Variation of Resistance Responses of Various Indicator Plants Against Infection *Odontoglossum ringspot virus* (ORSV)

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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 indicator plant against a single infection of ORSV trough mechanical inoculation. Futhermore, virus detection on plants tissue were done trough the DAS-ELISA and RT-PCR. The results showed that *Chenopodium amaranticolor* is the only indicator plant that shows a susceptible response with a characteristic symptom, namely necrotic local lesion. Whereas *Nicotiana tabaccum* and *Nicotiana benthamiana* showed susceptible responses to symptoms of severe ORSV infection, namely mosaic, chlorotic, and leaf malformations. The variation of resistance from several indicator plant against infection ORSV showed severe symptoms with the incubation periode was seen earlier. This indicate an infection of ORSV is a dangerous disease and require serious control.

Keywords: ORSV, *indicator plant*, *Chenopodium, Nicotiana*, DAS-ELISA

INTRODUCTION

Orchid is one type of ornamental plant that has a high aesthetic value and is the most desirable community (Kumalawati et al., 2011). Disease infection is still a major obstacle in the cultivation and development of natural orchid potential (Mahfut and Daryono, 2014). *Odontoglossum ringspot virus* (ORSV) is one of the orchids viruses that is reported to infect the most. This virus was first discovered in the United States (Corbett, 1967) and has spread to other countries including Indonesia. ORSV has quite extensive distribution areas in Java, Ujung Pandang, Kalimantan, Bali, and Papua (Inouye & Gara, 1996; Lakani et al., 2010; Mahfut and Daryono, 2014; Mahfut et al., 2016[a]; Mahfut et al., 2016[b]; Mahfut et al., 2017[a]; Mahfut et al., 2017[b]). In general, ORSV infection results in chloroplast damage (Mahfut et al., 2017[b]; Mahfut et al., 2019).

To facilitate disease control, an inventory of data regarding the infection should be carried out. Observing variations in symptoms in response to infectious diseases is the first

data needed for virus identification. This information is a very important aspect to determine disease management and control actions in the field.

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*, *Nicotiana tabaccum,* and *Nicotiana benthamiana*). Inoculation was carried out at the top of the leaf mechanically using a 600 mesh carborondum with the addition of a 0.05 M buffer solution pH 7.0. Inoculated plants are maintained in a greenhouse with conditions 25-30ºC 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 Indicator Plants

Indicator plant samples that have been inoculated by ORSV were detected by the DAS-ELISA method following the Clark and 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).

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	Spesies	Symptoms of ORSV infection[1]	Incubation period (days)
Chenopodiaceae	*Chenopodium amaranticolor*	NLL	5
		LM	11
Solanaceae	*Nicotiana tobacum*	K	5
		M	9
		LM	11
	Nicotiana benthamiana	K	7
		M	12
		LM	35

[1]NLL: *Necrotic local lesions*; K: Chlorotic; M: Mosaic; LM: Leaf Malformation

C. amaranticolor is an indicator plant in plant virus research. This plant responds in the form of a typical infection symptom that is a local necrotic spot (necrotic local lession) (**Figure 1**). These symptoms appear on day 5 and become clearer until the beginning of the second week.

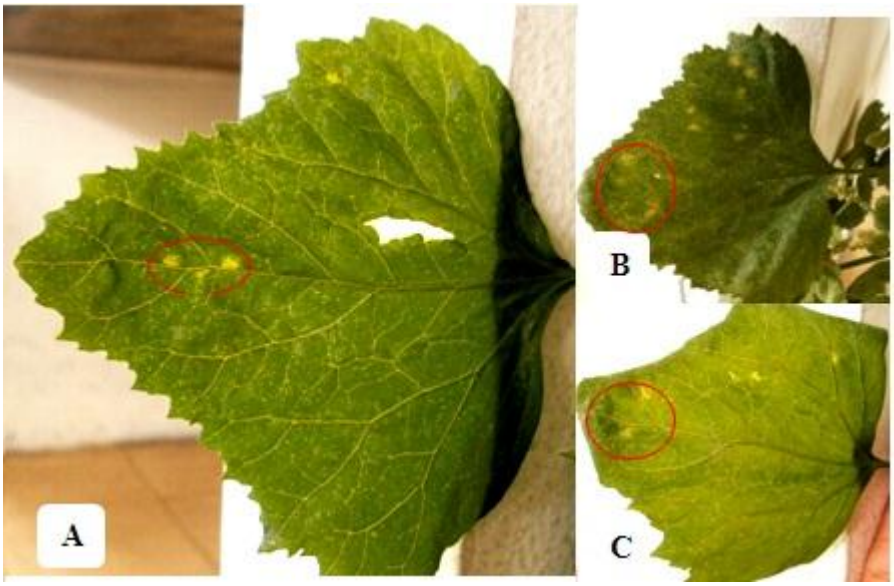

Figure 1. Symptoms necrotic local lesions on the leaf infections result ORSV *C. amaranticolor* (A,B,C: reiterative)

In contrast to *C. amaranticolor*, each tobacco plant has a variety of different

symptoms of infection. Specific symptoms in the *Nicotiana tabacum* plant are chlorotic which after a while (the first week) turn into systemic mosaic (**Figure 2**). This is caused by the condition of the host plant, the amount of virus in the inoculum, the content of inhibiting substances in the host plant that can eliminate the stability of the virus in cell fluids, and the ambient temperature.

