

Please rate the priority for publication of this article (10 is the highest priority, 1 is the lowest priority)

8

PART B: Comments per Section of Manuscript:

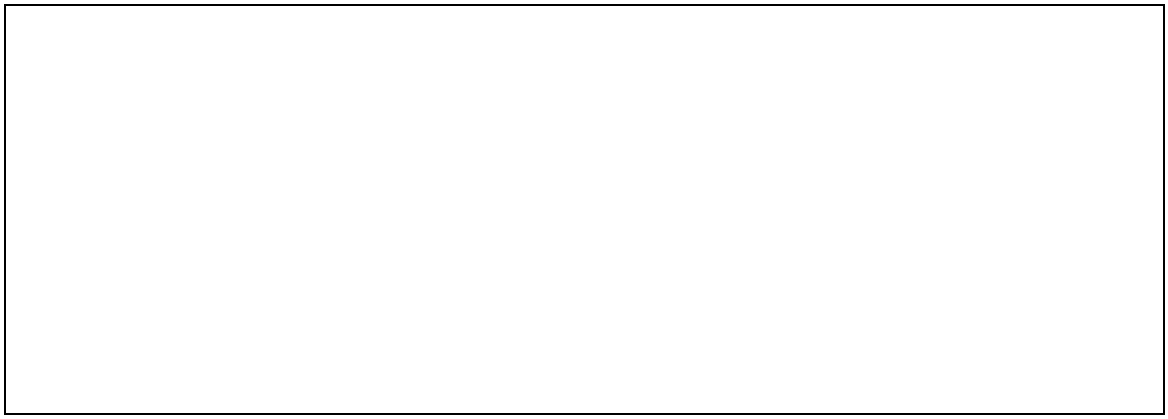
Abstract	Need English improvement
Introduction	Comments on text and need English improvement
Methodology	Comments on text and need English improvement
Results	Comments on text and need English improvement
Discussion and Conclusion	Comments on text and need English improvement
References	Need the check again and to follow JJBS instructions

PART C: Recommendation (Kindly Mark With An ✓)

Acceptable in its Present Form	
Acceptable with Minor Revision	✓
Reconsidered after Major Revision	
Reject on Ground of (Please be Specific)	

PART D: Additional Comments:

Please add any other additional comments or specific suggestions on the enclosed comments sheet:



Response to Reviewers 1 Comment

Manuscript ID: JJBS 69/21/A19

MS Title: Selection of Resistance and Physiological Analysis of Chlorophyll Orchid Against Infection of Odontoglossum ringspot virus

Section	Reviewer 1 Comment	Response to Reviewers 1 Comment
Title	The title needs to exclude resistance from and focus on the achieved study and I suggest it as ((Physiological Analysis of Chlorophyll Orchid Against Infection of *dontoglossum ringspot virus*))	Revision to the manuscript: Physiological Analysis of Chlorophyll Orchid Against Infection of *dontoglossum ringspot virus*
Abstract	It's been informative so far just needs to be improved in writing style	Revision to the manuscript: The manuscript has been revised according to the reviewer's comment (blue font)
Introduction	Almost in fair level.	Revision to the manuscript: The manuscript has been revised according to the reviewer's comment (blue font)
Methodology	This part in fair level and some details need more explanation like in 2.3 and 2.4.	Revision to the manuscript: The manuscript has been revised according to the reviewer's comment (blue font)
	Plantlets were immersed in a fungicide solution (Active ingredient better to be mentioned here)	Plantlets were immersed in a fungicide Benlate solution, with active ingredient Benomyl
Result	ORSV inoculation on each host plant was carried out at different times depending on the fast or slow growth of the plant and indicated whether or not the number of leaves was sufficient **(It's more likely suitable for material and methods).**	Revision to the manuscript: The manuscript has been revised according to the reviewer's comment (blue font)
	The results of the physiological response analysis showed that the chlorophyll a, b, **(I suggest to change these little numbers with capital ones (A and B) just for differentiation)**	Revision to the manuscript: The manuscript has been revised according to the reviewer's comment (blue font)
	Analysis of variance at 5% significance level showed that the virus treatment had no significant effect (p=0.34> 0.05) but the type of plant also had no significant effect on the chlorophyll b content (p=0.32>0.05) (please avoid repetitive style in writing sentences so	The results showed that the virus treatment had no significant effect (p=0.34>0.05) and the type of plant also had no significant effect on the levels of chlorophyll B (p=0.32>0.05) in the analysis of variance at a significant level of 5%.

	please rewrite it).	
References	So far match journal criteria.	Revision to the manuscript: The reference writing has been revised according to the JJBS

Response to Reviewers 2 Comment

Manuscript ID: JJBS 69/21/A19

MS Title: Selection of Resistance and Physiological Analysis of Chlorophyll Orchid Against Infection of Odontoglossum ringspot virus

Section	Reviewer 2 Comment	Response to Reviewers 2 Comment
Introduction	Add scientific name please: Orchids are ornamental plants that has a high aesthetic value (Mose et al., 2020)	Revision to the manuscript: Orchidaceae are ornamental plants that has a high aesthetic value (Mose et al., 2020)
Methodology	-it must be 2 orchid species -lease specify it? -where is your refrence? This study used two orchid plants, *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy on six replicates. Plantlets were immersed in a fungicide solution (2 grams/l water) for 20 minutes and then planted in plastic pots containing sterile of moss media.	his study used two orchid species, *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy on six replicates–. Plantlets were immersed in a fungicide Benlate solution, with active ingredient Benomyl (2 grams/l water) for 20 minutes and then planted in plastic pots containing sterile of moss media (Mahfut et al., 2021).
	need reference here 10 ml of phosphate buffer solution or 0.01 M phosphate buffer (pH 7) (w : v = 1 : 10). Before being inoculated, 100 mess carborundum powder was sprinkled on the upper surface of the leaves, then the virus was applied to the two youngest leaf surfaces that were fully opened. After the viral swab dries, the carborundum attached to the leaf surface of the test plant is rinsed by spraying sterile water.	10 ml of phosphate buffer solution or 0.01 M phosphate buffer (pH 7) (w : v = 1 : 10). Before being inoculated, 100 mess carborundum powder was sprinkled on the upper surface of the leaves, then the virus was applied to the two youngest leaf surfaces that were fully opened. After the viral swab dries, the carborundum attached to the leaf surface of the test plant is rinsed by spraying sterile water (Mahfut et al., 2016).
Result	infected by virus??? The results of the physiological response analysis showed that the chlorophyll a, b, and total content of *Dendrobium* Salaya Fancy was higher than *Phalaenopsis amabilis* after being infected virus.	The results of the physiological response analysis showed that the chlorophyll a, b, and total content of *Dendrobium* Salaya Fancy was higher than *Phalaenopsis amabilis* after being infected by virus.
	regardingbthe figure please remove the nubers above the columns (0.36 and 0.18)	Revision to the manuscript: The manuscript has been revised according to the reviewer's comment (blue font)
	it should written P>0.05	

	Analysis of variance at 5% significance Revision to the manuscript: The manuscript has been revised according to the reviewer's comment (blue font)level showed that the virus treatment had no significant effect (p=0.15>0.05) but the type of plant also had no significant effect on the chlorophyll b content (P>0.05). Likewise, the interaction between virus inoculation and plant species did not significantly affect the chlorophyll b content (P>0.05).	
References	So far match journal criteria.	Revision to the manuscript: The reference writing has been revised according to the JJBS

Selection of Resistance and Physiological Analysis of Chlorophyll Orchid Against Infection of *Odontoglossum ringspot virus*

Mahfut[1*], Irni Yuni Minarni[2], Sri Wahyuningsih[1], Tundjung Tripeni Handayani[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia
[2]Undergraduate Student, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia

Corresponding author e-mail: mahfut.mipa@fmipa.unila.ac.id

Abstract
Orchids (Orchidaceae) are one of the most popular ornamental plants ~~because of their~~ having diverse flower shapes and colors~~, and can be used~~. They ~~vastly~~ applied as cut flowers, potted plants, ~~or~~ and garden elements. Infectious diseases are still a major obstacle in the cultivation of orchids in Indonesia. *Odontoglossum ringspot virus* (ORSV) is one of the most widely reported ~~types of~~ viruses that infect orchids worldwide ~~and is widespread in the world~~, including ~~in~~ Indonesia. This research was done by mechanically injecting the virus on *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy. This study aimed to determine the symptoms of the disease, plant resistance, and chlorophyll content~~, as well as the resistance of orchids to ORSV~~. The results showed that each orchid had severe disease symptoms, the incidence of both orchids was 80%, and *Phalaenopsis amabilis* reaction was ~~very~~ more susceptible ~~and~~ than *Dendrobium* Salaya Fancy that showed tolerant response to ORSV. ~~The results of p~~ Physiological response analysis also showed that the content of chlorophyll a, b, and total *Dendrobium* Salaya Fancy was higher (0.35 ± 0.02; 0.29 ± 0.05; 0.63 ± 0.05) than *Phalaenopsis amabilis* (0, 18 ± 0.0; 0.31 ± 0.06; 0.48 ± 0.04) respectively, after viral infection. This proves that *Phalaenopsis* is the most susceptible type of orchid virus compared to *Dendrobium*.

Keywords: selection of resistance; analysis of chlorophyll; orchid; ORSV

1. Introduction

Orchids are ornamental plants that ~~has~~ have a high aesthetic value (Mose et al., 2020), because it has a variety of colors and flower shapes. For that, high demands of markets on orchids have been raised ~~This causes the need for orchids~~ in the form of cut flowers and potted ~~ornamental~~ plants ~~in pots is very high in the market~~ (Mahfut et al., 2016). One of the obstacles in cultivating orchids is infectious diseases that affect flower quality. Orchids can be infected with 50 types of viruses (He et al., 2019), including *Odontoglossum ringspot virus* (ORSV) (Pai et al., 2019). The virus is an important type ~~of virus~~ that attacks orchids and is ~~widespread~~ popular in the world.

ORSV, also known as *Tobacco mosaic virus* orchid strain (TMV-O), belongs to the genus ~~Tobamovirus~~ *Tobamovirus* and the family Virgaviridae (Forterre et al., 2017). There is very little information about ORSV infection in Indonesia. The ~~is~~ virus is reported to be able to infect *Phalaenopsis* and *Dendrobium* orchids in West Java, Central Java, East Java, Banten, Yogyakarta, and Bali (Mahfut et al., 2016). ORSV infection causes damage to chlorophyll and ~~so that it~~ affects the growth and development of orchids.

Efforts to protect orchids against viral infections need to be carried out to reduce the spread and preserve orchids in Indonesia. The initial stage of protection is considered ~~carried out~~ through observing symptoms to determine the type and nature of a disease (He et al., 2017; Ko et al., 2020). This data is then used in determining plant resistance. In addition, ~~an analysis of the~~ chlorophyll analysis ~~content~~ was also carried out, and so ~~that~~ the physiological response of plants due to viral infection was collected ~~known~~.

This study was conducted to distinguish plant responses in the form of disease symptoms, plant resistance, and chlorophyll content between *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy against ORSV infection. The results of this study are expected to provide information about the response and level of resistance of orchids to ORSV infection, ~~so that they~~ and it could ~~can~~ be used as a reference for the right type of orchid to be cultivated in disease endemic areas or there has been a history of previous ORSV infection.

