

Incidence of Cymbidium Mosaic Virus (CYMV), Odontoglossum Ring Spot Virus (ORSV), Tomato Spotted Wilt Virus (TSWV), Potex Virus and Poty Virus on Dendrobium

S. Sundaramoorthy, D. R. Sudha, J. Johnny Subakar Ivin,
 P. Sivasakthivelan

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ABSTRACT

Orchid plants collected from different nurseries were tested for Cymbidium Mosaic Virus (CYMV), Odontoglossum ring spot virus (ORSV), Tomato Spotted Wilt Virus (TSWV), Potex virus and Poty virus with Enzyme Linked Immunosorbent Assay (ELISA). Positive samples were tested for presence of Cymbidium Mosaic Virus and ORSV, Tomato Spotted Wilt Virus (TSWV), Potex Virus and Poty virus through transmission electron microscopy (TEM). In this comparative study, compared to other virus

Cymbidium Mosaic Virus (CYMV) infected more, in Dendrobium orchids than Odontoglossum ring spot virus (ORSV), Tomato Spotted Wilt Virus (TSWV), Potex virus, Poty virus.

Keywords Cymbidium Mosaic Virus (CYMV), Odontoglossum ring spot virus (ORSV), Tomato Spotted Wilt Virus (TSWV), Potex virus and Poty virus, Orchid, *Dendrobium* spp.

INTRODUCTION

The Orchidaceae is conceivably the largest and most varied plant family, and it includes the significant cultivated orchid species *Arachnis*, *Ascocentrum*, *Cattleya*, *Cymbidium*, *Dendrobium*, *Laelia*, *Oncidium*, *Paphiopedilum*, *Phalaenopsis*, *Renanthera*, and *Vanda* as well as their hybrids (De 2020). The two principal genera of orchids grown commercially in Taiwan and exported mostly to Japan, the United States, and Europe are *Dendrobium* and *Vanda* (Yuan *et al.* 2021).

Orchids form a large part of the floral trade in ornamental plants and cut flowers and are the largest family of flowering plants with more than 35,000 species (Lee and chang 2008). Orchids grow naturally in a wide range of habitats in many parts of the world. Grown commercially in many countries, orchids have perhaps the highest unit value of any commercial pot

S. Sundaramoorthy^{1*}, D. R. Sudha², J. Johnny Subakar Ivin³,
 P. Sivasakthivelan⁴

¹Assistant Professor
 Department of Plant Pathology, Faculty of Agriculture,
 Annamalai University, Annamalai Nagar 608002, Tamil Nadu,
 India

^{2,4}Assistant Professor
 Department of Microbiology, Faculty of Agriculture,
 Annamalai University, Annamalai Nagar 608002, Tamil Nadu,
 India

³Assistant Professor
 Mother Teresa College of Agriculture, Mettusalai, Illupur,
 Pudukkottai 622102, Tamil Nadu, India
 Faculty of Agriculture, Annamalai University, Annamalai Nagar
 608002, Tamil Nadu, India.

Email : sundardivi@gmail.com

*Corresponding author

plant (Sudha and Rani 2020) *Dendrobium* orchid hybrids, one of the most economically important cut and potted floricultural crops grown in Hawaii, commanded a wholesale value of US\$ 6.3 million in 1990 (Sen 2006).

Orchids are affected by more virus disease problems than most crops, reducing their commercial values considerably. Liu *et al.* (2013) Orchid viruses are widespread in cultivated orchids (Moraes *et al.* 2017), with Cymbidium Mosaic Potex Virus (CYMV) and Odontoglossum Ring Spot tobamo virus (ORSV) being the most prevalent (Hossain *et al.* 2009). Neither virus is known to be transmitted via seed or insect vectors (Pagan 2022). Their high incidence in cultivated orchids has been attributed to the stability and ease of transmission of these two viruses through cultural practices (Sherpa *et al.* 2012).

Orchids have been reported to be infected with more than 50 viruses (Bratsch *et al.* 2021, Lee *et al.* 2021, Fogell *et al.* 2019). Of several Orchid-infecting viruses, Cymbidium mosaic virus (CYMV) and Odontoglossum ring spot tobamovirus (ORSV) have been reported to be two of the most prevalent and important viruses which have attained worldwide distribution (Ajjikuttira and Wong 2009, Chen *et al.* 2019, Umikalsum *et al.* 2006). Mixed infection with both the viruses can cause blossom brown necrotic streak (Sherpa *et al.* 2007).

