Plant Pathol. J. 18(2) : 85-92 (2002)

The Plant Pathology Journal

Characterization and Sequence Analysis of a Lily Isolate of *Cucumber mosaic virus* from *Lilium tsingtauense*

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(Received on February 27, 2002)

A new isolate of *Cucumber mosaic virus* (CMV), identified as Li-CMV, was isolated from a diseased Korean native lily (*Lilium tsingtauense* Gilg). Biological and serological properties of Li-CMV were characterized, and reverse transcription-polymerase chain reaction (RT-PCR) analysis, restriction enzyme profiling of RT-PCR products, and nucleotide sequence analysis of RNA3 of the virus were performed in this study. Remarkable differences in symptoms between Li-CMV and ordinary CMV strains were found in tobacco plants and *Datura stramonium*. Li-CMV-infected tobacco plants (cv. Xanthi-nc and cv. Samsun) induced chlorotic ringspots on uninoculated upper leaves, and the symptom expression was delayed or faint, whereas, ordinary CMV strains induced green mosaic symptoms on the plant. Systemic infections were observed on *Nicotiana benthamiana* with severe mosaic symptom. Restriction mapping analysis of RT-PCR products using *Msp*I showed that Li-CMV belonged to CMV subgroup I. A full-length cDNA copy of RNA3 for the virus was amplified by RT-PCR, cloned, and its complete nucleotide sequence was determined. The RNA3 of Li-CMV was 2,232 nucleotides long, and consisted of two open reading frames of 843 and 657 bases encoding 3a protein (movement protein) and coat protein, respectively. Results of this study indicate that Li-CMV is a novel strain and belongs to subgroup I of CMV in the genus *Cucumovirus*.

Keywords : *Cucumber mosaic virus*, lily, *Lilium tsingtauense*, RFLP, sequence analysis, subgroup.

Lilies are indigenous to Europe (c. 12 species), Asia (50-60 species) and north America (c. 24 species), while some are native to the tropics at higher elevations (Beattie and Whittle, 1993). Over 10 viruses have been reported to infect lilies worldwide. *Lily symptomless virus* (LSV) is a carlavirus with particles of 640-650 nm long and occurs wherever lilies are grown (Allen, 1972). Leaves infected by the virus show vein-clearing of light green stripes between the veins. Many cultivars remain symptomless when singly infected with LSV. Plants often show diminished growth, smaller flowers, pronounced lower bulb yield, and shorter vase life as cut flowers. *Lily mottle virus* (LMoV) is a potyvirus with particles 750-770 nm long that also occurs wherever lilies are grown. LMoV-infected leaves show light to yellowish green mottle or mosaic and become twisted or tapered. Diseased plants are often shorter and die prematurely, and have shorter vase life as cut flower. *Cucumber mosaic virus* (CMV), a species of the genus *Cucumovirus* in the family *Bromoviridae*, is found in many cultivated lily plants (Chen et al., 2001; Jung et al., 2000). Leaves of diseased lily show chlorotic or yellow spots of stripes or vein-clearing. Later, grey or brown necrotic spots may develop. A coarse breaking pattern in the flowers is possible in some cultivars and leaves and petals may curl or may be malformed.

The genome of CMV consists of three capped plus-sense single strand genomic RNAs (Palukaitis et al., 1992). Proteins translated from RNA1 and RNA2 are associated with viral genome replication. Meanwhile, the bicistronic RNA3 encodes the 3a protein known as viral movement protein (MP), shown to be involved in cell-to-cell movement, and the viral coat protein (CP) for encapsidation and long-distance movement (Palukaitis et al., 1992; Li et al., 2000). The CP is translated from the subgenomic RNA4, which is encoded by the 3'-half of RNA3. In addition to the four major viral genes, a small overlapping gene (2b), encoded by RNA2, has been discovered (Ding et al., 1995), which is most likely expressed from a second subgenomic mRNA (RNA4A). The product of the 2b gene is involved in the virulence of the virus, possibly by suppressing gene silencing (Mayers et al., 2000). There are many strains of CMV, which differ in host range and pathogenicity (Kaper and Waterworth, 1981; Palukaitis et al., 1992).

