Kasetsart J. (Nat. Sci.) 40 : 49 - 57 (2006)

Incidence of *Cymbidium Mosaic Virus* and *Odontoglossum Ringspot Virus* on *In Vitro* Thai Native Orchid Seedlings and Cultivated Orchid Mericlones

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ABSTRACT

Incidence of *Cymbidium mosaic virus* (CymMV) and *Odontoglossum ringspot virus* (ORSV) in 50 species, 16 genera of *in vitro* Thai native orchid seedlings and 44 cultivars, 12 genera of tissue-cultured orchid plantlets was investigated using indirect enzyme-linked immunosorbent assay (ELISA). CymMV and ORSV were not detected in any of 1,000 axenic Thai native orchid seedlings. CymMV was detected in 6 genera namely *Brassolaeliocattleya, Cattleya*, *Dendrobium*, *Epicattleya, Oncidium* and *Mokara* at 27.6 % of 880 micropropagated cultivars of orchid samples. ORSV was not detected in any orchid sample.

Key words: *Cymbidium mosaic virus* (CymMV), *Odontoglossum ringspot virus* (ORSV), orchid, ELISA

INTRODUCTION

Orchid plants are the members of Orchidaceae consisting 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 (Nanakorn and Indhamusika, 2000). Although there are large numbers of Thai wild orchids, deforestation and over-collection for commercial purposes have made many orchid species at risk of extinction. The genus *Paphiopedilum* spp. and *Dendrobium cruentum* are now included in Appendix I of Conservation on International Trade in Endangered Species of Wild Flora and Fauna (CITES).

In vitro seed germination is being used for germplasm conservation and large-scale commercial propagation of orchid species. Furthermore, the tissue culture techniques, especially meristem culture technique have been employed for commercial multiplication of cultivated orchids. Tissue culture was introduced into the Thai orchid industry in late 1960's and has become more and more important in Thailand. Moreover, it helps Thailand to continue to be the leader in tissue culture in Southeast Asia and the world leader in orchid export. The total value of fresh cut orchids, which were exported to 87 countries in 2004, was 2,136 millions Baht (Office of Agricultural Economic, 2005). Orchids are also shipped out as pot plants and seedlings in flasks.

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Received date : 29/08/05 Accepted date : 06/02/06

There are approximately 60 commercial orchid micropropagation laboratories in Thailand. Their capacity may range from a few hundred thousand plants to over 50 million plants per year (Wannakrairoj, 2004). Currently, the international trade competition is increasing. Many countries restrict imports based on quality and particularly demand pathogen-free orchids.

In Thailand and in many other orchid-exporting countries, CymMV and ORSV are the most economically important viruses. These viruses reduce the growth of infected orchid plants as well as the quality of flowers, which effect to orchid industry (Person and Cole, 1986). The most efficient way for the spread of CymMV and ORSV in the orchid industry is by mass propagation of orchid plantlets from an infected mother stock through mericloning tissue culture process (Chang, 2004). Many investigations have shown that orchid seedlings exported from Thailand are infected with these viruses (Chang *et al.*, 2003). In order to produce high quality virus-free orchid plants for the domestic and international market, indexing for the absence viruses in the propagation plant material is an important approach.

In the purpose of this study, the status of CymMV and ORSV in axenic Thai native orchid seedlings and cultivated orchid mericlones was determined using indirect enzyme-linked immunosorbent assay **(**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. ELISA has been used to detect CymMV and ORSV. It could detect purified CymMV at a low concentration of 50-100 ng/ml (Vejaratpimol *et al.*, 1998) and 2.5 ng/ml of purified ORSV (Wong and Lim, 1994).

