

## Rapid Screening of *Apple mosaic virus* in Cultivated Apples by RT-PCR

Sun Hee Choi and Ki Hyun Ryu\*

Plant Virus GenBank, College of Natural Science, Seoul Women's University, Seoul 139-774, Korea

(Received on April 28, 2003; Accepted on June 10, 2003)

The coat protein (CP) gene of *Apple mosaic virus* (ApMV), a member of the genus *Ilarvirus*, was selected for the design of virus-specific primers for amplification and molecular detection of the virus in cultivated apple. A combined assay of reverse transcription and polymerase chain reaction (RT-PCR) was performed with a single pair of ApMV-specific primers and crude nucleic acid extracts from virus-infected apple for rapid detection of the virus. The PCR product was verified by restriction mapping analysis and by sequence determination. The lowest concentration of template viral RNA required for detection was 100 fg. This indicates that the RT-PCR for detection of the virus is a  $10^3$  times more sensitive, reproducible and time-saving method than the enzyme-linked immunosorbent assay. The specificity of the primers was verified using other unrelated viral RNAs. No PCR product was observed when *Cucumber mosaic virus* (*Cucumovirus*) or a crude extract of healthy apple was used as a template in RT-PCR with the same primers. The PCR product (669 bp) of the CP gene of the virus was cloned into the plasmid vector and resultant recombinant (pAPCP1) was selected for molecule of apple transformation to breed virus-resistant transgenic apple plants as the next step. This method can be useful for early stage screening of *in vitro* plantlet and genetic resources of resistant cultivar of apple plants.

**Keywords :** *Apple mosaic virus*, apple, coat protein, detection, *Ilarvirus*, RT-PCR

Control of viral diseases in woody plants is best accomplished by establishing new virus-free plantings from virus-tested plants. The certification of nursery stock requires fast and sensitive screening methods for detection of viruses. Bioassays such as grafting method with sensitive woody indicator species and enzyme-linked immunosorbent assay (ELISA) are the routine methods used for detecting viruses. However, these techniques are not sufficient to detect low concentrations of virus in the plant tissues, and sometimes variations occur in the responsiveness of indicator host plants to various isolates of virus (Park et al., 1990). Thus,

reliable virus detection method is required and this is important to maintain and disseminate virus-free germ-plasm of crops that are vegetatively propagated.

Reverse transcription-polymerase chain reaction (RT-PCR) has the potential to be an extremely sensitive method in some woody plants even during seasons of low titer of virus (Kinard et al., 1996). *Apple mosaic virus* (ApMV), a member of the genus *Ilarvirus* (Fulton, 1972), is one of the major pathogens infecting apple trees (Fulton, 1983). ApMV occurs worldwide and infects a number of woody plants of over 65 species in 19 families including rose, hop, hazelnut, birch, and raspberry (Aramburu and Rovira, 1998; Brunt et al., 1996; Gottlieb and Berbee, 1973; Postman and Cameron, 1987; Sweet and Barbara, 1979; Wong and Horst, 1993). ApMV can be transmitted by mechanical inoculation and by grafting but not through seeds (Fulton, 1983). In this study, ApMV-specific oligonucleotide primers were selected from a region of coat protein (CP) of the ApMV RNA3 (Shiel et al., 1995), and the ability of RT-PCR to detect the virus from cultivated apple tissue was examined.

### Materials and Methods

**Source of plants, virus, and antiserum.** Leaf tissues were obtained from diseased apple (*Malus domestica*) trees (cv. Fuji) in the experimental fields at the National Horticultural Research Institute (NHRI) in Suwon, Korea, and used for samples of RT-PCR to detect ApMV (Fig. 1). ApMV-F strain (PV-382) was obtained from ATCC (USA) and used for positive control. The ApMV antibody was kindly provided by Dr. Berger, P.H. (University of Idaho, USA).

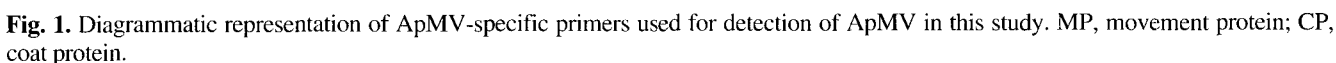
**Extraction of total nucleic acids.** Total nucleic acids were extracted from ApMV-infected leaf tissues by the SDS/proteinase K methods (Ryu and Park, 1995). RNA was extracted from 0.1 g of leaf blade tissue. Final preparations were dissolved in 20  $\mu$ l of DEPC-treated water and stored at  $-70^{\circ}\text{C}$  until use.

**Design of primer.** A set of ApMV-specific primers (PAPMCP5-2 and PAPMCP3-2) was designed based on the nucleotide sequences of CP gene of various ApMV strains in the GenBank database. PAPMCP5-2 upstream primer (GenBank accession no. AJ010123) was 5'-gcggtcaacatggtctgcaagtac-3', and PAPMCP3-2 downstream reverse primer (GenBank accession no. AJ243906) was 5'-ctaacaatcttcacgataaggcgg-3'.

\*Corresponding author.

