Resistance Response of Indonesian Native Orchids to *Odontoglossum ringspot virus* Involves Expression of the *CP* and *rbcL*

**Mahfut**1**, Budi Setiadi Daryono**2**, Susamto Somowiyarjo**3

1Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Jl. Sumantri Brojonegoro no 1, Bandar Lampung, Indonesia

2 Department of Biology, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta, Indonesia

5 Department of Virology, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta,, Indonesia

**email:** mahfut.mipa@fmipa.unila.ac.id1, bs\_daryono@**mail**.ugm.ac.id2, soesamto@faperta.ugm.ac.id3

**Abstract.** *Odontoglossum ringspot virus* (ORSV) is an orchid virus that is reported to be the most widely infected and has a wide spread in the world, including Indonesia. Biotic stress problems cause a decrease in the rate of photosynthesis. This study aims to determine the resistance response of Indonesian native orchids to viruses involved in the molecular analysis of the *CP* (coat protein) gene of ORSV and the *rbcL* (ribulose-1,5-bisphosphate carboxylase) gene of chloroplasts. Infected orchid leaf isolates were collected from nursery orchid cultivation in Cianjur. Amplification of the *CP* and *rbcL* genes succeeded in obtaining amplitudes measuring ± 474 bp and ± 599 bp, respectively. The results of the sequence analysis show that there are point mutations, namely deletions, insertions and transitions. The change is thought to be an adaptation and physiological response of plants to the environment in Indonesia.

**Keyword:** ORSV, *coat protein*, *rbcL*, orchid, Indonesia

1. Introduction

Pests and diseases are still a major obstacle in the cultivation of orchids. Viral infection is the most important limiting factor because it can reduce plant quality [1;2;3;4;5;6;7]. One of the most widely reported viruses that infect with widespread spread in Indonesia is *Odontoglossum ringspot virus* (ORSV[1;5]. Symptoms in the form of mosaics and chlorotics with a line or ring pattern (ringspot) on the leaf surface [1;4;5;6], as well as flower breaking.

Areas of spread of ORSV infections include natural forests, botanical gardens, and nurseries [1;5]. Based on molecular detection and characterization, it is known that ORSV has changes in genome sequences as a process of natural adaptation to different environments. ORSV that infects orchids in nurseries has more genome changes as a result of human treatment in the process of orchid cultivation and breeding. Fertilization and spraying treatment of pesticides have a viral resistance effect. This is compounded by minimal knowledge in plant breeding activities. Hybridization of orchids that have a close kinship can reduce genetic variation. The impact of the recessive properties of orchids will arise, including not resistant to biotic stress and environmental changes [2;4;6].

The problem of biotic stress further decreases the ability of plant photosynthesis [2;4]. This is caused by damage to chloroplasts followed by disruption of the synthesis of the rubisco enzyme (ribulose 1,5-bisphosphate carboxylase). This enzyme is produced by the *rbcL* gene and functions in the RuBP carboxylation reaction into 2 3-PGA molecules (phosphoglyceric acid) in the Calvin cycle. This study aims to determine the resistance response of Indonesian native orchids to viruses involved in the molecular analysis of the *CP* ORSV gene and the *rbcL* chloroplast gene through host-virus interaction patterns as a process of plant adaptation to environmental changes..

1. Materials and Methods
	1. Plants Materials

Samples of positive orchid leaves infected with ORSV were collected from the Balai Tanaman Hias, Cianjur. Samples were leaves of *Phalaenopsis amabilis* with mixed mosaic and chlorotic symptoms. As a comparison, *P. amabilis* leaves were infected with ORSV from Magelang as a positive control and *P. amabilis* results from in vitro culture as a negative control.