MATERIALS AND METHODS

1. Detection of CymMV and ORSV in *in vitro* Thai native orchids plantlets

Forty-five species of Thai native seedlings, grown *in vitro*, were kindly supplied by the 'Production of Good Varieties and Disease-free Plants for Export with Emphasis on Orchids Project', Department of Plant Pathology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. Five species were obtained from tissue culture laboratories in Bangkok and Ratcha Buri province. One leaf from each of 20 plants, of each species, was randomly selected, cut with sterilized scissor and individually assayed for CymMV and ORSV by indirect-ELISA technique as described by Clark and Adams (1977) with slight modifications. A 100 µg of leaf tissue was ground in 1 ml of 0.5M carbonate-coating buffer, pH 9.6. A 100 ml of each sample (without filtration) were loaded into ELISA wells (Costar, USA). The negative control was done with *in vitro* seedlings of *Dendrobium* hybrids. The positive control of CymMV was obtained from CymMV infected *Dendrobium* Sakura which showing chlorotic mosaic on leaves (Figure. 1A) and partially purified CymMV (Figure 1C). The positive controls of ORSV were obtained from ORSV-infected *Cattleya* spp. showing color breaking on flower (Figure 1B) and partially purified ORSV (Figure 1D). 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). The healthy plants were ground in PBST containing 2% ovalbumin (Sigma # 5253, Sigma Chemical, St. Louis, USA), in a ratio of 1:30 (w/v) and filtrated. Polyconal antibodies against CymMV and ORSV (provided by P. Hamelink, Department of Plant Pathology, Kasetsart University, Bangkok, Thailand) were diluted to 1:1000 in the filtrated solution. A 100 µl of the diluted antiserum was added to each well and incubated at 37°C for 1 h after that rinsing three times with PBST at 3-minutes intervals. Goat-anti-rabbit gamma immunoglobulin alkaline phosphatase conjugate (Sigma # A 8025, Sigma Chemical, St. Louis, USA) was diluted to 1:2000

