Plant Pathol. J. 18(1) : 6-11 (2002)

The Plant Pathology Journal

Characterization of *Cucumber mosaic virus* Subgroup II Isolated from Paprika (*Capsicum annuum* var. *grossum*) in Korea

Jae Hyun Kim, Gug Seoun Choi* and Jang Kyung Choi[1]

National Horticultural Research Institute, Rural Development Administration, Suwon 441-440, Korea

[1]Division of Biological Environment, College of Agriculture and Life Science, Kangwon National University, Chunchon 200-701, Korea

(Received on December 10, 2001)

An isolate of *Cucumber mosaic virus* (CMV), PaFM1-CMV, causing malformation on the fruit of paprika (*Capsicum annuum* var. *grossum*) was characterized based on biological reactions, serological relationships, and partial nucleotide sequence analyses. PaFM1-CMV was distinguishable from other isolates of CMV, Mf- (subgroup I) and LS-CMV (subgroup II), in terms of its reactions to some host plants. Polyclonal antibody against PaFM1-CMV showed homologous antigenic relationship with LS-CMV, however, the antibody formed a spur between PaFM1- and Mf-CMV. In the comparison of molecular size of dsRNAs of PaFM1-CMV with Mf- and LS-CMV, PaFM1-CMV had a slightly smaller RNA1 and larger RNA2, RNA3, and RNA4. When the cDNA product of PaFM1-CMV coat protein (CP) gene was digested with some restriction enzymes, the fragment pattern was identical with that of LS-CMV. The nucleotide and amino acid sequences of PaFM1-CMV CP gene were 99.5% and 98.6% identical with LS-CMV, respectively. The data indicate that PaFM1-CMV belongs to subgroup II of CMV, which is the first report in Korea.

Keywords : *Cucumber mosaic virus*, paprika, subgroup.

Cucumber mosaic virus (CMV), the type member of the genus *Cucumovirus* of tripartite RNA virus, is characterized by an extremely broad host range and high variability. Hundreds of isolates have been identified from a number of plant sources. CMV isolates are classified into two subgroups, I and II, according to various converging criteria which include symptomatology, serology, and nucleic acid homology (Palukaitis et al., 1992). CMV RNA1 and RNA2 are associated with the replication of viral genome (Nitta et al., 1988), while RNA3 contains both the viral coat protein (CP) gene and movement protein gene involved in the cell-to-cell and long-distance movements (Davies and Symons, 1988). After mapping for the determination of CMV pathogenicity (Mossop and Francki, 1977), pseudorecombinants created by exchanging gel-eluted genomic RNAs revealed that each of the three genomic RNAs plays its determinant role by various host-strain interactions (Palukaitis et al., 1992).

Paprika was first cultivated in 1994 in Jeju Island, southern part of Korea. An economically important crop in the country, its production area increased to 36.3 ha in 1998. However, virus diseases threatening yield and quality of paprika fruit have not been studied until now. In 1999, a CMV was isolated from paprika fruit showing malformation in a plastic house in Pyungchang, alpine area of Korea. Some characteristics of PaFM1-CMV different from the other isolates of CMV are reported and discussed in this study.

Materials and Methods

Virus source and isolation. In 1999, paprika (*Capsicum annuum* var. *grossum*) fruits showing malformation were collected from a plastic house in Pyungchang, alpine area. A virus, PaFM1-CMV, was isolated from the paprika fruits. After three repetition of single local lesion transfers on *Chenopodium quinoa*, biologically pure isolate was maintained in *Nicotiana benthamiana*. Two typical isolates of Mf-CMV in subgroup I (Choi et al., 1998) and LS-CMV in subgroup II (Wahyuni et al., 1992) obtained from Dr. Choi, J. K. (Kangwon National University) and from the Plant Virus GenBank (http://www.virusbank.org, Seoul Women's Univ.), respectively, were used as control.

Host range and symptomatology. Inoculations for host range tests were conducted in a glasshouse at 25±2°C. Inoculum was extracted from *N. benthamiana* infected with PaFM1-CMV in 0.01M phosphate buffer (pH 7.0). After mechanical inoculation using Carborundum (600 mesh), the 20 species of plants were maintained for visual inspection of virus symptoms in the glasshouse for at least 3 weeks.

