

Full-Length cDNA Cloning and Nucleotide Sequence Analysis of Cucumber Mosaic Virus (Strain Kor) RNA2

Kwon, Chang Seob, Kyung Hee Paek¹, and Won Il Chung*

Department of Biological Sciences, Korea Advanced Institute of Science and Technology, Yu-Sung Gu, Taejeon, 305-701, Korea

¹Department of Agricultural Biology, College of Natural Resources and Graduate School of Biotechnology, Korea University, Seoul, 136-701, Korea

Full-length cDNA for RNA2 of cucumber mosaic virus strain Kor (Kor-CMV) was cloned downstream of synthetic T7 promoter by reverse transcriptase-polymerase chain reaction (RT-PCR). The clone could generate a full-length transcript corresponding to RNA2 in size when synthesized by T7 RNA polymerase. The complete nucleotide sequence has shown that the RNA2 is composed of 3,049 nucleotides and contains one functional open reading frame (ORF) of 2,574 nucleotides encoding 2a protein. The deduced translation product of the 2,574 nucleotides contains GDD motif which is a characteristic of RNA-dependent RNA polymerase (RdRp). The amino acid sequence analysis of the 2a protein has shown that the homology is found in decreasing order with O-CMV (98.8%), Y-CMV (98.7%), Fny-CMV (98.3%), K-CMV (94.9%), Ix-CMV (91.9%), and Q-CMV (74.9%). Kor-CMV is suggested to belong to subgroup I in the aspect of nucleotide sequence homology of RNA2.

Keywords : CMV, cucumber mosaic virus, RNA2, 2a protein, RdRp

To protect economically important crops against plant RNA viruses, several pathogen-derived virus resistance strategies have been developed (reviewed by Wilson, 1993). The resistance strategies were to express virus-encoded replicase protein gene, coat protein gene or antisense RNA in transgenic plants. The strongest resistance was often shown in the plants expressing an intact or mutant form of the replicase protein (reviewed by Wilson, 1993; Anderson *et al.*, 1992), but the resistance was strain-specific as well as virus-specific (Zaitlin *et al.*, 1994). Therefore, it is necessary to clone and characterize the replicase gene of specific virus strain of interest.

Cucumber mosaic virus (CMV) infects a wide range of hosts exceeding 800 species including cucumber, cabbage, and pepper plants (reviewed by Palukaitis *et al.*, 1992). CMV is a single-stranded and plus-sense RNA virus of which genome is composed of RNAs 1, 2 and 3. RNAs 1 and 2 express 1a and 2a proteins, respectively, both of which are virus-encoded replicase components (reviewed by

Palukaitis *et al.*, 1992). The purified viral RNA replicase from CMV-infected plants showed polymerase activity upon addition of CMV RNA as template (Hayes and Buck, 1990). The 2a protein contains GDD motif that is highly conserved in RNA replicase (RNA-dependent RNA polymerase) which is essential in the life cycle of animal and plant RNA viruses (Kamer and Argos, 1984). The transgenic tobacco plants expressing a mutant form of the 2a protein could show effective resistance against CMV infection (Anderson *et al.*, 1992).

Therefore, the cloning of full-length cDNA for CMV will be very useful in the development of replicase-mediated resistance strategy and in the molecular study of RNA replicase *per se*. Additionally, it is also necessary that the cloned cDNA should be transcribed to generate an intact and functional viral RNA for the study of virus replication (Rizzo and Palukaistis, 1990).

In this study, RNA2 in Kor-CMV (Korean isolate) was cloned in the form of full-length cDNA that can generate an intact RNA2 by T7 RNA polymerase. Sequencing analysis of the cDNA revealed the complete nucleotide sequence of Kor-CMV RNA2, and

*Corresponding author: Fax +82-42-869-2610
© 1996 by Botanical Society of Korea, Seoul

phylogenetic relationships between Kor-CMV and other CMV strains are presented here.

MATERIALS AND METHODS

Virus strain and RNA extraction

Kor-CMV used in this study was supplied from Korea Ginseng and Tobacco Research Institute. The propagation and purification of Kor-CMV were described in the previous paper (Kim *et al.*, 1996). Kor-CMV RNAs were extracted with phenol buffered with TNE (10 mM Tris-HCl, pH 7.5, 100 mM NaCl, 10 mM Na₂-EDTA) from virus particles, and then precipitated by ethanol. The resulting RNAs were dissolved in diethylpyrocarbonate-treated water and quantitated by measuring absorbance at 259 nm.

cDNA synthesis

The cDNA of Kor-CMV RNA2 was synthesized using AMV reverse transcriptase (Poscochem.). The reverse transcription reaction was performed at 40°C for 3 h from 10 µg of purified RNA in a 50 µl reaction volume which contained 500 µM dNTP, 100 pmole of 3' MB primer [GACA(GGATCC:*Bam*HI) (ACGCGT : *Mlu*I)GGTCTCCTTTTGGAGG], RNasin (0.5 U/µl), AMV reverse transcriptase (1.0 U/µl), and reaction buffer supplied from manufacturer. Then, the reaction mixture was extracted with phenol/chloroform, and precipitated with ethanol. The resulting pellet was dissolved in 20 µl water which could be used at least 4 times for polymerase chain reaction.

Polymerase chain reaction and cloning

Five µl of the obtained cDNA mixture was amplified using *Dynazyme* (GENOMED). Polymerase chain reaction (PCR) was carried out in a 50 µl containing additional 100 pmole of 3' MB primer, 100 pmole of 1,2-5' primer [ACG(GGATCC:*Bam*HI) (TAATACGACTCACTATA : T7 promoter) GTTTTATTACAA-GAGCGT], 500 µM dNTP, 5 unit of *Dynazyme*, and reaction buffer supplied from manufacturer. PCR was performed in a thermal cycler (Perkin Elmer Cetus) for 20 cycles with each cycle consisting of 94°C for 1 min, 60°C for 0.5 min, and 72°C for 3.5 min. The amplified products were digested with *Bam*HI, and then electrophoresed on 0.8% agarose gel. The PCR product corresponding to approximately 3 kb was eluted with JETSORB kit (GENOMED), and then li-

gated into pUC18. The resulting plasmid was designated as pT7Kor2T.

In vitro transcription

The pT7Kor2T purified with Wizard Midipreps DNA purification system (Promega) was linearized with *Mlu*I digestion, and then transcribed by T7 RNA polymerase (Promega). *In vitro* transcription was performed at 37°C for 1 h from the linearized template in a 50 µl reaction volume that contained T7 RNA polymerase (1.0 U/µl), 500 µM NTP, and RNasin (0.5 U/µl). The heat-denatured transcripts were electrophoresed on TAE-buffered agarose gel, and stained with ethidium bromide.