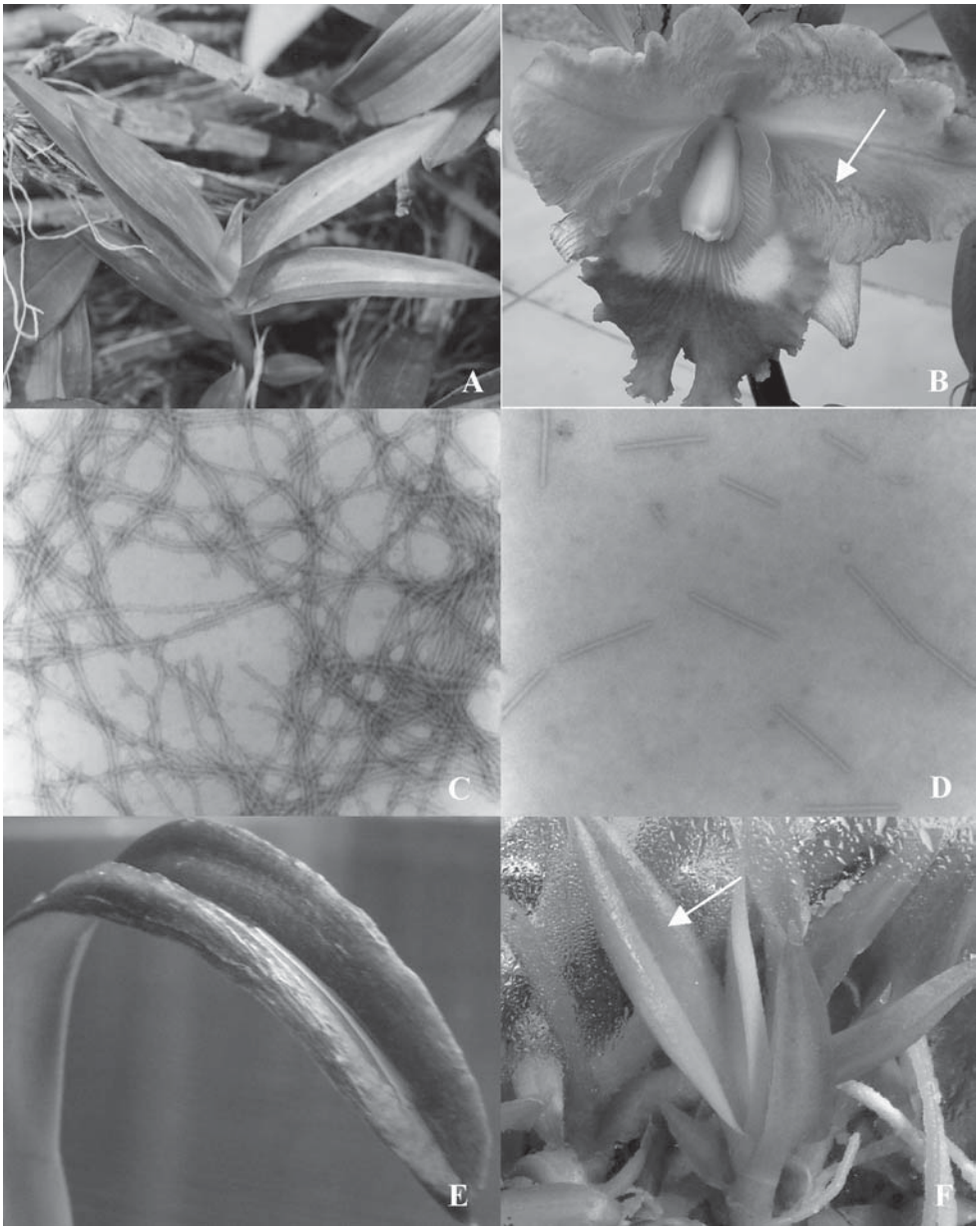

Figure 1 CymMV-infected *Dendrobium* Sakura showing mosaic on leaves (A), ORSV-infected *Cattleya* spp. showing color breaking on flower (arrow)(B), electron micrographs of CymMV particles (C), electron micrographs of ORSV particles (D), symptom on leaves of infected CymMV *in vitro* plantlets are not smooth, dark green areas raised somewhat above the light green tissue as longitudinal ridges and bumps (E) and CymMV infected plantlets showing mosaic on leaves (arrow)(F).

in PBST containing 2% ovalbumin and added to each well, incubated at 37°C for 1 hour and then, repeatedly washed as above. A 100 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 in a moist chamber at 37°C for 30 minute, 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 (GDV model DV 990BV4, Italy). The color reactions produced by tested samples were compared with known negative control wells. The mean absorbance of infected samples that exceeded two fold of mean absorbance of the healthy samples was considered as a positive reaction (Satula *et al*., 1986).

2. Detection of CymMV and ORSV in *in vitro*-cultivated orchids plantlets

Forty-four cultivars of *in vitro*-cultivated orchid plantlets were obtained from commercial tissue culture laboratories in Bangkok, Non Thaburi, Pathum Thani and Nakhon Pathom provinces. One leaf from each of 20 plants of each cultivar was randomly selected and separately assayed for CymMV and ORSV by indirect-ELISA as described previously.

3. Bioassay

Bioassay was used to confirm the ELISA results. Eighty samples that gave positive (66) and negative (14) reactions by indirect-ELISA were examined for presence of CymMV. Forty-five-day-old *Cassia occidentalis* plants were used as indicator plants for CymMV. The orchid leaves were ground in a 0.01 M phosphate pH 7.0 buffer solutions at ratio 1:2 and used as inoculum. The inoculum was kept cool and used immediately. The *C. occidentalis* leaves were dusted with 600-mesh carborundum. The inoculum was rubbed gently onto the leaf surface with a sterile cotton bud until leaves appears wetted. The inoculated leaves were rinsed with distilled water. Inoculum from infected CymMV orchid plants and plain buffer solution were included as positive and negative controls, respectively. The indicator plants were maintained in a greenhouse and symptoms were observed after incubation for 3-5 days, specifically for small, discrete, brown lesions, which indicated infection. The experiment was triplicate.