On the bases of serological relationships, peptide mapping of the viral coat protein and nucleic acid hybridization analysis, strains of CMV fall into two major subgroups (Gonda and Symons, 1978; Owen and Palukaitis, 1988).

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The relationships of virus strains within each subgroup are very close, and less so between subgroups, as determined by each of the above techniques. There are no clear differences in the host range of strains of subgroup I (S-I) and subgroup II (S-II). The two subgroups are closely related serologically with polyclonal antibodies, although monoclonal antibodies raised against S-I and S-II isolates could differentiate the two subgroups. Percentages of nucleotide sequence homologies of RNA3 between S-I and S-II range from 64 to 67%, and above 85% within each subgroup. Recently, a new grouping for the subgroup S-I, designated as S-IB (Asian isolates) and S-IA (all other isolates), has been proposed on the basis of sequence data (Roossinck et al., 1999). More recently, the subgroup S-I can be separated into four subgroups on the basis of electrophoretic patterns of restriction fragments of reverse transcription-polymerase chain reaction (RT-PCR) products digested with *Msp*I restriction enzyme (Anonymous, 1998).

CMV infections cause great damage to a number of ornamental crops singly or in combination with other viruses. Floricultural plants greatly depend on clean practice or on virus resistant varieties, as minor infection damage leads to greatly reduced market value. In particular, lily is one of the most popular ornamental plants as a cut flower not only in Korea but also in other countries. It is easily propagated vegetatively for mass production in commercial purpose, and virus diseases have caused serious problems for growers. CMV was isolated from a diseased Korean native lily (*Lilium tsingtauense* Gilg) showing severe mosaic symptom. The lily isolate of CMV showed different host reactions in some indicator plants and distinct symptom expression occurred in tobacco plants.

In this study, the biological, serological and molecular properties of the virus were investigated. Phylogenetic analysis, as well as, sequence alignments of the coding and noncoding regions of the lily isolate of CMV (Li-CMV) RNA3 was compared with those of other known strains of CMV.

Materials and Methods

Virus source, virus purification and antibody production. A diseased plant of lily (*Lilium tsingtauense* Gilg) was maintained in the experimental field of the Department of Horticultural Science, Seoul Women's University and used for the isolation of CMV. The virus, Li-CMV, was isolated from the diseased *L. tsingtauense* Gilg (Fig. 1). Fny-CMV (subgroup I) was obtained from the Plant Virus GenBank (http://www.virusbank.org), which was originally obtained from Dr. Peter Palukaitis (Scottish Crop Research Institute, UK), and was used as control for analysis of CP and RT-PCR. Virus was maintained in *Nicotiana tabacum* cv. Xanthi-nc by mechanical inoculation during the experimental period. These plants were used as source of plant for virus in this study. Virions were purified from infected leaves as described by Roossinck (1998). UV absorption spectra of virions were determined using a UV spectrophotometer (UV2000, Phamacia). Antibody for Li-CMV was produced from immunized white rabbit and used for western blot.

Fig. 1. Severe mosaic foliar symptom in *Lilium tsingtauense* Gilg. used as a source of plant for isolation of *Cucumber mosaic virus* in this study.

Host range test of Li-CMV. Virus inoculation to the plant was made by rubbing sap of inoculum with a sterilized cotton-ball onto Carborundum (400 mesh)-dusted leaves of the following test plants with three replications: *Nicotiana benthamiana, N. glutinosa, N. tabacum* cv. Xanthi-nc, *N. tabacum* cv. Samsun, *Datura stramonium, Cucurbita pepo* cv. Black Beauty and *Vigna unguiculata.* These plants were maintained in a glasshouse and observed for symptom development. Infection of the virus was checked by RT-PCR with CMV-specific primers 20-30 days post-inoculation.

