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Rapid Detection and Identification of Cucumber Mosaic Virus by Reverse Transcription and Polymerase Chain Reaction (RT-PCR) and Restriction Analysis

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Based upon the nucleotide sequence of As strain of cucumber mosaic virus (CMV-As) RNA4, coat protein (CP) gene was selected for the design of oligonucleotide primers of polymerase chain reaction (PCR) for detection and identification of the virus. Reverse transcription and polymerase chain reaction (RT-PCR) was performed with a set of 18-mer CMV CP-specific primers to amplify a 671 bp fragment from crude nucleic acid extracts of virus-infected leaf tissues as well as purified viral RNAs. The minimum concentrations of template viral RNA and crude nucleic acids from infected tobacco tissue required to detect the virus were 1.0 fg and 1 : 65,536 (w/v), respectively. No PCR product was obtained when potato virus Y-VN RNA or extracts of healthy plants were used as templates in RT-PCR using the same primers. The RT-PCR detected CMV-Y strain as well as CMV-As strain. Restriction analysis of the two individual PCR amplified DNA fragments from CMV-As and CMV-Y strains showed distinct polymorphic patterns. PCR product from CMV-As has a single recognition site for *Eco*RI and *Eco*RV, respectively, and the product from CMV-Y has no site for *Eco*RI or *Eco*RV but only one site for *Hin*dIII. The RT-PCR was able to detect the virus in the tissues of infected pepper, tomato and Chinese cabbage plants.

Keywords : cucumber mosaic virus (CMV), coat protein gene, RT-PCR, restriction analysis, molecular detection

Cucumber mosaic virus (CMV) is the type species of the Cucumovirus genus in the plant viruses (Palukaitis *et al.*, 1992). It is one of the well characterized and the most economically important plant viruses (Francki *et al.*, 1979; Palukaitis *et al.*,1992). CMV is known to have a very broad host range including both monocotyledonous and dicotyledonous plants, and easily transmitted by several species of aphids. Sometimes it results in viral epidemics in many plants all over the world (Francki *et al.*, 1979). The occurrences of viral epidemics in vegetables caused by CMV and turnip mosaic virus (TuMV) in Daekwallyeong area in Korea have been reported (Choi *et al.*, 1992). Outbreaks of the virus diseases occur 2 to 3-years intervals according to the populations of vectors and the initial inoculum sources.

The genome of CMV consists of three positive-sense single-stranded genomic RNAs (RNA1, 2, and 3) and the fourth RNA (RNA4), a subgenomic RNA derived from RNA3 (Palukaitis *et al.*, 1992). Their expressions provide four viral proteins. CMV genomic RNA1 and RNA2 encode two polypeptides, so called the 1a and 2a proteins, respectively, involved in virus replication. RNA3 encodes the 3a protein and coat protein. The 3a protein, movement protein, is thought to involve in cell-to-cell movement function (Hayakawa *et al.*, 1989). The coat protein is translated from the subgenomic RNA, RNA4, which is transcribed from RNA3(Hayakawa *et al.*, 1988). These Cucumoviral characteristics are shared with Bromoviruses, Ilarviruses and Alfamoviruses. They

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are now classified into the family *Bromoviridae* which is previously proposed as the family *Tricornaviridae* (Van Vloten-Doting *et al.*, 1981). A satellite RNA is found in most of the CMV strains, which is a distinct property of the *Cucumoviruses* (Palukaitis *et al.*, 1992).

The As strain of CMV, originally isolated from *Aster yomena* Makino in Korea, has been described and characterized (Choi *et al.*, 1990; Park *et al.*, 1990 a; Ryu and Park, 1995). The virus can be diagnosed by its ability to induce necrotic local lesions in *Vigna unguiculata* (Choi *et al.*, 1990), by peptide mapping and immunological analysis (Edwards and Gonsalves, 1983; Park *et al.*, 1990a) and by molecular biological methods (Choi and Sano, 1990; Park *et al.*, 1990 b; Ryu *et al.*, 1993). Nucleotide sequences of some strains of CMV have been determined (Cuozzo *et al.*, 1988; Hayakawa *et al.*, 1988, 1989; Kim *et al.*, 1992; Quemada *et al.*,1989).

