HAYATI Journal of Biosciences June 2010
Vol. 17 No. 2, p 101-104
EISSN: 2086-4094

Available online at:
http://journal.ipb.ac.id/index.php/hayati
DOI: 10.4308/hjb.17.2.101

SHORT COMMUNICATION

Identification and Molecular Characterization of *Odontoglosum Ringspot Virus* (ORSV) from Bogor, Indonesia

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Received January 5, 2010/Accepted June 4, 2010

Recently, field surveys were conducted in several orchid nurseries in Bogor (West Java), Magelang (Central Java) and Malang (East Java). We found that most of the commercially orchids sampled from Bogor was infected by virus-like disease. The symptoms depend on the orchid species. Thus, the symptoms varied such as mottle, mosaic and necrotic flecks either on the leaves or on the flowers. Similiar symptoms were not found in samples obtained from Central Java and East Java. A Tobamo-like virus was inferred to be possible cause of the viral disease-like symptoms. Serological test of the samples by ELISA showed positive against *Odontoglosum Ringspot Virus* (ORSV) antibody and was negative against *Cymbidium Mosaic Virus* (CyMV) antibody. Total RNA was extracted from symptomatic plants and RT-PCR was carried out by using a pair of coat protein (CP) gene primer of ORSV. It was successfully amplified a 500 bp of DNA fragment and it was directly sequenced. The nucleotide sequence of CP gene had confirmed the identity of ORSV. Phylogenetic analysis based on the CP nucleotide sequences were grouped into only one major cluster. The ORSV isolate from Bogor (Sd 21) and the other isolates were clustered in the same group and had highest nucleotide homology (99%). These results provide first evidence of ORSV infecting orchids in Bogor, Indonesia.

Key words: *Odontoglosum Ringspot Virus,* orchid, *Tobamovirus*

INTRODUCTION

Orchids can be infected by approximately 50 viruses (Zettler *et al*. 1990; Chang *et al*. 2005; Navalienskiene *et al*. 2005). The most important type of virus infecting orchids in the world is *Odontoglosum ringspot virus* (ORSV) (Zettler *et al*. 1990; Sherpa *et al*. 2004). It is also reported that the virus infects vanilla plant (Grisoni *et al*. 2004), and is considered as the most prevalent and economically important virus infecting orchids in Florida as well as worldwide (Jensen & Gold 1951; Zettler *et al*. 1990). The ORSV infecting orchid showed various symptoms such *Odontoglosum grande* ringspot on leaves, also stripe breaking (mottle), diamond-shaped, mosaic, color burst on *Cymbidium* flowers and also color breaking on *Cattleya*. The symptoms were depend on orchid spesies. The ORSV infects approximately 31 orchid genera (Jensen & Gold 1951; Zaitlin 1976).

Recent field study conducted between 2007-2008 at several orchid nurseries in the Java island, we found that most of the orchids were infected by virus-like disease. The infected leaves and flowers showed various symptoms which were appeared as concentric mosaic, necrotic on the lower part of leaf, leaf malformation, necrotic and some were symptomless (Figure 1), especially those samples from Gunung Sindur-Bogor.

The aim of this research was to identify the cause of those symptoms on orchids from Bogor. Basic information obtained from this study will contribute to find out appropriate management strategies of the disease.

MATERIALS AND METHODS

Source of Inoculum. Thirty symptomatic *Dendrobium* orchids were collected from the orchid seedlings in Gunung Sindur-Bogor. Samples were subjected to serological test, viral purification, and molecular detection by Reverse Transcription-Polymerase Chain Reaction (RT-PCR).

Serological Test. Samples were tested with Enzyme-linked-immunoabsorbent assay (ELISA) by using antibodies against ORSV and *Cymbidium Mosaic Virus* (CyMV) (DSMZ; German Resource For Biological Material, Braunschweig, German). Direct Double Antibody Sandwich-Enzyme Linked Immunoabsorbent assay (DAS-ELISA) was carried out according to protocol recomended by DSMZ.

