Detection of Cymbidium Mosaic Potexvirus and Odontoglossum Ringspot Tobamovirus from Thai Orchids by Rapid Immunofilter Paper Assay

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ABSTRACT

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During 1992 to 1994, 442 orchid plants from 24 genera in 43 nurseries and locations in eight provinces of northern, central, and southern regions of Thailand were examined for Cymbidium mosaic potexvirus (CyMV) and Odontoglossum ringspot tobamovirus (ORSV) with rapid immunofilter paper assay (RIPA). Both viruses were confirmed in all three regions. However, CyMV was detected in 17 genera in 93% nurseries, while ORSV was detected in only four genera (*Arachnis*, *Cattleya*, *Oncidium*, and *Vanda*) in 40% of nurseries tested. These results indicate that CyMV is more prevalent than ORSV in Thailand. Mixed infections of ORSV and CyMV were often found, and double infections were observed in 95% of ORSV-infected plants. The virus infections were divided into two categories: infection by CyMV alone and double infection by both viruses in *Cattleya* and *Oncidium*. CyMV was often detected alone from symptomatic or asymptomatic plants of certain orchid genera. In *Oncidium*, however, CyMV was detected alone from 79% of plants without symptoms, while both viruses were detected from 90% of plants with mosaic and necrotic spot symptoms on leaves. Expression of symptoms may be promoted by the double infection of CyMV and ORSV in *Oncidium*.

Among Southeast Asian countries that form a large production area of tropical orchids, Thailand is the largest producer and exporter of cut-flower orchids and had an 800-million baht (US\$ 32 million) export industry in 1994 (2,3). Thai cut-flower orchids are exported all over the world. In particular, Japan imports 120 million orchid cut-flowers annually, about 90% of which come from Thailand. Orchid diseases in Thailand are also important for Japan, because orchid diseases prolong the handling time due to plant quarantine at airports in Japan, which delays the flowers from reaching markets.

Various diseases affect cultivated orchids in Thailand as well as in other many countries (10). Cymbidium mosaic potexvirus (CyMV) and Odontoglossum ringspot tobamovirus (ORSV), the most prevalent and economically important viruses in cultivated orchids (17), have also been recorded in Thailand (10). Little detailed information is available, however, on occurrence and distribution of these viruses in Thai orchids. There are reports of CyMV and ORSV infections in orchids in Malaysia (1,11), Singapore (15), and Indo-

nesia (7) in Southeast Asia.

Orchids infected with CyMV and/or ORSV exhibit various symptoms, and can be symptomless (17). Such asymptotically infected plants are visually indistinguishable from healthy ones. As CyMV and ORSV are easily transmitted by horticultural tools used for harvest of flowers and dividing the plant, these viruses are often spread rapidly and widely in nurseries (17). Diagnoses of virus diseases are commonly conducted by enzyme-linked immunosorbent assay and electron microscopy. However, these methods are not practical for rapid diagnosis by growers, breeders, and nurseries, since expensive and specialized equipment is required. The rapid immunofilter paper assay (RIPA) has recently been established by Tsuda et al. (13). The technique has been used to successfully detect many kinds of viruses including CyMV and ORSV from infected plants, with high sensitivity and without specialized equipment (12,13). In addition, RIPA can be used to simultaneously diagnose plants infected with multiple viruses (12).

In this work, we surveyed the occurrence and distribution of CyMV and ORSV in cultivated orchids in northern, central, and southern regions of Thailand in 1992 to 1994. RIPA was effectively used to detect these viruses from orchid plants in the survey.

MATERIALS AND METHODS

Sampling. During a 3-year survey (16 November to 4 December 1992, 8 to 28

August 1993, and 25 July to 13 August 1994), leaf and flower samples were collected from 442 plants from 24 genera and their hybrids of cultivated orchids at 43 nurseries and locations in eight provinces (Chiang Mai, Kamphaeng Phet, Nakhon Sawan, Pathum Thani, Bangkok, Nakhon Pathom, Chon Buri, and Songkhla) of northern, central, and southern regions in Thailand. Diseased leaf and flower samples with viruslike symptoms such as mosaic, necrosis, mottle, and color breaking were collected in the first 2-year survey. In addition, leaf samples of five orchid genera with and without symptoms were randomly collected at several nurseries in the last year of the survey.

Preparation of multi-RIPA system.

The RIPA recently established by Tsuda et al. (12,13) was used to enable simultaneous diagnosis of CyMV and ORSV infections without specialized equipment at nurseries. RIPA for simultaneous diagnosis has been named multi-RIPA (12). The two-step method of multi-RIPA (12) was adopted in the present work. Materials of the RIPA system were prepared in Japan as follows and transported to Thailand.