Sequencing analysis

The pT7Kor2T was digested with *Sal*I and *Sph*I, and serially deleted with Erase-a-Base system (Promega). The deleted cDNAs of various length were sequenced by dideoxy chain termination method using Sequenase Version 2.0 DNA Sequencing Kit (USB). For the sequencing of the other strand, the insert cDNA of the pT7Kor2T was subcloned in the opposite orientation by ligation after *Bam*HI digestion, resulting in pT7Kor2TR. The pT7Kor2TR was digested with *Sph*I and *Mlu*I, serially deleted from the 3' terminus of RNA2 cDNA, and then sequenced.

Computer analysis

The PC/Gene (Intelligenetics) package programs were used in the analysis of CMV genomic RNAs and phylogeny. The published sequence sources of RNA2s used in computer analysis are following: strain Q (Rezaian *et al.*, 1984), Fny (Rizzo and Palukaitis, 1988), Y (Kataoka *et al.*, 1990), O (Hase *et al.*, 1992), K (Hellwald and Palukaitis, 1994), and Ix (McGarvey *et al.*, 1995).

RESULTS AND DISCUSSION

Strategy for the cloning of the full-length cDNA

Two primers used in the reverse transcriptase-polymerase chain reaction (RT-PCR) were specifically designed to clone the complete cDNA for RNAs 1 and 2 of Kor-CMV (Hays and Buck, 1993). The one is 1,2-5' primer that contains *Bam*HI site, T7 promoter sequence, and 5' terminal sequence. The 5'

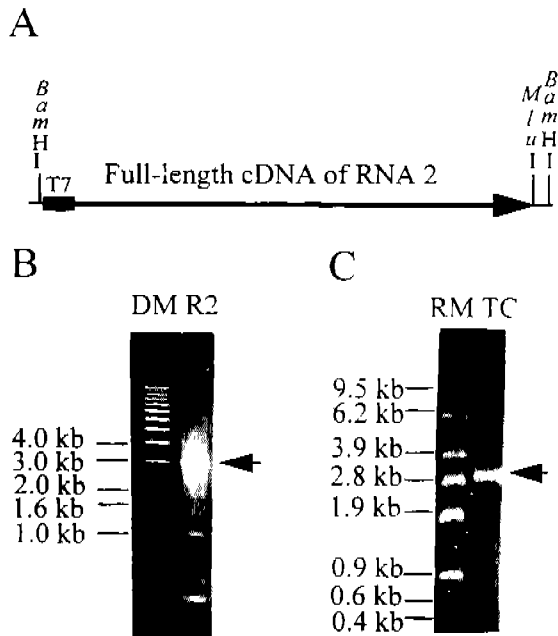


Fig. 1. Full-length cDNA cloning of Kor-CMV RNA2. (A) Schematic representation of pT7Kor2T that contains the cloned full-length cDNA of Kor-CMV RNA2. T7 indicates synthetic T7 promoter. (B) Agarose gel (0.8%) electrophoresis of RT-PCR products amplified from the first strand cDNA synthesized from CMV RNAs. 1,2-5' primer and 3' MB primer were used in the PCR amplification. DM and R2 indicates 1 kb size marker and RT-PCR products, respectively. (C) Agarose gel electrophoresis of *in vitro* transcripts from the pT7Kor2T. *In vitro* transcription was performed by T7 RNA polymerase from the *MluI*-linearized pT7Kor2T, and the resulting transcripts were electrophoresed on 1.0% agarose gel. RM indicates RNA size marker; TC, *in vitro* transcripts.

terminal sequence is invariably conserved among various CMV strains and highly conserved between RNAs 1 and 2. The 1,2-5' primer was thought to favor the amplification of the cDNA of RNA1 rather than RNA2 because the primer would have perfect match with the 5' terminus of the RNA1 in comparison with one T insertion (5'-GTTTTA-) at 5'-GTTTA- of 5' terminus of the RNA2. Interestingly, the major PCR product was corresponding to RNA2 (3.0 kb) rather than RNA1 (3.4 kb) in our PCR condition (Fig. 1B). This reflects that the size difference rather than the slight T_m difference might be an important factor in the first-strand cDNA synthesis and PCR. The 5' half cDNA fragment of RNA1 could be amplified selectively with the use of 3' primer which is highly conserved minus-sense sequence in the middle of RNA1 (unpublished result). The T7 promoter sequence in the 1,2-5' primer was designed

to initiate transcription at the G of the 5'-GTTTTA- which is thought to be the 5' proximal sequence of Kor-CMV RNA2 except viral cap structure (Fig. 2).

The other is 3' MB primer that contains *BamHI* site, *MluI* site, and the complementary sequence of 3' terminus. The *BamHI* sites in the 1,2-5' primer and 3' MB primer were inserted on purpose for the easy cloning of the amplified cDNA with *BamHI* digestion. The complementary sequence to the 3' MB primer was derived from the published sequence of Kor-CMV RNA3 because the 3' proximal sequence was thought to be highly conserved among RNAs 1, 2, 3, and 4 in the same virus strain (Kim *et al.*, 1996). The *MluI* cleavage of the cloned cDNA of RNA2 will terminate *in vitro* transcription at A of -CCA-3' which is thought to be the 3' proximal sequence of Kor-CMV RNA2. Therefore, it is possible to generate only virus sequence by *in vitro* transcription with the *MluI*-linearized template (Fig. 1A). Fig. 1C shows that the full-length transcript corresponding to RNA2 (3.0 kb) was generated by *in vitro* transcription from the pT7Kor2T which has the complete cDNA of RNA2.

Analysis of 5' and 3' untranslated sequences (UTRs) and coding regions

Kor-CMV RNA2 has 5' untranslated region (UTR) of 86 nucleotides, one functional coding region of 2574 nucleotides, and 3' untranslated region (UTR) of 389 nucleotides (Fig. 2). The 5' UTR contains conserved sequences homologous with internal control regions (ICRs) motifs that exist in the promoters of tRNA genes (Fig. 2; Marsh *et al.*, 1989). The ICRs motifs are also found in brome mosaic virus (BMV) which is another member of tripartite plant RNA viruses (Marsh *et al.*, 1989). The mutational analysis around the ICRs 1 and 2 in BMV has shown that the ICRs 1 and 2 are essential with the sequence- and structure-specific manner in the amplification of plus-sense RNA from minus-sense RNA (Pogue and Hall, 1992). From the viewpoint of similarity between CMV and BMV, the proposed structure of the 5' terminus is likely to be important in the replication of CMV RNAs 1 and 2 (Fig. 3A).