2. Materials and Methods

2.1. Plantlet Acclimatization

This study used two orchid plants, *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy on six replicates. Plantlets were immersed in a fungicide solution (Active ingredient better to be mentioned here) (2 grams/l water) for 20 minutes and then planted in plastic pots containing sterile of moss media. Orchids were well cared for before treatment in green house.

2.2. Virus Inoculation

The inoculum ~~selection~~ used was ~~the result~~ prepared from ~~of~~ inoculation of the Magelang isolate virus on tobacco plants that had been previously analyzed (Mahfut et al., 2016). The inoculum was then mechanically inoculated. The initial stage of inoculation is to weigh 1 gram of tobacco leaves which ~~are~~ were the source of virus inoculation, then the leaves ~~are~~ were ground in a sterile mortar by adding 10 ml of phosphate buffer solution or 0.01 M phosphate buffer (pH 7) (w : v = 1 : 10). Before being inoculated, 100 mess carborundum powder was sprinkled on the upper surface of the leaves, then the virus was applied to the two youngest leaf surfaces that were fully opened. After the viral swab dries, the carborundum attached to the leaf surface of the test plant ~~is~~ was rinsed by spraying sterile water.

2.3. Observation of Infection Symptoms

The results of the inoculation test on plants were noted for variations in symptoms and incubation time. ~~ORSV inoculation on various types of orchid plants will show different responses.~~ Observations were made every three days for one month to determine the response among host plants that were more quickly infected with symptoms of the disease.

2.4 Plant Resistance

Determination of disease resistance criteria for various types of plants against ORSV infection ~~is~~ was based on several factors, including symptoms of viral infection and the percentage of disease incidence. ~~Determination of p~~ Plant resistance was grouped into very resistant, resistant, moderately resistant, tolerant, susceptible, and very susceptible, following the method of Dwipa et al. (2018).

2.5 Chlorophyll Content Test

This test was carried out following Sedjati et al. (2020) using a spectrophotometer. ~~The test sample f~~ For measuring chlorophyll content, the sample was applied an orchid leaf that had been identified as infected with ORSV. In the first step, 1 gram of treated orchid leaves were weighed, the leaves had been removed, then crushed with a mortar and added 10 ml of ethanol. The solution was filtered with Whatman paper no. 1 and put into a flakon, then tightly closed. Sample solution and standard solution (ethanol) 1 ml were put into different cuvettes. Furthermore, absorption readings were carried out with a UV spectrophotometer at wavelengths (λ) 648 nm and 664 nm, the measurements were carried out three times for sample replication.

3. Results

3.1. Observation of Infection Symptoms

ORSV inoculation on each host plant was carried out at different times depending on the fast or slow growth of the plant and indicated whether or not the number of leaves was sufficient **(It's more likely suitable for material and methods).** The response of plants after virus inoculation showed that ORSV could infect all types of host plants with variations in symptoms and different incubation times. The results showed that in general the response began to appear about 2-3 weeks after inoculation.

The ~~results of the observation of infection~~ symptoms showed necrotic *Phalaenopsis amabilis*, while *Dendrobium* Salaya Fancy showed necrotic and mosaic symptoms. Variations in response to symptoms of viral infection in both host plants are shown on (Figure 1).

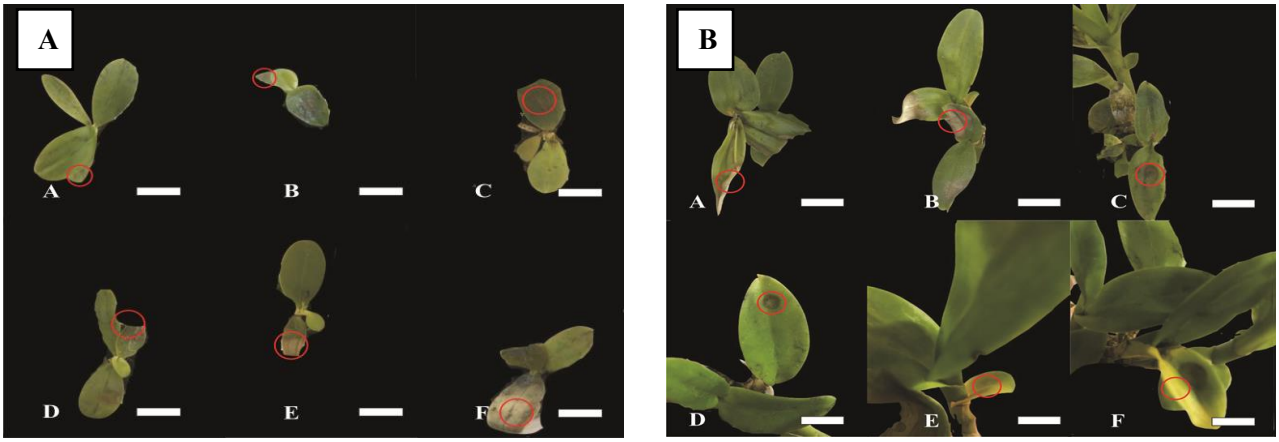


Figure 1. Symptoms of virus infection on (A) *Phalaenopsis amabilis* and (B) *Dendrobium* Salaya Fancy. Bar= 1cm

3.2. Plant Resistance Analysis

The results of the analysis of plant resistance to ORSV infection were based on variations in symptoms, incubation times, and disease incidence. The results of the analysis showed that the level of resistance is very susceptible to symptoms of very severe infection. Meanwhile, *Dendrobium* Salaya Fancy showed a level of resistance that was tolerant to a fairly severe variety of symptoms, but not as severe as the symptoms of infection in *Phalaenopsis amabilis*.

3.3. Chlorophyll Content Test

The results of the physiological response analysis showed that the chlorophyll a, b, **(I suggest to change these little numbers with capital ones (A and B) just for differentiation)** and total content of *Dendrobium* Salaya Fancy was higher than *Phalaenopsis amabilis* after being infected with the virus. The complete test results for the content of chlorophyll on both types of host plants for 30 days after ORSV inoculation are shown in Table 1.

Table 1. Tukey's test of chlorophyll a, b, total content of two types of host plants 30 days after inoculation

Treatment	Type of Chlorophyll	Species of Host Plant	
		Phalaenopsis amabilis	***Dendrobium* Salaya Fancy**
Control	Chlorophyll a	0,17 ± 0	0,37 ± 0
	Chlorophyll b	0,24 ± 0,01	0,28 ± 0
	Chlorophyll Total	0,41 ± 0,01	13,9 ± 13,27
Virus Inoculated	Chlorophyll a	0,18 ± 0,01	0,35 ± 0,02
	Chlorophyll b	0,31 ± 0,06	0,29 ± 0,05
	Chlorophyll Total	0,48 ± 0,04	0,63 ± 0,05
Total of Average	Chlorophyll a	$0,18^{a}$ ± 0,05	$0,36^{b}$ ± 0,01
	Chlorophyll b	0,28 ± 0,04	0,29 ± 0,03
	Chlorophyll Total	0,45 ± 0,025	7,27 ± 6,66

Note: The values followed by the same letter are not significantly different at the 5% level. Chlorophyll a : HSD Cell [.05] = 0.05. HSD Columns [.05]= 0.02. Chlorophyll b: Nilai-nilai yang diikuti oleh huruf yang sama tidak berbeda nyata pada taraf 5%. HSD Cell [0.05] = 0.12. HSD Columns [.05]= 0.06. Chlorophyll Total: HSD Cell [0.05] = 26.86. HSD Columns [.05]= 14.5

The homogeneity of Levene's test variance at 5% significance level showed that the variance of the samples of the two host plants was homogeneous (p-value = 0.201>0.05). Analysis of variance at the 5% level of significance showed that the virus treatment had no significant effect on the chlorophyll a content of the host plant (p=1>0.05), but the type of plant had a significant effect on the chlorophyll a content (p<0.0001). Thus, the interaction between virus inoculation and plant species did not significantly affect the chlorophyll a content (p=1>0.05). The content of chlorophyll a after virus inoculation with two types of host plants is shown on (Figure 2).

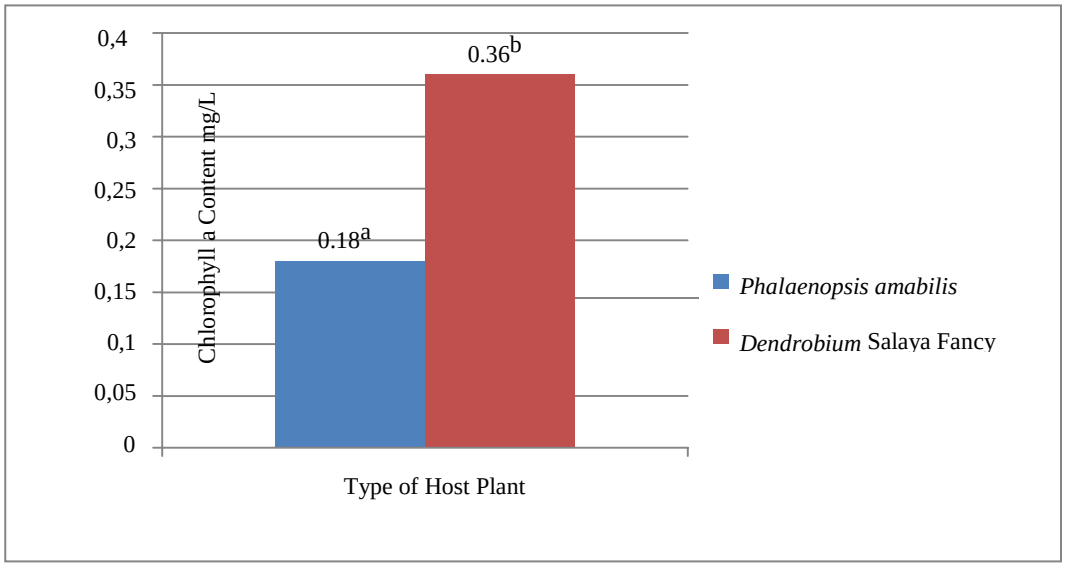


Figure 2. Curves of chlorophyll a content after virus inoculation in two types of host plants

Chlorophyll b is one of the parameters that affect plant metabolism through photosynthesis. The homogeneity of Levene's test variance at 5% significance level showed that the variance of the samples of the two host plants was homogeneous (p-value = 0.076>0.05). Analysis of variance at 5% significance level showed that ~~the virus treatment had~~ no significant effect for the virus treatment(p=0.15>0.05), and type of plant ~~but the type of plant also had no significant effect on the chlorophyll b content~~ (p=1>0.05). Likewise, the interaction between virus inoculation and plant species did not significantly affect the chlorophyll b content (p=1>0.05). Virus inoculation and plant species ~~on host plants~~ did not significantly affect chlorophyll b. ORSV ~~virus~~ inoculation on chlorophyll b content on *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy is showed on (Figure 3).