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Virus Isolation and Propagation. To isolate ORSV and separate it from other viruses which may present in the same sample, symptomatic leaves were infected to local lesions of *Datura stramonium* as the host. Leaves were homogenized in 0.05 M phosphate buffer pH 7.5, the sap was inoculated to *D. stramonium* mechanically (Sambrook *et al*. 1989). Leaves were dusted with carborundum 600 mesh prior to the mechanical inoculation. The inoculated plants were maintained in an insect-proof house for symptomatic development. After 3-21 days post inoculation, *D. stramonium* showed local lesions. Part of the local lesion was used as inoculum to inoculate *D. stramonium* serially. Similar procedure was repeated three times to obtain a pure virus source. Finally, the virus was propagated on *Nicotiana tabacum* by mechanical inoculation using the local lesion from last passage and the inoculum were used for further test.

Molecular Detection. Total RNA was extracted from a 0.1 g symptomatic leaves of *Nicotiana tabacum* using Total RNA extraction kit (Rneasy Plant Mini Kit, Qiagen, USA). Total RNA was used as a template for complementary DNA (cDNA) construction by using reverse transcriptase enzyme M-MULV (NEB) according to manufacture protocol. RT-PCR was carried out by using a specific primer for ORSV CP gene (forward primer ORSV-cpF 5-'GCTCTAGAATGTCTTACACTATTACAGACC-3') and reverse primer ORSV-cpR (5'-GCTCTAGAATGGGT CGTTTRGCGTTTTGTAG-3') (Ajjikuttira *et al*. 2005). PCR conditions for DNA amplification were 35 cycles at 95 °C for 30 sec, 50 °C for 45 sec, 72 °C for 1 min, and final extension at 72 °C for 10 min. The 500 bp DNA product was analyzed on 1.2% agarose gel containing 0.5% ethidium bromide under UV illuminator.

DNA Sequencing and Phylogenetic Analysis. RT-PCR product was subjected to nucleotide sequencing at PT Charoen-Phokphand, Jakarta, Indonesia. The CP sequence was aligned with ClustalW software of BioEdit program Version 7.0.0 (Isis Pharmaceuticals, Inc). The phylogenetic tree was constructed by using MEGA version 4.0 software (Tamura *et al*. 2007), with neighbour-joining method and maximum composite likelihood model to estimate the distance and bootstrap support was estimated with 1000 replicates.

RESULTS

Serological Test. Samples from Gunung Sindur showed a positive reaction against ORSV antisera and some of them also positive against CyMV. Typical symptomatic plants with positive reaction against antisera ORSV used as a source of inoculum. The infected orchid plants showed variation of symptoms such as mosaic, concentric, necrotic spots on the lower part of the leaf and leaf malformation, and the flowers found in the necrotic symptoms (Figure 1).

Molecular Detection and Phylogenetic Analysis. RT-PCR confirmed the existence of ORSV, a 500 bp DNA was succesfully amplified (Figure 2). Phylogenetic analysis of the DNA compared with those other ORSV showed

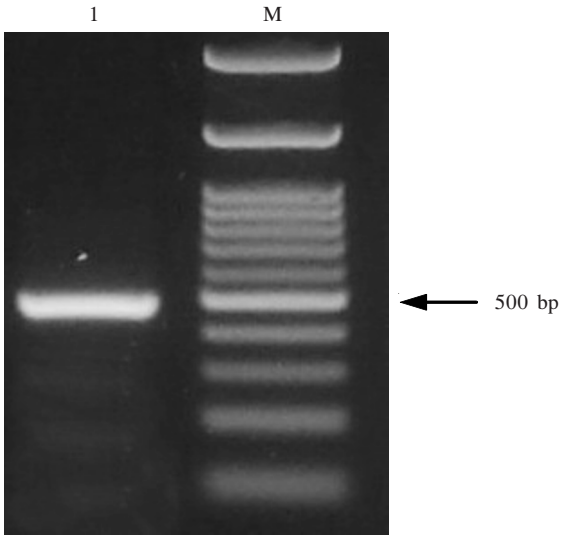

Figure 2. RT-PCR detection of ORSV with specific primer of CP gene. Lane 1: ORSV Bogor; lane M: represents molecular weight marker 100 bp (NEB).

Figure 1. Symptom Variation of orchids infected by ORSV: (a) mosaic; (b) concentric mosaic; (c) necrotic under leaf; (d) leaf malformation; (e) necrotic on flower; and (f) symptomless.