Antisera to CyMV and ORSV were provided by ATCC, and Japan Plant Protection Association, Komagome, Toshima-ku, Tokyo, Japan, respectively. Extraction buffer and dyed latex solutions coated with antibodies to CyMV and ORSV were prepared according to procedures described by Tsuda et al. (13). These two kinds of dyed latex solutions were mixed at final concentrations of 0.02% (vol/vol) for CyMV and 0.01% for ORSV, respectively, with Tris-buffered saline-bovine serum albumin (TBS-BSA; 0.05 M Tris-HCl, pH 7.2, containing 0.15 M NaCl and 0.1% [wt/vol] BSA). Two kinds of white latex, one coated with antibody to CyMV and another with antibody to ORSV, were gently applied with a fine, soft-pointed paint brush 1.5 and 1.2 cm, respectively, from the lower end of an 8 × 0.5 cm glass filter paper strip (Whatman GA/A) overlaid with a clear plastic sheet for reinforcement. Then, the strip was dried naturally for several minutes.

Extraction buffer (500 µl) and dyed latex solution (150 µl) were stored in 1.5- and 0.7-ml microfuge tubes, respectively, which were sealed with Parafilm for use in the field. Immobilized filter paper strips were placed into a small plastic box with

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desiccating agent until use in Thailand. When preliminary tests were carried out with saps of some orchid plants infected with CyMV and ORSV to determine the sensitivity of RIPA, the dilution-end-point of sap was $\times 10,000$ for both viruses.

Detection of viruses by multi-RIPA. A small piece of orchid leaf tissue (about 2×2 to 5×5 mm, 10 to 20 mg of fresh weight

per piece) obtained from each orchid sample was homogenized with 500 μ l of extraction buffer in a microfuge tube, using a plastic pestle. The filter paper strip was first wetted in the tissue extract by dipping its lower end for 1 min. The dipped end of the strip (1 to 2 mm) was cut off with scissors, and the remaining strip transferred to dyed latex solution mixture in a microfuge

tube and dipped at about a 3 to 5 mm depth for 2 min. When the red color band appeared on a zone previously treated with white latex, infection with a given virus was considered to be positive. In the present work, the upper and the lower bands represent CyMV and ORSV detections, respectively (Fig. 1). The RIPA was carried out immediately at nurseries visited or later during the survey.

Electron microscopy. In the first year's survey, 20 samples that gave positive and negative reactions by RIPA were randomly selected and examined for the presence of virus particles in their sap by negative staining with 2% phosphotungstic acid (pH 6.0), using a JEOL 100C transmission electron microscope.

RESULTS

Occurrence of CyMV and ORSV in Thai orchids. *Cattleya*, *Dendrobium*, *Oncidium*, and *Vanda* were surveyed extensively in many nurseries because these are the most common, important orchids in Thailand. A list of viruses and their host orchid genera confirmed in this survey is shown in Table 1. Virus diseases were seen in various genera in most of the nurseries surveyed. Extremely high incidences of virus diseases were often observed, especially in *Cattleya* and *Oncidium* in many nurseries. In some nurseries, at least 80 to 90% of plants of these genera were presumed to be infected with viruses on the basis of symptoms. When orchid plants selected randomly in the nursery were examined with RIPA, CyMV and/or ORSV were detected from almost all plants. Incidences of infected plants were generally high in nurseries retaining many older plants and in small nurseries producing orchids for domestic use. Although a few plants of a wild *Cymbidium* orchid were also examined for CyMV and ORSV infections with RIPA, in two locations in southern Thailand, neither virus was detected from these orchids.

Geographical distribution of CyMV and ORSV. Occurrence of CyMV and ORSV was confirmed in all of the northern, central, and southern regions of Thailand (Table 2). However, CyMV was detected in 37 (93%) of 40 nurseries surveyed, while ORSV was detected in 16 (40%) of them, showing that CyMV was more prevalent than ORSV in these regions.

Distribution of CyMV and ORSV in Thai orchids. CyMV was detected as single infections from 17 (71%) of 24 orchid genera while ORSV was detected as single infections from only three genera (13%), *Arachnis*, *Oncidium*, and *Vanda* (Table 1). Even in nurseries where ORSV was detected from these genera, the virus was not detected from any other genera. Although many *Dendrobium* plants growing in 25 nurseries were examined, ORSV was never detected from this genus. ORSV was often

Fig. 1. Detection of (A) Cymbidium mosaic virus (CyMV), and (B) CyMV and Odontoglossum ring-spot (ORSV) from *Oncidium* plants without and with virus symptoms (mosaic and necrotic spot), respectively, by rapid immunofilter paper assay (RIPA). Arrows C and O represent bands showing detection of CyMV and ORSV, respectively.