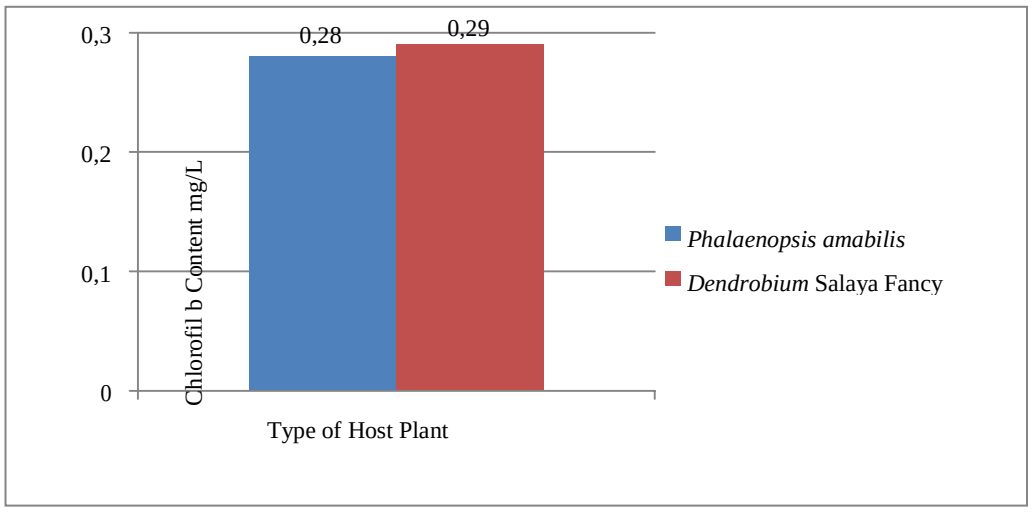


Figure 3. Curves of chlorophyll b content after virus inoculation in two types of host plants

The results of the total chlorophyll content test in both host plant samples also showed ~~the~~ homogeneity of the Levene test variance at the level of significance 5 ~~was homogeneous~~ (p-value = 0.224>0.05). Analysis of variance at 5% significance level showed that the virus treatment had no significant effect (p=0.34> 0.05) but the type of plant also had no significant effect on the chlorophyll b content (p=0.32>0.05) (please avoid repetitive style in writing sentences so please rewrite it). Likewise, the interaction between virus inoculation and plant species did not significantly affect the total chlorophyll content (p=0.33>0.05). ORSV virus inoculation, plant species and interactions on host plants did not significantly affect total chlorophyll. ORSV virus inoculation on total chlorophyll content in *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy is presented in (Figure 4).

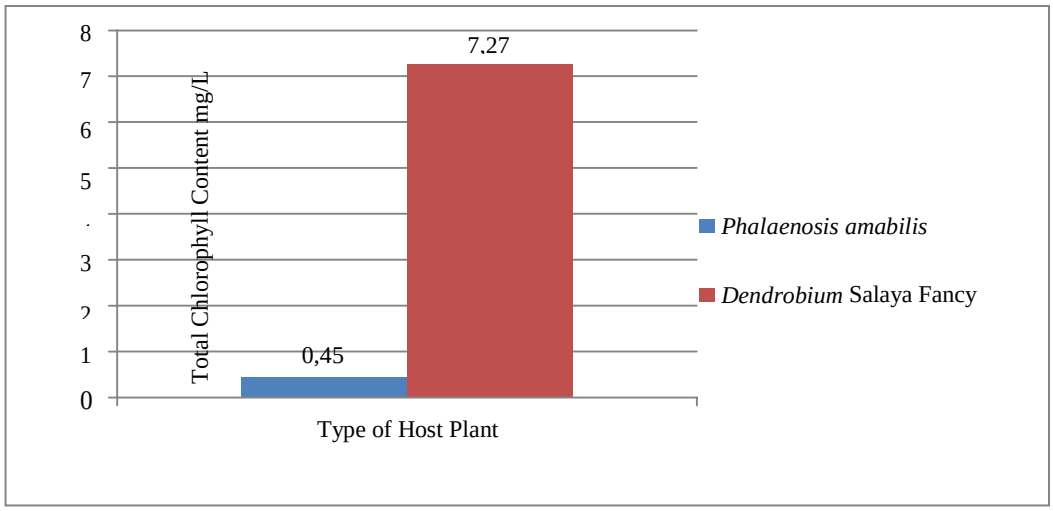


Figure 4. Curves of total chlorophyll content after virus inoculation in two types of host plants

4. Discussion

The symptomatic response of ORSV in host plants shows a wide range of symptoms~~.~~ ~~T~~ on the examined host plants ~~used were~~ *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy. Based on the results of the virus inoculation ~~injection~~ on the two orchids did not show symptoms until the 30th day. Then reinoculation is performed and observed again until symptoms can be seen. *Phalaenopsis amabilis* showed necrotic symptoms on the 18th day. Observations continued until day 30 and showed worsening necrotic symptoms. In a previous study~~(~~ of Mahfut et al.~~,~~ (2020[a]), it was also known that ORSV infection in *Phalaenopsis* sp. showed necrotic symptoms on 23rd day.

Dendrobium Salaya Fancy also showed necrotic symptoms on the 15th day. Necrotic symptoms in *Dendrobium* ~~are~~ were shown ~~seen~~ more rapidly than *Phalaenopsis*. Based on the observation of symptoms up to day 25, necrotic symptoms turned into a mosaic indicating that ORSV infection in *Dendrobium* was getting worse. In ~~a~~ previous study~~y~~ies ~~(Mahfut, 2020; Mahfut et al.,~~

~~2020[a]; Mahfut et al., 2020[b]; Mahfut et al., 2021)~~, it was known that ORSV infection in *Dendrobium* sp. also appeared necrotic and mosaic symptoms on 15th and 23rd day (Mahfut, 2020; Mahfut et al., 2020[a]; Mahfut et al., 2020[b]; Mahfut et al., 2021).

The results of disease incidence analysis showed that each host plant had the same response that the disease incidence was> 40% and disease infection was found. This proves that the inoculation of ORSV on the whole host plant was successful. In previous research (Mahfut, 2020), it is also known that ORSV inoculation in *Phalaenopsis* sp. and *Dendrobium* sp. showed an incidence of disease> 40% and found the presence of disease infection.

Based on the results of the study, it is known that the host plant ~~is~~ *Phalaenopsis amabilis* which shows a very susceptible level of resistance with very severe symptoms of infection. Meanwhile, *Dendrobium* Salaya Fancy orchid showed a tolerant level of resistance with a fairly severe variation of symptoms, but not as severe as the symptoms of infection in *Phalaenopsis amabilis*. This means that *Phalaenopsis amabilis* orchids are more susceptible to ORSV than *Dendrobium* Salaya Fancy.

In previous research (Mahfut, 2020) reported that *Phalaenopsis* is a highly susceptible host orchid plant and most susceptible to ORSV. In others research (Mahfut et al., 2020[a]; Mahfut et al., 2020[b]; Mahfut et al., 2021), it was also known that the plants *Phalaenopsis amabilis*, *P. small* Red White Lips x, *D. nindii*, *D. kyosimori*, *D. liniae*, *D. schulerii* had a response i.e. susceptible to ORSV.

Chlorophyll content analysis aims to determine the chlorophyll content in the host plant (Jaelani et al., 2016; Alananbeh et al., 2018; Saeed, 2019). Result of Tukey's test at the 5% significance level showed that two host plants, namely *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy, had a significant effect only on plant species but had no significant influence on viral treatment on chlorophyll a. The content of chlorophyll b in the leaves of *Dendrobium* Salaya Fancy was not much different from the content of chlorophyll b on *Phalaenopsis amabilis* leaves. This shows that the plants *Dendrobium* Salaya Fancy and *Phalaenopsis amabilis* have the same level of resistance, which is very susceptible to diseases not only caused by ORSV virus but can be caused by other factors such as fungi, bacteria, nutrients found in the media.

Total chlorophyll content in *Dendrobium* Salaya Fancy orchid leaves is relatively higher than the total chlorophyll content in *Phalaenopsis amabilis* orchid leaves. This indicates that the *Dendrobium* Salaya Fancy orchid is more resistant than the *Phalaenopsis amabilis* orchid to disease. Chlorophyll is a green pigment found in chloroplastide. In general, chlorophyll is found in leaf mesophyll cell chloroplasts, i.e. in palisade parenchyma cells and parenchyma sponge cells. In chloroplasts, chlorophyll is present in the gamma thylakoid membrane. In higher plants, the types of chlorophyll are chlorophyll a and chlorophyll b. Under normal circumstances, the proportion of chlorophyll a is much greater than that of chlorophyll b (Sedjati et al., 2020).

According to the chlorophyll data in this study, it was found that the chlorophyll data on *Dendrobium* Salaya Fancy plants are more than *Phalaenopsis amabilis* plants because seen morphologically, *Phalaenopsis amabilis* orchid plants have more severe symptoms than *Dendrobium* Salaya Fancy. It is possible that the leaf mesophyll tissue in *Dendrobium* Salaya Fancy is not damaged by the virus, and can produce more chlorophyll for photosynthesis. Therefore, it can be concluded that *Dendrobium* Salaya Fancy is more resistant to ORSV virus or other viruses than *Phalaenopsis amabilis*. Data on chlorophyll b and total chlorophyll in both host crops are stated to be statistically similar because usually the amount of chlorophyll b is less than that of chlorophyll a (Jaelani et al., 2016; Sedjati et al., 2020). Virus treatment and plant species interactions did not significantly affect the two host plants, so further testing was not performed. Therefore, it can be concluded that *Dendrobium* Salaya Fancy have a higher level of resistance than *Phalaenopsis amabilis*.

5. Conclusion

The results showed that the indicator crops and the host crops had quite severe disease ~~symptoms~~ with various symptoms. Each crop had the same response, the indicator crop had a disease incidence of > 40% i.e. 75% while the host crop with a disease incidence of> 40% i.e. 80%. Indicator and host plant responses to ORSV are highly susceptible, except that *Dendrobium* Salaya Fancy orchid host plants have a tolerant response to ORSV. The results of physiological response analysis showed that the content of chlorophyll a, b, and total *Dendrobium* Salaya Fancy were higher (0.35 ± 0.02; 0.29 ± 0.05; 0.63 ± 0.05) than *Phalaenopsis amabilis* (0.18) ± 0.0; 0.31 ± 0.06; 0.48 ± 0.04) respectively, after being infected with the virus. This proves that Phalaenopsis is the most susceptible type of orchid virus compared to *Dendrobium*.

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Selection of Resistance and Physiological Analysis of Chlorophyll Orchid Against Infection of *Odontoglossum ringspot virus*

Mahfut[1*], Irni Yuni Minarni[2], Sri Wahyuningsih[1], Tundjung Tripeni Handayani[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia
[2]Undergraduate Student, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia

Corresponding author e-mail: mahfut.mipa@fmipa.unila.ac.id

Abstract
Orchids (Orchidaceae) are one of the most popular ornamental plants because of their diverse flower shapes and colors and can be used as cut flowers, potted plants, or garden elements. Infectious diseases are still a major obstacle in the cultivation of orchids in Indonesia. *Odontoglossum ringspot virus* (ORSV) is one of the most widely reported types of viruses that infect orchids and is widespread in the world, including in Indonesia. This research was done by mechanically injecting the virus on *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy. This study aimed to determine the symptoms of the disease, plant resistance, and chlorophyll content, as well as the resistance of orchids to ORSV. The results showed that each orchid had severe disease symptoms, the incidence of both orchids was 80%, and *Phalaenopsis amabilis* reaction was very susceptible and *Dendrobium* Salaya Fancy tolerant response to ORSV. The results of physiological response analysis also showed that the content of chlorophyll a, b, and total *Dendrobium* Salaya Fancy was higher (0.35 ± 0.02; 0.29 ± 0.05; 0.63 ± 0.05) than *Phalaenopsis amabilis* (0, 18 ± 0.0; 0.31 ± 0.06; 0.48 ± 0.04) respectively, after viral infection. This proves that *Phalaenopsis* is the most susceptible type of orchid virus compared to *Dendrobium*.