RESULTS

A total of 1,000 tissue-cultured seedlings of Thai native orchid and 880 *in vitro* cultivated orchid plantlets were assayed for CymMV and ORSV using indirect ELISA. It was found that CymMV and ORSV were not detected in any axenic Thai native orchid seedlings (Table 1). Out of 880 cultivated orchid samples, 243 (27.6%) were positive for CymMV while ORSV was negative. CymMV was detected in 6 out of 10 genera namely *Brassolaeliocattleya*, *Cattleya*, *Dendrobium*, *Epicattleya***,** *Oncidium* and *Mokara*. The incidence of CymMV infection was in ranged between 50 % and 100 %. High infection rates were observed in *Brassolaeliocattleya* Alma Kee, *Dendrobium* Chanel, *Dendrobium* Chao Praya, *Dendrobium* Pravit White, *Dendrobium* Sakura and *Dendrobium* Shavin White (Table 2). Leaves of infected CymMV *in vitro* plantlets are not smooth, dark green areas raised somewhat above the light green tissue as longitudinal ridges and bumps (Figure 1E). CymMV-infected plantlets also showed mosaic on leaves (Figure 1F).

All sixty-six positive samples (of eighty) were screened for CymMV using ELISA also positive by bioassay. Local, necrotic lesions appeared on the inoculated leaves of *C. occidentalis* after 4-6 days of incubation in the greenhouse (Figure 2).

Table 1 Incidence of *Cymbidium mosaic virus* (CymMV) and *Odontoglossum ringspot virus* (ORSV) in 50 species of *in vitro* seedlings of Thai native orchids using indirect enzyme-linked immunosorbent assay (ELISA).

Species	No. of plants infected with		No. of non-infected plants	Percentage of infection
	CymMV	ORSV		
1. *Aerides falcata* Lindl.	0	0	20	0
2. *Aerides houlettiana* Rchb.f.	0	0	20	0
3. *Aerides multiflora* Roxb.	0	0	20	0
4. *Ascocentrum ampullaceum* Schltr.	0	0	20	0
5. *Ascocentrum miniatum* Schltr.	0	0	20	0
6. *Bulbophyllum morphologorum* Krzl.	0	0	20	0
7. *Coelogyne cumingii* Lindl.	0	0	20	0
8. *Coelogyne rochussenii*	0	0	20	0
9. *Cymbidium aloifolium* (Linn.) Sw.	0	0	20	0
10. *Dendrobium aggregatum* Roxb.	0	0	20	0
11. *Dendrobium anosmum* Lindl.	0	0	20	0
12. *Dendrobium bellatulum* Rolfe	0	0	20	0
13. *Dendrobium chrysotoxum* Lindl.	0	0	20	0
14. *Dendrobium crepidatum* Lindl.	0	0	20	0
15. *Dendrobium cruentum* Rchb. f.	0	0	20	0
16. *Dendrobium crystallinum* Rchb. f.	0	0	20	0
17. *Dendrobium draconis* Rchb. f.	0	0	20	0
18. *Dendrobium farmeri* Paxt.	0	0	20	0
19. *Dendrobium formosum* Roxb.	0	0	20	0
20. *Dendrobium friedricksianum* Rchb. f.	0	0	20	0
21. *Dendrobium hercoglossum* Rchb. f.	0	0	20	0
22. *Dendrobium infundibulum* Lindl.	0	0	20	0
23. *Dendrobium lindleyi* Steud.	0	0	20	0
24. *Dendrobium nobile* Lindl.	0	0	20	0
25. *Dendrobium palpebrae* Lindl.	0	0	20	0
26. *Dendrobium pendulum* Roxb.	0	0	20	0
27. *Dendrobium primulinum* Lindl.	0	0	20	0
28. *Dendrobium pulchellum* Roxb. ex Lindl.	0	0	20	0
29. *Dendrobium scabrilingue* Lindl.	0	0	20	0
30. *Dendrobium secundum* (Blume) Lindl.	0	0	20	0
31. *Dendrobium sulcatum* Lindl.	0	0	20	0
32. *Doritis pulcherrima* Lindl.	0	0	20	0
33. *Eulophia andamanensis* Rchb.f	0	0	20	0
34. *Gastrochillus calceolaris*	0	0	20	0
35. *Grammatophyllum speciosum* Blume.	0	0	20	0
36. *Paphiopedellum coerulea* Griff	0	0	20	0
37. *Paphiopedellum concolor*	0	0	20	0
38. *Phalaenopsis cornucervi* Pfitz.	0	0	20	0
39. *Rhynchostylis coelestis* Rchb.f.	0	0	20	0
40. *Rhynchostylis gigantea* (Lindl.) Ridl.	0	0	20	0
41. *Rhynchostylis gigantea* var. harrisonianum	0	0	20	0
42. *Rhynchostylis gigantea* var. petotiana	0	0	20	0
43. *Rhynchostylis gigantea* var. rubra Sagarik	0	0	20	0
44. *Rhynchostylis retusa* (L.) Blume.	0	0	20	0
45. *Spathoglottis plicata* Blume.	0	0	20	0
46. *Vanda coerulea* Griff. Ex Lindle.	0	0	20	0
47. *Vanda brunnea* Rchb.f	0	0	20	0
48. *Vanda denisoniana* Bens. & Rchb.	0	0	20	0
49. *Vanda livouvillei* Finet.Benson & Rchb.f	0	0	20	0
50. *Vandopsis gigantea* (Lindl.) Pfitz.	0	0	20	0
Total	0	0	1,000	0