The translation product of the functional coding region of Kor-CMV RNA2 is 2a protein of 96.7 kDa which is a virus-encoded component of RNA-dependent RNA polymerase (RdRp). The 2a protein of Kor-CMV RNA2 contains "polymerase site" which is characterized by GDD and TGxxxTxxxNT motifs in RNA-dependent polymerases (Fig. 2; Morch *et al.*,

___ICR 2___
gt tta ttt aca aga gcg tAC GGT TCA ACC CCT GCC TCC CCT GTA AAA CTC CCT AGA CTT TAA AAC TTT CTT TCT AGT ATC TTT TCT TCT GCT TCC CCT GCC CCC GCA
Za: MET Ala Ser Pro Ala Pro Ala

108 TTC TCA CTA GCC AAT CTT TTG AAC GGT AGT TAT GGT GTC GAC ACT CCC GAG GAT GTG GAA CGT TTG CGA TCT GAG CAA CGT GAA GAG GCT GCT GCG GCC TGT CGT AAC
Phe Ser Leu Ala Asn Leu Leu Asn Gly Ser Tyr Gly Val Asp Thr Pro Glu Asp Val Glu Arg Leu Arg Ser Glu Gln Arg Glu Glu Ala Ala Ala Cys Arg Asn

216 TAC AGG CCC CTA CCC GCT GTG GAT GTC AGC GAG AGT GTC ACA GAG GAC GCG CAT TCC CTC CGA ACT CCT GAC GGA GCT CCC GCT GAA GCG GTG TCT GAT GAG TTT GTA
Tyr Arg Pro Leu Pro Ala Val Asp Val Ser Glu Ser Val Thr Glu Asp Ala His Ser Leu Arg Thr Pro Asp Gly Ala Pro Ala Glu Ala Val Ser Asp Glu Phe Val

324 ACT TAT GGT GCT GAA GAT TAC CTT GAA AAA TCT GAT GAT GAG CTC CTT GTC GCT TTT GAG ACS ATG GTC AAA CCC ATG CGT ATC GGA CAA CTA TGG TGC CCC GCG TTT
Thr Tyr Gly Ala Glu Asp Tyr Leu Glu Lys Ser Asp Asp Glu Leu Leu Val Ala Phe Glu Thr MET Val Lys Pro MET Arg Ile Gly Gln Leu Trp Cys Pro Ala Phe

432 AAT AAA TGT TCT TTT ATT TCC AGC ATT GCT ATG GCC AGA GCT TTG CTG TTG GCA CCT AGA ACA TCC AAC CGA ACC ATG AAG TGT TTT GAA GAC CTG GTC GCG GCT ATT
Asn Lys Cys Ser Phe Ile Ser Ser Ile Ala MET Ala Arg Ala Leu Leu Leu Ala Thr Leu Leu Ser Asn Arg Thr MET Lys Cys Phe Glu Asp Leu Val Ala Ala Ile

540 TAC ACT AAA TCT GAT TTC TAT TAT GGT GAA GAG TGT GAA GCT GAC GAC GTT CAG ATG GAT ATC TCG TCT CSC GAT GTA CCC GGT TAT TCT TTC GAA CCG TGG TCC CGA
Tyr Thr Lys Ser Asp Phe Tyr Tyr Gly Glu Glu Cys Glu Ala Asp Asp Val Gln MET Asp Ile Ser Ser Arg Asp Val Pro Gly Tyr Ser Phe Glu Pro Trp Ser Arg

648 ACG TCT GGA TTC GAA CCG CCG CCC ATT TGT GAA GCG TGC GAC ATG ATC ATG TAC CAG TGC CCG TGT TTC GAT TTC AAT GCT TTA AAG AAA TCG TGC GCT GAG AGG ACT
Thr Ser Gly Phe Glu Pro Pro Pro Ile Cys Glu Ala Cys Asp MET Ile MET Tyr Gln Cys Pro Cys Phe Asp Phe Asn Ala Leu Lys Lys Ser Cys Ala Glu Arg Thr

756 TTC GCT GAT GAC TAT GTT ATT GAA GGT TTA GAT GGT GTT GTT GAT AAT GCG ACT CTG TTG TCG AAT TTG GGT CCA TTT TTG GTA CCC GTG AAG TGC CAA TAT GAA AAT
Phe Ala Asp Val Tyr Val Ile Val Ser Asp Thr Leu Asp Gly Val Val Asp Asn Ala Thr Leu Leu Ser Asn Gly Asn Pro Phe Leu Val Lys Glu Asp Leu Val Ala Ile

854 TGT CCA ACA CCA ACC CTC GCG AIT CCT CCG GAT TTA AAT GGT GCT ACT GAT CGT GTT GAT ATT AAT TTA GTT CAA TCC ATT TGT GAC TCG ACT CTG CCC ACT CAT AGT
Cys Pro Thr Pro Thr Leu Ala Ile Pro Pro Asp Leu Asn Arg Ala Thr Asp Arg Val Asp Ile Asn Leu Val Gln Ser Ile Cys Asp Ser Thr Leu Pro Thr His Ser

972 AAT TAC GAC GAC TCT TTT CAT CAA GTG TTT GTC GAA AGT GCA GAC TAC TCT ATA GAT CTG GAT CAT GTT AGA CTT CGA CAG TCT GAT CTT ATC GCG AAA ATT CCA GAT
Asn Tyr Asp Asp Ser Phe His Gln Val Phe Val Glu Ser Ala Asp Tyr Ser Ile Asp Leu Asp His Val Arg Leu Arg Gln Ser Asp Leu Ile Ala Lys Ile Pro Asp

1080 TCA GGG CAT ATG ATA CCG GTT CTG AAC ACC GGG AGC GGT CAC AAG AGA GTA GGT ACA CCG AAG GAG GTC CTT ACA GCA ATT AAG AAA CGT AAT GCT GAC GTT CCA GAG
Ser Gly His MET Ile Pro Val Leu Asn Thr Gly Ser Gly His Lys Arg Val Gly Thr Thr Lys Glu Val Leu Thr Ala Ile Lys Lys Arg Asn Ala Asp Val Pro Glu

1188 CTA GGT GAT TCC GTT AAT CTG TCT AGA TTG AGT AAA GCT GTG GCT GAG AGA TTC TTC ATT TCA TAC ATC AAT GGT AAC TCT CTA GCA TCC AGT AAC TTT GTC AAT GTC
Leu Gly Asp Ser Val Asn Leu Ser Arg Leu Ser Lys Ala Val Ala Glu Arg Phe Phe Ile Ser Tyr Ile Asn Gly Asn Ser Leu Ala Ser Ser Asn Phe Val Asn Val

1296 GTT AGT AAC TTC CAC GAT TAC ATG GAA AAA TGG AAG TCC TCA GGT CTT TCT TAT GAT GAT CTC CCA GAT CTT CAT GCT GAG AAT TTA CAG TTT TAT GAT CAC ATG ATA
Val Ser Asn Phe His Asp Tyr MET Glu Lys Trp Lys Ser Ser Gly Leu Ser Tyr Asp Asp Leu Pro Asp Leu His Ala Glu Asn Leu Gln Phe Tyr Asp His MET Ile

