

SYMPTOMS AND RESPONSE RESISTANCE TO DIFFERENT TYPES OF ORCHIDS (ORCHIDACEAE) AGAINST *ODONTOGLOSSUM RINGSPOT VIRUS* (ORSV) INFECTION

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ABSTRACT

Odontoglossum ringspot virus (ORSV) is a orchid virus which infection most widely reported and has spread widely in the world, including in Indonesia. Viral infections can degrade the quality of the orchid plant so it is economically detrimental. The early stages of disease control can be done through the introduction of infection symptoms that appear. This research was conducted to study the response of some plant of orchid (*Orchidaceae*) against a single infection of ORSV trough mechanical inoculation. Futhermore, virus detection on orchid tissue was done trough the DAS-ELISA and RT-PCR. The results showed that *Pectelis sussanae* (L.) Raf. was the only orchid plant that was immune against ORSV approached. *Dendrobium* sp., *Cymbidium* sp., *Chattleya* sp., *Phalaenopsis* sp., and *Spathoglotis* sp. showed a response of susceptible of ORSV severe infection symptoms, while *Liparis* sp. were resistant. The variation of resistance from several orchid plant against infection ORSV showed severe symptoms with the incubation periode was seen earlier. The observation of virus structure using TEM showed rigid road shape particle, 300 x 18 nm in size, which is general characteristic of *Tobamovirus*. This indicate an infection of ORSV is a dangerous disease and require serious control.

Keywords: ORSV, *Orchidaceae*, DAS-ELISA, TEM

INTRODUCTION

Orchid is one type of ornamental plant that has a high aesthetic value and is most in demand by the community. Disease infections are still the main obstacle in the cultivation and development of the potential of natural orchids (Aruni et al., 2011). The *Odontoglossum ringspot virus* (ORSV) is one of the most infectious orchid viruses. This virus was first discovered in the United States (Corbett, 1967) and has spread to other countries including Indonesia (Suseno, 1979). ORSV has a wide spread in Java, Ujung Pandang, Kalimantan, Bali and Papua (Inouye & Gara, 1996; Lakani et al., 2010; Mahfut & Daryono, 2014; Mahfut et al., 2016^a; Mahfut et al., 2016^b; Mahfut et al., 2017^a). In general,

ORSV infection can reduce photosynthetic ability because of damage to chloroplasts (Mahfut et al., 2017^b), inhibiting plant growth and resilience, and decreasing aesthetic value and selling power on a regular basis.

To facilitate disease control, it is better to do an inventory of data regarding the infection. Observation of variations in symptoms in response to disease infection is the first data needed for virus identification. This information is a very important aspect to determine disease management and control measures in the field. In line with the knowledge of the response of various types of orchid plants can also be the basis for selecting orchids that will be developed in areas endemic to ORSV so that possible diseases can be avoided.

MATERIALS AND METHODS

Propagation Test

This method aims to purify and multiply the virus through mechanical inoculation of the test plants. The source of the virus is orchid leaves *Phalaenopsis amabilis* positively infected by ORSV collected from Borobudur Orchids Center, Magelang. Virus isolation was carried out by transmission to indicator plants (*Chenopodium amaranticolor* and *Nicotiana tabacum*). Furthermore, the results of inoculation were multiplied using various types of orchids (*Dendrobium*, *Cymbidium*, *Chattleya*, *Phalaenopsis*, *Spathoglottis*, *Liparis*, and *Pecteilis*). Inoculation was carried out at the top of the leaf mechanically using a 600 mesh carborundum with the addition of a 0.05 M buffer solution pH 7.0. Inoculated plants are maintained in a greenhouse with conditions 25-30° C (Hu et al., 1994) and carried out observations every day. Observations were made on the variation of symptoms that arise, the incubation period, and the number of symptomatic plants or the percentage of disease occurrences.

