

Detection and Identification of Viruses of Orchids in Indonesia

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Three viruses, cymbidium mosaic virus (CyMV), odontoglossum ring-spot virus (ORSV) and an unidentified potyvirus were found in the orchids in Indonesia. CyMV was detected from orchids in 8 genera, namely *Aranthera*, *Calanthe*, *Cattleya*, *Cymbidium*, *Gromatophyllum*, *Phalaenopsis*, *Oncidium* and *Vanda*. The virus was widespread in many orchids in Indonesia and was common in *Aranthera* and *Calanthe*, thus being an economically important virus in Indonesia. ORSV was also detected in orchids of 5 genera, namely *Bulbophyllum*, *Calanthe*, *Cattleya*, *Oncidium* and *Phalaenopsis*. The unidentified potyvirus was found in *Aranthera*.

Key words: Cymbidium mosaic virus, Odontoglossum ringspot virus, Unidentified potyvirus, Identification, Orchids in Indonesia.

INTRODUCTION

Orchids have been cultivated intensively as an economically important flower crops in Indonesia, but very little research has been devoted to their virus diseases¹⁵⁾. The senior author visited Indonesia to survey viruses in the orchids during August 2-12, 1989¹³⁾ and July 21-Aug. 1, 1990¹²⁾, under the research project of "Cooperative Research on Virus-Free Tropical Plant Production" and examined plants for identification of the viruses in diseased orchids collected at various localities. In this present paper, we describe the results of this survey.

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This study was a part of the project carried out by the NODAI Center for International Programs for the JSPS-DGHE Program¹¹⁻¹³⁾.

MATERIALS AND METHODS

Viruses isolated from surveyed orchid plants were tested by symptomatology, serology and electron microscopy on 43 samples of 14 genera of orchids collected directly from 12 orchid nurseries or fields in the districts of Jakarta Timur, Bogor, Sukabumi, Yogyakarta, Ujung Pandang and Bali. Antisera^{5,6,8)} to cymbidium mosaic virus (CyMV) and odontoglossum ringspot virus (ORSV) originally from our laboratory were used for slide agglutination tests. For electron microscopy, all samples were stained negatively on formvar-coated grids with 2% aqueous phosphotungstic acid, pH 6.5, soon after collection of the samples in Indonesia. The stained preparations were brought to Japan, and examined electron microscopically (Hitachi HU-12 or H-7100). The length of virus particles was determined by using micrographs of a 1/2000 mm grating as a length standard. For thin sectioning, tissue samples of diseased orchids were immersed in 6% glutaraldehyde in 0.1 M potassium phosphate buffer, pH 7.0, soon after the collection in Indonesia, and were brought to Japan. The tissue samples were fixed with 1% osmium tetroxide, and embedded in Epoxy medium. Thinsections were cut with glass knives on an LKB Ultrame and stained with uranyl acetate and lead citrate.

RESULTS

1. *Diagnosis of viruses by serology and electron microscopy*

The results of diagnosis on 43 samples of 14 genera of orchids are shown in Table 1.

On serological test, the orchids of 8 genera, namely *Aranthera*, *Calanthe*, *Cattleya*, *Cymbidium*, *Grommatophyllum*, *Phalaenopsis*, *Oncidium* and *Vanda* out of tested 14 genera reacted strongly with CyMV antiserum, and positive *Aranthera* samples were found at all nurseries in 5 districts. Many plants that reacted positively to ORSV antiserum were found dually infected with CyMV, and the reaction to ORSV-antiserum was weak.

In electron microscopy, flexuous particles or straight rod particles were observed in crude sap preparations from diseased orchids which were found positive with CyMV-antiserum or ORSV antiserum. The modal length of the flexuous particles in all the samples reacted with CyMV-antiserum was approximately 480 nm (Plate II-3). The straight rod particles were found in the preparations from *Bulbophyllum*, *Calanthe*, *Oncidium* and *Phalaenopsis*, but samples from the 3 genera without *Bulbophyllum* were found to be dually infected with CyMV. The modal length of the straight rod particles was

