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Research Article

Incidence and Diagnosis of Cymbidium Mosaic Virus (CYMV) on Orchids

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Abstract

Orchids are 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. Viruses are constantly infecting orchids. The most important type of virus infecting orchids in the world is Cymbidium mosaic virus (CYMV). Orchids with mosaic and necrotic spots on the leaves were showing positive infection with CYMV. The symptomatic plants were collected and tested for CYMV. 1110 plants of three stages like seedling stage, medium stage and matured stage were assayed for CYMV using DAC- ELISA and confirmed with Electron Microscope. When compared to seedling stage matured plant infected highly with CYMV.

Keywords: Orchids, Cymbidium Mosaic Virus, ELISA and Electron Microscope

Introduction

The Orchidaceae is possibly the largest and most diverse plant family and contains important cultivated orchid species of *Arachnis, Aranda, Asocentrum, Calanthe, Cattleya, Cymbidium, Dendrobium, Epidendrum, Masdevallia, Oncidium, Paphiopedilum, Phalaenopsis, Vanda, Renanthera and* intergeneric *Odontoglossum* hybrids (Zettler et al. 1990). 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 plant (USDA, ERS, 2004).

Orchids have been reported to be infected with more than 50 viruses (Hu et al., 1998; Wong et al., 1994; Chang *et al* 2005; Zettler *et al* 1990). The most important type of virus infecting orchids in the world is Cymbidium mosaic virus (CYMV) (Zettler *et al.* 1990).

This study provides evidence of CYMV presence in the worldwide. In India, most of orchids were imported from other countries. Thus, the presence of CYMV infecting the orchids might occur accidentally through vegetative materials from abroad. Member of *Potexvirus* has stable viral particles, easily transmitted mechanically and some of them are seed transmitted, including through vegetative materials. Serological test of samples showed that CYMV could infect orchids on its own or together with ORSV. It might cause various symptom on orchids such as mosaic and necrotic spots on the leaf when single infection of CYMV occur and upward-rolling and stunting leaf when occur dual infection of CYMV and ORSV.

One of the detection methods frequently used in the certification programme is ELISA. The detection specificity and sensitivity of ELISA depend on the property of the antibodies. The commercial antibodies prepared by purified virions of CYMV or ORSV have been used for surveys of virus infection (Zettler et al. 1990; Wong et al. 1994; Ryu et al. 1995; Elliott et al. 1996) and ORSV surveys in Dendrobium samples imported from Thailand (Wisler et al. 1979; Zettler et al. 1990; Hu et al. 1993; Wong et al. 1994; Ryu et al. 1995; Grisoni et al. 2004; Khentry et al. 2006).

In this work, the incidence of CYMV was determined using Direct Antigen Coated (DAC) ELISA, ELISA is still the most widely used method for practical plant virus detection throughout the world because of its accuracy, simplicity and low cost.

Materials and Methods

Sampling

Orchid samples mainly Dendrobium Hybrids were collected from orchid nurseries. Diseased leaf and flower samples with virus like symptoms such as mosaic, necrosis, mottle and color breaking were collected (Fig.1.).

Fig.1. Infected Orchids for Cymbidium Mosaic Virus

Antisera

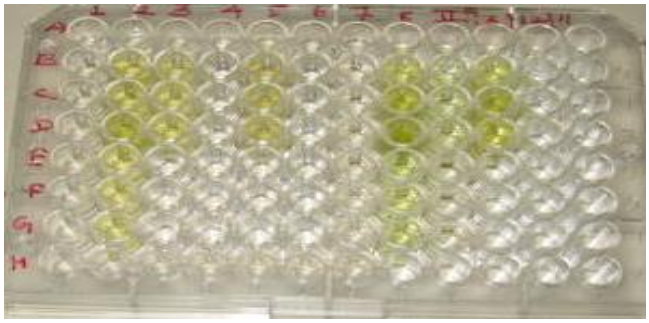
Antiserum for CYMV (ATCC-PVAS-355) was purchased from American Type Cell Culture (ATCC).