Since the polymerase chain reaction (PCR) for DNA amplification was first introduced in 1985 (Saiki *et al.*, 1985), the combination of reverse transcription (RT) with subsequent PCR amplification of the cDNA (RT-PCR) has been used for analysis of gene expression of eukaryotes (Saiki *et al.*, 1985; Wang *et al.*, 1989), cloning purpose (Hayes and Buck, 1990; Naidu *et al.*, 1995; Nicolas and Laliberte, 1991), and diagnosis of virus diseases (Barbarossa *et al.*, 1994; Colinet *et al.*, 1994).

In this study, a set of CMV-specific oligonucleotide primers was selected from the region of coat protein gene of the CMV-As RNA, and the ability of RT-PCR to detect the virus in crude nucleic acid extracts from some plant tissues infected with the virus was examined.

MATERIALS AND METHODS

Virus sources and purification of viral RNAs

The CMV-As and CMV-Y strains were used for sources of virus (Choi *et al.*, 1990; Park *et al.*, 1990a). PVY-VN strain, kindly provided by Dr. E. K. Park (Korea Ginseng & Tobacco Research Institute), was used as a negative control. The CMVs were maintained in *Nicotiana tabacum* cv. Xanthi-nc and PVY-VN in *N. tabacum* cv. Burley 21. Virus particles were purified from individually propagating leaves as described previously (Park *et al.*, 1990a). Viral genomic RNAs were extracted from purified virus particles by using SDS/proteinase K disruption and phenol extraction. The purity and yield of the recovered viral RNAs were determined by spectrophotometry (1 A_{260}=40 μg/mL). The final concentrations of the viruses were adjusted to 0.1 μg/μL. They were stored at −70℃ until use.

Plant sources

Pepper (*Capsicum annuum*), tomato (*Lycopersicon esculentum*) and Chinese cabbage (*Brassica campestris* ssp. *pekinensis*), which were randomly collected from fields in Kyungki and Kangwon Provinces in 1994, were used as test plants in this study.

Extraction of total nucleic acids from plants

Samples of fresh leaf tissue were homogenized in a sterilized pestle and mortar with two volumes of extraction buffer (50 mM Tris-HCl pH 7.6, 10 mM EDTA, 1.0 mM DTT and 20 mM NaCl). Proteinase K and SDS were added to the extract to the final concentrations of 50 μg/mL and 0.1%, respectively. It was gently mixed and incubated at 42℃ for 10 min. The mixture was extracted once with phenol and chloroform/isoamyl alcohol and total nucleic acid was ethanol-precipitated at −70℃ . Following centrifugation and washing steps, resultant pellet of total nucleic acid extract was resuspended in 20 μL of diethylpyrocarbonate-treated water and stored at −70℃ until use.

Oligonucleotide primers

A set of CMV-coat protein (CP) specific oligonucleotide primers was designed to amplify a 671 bp DNA fragment based upon the nucleotide sequence of CMV-As CP gene (Kim *et al.*, 1992). The downstream and upstream primers, named as P1 and P2, respectively, were synthesized and purified by HPLC.

Reverse transcription (RT) reaction

Synthesis of the first strand cDNA of CP gene from the CMV RNA was performed as follows. RT

reaction was carried out at 42℃ for 30 min in 10 mM Tris-HCl buffer (pH 8.3) containing 10 ng viral RNA or 0.1 μg of total crude nucleic acids from leaf tissue, 5.0 mM $MgCl_2$, 50 mM KCl, 1.0 mM of each dATP, dCTP, dGTP and dTTP, 50 pmol primer P1, 1 unit of RNase inhibitor (BM) and 2.5 units Moloney murine leukemia virus reverse transcriptase (GIBCO BRL). After the reaction, the reverse transcriptase was heat-denatured at 99℃ for 5 min. The sensitivity for detection of the virus was determined by using serially diluted preparations of purified total RNAs of CMV (1 ng-0.5 fg) and crude nucleic acid from CMV-infected tobacco leaf tissue. Crude nucleic acid extracts from healthy leaves and PVY-VN RNA were also used as negative control templates.