Viral RNA preparation. Equal volumes of purified virus preparation and VEBA extraction buffer (0.2 M Tris-HCl (pH 8.5), 1 M NaCl, 1% SDS and 2 mM EDTA) were mixed and pulverized. This was extracted twice with phenol: chloroform: isoamyl alcohol (PCI, 25:24:1, v/v/v) (Roossinck, 1998), and was centrifuged at 15,000 rpm for 15 minutes at room temperature. The aqueous phase was recovered, and re-extracted once with chloroform: isoamyl alcohol (CI, 24:1, v/v). Viral RNA was precipitated with half volume of 7.5 M ammonium acetate and 2.5 volumes of cold absolute ethanol and incubated at -70°C for 20 minutes. After centrifugation, precipitate of viral RNA was rinsed with 80% cold ethanol, and precipitates were dried to remove residual ethanol under speed vacuum machine. The resulting precipitates of viral RNA were dissolved in RNase-free distilled water and used for subsequent procedure.

Coat protein and viral RNA analysis. Coat protein of CMV was separated in the 12.5% SDS-PAGE and visualized by Coomassie

blue R-250 staining (Laemmli, 1970). SDS-PAGE separated coat proteins were transferred onto nitrocellulose membrane and immuno-probed with CMV antibody for Western analysis. Purified viral RNAs from virions were separated in a formaldehyde-denatured 1.2% agarose gel under denatured condition in MOPS buffer (Sambrook et al., 1989) at 90 volts for 40 minutes. Size and integrity of viral genomic RNAs were estimated by using the comigrated RNA size markers (GIBCO BRL, USA). The RNA bands were visualized on the UV transilluminator, after staining gel in ethidium bromide solution (100 ng/ml) for 3 minutes and destaining in DEPC-treated distilled water for 5 minutes, and then photographed with Polaroid camera (type 667 film).

RT-PCR detection and cloning of RNA3 gene of Li-CMV. RT-PCR was performed for molecular characterization of CMV (Choi et al., 1999). Reverse transcription (RT) reaction was carried out at 42°C for 30 minutes in 20 mM Tris-HCl buffer (pH 8.0) containing 20 μl sample RNA, 5 mM $MgCl_2$, 50 mM KCl, 1.0 mM each of four dNTPs, 50 pcM primer CMDN (5'-GGAACACGGAATCAGACTGG-3', 1,912nd-1,931st), 1 unit of RNase inhibitor, and 2.5 units MuLV reverse transcriptase (Roche, USA). The RT reaction mixture was mixed with 1 unit of *Tag* DNA polymerase (Roche) in 30 μl of PCR buffer (20 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 100 mM KCl) containing 50 pcM of the two primers (CMUP, 5'-GAGTCATGGACAAATCTGAATC-3', 1,259th-1,279th; CMDN) and four dNTPs. For amplification of full-length cDNA of RNA3 for the virus, the two primers were used as follows: CMR3T7, 5'-GGGGATCCTAATACGACTCACTATAGTAATCTTACCACTGTGTG-3', and CM3allPst, 5'-GGGGATCCCTGCAGTGGTCTCCTTTTRG-3'. Restriction enzymes used were as follows: *Bam*HI, *Eco*RI, *Hin*dIII, *Sac*I, *Sal*I and *Xho*I. Restriction enzyme digestions were performed to estimate the region of RNA3 of Li-CMV. A reaction mixture consisted of PCR-amplified DNA products (500 ng), 2 μl of 10x reaction buffer and restriction enzyme (10 units) in a total volume of 20 μl according to the conditions recommended by the manufacturers. Digested DNA fragments were separated in a 1.2% agarose gel electrophoresis in TAE buffer at 100 volts for 20 minutes. Sizes of DNA restriction fragments were estimated using 1 kb plus DNA ladder (GIBCO BRL, USA). Double-stranded cDNA molecules were cloned into *Bam*HI/*Pst*I site of pUC18 vector (Gibco BRL, MD). The recombinant plasmids were transfected into competent *Escherichia coli* strain *JM109* (Sambrook et al., 1989).

