IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
e ISSN-0976-593X Volume **11** | Issue 2 | July, 2016 | 302-306

DOI: 10.15740/HAS/IJPS/11.2/302-306
Visit us - *www.researchjournal.co.in*

RESEARCH ARTICLE

Detection, diagnosis of orchid virus and inactivation of cymbidium mosaic virus (CYMV) on plants

■ **D.R. SUDHA AND G. USHA RANI**

SUMMARY

Floriculture is one of the disciplines of Horticulture which is dealing with growing of ornamental plants flowering plants and garden maintenance etc. orchids are one of the floriculture plant. It is a member of Orchidaceae family consisting of more than 25,000 species, which are distributed almost all over the world. Vanda Orchid plant is collected from different nurseries showing chlorotic and mosaic symptoms were observed and it was suspected to infect with virus. So the symptomatic plants were tested with Direct Antigen Coating- Enzyme Linked Immunosorbent Assay (DAC-ELISA) for Cymbidium Mosaic Virus (CYMV), Odontoglossum ring spot virus (ORSV), Poty virus and Tomato Spotted Wilt Virus (TSWV) and further confirmed by Transmission Electron Microscopy (TEM). With the two methods CYMV were detected positively from the samples and low positive results were observed for ORSV, Potex, Poty virus and Tomato Spotted Wilt Virus (TSWV). High incidence of Cymbidium Mosaic Virus (CYMV) was observed. Chemicals were evaluated for inactivation of CYMV on pruning tools for disease control. Skimmed milk was found to be effective, non caustic and inexpensive for inactivation of CYMV inoculated on local lesion indicator host plants. However, systemic host plants (Orchids) were used in evaluation.

Key Words : Orchids, ELISA and electron microscope, Cymbidium mosaic virus, Odontoglossum ring spot virus, Poty virus, Tomato spotted wilt virus, Skimmed milk

How to cite this article : Sudha, D.R. and Rani, G. Usha (2016). Detection, diagnosis of orchid virus and inactivation of cymbidium mosaic virus (CYMV) on plants. *Internat. J. Plant Sci.*, **11** (2): 302-306, **DOI: 10.15740/HAS/IJPS/11.2/302-306**.

Article chronicle : Received : 08.02.2016; **Revised :** 14.05.2016; **Accepted :** 19.06.2016

India has long tradition of flowers. All occasions are celebrated by flowers and it has occupied a prominent place in human's life style. There are important factors for a successful floriculture venture such as favourable climatic conditions for growing of wide range of flowers (Korikanthimath, 2009). The annual domestic demand for the flowers in growing at the rate of 25 per cent and the international floriculture demand is at around Rs.90, 000 Crores, but India's share in the International market is negligible.

Orchids are affected by more virus disease problems than most crops, reducing their commercial values considerably. Orchid viruses are widespread in cultivated orchids (Sherpa *et al.*, 2007). Orchids have been reported to be infected with more than 50 viruses of (Wong *et al.*,1996 and Chang *et al.*, 2005) several

MEMBERS OF THE RESEARCH FORUM

Author to be contacted :
D.R. SUDHA, Department of Microbiology, Annamalai University, Annamalainagar, CHIDAMBARAM (T.N.) INDIA
Email: sudhadrv@gmail.com

Address of the Co-authors:
G. USHA RANI, Department of Microbiology, Annamalai University, Annamalainagar, CHIDAMBARAM (T.N.) INDIA

Orchid-infecting viruses, Cymbidium mosaic virus (CYMV) and Odontoglossum ringspot tobamovirus (ORSV) have been reported to be two of the most prevalent and important viruses which have attained worldwide distribution (Wong *et al.*, 1996 and Sherpa *et al.*, 2004). Cymbidium Mosaic Potexvirus (CYMV) and Odontoglossum ringspot tobamovirus (ORSV) being the most prevalent (Wong *et al.*, 1996). Neither virus is known to be transmitted via seed or insect vectors (Porter *et al.*, 1996). Their high incidence in cultivated orchids has been attributed to the stability and ease of transmission of these two viruses through cultural practices.

Current recommended control methods for CYMV involve sanitation practices and use of chemicals to sterilize pruning tools, cutting tools to inactivate CYMV.

MATERIAL AND METHODS

Sampling and testing :

Totally 80 numbers of Vanda seedling plants were collected for chlorotic and mosaic symptoms in this four varieties were collected (Fig.A). 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 and Reddy, 1989) and further confirmed by Transmission Electron Microscopy (TEM).