Table 2 Incidence of *Cymbidium mosaic virus* (CymMV) and *Odontoglossum ringspot virus* (ORSV) in 44 cultivars of *in vitro* orchid plantlets using indirect enzyme-linked immunosorbent assay (ELISA).

Cultivars	No. of plants infected with		No. of non-infected plants	Percentage of infection
	CymMV	ORSV		
1. *Brassolaeliocattleya* Alma Kee	20	0	0	100
2. *Brassolaeliocattleya* Arom Gold	0	0	0	0
3. *Brassolaeliocattleya* Elizabeth Hearns	10	0	10	50
4. *Brassolaeliocattleya* Free spirit	0	0	20	0
5. *Brassolaeliocattleya* Golden Zell	0	0	20	0
6. *Brassolaeliocattleya* Green Wich	13	0	7	65
7. *Brassolaeliocattleya* Haad Yai	0	0	20	0
8. *Brassolaeliocattleya* Hawaiian Passion	0	0	20	0
9. *Brassolaeliocattleya* Lucky Strike 'Mongkorn'	0	0	20	0
10. *Brassolaeliocattleya* Mem Tiang	0	0	20	0
11. *Brassolaeliocattleya* White Diamond	2	0	18	10
12. *Brassolaeliocattleya* White Diamond x *Cattleya* Gertrude Hausermann	0	0	20	0
13. *Cattleya* Sea Breeze	10	0	10	50
14. *Cattleya* hybrid	0	0	20	0
15. *Cattleya* hybrid	0	0	20	0
16. *Cattleytonia* Starrlyn	0	0	20	0
17. *Colmanara* Wildcat 'Bobcat'	0	0	20	0
18. *Dendrobium* Blushing White	18	0	2	90
19. *Dendrobium* Burana Jade x *D.* Bertha Chong	0	0	20	0
20. *Dendrobium* Chanel	20	0	0	100
21. *Dendrobium* Chao Praya	20	0	0	100
22. *Dendrobium* Earsakul	0	0	20	0
23. *Dendrobium* Emma White	0	0	20	0
24. *Dendrobium* Honey	0	0	20	0
25. *Dendrobium* hybrid	15	0	5	75
26. *Dendrobium* hybrid	0	0	20	0
27. *Dendrobium* hybrid	0	0	20	0
28. *Dendrobium* Juree Red	0	0	20	0
29. *Dendrobium* Madame Vipa x *Dendrobium* Burana Green	0	0	20	0
30. *Dendrobium* Pravit White	20	0	0	100
31. *Dendrobium* Pigasus	0	0	20	0
32. *Dendrobium* Sakura	20	0	0	100
33. *Dendrobium* Shavin White	20	0	0	100
34. *Dendrobium* Thongchai Gold	0	0	20	0
35. *Epicattleya* Landwood	18	0	2	90
36. *Grammatocymbidium* Lovely Melody	0	0	20	0
37. *Mokara* Bota Gold	19	0	1	95
38. *Laeliocattleya* Secret Love x *Cattleya* Queen Sirikit	0	0	20	0
39. *Oncidium* Gower Ramsey	0	0	20	0
40. *Oncidium* Sharry Baby	0	0	20	0
41. *Oncidium* hybrid	18	0	2	90
42. *Phalaenopsis* hybrid	0	0	20	0
43. *Vanda* Motes Butterscotch x *Vanda* Doctor Anek	0	0	20	0
44. *Vanda* Sanderiana x *Vanda* Tubtim	0	0	20	0
Total	243	0	637	27.6