Nucleotide sequencing and sequence analysis. Subclones containing cDNA molecules of RNA3 of Li-CMV were generated by restriction enzyme hydrolysis. Overlapped cDNA clones containing the viral genes were sequenced in both directions by the dideoxynucleotide chain termination method (Sanger et al., 1977) using the Model 377 automatic DNA sequencer (ABI). Nucleotide sequences and deduced amino acid sequences of the RNA3 were analyzed using the DNASTAR software package (Madison, WI). Phylogenetic tree and multiple alignment of the sequences were analyzed by the DNASTAR software package (Madison, WI). Nucleotide sequences of RNA3 from 14 strains of CMV, *Peanut stunt virus* (PSV-ER), and *Tomato aspermy virus* (TAV-KC) were retrieved from the GenBank/EMBL databases and used for sequence analysis (Table 1).

Table 1. *Cucumber mosaic virus* (CMV), *Peanut stunt virus* (PSV), and *Tomato aspermy virus* (TAV) and their GenBank accession numbers used for sequence analysis in this study

Virus	Accession no.	Subgroup	Country of origin
Li-CMV	in this study	IA	Korea
D8-CMV	AB004781	IA	Japan
E5-CMV	D42080	IA	Japan
Fny-CMV	D10538	IA	USA
O-CMV	D00385	IA	Japan
Y-CMV	D12499	IA	Japan
LiNB-CMV	AJ131618	IA	Taiwan
Ly2-CMV	AJ296154	IA	Korea
C7-2-CMV	D42079	IB	Japan
M48-CMV	D49496	IB	Taiwan
NT9-CMV	D28780	IB	Taiwan
SD-CMV	AB008777	IB	China
Q-CMV	M21464	II	Australia
Kin-CMV	Z12818	II	UK
LS-CMV	AF127976	II	USA
ER-PSV	U15730		USA
KC-TAV	AJ237849		Korea

Table 2. Comparison of reactions of indicator plants induced by four different isolates of *Cucumber mosaic virus*

Indicator plants	Reaction[a]			
	Li-CMV	Ly2-CMV	Fny-CMV	LS-CMV
Nicotiana benthamiana	−/M[b]	−/M	−/M	−/M
N. glutinosa	−/−	−/−	−/M	−/M
N. tabacum cv. Xanthi-nc	−/mM, RS	−/−	−/M	−/M
N. tabacum cv. Samsun	−/mM, RS	−/M	−/M	RS/M
Vigna unguiculata	L/−	L/−	L/−	L/−
Cucurbita pepo	−/CS	−/−	−/M	−/M
Datura stramonium	L/−	L/−	−/M	−/M

[a] Abbreviations: M; mosaic, mM; mild mosaic, CS; systemic chlorotic spot, L; local lesion, RS; chlorotic ringspot.
[b] Inoculated leaf/upper uninoculated leaves.

Results

Host reactions and symptomatology of Li-CMV. The host range and symptomathology of Li-CMV are presented in Table 2. Of the indicator plants tested, Li-CMV systemically infected *N. benthamiana* and some tested plants. Systemic infections were observed on the leaves of *N. tabacum* cv. Xanthi-nc, *N. benthamiana, N. tabacum* cv. Samsun and zucchini squash. Tobacco plants and zucchini squash plants showed systemic symptoms of chlorotic ring

