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Detection of Cymbidium Mosaic Virus (CYMV) on Vanda Plants

D. R. Sudha[1], G. Usha Rani[2]

[1,2]Department of Microbiology, Annamalai University, Chidambaram, Tamil Nadu

Abstract: ***Orchid plants are the members of Orchidaceae consisting of more than 25,000 species, which are distributed almost all over the world but more abundantly in the tropics. There are 177 genera, 1,125 species of orchids that originated in Thailand. Orchid plant collected from different nurseries showing Chlorotic and mosaic symptoms were observed on Vanda plants and it was suspected to infect with virus. So the symptomatic plants were tested for Cymbidium Mosaic Virus (CYMV), Odontoglossum ring spot virus (ORSV), Poty virus and Tomato Spotted Wilt Virus (TSWV) with Direct Antigen Coating- Enzyme Linked Immunosorbent Assay (DAC-ELISA) and further confirmed by Transmission Electron Microscopy (TEM). With the two methods CYMV and ORSV were detected positively from the suspected imported samples and low positive results were observed for Potex, Poty virus and Tomato Spotted Wilt Virus (TSWV).***

Keywords: Cymbidium Mosaic Virus, Odontoglossum ring spot virus , Poty virus and Tomato Spotted Wilt Virus.

1. Introduction

Orchids, the most beautiful flowers in god's creation, comprise a unique group of plants. Taxonomically, they represent the most highly evolved family among monocotyledons with 600-800 genera and 25,000-35,000 species all over the world (http://www.orchidsasia.com.). Among all viruses Cymbidium Mosaic Virus (CYMV) and Odontoglosum ringspot virus (ORSV) are the most prevalent and economically important viruses infecting Orchids worldwide [Okemura,1984, Lawson *et al.*,1986].CYMV induces floral [Cho et al.,1989] and foliar [Sudarshana *et al.*, 1989] necrosis, and ORSV causes ring spots on leaves and color breaking on flowers [Gibbs *et al.*, 1966].Mixed infection of both viruses can cause blossom brown necrotic streak [Shiv Sagar Verma *et al.*, 2010].The viruses also reduce plant vigour and lower flower quality, which affects economic value [Kaper *et al.*, 1981].These viruses are very stable and are spread by contaminated tools and pots [8] These viruses reduce the growth of infected orchid plants as well as the quality of flowers, which effect to orchid industry. The most efficient way for the spread of CYMV and ORSV in the orchid industry is by mass propagation of orchid plantlets from an infected mother stock through mericloning tissue culture process. Orchid viruses such as Cymbidium mosaic, Odontoglosum ring spot, Vanilla necrosis and Orchid fleck and also several Poty viruses such as Bean yellow Mosaic Virus, Turnip Mosaic Virus and Dendrobium mosaic Virus infect orchids, Tomato spotted wilt virus is the most devastating and wide spread plant virus in orchids. It has wide host range, infecting 192 dicotyledonous species in 33 families and eight monocotyledons in five families [9].Therefore Poty viruses and Tomato spotted wilt virus are included in this study.

Finally the study was undertaken for the presence of Cymbidium Mosaic Virus (CYMV), Odontoglosum ringspot virus (ORSV), Poty viruses and Tomato spotted wilt virus from Orchids which are collected from different nurseries.

2. Materials and Methods

ANTISERA

Antisera to CYMV (ATCC-PVAS-355), ORSV (ATCC-PVAS-497), TSWV (ATCC-PVAS-731), and Poty viruses (ATCC-PVAS-50A) were purchased from American Type Cell Culture (ATCC).

Sampling and Testing

Totally 675 numbers of Vanda plants were collected for chlorotic and mosaic symptoms in this four varieties were collected. And symptomatic *Vanda* samples were screened against Cymbidium Mosaic Virus (CYMV), Odontoglossum ring spot virus (ORSV), Poty virus and Tomato Spotted Wilt Virus (TSWV) with Direct Antigen Coating- Enzyme Linked Immunosorbent Assay (DAC-ELISA) technique (Sudarshana *et al.*,1989) and further confirmed by Transmission Electron Microscopy (TEM).