Polymerase chain reaction

First strand cDNA generated in RT reaction was used as a template for the PCR. The cDNA was amplified using 5 units of *Taq* DNA polymerase (Perkin Elmer Cetus) in PCR buffer (10 mM Tris-HCl, pH 8.3, 2.0 mM $MgCl_2$, 50 mM KCl) containing 50 pmol of each two CMV-specific primers (P1, P2) and 0.2 mM of each four dNTPs per 50 μL reaction volume. Each 0.5 mL PCR reaction tube was covered with a drop of light mineral oil (Sigma, U.S.A.) before amplification reaction. The cDNA was then amplified by PCR in a programmable DNA Thermal Cycler (Perkin Elmer Cetus, U.S.A.) for 35 cycles. To determine the optimum temperature for primer and template annealing, three different temperatures, 52℃, 42℃ and 32℃, were examined. The optimum PCR program for CMV detection was designed as denaturation at 94℃ for 60 sec, primer annealing at 42℃ for 60 sec and DNA extension at 72℃ for 120 sec. In the final 35th cycle, the elongation at 72℃ was for 10 min. The optimum concentration of $MgCl_2$ for maximal sensitivity of the assay was determined by adjusting the final concentration from 0.0 to 10.0 mM in the reaction mixture (Innis and Gelfand, 1990).

Restriction analysis of PCR product

Ten microliters of the PCR amplified products from CMV-As RNA and CMV-Y RNA were directly treated with 5 units of *Bam*HI, *Eco*RI, *Eco*RV, *Hin*dIII or *Xba*I to complete digestion, and electrophoresis was followed to determine the restriction site and the size of the digested fragments.

Electrophoresis

Amplified PCR products of 10 μL aliquot from each reaction mixture were analyzed by electrophoresis. Electrophoresis was carried out in 1.2% agarose gels in 20 mM Tris-acetate, 0.5 mM EDTA buffer (Sambrook *et al.*, 1989). Gels were stained with ethidium bromide (100 ng/mL) and destained in tap water for 5 min. The DNA bands on the gels were visualized on a UV transilluminator and photographed. Molecular size markers, 1 kb DNA ladder (GIBCO BRL, U.S.A.), were loaded in each gel to estimate the size of the PCR products.

RESULTS

Design of the PCR primers for CMV detection

Two highly conserved regions, N-terminal and C-terminal parts of the CMV coat protein (CP), were searched for the design of primers based on the known nucleotide sequences of some CMV strains. Downstream primer, 5′-AACACGGATTCAAACTGG-3′, was denoted as P1 and was complementary to nucleotides 722 to 739. Upstream primer P2, 5′-GAGTCATGGACAAATCTG-3′, corresponded to nucleotides 69 to 86 of the CMV-As RNA4. The nucleotide sequence between the two primers was designed to span the entire region of the viral CP containing translational start and stop codons (Fig. 1). Calculated *Tm* value of the two primers was 52℃. A set of CMV-CP specific primers was designed to amplify a 671 bp DNA fragment.

Optimization of the PCR

The RT-PCR assay for the detection of CMV from plants using total nucleic acid extracts was modified to enhance its sensitivity by adjusting the template RNA concentration, $MgCl_2$ concentration, annealing temperature and time. Total nucleic acid extracts of healthy and CMV-infected tobacco leaves were amplified in the presence of the two primers.

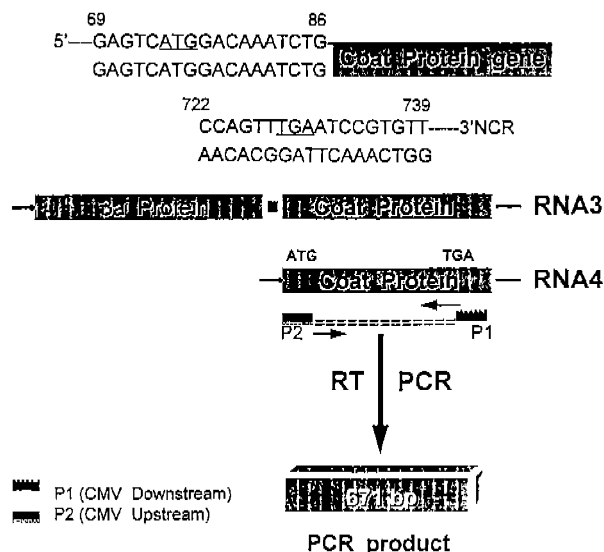

Fig. 1. Schematic representation of the PCR amplified fragment of the coat protein gene of cucumber mosaic virus (CMV). The partial nucleotide sequences of two primers are shown with the start and stop codons (underlined) of the gene together with the two oligonucleotide primer binding sites.