Keywords: selection of resistance; analysis of chlorophyll; orchid; ORSV

1. Introduction

Orchids are ornamental plants that has a high aesthetic value (Mose et al., 2020) because it has a variety of colors and flower shapes. This causes the need for orchids in the form of cut flowers and ornamental plants in pots is very high in the market (Mahfut et al., 2016). One of the obstacles in cultivating orchids is infectious diseases that affect flower quality. Orchids can be infected by 50 types of viruses (He et al., 2019), including *Odontoglossum ringspot virus* (ORSV) (Pai et al., 2019). The virus is an important type of virus that attacks orchids and is widespread in the world.

ORSV, also known as Tobacco mosaic virus orchid strain (TMV-O), belongs to the genus Tobamovirus and the family Virgaviridae (Forterre et al., 2017). There is very little information about ORSV infection in Indonesia. This virus is reported to be able to infect *Phalaenopsis* and *Dendrobium* orchids in West Java, Central Java, East Java, Banten, Yogyakarta, and Bali (Mahfut et al., 2016). ORSV infection causes damage to chlorophyll so that it affects the growth and development of orchids.

Efforts to protect orchids against viral infections need to be carried out to reduce the spread and preserve orchids in Indonesia. The initial stage of protection is carried out through observing symptoms to determine the type and nature of a disease (He et al., 2017; Ko et al., 2020). This data is then used in determining plant resistance. In addition, an analysis of the chlorophyll content was also carried out, so that the physiological response of plants due to viral infection was known.

This study was conducted to distinguish plant responses in the form of disease symptoms, plant resistance, and chlorophyll content between *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy against ORSV infection. The results of this study are expected to provide information about the response and level of resistance of orchids to ORSV infection, so that they can be used as a reference for the right type of orchid to be cultivated in disease endemic areas or there has been a history of previous ORSV infection.

2. Materials and Methods

2.1. Plantlet Acclimatization

This study used two orchid plants, *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy on six replicates. Plantlets were immersed in a fungicide solution (2 grams/l water) for 20 minutes and then planted in plastic pots containing sterile of moss media. Orchids were well cared for before treatment in green house.

2.2. Virus Inoculation

The inoculum selection used was the result of inoculation of the Magelang isolate virus on tobacco plants that had been previously analyzed (Mahfut et al., 2016). The inoculum was then mechanically inoculated. The initial stage of inoculation is to weigh 1 gram of tobacco leaves which are the source of virus inoculation, then the leaves are ground in a sterile mortar by adding

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10 ml of phosphate buffer solution or 0.01 M phosphate buffer (pH 7) (w : v = 1 : 10). Before being inoculated, 100 mess carborundum powder was sprinkled on the upper surface of the leaves, then the virus was applied to the two youngest leaf surfaces that were fully opened. After the viral swab dries, the carborundum attached to the leaf surface of the test plant is rinsed by spraying sterile water.

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2.3. Observation of Infection Symptoms

The results of the inoculation test on plants were noted for variations in symptoms and incubation time. ORSV inoculation on various types of orchid plants will show different responses. Observations were made every three days for one month to determine the response among host plants that were more quickly infected with symptoms of the disease.

2.4 Plant Resistance

Determination of disease resistance criteria for various types of plants against ORSV infection is based on several factors, including symptoms of viral infection and the percentage of disease incidence. Determination of plant resistance was grouped into very resistant, resistant, moderately resistant, tolerant, susceptible, and very susceptible, following the method of Dwipa et al. (2018).

2.5 Chlorophyll Content Test

This test was carried out following Sedjati et al. (2020) using a spectrophotometer. The test sample for chlorophyll content was an orchid leaf that had been identified as infected with ORSV. In the first step, 1 gram of treated orchid leaves were weighed, the leaves had been removed, then crushed with a mortar and added 10 ml of ethanol. The solution was filtered with Whatman paper no. 1 and put into a flakon, then tightly closed. Sample solution and standard solution (ethanol) 1 ml were put into different cuvettes. Furthermore, absorption readings were carried out with a UV spectrophotometer at wavelengths (λ) 648 nm and 664 nm, the measurements were carried out three times for sample replication.

3. Results

3.1. Observation of Infection Symptoms

ORSV inoculation on each host plant was carried out at different times depending on the fast or slow growth of the plant and indicated whether or not the number of leaves was sufficient. The response of plants after virus inoculation showed that ORSV could infect all types of host plants with variations in symptoms and different incubation times. The results showed that in general the response began to appear about 2-3 weeks after inoculation.

The results of the observation of infection symptoms showed necrotic *Phalaenopsis amabilis*, while *Dendrobium* Salaya Fancy showed necrotic and mosaic symptoms. Variations in response to symptoms of viral infection in both host plants are shown on (Figure 1).

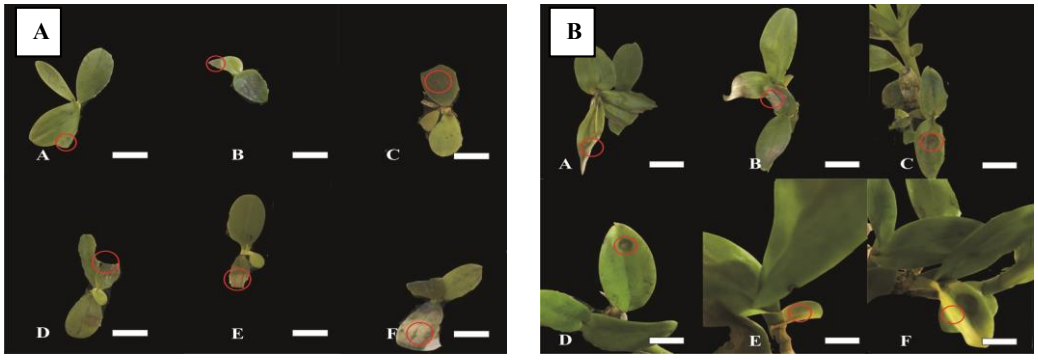

Figure 1. Symptoms of virus infection on (A) *Phalaenopsis amabilis* and (B) *Dendrobium* Salaya Fancy. Bar= 1cm

3.2. Plant Resistance Analysis

The results of the analysis of plant resistance to ORSV infection were based on variations in symptoms, incubation times, and disease incidence. The results of the analysis show that the level of resistance is very susceptible to symptoms of very severe infection. Meanwhile, *Dendrobium* Salaya Fancy showed a level of resistance that was tolerant to a fairly severe variety of symptoms, but not as severe as the symptoms of infection in *Phalaenopsis amabilis*.

3.3. Chlorophyll Content Test

The results of the physiological response analysis showed that the chlorophyll a, b, and total content of *Dendrobium* Salaya Fancy was higher than *Phalaenopsis amabilis* after being infected virus. The complete test results for the content of chlorophyll on both types of host plants for 30 days after ORSV inoculation are shown in Table 1.

Table 1. Tukey's test of chlorophyll a, b, total content of two types of host plants 30 days after inoculation

Treatment	Type of Chlorophyll	Species of Host Plant	
		Phalaenopsis amabilis	***Dendrobium* Salaya Fancy**
Control	Chlorophyll a	0,17 ± 0	0,37 ± 0
	Chlorophyll b	0,24 ± 0,01	0,28 ± 0
	Chlorophyll Total	0,41 ± 0,01	13,9 ± 13,27
Virus Inoculated	Chlorophyll a	0,18 ± 0,01	0,35 ± 0,02
	Chlorophyll b	0,31 ± 0,06	0,29 ± 0,05
	Chlorophyll Total	0,48 ± 0,04	0,63 ± 0,05
Total of Average	Chlorophyll a	$0,18^{a}$ ± 0,05	$0,36^{b}$ ± 0,01
	Chlorophyll b	0,28 ± 0,04	0,29 ± 0,03
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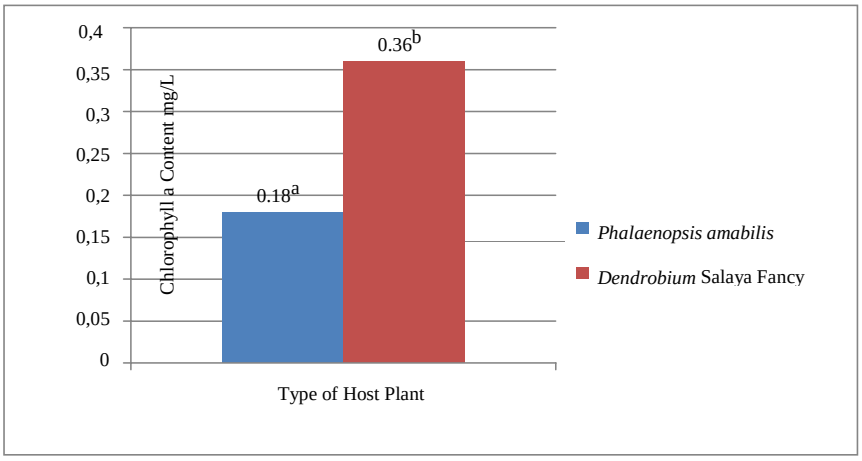

Figure 2. Curves of chlorophyll a content after virus inoculation in two types of host plants

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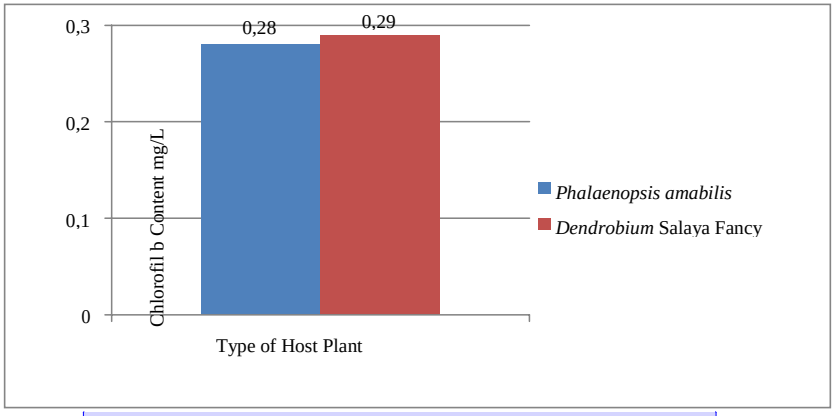

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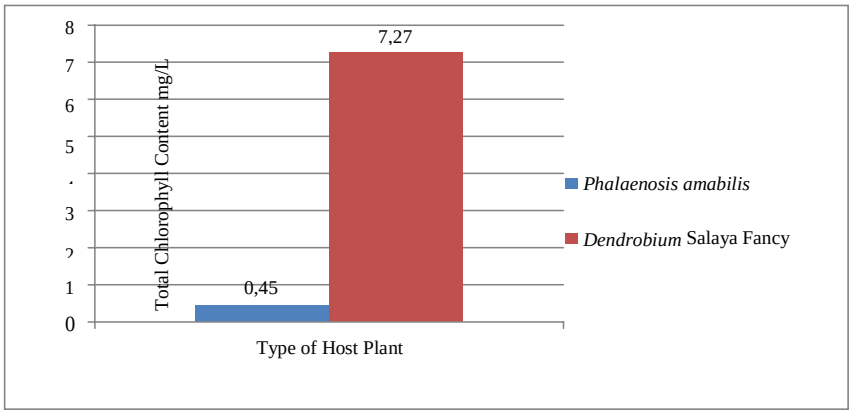

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4. Discussion

The symptomatic response of ORSV in host plants shows a wide range of symptoms. The host plants used were *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy. Based on the results of the virus injection on the two orchids did not show symptoms until the 30th day. Then reinoculation is performed and observed again until symptoms can be seen. *Phalaenopsis amabilis* showed necrotic symptoms on the 18th day. Observations continued until day 30 and showed worsening necrotic symptoms. In a previous study (Mahfut et al., 2020[a]), it was also known that ORSV infection in *Phalaenopsis* sp. showed necrotic symptoms on 23rd day.