ELISA

The standard One day procedure DAC – ELISA (Direct Antigen Coated- Enzyme Linked ImmunoSorbant Assay) used for detection of Cymbidium Mosaic Virus (CYMV), Odontoglossum ring spot virus (ORSV), Poty virus and Tomato Spotted Wilt Virus (TSWV). An Antibody specific to Cymbidium Mosaic Virus (CYMV), Odontoglossum ring spot virus (ORSV), Poty virus and Tomato Spotted Wilt Virus (TSWV) was obtained from ATCC were used. Briefly 500 µg of leaf tissue was ground in 3 ml of 0.5M carbonate-coating buffer, pH 9.6 and centrifuge the sample. A 200 µl of each sample (without filtration) were loaded into ELISA wells (Tarsons) The coating plates were incubated in a moist chamber at 37°C for 1 hour and then, these plates were decanted and washed with phosphate buffered saline, containing 0.05% (v/v) Tween 20 (PBST). Antibodies against Cymbidium Mosaic Virus (CYMV), Odontoglossum ring spot virus (ORSV), Poty virus and Tomato Spotted Wilt Virus (TSWV) and antisera were added to the wells were diluted to 1:78000,1:6000,1:256000,1:1000 respectively in the Antibody buffer solution. A 200 µl of the diluted

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antiserum was added to each well and incubated at 37°C for 1 h after repeat the washings. Goat-antirabbit gamma immunoglobulin alkaline phosphatase conjugate (Sigma, Sigma Chemical, St. Louis, USA) was diluted to 1:30000 in PBST containing 2% ovalbumin and added to each well, incubated at 37°C for 1 hour and then, repeatedly washed as above. A 200 µl aliquot of freshly prepared substrate (10 mg p-nitrophenyl phosphate; Sigma # N 6260, Sigma Chemical, St.Louis, USA) was dissolved in 10 ml of substrate buffer (9.7% diethanolamine, 0.02% NaN3, pH 9.6) and added to each well. They were incubated at room temperature for 1 hour in dark for colour development. After that 50 µl of 3 M NaOH was added to all the wells to stop further enzymatic reactions. Absorbance value of each well was measured at 405 nm with an ELISA microplate reader (Biorad). The color reactions produced by tested samples were compared with known negative control wells.

Electron Microscopy

ELISA results were further confirmed by electron microscopy. The sample was tested in Transmission Electron Microscope by Leaf dip method (*Gibbs et al.,* 1966). A small piece of symptomatic tissue is placed on a glass slide and a few drops of sample buffer are added. Using a glass rod, the leaf material is macerated in the buffer and the extruding plant sap is used for adsorption preparates. Carbon coated grids, used in electron microscopy, are incubated for 5 min with a drop of homogenates prepared from virus infected plant samples and subsequently washed with a gentle stream of approximately 10-15 drops of Sterile Double distilled water to remove buffer salts. For negative staining – contrasting 3-5 drops of a 1% Uranyl acetate solution in Sterile Double distilled water are applied after which the grids are dried by gently tapping on a piece of Whatman filter paper and examined in the electron microscope (Fig.3.)

By referring the Guide for Identification of Plant Quarantine Pathogens (Shiv Sagar Verma, *et al.,* 2010) it was suspected to be infected with Cymbidium Mosaic Virus (CYMV) and Odontoglosum Ring Spot Virus (ORSV) (Fig 1). Hence, symptomatic *Vanda* leaf samples (4 Hybrids) were screened against Cymbidium Mosaic Virus (CYMV), Odontoglossum ring spot virus (ORSV), Poty virus and Tomato Spotted Wilt Virus (TSWV) with Direct Antigen Coating- Enzyme Linked Immunosorbent Assay (DAC-ELISA) technique (*Sudarshana et al.,* 1989) and further confirmed by Transmission Electron Microscopy (TEM).

Figure 1: Infected Vanda Orchids for Cymbidium Mosaic Virus and Odontoglossum ring spot virus

