

Evolution of Indonesian Orchid Virus

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ABSTRACT

Odontoglossum ringspot virus (ORSV) is an important virus infecting in the world. This virus has entered and infected orchids in Indonesia, both natural orchids and hybrid orchid. Detection result indicates that the virus is found in various locations of natural forests, botanical gardens, and nurseries in Java, Kalimantan, Sulawesi, Bali and Papua.

Result showed that hybrid orchids in nurseries were more susceptible to ORSV infection compared to natural orchids in natural forests and botanical gardens. ORSV was found to infect hybrid orchids in eight nurseries, compared to natural orchids in natural forests and botanical gardens, each of which was found in only 3 location. The DAS-ELISA serological test showed higher mean value of absorbance in samples collected from hybrid orchids at nurseries (1.125-1.152), compared to samples collected from natural orchids in botanical gardens and natural forests (0.520-0.918 and 0.520, respectively). *Phalaenopsis* was the most suitable and the most vulnerable host to be infected with ORSV (incidence rate at 57%), followed by *Calanthe* (14%), *Dendrobium* (9%), and *Bulbophyllum*, *Cattleya*, *Oncidium*, and *Liparis* with incidence rate at 5%. Sequence analysis of coat protein genes showed that Indonesian ORSV isolates from natural forests and botanical gardens had higher similarity index value with isolates from other countries (100%), compared with Indonesian ORSV isolates from nurseries (99.3%). ORSV isolates from natural forests and botanical gardens had fewer mutation rate of 18% and 36%, compared to ORSV isolates from nurseries with mutation rate of 45%. The sequence analysis also indicated that the virus has evolved, even leading to speciation. Reconstruction of the phylogenetic tree showed that Indonesian ORSV was thought to originate from Germany.

Keywords: ORSV, orchid, coat protein, evolution, Indonesia

Introduction

Orchids are ornamental plants with the highest demand in the market and consumer interest (Mahfut *et al.*, 2013) in Southeast Asian countries. Orchid demands tend to increase every year. This spurred the high interest of the people to maintain and develop orchids as commercial plants.

Pest and disease attacks are one of the obstacles in the orchid cultivation business. Viral infection is the most important limiting factor because it may reduce plant quality (Pataky, 1990). Orchids are reported to be infected by more than 30 types of viruses (Zettler *et al.*, 2010; Navalinskiene and Samuitiene, 2005; Chang *et al.*, 2005). One of the most reported infecting virus with worldwide spread, including Indonesia, is the *Odontoglossum ringspot virus* (ORSV) (Wisler, 1989; Zettler *et al.*, 1990; Sherpa *et al.*, 2004).

***Odontoglossum ringspot virus* (ORSV)**

ORSV or also known as *Tobacco mosaic orchid strain* (TMV-O), is a member of Tobamovirus genus (Jensen and Gold, 1951) and Virgaviridae Familia (Adams *et al.*, 2009). ORSV is relatively new in Indonesia even though it has been known since 1951. This virus was first reported in America to infect *Odontoglossum grande* orchids which showed symptoms of ring spots (Jensen and Gold, 1951). Research of ORSV in Indonesia only started in 1996 and continues until now. In this research ORSV was analyzed by virus detection, identification (Inouye & Gara, 1996; Lakani *et al.*, 2010; Mahfut, 2011; Mahfut *et al.*, 2016 a ; Mahfut, 2018) and molecular characterization (Lakani *et al.*, 2010; Mahfut *et al.*, 2016 b ; Mahfut *et al.*, 2017 a ; Mahfut *et al.*, 2017 b ; Mahfut, 2018). Virus detection and identification were aimed to study the pattern of ORSV infection, while molecular characterization was used to determine the nucleotide changes. This changes were then used as the basis to study the evolution of ORSV in Indonesia. The result will be used to study the spread patterns of ORSV and if possible, to anticipate the ORSV infection in orchids in Indonesia.