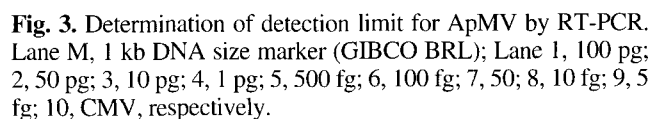
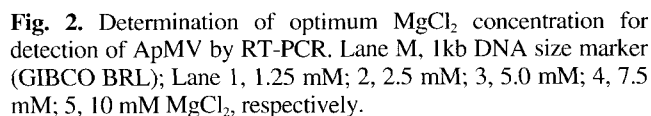
Phone) +82-2-970-5618, FAX) +82-2-970-5610

E-mail) ryu@swu.ac.kr

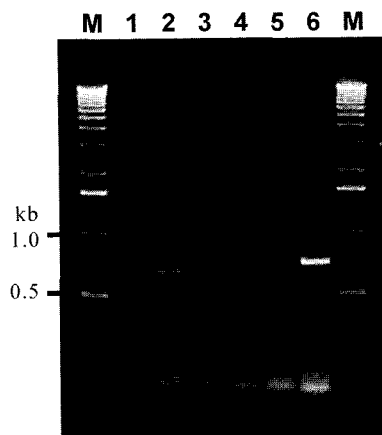


## Results and Discussion

The RT-PCR for ApMV could be sensitively detected as low as 100 fg of viral RNA (Fig. 3). No PCR product was observed when *Cucumber mosaic virus* (genus *Cucumovirus*) or a crude extract of healthy apple was used as a template in RT-PCR with the same primers, and this demonstrated that the primers were only specific for ApMV. This indicates



that the RT-PCR for detection of the virus is a  $10^3$  times more sensitive, reproducible, and time-saving method than the enzyme-linked immunosorbent assay (data not shown).



**Fig. 4.** Analysis of PCR product for ApMV by restriction endonucleases digestion. Lane M, 1 kb DNA size marker (GIBCO BRL); Lane 1, *Apa*I; 2, *Cla*I; 3, *Hae*III; 4, *Hinc*II; 5, *Taq*I; 6, uncut, respectively.

This method was applied for other tissues, such as stem, root and dried fallen leaves, and ApMV was successfully detected in all the tissues (data not shown).

A single PCR product of the virus was digested by *Apa*I, *Cla*I, *Hae*III, *Hinc*II, and *Taq*I restriction endonucleases (Fig. 4). Restriction mapping analysis indicated that the sequence of ApMV isolated from Korea was not identical with those of other previously reported ApMVs (Lee et al., 2002). The PCR product of the CP gene of the virus was cloned into the phagemid vector (pCRII, Invitrogen) and the cloned recombinant plasmid (pAPCP1) was selected and the CP sequence determined. ApMV-K CP consisted of 218 amino acid residues, and this indicated that it was 3-5 amino acids shorter than that of other strains of ApMV. In the BLAST search analysis, the virus showed 83.0-97.7% and 85.3-100.0% identical at the nucleotide and amino acid levels, respectively, to that of all the known ApMV strains (data not shown). The plasmid containing the viral CP can be applied in breeding virus-resistant transgenic apple plants. This method can be useful for early stage screening of *in vitro* plantlet and genetic resources of resistant cultivar of apple plants.

### Acknowledgment

This study was supported by a Research Grant from the

Natural Science Institute, Seoul Women's University in 2003 to KHR.

### References

- Aramburu, J. and Rovira, M. 1998. The effects of apple mosaic ilarvirus (ApMV) on hazelnut (*Corylus avellana* L.). *J. Hort. Sci. Biotech.* 73:97-101.
- Brunt, A. A., Crabtree, K., Dallwitz, M. J., Gibbs, A. J., Watson, L. and Zurcher, E. J. 1996. Plant Viruses Online: Descriptions and Lists from the VIDE Database. <http://image.fs.uidaho.edu/vide/descr024.htm>
- Fulton, R. W. 1983. Iilarvirus Group. CMI/AAB Descriptions of Plant Viruses No. 213, Kew, Surrey, UK.
- Fulton, R. W. 1972. Apple mosaic virus. CMI/AAB Descriptions of Plant Viruses No. 83, Kew, Surrey, UK.
- Gottlieb, A. R. and Berbee, J. G. 1973. Line pattern of birch caused by apple mosaic virus. *Phytopathology* 63:1470-1477.
- Kinard, G. R., Scott, S. W. and Barnett, O. W. 1996. Detection of apple chlorotic leaf spot and apple stem grooving viruses using RT-PCR. *Plant Dis.* 80:616-621.
- Lee, G. P., Ryu, K. H., Kim, H. R., Kim, C. S., Lee, D. W., Kim, J. S., Park, M. H., Noh, Y. M., Choi, S. H., Han, D. H. and Lee, C. H. 2002. Cloning and phylogenetic characterization of coat protein genes of two isolates of Apple mosaic virus from 'Fuji' apple. *Plant Pathol J.* 18:259-265.
- Park, W. M., Ryu, K. H. and Choi, J. K. 1990. Serological diagnosis of cucumber mosaic virus. *Korean J. Plant Pathol.* 6:402-408.
- Postman, J. D. and Cameron, H. R. 1987. Apple mosaic virus in U.S. filbert germplasm. *Plant Dis.* 71:944-945.
- Ryu, K. H. and Park, W. M. 1995. Rapid detection and identification of odontoglossum ringspot virus by polymerase chain reaction amplification. *FEMS Microbiol. Lett.* 133:265-269.
- Ryu, K. H., Yoon, K. E. and Park, W. M. 1995. Detection by RT-PCR of cymbidium mosaic virus in orchids. *J. Phytopathol.* 143:643-646.
- Shiel, P. J., Alrefai, R. H., Domier, L. L., Korban, S. S. and Berger, P. H. 1995. The complete nucleotide sequence of apple mosaic virus RNA-3. *Arch. Virol.* 140:1247-1256.
- Sweet, J. B. and Barbara, D. J. 1979. A yellow mosaic disease of horse chestnut caused by apple mosaic virus. *Ann. Appl. Biol.* 92:335-341.
- Wong, S. M. and Horst, R. K. 1993. Purification and characterization of an isolate of apple mosaic virus from rose in the USA. *J. Phytopathol.* 139:33-47.