ELISA

The standard procedure of direct antigen-coated enzyme-linked immunosorbent assay was used for the detection of CYMV. One hundred mg of plant tissue was ground in 1 ml of sample extraction buffer. Two hundred micro litre of the extracts were coated to the 96-well ELISA plate and incubated at 37°C for 1 h. Each sample had triplicated wells. After two to three washings with phosphate-buffered saline + Tween-20.200 µl of CYMV antibodies diluted in antibody buffer was added to the ELISA plates and incubated at 37°C for 1 h.

The antibodies purchased from ATCC the antisera produced in this study were diluted 78,000- fold. After two to three washings with PBST buffer, 100 µl of diluted 30,000-fold 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-nitrophenylphosphate (PNP) solution (1 mg ml−1), dissolved in PNP buffer was added after two to three washings with PBST buffer. The values of OD405 of each sample were measured by the Biorad (Molecular Devices Co., Berkeley, CA, USA) after 60 min incubation. A sample was considered positive if the absorbance value was greater than twice the mean value of the healthy controls (Fig.2.).

Fig.2. DAC-ELISA Microplate showing positive reaction (yellow colour) & known positive control

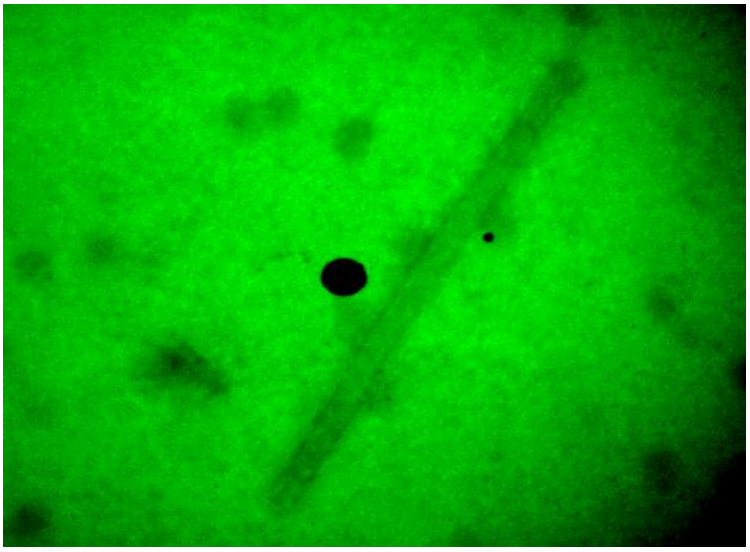


Electron microscopy

ELISA results were confirmed by leaf-dip electron microscopy (Gibbs *et al.* 1966). Leaves showing chlorotic rings and mosaic mottling were tested in Transmission Electron Microscope by standard method (leaf dip assay- Gibbs et al. 1966). Leaves were homogenized with extraction buffer (Phosphate Buffer, pH 6.4). 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.

A 10-µl aliquot from each of the Dendrobium 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% 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 CYMV (Fig.3.).

Fig.3. Transmission Electron microscope picture for CYMV



Results and Discussion

A total of 1110 plants in three stages like seedling stage, medium stage and matured stage were assayed for CYMV using DAC- ELISA. It was found that detected all the stages of Dendrobium plants. Out of 370 plants 111 number of plants affected with Cymbidium mosaic virus (CYMV) which is an average of 22% (Table.1.).The incidence of Cymbidium mosaic virus (CYMV) infection was ranged in between 22% to 58%.The high infection rate was observed in the matured stage of the Dendrobium hybrids. Orchids with medium stage showing 31% of infection (Table.2.).. Leaves of infected Cymbidium mosaic virus (CYMV) are not smooth, dark green areas raised above the light green tissue as longitudinal ridges and bumps. CYMV infected plantlets also showed mosaic on leaves. Out of 1110 plant samples 439 samples were reacted positively with Cymbidium mosaic virus (CYMV). By comparing the three stages of Dendrobium hybrids with CYMV infection, maturity stage of Dendrobium hybrids showing high degree of infection upto 58% (Table.3.).

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 programme.