1404 AAA TCT GAT GTG AAA CCT GTG GTG AGC GAC ACA CTC AAT ATC GAC AGA CCG GTT CCA GCT ACT ATA CCG TAT CAT AAG AAG AGT ATA ACC TCC CAG TTC TCA CCG TTA
Lys Ser Asp Val Lys Pro Val Val Ser Asp Thr Leu Asn Ile Asp Arg Pro Val Pro Ala Thr Ile Thr Tyr His Lys Lys Ser Ile Thr Ser Gln Phe Ser Pro Leu

1512 TTC ACA GCG CTA TTC GAG CGT TTC CAG AGA TGC CTT CGA GAA CGT ATT ATT CTT CCT GTT GGT AAG ATT TCA TCC CTT GAG ATG GCA GGA TTT GAT GTC AAA AAC AAG
Phe Thr Ala Leu Phe Glu Arg Phe Gln Arg Cys Leu Arg Glu Arg Ile Ile Leu Pro Val Gly Lys Ile Ser Ser Leu Glu MET Ala Gly Phe Asp Val Lys Asn Lys

1620 CAC TGC CTC GAA ATT GAT CTG TCT AAG TTT GAT AAA TCT CAA GGT GAA TTC CAT TTG CTA ATT CAG GAA CAT ATT TTG AAT GGT CTA GGA TGT CCA GCT CCG ATA ACT
His Cys Leu Glu Ile Asp Leu Ser Lys Phe Asp Lys Ser Gln Gly Glu Phe His Leu Leu Ile Gln Glu His Ile Leu Asn Gly Leu Gly Cys Pro Ala Pro Ile Thr

1728 AAG TGG TGG TGT GAT TTT CAC CGA TTC TCT TAC ATT AGA GAC CGT AGA GCT GGT GTT GGT ATG CCT ATT AGT TTC CAG AGA CGA ACT GGT GAT GCA TTC ACT TAT TTT
Lys Trp Trp Cys Asp Phe His Arg Phe Ser Tyr Ile Arg Asp Arg Arg Ala Gly Val Gly MET Pro Ile Ser Phe Gln Arg Arg Thr Gly Asp Ala Phe Thr Tyr Phe
(S/T)GxxxTxxxN(S/T)
GDD

1836 GGC AAT ACC ATT GTC ACC ATG GCA GAG TTT GCC TGG TGT TAC GAC ACC GAC CAA TTC GAA AAG CTT TTA TTC TCG GGC GAT GAC TCT CTA GGA TTT TCA TTG CTT CCC
Gly Asn Thr Ile Val Thr MET Ala Glu Phe Ala Trp Cys Tyr Asp Thr Asp Gln Phe Glu Lys Leu Leu Phe Ser Gly Asp Asp Ser Leu Gly Phe Ser Leu Leu Pro

1944 CCT GTT GGT GAT CCG AGT AAA TTC ACA ACT CTT TTC AAC ATG GAA GCT AAG GTG ATG GAA CCT GCA GTA CCA TAT ATT TGT TCG AAG TTC TTA CTC TCT GAC GAG TTC
Pro Val Gly Asp Pro Ser Lys Phe Thr Thr Leu Phe Asn MET Glu Ala Lys Val MET Glu Pro Ala Val Pro Tyr Ile Cys Ser Lys Phe Leu Leu Ser Asp Glu Phe

2052 GGT AAC ACA TTT TCC GTT CCA GAT CCA TTG CSC GAG GTT CAG CCG TTA GGA ACA AAG AAA ATT CCC TAT TCT GAC AAT GAT GAA TTC TTG TTT GCT CAC TTC ATG AGC
Gly Asn Thr Phe Ser Pro Leu Arg Glu Val Gln Arg Leu Gly Thr Lys Lys Ile Pro Tyr Ser Asp Asn Asp Glu Phe Leu Phe Ala His Phe MET Ser

2160 TTT GTT GAT CGA TTG AAG TTT TTG GAT CGA ATG TCT CAG TCG TGT ATC GAT CAG CTT TCA ATT TTC TTT GAA TTG AAA TAC AAG AAG TCT GGG GAA GAG GCT GCT TTA
Phe Val Asp Arg Leu Lys Phe Leu Asp Arg MET Ser Gln Ser Cys Ile Asp Gln Leu Ser Ile Phe Phe Glu Leu Lys Tyr Lys Lys Ser Gly Glu Glu Ala Ala Leu

2268 ATG TTA GGC GGC TTT AAG AAA TAT ACC GCT AAT TTT CAG TCC TAC AAG GAA CTC TAC TAT TCA GAT CGT CGT CAG TGC GAA TTG ATC AAT TCG TTT TGT AGT ACA GAG
MET Leu Gly Ala Phe Lys Lys Tyr Thr Ala Asn Phe Gln Ser Tyr Lys Glu Leu Tyr Tyr Ser Asp Arg Arg Gln Cys Glu Leu Ile Asn Ser Phe Cys Ser Thr Glu

2376 TTC AGG GTT GAG CGT GTA AAT TCC AAT AAA CAG CGA AAG AAA TAT GGA ATT GAA CGT AGG TGC AAT GAC AAA CGT CGA ACT CCA ACT GGC TCG TAT GGT GGA GGC GAA
Phe Arg Val Glu Arg Val Asn Ser Asn Lys Gln Arg Lys Lys Tyr Gly Ile Glu Arg Arg Cys Asn Asp Lys Arg Arg Thr Pro Thr Gly Ser Tyr Gly Gly Gly Glu
Zb: MET Glu Leu Asn Val Gly Ala MET Thr Asn Val Glu Leu Gln Leu Ala Arg MET Val Glu Ala Lys

2484 GAA GCA GAG ACG AAG ATC TCA CAA GCA GAA TCG ACG GGA ACG AGG TCA CAA AAG TCC CAG CGA GAG AGC GCG TTC AAA TCT CAG ACT GTT CCG CTT CCT ACC GTT CTA
Glu Ala Glu Thr Lys Ile Ser Gln Ala Glu Ser Thr Gly Thr Arg Ser Gln Lys Ser Gln Arg Glu Ser Ala Phe Lys Ser Gln Thr Val Pro Leu Pro Thr Val Leu
Lys Gln Arg Arg Arg Ser His Lys Gln Asn Arg Arg Glu Arg Gly His Lys Ser Pro Ser Glu Arg Ala Arg Ser Asn Leu Arg Leu Phe Arg Phe Leu Pro Phe Tyr