* 1. Molecular Detection of Coat Protein Gene

RNA isolation on a sample positive infected ORSV based on ELISA, using a Total RNA isolation kit (SBS Genetech Co., Ltd., China). According to Mahfut et al. (2016a), amplification of RNA by RT-PCR was performed with a separate method using specific primer, i.e. ORSV CP-F1 (5'ATGTCTTACACTATTACAGACCCG-3') and ORSV CP-R1 (5'GGAAGAGGTCCAAGTAAGTCC-3'). The process of RT using the first strand cDNA synthesis kit (Thermo scientific, USA), subsequently cDNA molecules that form is used as the mold in the process of PCR using GoTaq GreenMaster Mix (Promega, USA). RT reaction performed at temperature 37° C for 60 minutes, followed by incubation 96° C for 5 minutes and terminated at a temperature of 4° C. Amplification of cDNA are begining with predenaturasi at 95° C for 5 minutes, followed by 34 cycle, including denaturation temperature 95° C for 30 seconds at a temperature of 50° C, annealing for 45 seconds, and extension at a temperature of 70° C for 1 minute. PCR products were analyzed using electrophoresis on agarosa gel 2%, in 1x TBE buffer previously soaked in ethidium bromide. DNA visualization on UV transluminator (Bio-Rad Transilluminator 2000).

* 1. Molecular Detection of rbcL Gene

Genomic DNA isolation was carried out according to the DNA isolation kit (Phytopure) protocol. DNA amplification by Polymerase Chain Reaction (PCR) was carried out using a pair of primers, namely rbcLa-F (5'-ATGTCACCACAAACAGAGACTAAAGC-3 ') and rbcLa-R (5'-GTAAAATCAAGTCCACCGCG-3') [2;5]. The reaction was carried out using Thermocycler (Boeco) with a GoTaq GreenMaster Mix PCR kit (Promega, USA). DNA amplification begins with the predenaturation stage at 94oC for 3 minutes, followed by 34 cycles, including denaturation at 94oC for 30 seconds, annealing at 55oC for 30 seconds, and extension at 72oC for 1 minute. After the cycle is complete, then followed by 10 minutes post-elongation at 70oC and cooling at 4oC. PCR products were analyzed using electrophoresis on 2% agarose gel. The visualized DNA band shows the length of the base pair of the target gene, *rbcL* gene.

* 1. Analysis of DNA Sequencing

Sequencing of nucleotide sequences to amplified DNA is carried out by sending amplified samples to the 1st Base company, Malaysia. Data were analyzed and combined using DNASTAR Lasergene DM Version 3.0.25 Software Suite for Sequence Analysis. Sequence alignment analysis is performed with the Molecular Evolutionary Genetics Analysis (MEGA) program.

1. Results and Discussion
	1. Molecular Detection of CP Gene ORSV and rbcL Gene Chloroplast

The results of PCR detection of control and positive ORSV samples showed amplification of DNA fragments measuring ± 474 bp dan ± 599 bp which is *CP* gene and *rbcL*, as reported [1;4].

* 1. DNA Sequence Analysis

Nucleotide sequence analysis results of total nucleotides obtained in controls and isolates of 603 and 602 bases. The percentage homology of the two isolates of the isolates was 99.3%. Magelang isolate has a lower GC percentage base content than the control that is 20.25% of the average of 20.35. While the percentage of AT base content is higher at 29.75% from the average 29.65%. Based on the analysis of elbow alignment, it was found that there were point mutations in the form of deletions, insertions, and transition substitutions in Magelang isolates. In this study no transversion substitution was found. The most occurrence of mutations is a transition that is as much as 4 times, while deletions and insertions 1 time each.

The effect of mutations that occur is able to cause changes in the amino acid encoding codlet triplets. The percentage of amino acids translated in the control and isolates of Magelang were 191 and 192 amino acids. The presence of base C deletions in the 11th nucleotide sequence causes the formation of Pro amino acids in the control. The insertion of bases G and T in the 49th and 54th nucleotide sequences respectively led to the formation of the amino acids Val and Phe in Magelang isolates. Whereas the G, T, C, T transition transitions that occur in the order of 68, 134, 201, and 202 only cause changes in amino acids at only two points. The changes that occur are amino acids Ser into Pro and Ser into Phe. Subsequent analysis of amino acid frequencies showed that Magelang isolates had decreased amino acids Ser and Pro respectively 0.55% and 0.01% and an increase in Phe and Val of 0.51% and 0.25%.