DsRNA analysis. Viral double-stranded RNA (dsRNA) was extracted from the leaf tissues of the *N. tabacum* cv. Ky-57 infected with the PaFM1-CMV by the procedure of Morris and Dodds (1979) using Whatman CF-11 cellulose column chromato-

*Corresponding author.
Phone) +82-31-290-6224, FAX) +82-31-295-9548
E-mail) choigs@rda.go.kr

graphy. DsRNA of the *Rice dwarf virus* was used as molecular size markers. DsRNA was analyzed by electrophoresis through 6% polyacrylamide (arcrylamide: bisacrylamide, 30: 1) slab gel containing 1 × TBE buffer (1 M Tris, 0.83 M boric acid and 10 mM EDTA, pH 8.0). The dsRNA bands were visualized by silver staining with silver stain plus kit (Bio-Rad, USA).

Virus purification and serological test. The PaFM1-CMV was purified from the infected leaves of *N. tabacum* cv. Ky-57 by the method of Takanami (1981), followed by an additional 10-40% sucrose density gradient centrifugation for 180 minutes at 22,000 rpm (SW-28, Beckman). Concentration of the purified virus was determined spectrophotometrically with extinction coefficient of 5.0 (Francki et al., 1966). The immunodiffusion test (Ouchterlony, 1968) was conducted in the 1% agar gel medium. Precipitin lines were observed after incubation for 12 h at room temperature.

Electrophoresis of coat protein. The purified virus (1 mg/ml) was mixed with an equal volume of sample buffer (62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 2% SDS, 1% 2-mercaptoethanol, and 0.002% bromophenol blue) and heated for 3 minutes at 100°C. The sample was electrophoresed at 30 mA for 4 h on 3% and 7.5% discontinuous polyacrylamide slab gel containing 0.1% SDS following the method of Laemmli and Favre (1973). Coat protein bands on the gel were stained with Coomassie brilliant blue R 250.

RT-PCR and treatment of restriction enzymes. Total nucleic acids were extracted from the infected leaves of *N. tabacum* cv. Ky-57 by the method of Choi et al. (1998). The pair of PCR primers (CPTALL-3 and CPTALL-5) for CMV coat protein genes was used following the same sequence as described by Choi et al. (1999). Reverse trancriptase (RT) reaction was carried out with one cycle at 42°C for 45 minutes and 35 cycles of PCR amplification using the step programme (94°C, 30 seconds; 40°C, 60 seconds; and 72°C, 60 seconds) followed by a final extension at 72°C for 10 minutes. For the differentiation of amplified PCR products, these were digested with restriction enzymes, *Ava*I, *Eco*RI, *Eco*RV, *Hin*cII, *Hin*dIII, *Sal*I, and *Xho*I (Promega) and analyzed by electrophoresis in 1.5% agarose gel.

Cloning and sequencing of coat protein gene. The cDNA of the 3' half region of PaFM1-CMV RNA 3 was cloned into the pGEM-T-easy vector (Promega). The nucleotide sequence was determined using a BigDye DNA sequencing kit (Perkin-Elmer Corp., Norwalk, CT, USA) on an ABI 377 DNA sequencer (PE Applied Biosystems, Foster City, CA USA). All sequences were analyzed using the program of DNASTAR, Madison, Wis., USA. Homologies of nucleotide and amino acid sequences were performed using a BLAST in the GenBank database (Mf-CMV: GenBank accession no. AJ276481, Choi et al., 2000; and LS-CMV: GenBank accession no. AF127976, Roossinck et al., 1999).

Results

Host reaction and morphology of the virus. The reactions of the test plants inoculated mechanically with PaFM1-CMV isolated from paprika fruit showing malformation (Fig. 1A) are listed in Table 1. PaFM1-CMV did not infect *Cucumis sativus*, *Lycopersicon esculentum*, *Nicotiana rustica*, *N. tabacum* cvs. (Samsun, Xanthi-nc, and White Burley), and *Solanum melongena.* However, Mf-CMV and LS-CMV, used as control virus in this study, infected those plants. PaFM1-CMV caused mild mosaic symptom in upper leaves of *Datura stramonium, Capsicum annuum, N. benthamiana, N. occidentalis, N. tabacum* cv. Ky-57, and *Cucubita pepo,* whereas, LS- and Mf-CMV produced severe mosaic symptom in those plants. Typically, spherical particles with 28-30 nm in diameter were observed in the purified Mf-CMV (Fig 1-B).