Fig. A : Infected vanda orchids for cymbidium mosaic virus

Antisera used :

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) (Table A).

Antisera preparation :

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) and were diluted 1:78000, 1:6000, 1:256000, 1:1000, respectively.

Detection of Cymbidium mosaic virus, Odontoglossom virus, Poty virus and Tomato spotted wilt virus on orchid plants :

Detection of Cymbidium Mosaic Virus,

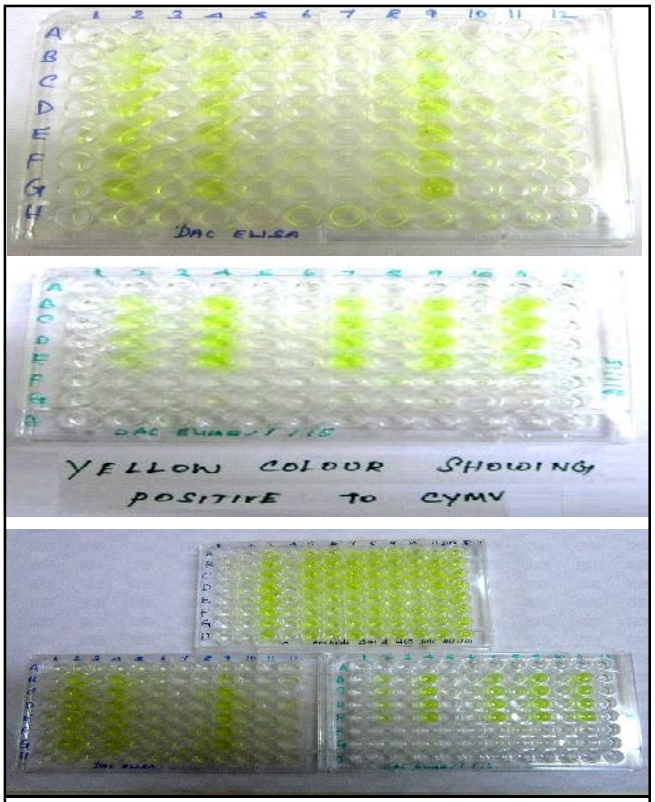

Fig. B : DAC-ELISA Microplate showing positive reaction in 4 samples (yellow colour) and known positive control

Table A: Antisera used for direct antigen coated -ELISA for detection of virus

Sr. No.	Name of the antisera	ATCC number	Dilution concentration
1.	Cymbidium mosaic virus	ATCC-PVAS-355	1:78000
2.	Odontoglosum ringspot virus	ATCC-PVAS-497	1:6000
3.	Poty virus	ATCC-PVAS-50A	1:256000
4.	Tomato spotted ringspot virus	ATCC-PVAS-731	1:1000

Odontoglossom virus, Poty virus and Tomato spotted wilt virus were detected with DAC-ELISA (Fig.B).

DAC-ELISA :

The standard One day procedure DAC – ELISA (Direct antigen coated- enzyme linked immuno Sorbant 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 per cent (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 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 per cent 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% NaN_3, 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 yellow colour reactions produced by tested samples were compared with known negative control wells. Negative controls also were maintained in the ELISA micro plates. A sample was considered positive if the absorbance value was greater than twice the mean value of the healthy controls. 80 Vanda plants at seedling stage were detected and the results presented in Table 1.

By referring the guide for identification of plant quarantine pathogens (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) 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 and further confirmed by Transmission electron microscopy (TEM).

Transmission electron microscopy :

DAC-ELISA results were confirmed by electron microscopy leaves showing chlorotic rings and mosaic mottling were tested by Transmission electron microscope and were used to detect CYMV by leaf-dip method (Gibbs *et al.*, 1966). The infected leaves were used as positive control to detect of CYMV in orchids by direct ELISA technique. Leaves were homogenized with extraction buffer (Phosphate Buffer, pH 6.4). The plant extracts were adsorbed on carbon coated grid and were dried for 5-10 mins and washed with sterile distilled water. They were negatively stained with Uranyl acetate 2 per cent and absorbed under electron microscope.

An 10-µl aliquot from each of the fractions was loaded onto a carbon-coated copper grid. After 1-min incubation, excess liquid was removed with filter paper and 10 µl of 2 per cent 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 JEOL-10 TEM (Japan). TEM analyses were performed to confirm the identities of the observed in the electropherograms as well as the presence of Cymbidium mosaic virus (CYMV), Odontoglossum ring spot virus (ORSV), Poty virus and Tomato spotted wilt virus (TSWV). The grid was further examined under a JEOL model transmission electron microscope at a 200000-fold magnification. The presence of flexuous shape of virus particles was the indication of CYMV positive (Fig.C).

