# Use of Polymerase Chain Reaction (PCR) for Cymbidium Mosaic Virus (CYMV) Detection and a Comparison with DAS-ELISA

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## Introduction

Both *Cymbidium* mosaic virus (CyMV) and *Odontoglossum* ring spot virus (ORSV) are known to occur worldwide in cultivated orchids; however CyMV seems to be more common and widespread (1). Identification and detection of CyMV had been done by bioassay, electron microscopy and several serological techniques. Recently an enzymatic procedure, the polymerase chain reaction (PCR) has been developed which allows the amplification of very low amounts of target nucleic acids (2). To date PCR was found to be more sensitive and powerful in detecting animal and plant pathogens than other methods. In the present study PCR was applied in detection of CyMV and comparison will be made with DAS-ELISA.

## Materials and Methods

The viral RNA was isolated and purified by Tri Reagent LS-RNA/DNA/Protein isolation reagent of Life Technologies, USA according to the manufacturer's protocols. Three oligonucleotide primers, 20-mer corresponding to the C-terminal of the coat protein region of CyMV, were selected according to the published CyMV coat protein sequences by using "ClustalW'. All were custom made by Gibco BRL, Life Technologies, USA. The CyMV RNA was reverse-transcribed to complimentary DNA before being subjected to amplification process. The PCR amplification procedures were done according to standard protocols. The PCR-amplified products were directly detected by agarose gel electrophoresis. The DAS-ELISA procedures were done according to the methods of Clark et al. and Adams (1977).

## Results and Discussion

The two *oligonucleotide* primers that was selected from the conserved region of the virus were able to amplify approximately 497 bp fragments using the optimum condition of 0.8mM $MgCl_2$, annealing temperature at 48°C and 35 cycles of amplification. By using RT-PCR, minimum quantity of purified RNA and virion that could be detected was 10 ng and 2 ng, respectively. This result was identical with the detection limit of DAS-ELISA in parallel experiments. With crude sap of CyMV infected samples, both PCR and DAS-ELISA techniques were comparable in sensitivity. Since PCR involves higher cost, DAS-ELISA seems to be more applicable and practical when dealing with large number of samples.

## Conclusions

The sensitivity of RT-PCR and DAS-ELISA was found to be identical for the detection CyMV. However, DAS-ELISA procedure is more applicable and practical for routine, large-scale testing of field collected samples.

## Benefits from the study

The study is useful for routine indexing of commercial orchid varieties so as to produce healthy and high quality planting materials.

## Literature cited in the text

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## Graduate Research

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