Serological relationship. Antiserum against PaFM1-CMV showed positive reactions serologically with LS-CMV and

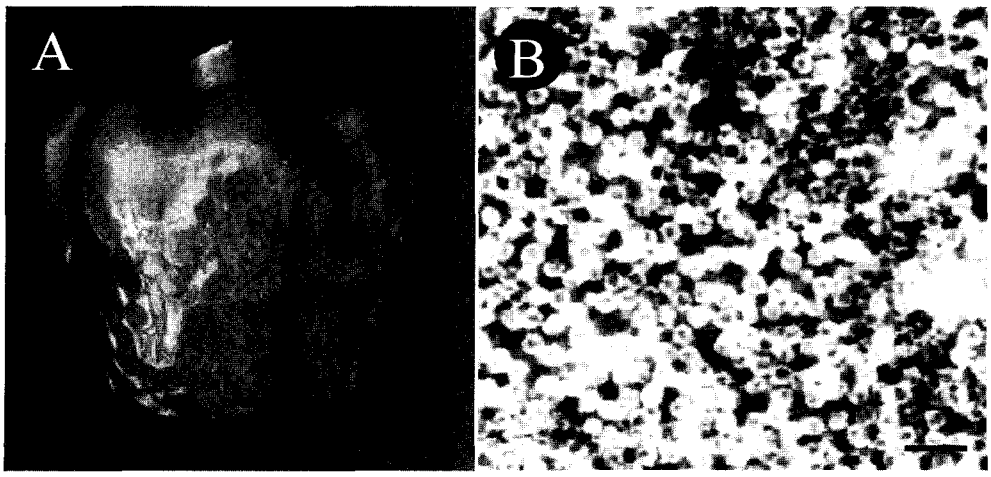

Fig. 1. Symptoms on paprika fruit naturally infected with PaFM1-CMV (**A**) and electron micrograph of purified particles of PaFM1-CMV (**B**). The bar represents 100 nm.

Table 1. Reactions of *Cucumber mosaic virus* (CMV) isolates to different indicator plants

Indicator plants	Host reactions of CMV isolates[a]		
	Mf	LS	PaFM1
Chenopodium amaranticolor	NL/-[b]	NL/-	NL/-
C. quinoa	NL/-	NL/-	NL/-
Gomphrena globosa	-/-	-/-	-/-
Datura stramonium	-/M	-/SM	-/mM
Physalis floridana	-/M	-/-	-/-
Tetragonia expensa	-/M	-/-	-/-
Capsicum annuum	-/M	-/M	-/mM
Lycopersicon esculentum	-/M	-/M	-/-
Nicotiana benthamiana	-/M	-/M	-/mM
N. occidentalis	-/M	-/M	-/mM
N. rustica	-/M	-/M	-/-
N. tabacum cv. Samsun	-/M	-/M	-/-
Ky-57	-/M	-/M	-/mM
Xanthi-nc	-/M	-/M	-/-
White Burley	-/M	-/M	-/-
Cucubita pepo	-/M	-/M	-/mM
Cucumis sativus	-/M	-/M	-/-
Brassica campestris	-/-	-/-	-/-
Raphanus sativus	-/-	-/-	-/-
Solanum melongena	-/M	-/-	-/-

[a] Mf-CMV (Choi et al., 1998) and LS-CMV (Wahyuni et al., 1992) isolates were kindly provided by Dr. Choi, J. K. (Kangwon National University) and the Plant Virus GenBank (www.virusbank.org), respectively, and used as control. PaFM1-CMV was isolated from paprika fruit showing malformation in the experiment.

[b] Inoculated leaves/upper leaves, NL: necrotic local, M: mosaic, mM: mild mosaic, SM; severe mosaic, -: no reaction.

Mf-CMV in the agar gel double diffusion test. Both antigens of PaFM1- and LS-CMV (subgroup II) formed homologous one precipitin line, however, PaFM1- and Mf-CMV (subgroup I) formed a spur line (Fig. 2A).