Since Victorian times, when orchids were grown in greenhouse environments, viral illnesses affecting them have been known, but it wasn't until 1950 that these viruses were accurately identified. Odontoglossum ring spot virus (ORSV) and Cymbidium mosaic

virus (CYMV) were first described by Jensen (1951), Jensen and Gold (1951) in the same year. Contemporary there have been identified numerous viruses infecting orchids, nevertheless CYMV and ORSV are commonly diagnosed in most genera belonging to the family Orchidaceae brought under cultivation (Ajjikuttira and Wong 2009). As a result, Cymbidium mosaic and Odontoglossum ring spot viruses are considered to be a significant problem for horticulture production (Milosevic *et al.* 2015). The negative impact of these viruses on cultivated orchids, especially the more common CYMV, has been reported in the orchid growing areas of the world. Orchid cultivars infected with CYMV often show smaller flower size, poor quality, necrosis and flower disfigurement as well as foliage symptoms, resulting in great economic losses (Sherpa *et al.* 2007).

In this comparative study, compared the infection of Cymbidium Mosaic Virus (CYMV), Odontoglossum ring spot virus (ORSV), Tomato Spotted Wilt Virus (TSWV), Potex virus, Poty virus on *Dendrobium* Orchid. Cymbidium Mosaic Virus (CYMV) infection was more when compared to Odontoglossum ring spot virus (ORSV), Tomato Spotted Wilt Virus (TSWV), Potex virus, Poty virus.

MATERIALS AND METHODS

Orchid samples were collected from orchid collections imported from South India, India. Diseased leaf and flower samples with virus like symptoms such as mosaic, necrosis, mottle, and color breaking were collected (Fig.1). When compared to other orchids *Dendrobium* infection was more with other orchid. So, *Dendrobium* plants were taken for further virus

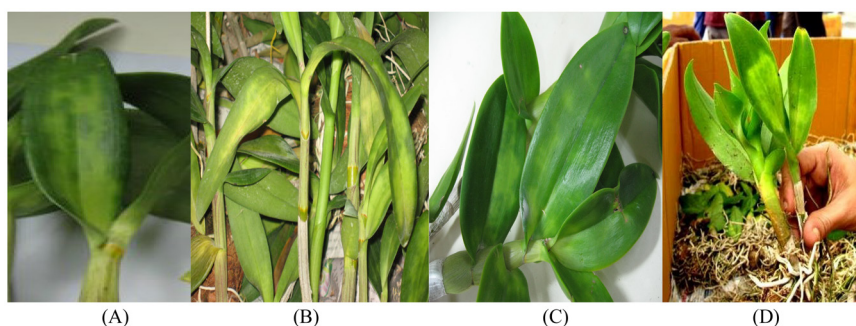


Fig. 1. Showing the Mosaic symptoms of virus.

Table 1. Antisera used for Direct Antigen coated -ELISA for detection of virus.

Sl. No.	Name of the Antisera	ATCC number
1	Cymbidium Mosaic Virus	ATCC-PVAS-355
2	Odontoglossum Ring Spot Virus	ATCC-PVAS-497
3	Poty virus	ATCC-PVAS-50A
4	Tomato Spotted Ring Spot Virus	ATCC-PVAS-731

analysis. In virus infection analysis of Cymbidium Mosaic Virus (CYMV), Odontoglossum ring spot virus (ORSV), Tomato Spotted Wilt Virus (TSWV), Potex virus, Poty virus on Dendrobium Orchid. Cymbidium Mosaic Virus (CYMV) infection was more when compared to Odontoglossum ring spot virus (ORSV), Tomato Spotted Wilt Virus (TSWV), Potex virus, Poty virus.

Dendrobium plants exhibiting virus like symptoms such as chlorotic or necrotic steaks on leaves were collected and were tested by direct antigen-coated enzyme-linked immunosorbent assay (DAC-ELISA) using virus-specific antibodies purchased from ATCC (USA) (Table 1).

Antisera to Cymbidium Mosaic Virus (CYMV), Odontoglossum Ring Spot Virus (ORSV), Tomato Spotted Wilt Virus (TSWV), Potex virus and Poty virus were purchased from ATCC (Table 1).

The standard procedure of direct antigen-coated enzyme-linked immunosorbent assay was used for the detection of viruses. One hundred mg of Dendrobium plant tissue was ground in 1 ml of sample extraction buffer (Phosphate Buffer Saline- Na₂HPO₄ 12H₂O-90g, KH₂PO₄-0.20g, KCl-0.20g, 2% polyvinyl pyrrolidone (MW 40,000), pH 7.4). Two hundred micro liter of the extracts were coated to the 96-well ELISA plate (Tarson) and incubated at 37°C for 1 h. Each sample had triplicated wells. After two to three washings with phosphate-buffered saline + Tween-20 (PBST; 137 mM NaCl, 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, 2.7 mM KCl, 0.05% Tween-20, pH 7.4), 200 µl of CYMV or ORSV antibodies diluted in antibody buffer (0.2% bovine serum albumin, 2% polyvinylpyrrolidone in PBST buffer, pH 7.4) was added to the ELISA plates and incubated at 37°C for 1 h. The antibodies purchased from ATCC

Table 2. Detection of CYMV infection from Orchid genera collected from South India, India.