Virus Detection on Orchid Plants

Orchid plant samples that have been inoculated by ORSV were detected by the DAS-ELISA method following the Clark & Adams (1977) method. Enzyme-linked immunosorbent assay (ELISA) is a virus detection method using specific antibodies. In Wahyuni (2005) it was explained that the method commonly used to detect plant viruses is direct ELISA (direct-ELISA) such as double antibody sandwich (DAS) where antigens are

flanked by one type of antibody. The advantage of the ELISA technique is that it can identify multiple samples at once with relatively low costs and relatively short time.

Determination of Plant Resistance

Determination of response criteria for various types of orchids against ORSV infection is based on several factors, including the percentage of occurrence of diseases and viral infections. The response of orchid plants is grouped to be close to immune, tolerant, somewhat resistant and vulnerable (Matthews, 1992).

Virus Particle Analysis

Leaf samples of infected indicator plants were cut into small pieces and crushed for viral extraction. Prepared petri dishes which have been placed in the base by parafilm. Then a drop of sap is extracted on the surface of the parafilm, then the petri dish is closed to avoid evaporation. Samples were incubated for 30 minutes at 25 ° C and then washed using 30 drops of aquades. The sample is dried with filter paper for a few seconds and drops of a negative 2% phosphotungstic acid dye solution of 7 drops. The sample is dried again using filter paper. The next sample was observed under the JEOL JEM-1400 (TEM) electron microscope at a magnification of 20000. The presence of a rigid rod-shaped morphology was an indication of the presence of ORSV (Choi et al., 2002).

RESULTS AND DISCUSSION

Propagation Test

The response of host plants to the transmission test shows that ORSV can infect almost all plants tested with different variations in symptoms and incubation time (**Table 1**). The response generally starts to appear around 2-3 weeks after inoculation in all host plants.

Table 1. Variation in symptoms of ORSV infection in test plants

Familia	Species	Symptoms of ORSV infection¹	Incubation period (days)
<i>Chenopodiaceae</i>	<i>Chenopodium amaranticolor</i>	NLL, LD	5, 11
<i>Orchidaceae</i>	<i>Dendrobium</i> sp.	N, M	15, 23
	<i>Cymbidium</i> sp.	Ns	65

	<i>Cattleya</i> sp.	M, Ns	35, 32
	<i>Phalaenopsis</i> sp.	N	23
	<i>Liparis</i> sp.	C	62
	<i>Pecteilis sussanae</i> (L.) Raf.	O	69
	<i>Spathoglottis</i> sp.	M, C	65, 30
<i>Solanaceae</i>	<i>Nicotiana tabaccum</i>	C, M, LD	5, 9, 12

¹M: Mosaic; NLL: *Necrotic local lesions*; N: Necrotic; Ns: Necrosis; LD: Leaf Demalformation; K: Chlorotic; O: No symptom.

Specific symptoms commonly caused by indicator plants are symptoms of necrotic local lesions on *Chenopodium amaranticolor* on the 5th days and become clearer until the beginning of the second week, and chlorotic symptoms in the *Nicotiana tabacum* plant which after some time (first week) turn into a systemic mosaic (**Figure 1**). Another symptom in the two plants is the leaf edge that rolls down on the young leaves as the initial symptom of ORSV infection. *Chenopodiaceae* is the fastest symptomatic plant with a short incubation period and a high percentage of events reaches 80%. Necrotic local lesions that appear are a symptom of the results of a hypersensitive response that is responsible for limiting pathogens so that the plant becomes more resistant to disease (Milne, 1965).