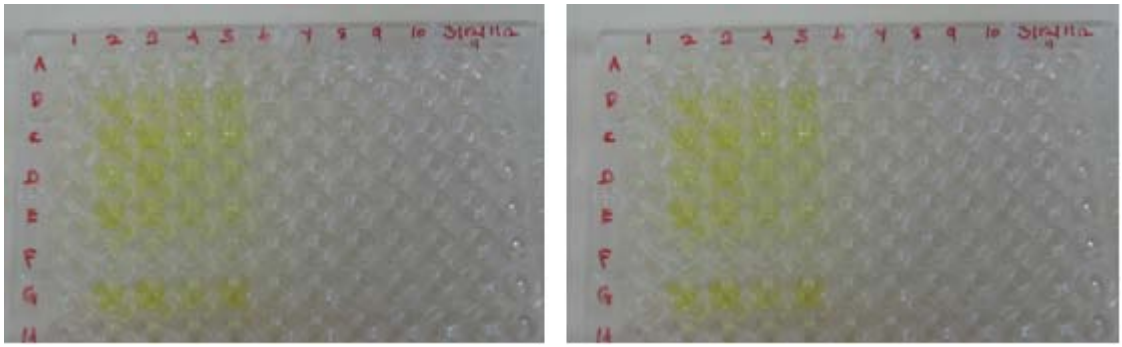

Figure 2: DAC-ELISA Microplate showing positive reaction in 4 samples (yellow colour) & known positive control

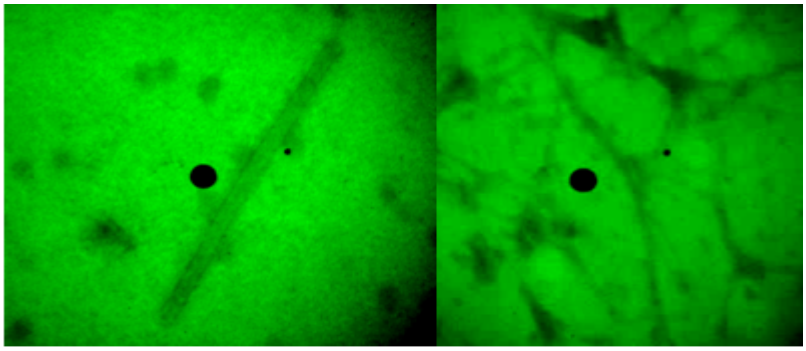

Figure 3: Transmission Electron microscope picture for CYMV and ORSV respectively

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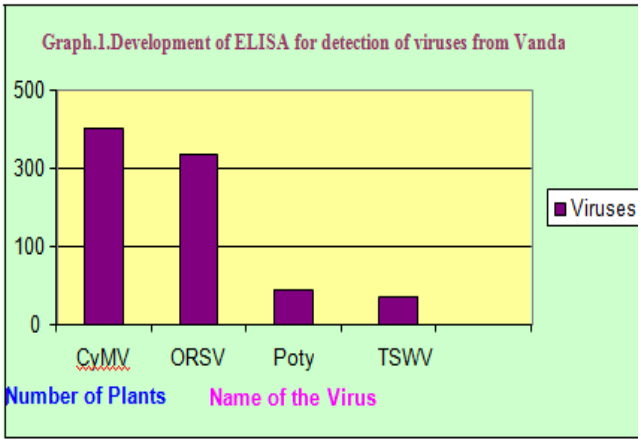
3. Results and Discussion

Detection by DAC ELISA

The present study reveals that CYMV is prevalent virus in orchid. It has spreaded widely in many cultivated orchid genera in Thailand. A total of 675 Vanda Thai native orchids were assayed for CYMV and ORSV, Poty viruses, and TSWV using Direct Antigen Coating ELISA. But Vanda was reacted positively with CYMV and ORSV while Poty viruses, and TSWV were shown only slight reaction. The incidence of CYMV infection was in ranged between 50 % and 100 % and ORSV was in ranged between 30% to 40 %. Leaves of infected CYMV *in vitro* plantlets are not smooth, dark green areas raised somewhat above the light green tissue as longitudinal ridges and bumps (Fig.2.). But ORSV has not shown any symptom on host. CYMV-infected plantlets also showed mosaic on leaves. CYMV and ORSV were found in all of 675 in vitro Thai native orchid seedlings. The details of number of plants infected with CYMV and ORSV while Poty viruses, and TSWV detected by ELISA micro plate reader (Biorad, U.S. 550). (Table.1. Graph1.) given below.



Detection by Transmission Electron Microscope:

After getting positive results from DAC –ELISA, The Positive samples were again confirmed with our Transmission Electron Microscope from Regional Plant Quarantine Station, Chennai. We are confirmed with rods and filamentous rods (Table.2.) On confirmation of CYMV and ORSV by Transmission Electron Microscope after DAC –ELISA on Vanda plants which are imported from Thailand were undertaken segregation of symptoms showing plants. Totally 675 Vanda plants were incinerated.