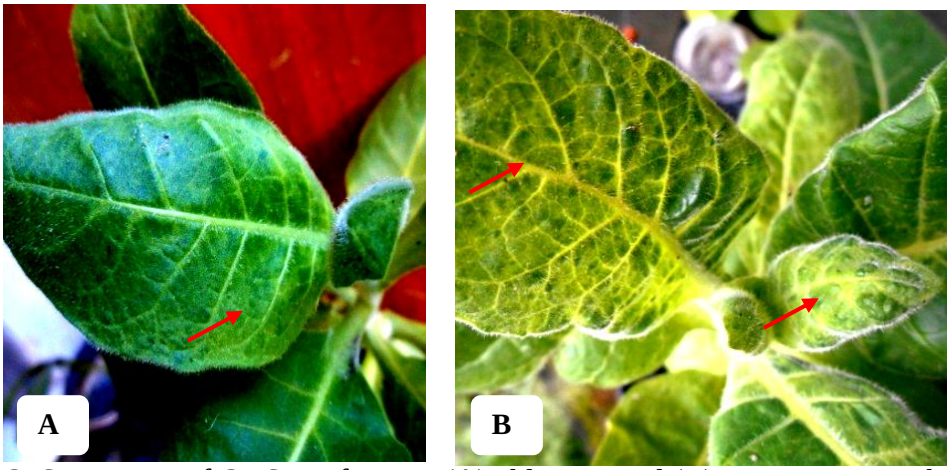

Figure 2. Symptoms of ORSV infection: (A) chlorotic and (B) mosaic on *N. tabacum*

Chlorotic is a type of symptom caused by damage to chloroplasts which results in plant parts which are normally green to turn yellow. Chloroplast damage can be caused by the lack or formation 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 turn brown in time. Sometimes chlorotic symptoms are often associated with necrotic where chlorotic surrounds necrotic called "halo" (Purnomo, 2006). These symptoms subsequently turn into mosaic symptoms. Akin (2006) explains that the symptoms of mosaics are marked in the form of "green islands" (green islands) where a mixture of leaf parts that are yellow or light green with green.

Another symptom in both plants is the leaf edge curling down on young leaves as an initial symptom of ORSV infection (**Figure 3**). Chenopodiaceae is the fastest growing symptom with a short incubation period and a high percentage of events is 80%. Necrotic local lesions that appear are symptoms of hypersensitive response results that are responsible for limiting pathogens so that plants become more resistant to disease (Akin, 2006).

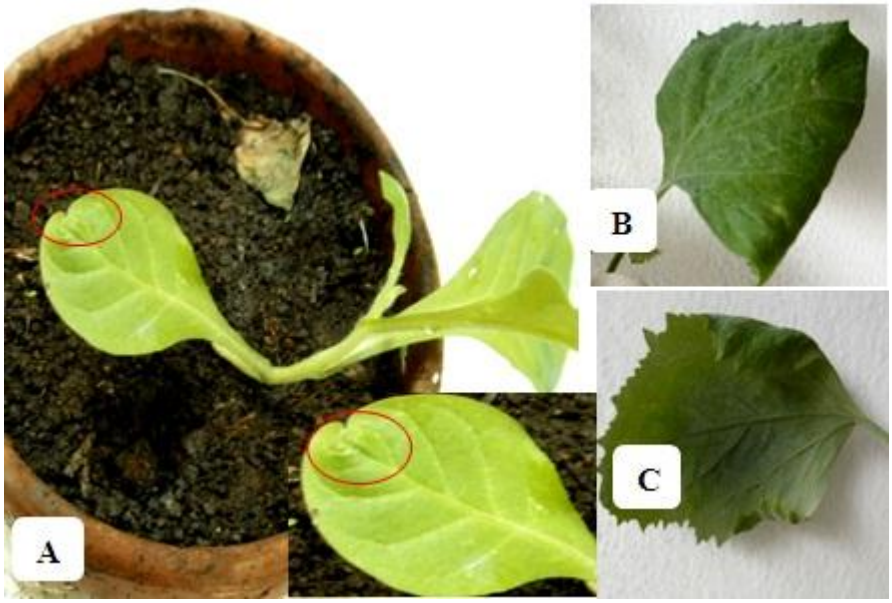

Figure 3. Leaf deformation symptoms are leaf edges curl (curling leaf) in *Nicotiana tobaccum* (A) and *C. amaranticolor* (B and C)

Other symptoms of infection with *N. tobaccum* and *N. benthamiana* are similar: chlorotic and leaf malformation (**Figure 4**). Malformation is a symptom of leaf surface changes becoming abnormal. This symptom begins with changes in the surface of the leaf to become bumpy (regose), resembling crackers (regulose), and curling leaf.