Table 1. Detection of viruses in orchids surveyed in Indonesia

District	Orchid (genus)	No. of sample tested	No. of sample containing		
			CyMV ^{a)}	ORSV ^{a)}	FP ^{b)}
Jakarta	<i>Aranda</i>	1	0	0	0
Timur (1) ^{c)}	<i>Aranthera</i>	3	2	0	(1) ^{d)}
	<i>Den. Phalaenopsis</i>	1	0	0	0
	<i>Vanda</i>	1	1	0	0
Bogor and Sukabumi (4)	<i>Aranthera</i>	1	1	0	0
	<i>Bulbophyllum</i>	1	0	1	0
	<i>Calanthe</i>	4	4	(3) ^{d)}	0
	<i>Cattleya</i>	5	5	1	0
	<i>Cymbidium</i>	2	2	0	0
	<i>Den. Phalaenopsis</i>	2	0	0	0
	<i>Eria</i>	1	0	0	0
	<i>Grommatophyllum</i>	1	1	0	0
	<i>Paphiopedilum</i>	1	0	0	0
	<i>Phalaenopsis</i>	2	2	(1)	0
	<i>Vanda</i>	1	1	0	0
Yogyakarta (2)	<i>Aranthera</i>	5	5	0	0
	<i>Cattleya</i>	1	0	0	0
	<i>Dendrobium</i>	2	0	0	0
	<i>Vanda</i>	1	0	0	0
Ujung Pandang(3)	<i>Aranthera</i>	2	2	0	0
	<i>Oncidium</i>	2	2	(1)	0
Bali (2)	<i>Aranthera</i>	1	1	0	0
	<i>Oncidium</i>	1	1	0	0
	<i>Vanda</i>	1	1	0	0
	Total	43	31	2(5)	(1)

a): The results were based on symptomatology, serological test and electron microscopic findings.

b): FP: Filamentous particles of unidentified Potyvirus (Plate II-4, 5).

c): The number in the parentheses on the districts column indicates the number of nurseries and fields of orchid surveyed.

d): The number in the parentheses on the results tested indicates double infections with CyMV.

300-320 nm (Plate II-3, arrow). Other filamentous virus particles were observed in dip preparations from diseased *Aranthera* collected at Jakarta Timur (Plate II-4).

From the results mentioned above, the flexuous virus was identified as CyMV^{1-4,6,8,10}, and the virus of the straight rod particles was identified as ORSV^{2-5,8-10,14}. The filamentous virus particles found in *Aranthera* was considered to belong in *Potyvirus* genus. CyMV was found in *Aranthera* (11 infected plants/12 tested plants), *Calanthe* (4/4), *Cattleya* (5/6), *Cymbidium* (2/2), *Grommatophyllum* (1/1), *Phalaenopsis* (2/2), *Oncidium* (3/3) and *Vanda* (3/4) of 43 samples of orchids tested indicating that they were widespread in

Indonesia. ORSV was found in 7 plants of *Bulbophyllum*, *Cattleya*, *Calanthe*, *Oncidium* and *Phalaenopsis*. A *Cattleya* plant showing reddish-purple patches on the leaves and flower color breaking was found to be infected by ORSV. CyMV and ORSV were found to incite mosaic or necrosis in orchids.

2. *Symptoms in naturally infected orchids*

Symptoms on orchids associated with infections by CyMV, ORSV and/or others were as follows:

1) *Symptoms caused by CyMV.*

Aranthera: Chlorotic and/or necrotic spots were observed on the leaves. On some other plants, chlorosis and chlorotic streaks were formed in the mesophyll (Plate I-1, 2).

Calanthe: Conspicuous mosaic and chlorotic streaks were observed on the leaves (Plate I-6).

Cattleya: Light brown necrotic spots or sunken brownish necrotic streaks were formed in the mesophyll. Chlorotic or necrotic wave patterns and dents appeared on the surface of the leaves (Plate I-3, 4). On some other plants, chlorotic mosaic developed on the leaves (Plate I-5).

Cymbidium: Chlorotic patches and elongated chlorotic areas were formed on the leaves. Chlorotic patches and black spots or streaks appeared on the under side of the older infected leaves.

Grommatophyllum: Mosaic appeared on the leaves (Plate I-7).

Phalaenopsis: Light green mosaic, green island spots and necrosis were appeared on the leaves (Plate I-8).

Vanda: Leaf tips had a dark and light green mottling accompanied by irregular purple blotches, with some brown necrosis (Plate I-9). The leaves of mosaic plants were often noticeably short in length and irregular in shape.

2) *Symptoms of orchids caused by ORSV.*

Bulbophyllum: Chlorotic patterns appeared on the young leaves, and reddish purple blotches on the old leaves.

Cattleya: Faint chlorotic streaks and mosaic appeared on the young leaf, and reddish purple blotches on the old leaves. Color-breaking was seen on the flowers.