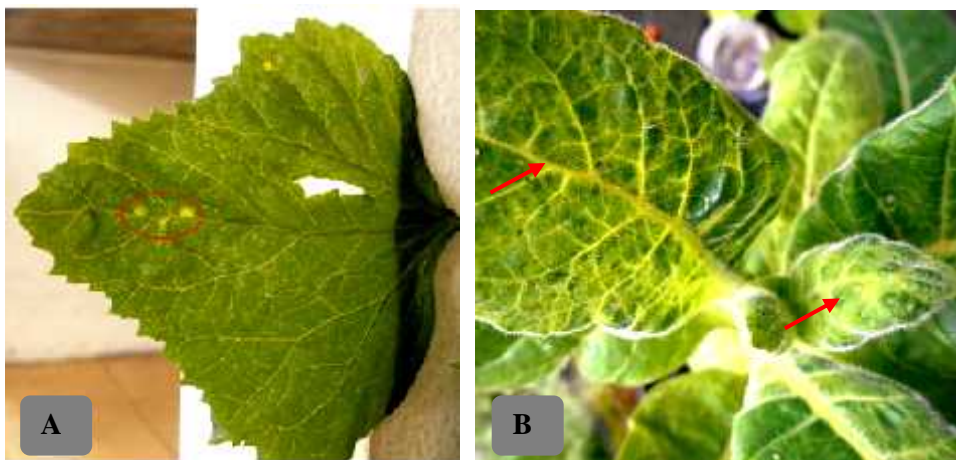


Figure 1. Symptoms of ORSV infection: (A) Necrotic local lesion in *C. amaranticolor*, (B) mosaic and vein clearing on *N. tabacum*

Symptoms of viral disease in host plants can occur due to the use of metabolic results of plants for viral synthesis, buildup of virions or parts of viruses and the effects of typical polypeptides that are encoded by viral genes (Akin, 2006). The response to ORSV infection shows that variations in symptoms between orchids are necrotic, necrotic, mosaic, chlorotic, and symptomless. Based on the symptoms shown, the ORSV inoculum used in the study was very infective. For necrotic symptoms appear in *Phalaenopsis* sp. namely day

23 and *Dendrobium* sp on day 15 after inoculation (**Figure 2 A & B**).