Virus Collection

Based on collection site, ORSV samples were divided into three location groups: nurseries, botanical gardens, and natural forests. Result of detection and identification showed that ORSV had infected orchids at nurseries in Ujung Pandang (Inouye & Gara, 1996), West Java (Lakani *et al.*, 2010), Cianjur (Mahfut, 2018), Magelang (Mahfut *et al.*, 2016a), orchids at Botanical Gardens in Bogor (Mahfut *et al.*, 2016 a ; Mahfut *et al.*, 2017 a ; Mahfut, 2018), Purwodadi (Mahfut *et al.*, 2017 a), Balikpapan (Mahfut, 2018), and orchids in natural forests in Yogyakarta (Mahfut *et al.*, 2016 a), Jayapura (Mahfut, 2018). These data of locations presents infection and spread patterns of ORSV which is quite extensive in Indonesia.

Collection sites were chosen based on orchid vegetation type. Botanical gardens are conservation area for natural or non-natural, original and/or non-original (introduced) plant

collection, which are used for various interests such as research, science, tourism, and recreation (Napitu, 2007), thus, many native and introduced orchids can be found. In natural forests, the whole vegetation is still natural. These natural orchids are the native vegetation allowed to grow naturally without human intervention (Napitu, 2007) while in nurseries as an orchid farms, the whole vegetation is hybrid orchids traded commercially.

ORSV Detection

Detection result indicated that ORSV infected natural orchids in Indonesia although the incidence rate is still low: 7 samples from 3 botanical gardens (Mahfut, 2011; Mahfut *et al.*, 2016 a; Mahfut *et al.*, 2016 b) and 3 samples from 3 natural forests (Mahfut & Daryono, 2014; Mahfut *et al.*, 2016 a; Mahfut *et al.*, 2016 b). More ORSV infected hybrid orchids found in 8 nursery locations (11 samples (Inouye & Gara, 1996; Lakani *et al.*, 2010; Mahfut *et al.*, 2016 a; Mahfut *et al.*, 2016 b). Virus titer observation using DAS-ELISA test also showed higher mean value of absorbance in samples collected from hybrid orchids at nurseries nurseries (1.125-1.152) (Mahfut *et al.*, 2016 b), compared to samples collected from natural orchids in botanical gardens and natural forests (0.520-0.918 and 0.520, respectively) (Mahfut, 2018). This was caused by the loss of genetic variation due to repeated crossing of hybrid orchids making them more susceptible. Frankham *et al.* (2002) explain that this process causes inequality biochemical or morphological functions that result in differences in the level of reproduction and survival ability. Low genetic variation gives a decreased response of survival ability against environmental changes such as global warming, pollution, presence of competitors, pests and diseases.

In Indonesia, ORSV infection extends from natural orchids in natural forests and botanical gardens to hybrid orchids at nurseries. ORSV enter the nurseries and botanical gardens by human intervention through activities of introducing and purchasing infected orchid seeds from other regions and countries. Those are added with the presence of human contact between healthy and sick plants alternately, and contaminated equipment during vegetative propagation and flower harvesting. While in natural forests, infections occur usually through infected seeds, water, and soil, or mechanical transmission by animal (non-vector) although this does not rule out the possibility of ORSV spread by human through infected soil carried by shoes when entering natural forests.

ORSV Host Range

Several studies reported that ORSV has been able to infect 27 genera orchid in the world. In Indonesia, ORSV is reported to have infected 7 genera of orchids, namely: *Dendrobium* (Lakani *et al.*, 2010; Mahfut, 2011), *Phalaenopsis* (Inouye & Gara, 1996; Mahfut, 2011; Mahfut *et al.*, 2016 a; Mahfut *et al.*, 2016 b; Mahfut *et al.*, 2017 a; Mahfut, 2018), *Liparis* (Mahfut & Daryono, 2014), *Bulbophyllum*, *Calanthe*, *Cattleya*, and *Oncidium* (Inouye & Gara, 1996). According to those studies, the total of positive samples infected with ORSV were 21 samples with percentages: *Phalaenopsis* (57%), *Calanthe* (14%), *Dendrobium* (9%), and *Bulbophyllum*, *Calanthe*, *Cattleya*, *Oncidium*, and *Liparis* (5% each). This shows that *Phalaenopsis* has the highest percentage of infected with ORSV. Mahfut