Table 1. Detection of Cymbidium mosaic virus (CyMV) and Odontoglossum ringspot virus (ORSV) from cultivated orchids in Thailand (1992 to 1994)^a

Genus	Infected plants (no.)				Total plants
	CyMV only	ORSV only	CyMV + ORSV	ND ^b	
<i>Arachnis</i>	7	1	1	0	9
<i>Arachnostylis</i>	7	0	0	1	8
<i>Aerides</i>	0	0	0	1	1
<i>Ascocenda</i>	15	0	0	0	15
<i>Calanthe</i>	1	0	0	0	1
<i>Cattleya</i>	45	0	43	11	99
<i>Christeara</i>	1	0	0	0	1
<i>Cymbidiella</i>	1	0	0	0	1
<i>Cymbidium</i>	2	0	0	1	3
<i>Dendrobium</i>	69	0	0	36	105
<i>Doritis</i>	0	0	0	3	3
<i>Gramanthophyllum</i>	0	0	0	1	1
<i>Kagawara</i>	0	0	0	1	1
<i>Lowana</i>	1	0	0	0	1
<i>Mokara</i>	16	0	0	13	29
<i>Oncidium</i>	34	1	51	11	97
<i>Paphiopedilum</i>	0	0	0	4	4
<i>Phalaenopsis</i>	1	0	0	3	4
<i>Renanthera</i>	1	0	0	0	1
<i>Rhynchostylis</i>	1	0	0	3	4
<i>Thunia</i>	0	0	0	1	1
<i>Vanda</i>	25	3	2	19	49
<i>Vandopsis</i>	0	0	0	1	1
<i>Vascostylis</i>	1	0	0	2	3
Total plants	228	5	97	112	442
Total genera (%)	17 (71)	3 (13)	4 (17)	17 (–)	24 (100)

^a Plants with viruslike symptoms were examined in the first 2-year survey (1992 to 1993), and plants with and without viruslike symptoms were separately surveyed in 1994.

^b Neither virus was detected.

detected together with CyMV from *Cattleya* and *Oncidium* plants. Double infections were confirmed in 97 of 102 plants (95%) from which ORSV was detected. On the other hand, single infections by ORSV were observed only in 5 plants (5%) of *Arachnis*, *Oncidium*, and *Vanda* from three nurseries (Table 1). In *Cattleya*, virus infection was clearly divided into two types: single infection by CyMV and double infection by CyMV and ORSV. A similar result was also obtained in *Oncidium*.

Detection of viruses from plants without symptoms. In the last year's survey (1994), orchid plants with and without symptoms were separately examined for virus infection in five orchid genera by multi-RIPA. The result is shown in Table 3 and Figure 1. CyMV was often detected not only from plants with symptoms but also from healthy-looking plants without symptoms in all of these genera. ORSV was detected only from symptomatic plants of these genera. Double infection by these viruses in *Oncidium* was observed in 27 of 30 plants (90%) with symptoms, while single infections by CyMV were detected in 19 of 24 plants (79%) without symptoms. Neither virus was detected from another three and five plants with and without symptoms, respectively. Both double and single infections were often observed in *Oncidium* growing in a particular nursery.

Electron microscopy. CyMV-like, flexuous, filamentous particles and/or ORSV-like, rod-shaped ones were detected from nine of 11 samples giving positive reactions by RIPA, while neither virus was detected from nine samples that gave a negative reaction by RIPA. Similar results were obtained by RIPA and electron microscopy for all samples excluding two samples with a weak positive reaction in RIPA.

DISCUSSION

CyMV and ORSV are distributed widely and cause economical losses in almost all orchid-growing areas in northern, central, and southern regions of Thailand (Tables 1 and 2). Occurrences of CyMV and ORSV were recorded in 17 genera from 37 nurseries and four genera from 16 nurseries, respectively, in this survey. The results confirm that CyMV is more common than ORSV in Thailand, as it is in other many countries (1,4,6–8,14–17). ORSV was detected in the orchids *Arachnis*, *Cattleya*, *Oncidium*, and *Vanda*. Occurrence of ORSV has been reported in 11 genera in Hawaii (4), more than nine genera in Florida (17), 17 genera in Japan (6), five genera in Taiwan (8), and three genera in Indonesia (7). Although ORSV has not been detected in many genera in Thailand, it is not known whether ORSV naturally infects these genera in this country. In *Dendrobium*, one of the most economically important orchids in Thailand, ORSV was not also detected despite examination of a large number of plants with viruslike

symptoms and testing in nurseries where the virus was detected in other orchids. Hu et al. (5) reported that attempts to infect University of Hawaii *Dendrobium* hybrids with CyMV by rub, cut, and slash inoculation methods had been successful, but attempts to infect these hybrids with ORSV by cut and slash inoculation methods were not successful. They also suggested that although the *Dendrobium* hybrids were not resistant to infection by ORSV, they were resistant to systemic movement of the virus in infected plants. Additional surveys should be conducted on natural infection of *Dendrobium* and other genera by ORSV in Thailand. However, natural infections of ORSV may be generally less common than those of CyMV, not only in *Dendrobium* but also in other orchid genera.