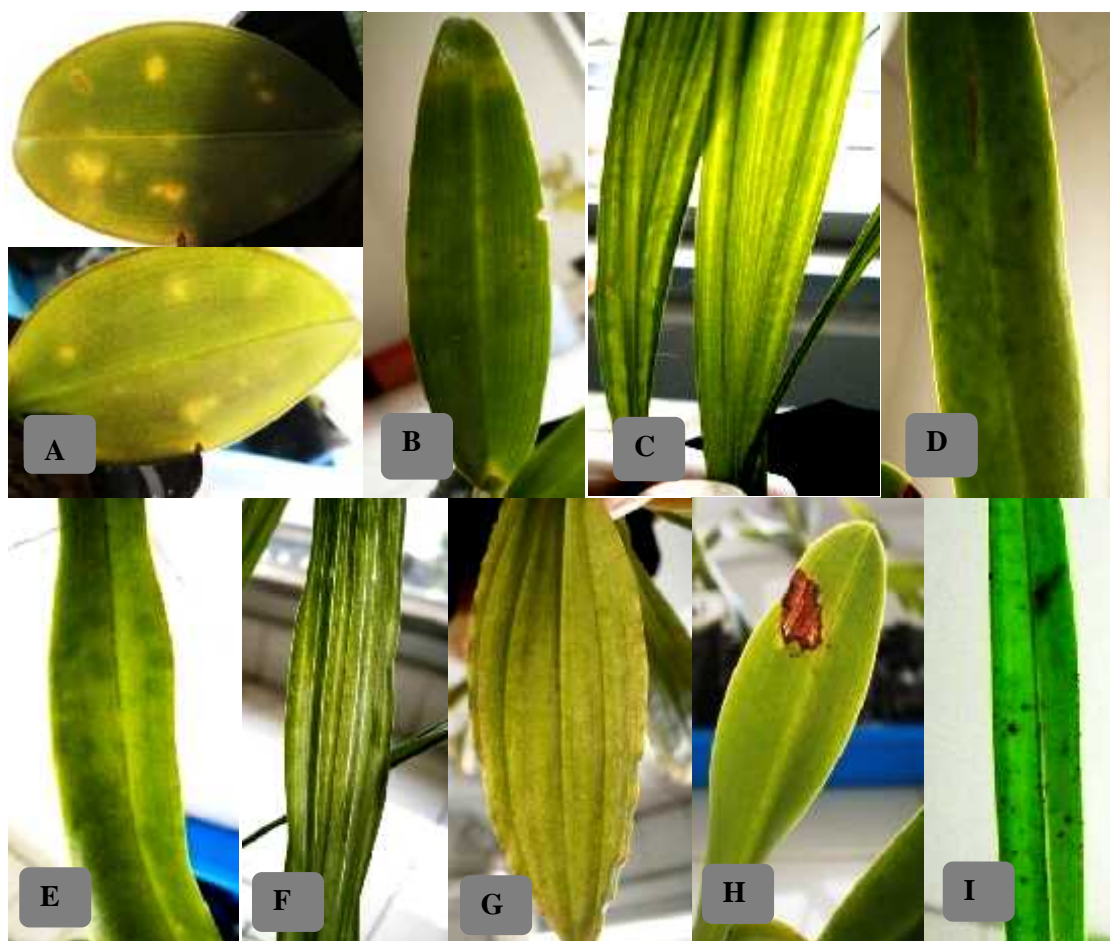


Figure 2. Variations in symptoms of ORSV infection in several types of orchids: Necrotic in (A) *Phalaenopsis* sp. and (B) *Dendrobium* sp. ; Mosaic on (C) *Spathoglottis* sp., (D) *Dendrobium* sp., and (E) *Cattleya* sp. ; Chlorotics in (F) *Spathoglottis* sp., and (G) *Liparis* sp. ; and Necrosis in (H) *Cattleya* sp. and (I) *Cymbidium* sp.

Dendrobium sp. besides showing necrotic symptoms, another symptom that appears is mosaic on the 23rd day. Mosaic symptoms are also seen in *Cattleya* sp. Orchids. on day 35 and *Spathoglottis* sp. on day 65 (**Figure 2 C, D, & E**). Akin (2006) explains that the symptoms of mosaic are marked in the form of "green islands" where there is a mixture of yellow or light green leaves in green. Another symptom is chlorotic in *Spathoglottis* sp. Orchids. appears earlier than the symptoms of mosaic which is on the 30th day. Other orchids that show chlorotic symptoms are *Liparis* sp. on day 62 (**Figure 2 F & G**). Chlorotic is a type of symptom caused by damage to chloroplasts which causes yellow parts of plants that are normally normal to turn yellow. Chloroplast damage can be caused by lack or absence of chlorophyll due to pathogenic poisons, mineral deficiencies, air pollution, lack of water, or due to chemicals. Chlorotic symptoms often precede necrotic

symptoms so that they gradually turn brown. Sometimes chlorotic symptoms are often associated with necrotics where chlorotic surrounds the necrotic called "halo" (Purnomo, 2006).

Symptoms of necrosis are black spots where cell death and leaf tissue occur only in *Cattleya* sp. and *Cymbidium* sp. (**Figure 2 H & I**). The difference is necrosis in *Cattleya* sp. greater, clearer, and shorter incubation period of 32 days compared to *Cymbidium* sp, which is 65 days after inoculation. This is because the leaves of *Cymbidium* sp. have thicker and harder leaves because of the layers of lignin and wax. Purnomo (2006) explains that necrosis is a type of symptom caused by physical damage or death to cells, cell parts, or tissues. Some symptoms including necrotic types are specks (necrose), rot (rot), die-back (die back), and cancer (dead bark dries with clear boundaries).

Pectelis sussanae L. (Raff.) was the only test plant that showed no symptoms after being inoculated until the 69th day. This shows that this type of orchid has a resistant response to ORSV. Latent symptoms (symptomless) are systemic symptoms of infection (Srinivasan et al., 2004). Bos (1990) explains that viral propagation of test plants that are resistant to ORSV attacks produces latent symptoms (symptomless) caused by environmental factors such as the state of the environment in which they grow and temperature treatment. In addition, other factors that also play a role are the test plant is not an ORSV host, the amount of virus in the inoculum is insufficient, and the content of inhibitors in the test plant can eliminate the stability of the virus in sap.