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Based on the results of the study, it is known that the host plant is *Phalaenopsis amabilis* which shows a very susceptible level of resistance with very severe symptoms of infection. Meanwhile, *Dendrobium* Salaya Fancy orchid showed a tolerant level of resistance with a fairly severe variation of symptoms, but not as severe as the symptoms of infection in *Phalaenopsis amabilis*. This means that *Phalaenopsis amabilis* orchids are more susceptible to ORSV than *Dendrobium* Salaya Fancy.

In previous research (Mahfut, 2020) reported that *Phalaenopsis* is a highly susceptible host orchid plant and most susceptible to ORSV. In others research (Mahfut et al., 2020[a]; Mahfut et al., 2020[b]; Mahfut et al., 2021), it was also known that the plants *Phalaenopsis amabilis*, *P. small* Red White Lips x, *D. nindii*, *D. kyosimori*, *D. liniae*, *D. schulerii* had a response i.e. susceptible to ORSV.

Chlorophyll content analysis aims to determine the chlorophyll content in the host plant (Jaelani et al., 2016; Alananbeh et al., 2018; Saeed, 2019). Result of Tukey's test at the 5% significance level showed that two host plants, namely *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy, had a significant effect only on plant species but had no significant influence on viral treatment on chlorophyll a. The content of chlorophyll b in the leaves of *Dendrobium* Salaya Fancy was not much different from the content of chlorophyll b on *Phalaenopsis amabilis* leaves. This shows that the plants *Dendrobium* Salaya Fancy and *Phalaenopsis amabilis* have the same level of resistance, which is very susceptible to diseases not only caused by ORSV virus but can be caused by other factors such as fungi, bacteria, nutrients found in the media.

Total chlorophyll content in *Dendrobium* Salaya Fancy orchid leaves is relatively higher than the total chlorophyll content in *Phalaenopsis amabilis* orchid leaves. This indicates that the *Dendrobium* Salaya Fancy orchid is more resistant than the *Phalaenopsis amabilis* orchid to disease. Chlorophyll is a green pigment found in chloroplastide. In general, chlorophyll is found in leaf mesophyll cell chloroplasts, i.e. in palisade parenchyma cells and parenchyma sponge cells. In chloroplasts, chlorophyll is present in the gamma thylakoid membrane. In higher plants, the types of chlorophyll are chlorophyll a and chlorophyll b. Under normal circumstances, the proportion of chlorophyll a is much greater than that of chlorophyll b (Sedjati et al., 2020).

According to the chlorophyll data in this study, it was found that the chlorophyll data on *Dendrobium* Salaya Fancy plants are more than *Phalaenopsis amabilis* plants because seen morphologically, *Phalaenopsis amabilis* orchid plants have more severe symptoms than *Dendrobium* Salaya Fancy. It is possible that the leaf mesophyll tissue in *Dendrobium* Salaya Fancy is not damaged by the virus, and can produce more chlorophyll for photosynthesis. Therefore, it can be concluded that *Dendrobium* Salaya Fancy is more resistant to ORSV virus or other viruses than *Phalaenopsis amabilis*. Data on chlorophyll b and total chlorophyll in both host crops are stated to be statistically similar because usually the amount of chlorophyll b is less than that of chlorophyll a (Jaelani et al., 2016; Sedjati et al., 2020). Virus treatment and plant species interactions did not significantly affect the two host plants, so further testing was not performed. Therefore, it can be concluded that *Dendrobium* Salaya Fancy have a higher level of resistance than *Phalaenopsis amabilis*.

5. Conclusion

The results showed that the indicator crops and the host crops had quite severe disease symptoms with various symptoms. Each crop had the same response, the indicator crop had a disease incidence of > 40% i.e. 75% while the host crop with a disease incidence of> 40% i.e. 80%. Indicator and host plant responses to ORSV are highly susceptible, except that *Dendrobium* Salaya Fancy orchid host plants have a tolerant response to ORSV. The results of physiological response analysis showed that the content of chlorophyll a, b, and total *Dendrobium* Salaya Fancy were higher (0.35 ± 0.02; 0.29 ± 0.05; 0.63 ± 0.05) than *Phalaenopsis amabilis* (0.18) ± 0.0; 0.31 ± 0.06; 0.48 ± 0.04) respectively, after being infected with the virus. This proves that Phalaenopsis is the most susceptible type of orchid virus compared to *Dendrobium*.

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7.

Review 3

Physiological Analysis of Chlorophyll Orchid Against Infection of *Odontoglossum ringspot virus*

Mahfut[1*], Irni Yuni Minarni[2], Sri Wahyuningsih[1], Tundjung Tripeni Handayani[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia
[2]Undergraduate Student, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia

Corresponding author e-mail: mahfut.mipa@fmipa.unila.ac.id

Abstract
Orchids (Orchidaceae) are one of the most popular ornamental plants having diverse flower shapes and colors. They vastly applied as cut flowers, potted plants, and garden elements. Infectious diseases are still a major obstacle in the cultivation of orchids in Indonesia. *Odontoglossum ringspot virus* (ORSV) is one of the most widely reported viruses that infect orchids worldwide, including Indonesia. This research was done by mechanically injecting the virus on *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy. This study aimed to determine the symptoms of the disease, plant resistance, and chlorophyll content. The results showed that each orchid had severe disease symptoms, the incidence of both orchids was 80%, and *Phalaenopsis amabilis* reaction was more susceptible than *Dendrobium* Salaya Fancy that showed tolerant response to ORSV. Physiological response analysis also showed that the content of chlorophyll A,B, and total *Dendrobium* Salaya Fancy was higher (0.35 ± 0.02; 0.29 ± 0.05; 0.63 ± 0.05) than *Phalaenopsis amabilis* (0, 18 ± 0.0; 0.31 ± 0.06; 0.48 ± 0.04) respectively, after viral infection. This proves that *Phalaenopsis* is the most susceptible type of orchid virus compared to *Dendrobium*.

Keywords: selection of resistance; analysis of chlorophyll; orchid; ORSV

1. Introduction

Orchidaceae are ornamental plants that have a high aesthetic value (Mose et al., 2020), because it has a variety of colors and flower shapes. For that, high demands of markets on orchids have been raised in the form of cut flowers and potted plants (Mahfut et al., 2016). One of the obstacles in cultivating orchids is infectious diseases that affect flower quality. Orchids can be infected with 50 types of viruses (He et al., 2019), including *Odontoglossum ringspot virus* (ORSV) (Pai et al., 2019). The virus is an important type that attacks orchids and is popular in the world.

ORSV, also known as *Tobacco mosaic virus* orchid strain (TMV-O), belongs to the genus *Tobamovirus* and the family Virgaviridae (Forterre et al., 2017). There is very little information about ORSV infection in Indonesia. The virus is reported to be able to infect *Phalaenopsis* and *Dendrobium* orchids in West Java, Central Java, East Java, Banten, Yogyakarta, and Bali (Mahfut et al., 2016). ORSV infection causes damage to chlorophyll and affects the growth and development of orchids.

Efforts to protect orchids against viral infections need to be carried out to reduce the spread and preserve orchids in Indonesia. The initial stage of protection is considered through observing symptoms to determine the type and nature of a disease (He et al., 2017; Ko et al., 2020). This data is then used in determining plant resistance. In addition, chlorophyll analysis was also carried out, and so the physiological response of plants due to viral infection was collected.

This study was conducted to distinguish plant responses in the form of disease symptoms, plant resistance, and chlorophyll content between *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy against ORSV infection. The results of this study are expected to provide information about the response and level of resistance of orchids to ORSV infection, and it could be used as a reference for the right type of orchid to be cultivated in disease endemic areas or there has been a history of previous ORSV infection.

2. Materials and Methods

2.1. Plantlet Acclimatization

This study used two orchid species, *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy on six replicates. Plantlets were immersed in a fungicide Benlate solution, with active ingredient Benomyl (2 grams/l water) for 20 minutes and then planted in plastic pots containing sterile of moss media (Mahfut et al., 2021). Orchids were well cared for before treatment in green house.

2.2. Virus Inoculation

The inoculum used was prepared from inoculation of the Magelang isolate virus on tobacco plants that had been previously analyzed (Mahfut et al., 2016). The inoculum was then mechanically inoculated. The initial stage of inoculation is to weigh 1 gram of tobacco leaves which were the source of virus inoculation, then the leaves were ground in a sterile mortar by adding 10 ml of phosphate buffer solution or 0.01 M phosphate buffer (pH 7) (w : v = 1 : 10). Before being inoculated, 100 mess

carborundum powder was sprinkled on the upper surface of the leaves, then the virus was applied to the two youngest leaf surfaces that were fully opened. After the viral swab dries, the carborundum attached to the leaf surface of the test plant was rinsed by spraying sterile water (Mahfut et al., 2016).

2.3. Observation of Infection Symptoms

The results of the inoculation test on plants were noted for variations in symptoms and incubation time. Observations were made every three days for one month to determine the response among host plants that were more quickly infected with symptoms of the disease. ORSV inoculation on each host plant was carried out at different times depending on the fast or slow growth of the plant and indicated whether or not the number of leaves was sufficient.

2.4 Plant Resistance

Determination of disease resistance criteria for various types of plants against ORSV infection was based on several factors, including symptoms of viral infection and the percentage of disease incidence. Analysis of plant resistance was used to determine the development of the observed disease, namely disease incidence. Disease incidence was carried out by counting the number of infected plant parts from all plant samples, namely by calculating the scale of damage (%) of diseases that appear on the host plant. Plant resistance was grouped into very resistant, resistant, moderately resistant, tolerant, susceptible, and very susceptible, following the method of Dwipa et al. (2018).