et al. (2016 b) and Mahfut (2018) considers that *Phalaenopsis* orchids are the most suitable and the most vulnerable hosts for ORSV, especially the species of *Phalaenopsis amabilis*.

The susceptibility of *Phalaenopsis amabilis* to ORSV infection can be induced by the host response that supports growth and virus development. According to Hull (2002) the main factors that can influence plant susceptibility to disease infection is the genetic composition of host plants and environmental conditions. The content of chemical compounds found in *Phalaenopsis* plant can also play a role in the process of genome replication and development of the virus. Certain polypeptides compounds that are encoded by viral genes containing nitrogen, such as in growth regulator substances and phenol compounds, play an important role in the presence of systemic symptoms as the result of virus-host interaction. The abundance of metabolic compounds in *Phalaenopsis* sp. is also helping the virus synthesis. Mahfut *et al.* (2016 b) mention that it is also related to the texture of *Phalaenopsis* leaves of sp. which are wide and soft due to the high content of water and its role as food reserve.

Analysis of Coat Protein Genes

Molecular characterization was done based on sequences analysis of CP gene and used to analyze the sequence similarity of ORSV isolates from Indonesia and other countries. Mahfut *et al.* (2016 a) report that Indonesian ORSV isolates have high sequence similarity (99-100%) with 7 isolates from other countries in Asia, America, and Europe. Indonesian ORSV isolates from natural forests and botanical gardens, show a higher similarity index (SI) value with isolates from other countries (100%), compared to Indonesian ORSV isolates from nurseries (99.3%) (Mahfut, 2011). This means that Indonesian ORSV isolates from nurseries have a significant amount of nucleotide mutations compared to Indonesian ORSV isolates from natural forests and botanical gardens. Lakani *et al.* (2010) also report that Indonesian ORSV isolates from nurseries in Bogor show similarity with 55 ORSV isolates from other countries at the value of 98-99%.

Sequence analysis of CP gene was also used to determine viral nucleotide mutations which caused changes in genome composition as a result of virus natural adaptation to the environment in Indonesia (Mahfut & Daryono, 2014; Mahfut *et al.*, 2016 a; Mahfut *et al.*, 2016 b ; Mahfut *et al.*, 2017 a; Mahfut *et al.*, 2017 b). According to Mahfut (2011) Indonesian ORSV isolates showed a total of 11 times of point mutations, with fewer mutations percentage in ORSV isolates from natural forests and botanical gardens (18% and 36%, respectively) compared to ORSV isolates from nurseries (45%). The research showed that hybrid orchids at nurseries are more susceptible to infection due to changes in genetic variation.

Mahfut *et al.* (2017 a) explain that the CP gene is a conserved sequence and has a proofreading mechanism which corrects errors that occur during genome replication process. However, the size of the viral genome is relatively small and a slight error will have a significant effect on the mutation rate. Higher mutation rate will produce viral genetic variation thus increasing the probability of evolution. Sequence analysis result also indicated that the virus has evolved, which can lead to speciation.

Analysis of Phylogenetic Relationship

The nucleotide sequence analysis was used to determine the relationship between isolates through reconstruction of phylogenetic trees. Mahfut *et al.* (2016 a) and Mahfut *et al.* (2017 a) report that Indonesian ORSV isolates are in the same branch as the German ORSV isolates which means that these two isolates have a high similarity, thus a closer relationship. Indonesian isolates also share a high similarity with isolates from China, Taiwan, Brazil, and America. This

is similar to the result of Lakani *et al.* (2010) in addition to ORSV isolates from Thailand, India, South Korea, Japan and Singapore.