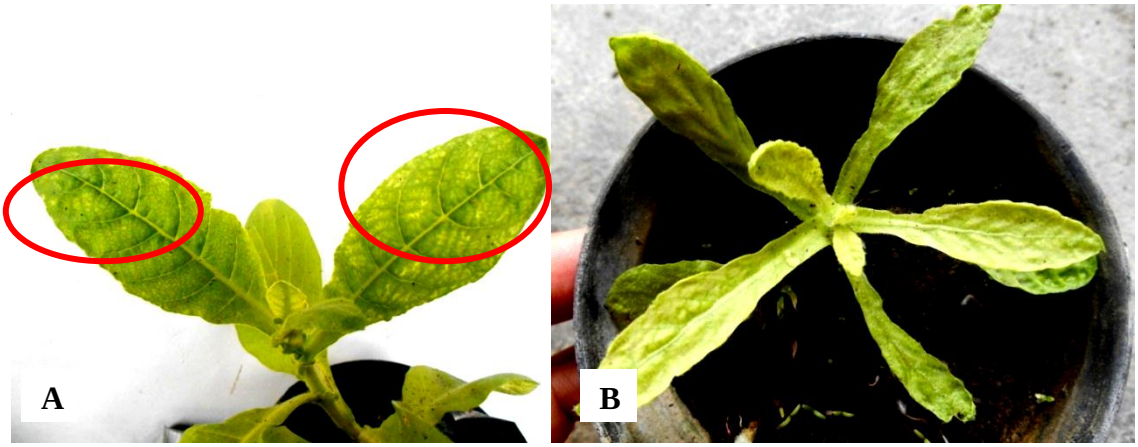

Figure 4. Symptoms of infection appearing on the leaves of the *N. benthamiana* plant; (A) chlorotic, (B) leaf malformation

Symptoms of viral diseases in host plants can occur due to the use of plant metabolic products for viral synthesis, accumulation of virions or parts of viruses and the effects of typical polypeptides encoded by viral genes (Akin, 2006). The response to ORSV infection shows a variety of symptoms between types of host plants in the form of necrotic

local lesions, mosaics, chlorotics, and leaf malformations. Based on the symptoms seen, it shows that the ORSV inoculum used in the study is very infective.

Virus Detection in Indicator Plants

DAS-ELISA serological test results (**Table 2**) indicate that the overall sample of inoculation plant leaves showed positive ORSV infection with an average absorbance value of 1.125-1.152. Daryono and Natsuaki (2009) suggested that a test sample was said to be positively infected by DAS-ELISA if the absorbance value at the wavelength of 405 nm was close to the positive control absorbance value, or had a value of 2-3 times the absorbance value of the control buffer.

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

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

Read by ELISA-reader BioTek, FALITMA Faculty of Biology UGM

The results of this serological detection reinforce the evidence that the method can be used to detect ORSV which causes indicator plants with symptomatic necrotic local lesions, mosaics, chlorotics, and leaf malformations in plants infected with ORSV.

Determination of Plant Resistance

Matthews (1992) explains that plant responses to pathogens can be grouped into immune, resistant, tolerant, and vulnerable. Based on observations of plant response indicators to a single ORSV infection by observing the incidence of disease and viral infections, the response of plants is grouped into near immune, somewhat resistant, and vulnerable. The results showed that the Chenopodiaceae plant was an indicator plant that was close to immune to ORSV infection. Overall tobacco plants provide susceptible responses to ORSV infections (**Table 2**).

Table 2. The level of resistance of various types of plants is an indicator of ORSV infection

Types of Plants	Host Plant Reaction		
	Disease Incidence[1)]	Virus Infection[2)]	Plant Response Criteria
C. amaranticolor	++	+	Susceptible
N. tabacum	++	+	Susceptible
N. benthamiana	++	+	Susceptible

[1)] Disease Incidence: - : There was no disease occurrence
\+ : Disease Incidence $0 < x \leq 40$
++ : Disease Incidence $40 \leq x < 100$
[2)] Virus Infection: - : Based on the ELISA watershed there is no virus infection
\+ : Based on the ELISA watershed there is a viral infection

Response plants that have a response are plants that support the growth and development of viruses, and viruses can cause damage. Based on the severity of the symptoms that are seen and the incubation period, the interactions that occur in most of the inoculated orchid plants cause symptoms of infection that are quite severe. The difference in the severity of disease symptoms is related to the process of development and spread of the virus in plant cells. The severity will be higher with the faster process of virus development and spread in infected plant cells (Hull, 2002).

The virus moves into plant tissue through phloem vessels and interferes with the plant's biological function by utilizing existing amino acids for their replication processes. These physiological disorders cause systemic symptoms that appear on young leaves. So, the faster the process of development and spread of both viruses in plant cells, the systemic symptoms appear more quickly and the higher the severity.

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

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