2.5 Chlorophyll Content Test

This test was carried out following Sedjati et al. (2020) using a spectrophotometer. For measuring chlorophyll content, the sample was applied an orchid leaf that had been identified as infected with ORSV. In the first step, 1 gram of treated orchid leaves were weighed, the leaves had been removed, then crushed with a mortar and added 10 ml of ethanol. The solution was filtered with Whatman paper no. 1 and put into a flakon, then tightly closed. Sample solution and standard solution (ethanol) 1 ml were put into different cuvettes. Furthermore, absorption readings were carried out with a UV spectrophotometer at wavelengths (λ) 648 nm and 664 nm, the measurements were carried out three times for sample replication.

3. Results

3.1. Observation of Infection Symptoms

The response of plants after virus inoculation showed that ORSV could infect all types of host plants with variations in symptoms and different incubation times. The results showed that in general the response began to appear about 2-3 weeks after inoculation. The symptoms showed necrotic *Phalaenopsis amabilis*, while *Dendrobium* Salaya Fancy showed necrotic and mosaic symptoms. Variations in response to symptoms of viral infection in both host plants are shown on (Figure 1).

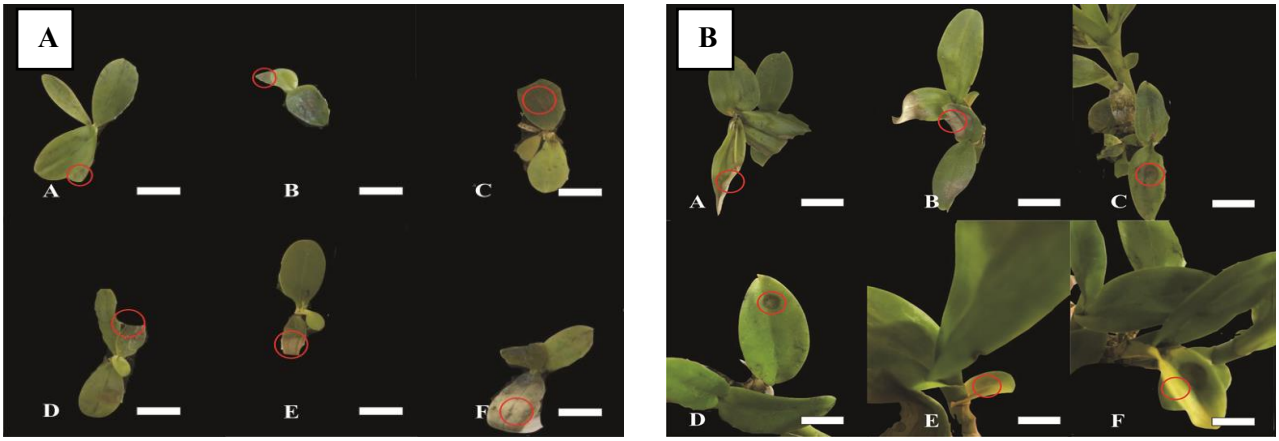

Figure 1. Symptoms of virus infection on (A) *Phalaenopsis amabilis* and (B) *Dendrobium* Salaya Fancy. Bar= 1cm

3.2. Plant Resistance Analysis

The results of the analysis of plant resistance to ORSV infection were based on variations in symptoms, incubation times, and disease incidence. The results of the analysis showed that the level of resistance is very susceptible to symptoms of very severe infection. Meanwhile, *Dendrobium* Salaya Fancy showed a level of resistance that was tolerant to a fairly severe variety of symptoms, but not as severe as the symptoms of infection in *Phalaenopsis amabilis*.

3.3. Chlorophyll Content Test

The results of the physiological response analysis showed that the chlorophyll A, B, and total content of *Dendrobium* Salaya Fancy was higher than *Phalaenopsis amabilis* after being infected with the virus. The complete test results for the content of chlorophyll on both types of host plants for 30 days after ORSV inoculation are shown in Table 1.

Table 1. Tukey's test of chlorophyll A, B, total content of two types of host plants 30 days after inoculation

Treatment	Type of Chlorophyll	Species of Host Plant	
		Phalaenopsis amabilis	***Dendrobium* Salaya Fancy**
Control	Chlorophyll A	0,17 ± 0	0,37 ± 0
	Chlorophyll B	0,24 ± 0,01	0,28 ± 0
	Chlorophyll Total	0,41 ± 0,01	13,9 ± 13,27
Virus Inoculated	Chlorophyll A	0,18 ± 0,01	0,35 ± 0,02
	Chlorophyll B	0,31 ± 0,06	0,29 ± 0,05
	Chlorophyll Total	0,48 ± 0,04	0,63 ± 0,05
Total of Average	Chlorophyll A	$0,18^{a}$ ± 0,05	$0,36^{b}$ ± 0,01
	Chlorophyll B	0,28 ± 0,04	0,29 ± 0,03
	Chlorophyll Total	0,45 ± 0,025	7,27 ± 6,66

Note: The values followed by the same letter are not significantly different at the 5% level. Chlorophyll A: HSD Cell [.05] = 0.05. HSD Columns [.05]= 0.02. Chlorophyll B: Values followed by the same letter are not significantly different at the 5% level. HSD Cell [0.05] = 0.12. HSD Columns [.05]= 0.06. Total Chlorophyll: HSD Cell [0.05] = 26.86. HSD Columns [.05]= 14.5

The homogeneity of Levene's test variance at 5% significance level showed that the variance of the samples of the two host plants was homogeneous (P=0.201>0.05). Analysis of variance at the 5% level of significance showed that the virus treatment had no significant effect on the chlorophyll a content of the host plant (P=1>0.05), but the type of plant had a significant effect on the chlorophyll a content (P<0.0001). Thus the interaction between virus inoculation and plant species did not significantly affect the chlorophyll a content (P=1>0.05). The content of chlorophyll A after virus inoculation with two types of host plants is shown on (Figure 2).

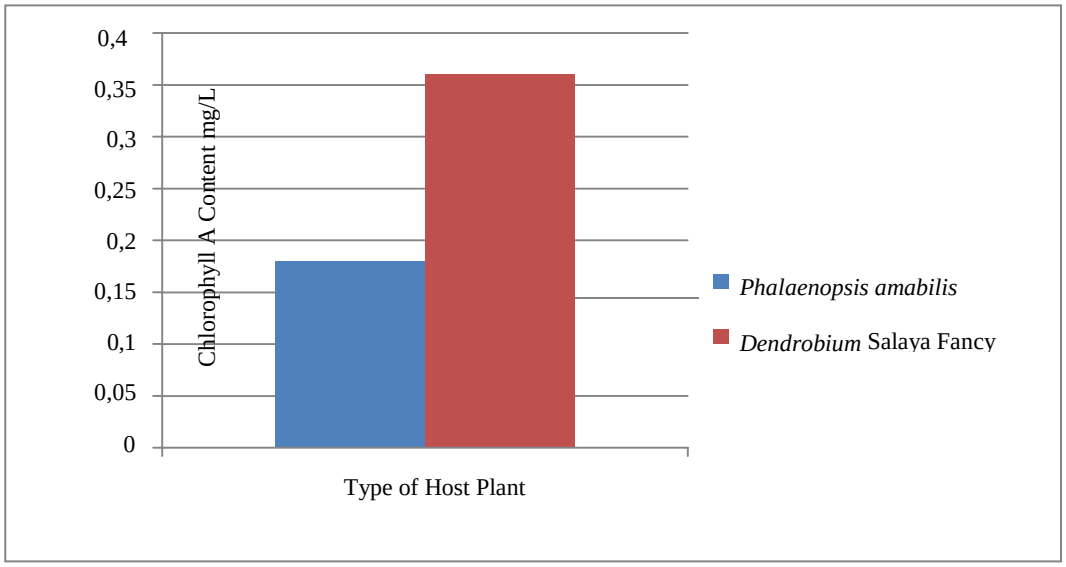

Figure 2. Curves of chlorophyll A content after virus inoculation in two types of host plants

Chlorophyll B is one of the parameters that affect plant metabolism through photosynthesis. The homogeneity of Levene's test variance at 5% significance level showed that the variance of the samples of the two host plants was homogeneous (P= 0.076>0.05). Analysis of variance at 5% significance level showed that no significant effect for the virus treatment (P=0.15>0.05), and type of plant (P>0.05). Likewise, the interaction between virus inoculation and plant species did not significantly affect the chlorophyll B content (P>0.05). Virus inoculation and plant species did not significantly affect chlorophyll B. ORSV inoculation on chlorophyll B content on *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy is showed on (Figure 3).

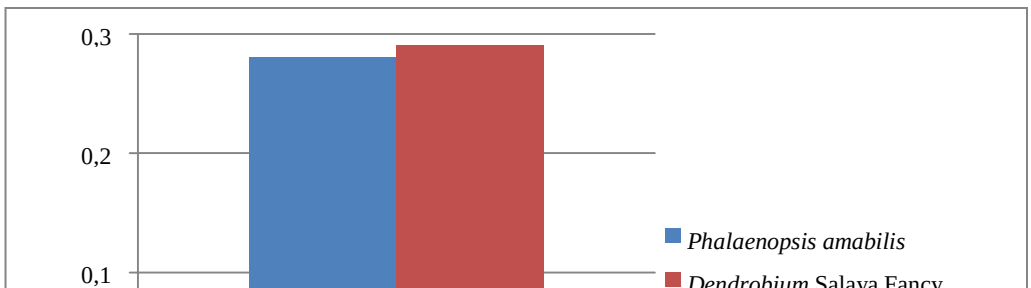

Figure 3. Curves of chlorophyll B content after virus inoculation in two types of host plants

The results of the total chlorophyll content test in both host plant samples also showed homogeneity of the Levene test variance at the level of significance 5 (P=0.224). The results showed that the virus treatment had no significant effect (P=0.34) and the type of plant also had no significant effect on the levels of chlorophyll B (P=0.32) in the analysis of variance at a significant level of 5%. Likewise, the interaction between virus inoculation and plant species did not significantly affect the total chlorophyll content (P=0.33). ORSV virus inoculation, plant species and interactions on host plants did not significantly affect total chlorophyll. ORSV virus inoculation on total chlorophyll content in *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy is presented in (Figure 4).