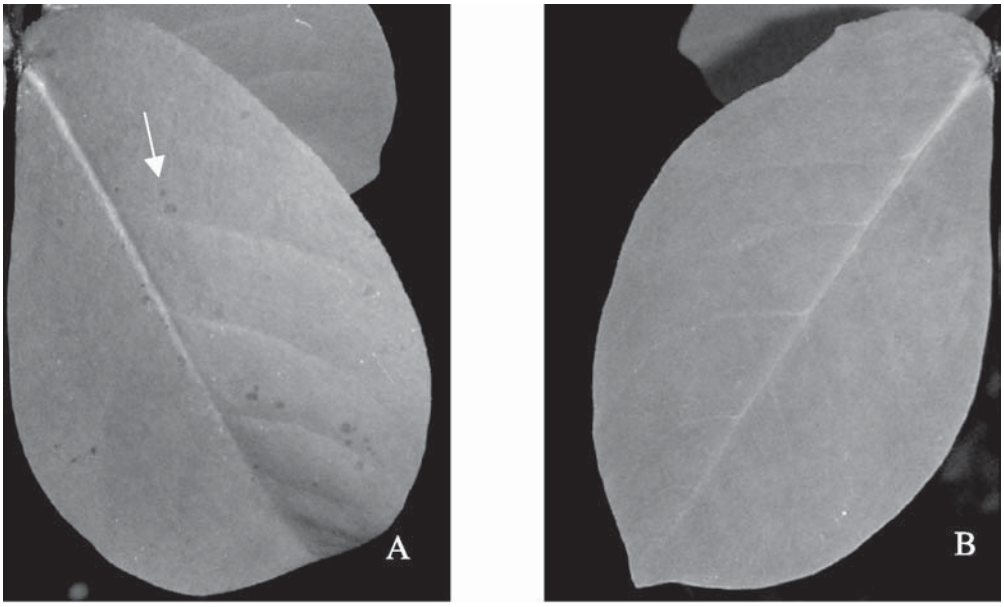

Figure 2 *Cassia occidentalis* leaf infected with CymMV showing necrotic lesions (arrow), 5 days of inoculation (A) and the normal control leaf of *Cassia occidentalis* (B).

DISCUSSION

CymMV and ORSV were not found in all of 1,000 *in vitro* Thai native orchid seedlings. Similarly, Porter *et al.* (1996) found that CymMV was not transmitted from CymMV-infected pod and pollen parents of 7,050 University of Hawaii (UH) *Dendrobium* hybrids seedlings and concluded that CymMV was not seed transmitted. Therefore, the use of seed-propagated cultivars and species shall be one of the most promising approaches to establish virus-free orchid plants and high quality germplasm. However genetic variation within offspring is unexpected.