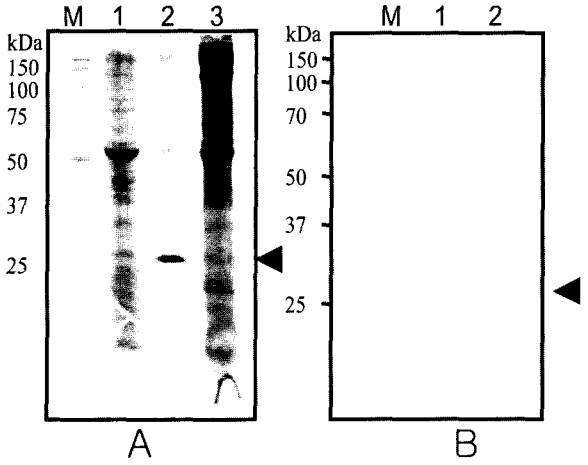

Fig. 2. SDS-PAGE and Western blot analysis of viral coat proteins of CMV. Coomassie stained gel (A) and immuno-probed NC membrane with CMV CP antibody (B). Photo (A) Lane M: pre-stained SDS protein markers, 1: total proteins from Li-CMV-infected tobacco, 2: purified Li-CMV, 3: healthy tobacco plant. Photo (B) Lane M: pre-stained SDS protein markers, 1: purified Li-CMV, 2: Fny-CMV.

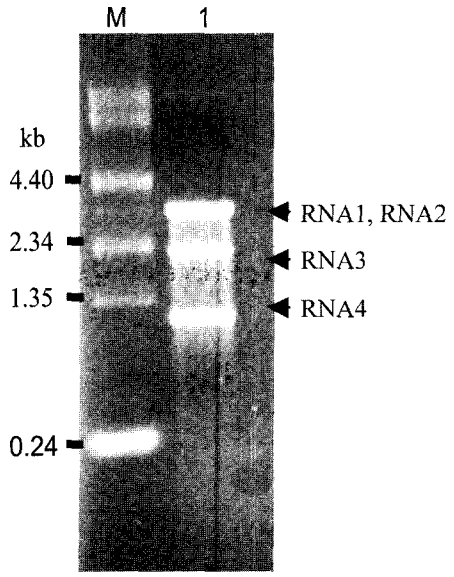

Fig. 3. Electrophoretic pattern of genomic RNAs of Li-CMV in 1.2% formaldehyde-denatured agarose gel with MOPS buffer system. Lane M: RNA ladder (GIBCO BRL), 1: Li-CMV genomic RNAs purified from virions.

spot. Li-CMV-infected tobacco plants (cv. Xanthi-nc and cv. Samsun) induced chlorotic ringspots on uninoculated upper leaves and the symptom expression was delayed or faint, whereas, ordinary CMV strains induced green mosaic symptoms on the plant. Small necrotic ring spots were expressed on the inoculated leaves of *Datura stramonium*. Li-CMV induced necrotic local lesions on the inoculated leaves of *V. unguiculata*. The virus was able to systemically infect *Lilium* plants (*L. logiflorum* and *L. tsingtauense*), but failed to infect *Datura stramonium* systemically.

Coat protein and viral RNA analysis. The coat protein of Li-CMV revealed electrophoretic homogeneity on SDS-PAGE. There was a single protein band with molecular weight of 26 kDa estimated by comigrated molecular marker proteins (Fig. 2A). Western blot analysis was performed using polyclonal antiserum against CMV. The protein band of the virus reacted strongly with its homologous antiserum and this indicates that the 26 kDa protein was a coat protein of the virus (Fig. 2B). The genomic RNAs of the virus were extracted from the purified virus particles by the VEBA and phenol extraction methods, and then analyzed. Ratio of A260/A280 was 1.8 (data not shown). When purified viral RNA preparation was separated in formaldehyde-denatured MOPS agarose gel, four ethidium bromide-stained RNA bands were