Virus Detection on Orchid Plants

The results of the DAS-ELISA serological test (**Table 2**) indicate the overall sample of plant leaves from inoculation, except for the sample of *Pectelis sussanae* leaves, showed positive infection with ORSV with an average absorbance value of 1.125-1.152. Daryono & Natsuaki (2009) suggested that a test sample is said to be positively infected based on DAS-ELISA if the absorbance value at 405 nm wavelength approaches the absorbance value of positive control, or has a value 2-3 times the absorbance value of the control buffer.

Table 2. The range of absorbance mean values is based on DAS-ELISA at wavelength 405 nm

Buffer	Positive Control	Negative Control	Positive Sample	Negative Sample
0,129	1,515	0,129	1,125-1,152	0,126-0,227

Read by ELISA-reader BioTek, FALITMA Faculty of Biology UGM, on August 7, 2016

This serological detection result reinforces evidence that the method can be used to detect ORSV which causes mosaic, chlorotic, necrotic, necrotic, and malformed orchid plants in plants infected with ORSV.

Determination of Plant Resistance

According to Matthews (1992) plant responses to pathogens can be classified as immune, resistant, tolerant, and vulnerable. Based on observations of orchid plants' responses to single ORSV infections by observing the occurrence of diseases and viral infections, the response of orchid plants is grouped to be close to immune, somewhat resistant, and vulnerable. The results showed that *Pecteilis sussanae* plants were orchids that were close to the immune system against ORSV infection. *Dendrobium*, *Cymbidium*, *Chattleya*, *Phalaenopsis*, and *Spathoglottis* are susceptible and *Liparis* is resistant to ORSV infection (Table 3).

The response of *Pecteilis sussanae* to ORSV is close to the immune system, presumably because ORSV cannot replicate in plant cells. Orchids that have a resistant response to ORSV are orchid plants that can be infected with viruses, but plant cells do not support the growth and development of viruses, so that no disease occurs. In orchids that have a response. While orchids that have a susceptible response to ORSV are orchids that support the growth and development of viruses, and viruses can cause damage (Hull, 2002).

Table 3. The level of resistance of various types of orchids to ORSV infections

Orchid	Host Plant Reactions		
	Disease Incidence ¹⁾	Virus infection ²⁾	Plant Response Criteria
<i>Dendrobium</i>	++	+	Susceptible
<i>Cymbidium</i>	++	+	Susceptible
<i>Chattleya</i>	++	+	Susceptible
<i>Phalaenopsis</i>	++	+	Susceptible

<i>Spathoglottis</i>	++	+	Susceptible
<i>Liparis</i>	+	+	Resistant
<i>Pecteilis sussanae</i>	-	-	Immune

¹⁾ Disease Incidence: - : No disease incidence

+ : Disease Incidence $0 < x < 40$

++ : Disease Incidence $40 < x < 100$

²⁾ Virus Infection: - : Berdasarkan DAS ELISA tidak ada infeksi virus

+ : Berdasarkan DAS ELISA ada infeksi virus

Based on the severity of the symptoms seen and the incubation period, interactions that occur in the majority of orchid plants resulting from inoculation cause severe symptoms of infection. The difference in the severity of the symptoms of the disease is related to the process of development and spread of the virus in plant cells. The severity will be higher with the rapid process of virus development and spread on infected plant cells (Hull, 2002). Viruses move into plant tissue through phloem vessels and interfere with plant's physiological function by utilizing the existing amino acids for the replication process. These physiological disorders cause systemic symptoms that appear on young leaves. So, the faster the process of development and spread of the two viruses in plant cells, then the systemic symptoms appear more quickly and the severity increases.

Unlike other orchid species, *Liparis* and *Pecteilis sussanae* exhibit less severe symptoms. In both of these orchids, the interaction of viral inoculation is interference and it is thought that the plant has resistance to viral infection so that the virus cannot cause significant damage. When the virus enters plant cells, the elicitor in the virus will be associated with receptors on plant cells to determine the relationship of subsequent infections. If it occurs incompatible, then all parts of the plant will provide a systemic reaction of resistance when infected with the virus, so that the virus cannot do multiplication and cause symptoms. If the interaction is compatible, the virus can infect host plants.