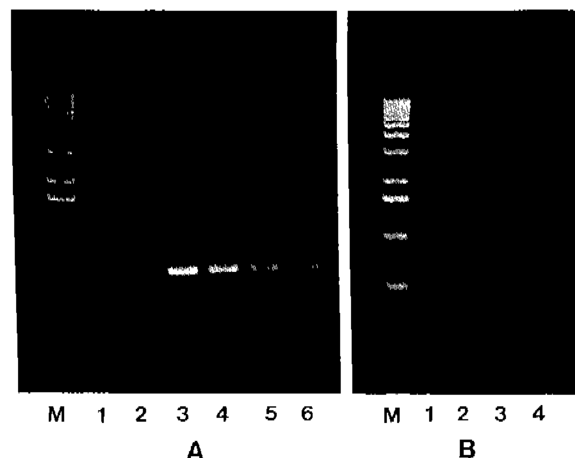

Fig. 2. Agarose gel (1.2%) electrophoresis of RT-PCR amplified products stained with ethidium bromide. Photo A: Determination of optimum $MgCl_2$ concentration for the detection of CMV by RT-PCR. Lanes M, 1 kb DNA ladder as size marker; Lanes 1, 0.0 mM; 2, 1.5 mM, 3, 2.5 mM; 4, 5.0 mM; 5, 7.5 mM and 6, 10.0 mM $MgCl_2$, respectively. Photo B: Lane 1, PCR product from CMV-As; 2, PCR product from CMV-Y; 3, PCR product from healthy-control; 4, PCR product from PVY-VN and M, 1 kb DNA ladder.

From the total nucleic acids of CMV-infected leaf or the purified CMV RNA, single 671 bp DNA product was successfully amplified after RT-PCR corresponding to the CMV CP cistron. The optimized conditions of RT-PCR were 94℃ and 60 sec for denaturation, 42℃ and 60 sec for annealing, and 72℃ and 120 sec for extension cycle, respectively. The optimum concentration of the $MgCl_2$ was 2.5 mM (Fig. 2-A). The RT-PCR amplifications with the primers for CMV-As and -Y strains yielded the product of approximately 691 bp (Fig. 2-B).

Determination of minimum amount of CMV RNA for detection

Positive results were obtained at as little as 1.0 fg for the purified CMV RNA and 1: 65,536 (w/v) dilution of the crude nucleic acids (Fig. 3). One microliter aliquot of total nucleic acids extracted from the 20 μL volume for 0.1 g leaf tissue was sufficient to produce the diagnostic PCR fragment in the RT-PCR. Extracts from all the CMV infected samples including pepper,tomato and cucumber gave positive results in the RT-PCR assay,while no PCR products were obtained from healthy plants with the same primers (Fig. 4).

Restriction analysis of PCR products

Digestions of the PCR product of CMV-As with *Eco*RI or *Eco*RV yielded single fragment for each digest. The PCR product of CMV-Y strain was digested with *Hin*dIII but not with *Eco*RI and *Eco*RV. As shown in Fig. 5, the restriction patterns of the PCR products for the two strains were concordant with the predicted maps. Restriction analyses of the RT-PCR products from CMV-As and -Y strains generated distinct restriction patterns that clearly differentiated the two strains.

DISCUSSION

This study showed that RT-PCR using the CMV-CP gene specific primers, PCMCP1 and PCMCP2. was very useful for the diagnosis of the virus. In general, detection and identification of viruses are based on symptomatology, electron microscopy and serological assays. Molecular hybridization and double-stranded RNA detection have permitted the detection of both single-stranded and double-stranded viral genomes with high specificity and sensitivity. Recently, RT-PCR has been developed for the