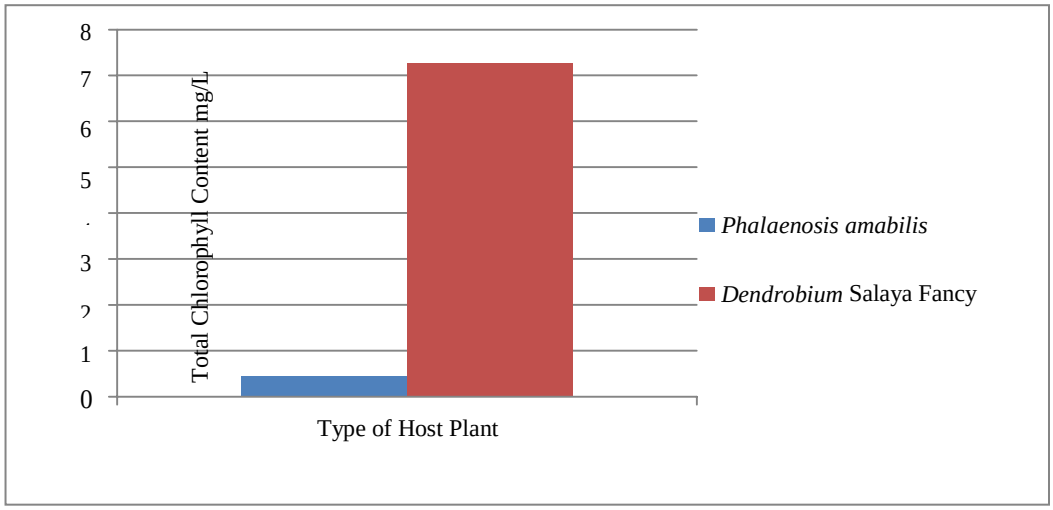

Figure 4. Curves of total chlorophyll content after virus inoculation in two types of host plants

4. Discussion

The symptomatic response of ORSV in host plants shows a wide range of symptoms on the examined host plants *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy. Based on the results of the virus inoculation on the two orchids did not show symptoms until the 30th day. Then reinoculation is performed and observed again until symptoms can be seen. *Phalaenopsis amabilis* showed necrotic symptoms on the 18th day. Observations continued until day 30 and showed worsening necrotic symptoms. In a previous study of Mahfut et al. (2020[a]), it was also known that ORSV infection in *Phalaenopsis* sp. showed necrotic symptoms on 23rd day.

Dendrobium Salaya Fancy also showed necrotic symptoms on the 15th day. Necrotic symptoms in *Dendrobium* were shown more rapidly than *Phalaenopsis*. Based on the observation of symptoms up to day 25, necrotic symptoms turned into a mosaic indicating that ORSV infection in *Dendrobium* was getting worse. In previous studies, it was known that ORSV infection in *Dendrobium* sp. also appeared necrotic and mosaic symptoms on 15th and 23rd day (Mahfut, 2020; Mahfut et al., 2020[a]; Mahfut et al., 2020[b]; Mahfut et al., 2021).

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Volume 14, Supplementary Issue,December 2021
ISSN 1995-6673
Pages xxx – xxx

Jordan Journal of Biological Sciences

Physiological Analysis of Chlorophyll Orchid Against Infection of *Odontoglossum ringspot virus*

Mahfut[1,*], Irni Yuni Minarni[2], Sri Wahyuningsih[1], Tundjung Tripeni Handayani[1]

[1]*Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia;* [2]*Undergraduate Student, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung, Indonesia*

NO Date

Abstract

Orchids (Orchidaceae) are one of the most popular ornamental plants having diverse flower shapes and colors. They are vastly applied as cut flowers, potted plants, and garden elements. Infectious diseases are still a major obstacle in the cultivation of orchids in Indonesia. *Odontoglossum ringspot virus* (ORSV) is one of the most widely reported viruses that infect orchids worldwide, including Indonesia. This research was done by mechanically injecting the virus on *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy. This study aimed to determine the symptoms of the disease, plant resistance, and chlorophyll content. The results showed that each orchid had severe disease symptoms, the incidence of both orchids was 80%, and *Phalaenopsis amabilis* reaction was more susceptible than *Dendrobium* Salaya Fancy that showed tolerant response to ORSV. Physiological response analysis also showed that the content of chlorophyll A,B, and total *Dendrobium* Salaya Fancy was higher (0.35 ± 0.02; 0.29 ± 0.05; 0.63 ± 0.05) than *Phalaenopsis amabilis* (0, 18 ± 0.0; 0.31 ± 0.06; 0.48 ± 0.04) respectively, after viral infection. This proves that *Phalaenopsis* is the most susceptible type of orchid virus compared to *Dendrobium*.

Keywords: selection of resistance; analysis of chlorophyll; orchid; ORSV

1. Introduction

Orchidaceae are ornamental plants that have a high aesthetic value (Mose et al., 2020), because they have a variety of colors and flower shapes. For that, high demands of markets on orchids have been raised in the form of cut flowers and potted plants (Mahfut et al., 2016). One of the obstacles in cultivating orchids is infectious diseases that affect flower quality. Orchids can be infected with 50 types of viruses (He et al., 2019), including *Odontoglossum ringspot virus* (ORSV) (Pai et al., 2019). The virus is an important type that attacks orchids and is popular in the world.

ORSV, also known as *Tobacco mosaic virus* orchid strain (TMV-O), belongs to the genus *Tobamovirus* and the family Virgaviridae (Forterre et al., 2017). There is very little information about ORSV infection in Indonesia. The virus is reported to be able to infect *Phalaenopsis* and *Dendrobium* orchids in West Java, Central Java, East Java, Banten, Yogyakarta, and Bali (Mahfut et al., 2016). ORSV infection causes damage to chlorophyll and affects the growth and development of orchids.

Efforts to protect orchids against viral infections need to be carried out to reduce the spread and preserve orchids in Indonesia. The initial stage of protection is considered through observing symptoms to determine the type and nature of a disease (He et al., 2017; Ko et al., 2020). This data is then used in determining plant resistance. In addition, chlorophyll analysis was also carried out, and so the physiological response of plants due to viral infection was collected.

This study was conducted to distinguish plant responses in the form of disease symptoms, plant resistance, and chlorophyll content between *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy against ORSV infection. The results of this study are expected to provide information about the response and level of resistance of orchids to ORSV infection, and it could be used as a reference for the right type of orchid to be cultivated in disease endemic areas or there has been a history of previous ORSV infection.

2. Materials and Methods

2.1. Plantlet Acclimatization

This study used two orchid species, *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy on six replicates. Plantlets were immersed in a fungicide Benlate solution, with active ingredient Benomyl (2 grams/l water) for 20 minutes and then planted in plastic pots containing sterile of moss media (Mahfut et al., 2021). Orchids were well cared for before treatment in a green house.

2.2. Virus Inoculation

The inoculum used was prepared from inoculation of the Magelang isolate virus on tobacco plants that had been previously analyzed (Mahfut et al., 2016). The inoculum was then mechanically inoculated. The initial stage of inoculation is to weigh 1 gram of viral inoculum, then

* Corresponding author e-mail: mahfut.mipa@fmipa.unila.ac.id.

grind in a sterile mortar by adding 10 ml of 0.01 M phosphate buffer solution (pH 7) (w : v = 1 : 10). Before being inoculated, 100 mess of carborundum powder was sprinkled on the upper surface of the leaves, then the virus was applied to the two youngest leaf surfaces that were fully opened. After the virus sap dries, the carborundum that remains attached to the leaf surface of the test plant was cleaned by spraying sterile water (Mahfut et al., 2016).

2.3. Observation of Infection Symptoms

The results of the inoculation test on plants were noted for variations in symptoms and incubation time. Observations were made every three days for one month to determine the response among host plants that were more quickly infected with symptoms of the disease. ORSV inoculation on each host plant was carried out at different times depending on the fast or slow growth of the plant and indicated whether or not the number of leaves was sufficient.

2.4. Plant Resistance

Determination of disease resistance criteria for various types of plants against ORSV infection was based on several factors, including symptoms of viral infection and the percentage of disease incidence. Analysis of plant resistance was used to determine the development of the observed disease, namely disease incidence. The incidence of disease is carried out by calculating the scale of damage (%) of the disease that appears on the host plant. Plant resistance was grouped into very resistant, resistant, moderately resistant, tolerant, susceptible, and very susceptible, following the method of Dwipa et al. (2018).

2.5. Chlorophyll Content Test

This test was carried out following Sedjati et al. (2020) using a spectrophotometer. For measuring chlorophyll content, the sample was applied an orchid leaf that had been identified as infected with ORSV. In the first step, 1 gram of treated orchid leaves were weighed, the leaves had been removed, then crushed with a mortar and added 10 ml of ethanol. The solution was filtered with Whatman paper no. 1 and put into a flakon, then tightly closed. Sample solution and standard solution (ethanol) 1 ml were put into different cuvettes. Furthermore, absorption readings were carried out with a UV spectrophotometer at wavelengths (λ) 648 nm and 664 nm, the measurements were carried out three times for sample replication.

3. Results

3.1. Observation of Infection Symptoms

The response of plants after virus inoculation showed that ORSV could infect all types of host plants with variations in symptoms and different incubation times. The results showed that in general the response began to appear about 2-3 weeks after inoculation. The symptoms showed necrotic *Phalaenopsis amabilis*, while *Dendrobium* Salaya Fancy showed necrotic and mosaic symptoms. Variations in response to symptoms of viral infection in both host plants are shown on (Figure 1).

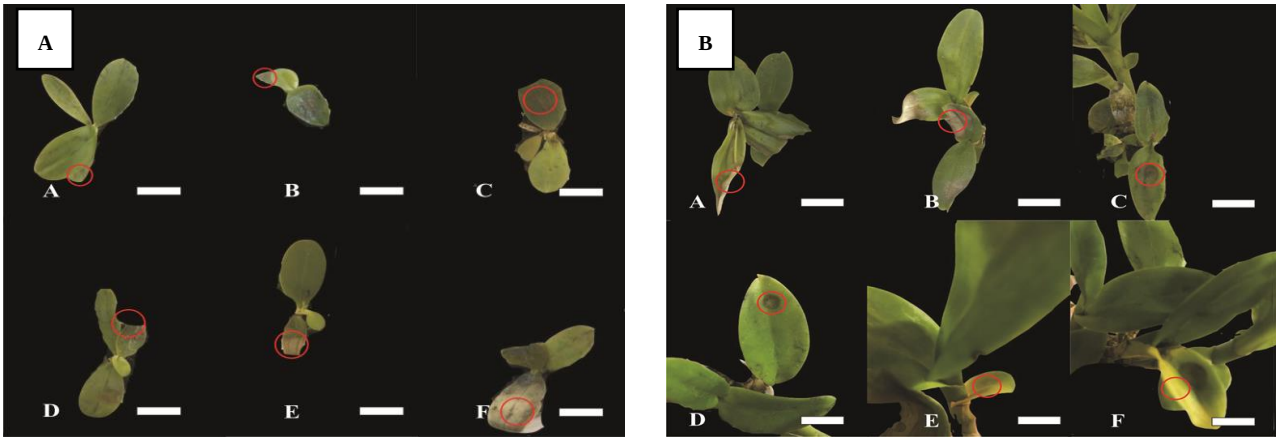

Figure 1. Symptoms of virus infection on (A) *Phalaenopsis amabilis* and (B) *Dendrobium* Salaya Fancy. Bar= 1cm

3.2. Plant Resistance Analysis

The results of the analysis of plant resistance to ORSV infection were based on variations in symptoms, incubation times, and disease incidence. The results of the analysis showed that the level of resistance is very susceptible to symptoms of very severe infection. Meanwhile, *Dendrobium* Salaya Fancy showed a level of resistance that was tolerant to a fairly severe variety of symptoms, but not as severe as the symptoms of infection in *Phalaenopsis amabilis*.

3.3. Chlorophyll Content Test

The results of the physiological response analysis showed that the chlorophyll A, chlorophyll B, and chlorophyll total content of *Dendrobium* Salaya Fancy was higher than *Phalaenopsis amabilis* after being infected with the virus. The complete test results for the content of chlorophyll on both types of host plants for 30 days after ORSV inoculation are shown in Table 1.