Lakani *et al.* (2010), Mahfut *et al.* (2016 a), and Mahfut (2018) mention that Indonesian ORSV isolates are thought to have originated from Germany. BPPP (2005) records Germany ranked 14th as an importer of orchid seeds and plants to Indonesia since 1997-2001, besides United States, Brazil, South Korea, China, Japan, Taiwan, and several West Asian countries. This is also confirmed by reports of ORSV infection in Germany, United States, Japan (Lawson 1990), Brazil (Freitas *et al.*, 1999), India (Sherpa *et al.*, 2006), Singapore (Wong *et al.*, 1994), Taiwan (Chang, 2008; Zheng *et al.* 2008), Korea (Chang *et al.*, 1991), and China (Rao *et al.*, 2015). There was also possibility that ORSV infection in these countries was due to its relatively close geographical location. Thus, one of effective ways to protect and maintain the health status of native orchids in Indonesia is by limiting and controlling the import of orchids from other countries.

Human-Induced Rapid Evolutionary Change (HIREC)

The evolution of the orchid virus in Indonesia occurs through human intervention. ORSV responds quickly to changes in its environment through CP gene mutations. The response of this virus includes Indonesia natural environment which is different from the country of origin of the virus and the different types of host. This was compounded by human intervention through hybridization and maintenance of plants using fertilizers and pesticides. So that the viruses in hybrid orchids in nurseries become more adaptive by experiencing more genomic changes. Sih *et al.* (2011) revealed almost all live organisms in environments that have been altered by human activities. The impact of this human activity provides rapid environmental changes or what is referred to as Human-Induced Rapid Environmental Change (HIREC).

No other agent is changing the environment as fast and drastically as humans are (Schilthuizen, 2018). We introduce exotic species and pets into the environment. In areas that are strongly influenced by humans, and by the middle of this century that will be almost anywhere on Earth, wild animals and plants will need to either adapt or go extinct. Sih *et al.* (2010) mentions five major types of HIREC have been identified: habitat loss/fragmentation, the spread of exotic species, harvesting by humans, pollutants of various sorts, and climate change. The results of the phylogenetic tree analysis showed that Indonesian ORSV isolates formed separate branches with ORSV isolates from other countries. HIREC causes the evolutionary probability of the Indonesian orchid virus to be faster and leads to speciation. Schilthuizen (2018) revealed speciation is the evolution of two species where first there was just one. Speciation is what creates splits in the branches of the evolutionary tree of life. And normally it is a slow process, but when humans are involved, it can happen much faster. Evolution can go very, very quickly if the selection pressure is strong. And human action creates such strong selection pressures. This means that humans are influencing the evolution of life on this planet in irreversible ways. But it also means that some species may be able to survive environmental change by evolving adaptations, a kind of evolutionary rescue. The classification of speciation mechanisms by geographical context into allopatric, parapatric and sympatry classes no longer yield satisfactory results (Butlin *et al.*, 2012). This is due to the very fast rate of evolution. The influence of human activity has an effect on adaptive selection. This means that only those species that can evolve fast enough to keep up with the changes will survive. And Indonesian ORSV is included.

Conclusion

ORSV was detected in natural orchids and hybrid orchids in Indonesia. This virus was found in samples from natural forests, botanical gardens, and nurseries in Java, Kalimantan, Sulawesi, Bali, and Papua. Results showed that hybrid orchids at nurseries were more susceptible to infection with ORSV compared to natural orchids in natural forests and botanical gardens. This is related to changes in genetic variation in hybrid orchids that result in susceptible viral infections. The Indonesian ORSV is thought to have originated from Germany through the import of orchid seeds and plants. To protect natural orchids in Indonesia, orchid import activities from other countries should be limited and controlled.

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