3) *Symptoms of orchids caused by double infection with ORSV and CyMV.*

Double infection with CyMV and ORSV was found on some *Calanthe*, *Oncidium* and *Phalaenopsis*. Their symptoms were as follows:

Calanthe: Conspicuous mosaic and chlorotic streaks appeared on the leaves

(Plate I-10).

Oncidium : Mosaic and necrosis were seen on the leaves (Plate II-1).

Necrosis was severe on the lower surface of the infected leaves.

Phalaenopsis : Chlorotic spots and mosaic appeared on the leaves.

4) *Symptoms of orchid caused by double infection with CyMV and flexuous particles.*

Aranthera : Symptoms on young leaves were characterized by chlorotic spots and chlorotic streaks (Plate II-2). Symptoms in the older leaves were chlorotic patterns with light brown necrosis.

3. *Ultrathin sections of infected orchids.*

In ultrathin sections of mesophyll cells of diseased *Aranthera*, on which were found flexuous virus particles (Plate II-4), pinwheel inclusions were observed in the cytoplasm (Plate II-5). Therefore, the flexuous virus particles in *Aranthera* may belong to *Potyvirus* genus, but were not identified.

DISCUSSION

Many viruses have been reported to occur in orchids^{1-6,8-10} throughout the world. A survey was conducted to determine the identity and the distribution of orchid viruses in Indonesia. On the basis of symptomatology, electron microscopy and serology, CyMV was found in orchids of 8 genera in all 12 nurseries surveyed in 5 districts of Jakarta Timur, Bogor, Yogyakarta, Ujung Pandang and Bali. CyMV was found to be widespread in many orchids in wide areas of Indonesia. ORSV was found in orchids of 5 genera at districts of Bogor and Ujung Pandang. Thus, it is very likely that CyMV and ORSV cause severe damage in orchid production. An unidentified potyvirus was found in *Aranthera* of Jakarta Timur.

As orchid viruses are transmitted easily by mechanical contacts such as contaminated cutting tools and contaminated hands when infected orchids are transplanted⁷), these viruses will continue to be widespread in many orchids in Indonesia, unless certain measures are applied to control virus transmission.

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インドネシアのランに発生するウイルスの検索と同定

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1989年8月と1990年7月の2回にわたって、インドネシア国のランに発生するウイルス病の調査研究を行った。5地域12カ所のラン園または温室で採集したランのうち、特徴のある病斑を示した14属43個体について、病徴、血清反応、電顕によるウイルス粒子の形態を調べ、また一部の試料については病葉超薄切片の電顕観察を行ってウイルスの診断同定を行った。その結果、cymbidium mosaic virus (CyMV), odontoglossum ringspot virus (ORSV) および未同定 potyvirus の3種のウイルスの発生が確認された。CyMVは *Aranthera*, *Calanthe*, *Cattleya*, *Cymbidium*, *Grommatophyllum*, *Oncidium*, *Phalaenopsis*, *Vanda* の8属から、ORSVは *Bulbophyllum*, *Calanthe*, *Cattleya*, *Oncidium*, *Phalaenopsis* の5属から、また未同定のpotyvirusは *Aranthera* から検出された。ORSVおよび未同定のpotyvirusはCyMVと混合感染しているものが多かった。以上の調査研究から、インドネシアではCyMVが各地に広く発生分布し、ORSVとともに被害の大きいウイルス病の病原であることが認められた。

キーワード : Cymbidium mosaic virus, Odontoglossum ringspot virus,
未同定の potyvirus, 同定, インドネシアのラン

Explanation of plates

Plate I.

- 1, 2: Mosaic and necrotic spots or sunken necrosis in *Aranthera* caused by CyMV.
- 3, 4: Necrotic streaks in *Cattleya* caused by CyMV. 3: upper surface, 4: lower surface.
- 5: Chlorotic spots and mosaic in *Cattleya Browningiana* caused by CyMV.
- 6: Mosaic and chlorotic streaks in *Calanthe* caused by CyMV.
- 7: Mosaic in *Grommatophyllum* caused by CyMV.
- 8: Chlorotic spots and mosaic in *Phalaenopsis* infected by CyMV and ORSV.
- 9: Irregular purple blotches in *Vanda* caused by CyMV.
- 10: Mosaic in *Calanthe* infected by CyMV and ORSV.

Plate II.