2592 TCA AGT AGA TGG TTC GGA ACT GAC AGG GTC GAG CCG CCA TGT GAA CGT GGC GGA GTT ACC CGA GCC TGA GGC CTC TCG TTT AGA GTT ATC GGC GGA AGA CCA TGA GTT
Ser Ser Arg Trp Phe Gly Thr Asp Arg Val Glu Pro Pro Cys Glu Arg Gly Gly Val Thr Arg Ala
Gln Val Asp Gly Ser Glu Leu Thr Gly Ser Ser Arg His Val Asn Val Ala Glu Leu Pro Glu Pro Glu Ala Ser Arg Leu Glu Leu Ser Ala Glu Asp His Glu Phe

2700 TGA CGA TAC GGA TTG GTT CSC CCG TAA CGA ATG GGC GGA AAG TGC TTT CTG AAA CCT CCC CTT CCG CAA CTC CCT CCG GTC CTG TGG CCG GAG CTG AGT TGG CAG TAT
Asp Asp Thr Asp Trp Phe Ala Gly Asn Glu Trp Ala Glu Gly Ala Phe - -

2808 TGC TAT AAA CTG ICT GAA GTC ACT AAA CAC ATT GTG GTG AAC GGG TTG TCC ATC CAG CTT ACG GCT AAA ATG GTC AGT CGT AGA GAA ATC TAC GCC AGC AGA CTT ACA
2916 AGT CTC TGA GGC ACC TTT GAA ACC ATC TCC TAG GTT TCT TCG GAA GGA CTT CCG TCC GTG TAC TTC TAG CAC AAC GTG CTA GTT TAG GGT ACG GGT GCC CCC CCA CTT
3024 TCG TGG GGG cct cca aaa gga gac ca

Fig. 2. Nucleotide sequence of RNA2 and amino acid sequences of putative 2a and 2b proteins in the Kor-CMV. The conserved sequence showing homology with ICR 2 motif is indicated by ICR 2. The highly conserved 'polymerase site' is represented by GDD and (S/T)GxxxTxxxN(S/T) which are consensus sequences within RNA-dependent polymerases (Morch *et al.*, 1988). The nucleotide sequences written in lower case mean the sense sequences of the primer binding sites in RT-PCR for the cloning of Kor-CMV RNA2. The nucleotide and amino acid sequences are available in the GenBank under accession number U66287.

1988). Additionally, Kor-CMV RNA2 contains homologous region with overlapping gene encoding 2b protein that has been found in Q-CMV (Fig. 2; Ding *et al.*, 1994). The 2b protein of unknown function is expressed only from a subgenomic RNA4A (Ding *et al.*, 1995). However, RNA4A was not found in tobacco leaves infected with Kor-CMV (unpublished result), so that the putative 2b protein of Kor-CMV is not likely to be expressed.

The 3' UTRs of CMV RNAs 1, 2, and 3 contain the conserved sequences showing tRNA-like structure (Fig. 3B) that is thought to be the recognition site of viral RdRp. Although some base changes have been found in the 3' terminus of Kor-CMV RNA2 compared with those of Fny-, Y-, K-, Ix-, and O-CMV (data not shown), the whole structure of stem and loop is conserved well (Fig. 3B). Since the 3' terminal sequence of Q-CMV (subgroup II) is considerably different from that of Fny-CMV (subgroup I), it seems that the secondary structure is more important than the simple primary sequence in the function of 3' terminus (reviewed by Palukaitis *et al.*, 1992).

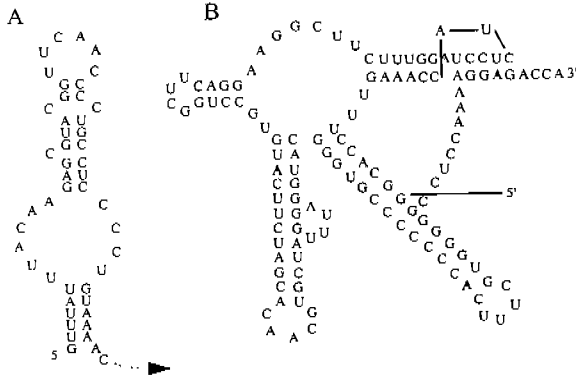


Fig. 3. Secondary structure models of plus-sense RNA sequences at the 5' (A) and 3' (B) termini of Kor-CMV RNA2. The structures were adapted from the similar models for Q-CMV RNAs by Joshi *et al.* (1983) and Pogue and Hall (1992).

Analysis of nucleotide and amino acid sequence homology

CMV has been divided into two subgroups based on serological relationships, peptide mapping, and nucleic acid hybridization (reviewed by Palukaitis *et al.*, 1992). Kor-CMV RNA2 shows nucleotide sequence homology in decreasing order with O- (97.5%), Y- (97.3%), Fny- (97.2%), K- (92.0%), Ix- (90.0%), and Q-CMV (72.5%). From the nucleotide sequence homology, Kor-CMV is confirmed to belong to subgroup I (For RNA3, refer to Kim *et al.*, 1996)