Table.No.1. Incidence of Cymbidium mosaic Virus for twenty species of orchid saplings by Direct Antigen Coated Enzyme Linked immunosorbant Assay (ELISA)

Sl.No	ORCHIDS VARIETIES	No of plants collected	No. of plants infected	Percentage of infection
	Seedling stage I			
1)	D.Aridang # 17	20	6	30
2)	D.Aridang #72	20	5	25
3)	D.Aridang Green	20	8	40
4)	D.Aridang Green x Burana stripe	20	7	35
5)	D. Atakit x Chaisri gold	10	2	20
6)	D.Beenan Sweet No.2	20	7	35
7)	D.Bermis ruby	20	0	0
8)	D. Bertha Chang x Chaisri gold	20	3	15
9)	D.Blue charm x Burana dark blue	20	7	35
10)	D.Blue dark blue x Burana Angel	20	6	30
11)	D.Burana peal jumbo	20	3	15
12)	D.Burana white	20	5	25
13)	D.Chaisri brow	20	4	20
14)	D.Charack red	20	2	10
15)	D.Copper queen x Bobby mesina	20	0	
16)	D.Dancing Girl	20	5	25
17)	D.Dragon Eye	10	30	3
18)	D.Give my god	10	0	0
19)	D.Kultana blue x Mollisa beauty	20	7	35
20)	D.Morning Sun	20	4	20
	Grand Total	**370**	**111**	**418**
	Mean		**5.55**	**22**
	Positive Control	**3**	**3**	**100**

Table.No.2. Incidence of Cymbidium mosaic Virus for twenty species of orchid plants by Direct Antigen Coated Enzyme Linked immunosorbant Assay (ELISA)

l.No	ORCHIDS VARIETIES	No of plants collected	No. of plants infected	Percentage of infection
	Medium stage			
1)	D.Aridang # 17	20	7	35
2)	D.Aridang #72	20	5	25
3)	D.Aridang Green	20	6	30
4)	D.Aridang Green x Burana stripe	20	5	25
5)	D. Atakit x Chaisri gold	10	3	30
6)	D.Beenan Sweet No.2	20	8	40
7)	D.Bermis ruby	20	5	25
8)	D. Bertha Chang x Chaisri gold	20	6	30
9)	D.Blue charm x Burana dark blue	20	6	30
10)	D.Blue dark blue x Burana Angel	20	8	40
11)	D.Burana peal jumbo	20	7	35
12)	D.Burana white	20	7	35
13)	D.Chaisri brow	20	5	25
14)	D.Charack red	20	6	30
15)	D.Copper queen x Bobby mesina	20	5	25
16)	D.Dancing Girl	20	7	35
17)	D.Dragon Eye	10	4	40
18)	D.Give my god	10	3	30
19)	D.Kultana blue x Mollisa beauty	20	6	30
20)	D.Morning Sun	20	6	30
	Grand Total	**370**	**115**	**625**
	Mean		**5.75**	**31.25**
	Positive Control	**3**	**3**	**100**

Table.No.3. Incidence of Cymbidium mosaic Virus for twenty species of orchid matured plants by Direct Antigen Coated Enzyme Linked immunosorbant Assay (ELISA)

l.No	ORCHIDS VARIETIES	No of plants collected	No. of plants infected	Percentage of infection
	Maturity stage			
1)	D.Aridang # 17	20	10	50
2)	D.Aridang #72	20	11	55
3)	D.Aridang Green	20	12	60
4)	D.Aridang Green x Burana stripe	20	12	60
5)	D. Atakit x Chaisri gold	10	6	60
6)	D.Beenan Sweet No.2	20	12	60
7)	D.Bermis ruby	20	11	55
8)	D. Bertha Chang x Chaisri gold	20	10	50
9)	D.Blue charm x Burana dark blue	20	11	55
10)	D.Blue dark blue x Burana Angel	20	10	50
11)	D.Burana peal jumbo	20	12	60
12)	D.Burana white	20	13	65
13)	D.Chaisri brow	20	12	60
14)	D.Charack red	20	13	65
15)	D.Copper queen x Bobby mesina	20	12	60
16)	D.Dancing Girl	20	11	55
17)	D.Dragon Eye	10	6	60
18)	D.Give my god	10	6	60
19)	D.Kultana blue x Mollisa beauty	20	12	60
20)	D.Morning Sun	20	11	55
	Grand Total	**370**	**213**	**1155**
	Mean		**10.65**	**57.75**
	Positive Control	**3**	**3**	**100**

Conclusion

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. This study suggests that the plant material must be examined for the existence of the virus before using them for mass multiplications. ELISA is a more rapid method and powerful tool for detecting CYMV than observation under Electron Microscope for large scale.

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