# Evaluation of several immunobinding assays and polymerase chain reactions (PCR) for the detection of plant virus infections

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## Introduction

Cymbidium mosaic virus (CyMV) and Odontoglossum ringspot virus (ORSV) are widespread in commercially grown orchids (Abdul-Samad, 1989; Abdul-Samad and Ari, 1990). These two viruses were found to occur separately and also together in various orchid genera and hybrids. Virus diseases are difficult to control and once infected, the plants remain diseased. The virus could be spread into healthy plants through routine horticultural practices such as vegetative propagation and flower harvesting. Hence it is essential to detect and identify the infected plants and separate them from the healthy ones. Identification and detection of CyMV has been carried out by bioassay and electron microscopy as well as the serological techniques such as gel immunodifusion, immunoelectron microscopy, latex agglutination and enzyme-linked immunosorbent assay (ELISA) have been widely used (Abdul-Samad, 1990; Abdul-Samad and Ari, 1993). The above methods have their own advantages and disadvantages. However, since the viruses which normally occurs in orchids are unevenly distributed in nature and sometimes present in very low concentration, a highly sensitive method is essential to overcome these problems. Beside these methods, the reverse transcription-polymerase chain reaction (RT-PCR) was used for the detection of CyMV in orchid hybrids.

## Materials and Methods

The oligonucleotide primers were selected from the conserved regions of a known coat protein sequences of CyMV and were able to amplify approximately 497 bp fragment using optimum reaction conditions of 0.8 mM MgCl2 at 48oC of annealing temperature and 35 cycles of amplification. The RT-PCR technique was also tested for detection CyMV in orchid crude sap. The virus was purified from infected orchids and the RNA was isolated by using the single step TRIZOL LS method (Bracete and Fox, 1999).

## Results and Discussion

The two oligonucleotide primers selected from the conserved region of the virus were able to amplify approximately 497 bp fragments using optimum reaction conditions of 0.8 mM MgCl2 at 48°C of annealing temperature and 35 cycles of amplification. The RT-PCR were also able to detect CyMV in orchid crude sap. Results of the study indicated that the RT-PCR is a highly sensitive mode of detection fir CyMV in orchid hybrids.

## Conclusions

Results of the study indicated that the RT-PCR technique is a highly sensitive mode of detection for CyMV in orchid hybrids.

## Benefits from the study

A fast and sensitive method for virus identification in ornamentals.

## Patent(s), if applicable :

Nil

## Stage of Commercialization, if applicable :

Nil

## Project Publications in Refereed Journals

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7.GIBBS, A., A. MaCKENZIE and N. ABDUL-SAMAD. 1997. A novel tymovirus from Calopogonium mucunoides in Malaysia. *Archives Virology* 142, 1697-1702.

## Project Publications in Conference Proceedings

1. Mohamed Ainee, S.S., M. Abdul Kadir, S. Kadzimin and N. Abdul Samad. 2001. Use of RT-PCR for detection of orchid viruses. Proc: 12th National Biotechnology Seminar, pp.445-447

*Graduate Research*

| Name of Graduate | Research Topic | Field of Expertise | Degree Awarded | Graduation Year |
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| Mazidah Mat. | Use of RT-PCR for CyMV detection in orchids. | Biotechnology | Master of Science | 1999 |
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