Table 1. Tukey's test of chlorophyll A, B, total content of two types of host plants 30 days after inoculation

Treatment	Type of Chlorophyll	Species of Host Plant	
		Phalaenopsis amabilis	*Dendrobium* Salaya Fancy
Control	Chlorophyll A	0,17 ± 0	0,37 ± 0
	Chlorophyll B	0,24 ± 0,01	0,28 ± 0
	Chlorophyll Total	0,41 ± 0,01	13,9 ± 13,27
Virus Inoculated	Chlorophyll A	0,18 ± 0,01	0,35 ± 0,02
	Chlorophyll B	0,31 ± 0,06	0,29 ± 0,05
	Chlorophyll Total	0,48 ± 0,04	0,63 ± 0,05
Total of Average	Chlorophyll A	$0,18^{a}$ ± 0,05	$0,36^{b}$ ± 0,01
	Chlorophyll B	0,28 ± 0,04	0,29 ± 0,03
	Chlorophyll Total	0,45 ± 0,025	7,27 ± 6,66

Note: The values followed by the same letter are not significantly different at the 5% level. Chlorophyll A: HSD Cell [.05] = 0.05. HSD Columns [.05]= 0.02. Chlorophyll B: Values followed by the same letter are not significantly different at the 5% level. HSD Cell [0.05] = 0.12. HSD Columns [.05]= 0.06. Total Chlorophyll: HSD Cell [0.05] = 26.86. HSD Columns [.05]= 14.5

The homogeneity of Levene's test variance at 5% significance level showed that the variance of the samples of the two host plants was homogeneous (P=0.201>0.05). Analysis of variance at the 5% level of significance showed that the virus treatment had no significant effect on the chlorophyll a content of the host plant (P=1>0.05), but the type of plant had a significant effect on the chlorophyll a content (P<0.0001). Thus, the interaction between virus inoculation and plant species did not significantly affect the chlorophyll a content (P=1>0.05). The content of chlorophyll A after virus inoculation with two types of host plants is shown in (Figure 2).

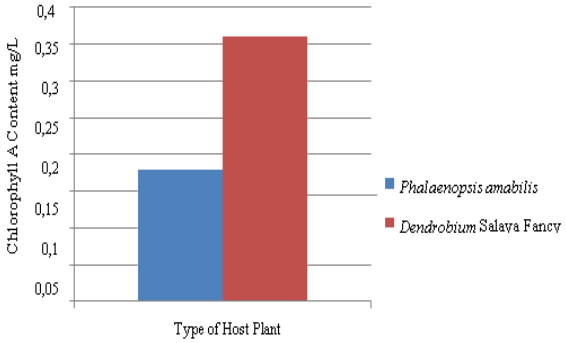

Figure 2. Curves of chlorophyll A content after virus inoculation in two types of host plants

Chlorophyll B is one of the parameters that affect plant metabolism through photosynthesis. The homogeneity of Levene's test variance at 5% significance level showed that the variance of the samples of the two host plants was homogeneous (P= 0.076>0.05). Analysis of variance at 5% significance level showed that the virus treatment (P=0.15>0.05) and plant species (P>0.05) had no significant effect on chlorophyll B levels, respectively. Likewise, the interaction between virus inoculation and plant species did not significantly affect the chlorophyll B content (P>0.05). Virus inoculation and plant species did not significantly affect chlorophyll B. ORSV inoculation on chlorophyll B content on *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy is shown in (Figure 3).

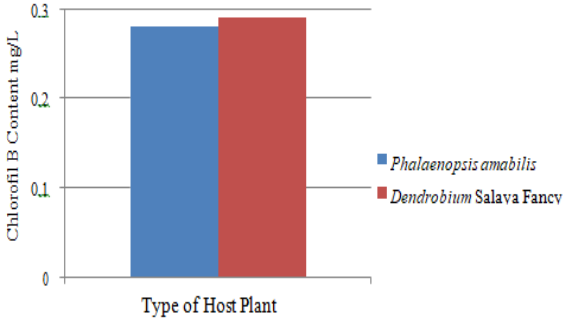

Figure 3. Curves of chlorophyll B content after virus inoculation in two types of host plants

The results of the total chlorophyll content test in both host plant samples also showed homogeneity of the Levene test variance at the level of significance 5 (P=0.224). The results showed that the virus treatment had no significant effect (P=0.34) and the type of plant also had no significant effect on the total chlorophyll content (P=0.32) in the analysis of variance at 5% significance level.

Likewise, the interaction between virus inoculation and plant species did not significantly affect the total chlorophyll content (P=0.33). ORSV virus inoculation, plant species and interactions on host plants did not significantly affect total chlorophyll. ORSV virus inoculation on total chlorophyll content in *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy is presented in (Figure 4).

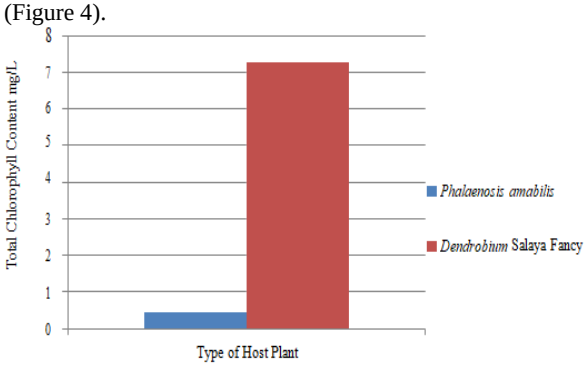

Figure 4. Curves of total chlorophyll content after virus inoculation in two types of host plants

4. Discussion

The symptomatic response of ORSV in host plants shows a wide range of symptoms on the examined host plants *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy. Based on the results of the virus inoculation on the two orchids did not show symptoms until the 30th day, so reinoculation was necessary. Then, reinoculation is performed and observed again until symptoms can be seen. *Phalaenopsis amabilis* showed necrotic symptoms on the 18th day. Observations continued until day 30 and showed worsening necrotic symptoms. In a previous study of Mahfut et al. (2020[a]), it was also known that ORSV infection in *Phalaenopsis* sp. showed necrotic symptoms on 23rd day.

Dendrobium Salaya Fancy also showed necrotic symptoms on the 15th day. Necrotic symptoms in *Dendrobium* were shown more rapidly than *Phalaenopsis*. Based on the observation of symptoms up to day 25, necrotic symptoms turned into a mosaic indicating that

ORSV infection in *Dendrobium* was getting worse. In previous studies, it was known that ORSV infection in *Dendrobium* sp. also appeared necrotic and mosaic symptoms on 15th and 23rd day (Mahfut, 2020; Mahfut et al., 2020[a]; Mahfut et al., 2020[b]; Mahfut et al., 2021).

The results of disease incidence analysis showed that each host plant had the same response that the disease incidence was > 40% and disease infection was found. This proves that the inoculation of ORSV on the whole host plant was successful. In previous research (Mahfut, 2020), it is also known that ORSV inoculation in *Phalaenopsis* sp. and *Dendrobium* sp. showed an incidence of disease > 40% and found the presence of disease infection.

Based on the results of the study, it is known that the host plant *Phalaenopsis amabilis* which shows a very susceptible level of resistance with very severe symptoms of infection. Meanwhile, *Dendrobium* Salaya Fancy orchid showed a tolerant level of resistance with a fairly severe variation of symptoms, but not as severe as the symptoms of infection in *Phalaenopsis amabilis*. This means that *Phalaenopsis amabilis* orchids are more susceptible to ORSV than *Dendrobium* Salaya Fancy.

Previous research (Mahfut, 2020) reported that *Phalaenopsis* is a highly susceptible host orchid plant and most susceptible to ORSV. In other research (Mahfut et al., 2020[a]; Mahfut et al., 2020[b]; Mahfut et al., 2021), it was also known that the plants *Phalaenopsis amabilis*, *P. small* Red White Lips x, *D. nindii*, *D. kyosimori*, *D. liniae*, *D. schulerii* had a response, i.e. are susceptible to ORSV.

Chlorophyll content analysis aims to determine the chlorophyll content in the host plant (Jaelani et al., 2016; Alananbeh et al., 2018; Saeed, 2019). Result of Tukey's test at the 5% significance level showed that two host plants, namely *Phalaenopsis amabilis* and *Dendrobium* Salaya Fancy, had a significant effect only on plant species but had no significant influence on viral treatment on chlorophyll A. The content of chlorophyll B in the leaves of *Dendrobium* Salaya Fancy was not much different from the content of chlorophyll B on *Phalaenopsis amabilis* leaves. This shows that the plants *Dendrobium* Salaya Fancy and *Phalaenopsis amabilis* have the same level of resistance, which is very susceptible to diseases not only caused by ORSV virus but can be caused by other factors such as fungi, bacteria, nutrients found in the media.

Total chlorophyll content in *Dendrobium* Salaya Fancy orchid leaves is relatively higher than the total chlorophyll content in *Phalaenopsis amabilis* orchid leaves. This indicates that the *Dendrobium* Salaya Fancy orchid is more resistant than the *Phalaenopsis amabilis* orchid to disease. Chlorophyll is a green pigment found in chloroplastide. In general, chlorophyll is found in leaf mesophyll cell chloroplasts, i.e. in palisade parenchyma cells and parenchyma sponge cells. In chloroplasts, chlorophyll is present in the gamma thylakoid membrane. In higher plants, the types of chlorophyll are chlorophyll A and chlorophyll B. Under normal circumstances, the proportion of chlorophyll A is much greater than that of chlorophyll B (Sedjati et al., 2020).

According to the chlorophyll data in this study, it was found that the chlorophyll data on *Dendrobium* Salaya Fancy plants are more than *Phalaenopsis amabilis* plants because seen morphologically, *Phalaenopsis amabilis* orchid plants have more severe symptoms than *Dendrobium* Salaya Fancy. It is possible that the leaf mesophyll tissue in *Dendrobium* Salaya Fancy is not damaged by the virus, and can produce more chlorophyll for photosynthesis. Therefore, it can be concluded that *Dendrobium* Salaya Fancy is more resistant to ORSV virus or other viruses than *Phalaenopsis amabilis*. Data on chlorophyll B and total chlorophyll in both host crops are stated to be statistically similar because usually the amount of chlorophyll B is less than that of chlorophyll A (Jaelani et al., 2016; Sedjati et al., 2020). Virus treatment and plant species interactions did not significantly affect the two host plants, so further testing was not performed. Therefore, it can be concluded that *Dendrobium* Salaya Fancy have a higher level of resistance than *Phalaenopsis amabilis*.

5. Conclusion

The results showed that the indicator crops and the host crops had quite severe disease with various symptoms. Each crop had the same response, the indicator crop had a disease incidence of > 40% i.e. 75% while the host crop with a disease incidence of > 40% i.e. 80%. Indicator and host plant responses to ORSV are highly susceptible, except that *Dendrobium* Salaya Fancy orchid host plants have a tolerant response to ORSV. The results of physiological response analysis showed that the content of chlorophyll A, B, and total *Dendrobium* Salaya Fancy were higher (0.35 ± 0.02; 0.29 ± 0.05; 0.63 ± 0.05) than *Phalaenopsis amabilis* (0.18) ± 0.0; 0.31 ± 0.06; 0.48 ± 0.04) respectively, after being infected with the virus. This proves that *Phalaenopsis* is the most susceptible type of orchid virus compared to *Dendrobium*.

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