4. Discussion

Viruses are constantly infecting plants. Once infected a plant can never be cured except by tissue culture where the material of the plant can be rescued but the drawback is that it is costly in terms of time and money (Kaper and Water worth, 1981; Arditti, 1992; Sutic *et al.*, 1999). CYMV and ORSV are widespread in world, with CYMV being prevalent. About 45% of cloned orchids were infected by CYMV. Because of the level of incidence, it is necessary to index orchid materials before vegetatively propagating plants. Orchids from other countries should be tested with rapid and sensitive assays before their introduction into the India. ELISA is a more rapid method for detecting CYMV and ORSV than mechanical inoculation bioassay, and it may replace bioassay in regular indexing programmes.

There are four main virus detection methods which have been developed, i.e. serological, electron microscopy, hybridization, and polymerase chain reaction (PCR) (Gibbs and Harrisson, 1976; Seoh *et al.*, 1998). Serological techniques (Gibbs and Harrisson, 1976) are based on the utilization of the nature of antibodies, which bind specifically to their antigens; Electron microscopy (Matthews, 1991) is based on the electron beam passing from an electrode through the specimen to the anode where a image of the specimen will be produced when the electrons are blocked by the specimen, viruses can then be identified by their distinct morphological characteristics and size;

The present study reveals that CYMV is prevalent virus in orchid. It has spreaded widely in many cultivated orchid genera in Thailand. Plants must test for viral contamination before cloning to prevent the viral spreading. After tissue proliferation and plant differentiation, another test for viral infection has to be conducted before releasing the material from flask to further multiply or to transfer to community pots in greenhouses. It is essential to produce disease-free plantlets for export, especially to countries that impose strict plant quarantine conditions. Poty viruses, TSWV are not prevalent virus in cultivated orchids in this test but screening regimes should be included to determine its existence.

5. Conclusion

CYMV and ORSV were found in Thai native orchids seedlings. CYMV was detected more than 50% of imported orchid's samples. CYMV is the most prevalent virus in cultivated orchids in Thailand. This study suggests that the plant material must be examined for the existence of the virus before using them for mass production by tissue culture techniques. Use of seed-propagated cultivars provides a most suitable mechanism to establish virus-free plantings of orchids and high quality germplasm. It is believed that the results from this study are essential for tissue culture laboratories to change their practices for producing high-quality virus free plants in the very near future. The DAC ELISA will be a powerful tool for diagnosing virus in cultivated orchids by large-scale indexing program.

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Table 1: Incidence of CYMV, ORSV, Poty Virus and TSWV in 675 species Thai native orchids using direct antigen coating enzyme-linked immunosorbent assay (DAC-ELISA)

S. No	Name of the Vanda Hybrids	No. of plants	DAC-ELISA results			
			No. of plants infected for CYMV	No. of plants infected for ORSV	No. of plants infected for Poty Virus	No. of plants infected for TSWV
1	Hybrid 1	360	300	300	60	26
2	Hybrid 2 (Vanda Pachara Delight)	100	90	80	20	5
3	Hybrid 3 (Ascocenda Fuchs harvest moon x Ascocenda pralor)	100	85	70	20	7
4	Hybrid 4 (Ascocenda Fuchs harvest moon x Vanda Chao Praya Sapphire x Vanda Bitz's Heartthrob)	175	160	105	20	9
	Total	**675**	635	555	120	47

Table.2. Incidence of CYMV, ORSV, Poty Virus and TSWV in 675 species Thai native orchids using TEM

S. No	*Name of the Vanda Hybrids*	*No. of plants*	*TEM results*			
			No. of plants infected for CYMV	*No. of plants infected for ORSV*	*No. of plants infected for Poty Virus*	*No. of plants infected for TSWV*
1	Hybrid 1	360	300	300	60	26
2	Hybrid 2 (Vanda Pachara Delight)	100	90	80	20	5
3	Hybrid 3 (Ascocenda Fuchs harvest moon x Ascocenda pralor)	100	85	70	20	7
4	Hybrid 4(Ascocenda Fuchs harvest moon x Vanda Chao Praya Sapphire xVanda Bitz's Heartthrob)	175	160	175	20	9
	Total	**675**	635	555	120	47

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