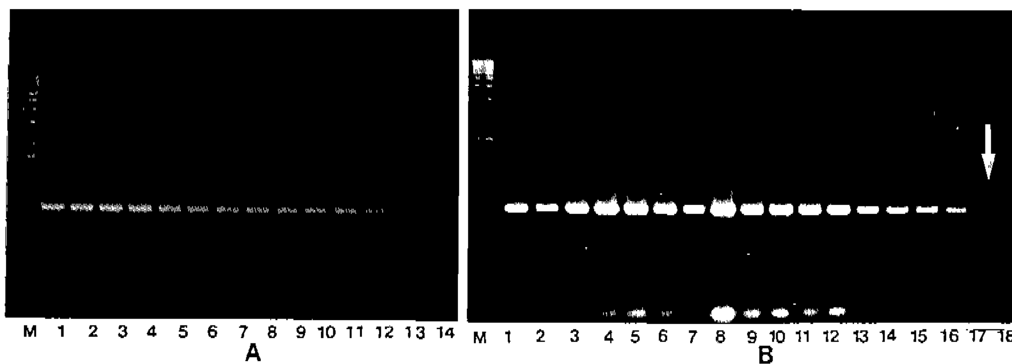

Fig. 3. Determination of the detection limit by RT-PCR of purified CMV RNA and total nucleic acid extracts from CMV-infected tobacco leaves. Photo A: Lane M, 1 kb DNA ladder for size marker; Lane 1, 1 ng; Lane 2, 500 pg; Lane 3, 100 pg; Lane 4, 50 pg; Lane 5, 10 pg; Lane 6, 5 pg; Lane 7, 1 pg; Lane 8, 500 fg; Lane 9, 100 fg; Lane 10, 50 fg; Lane 11, 10 fg; Lane 12, 5 fg; Lane 13, 1 fg; Lane 14, 0.5 fg of purified CMV-As RNA. Photo B: Lane M, 1 kb DNA ladder (GIBCO BRL) for size marker; Lane 1, 1; Lane 2, 2; Lane 3, 4; Lane 4, 8; Lane 5, 16; Lane 6, 32; Lane 7, 64; Lane 8, 128; Lane 9, 256; Lane 10, 512; Lane 11, 1,024; Lane 12, 2,048; Lane 13, 4,096; Lane 14, 8,192; Lane 15, 16,384; Lane 16, 32,768; Lane 17, 65,536; Lane 18, 131,072 and Lane 19, 262,144 (reciprocal dilution, v/w). Arrow indicate the dilution end point (1 : 65,536, w/v).

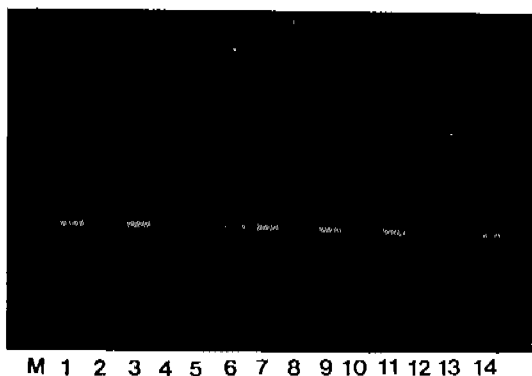

Fig. 4. Detection of CMV by RT-PCR in total nucleic acids extracts of leaves. Lane 1; CMV-As RNA as a positive control; Lane 2, PVY-VN RNA as a negative control; Lane 3-14, total crude nucleic acid extracts (Lanes 3-7, pepper plants; Lanes 8-11, cucumber plants and Lanes 12-14, Chinese cabbage plants) and Lane M, 1 kb DNA ladder as size marker, respectively.

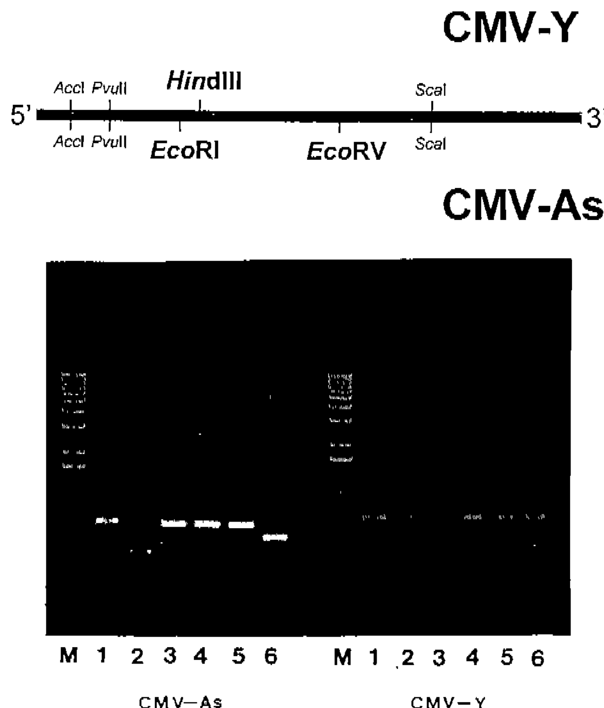

Fig. 5. Comparison of restriction endonuclease maps between CMV-As RNA4 and CMV-Y RNA4 (numbered from the 3'-end of the RNAs) and restriction patterns of RT-PCR amplified products from CMV-As and CMV-Y strains. Lane 1, no digest; 2, *Eco*RI; 3, *Hin*dIII; 4, *Xba*I; 5, *Bam*HI and 6, *Eco*RV digested and M, 1 kb DNA ladder as size marker, respectively.