Q	MISPPPTFSPANLLNGSYGVDTPPEEVERVRREQREDEAAALRNYKPLPAVDVSESVRDEPIVSQTVTAAPVTSVDDAFVFSGAEDYLEMSPSELLSAFE	100
K	MAFPAPAFSLANLLNGSYGVDTPPEEVERVRSEQREEAACRNYRPLPAVDVSESVDEDAHSL-RTPDGAPSEEVSVFEVTVYGAEDYLEKSDDELLVAFE	99
Ix	MAFPAPAFSLANLLNGTYGVDTPPEEVERVRSEQREEAACRNYRPLPAVDVSESVDEDAHSL-RTPDGAPSEEVSVFEVTVYGAEDYLEKSDDELLVAFE	99
Fny	MAFPAPAFSLANLLNGSYGVDTPEDVERLRSEQREEAACRNYRPLPAVDVSESVDEDAHSL-RTPDGAPAEAVSDEFVTVYGAEDYLEKSDDELLVAFE	99
O	MASPPAPAFSLANLLNGSYGVDTPEDVERLRSEQREEAACRNYRPLPAVDVSESVDEDAHSL-RTPDGAPAEAVSDEFVTVYGAEDYLEKSDDELLVAFE	99
Y	MAFPAPAFSLANLLNGSYGVDTPEDVERLRSEQREEAACRNYRPLPAVDVSESVDEDAHSL-RTPDGAPAEAVSDEFVTVYGAEDYLEKSDDELLVAFE	99
Kor	MASPPAPAFSLANLLNGSYGVDTPEDVERLRSEQREEAACRNYRPLPAVDVSESVDEDAHSL-RTPDGAPAEAVSDEFVTVYGAEDYLEKSDDELLVAFE	99
	* * * * *	
Q	LMWKPLRVGEVLCSSFDRLSFLISSVAMARTLLAPLTSRTLRKRFEDLVAAILVTKDFLEFD- - -DGPQTDSVQSDVPGYIFEPGQHSSEGFEPFICAK	196
K	TWVKPMRIGQLWCPAFNKCSFISSIAMARALLAPRTSHRTMKCFEDLVAAILYTKSDFYVDECEADDVQIDISSRDVPGYSEFPWSRTSGFEPFICEA	199
Ix	TWVKPMRIGQLWCPAFNKCSFISSIAMARALLVPNTPNRTMKCFEDLVAAILYTKSDFYVDECEADDVQINISSRDVPGYSEFPWSRTSGFEPFICEA	199
Fny	TWVKPMRIGQLWCPAFNKCSFISSIAMARALLAPRTSHRTMKCFEDLVAAILYTKSDFYVSECEADDAGIDISSRDVPGYSEFPWSRTSGFEPFICEA	199
O	TWVKPMRIGQLWCPAFNKCSFISSIAMARALLAPRTSHRTMKCFEDLVAAILYTKSDFYVSECEADDAGIDISSRDVPGYSEFPWSRTSGFEPFICEA	199
Y	TWVKPMRIGQLWCPAFNKCSFISSIAMARALLAPRTSHRTMKCFEDLVAAILYTKSDFYVSECEADDAGIDISSRDVPGYSEFPWSRTSGFEPFICEA	199
Kor	TWVKPMRIGQLWCPAFNKCSFISSIAMARALLAPRTSHRTMKCFEDLVAAILYTKSDFYVSECEADDVQMDISSRDVPGYSEFPWSRTSGFEPFICEA	199
	* * * * *	
Q	CDLILYQPCPCDFNALRESKAERTFADDYVIEGLDGVVDNATLLSNLGFPLVPVKQYKCPPTIAPPPDLNRRATDRVDINLVGSCIDSTLPTHSNYDD	296
K	CDMIMYQPCPCDFNALKKSCAERTFADDYVIEGLDGVVDNATLLSNLGFPLVPVKQYKCPPTIAPPPDLNRRATDRVDINLVGSCIDSTLPTHSNYDD	299
Ix	CNMIMYQPCPCDFNALKKSCAERTFADDYVIEGLDGVVDNATLLSNLGFPLVPVKQYKCPPTIAPPPDLNRRATDRVDINLVGSCIDSTLPTHSNYDD	299
Fny	CDMIMYQPCPCDFNALKKSCAERTFADDYVIEGLDGVVDNATLLSNLGFPLVPVKQYKCPPTIAPPPDLNRRATDRVDINLVGSCIDSTLPTHSNYDD	299
O	CDMIMYQPCPCDFNALKKSCAERTFADDYVIEGLDGVVDNATLLSNLGFPLVPVKQYKCPPTIAPPPDLNRRATDRVDINLVGSCIDSTLPTHSNYDD	299
Y	CDMIMYQPCPCDFNALKKSCAERTFADDYVIEGLDGVVDNATLLSNLGFPLVPVKQYKCPPTIAPPPDLNRRATDRVDINLVGSCIDSTLPTHSNYDD	299
Kor	CDMIMYQPCPCDFNALKKSCAERTFADDYVIEGLDGVVDNATLLSNLGFPLVPVKQYKCPPTIAPPPDLNRRATDRVDINLVGSCIDSTLPTHSNYDD	299
	* * * * *	
Q	SFHQVFVDSADYSTMDHVRRLRQSDLIAPDGGHMLPVLNTGSGHGRVGTTKVEVLTAIKKRNADVPELGDVSNLSRLSKAVAEFRFISYINGNSLASSN	396
K	SFHQVFVESADYSIDLHVRLRQSDLIAPDGGHMLPVLNTGSGHGRVGTTKVEVLTAIKKRNADVPELGDVSNLSRLSKAVAEFRFIPVINGNSLASSN	399
Ix	SFHQVFVESADYSIDLHVRLRQSDLIAPDGGHMLPVLNTGSGHGRVGTTKVEVLTAIKKRNADVPELGDVSNLPRLSKAVAEFRFIPVINGNSLASSN	399
Fny	SFHQVFVESADYSIDLHVRLRQSDLIAPDGGHMLPVLNTGSGHGRVGTTKVEVLTAIKKRNADVPELGDVSNLSRLSKAVAEFRFIPVINGNSLASSN	399
O	SFHQVFVESADYSIDLHVRLRQSDLIAPDGGHMLPVLNTGSGHGRVGTTKVEVLTAIKKRNADVPELGDVSNLSRLSKAVAEFRFIPVINGNSLASSN	399
Y	SFHQVFVESADYSIDLHVRLRQSDLIAPDGGHMLPVLNTGSGHGRVGTTKVEVLTAIKKRNADVPELGDVSNLSRLSKAVAEFRFIPVINGNSLASSN	399
Kor	SFHQVFVESADYSIDLHVRLRQSDLIAPDGGHMLPVLNTGSGHGRVGTTKVEVLTAIKKRNADVPELGDVSNLSRLSKAVAEFRFIPVINGNSLASSN	399
	* * * * *	

Fig. 4. Alignment of the deduced amino acid sequences in the 2a proteins from the published nucleotide sequences of Q-, K-, Ix-, Fny-, O-, Y-, and Kor-CMV. Stars indicate the positions in the perfectly conserved amino acids. The positions in the chemically similar amino acids are indicated by '!'. Four conserved motifs in RNA-dependent polymerases are represented by A, B, C, and D (Poch *et al.*, 1987) and eight conserved motifs in RNA-dependent RNA polymerases are designated by I, II, III, IV, V, VI, VII, and VIII (Koonin, 1991). The nearly invariant amino acids in RNA-dependent polymerases are underlined (Poch *et al.*, 1987).