Cultivated orchid plantlets derived from the micropropagation of axilliary bud and the shoot tip explants were infected with CymMV but non-infected with ORSV. It was found that orchid tissue cultured plantlets were infected with CymMV which similarly to Wong *et al.* (1994) reported that 50.5% of the thirteen orchid genera derived from tissue culture at the tissue culture laboratory of the Botanical Garden in Singapore were infected with CymMV. In Taiwan, some *in vitro* plantlets derived from meristem of commercial cut flower cultivars were infected with CymMV (Chia *et al.*, 1991). The widespread of CymMV in the orchid tissue culture plantlets resulted from the use of infected mother plants for mass clonal propagation. This might cause serious damage to Thai orchid industry in subsequent years. Therefore, it is critical to screen all the plants with a very sensitive technique such as reverse transcription-polymerase chain reaction (RT-PCR) or ELISA before the tissue is cultured. Otherwise, a large population of virus-infected plant will be produced. In this survey, some axenic cultivated orchid plantlets were not infected with CymMV. Virus-free mother stock orchids should be well maintained by separate planting. There are many available methods to produce virus-free orchids from virus-infected plant including culture of 0.1 mm apical tissue pieces of infected orchid plant (Morel, 1960) and, chemotherapy (Yab *et al.*, 1999) or chemotherapy and thermotherapy of infected tissue before culturing (Kim *et al.*, 1997).

Virus-free orchid plants show faster and healthier growth and produce much larger and longer inflorescences length than infected orchid plants (Chia and He, 1999).

The present study reveals that CymMV 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. ORSV is not prevalent virus in cultivated Thai orchids in this test, but screening regimes should be included to determine its existence.

The reliability of indirect-ELISA in detection of CymMV of *in vitro* orchid plantlets was similar to bioassay. Hu *et al.* (1994) detected CymMV in fifty orchid samples and found that the results of ELISA and bioassay for detection were similar. However bioassay bioassay is time-consuming and requires greenhouse space and it takes many days or weeks to get a conclusive result (Chang, 2004). Consequencely, ELISA is more rapid method for detecting CymMV than the mechanical inoculation bioassay (Hu *et al.*, 1994).

CONCLUSION

CymMV and ORSV were not found in micropropagated Thai native orchids seedlings. CymMV was detected at 27.6% of 880 axenic cultivated orchids samples. CymMV was detected in 6 genera namely *Brassolaeliocattleya***,** *Cattleya*, *Dendrobium*, *Epicattleya*, *Oncidium* and *Mokara*. ORSV was not detected in all orchid samples. This investigation revealed that CymMV was found in orchid mericlones. CymMV 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 indirect-ELISA will be a powerful tool for diagnosing CymMV in cultivated Thai orchids by large-scale indexing program.

ACKNOWLEDGMENTS

This research was granted by Graduate School, Kasetsart University, Bangkok, Thailand. We would like to express our gratitude to Laboratory of Dr. Pranee Hamelink, Department of Plant Pathology, Kasetsart University for providing CymMV and ORSV antisera. We also thank to all tissue culture laboratories for orchid samples. Dr. Srimek Chowpongpang, Department of Plant Pathology, Kasetsart University for the manuscript editing and helpful suggestion. A special thank goes to Dr. T. Hanselmann (University of Melbourne) for critically reading the manuscript.

LITERATURE CITED

Chang, C.A., H.H. Lee, C.C Chen, M.J. Lin and C.P. Wang. 2003. Phytosanitary certification program of oncidium seedlings and its future the development of ornamental industry in Taiwan**. Plant Pathol. Bulletin** 12(3): 141-148

Chang, C.A. 2004. Orchid virus disease in Taiwan and their control strategies, pp. 343-356. *In* Editorial Committee of Taiwan Orchid Growers Association (ed.). **Proceedings of 8th Asia Pacific Orchid Conference.** Liang

Hung Color printing Co., Ltd., Taiwan.