Cymbidium mosaic virus (CYMV) infection is high compared with all other four virus.

Inactivation of cymbidium mosaic virus by skimmed milk :

Ten ml of skimmed milk was mixed and grounded with one gram of Cymbidium mosaic virus infected leaves

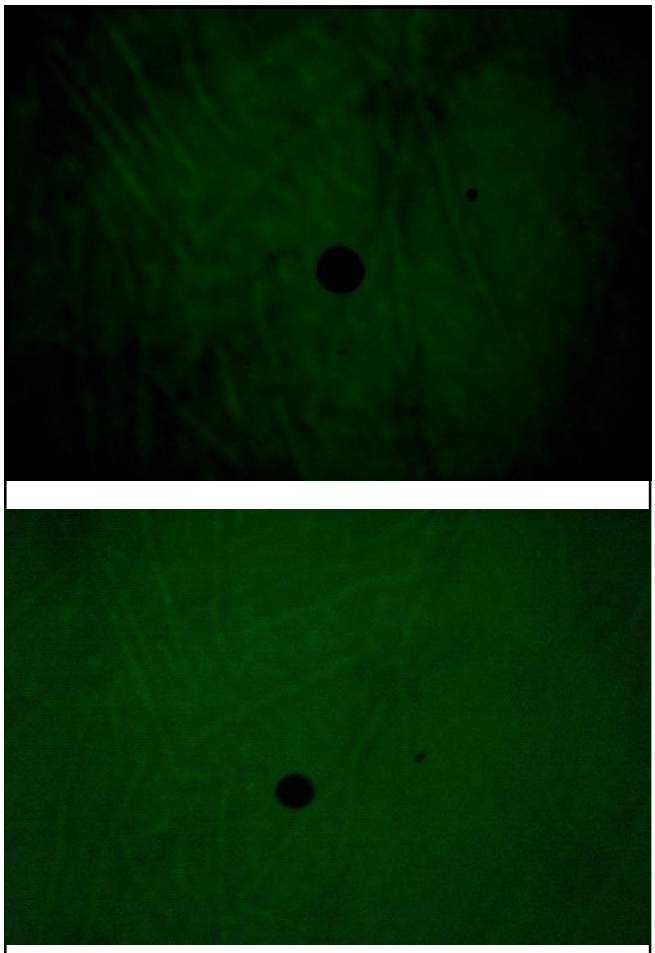

Fig. C : Transmission electron microscope picture for CYMV

and it has given the concentration of 1:1 dilution. The crude leaf extract 1:1 was diluted 10 times with 10 per cent skimmed milk that was 9 ml of 10 per cent skimmed milk added to the 1:1 dilution it has given the concentration of 1:10 likewise 20 per cent, 30 per cent, 40 per cent, 50 per cent, 60 per cent, 70 per cent, 80 per cent and 90 per cent skimmed milk was added to the virus dilution. In the same time the virus concentration was also diluted with required chemical and the concentration was made such a way 1:10, 1:100, 1: 500, and 1:1000. Both virus dilutions and skimmed milk concentration was taken to control in inactivation of Cymbidium mosaic virus. Diluted samples were inoculated on the plants.

Before the inoculation the leaves were lightly dusted with carborundum and inoculated by rubbing with a pestle dipped in virus extracts. After inoculation the leaves rinsed immediately with tap water for 15 sec. Plants were kept in green house at 25° to 30° C and periodically monitored for the symptoms from 7 to 14 days. These orchid plants were tested by ELISA for virus infection at 7 to 14 days.

RESULTS AND DISCUSSION

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 80 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 while ORSV, Poty viruses, and TSWV were shown only slight reaction. The incidence of CYMV infection was in ranged between 50 per cent and 100 per cent and ORSV was in ranged between 30 per cent to 40 per cent. 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.But ORSV has not shown any symptom on host. CYMV-infected plantlets also showed mosaic on leaves. CYMV was found positive on 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).

Transmission electron microscopy was used for the detection of CYMV, ORSV, TSWV and Poty virus. It was observed that all the 4 virus namely CYMV, ORSV, TSW, Poty virus were infected on Vanda Seedings. The CYMV flexious particles were recorded the highest infection rate 30 per cent followed by ORSV, TSW, Poty virus respecting the least particles.