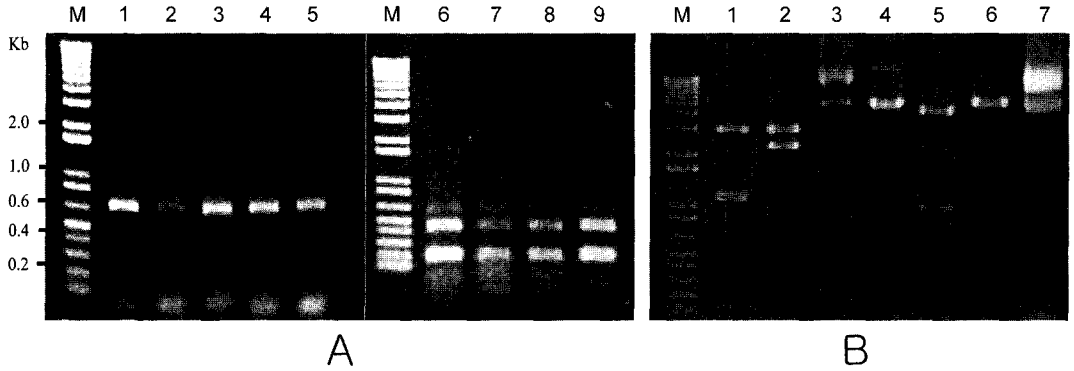

Fig. 4. Electrophoretic pattern of amplified RT-PCR products and their restriction mapping analysis for coat protein (A) and full-length RNA3 gene (B) of Li-CMV. Photo (A) Lane M: 1kb plus DNA ladder, 1-4: CMV isolates from *Lilium tsingtauense*, 5: Fny-CMV, 6-9: *Msp*I digested RT-PCR products of CMV isolates 1, 2, 3 and 4, respectively. Photo (B) Lane M: 1kb plus DNA ladder, 1: *Bam*HI, 2: *Eco*RI, 3: *Hin*dIII, 4: *Sac*I, 5: *Sal*I, 6: *Xho*I, 7: uncut PCR product of Li-CMV.

observed (Fig. 3). Sizes of the RNA1, RNA2, RNA3 and RNA4 of Li-CMV were about 3.4 kb, 3.1 kb, 2.2 kb and 1.0 kb, respectively.

RT-PCR detection and RFLP analysis for subgrouping. Total nucleic acids extracted from leaf tissues of collected lilies were amplified in a RT-PCR assay. Lily leaf tissue homogenates in 60 μl extraction buffer was sufficient to yield an intense PCR fragment in the RT-PCR. All the CMV-infected lily plants gave positive results in the RT-PCR assay. The virus was successfully detectable by the RT-PCR with infected lily leaf samples. These results were consistent through successive replications. After the PCR product has been digested with *Msp*I, two DNA fragments of about 200 bp and 450 bp were observed (Fig. 4A). This suggests that the PCR products originated from CMV RNA and the Li-CMV belonged to subgroup I of CMV (Anonymous, 1998; Jung et al., 2000).

The restriction profiles of the full-length cDNA clone of RNA3 of Li-CMV showed particular patterns in agarose gel electrophoresis (Fig. 4B). The RNA3 of Li-CMV endonuclease restriction patterns were very similar to Ly2-CMV restriction mapping but Ly2-CMV (Jung et al., 2000) had no *Eco*RI restriction site. While endonuclease restriction sites of other CMV strains had *Hin*dIII, Li-CMV did not have any. LS-CMV (subgroup II) was quite different to that of other CMV strains (subgroup I) (Fig. 5). This indicates that Li-CMV belongs to subgroup I.

Sequence analysis of RNA3 gene of Li-CMV. The clones containing sequences from the viral genomic RNA3 were selected and the nucleotide sequences of the inserts were determined in both strands. Sequences of RNA3 of Li-CMV were compared with several strains from subgroup I and subgroup II of CMV.