Molecular weight of coat protein and dsRNA analysis. The coat protein (CP) of the purified PaFM1-CMV migrated as a single band with molecular weight of about 26 kDa in SDS-PAGE. The molecular weight of PaFM1-CMV was similar with that of CP of LS-CMV (Fig. 2B). DsRNAs of the PaFM1-, Mf-, and LS-CMV are shown in Fig. 3. Molecular sizes of PaFM1-CMV dsRNAs were estimated at approximately 3.32, 3.23, 2.3 and 1.3 kbp. Comparing the molecular size of dsRNAs of PaFM1-CMV with Mf- and LS-CMV, PaFM1-CMV had a slightly smaller RNA1 and larger RNA2, RNA3, and RNA4 (Fig. 3).

RT-PCR and RFLP analysis. The amplified DNA products of the three CMV isolates corresponded to 3' half region of RNA3 covering full-length CP gene, and to the target size, approximately 960 bp long (Fig. 4A). When the cDNA of PaFM1-CMV CP gene was digested with *Ava*I, *Eco*RI, *Eco*RV, *Hin*cII, *Hin*dIII, *Sal*I, and *Xho*I, the fragment pattern obtained was identical with that of LS-CMV (Fig. 4B and C). However, the fragments of the cDNA of PaFM1-CMV CP gene differed with that of restriction enzyme sites of the cDNA of Mf-CMV (Fig. 4D).

Sequence analysis of coat protein gene. In 961 nucleotide sequence of the 3' half region of PaFM1-CMV RNA3, the virus CP gene consisted of 657 nucleotides which encoded 218 amino acid residues. Identity of PaFM1-CMV CP gene ranged from 72 to 99.5% with other CMV isolates in nucleotide and from 79.5 to 98.6% in amino acid level

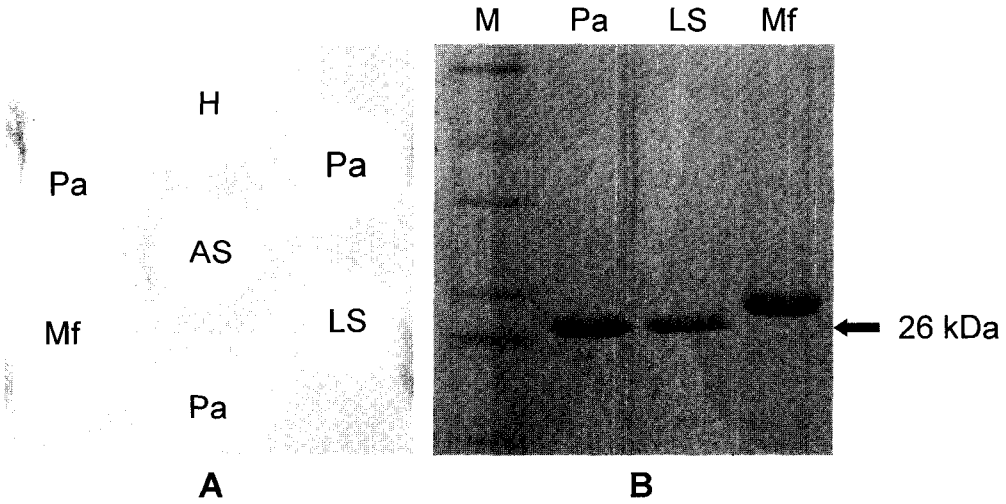

Fig. 2. A: Immunodiffusion test conducted with antigens of PaFM1- (Pa), LS- (LS) and Mf-CMV (Mf), using antiserum against PaFM1-CMV (central well). Healthy control was crude sap of healthy *N. tabacum* cv. Ky-57 (H). Molecular weights of the coat proteins of PaFM1- (Pa), LS- (LS) and Mf-CMV (Mf) on SDS-PAGE electrophoresed at 30 mA for 4 h (**B**). Lane M: molecular weight marker protein (Sigma, MW-SDS-200).