The inactivation of CYMV with skimmed milk at

Table 1 : Detection of CYMV, ORSV, POTY VIRUS and TSWV infection on vanda plants at seedling stage

Sr. No.	Name of the vanda hybrid	CYMV*	ORSV*	Poty virus*	TSWV*
1.	VH1	6	5	6	5
2.	VH2	6	6	5	4
3.	VH3	5	7	6	4
4.	VH4	7	6	5	3
5.	VH5	6	4	4	2
	Total	30	28	26	18
	SD	0.03	0.06	0.08	0.08

different concentration such as 10, 20, 30, 40, 50, 60, 70, 80 and 90 on the Vanda hybrid (4) on seedling stage was tested. It was found that all the Vanda plant VH4 Inactivated at different concentration of skimmed milk and the results were presented in (Table 2 and 3) Among the different concentration *viz*. 10, 20, 30, 40, 50, 60, 70, 80 and 90 per cent were tested and 30 per cent concentration highly inactive on CYMV while other concentrations were found to be intermediary inactive CYMV.

Table 2 : Inactivation of CYMV with skimmed milk at different concentration of vanda

Sr. No.	Skimmed milk at different concentration	No. of plants infected at 1:1 level
1.	10%	8
2.	20%	9
3.	30%	12
4.	40%	9
5.	50%	8
6.	60%	9
7.	70%	9
8.	80%	4
9.	90%	5

Table 3 : Inactivation of CYMV with 30 per cent skimmed milk at different dilutions of leaf extract of vanda

Sr. No.	Leaf extract dilutions	No. of plants infected at 30% skimmed milk
1.	1:1	12
2.	1:10	10
3.	1:100	9
4.	1:500	7
5.	1:1000	6

Viruses are constantly infecting plants. Plants must test for viral contamination before cloning to prevent the viral spreading. It is necessary to index orchid materials before vegetatively propagating. 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. 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 (Sutic *et al*., 1999). CYMV and ORSV are widespread in world, with CYMV being prevalent. About 45 per cent of cloned orchids were infected by CYMV. Because of the level of incidence, Poty viruses, TSWV are not prevalent virus in cultivated orchids in this test but screening regimes should be included to determine its existence.

REFERENCES

Chang, C., Chen, Y.C., Hsu, Y.H., Wu, J.T., Hu, C.C., Chang, W. and Lin, N.S. (2005). Transgenic resistance to *Cymbidium mosaic virus* in *Dendrobium* expressing the viral capsid protein gene. *Trans.Res.*,**14**(1):41-46.

Gibbs, A.J., Varma, A. and Woods, R.D. (1966). Viruses occurring in white clover (Trifolium repens) from permanent pastures in Britain.*Ann.Appl.Biol.*,**58**: 231.

Korikanthimath, V.S. (2009). News letter ICAR Prospects and problems of floriculture in Goa., **11** (1) :1-2.

Porter, K.G., Kuehnle A.R. and Hu J.S. (1996). Lack of seed transmission of cymbidium mosaic virus in *Dendrobium*. *Lindleyana*, **1**(4) : 211-213.

Sherpa, A.R., Hallan, V., Pathak, P. and Zaidi, A.A. (2007). Complete nucleotide sequence analysis of Cymbisium mosaic virus Indian isolate: further evidence for natural recombination among Potexviruses. *J. Biosci.*, **32** (4) : 663-667.

Sherpa, A.R., Hallan, V. and Zaidi, A.A. (2004). Cloning and sequencing of coat protein gene of an Indian *Odontoglossum ringspot virus* isolate; *Acta Virol*., **48** : 267-269.

Sudarshana, M.R. and Reddy, D.V.R. (1989). Penicillinase based enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Virolog. Methods*., **26**: 45-52.

Sutic, D.D., Ford, R.E. and Tosic, M.T. (1999). *Handbook of plant virus diseases* (pp. 56–142). Boca Raton, Florida: CRC Press.

Verma, Shiv Sagar, Latha, S., Ayswarya, R. and Sudha, D.R. (2010). In: *Guide for Identification of Plant Quarantine Pathogens.* 109-119 pp.

Wong, S.M., Chng C.G., Lee Y.H. and Lim T.M. (1996). An appraisal of the banded and paracrystalline cytoplasmic inclusions induced in Cymbidium Mosaic Potexvirus- and Odontoglossum Ringspot Tobamovirus infected orchid cells using confocal laser scanning microscopy. *Arch. Virol.,* **141**(2) : 231-242.