Cattleya and *Oncidium* are presumed to be especially important hosts of ORSV in Thailand, because high incidence of ORSV infection was often observed in these two genera collected from many nurseries. ORSV, however, was detected from co-infecting plants with CyMV with high frequency (95% of ORSV-infected plants). Although single infection by CyMV was often observed in these genera, single infection by ORSV was seen rarely in *Oncidium* and never in *Cattleya*. Similar results have been found in surveys of orchid genera in Hawaii (4), French Polynesia (14), Singapore (15), and Indonesia (7). These results suggest that ORSV infection follows that of CyMV in most incidences of double infection of orchid plants. We hypothesize that primary or systemic in-

fectivity of ORSV may be relatively weak but promoted by CyMV in some orchids. Contact transmission of cucumber mosaic virus is promoted by infection of potyviruses such as turnip mosaic and zucchini yellow mosaic viruses in cucumber (9). However, this hypothesis remains to be tested.

In *Oncidium*, CyMV was often detected as a single infection from asymptomatic plants while both CyMV and ORSV double infections were found at high frequency (90%) from plants with clear symptoms (Table 2). This suggests that symptoms on *Oncidium* plants become obvious after the double infection by these both viruses in Thailand. Mosaic and necrotic spot symptoms were observed on younger leaves and on under-surfaces of older leaves, respectively, in *Oncidium* plants infected with both viruses. Ting and Ho (11) described similar symptoms on *Oncidium* growing in Malaysia but did not mention a double infection. Inoue (7) reported mosaic and necrotic spot symptoms on leaves of *Oncidium* plants infected with both CyMV and ORSV in Indonesia but not the symptomless infection of the genus by CyMV. It is unknown whether these symptoms are induced by CyMV, ORSV, or by a mixed infection of the two viruses. Since *Oncidium* is one of the most important commercial orchids in Southeast Asia, additional work on the symptomatology of *Oncidium* and other orchids by CyMV and ORSV is needed.

Symptomless infections by CyMV were often observed in *Cattleya*, *Dendrobium*,

Table 2. Geographical distribution of Cymbidium mosaic virus (CyMV) and Odontoglossum ringspot virus (ORSV) in Thailand (1992 to 1994)

Region	Total nurseries surveyed	No. of nurseries where virus was confirmed (%)		
		CyMV	ORSV	ND ^a
Northern (1) ^b	10	9 (90) ^c	6 (60)	1 (10)
Central (6)	27	25 (93)	8 (30)	2 (7)
Southern (1)	3	3 (100)	2 (67)	0 (0)

^a Neither virus was confirmed.

^b Number of provinces surveyed.

^c Number of nurseries where virus was confirmed (percentage of nurseries where virus was found).

Table 3. Detection of Cymbidium mosaic virus (CyMV) and Odontoglossum ringspot virus (ORSV) from cultivated orchid plants with and without symptoms (1994)

Genus (no. of nurseries)	Symptom ^a	Total plants	Infected plants (no.)			
			CyMV only	ORSV only	CyMV + ORSV	ND ^b
<i>Cattleya</i> (6)	+	16	13	0	1	2
	–	5	3	0	0	2
	Random	35	20	0	15	0
<i>Dendrobium</i> (7)	+	16	13	0	0	3
	–	17	17	0	0	0
<i>Mokara</i> (2)	+	2	2	0	0	0
	–	10	2	0	0	8
<i>Oncidium</i> (7)	+	30	3	0	27	0
	–	24	19	0	0	5
<i>Vanda</i> (3)	+	9	6	0	0	3
	–	9	6	0	0	3

^a + = with viruslike symptoms; – = without symptoms; random = randomly selected plants.

^b Neither virus was detected.

Mokara, and *Vanda* in our survey. Infected plants without symptoms are also an important source of inoculum (17), and such infected plants are indistinguishable from healthy ones. The use of RIPA (12,13) enabled rapid, simultaneous diagnosis of virus diseases in the field without specialized equipment, and gave satisfactory results. The method was very easy and convenient. This technique can show results of diagnosis in a short time (about 5 min per sample) with high sensitivity, as shown by Tsuda et al. (13) and this work. The RIPA system for CyMV and ORSV was recently commercialized by Japan Plant Protection Association, Komagome, Toshima-ku, Tokyo, Japan.

This work indicated that CyMV and ORSV are important problems in orchid production in Thailand. Growers should prevent healthy orchids from being infected by these viruses in nurseries. RIPA is suitable for field diagnosis of infected orchids by growers, and could help to add to the knowledge of orchid virus diseases by growers.

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