Chia, T.F., Y.S. Chan and N.H. Chua. 1991. Survey of cymbidium mosaic and odontoglossum ringspot viruses in cultivated orchids by nucleic acid hybridization, pp. 56-59. *In* **Proceedings of The Nagoya International Orchid Show 91**. Naganae Printing Co., Ltd. Aichi-ken.

Chia, T.F. and J. He. 1999. Photosynthetic capacity in *Oncidium* (Orchidaceae) plants after virus eradication. **Environ. Exp. Bot.** 42: 11-16.

Clark, M.F. and A.N. Adams. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. **J.Gen.Virol.** 34: 475-483.

Hu, J.S, S. Ferreira, M.Q. Xu, M. Lu, M. Iha, E. Pflum and M. Wang. 1994. Transmission, movement, and inactivation of cymbidium mosaic and odontoglossum ringspot viruses. **Plant Dis.** 78(6): 633-636.

Kim, M.S., Y.R. Lee, C.H. Song, B.H. Kim, S.B. Kyun and J.S. Eun. 1997. Elimination of odontoglossum ringspot virus (ORSV) from orchid (*Cymbidium* spp.) by chemotherapy and thermotherapy. **RDA. J. Crop Protec.** 39(1): 19-24.

Morel, 1960. Producing virus-free cymbidiums. **Amer. Orchid Soc. Bull**. 29: 473-478.

Nanakorn, W. and S. Indhamusika. 2000. **Queen Sirikit Botanic Garden (Vol.6).** O.S. Printing House, Bangkok.

Office of Agricultural Economics. 2005. Quantity and Value of Exporting Agricultural Product 2004. Available source: http://www.oae.go.th/Import-Export.php, 15 January 2005.

Pearson, M.N. and J.S. Cole. 1986. The effect of cymbidium mosaic virus and odontoglossum ringspot virus on the growth of *Cymbidium* orchids. **J. of Phytopathology** 117: 193-197.

Porter, K.G., A.R. Kuehnle and J.S. Hu. 1996. Lack of seed transmission of cymbidium mosaic virus in dendrobium. **Lindleyana** 11(4): 211-213.

Satula, C.L., J.M. Gillett, S.M. Morrissey and D.C. Ramsdell. 1986. Interpreting ELISA data and establishing the positive-negative threshold. **Plant Dis**. 70: 722-726.

Vejaratpimol, R., C. Channuntapirat, T. Pewnim, K. Ito, M. Iizuka and N. Minamiura. 1998. Evaluation of enzyme-linked immunosorbent assays for the detection of cymbidium mosaic virus in orchids. **J. Ferment. Bioeng.** 86: 65-71.

Wannakrairoj, S. 2004. Orchid business of Thailand, pp.109-113. *In* Editorial Committee of Taiwan Orchid Growers Association (ed). **Proceedings of 8th Asia Pacific Orchid Conference.** Liang Hung Color Printing Co., Ltd., Taiwan.

Wong, S.M. and S.H. Lim. 1994. Detection of odontoglossum ringspot virus with and amplified enzyme-linked immunosorbent assay (ELISA) system employing a secondary cyclic enzyme reaction. **Acta Hort.** 377: 183-188.

Wong, S.M., C.G. Chng, Y.H. Lee, K. Tan and F.W, Zettler. 1994. Incidence of cymbidium mosaic and odontoglossum ringspot viruses and their significance in orchid cultivation in Singapore. **Crop. Prot.** 13(3): 235-239.

Yap, M.L., L.T. Lan-Chan, C.P. Yik, J. Kueh, S.M. Lee and H.M. Loh. 1999. Eradiation of viruses from commercial orchids. **Singapore J. of Pri. Ind.** 27: 11-19