Virus Particle Analysis

The leaves of *Nicotiana tabaccum* symptomatic infection resulting from inoculation were used in the analysis of virus particles using electron microscopy (TEM) with 1% ammonium molybdate as a negative dye and distilled water as a buffer. The TEM working principle is the conversion of electrical energy into electrons penetrated into specimens to

be emitted into light energy. The electron beam is transmitted and absorbed by all specimens, for the next monitor screen captures the specimen structure (Wahyuni, 2005).

Based on the results of the observations (**Figure 3**) showing the structure of rigid elongated stem particles which is a common character of Tobamovirus. ORSV particles have a length of 300 nm and a width of 18 nm (Paul, 1975). Choi et al. (2002) reported that observations of ORSV particles scattered in the test preparations would have different sizes. On further observation, infection with this virus causes form malformations in the chloroplasts, mitochondria, and other cell organelles. In addition, X-body formation and paramural bodies occur between cell membranes and cytoplasm. The stability of a virus is the result of interactions between subunits of proteins and viral genomes (Akin, 2006).

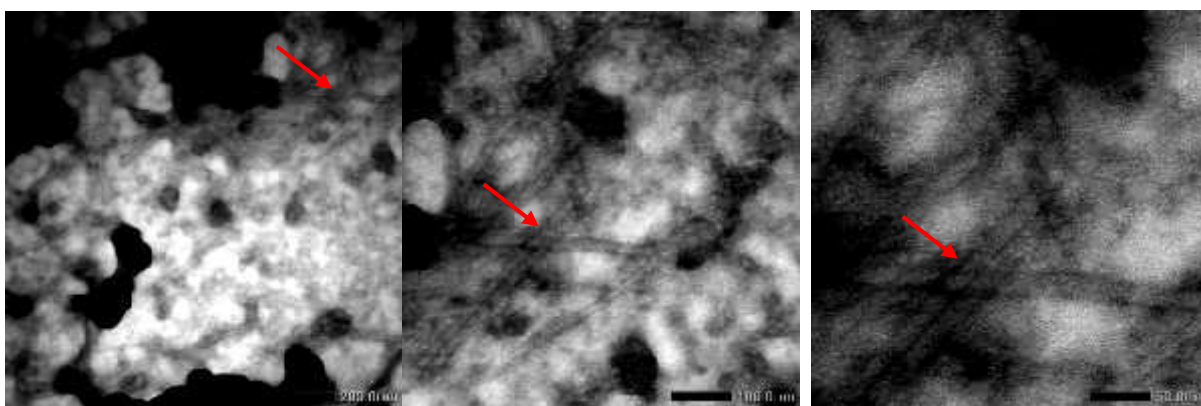


Figure 3. Particle structure of ORSV with TEM observations; arrow indicates virus particles

The results of this study as an initial inventory of ORSV infection in Indonesia. This data is then expected to be used as basic information in the application of the concept of natural orchid conservation in Indonesia through crop protection efforts.

CONCLUSION

The ability of ORSV to infect plants as a result of inoculation is very fast and shows symptoms of a fairly severe infection. Indicator plants show specific symptoms of necrotic local lesions in *Chenopodium amaraticolor* and chlorotic symptoms and mosaics in *Nicotiana tabacum*. Another symptom in both plants is leaf malformation. While the response in the host orchid plants showed a variety of symptoms, namely in the form of necrotic, necrotic, mosaic, chlorotic, and symptomless (symptomless). The response of orchids to a single infection of ORSV based on the incidence of disease and viral infection shows *Pecteilis sussanae* is an orchid plant that is close to the immune system against

ORSV infection. *Dendrobium*, *Cymbidium*, *Chattleya*, *Phalaenopsis*, and *Spathoglottis* are vulnerable, and *Liparis* is somewhat resistant to ORSV infection. The observation of the structure of the virus particles showed a rigid elongated rod measuring 300 nm long and 18 nm wide which is a common character of Tobamovirus.

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