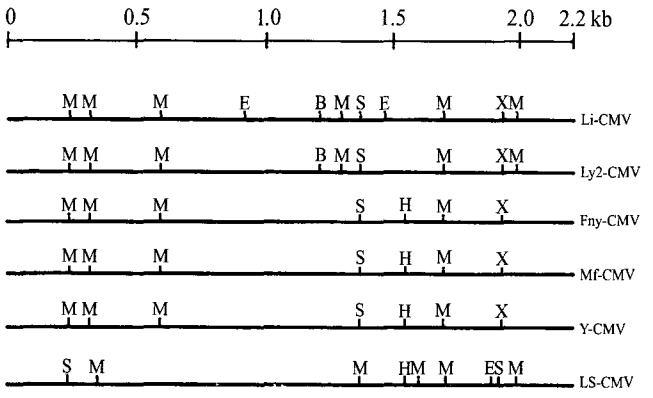

Fig. 5. Comparison of restriction endonuclease recognition sites of RNA3 gene of Li-CMV with that of other CMV strains generated by sequence analysis software package (DNASTAR) based on nucleotide sequences of selected CMV strains. Abbreviations of restriction endonucleases: B = *Bam*HI, E = *Eco*RI, H = *Hin*dIII, M = *Msp*I, S = *Sal*I, X = *Xho*I.

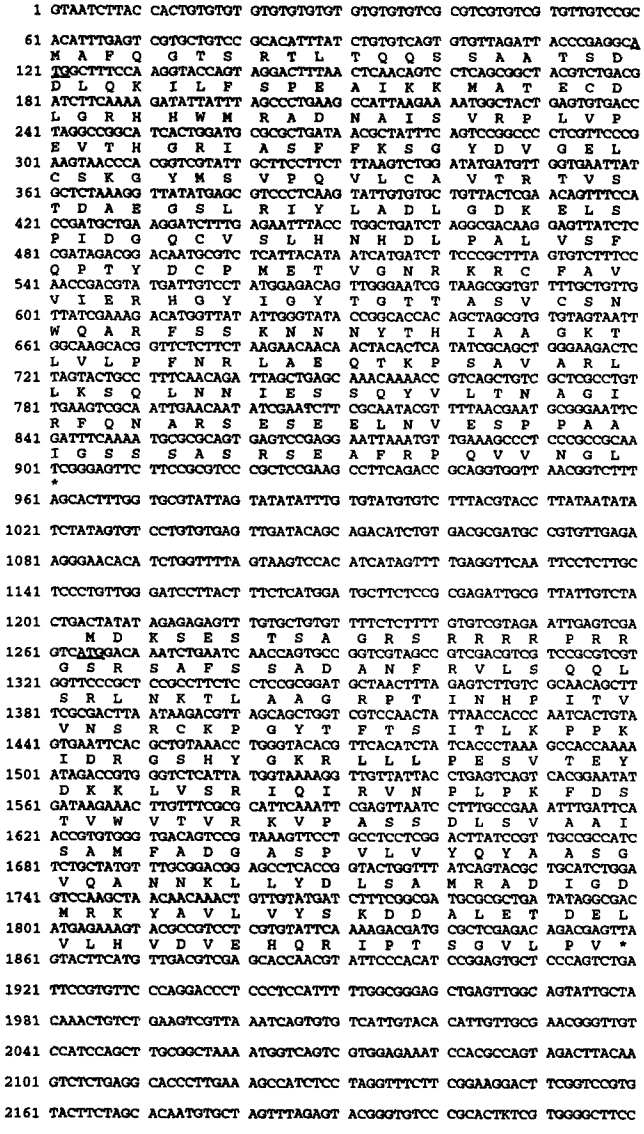

Fig. 6. Complete nucleotide and amino acid sequences of the RNA3 of Li-CMV.

The genome of the virus was 2,232 nucleotides long and contained two open reading frames (ORFs) (Fig. 6). ORFs encoded two proteins of 30 kDa MP (281 aa) and 26 kDa CP (219 aa) from the 5' to 3' end, respectively. The 5' non-coding region was 119 nucleotide long. The first open reading frame comprised 843 nucleotides, which coded the 281 amino acid long 3a protein. The intergenic region was 301 nucleotides long and the second open reading frame consisted of 657 nucleotides, which were translated into a 219 amino acid long coat protein.