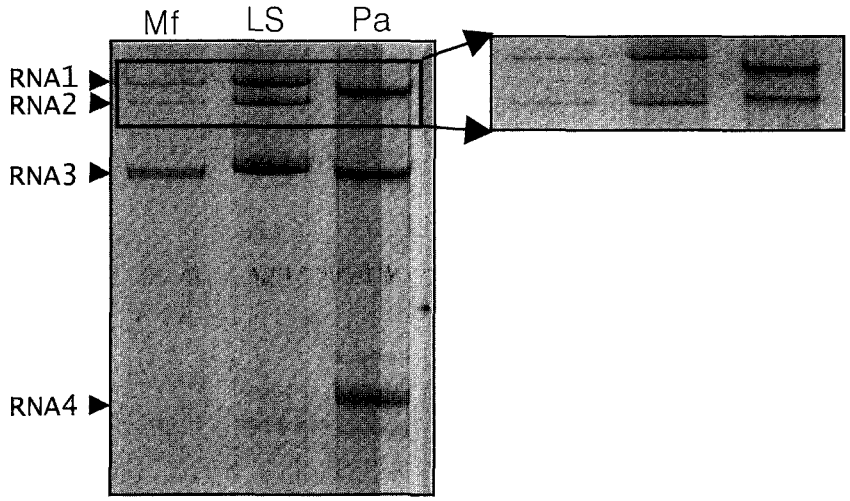

Fig. 3. DsRNA profiles of CMV isolates extracted from infected *Nicotiana tabacum* cv. Ky-57 plants. Electrophoresis was carried out with 6% polyacryamide gel and the gel was stained with silver stain plus kit (Bio-Rad, USA). Lane Mf: Mf-CMV, LS: LS-CMV, Pa: PaFM1-CMV.

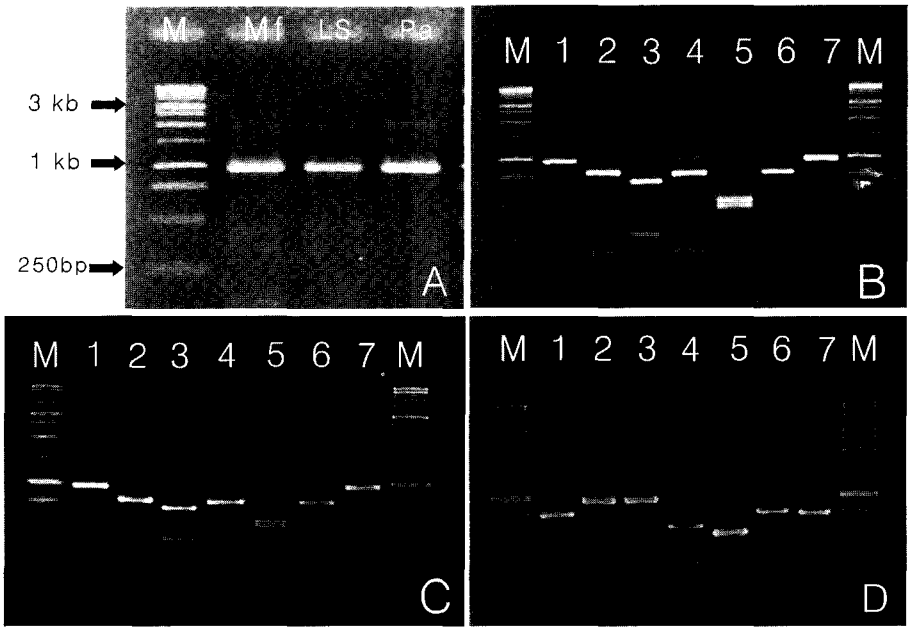

Fig. 4. Agarose gel (1.2%) electrophoresis of the amplified cDNA products obtained by RT-PCR with single pair of Cucumovirus-specific degenerate primers (A). RFLP analysis of RT-PCR amplified products of strain of PaFM1-CMV (B), LS-CMV (C) and Mf-CMV (D) with a set of the genus Cucumovirus-specific primers (Choi et al., 1999). Lane M: 1 kb DNA ladder (Promega), Mf: Mf-CMV, LS: LS-CMV, Pa: PaFM1-CMV. Lane 1: *Ava*I, 2: *Eco*RI, 3: *Eco*RV, 4: *Hinc*II, 5: *Hin*dIII. 6:*Sal*I, 7: *Xho*I digestions.