Sl. No.	Name of the genera	No. of plants collected	No. of plants infected	Percentage of CYMV infection (percentage)
1	Cattleya	20	6	30
2	Cymbidium	20	5	25
3	Dendrobium	20	9	45
4	Epidendrum	20	7	35
5	Mokara	20	6	30
6	Oncidium	20	6	30
7	Paphiopedilum	20	5	25
8	Phalaenopsis	20	3	15
9	Spathoglottis	20	4	20
10	Vanda	20	8	40

were diluted 100-fold and antisera produced in this study were diluted 10,000- fold. After two to three washings with PBST buffer, 100 µl of 30,000-fold diluted Alkaline Phosphatase (AP)-conjugated goat anti-rabbit secondary antibody (Sigma, San Diego, CA, USA) was added and incubated at 37°C for 1 h. Finally, 200 µl of p-Nitrophenyl phosphate (PNP) solution (1 mg ml⁻¹), dissolved in PNP buffer (9.7% Diethanola mine, 0.5 mM MgCl₂, pH 9.8), was added after two to three washings with PBST buffer. After each sample had been incubated for 60 minutes, the values of OD 405 were determined using a Biorad (Molecular Devices Co., Berkeley, CA, USA). When the absorbance value of a sample exceeded twice that of the healthy controls, it was deemed positive. The results or OD Value of Cymbidium Mosaic Virus (CYMV), Odontoglossum ring spot virus (ORSV), Tomato Spotted Wilt Virus (TSWV), Potex virus and Poty virus are given in (Table 2).

ELISA results were confirmed by leaf-dip electron microscopy (Ciuffo *et al.* 2009). Using a typical technique, leaves with chlorotic rings and mosaic mottling were examined with a transmission electron microscope (Vinodhini *et al.* 2021). With extraction buffer (phosphate buffer, pH 6.4), leaves were homogenized. The plant extracts were adsorbed on copper coated grid and were dried for 5-10 mins and washed with sterile distilled water. They were negatively stained with Uranyl acetate 2% and absorbed under electron microscope.

Table 3. Detection of ORSV* infection from Orchid genera collected from South India, India.

Sl. No.	Name of the genera	No. of plants collected	No. of plants infected	Percentage of ORSV* infection (percentage)
1	Cattleya	20	5	25
2	Cymbidium	20	4	20
3	Dendrobium	20	6	30
4	Epidendrum	20	6	30
5	Mokara	20	5	25
6	Oncidium	20	5	25
7	Paphiopedilum	20	4	20
8	Phalaenopsis	20	3	15
9	Spathoglottis	20	3	15
10	Vanda	20	6	30

A 10- μ l aliquot from each of the *Dendrobium* fractions was loaded onto a Formvar- and carbon-coated copper grid. After 1-min incubation, excess liquid was removed with filter paper and 10 μ l of 2% uranyl acetate was loaded onto the grid. Excess stain was removed with filter paper after 1-min incubation and the grid was air-dried. All specimens were examined in a JEOLCM-10 TEM (Japan). TEM analyses were performed to confirm the identities of the observed in the electropherograms as well as the presence of virus particles.

RESULTS AND DISCUSSION

This study provides evidence of Cymbidium Mosaic Virus (CYMV), Odontoglossum Ring Spot Virus

Table 4. Detection of Poty virus infection from Orchid genera collected from South India, India.

Sl. No.	Name of the genera	No. of plants collected	No. of plants infected	Percentage of Poty virus infection (percentage)
1	Cattleya	20	2	10
2	Cymbidium	20	2	10
3	Dendrobium	20	4	20
4	Epidendrum	20	3	10
5	Mokara	20	3	15
6	Oncidium	20	3	15
7	Paphiopedilum	20	2	10
8	Phalaenopsis	20	2	10
9	Spathoglottis	20	3	15
10	Vanda	20	2	10

**Fig. 2.** Samples loaded in ELISA plate.

(ORSV), Tomato Spotted Wilt Virus (TSWV), Potex virus and Poty virus in orchids. Thus, the presence of Cymbidium Mosaic Virus (CYMV), Odontoglossum Ring Spot Virus (ORSV), Tomato Spotted Wilt Virus (TSWV), Potex virus and Poty virus infecting the orchids might occur accidentally through vegetative materials from abroad. Member of Potex virus has stable viral particles, easily transmitted mechanically and some of them are seed transmitted, including through vegetative materials (Davino *et al.* 2020). According to samples' results from serological tests, CYMV could infect orchids either alone or in conjunction with ORSV (Lakani *et al.* 2010). When a single infection with CYMV occurs, it may result in mosaic and necrotic areas on the leaf, and when a dual infection with CYMV and ORSV occurs, it may result in upward-rolling and stunted leaves (Madhubala *et al.* 2005).