The *rbcL* gene (rubisco gene) is a gene producing rubisco enzyme which is a CO2 fixation enzyme. Judging from the composition of its nucleotide, the *rbcL* gene is conserved (sustainable) and has an important role to maintain the stability of the genetic material of the chloroplast genome to the activity of the nuclelease when the virus enters the host cell. Chloroplast DNA gene analysis can be used for phylogenetic studies, data collection on plant species diversity, observing intraspecific variations, as well as knowing plant physiological responses through host-virus interaction patterns.

Some of the nukeotide mutations that occur are able to cause changes in amino acids that are formed in the genome composition of the chloroplast DNA. Changes in amino acids will change the function of genes that are arranged, so that the infectivity also changes. Increased synthesis of the amino acid Phe is related to chloroplast synthesis. The presence of these amino acids is related to the formation and repair of damaged chloroplasts as a result of viral infections.

Most of the nuclear genes, including the DNA chloroplast gene, have proffreading mechanism capabilities. This causes the organism to make corrections and correct errors that occur during the genome replication process. With the relatively large size of the chloroplast DNA genome, a slight error (mutation) will not have a significant effect. This is different for viruses, mutations are common.

Many mutations in viruses are supported by the ability to adapt to the environment and broaden the host range [2;4]. The hypothesis states that the emergence of severe types of viral infections in a geographical location and achieving fitness to infect certain types of host will cause the loss of the virus's ability to re-infect its original host. In this study, the presence of host chloroplasts DNA mutations is a form of adaptation to viral infections leading to fitness due to selection.

The rate of mutation will produce genetic variations both viruses and the host. The magnitude of the mutation rate and genetic variation produced increases the probability of faster evolution [2;4]. The results of this study can be used as basic information to study plant physiological responses to viral infections through molecular identification of chloroplasts.

1. References

[1] Mahfut, Daryono BS and Somowiyarjo S 2017a Deteksi Odontoglossum ringspot virus (ORSV) yang Menginfeksi Anggrek Asli Koleksi Kebun Raya di Indonesia *Jurnal Fitopatologi Indonesia* **13** 1-8

[2] Mahfut, Daryono BS and Somowiyarjo S 2017b Identifikasi Molekuler DNA Kloroplas Pada Anggrek Terinfeksi Odontoglossum ringspot virus (ORSV) di Magelang, Jawa Tengah *Seminar Nasional Pengendalian Penyakit Pada Tanaman Pertanian Ramah Lingkungan II Perhimpunan Fitopatologi Indonesia Komisariat Daerah Yogyakarta, Solo, dan Semarang 2016* Yogyakarta Indonesia p 354-360

[3] Mahfut and Daryono BS 2019 Variation Symptoms and Resistance Response of Different Types on Orchids (Orchidaceae) Against Odontoglossum ringspot virus (ORSV) Infection *International Series on Interdisciplinary Science and Technology* **4** 246–249

[4] Mahfut, Daryono BS, Indrianto A and Somowiyarjo S 2019a Plant-Virus Interaction on Orchids Infected Odontoglossum ringspot virus (ORSV) in Bogor Botanical Garden, Indonesia *The 1st International Conference on Science and Technology (ICoST)* Makassar, Indonesia p 1-8

[5] Mahfut 2020a Indonesia Darurat Konservasi: Sudah Amankah Kebun Raya Kita? *Prosiding Seminar Nasional Biodiversitas Indonesia* Makassar, Indonesia p 1-6

[6] Mahfut 2020b Variation of Resistance Responses on Indicator Plants Against Odontoglossum ringspot virus (ORSV) Infection *International Journal of Advanced Science and Technology* **29** 11780-11785

[7] Mahfut, Indrianto A, Somowiyarjo S and Daryono BS 2020 Molecular phylogeny of Orchids Mycorrhiza Isolated From Native Tropical Orchids in Indonesia Malaysian Journal of *Microbiology* **16** 68-72