diagnosis and cloning of RNA-based molecules like RNA viruses or mRNA from eukaryotes (Rybicki and Hughes, 1990; Robertson *et al.*, 1991; Pappu *et al.*, 1993; Pennington *et al.*, 1993; Rojas *et al.*, 1993; Colinet *et al.*, 1994; De Blas *et al.*, 1994; Tsuda *et al.*, 1994).

We excluded the 3'-noncoding region of CMV RNA4 when designing the primers for PCR amplification. Like bromoviruses, all three genomic RNAs and a subgenomic RNA of CMV have common 3'-terminal sequence (Ahlquist *et al.*, 1981; Mitsuhashi *et al.*, 1994), which has a tRNA-like structure. This may interfere the specific amplification in PCR be-

cause of its secondary structures blocking the annealing reaction.

Detection limit for the maximum dilution of crude nucleic acids from CMV-infected tobacco leaf was 1 : 65,536 (w/v), and detection limit for the purified viral RNA was 1 fg. Significantly, virus infection was detectable by RT-PCR even with the samples which showed no foliage symptoms or with the samples which the serological methods did not give positive results on.

De Blas *et al.* (1994) used RT-PCR for detection of CMV by using a set of primers encoding CMV-CP and it resulted in a 540 bp product. They reported that detections of CMV were successful from tomato, cucumber and tobacco plants but not for pepper plants. They assumed that pepper plants contained inhibitors for the RT and/or PCR reaction. But, we successfully detected CMV from the infected pepper plants of more than 1 : 20 (w/v) diluted samples. We also tested the inhibitory effects of the extracts from pepper plants in RT and PCR reactions for the PVY, odontoglossum ringspot tobamovirus (ORSV) and cymbidium mosaic potexvirus (CymMV) and genomic DNAs from orchid plants. We did not observe any inhibition from the extracts of pepper plants in the reactions (data not shown).

The use of RT-PCR offers a simple and accurate means of detecting CMV and has some advantages over the serological methods. First, virus-specific sequences can be detected in small quantities of leaf tissue. About 0.1 g of infected tissue was sufficient for at least 20 amplification reactions. Second, crude nucleic acid extract from plant tissue could be used for RT-PCR without further purification. Third, RT-PCR amplified products are potentially useful for probe construction and for transformation of plants. Forth, many biological variants or strains of CMV can be identified by restriction mapping using the PCR product.

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LITERATURE CITED

Ahlquist, P., R. Dasgupta and P. Kaesberg. 1981. Near identity of 3'RNA secondary structure in bromoviruses and cucumber mosaic virus. *Cell* **23**: 183-189.

Barbarossa, L., F. Grieco, P. Iosco and D. Gallitelli. 1994. Use of the polymerase chain reaction and sandwich-hybridization for detecting artichoke mottled crinkle tombusvirus in artichoke. *J. Phytopathol.* **140**: 201-208.

Choi, C.W., K.H. Ryu, S.R. Choi and W.M. Park. 1992. Mixed infection of turnip mosaic virus and cucumber mosaic virus identified from vegetables in Daekwallyeong area. *Korean J. Plant Pathol. Newslett.* **3**: 85 (Abstr.).

Choi, J.K., S.B. Kwon, S.Y. Lee and W.M. Park. 1990. Some properties of two isolates of cucumber mosaic virus isolated from *Aster yomena* Makino and *Commelina communis* L. *Korean J. Plant Pathol.* **6**: 138-143.

Choi, J.K. and T. Sano. 1990. Hybridization detection of CMV satellite RNA using enhanced chemiluminescence. *Korean J. Plant Pathol.* **6**: 507-510.

Colinet, D., J. Kummert, P. Lepoivre and J. Semal. 1994. Identification of distinct potyviruses in mixed-infected sweetpotato by the polymerase chain reaction with degenerate primers. *Phytopathology* **84**: 65-69.

Cuozzo, M., K.M. O'Connel, W. Kaniewski, R.X. Fang, N. H. Chua and N.E. Tumer. 1988. Viral protection in transgenic tobacco plants expressing the cucumber mosaic virus coat protein or its antisense RNA. *Bio/Technology* **6**: 549-557.