(Table 2). In sequence similarity of CP genes, PaFM1-CMV showed 99.5% of the highest homology with LS-CMV. The putative amino acid sequence of CP is presented in Fig. 5. The variation of nucleotide sequence between PaFM1- and LS-CMV CP gene changes at position 122 (G → A), 131 (A → G), and 441 (T → C) in 697nt. The putative amino acid sequences of PaFM1- and LS-CMV change only at position 42 (Arg → Lys) and 44 (Lys → Arg) in 218 amino acid (Fig. 5).

Discussion

Cucumber mosaic virus (CMV) isolates are characterized into two subgroups based on serological relationship, host

Table 2. Percentage sequence identities between the complete coat protein of PaFM1-CMV and other CMV isolates

CMV sources[a]	Subgroups	Coat protein	
		Nucleotide	Amino acid
Fny-CMV	I	72.0	79.9
Mf-CMV	I	73.1	79.5
Kin-CMV	II	99.4	98.6
LS-CMV	II	99.5	98.6

[a]Homology of nucleotide and amino acid sequences of CMV coat protein genes were performed using a BLAST in the GenBank database (Fny-CMV: no. D10538, Mf-CMV: no. AJ276481, Kin-CMV: no. Z12818, and LS-CMV: no. AF127976).

```
PaFM1 MDKSGSPNASRTSRRRRPRRGSRSA SGADAGLRALTQQMLRLNKTLAIG 49
  LS  ------------------------ ----------K--R----- 49
  Mf  ----E-TS-G- N----------P-S---NF-V-S--LS-------A- 49

PaFM1 RPTLNHPTFVGSESCKPGYTFTSITLKPPEIEKGSYFGRRLSLPDSVTDY 99
  LS  ---------------------------------------------- 99
  Mf  ---I---------R-R----------K-DR---Y-K--L------E- 99

PaFM1 DKKLVSRIQIRINPLPKFDSTVWVTVRKVPSSSDLSVAAISAMFGDGNSP 149
  LS  ---------------------------------------------- 149
  Mf  ----------V----------------A------T------A--A-- 149

PaFM1 VLVYQYAASGVQANNKLLYDLSEMRADIGDMRKYAVLVYSKDDKLEKDEI 199
  LS  ---------------------------------------------- 199
  Mf  --------------------A----------------------A--T--L 199

PaFM1 VLHVDVEHQRIPISRMLPT. 218
  LS  ------------------. 218
  Mf  -----I------Y-GV--V. 218
```

Fig. 5. Alignment of the putative amino acid sequences of the coat proteins of PaFM1-CMV, LS-CMV (GenBank accession no. AF127976) and Mf-CMV (GenBank accession no. AJ276481). Identical sequences are indicated by dashed lines.

range, peptide mapping of viral CP, and nucleic acid hybridization (Edwards and Gonsalves, 1983; Gonda and Symons, 1978). The host range and symptomatology of PaFM1-CMV differed in some respect from those of other CMV isolates reported (Choi et al., 1998; Choi et al., 2001; Wahyuni et al., 1992). PaFM1-CMV induced systemic mild mosaic symptoms in several host plants for CMV diagnosis such as *D. stramonium*, *C. annuum*, *N. benthamina*, *N. occidentalis*, *N. tabacum* cv. Ky-57, and *C. pepo*. However, Mf- and LS-CMV induced severe mosaic symptoms in those plants. Of considerable interest are PaFM1-CMV's differences in terms of host reactions compared with well-known CMV isolates. Some of the differences in host range and symptoms may reflect differences in intrinsic property of PaFM1-CMV. Shintaku and Palukaitis (1990) reported that specific regions of CMV CP gene were delimited to affect systemic infection by CMV. Cucumoviruses were differentiated by PCR-restriction fragment length polymorphism with different enzymes (Choi et al., 1999). When the cDNA of PaFM1-CMV CP gene was digested with *Ava*I, *Eco*RI, *Eco*RV, *Hinc*II, *Hin*dIII, *Sal*I, and *Xho*I, the fragment pattern was identical with that of LS-CMV, which belonged to subgroup II (Fig. 5). In addition, results of sequence comparisons showed that CMV isolates within each subgroup share 91-99% amino acid and nucleotide sequence identity, whereas, isolates between subgroups share 76-84% sequence identity (Owen et al., 1990; Quemada et al., 1989; Rizos et al., 1992). There is a remarkably little sequence variation within the CP genes of the subgroup II strain of CMV (LS-CMV). In conclusion, results based on serological and molecular properties show that PaFM1-CMV belongs to CMV subgroup II, which is the first report in Korea. More detailed molecular characteristics such as pseudorecombinants analyses and full-length genomic RNA sequences will be required to define different pathological characteristics of the PaFM1-CMV.