- 1: Chlorotic spots and necrosis in *Oncidium* infected by CyMV and ORSV.
- 2: Chlorotic spots and mosaic in *Aranthera* by CyMV and flexuous virus particles.
- 3: Electron micrographs of CyMV and ORSV (arrow) from a leaf-dip preparation of *Calanthe*. Bar represents 100 nm.
- 4: Electron micrographs of flexuous particles (an unidentified Potyvirus) found in *Aranthera*. Bar represents 100 nm.
- 5: Pinwheel inclusions in mesophyll cells of *Aranthera*.

Plate I.

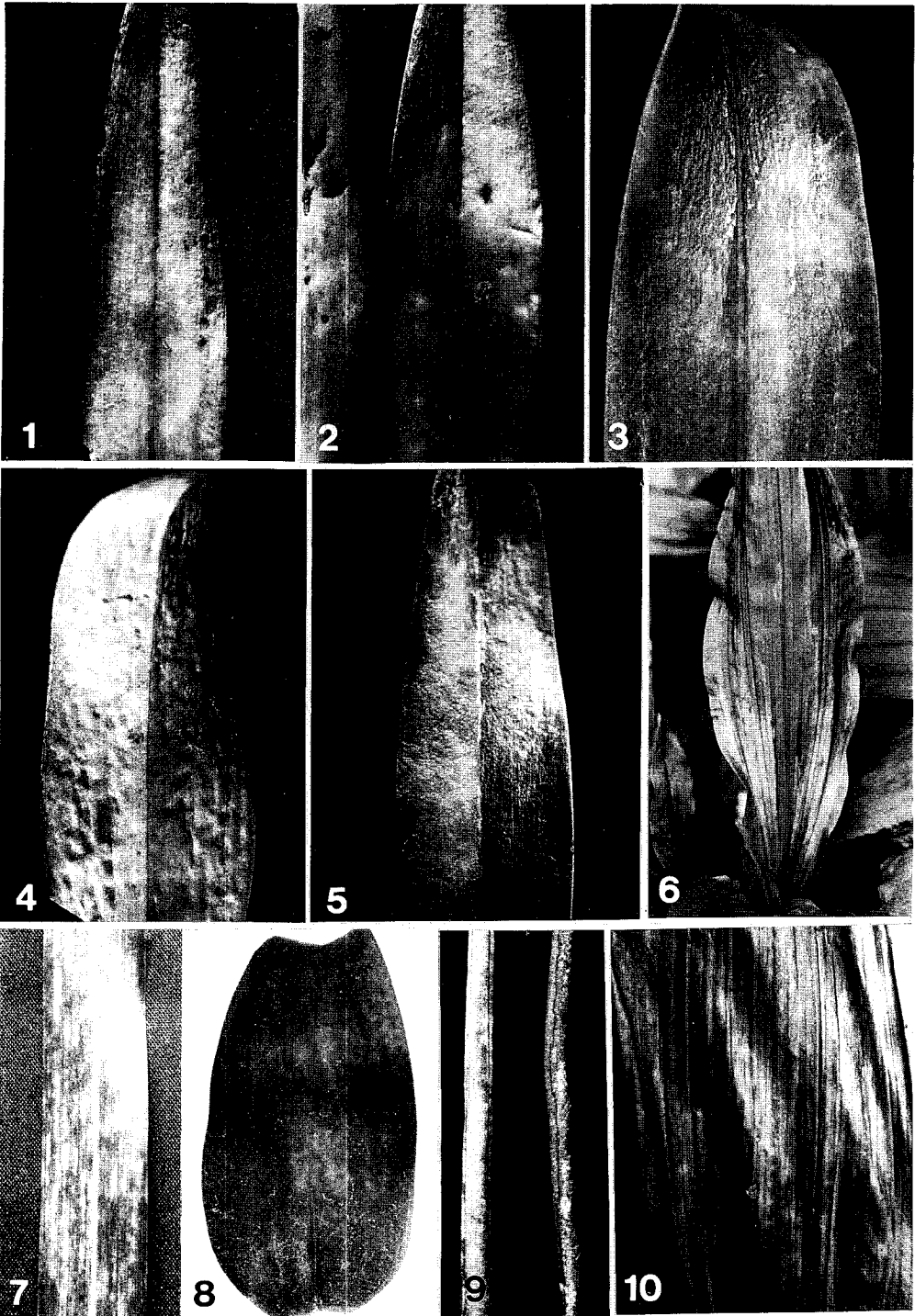


Plate II.