De Blas, C., M.J. Borja, M. Saiz and J. Romero. 1994. Broad spectrum detection of cucumber mosaic virus (CMV) using the polymerase chain reaction. *J. Phytopathol.* **141**: 323-329.

Edwards, M.C. and D. Gonsalves. 1983. Grouping of seven biologically defined isolates of cucumber mosaic virus by peptide mapping. *Phytopathology* **73**: 1117-1120.

Francki, R.I.B., D.W. Mossop and T. Hatta. 1979. Cucumber mosaic virus. CMI/AAB Descriptions of Plant Viruses No 213. Kew surrey, England.

Hayakawa, T., M. Hazama, H. Onda, T. Komiya, K. Mise, M. Nakayama and I. Furusawa. 1988. Nucleotide sequence analysis of cDNA encoding the coat protein of cucumber mosaic virus: genomic organization and molecular features of the protein. *Gene* **71**: 107-114.

Hayakawa, T., M. Mizukami, M. Nakajima and M. Suzuki. 1989. Complete nucleotide sequence of RNA 3 from cucumber mosaic virus (CMV) strain O: comparative study of nucleotide sequences and amino acid sequences among CMV strains O, Q, D and Y. *J. Gen. Virol.* **70**: 499-504.

Hayes, R.J. and K.W. Buck. 1990. Infectious cucumber mosaic virusRNA transcribed *in vivo* from clones obtained from cDNA amplified using the polymerase chain

reaction. *J. Gen. Virol.* **71**: 2503-2508.

Innis, M.A. and D.H. Gelfand. 1990. Optimization of PCRs. *In* PCR Protocols; a guide to methods and applications. M.A. Innis, D.H. Gelfand, J.J. Sninsky and White, T.J. (eds.), Academic Press, San Diego, California, pp. 3-12.

Kim, S.H., K.H. Ryu, W.M. Park, S.Y. Lee and Y.I. Park. 1992. Determination of nucleotide sequence of cDNA of the coat protein gene encoded by RNA4 of cucumber mosaic virus-As. *Plant Mol. Biol. Biotech. Res. Cen. Ann. Rept.* **3**: 153-165.

Mitsuhashi, M., A. Cooper, M. Ogura, T. Shinagawa, K. Yano and T. Hosokawa. 1994. Oligonucleotide probe design - a new approach. *Nature* **367**: 759-761.

Naidu, R.A., C.C. Hu, R.E. Pennington and S.A. Ghabrial. 1995. Differentiation of eastern and western strains of peanut stunt cucumovirus based on satellite RNA support and nucleotide sequence homology. *Phytopathology* **85**: 502-507.

Nicolas, O. and J.F. Lalibéte. 1991. The use of PCR for cloning of large cDNA fragments of turnip mosaic potyvirus. *J. Virol. Methods* **32**: 57-66.

Palukaitis, P., M.J. Roossinck, R.G. Dietzgen and R.I.B. Francki. 1992. Cucumber mosaic virus. *Adv. Virus Res.* **41**: 281-348.

Pappu, S.S., R. Brand, H.R. Pappu, E.P. Rybicki, G.H. Gough, M.J. Frenkel and C.L. Niblett. 1993. A polymerase chain reaction method adapted for selective amplification and cloning of 3'sequences of potyviral genomes: application to dasheen mosaic virus. *J. Virol. Methods* **41**: 9-20.

Park, W.M., K.H. Ryu and J.K. Choi. 1990a. Properties and purification of cucumber mosaic virus As strain. *Korean J. Plant Pathol.* **6**: 393-401.

Park, W.M., K.H. Ryu, H.K. Koh and S.Y. Lee. 1990b. Diagnosis of cucumber mosaic virus using ^{32}P and biotinylated cDNA probe. *Korean J. Plant Pathol.* **6**: 417-422.

Pennington, R.E., J.L. Sherwood and R.M. Hunger. 1993. A PCR-based assay for wheat soilborne mosaic virus in hard red winter wheat. *Plant Dis.* **77**: 1202-1205.

Quemada, H., C. Kearney, D. Gonsalves and J.L. Slightom. 1989. Nucleotide sequence of the coat protein genes and flanking regions of cucumber mosaic virus strains C and WL RNA3. *J. Gen. Virol.* **70**: 1065-1073.