	I	II	III	
Q	FVNVSNFHAYMCKWPKSSGLSYDDL	PDLHAENLQFYDHMKSVDKPVVSDTLNDRPVPATITVHKKGTISQFSPLFTALFERFORCLRERVILPVGKIS	496	
K	FVNVSNFHDYMEKWKSSGLSYDDL	PDLHAENLQFYDHMKSVDKPVVSDTLNDRPVPATITVHKKGTISQFSPLFTALFERFORCLRERILPVGKIS	499	
Ix	FVNVSNFHDYMEKWKSSGLSYDDL	PDLHAENLQFYDHMKSVDKPVVSDTLNDRPVPATITVHKKGTISQFSPLFTALFERFORCLRERVILPVGKIS	499	
Fny	FVNVSNFHDYMEKWKSSGLSYDDL	PDLHAENLQFYDHMKSVDKPVVSDTLNDRPVPATITVHKKGTISQFSPLFTALFERFORCLRERILPVGKIS	499	
O	FVNVSNFHDYMEKWKSSGLSYDDL	PDLHAENLQFYDHMKSVDKPVVSDTLNDRPVPATITVHKKGTISQFSPLFTALFERFORCLRERILPVGKIS	499	
Y	FVNVSNFHDYMEKWKSSGLSYDDL	PDLHAENLQFYDHMKSVDKPVVSDTLNDRPVPATITVHKKGTISQFSPLFTALFERFORCLRERILPVGKIS	499	
Kor	FVNVSNFHDYMEKWKSSGLSYDDL	PDLHAENLQFYDHMKSVDKPVVSDTLNDRPVPATITVHKKGTISQFSPLFTALFERFORCLRERILPVGKIS	499	
	IV	V		
Q	SLEMTGFSVLNKHKCLEIDL	SKFDKSGGEPHLLIQEHLNGLGCPAPITKWNCFHFRFSYIRDRRAGVGMPISEFQRRIT@DAFTYFGNTIVTMAEFAWCYDT	596	
K	SLEHAGFDVKNKHKCLEIDL	SKFDKSGGEPHLLIQEHLNGLGCPAPITKWNCFHFRFSYIRDRRAGVGMPISEFQRRIT@DAFTYFGNTIVTMAEFAWCYDT	599	
Ix	SLEMSGFDVKNKHKCLEIDL	SKFDKSGGEPHLLIQEHLNGLGCPAPITKWNCFHFRFSYIRDRRAGVGMPISEFQRRIT@DAFTYFGNTIVTMAEFAWCYDT	599	
Fny	SLEHAGFDVKNKHKCLEIDL	SKFDKSGGEPHLLIQEHLNGLGCPAPITKWNCFHFRFSYIRDRRAGVGMPISEFQRRIT@DAFTYFGNTIVTMAEFAWCYDT	599	
O	SLEHAGFDVKNKHKCLEIDL	SKFDKSGGEPHLLIQEHLNGLGCPAPITKWNCFHFRFSYIRDRRAGVGMPISEFQRRIT@DAFTYFGNTIVTMAEFAWCYDT	599	
Y	SLEHAGFDVKNKHKCLEIDL	SKFDKSGGEPHLLIQEHLNGLGCPAPITKWNCFHFRFSYIRDRRAGVGMPISEFQRRIT@DAFTYFGNTIVTMAEFAWCYDT	599	
Kor	SLEHAGFDVKNKHKCLEIDL	SKFDKSGGEPHLLIQEHLNGLGCPAPITKWNCFHFRFSYIRDRRAGVGMPISEFQRRIT@DAFTYFGNTIVTMAEFAWCYDT	599	
	VI	VII	VIII	
Q	DQFDRLLFSSGDDSLGFSVLPV	GGDPSKFTTLFNMEAQVMEPAVPI	CSKFLLSDEFGNITFSVPDPLREVQRLGTTKIPYSDNDEFLFAHFMSFVDRLKFL	695
K	DQFEKLLFSSGDDSLGFSVLPV	GGDPSKFTTLFNMEAQVMEPAVPI	CSKFLLSDEFGNITFSVPDPLREVQRLGTTKIPYSDNDEFLFAHFMSFVDRLKFL	699
Ix	DQFEKLLFSSGDDSLGFSVLPV	GGDPSKFTTLFNMEAQVMEPAVPI	CSKFLLSDEFGNITFSVPDPLREVQRLGTTKIPYSDNDEFLFAHFMSFVDRLKFL	699
Fny	DQFEKLLFSSGDDSLGFSVLPV	GGDPSKFTTLFNMEAQVMEPAVPI	CSKFLLSDEFGNITFSVPDPLREVQRLGTTKIPYSDNDEFLFAHFMSFVDRLKFL	699
O	DQFEKLLFSSGDDSLGFSVLPV	GGDPSKFTTLFNMEAQVMEPAVPI	CSKFLLSDEFGNITFSVPDPLREVQRLGTTKIPYSDNDEFLFAHFMSFVDRLKFL	699
Y	DQFEKLLFSSGDDSLGFSVLPV	GGDPSKFTTLFNMEAQVMEPAVPI	CSKFLLSDEFGNITFSVPDPLREVQRLGTTKIPYSDNDEFLFAHFMSFVDRLKFL	699
Kor	DQFEKLLFSSGDDSLGFSVLPV	GGDPSKFTTLFNMEAQVMEPAVPI	CSKFLLSDEFGNITFSVPDPLREVQRLGTTKIPYSDNDEFLFAHFMSFVDRLKFL	699
	C	D	E	
Q	DRMSQSCIDQLSIF	FELKYKKSSEAAALMLGAFK	KYTANFQSYKELYSDRRQCELINSFCSTEF	839
K	DRMTQSCIDQLSIF	FELKYKKSSEAAALMLGAFK	KYTANFQSYKELYSDRRQCELINSFCSTEF	858
Ix	DRMTQSCIDQLSIF	FELKYKKSSEAAALMLGAFK	KYTANFQSYKELYSDRRQCELINSFCSTEF	858
Fny	DRMSQSCIDQLSIF	FELKYKKSSEAAALMLGAFK	KYTANFQSYKELYSDRRQCELINSFCSTEF	857
O	DRMSQSCIDQLSIF	FELKYKKSSEAAALMLGAFK	KYTANFQSYKELYSDRRQCELINSFCSTEF	857
Y	DRMSQSCIDQLSIF	FELKYKKSSEAAALMLGAFK	KYTANFQSYKELYSDRRQCELINSFCSTEF	857
Kor	DRMSQSCIDQLSIF	FELKYKKSSEAAALMLGAFK	KYTANFQSYKELYSDRRQCELINSFCSTEF	857

Fig. 4. Continued.

because Kor-CMV shows higher homology with Fny-CMV (subgroup I) than Q-CMV (subgroup II).

The amino acid sequence of the 2a protein of Kor-CMV was aligned with those of Q-, K-, Ix-, Fny-, Y-, and O-CMV (Fig. 4). The 2a protein (polymerase) is the only viral protein containing motifs conserved throughout plus-sense RNA viruses. Eight conserved motifs were identified from the linear alignment of RNA-dependent RNA polymerases from plus-sense RNA viruses (Fig. 4; Koonin, 1991), whereas only four motifs were found in RNA-dependent polymerases including RNA-dependent DNA polymerases (Fig. 4; Poch *et al.*, 1989). The central part that contains conserved motifs is more conserved than N-terminal or C-terminal portion (Fig. 4). The 2a protein of Kor-CMV shows the highest homology with that of O-CMV (98.8%) and the lowest homology with that of Q-CMV (74.9%). Additionally, the 2a protein of Kor-CMV shows more homology with those of Y-CMV (98.7%) and Fny-CMV (98.3%) than those of K-CMV (94.9%) and Ix-CMV (91.9%). The phylogenetic tree based on the amino acid similarity reflects that the 2a protein of Kor-CMV is very closely related to those of O-, Y- and Fny-CMV (Fig. 5). Additionally, the tree represents that the 2a

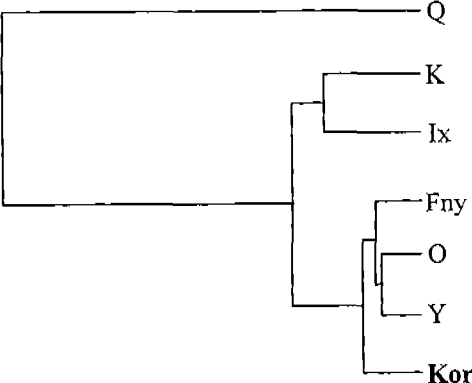


Fig. 5. Phylogenetic tree based on the amino acid similarities in the 2a proteins of Q-, K-, I-, Fny-, O-, Y-, and Kor-CMV RNA2.

protein of Q-CMV is distinctly different from the other CMV strains and the 2a proteins of Ix- and K-CMV are similar to each other.