One of the detection methods frequently used in the certification program is ELISA. (Fig. 2). The detection specificity and sensitivity of ELISA depend on the property of the antibodies (Lee and Chang 2008). The commercial antibodies prepared by purified virions of CYMV or ORSV have been used for surveys of virus infection (Lee and Chang 2008) and ORSV surveys in *Dendrobium* samples imported from Thailand (Rishi 2010, Pal *et al.* 2022). ELISA is a more rapid method for detecting Cymbidium Mosaic Virus (CYMV), Odontoglossum Ring Spot Virus (ORSV), Tomato Spotted Wilt Virus (TSWV), Potex virus and Poty virus than mechanical inoculation bioassay, and it may replace bioassay in regular

Table 5. Detection of TSWV infection from Orchid genera collected from South India, India.

Sl. No.	Name of the genera	No. of plants collected	No. of plants infected	Percentage of TSWV infection (percentage)
1	Cattleya	20	2	10
2	Cymbidium	20	2	10
3	Dendrobium	20	3	15
4	Epidendrum	20	2	10
5	Mokara	20	2	10
6	Oncidium	20	2	10
7	Paphiopedilum	20	1	5
8	Phalaenopsis	20	1	5
9	Spathoglottis	20	2	10
10	Vanda	20	3	15

indexing program (Tables 3–5).

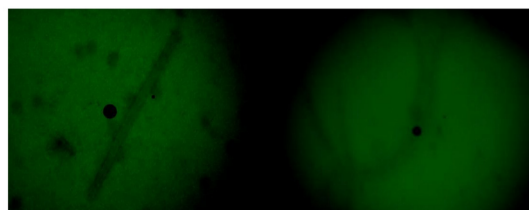
In the orchid industry, CYMV and ORSV are two covalent diseases that are both commercially significant and widely dispersed (Narayanasamy 2010). The world is affected by both CYMV and ORSV, with CYMV being more common. Approximately 45% of cloned orchids have CYMV infection. Before vegetatively propagating plants, it is required to index orchid materials due to the level of incidence (Nongsang and Das 2022). Orchids from other countries should be tested with rapid and sensitive assays before their introduction into the India. Dendrobium plants

Table 6. Direct antigen coated-ELISA (DAC-ELISA) for detection of viruses in Dendrobium orchid plants ^a.

Viruses	OD at 405nm in DAC-ELISA with antibodies against viruses ^b				
	CYMV	ORSV	Poty viruses	Potex viruses	TSWV
Dendrobium 1	0.324	0.227	0.190	0.018	0.034
Dendrobium 2	0.370	0.224	0.222	0.021	0.036
Dendrobium 3	0.354	0.233	0.224	0.016	0.038
Dendrobium 4	0.364	0.229	0.213	0.022	0.035
Dendrobium 5	0.348	0.231	0.192	0.018	0.034
Average	0.352	0.228	0.208	0.019	0.035

a) DAC-ELISA was used for detection of Cymbidium mosaic virus (CYMV), Odontoglossum ring spot virus (ORSV), Poty viruses, Potex viruses and Tomato spotted wilt viruses (TSWV) with specific polyclonal antibodies. Coating antibodies (1µg/ml) and conjugated antibodies (1:3,000) were used for all five antibodies (Antisra supplied by ATCC).

b) The absorbance readings were means for several tests, in each ELISA, homologous and heterologous controls were used.

**Fig. 3.** Single flexuous filamentous particles observed under Transmission Electron Microscope of C Virus.

were not infected by Potex Poty TSWV probably less infection for Dendrobium orchids (Huang *et al.* 2019). The results or OD value of Cymbidium Mosaic Virus (CYMV), Odontoglossum Ring Spot Virus (ORSV), Tomato Spotted Wilt Virus (TSWV), Potex virus and Poty virus revealed that compared to other virus Cymbidium Mosaic Virus (CYMV), infected more than Odontoglossum Ring Spot Virus (ORSV), Tomato Spotted Wilt Virus (TSWV), Potex Virus and Poty virus on Dendrobium plants (Fig. 3, Table 6).

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