Robertson, N.L., R. French and S.M. Gray. 1991. Use of group-specific primers and the polymerase chain reaction for the detection and identification of luteoviruses. *J. Gen. Virol.* **72**: 1473-1477.

Rojas, M.R., R.L. Gilbertson, D.R. Russell and D.P. Maxwell. 1993. Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. *Plant Dis.* **77**: 340-347.

Rybicki, E.P. and F.L. Hughes. 1990. Detection and typing of maize streak virus and other distantly related geminiviruses of grasses by polymerase chain reaction amplification of a conserved viral sequence. *J. Gen. Virol.* **71**: 2519-2526.

Ryu, K.H. and W.M. Park. 1995. Construction of a plant expression vector for the coat protein gene of cucumber mosaic virus-As strain for plant transformation. *Korean J. Plant Pathol.* **11**: 66-72.

Ryu, K.H., W.M. Park, C.W. Choi, S.R. Choi, K.E. Yoon and S.Y. Lee. 1993. Detection of double-stranded ribonucleic acid from virus infected plants. *Korean J. Plant Pathol.* **9**: 112-117.

Saiki, R.K., S. Scharf, F. Faloona, K.B. Mullis, G.T. Horn, H.A. Erlich and N. Arnheim. 1985. Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Science* **230**: 1350-1354.

Sambrook, J., E.F. Fritsch and T. Maniatis. 1989. Molecular cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory, New York.

Tsuda, S., I. Fujisawa, K. Hanada, S. Hidaka, K. Higo, M. Kameya-Iwakiand K. Tomaru. 1994. Detection of tomato spotted wilt virus S RNA in individual thrips by reverse transcription and polymerase chain reaction. *Ann. Phytopath. Soc. Japan* **60**: 99-103.

Van Vloten-Doting, L., R.I.B. Francki, R.W. Fulton, J.M. Kaper and L.C. Lane. 1981. Tricornaviridae: A proposed family of plant viruses with tripartite, single stranded RNA genomes. *Intervirology* **15**: 198-209.

Wang, A.M., M.V. Doyle and D.F. Mark. 1989. Quantitation of mRNA by the polymerase chain reaction. *Proc. Natl. Acad. Sci. USA* **86**: 9717-9721.

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逆轉寫 重合酵素連鎖反應(RT-PCR)과 制限酵素 分析을 이용한 오이 모자이크 바이러스의 신속한 檢定 및 同定

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적 요

오이 모자이크 바이러스 (CMV)의 신속한 검정 및 동정을 위해 CMV-As 계통 RNA4의 염기서열을 토대로 외피단백질 유전자 부위에 대응하는 중합효소연쇄반응 (PCR) primer를 제작하였다. 역전사 중합효소연쇄반응 (RT-PCR)은 바이러스의 외피단백질 유전자에 특이적인 18개 염기로 구성된 primer를 사용하여 순화바이러스 RNA와 감염식물 핵산추출액을 주형으로 하였을때 671 bp의 DNA 절편이 증폭되었다. 이 방법을 사용하여 검정가능한 바이러스의 최소농도를 조사한 결과 순화바이러스 RNA는 1.0 fg, 그리고 감염담배 조즙액의 경우 1 : 65,536 (w/v) 희석농도에서도 바이러스 검정을 할 수 있었다. 감자 바이러스 Y에 감염된 담배 및 건전담배의 조즙액과 CMV 특이적 primer를 사용한 결과에서는 PCR 증폭산물이 관찰되지 않았다. 이 RT-PCR은 CMV-As와 CMV-Y 계통 모두에서 검정이 가능하였다. 제한효소 분석을 사용하여 CMV-As 및 Y 계통에서 증폭된 PCR 산물을 비교한 결과 서로 구별되는 다형성을 보였다. 즉, CMV-As 계통을 주형으로 생성된 PCR 산물은 *Eco*RI과 *Eco*RV 제한효소 절단부위가 각각 1개씩 존재하였으며, CMV-Y로부터 증폭된 PCR 산물 DNA는 단지 1곳의 *Hin*dIII 부위만을 포함하였다. 한편 이 RT-PCR 방법을 포장에서 채집한 고추, 토마토 및 배추에 적용한 결과 이들로부터 CMV 감염개체를 검정할 수 있었다.

주요어 : 오이 모자이크 바이러스, 외피단백질 유전자, 역전사 중합효소연쇄반응, 제한효소분석, 바이러스 유전자 검정

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