ACKNOWLEDGMENTS

We thank Dr. Eun Kyung Park of Korea Ginseng and Tobacco Research Institute for the gift of Kor-CMV virions. This research was supported partly

from the Korea Research Foundation and from the Korea Ministry of Science and Technology.

LITERATURE CITED

- Anderson, J.M., P. Palukaitis and M. Zaitlin.** 1992. A defective replicase gene induces resistance to cucumber mosaic virus in transgenic tobacco plants. *Proc. Natl. Acad. Sci. USA* **89**: 8759-8763.
- Ding, S.-W., B.-J. Anderson, H.-R. Haase and R. Symons.** 1994. New overlapping gene encoded by the cucumber mosaic virus genome. *Virology* **198**: 593-601.
- Ding, S.-W., J.P. Rathjen, W.-X. Li, R. Swanson, H. Healy and R. Symons.** 1995. Efficient infection from cDNA clones of cucumber mosaic cucumovirus RNAs in a new plasmid vector. *J. Gen. Virol.* **76**: 459-464.
- Hase, S., A. Karasawa, H. Takahashi and Y. Ehara.** 1992. Nucleotide sequence of RNA2 from cucumber mosaic virus strain O. *Ann. Phytopath. Soc. Japan* **58**: 789-793.
- Hayes, R.J. and K.W. Buck.** 1990. Complete replication of a eukaryotic virus RNA *in vitro* by a purified RNA-dependent RNA polymerase. *Cell* **63**: 363-368.
- Hayes, R.J. and K.W. Buck.** 1993. Analysis of replication complexes of positive strand RNA plant viruses. In *Molecular Virology: A Practical Approach*, Davison, A. J. and Elliott, R. M. ed (Oxford, UK: IRL Press Limited): pp. 1-34.
- Hellwald, K.-H. and P. Palukaitis.** 1994. Viral RNA as a potential target for two independent mechanisms of replicase-mediated resistance against cucumber mosaic virus. *J. Gen. Virol.* **75**: 2121-2125.
- Joshi, R.L., S. Joshi, F. Chapeville and A.-L. Haenni.** 1983. tRNA-like structures of plant viral RNAs: conformational requirements for adenylation and aminoacylation. *EMBO J.* **2**: 1123-1127.
- Kamer, G. and P. Argos.** 1984. Primary structural comparison of RNA-dependent RNA polymerases from plant, animal and bacterial viruses. *Nucleic Acids Res.* **12**: 7269-7282.
- Kataoka, J., C. Masuta and Y. Takanami.** 1990. Complete nucleotide sequence of RNA2 of cucumber mosaic virus Y strain. *Ann. Phytopath. Soc. Japan* **56**: 495-500.
- Kim, S.J., H.S. Cho, J.S. You, S.Y. Kwon, E.K. Park, and K.H. Paek.** 1996. Complete nucleotide sequence and phylogenetic classification of the RNA3 from cucumber mosaic virus (CMV) strain: Kor. *Mol. Cells* **6**: 190-196.
- Koonin, E.V.** 1991. The phylogeny of RNA-dependent RNA polymerases of positive-strand RNA viruses. *J. Gen. Virol.* **72**: 2197-2206.
- Marsh, L.E., G.P. Pogue and T.C. Hall.** 1989. Similarities among plant virus (+) and (-) RNA termini imply a common ancestry with promoters of eukaryotic tRNAs. *Virology* **172**: 415-427.
- McGarvey, P., M. Tousignant, L. Geletka, F. Cellini, and J.M. Kaper.** 1995. The complete sequence of a cucumber mosaic virus from *Ixora* that is deficient in the replication of satellite RNAs. *J. Gen. Virol.* **76**: 2257-2270.
- Morch, M.D., J.C. Boyer and A.L. Haenni.** 1988. Overlapping open reading frames revealed by complete nucleotide sequencing of turnip yellow mosaic virus genomic RNA. *Nucleic Acids Res.* **16**: 6157-6173.
- Palukaitis, P., M.J. Roossinck, R.G. Dietzgen, and R.I. B. Francki.** 1992. Cucumber mosaic virus. *Adv. Virus Res.* **41**: 281-348.
- Poch, O., I. Sauvaget, M. Delarue and N. Tordo.** 1989. Identification of four conserved motifs among the RNA-dependent polymerase encoding elements. *EMBO J.* **8**: 3867-3874.
- Pogue, G.P., and T.C. Hall.** 1992. The requirement for a 5' stem-loop structure in brome mosaic virus replication supports a new method for viral positive-strand RNA infection. *J. Virol.* **66**: 674-684.
- Rezaian, M.A., R.H.V. Williams, K.H.J. Gordon, A.R. Gould and R.H. Symons.** 1984. Nucleotide sequence of cucumber mosaic virus RNA2 reveals a translation product significantly homologous to corresponding proteins of other viruses. *Eur. J. Biochem.* **143**: 277-284.
- Rizzo, T.M. and P. Palukaitis.** 1988. Nucleotide sequence and evolutionary relationships of cucumber mosaic virus (CMV) strains: CMV RNA 2. *J. Gen. Virol.* **69**: 1777-1787.
- Rizzo, T.M. and P. Palukaitis.** 1990. Construction of full-length cDNA clones of cucumber mosaic virus RNAs 1, 2 and 3: generation of infectious RNA transcripts. *Mol. Genet.* **222**: 249-256.
- Wilson, T.M.A.** 1993. Strategies to protect crop plants against viruses: pathogen-derived resistance blossoms. *Proc. Natl. Acad. Sci. USA* **90**: 3134-3141.
- Zaitlin, M., J.M. Anderson, K.L. Perry, L. Zhang and P. Palukaitis.** 1994. Specificity of replicase-mediated resistance to cucumber mosaic virus. *Virology* **201**: 200-205.

(Received December 5, 1996)