References

Choi, J. K., Kim, H. J., Hong, J. S., Kim, D. W. and Lee, S. Y. 1998. Identification and differentiation of cucumber mosaic virus isolates in Korea. *Korean J. Plant Pathol.* 14:7-12.

Choi, H. S., Ryu, J. K., Ahn, K. K., Cho, J. D. and Kim, J. S. 2001. Cucumber mosaic Cucumovirus-CARNA5 causing bud necrosis on table tomato. *Plant Pathol. J.* 17:169-173.

Choi, S. K., Choi, J. K., Park, W. M. and Ryu, K. H. 1999. RT-PCR detection and identification of three species of cucumovirus with a genus specific single pair of primers. *J. Virol. Methods* 83:67-73.

Davies, C. and Symons, R. H. 1988. Further implication for the evolutionary relationship between triparticle plant viruses based on cucumber mosaic virus RNA3. *Virology* 165:216-224.

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

Francki, R. I. B., Randles, J. W., Chambers, T. C. and Wilson, S. B. 1966. Some properties of purified cucumber mosaic virus (Q strain). *Virology* 28: 729-741.

Gonda, T. J. and Symons, R. H. 1978. The use of hybridization analysis with complementary DNA to determine the RNA sequence homology between strains of plant viruses: Its application to several strains of cucumoviruses. *Virology* 88:361-370.

Laemmli, U. K. and Favre, M. 1973. Maturation of the head of bacteriophage T4. I. DNA packaging event. *J. Mol. Biol.* 80: 575-599.

Morris, T. J. and Dodds, J. A. 1979. Isolation and analysis of double- stranded RNA from virus-infected plant and fungal tissue. *Phytopathology* 69:854-858.

Mossop, D. W., Francki, R. I. B. and Gribvell, C. J. 1976. Comparative studies on tomato aspermy and cucumber mosaic

viruses. V. Purification and properties of a cucumber mosaic virus inducing severe chlorosis. *Virology* 74:544-546.

Nitta, N., Masuta, C., Kuwata, S. and Takanami, Y. 1988. Inoculation with RNAs 1 and 2 of cucumber mosaic virus induces viral RNA replicase activity in tobacco mesophyll protoplasts. *J. Gen. Virol.* 69:2695-2700.

Ouchterlony, O. 1968. Handbook of immunodiffusion and immunoelectrophoresis. *Prog. Allergy. Ann. Arbor Sci. Publ.* 135 p.

Owen, J., Shintaku, M., Aeschleman, P., Tahar, S. B. and Palukaitis, P. 1990. Nucleotide sequence and evolutionary relationship cucumber mosaic virus (CMV) strains: CMV RNA3. *J. Gen. Virol.* 71:2243-2249.

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

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

Rizos, H., Gunn, L. V., Pares, R. D. and Gillings, M. R. 1992. Differentiation of cucumber mosaic virus isolates using the polymerase chain reaction. *J. Gen. Virol.* 73:2099-2103.

Takanami, Y. 1981. A striking change in symptoms on cucumber mosaic virus-infected tobacco plants induced by a satellite RNA. *Virology* 109:120-126.

Shintaku, M. and Palukaitis, P. 1990. Genetic mapping of cucumber mosaic virus. In *Viral Genes and Plant Pathogenesis*, ed. by Pirone, T. P. and Shaw, J. G., pp. 157-164, Spriger-Verlag, New York.

Wahyuni, W. S., Dietzgen, R. G., Hanada, K. and Francki, R. I. B. 1992. Serological and biological variation between and within subgroup I and II strains of cucumber mosaic virus. *Plant Pathol.* 41:282-297.