The 5' non-coding regions (NCR) of both Li- and Ly2-strains from subgroup I were identical (Table 3). The 5' NCRs of the subgroup I strains showed that homology varied from 68.5% to 100.0%. Li-CMV was the most

Table 3. Percentage sequence identities of coding and noncoding (5' and 3') regions between the RNA3 of lily isolate of *Cucumber mosaic virus* (Li-CMV) and other strains of CMV, *Tomato aspermy virus* (TAV) and *Peanut stunt virus* (PSV)

Virus	30 kDa aa	(MP) nt	26 kDa aa	(CP) nt	5'NCR nt	3'NCR nt
C7-2-CMV	94.2	92.5	93.6	91.0	74.8	74.1
D8-CMV	97.1	94.5	93.6	94.4	69.7	78.8
E5-CMV	97.5	94.4	93.2	94.1	70.3	77.5
Fny-CMV	97.1	93.9	94.5	95.1	75.6	75.2
O-CMV	97.1	94.2	94.1	95.0	73.1	79.7
Y-CMV	97.1	93.3	92.2	95.0	70.6	76.3
Ly2-CMV	97.5	98.2	95.9	97.9	100.0	95.5
M48-CMV	93.2	92.0	91.3	90.1	68.5	75.5
NT9-CMV	95.4	93.3	94.1	92.1	79.0	73.3
SD-CMV	93.3	91.6	94.1	91.0	84.0	78.7
Q-CMV	81.4	74.5	78.1	70.2	40.0	47.4
Kin-CMV	82.5	74.6	77.6	69.4	40.0	48.4
LS-CMV	81.4	74.3	77.2	69.9	38.5	47.6
LINB-CMV	–[a]	–	95.4	97.6	–	–
ER-PSV	58.2	64.6	43.4	39.9	27.7	46.6
KC-TAV	57.5	65.4	41.1	40.8	39.8	39.7

[a] –: not available.

divergent with subgroup II; the similarity was below 38.5%. In contrast, other *cucumoviruses*, TAV, PSV and the different CMV strains showed a nucleotide sequence similarity; for example, this value was 39.8% in the case of Li-CMV and TAV, and 27.7% for Li-CMV and PSV (TAV and PSV as the out-group in all cases, and different members of CMV subgroups IA, IB and II as the in-group).

The sequence variation among the 3a genes of the subgroup I strains of CMV was low. In this region, the similarity between the nucleotide sequences of the different strains was between 91.6% and 98.2% (Table 3). Phylogenetic tree analysis revealed that Li-CMV was subgrouped together with other known isolates of CMV belonging to subgroup I (Fig. 7). The two CMV subgroups showed an average homology of 89.3% at the nucleotide sequence level. The coat protein genes of the subgroup I strains showed that homology varied from 90.1% to 97.9%. The two subgroups displayed an average homology of 88.8% at the nucleotide sequence level. The comparison of the 3' non-coding regions revealed 73.3-95.5% homology among the subgroup I strains and an average homology of 69.5% between the two groups (Table 3).

Discussion

In this paper, characterization of a new strain of CMV, Li-CMV, from diseased lily (*Lilium tsingtauense* Gilg) was investigated. The virus had some of the main biological, serological, and molecular properties characteristic of subgroup I of CMVs. However, the host range and symptoms of Li-CMV differed in some respects from those reported for other subgroup I. Li-CMV induced systemic mosaic in *N. benthamiana* but did not induce systemic symptoms in *Datura stramonium*. Thus, of considerable interest is Li-CMVs difference in host reaction to the well-known CMV strain, and some differences in host range and symptoms may reflect differences in the intrinsic properties of the Li-CMV.

The CP gene from Li-CMV was successfully amplified using RT-PCR on a total RNA template from infected plants. Present *Msp*I analysis of PCR products provided a simple routine method to determine the CMV subgroup (Anonymous, 1998; Jung et al., 2000; Rizos et al., 1992). In the analysis of the PCR products using *Msp*I digestions, CMV was divided into two groups as described by Rizos et

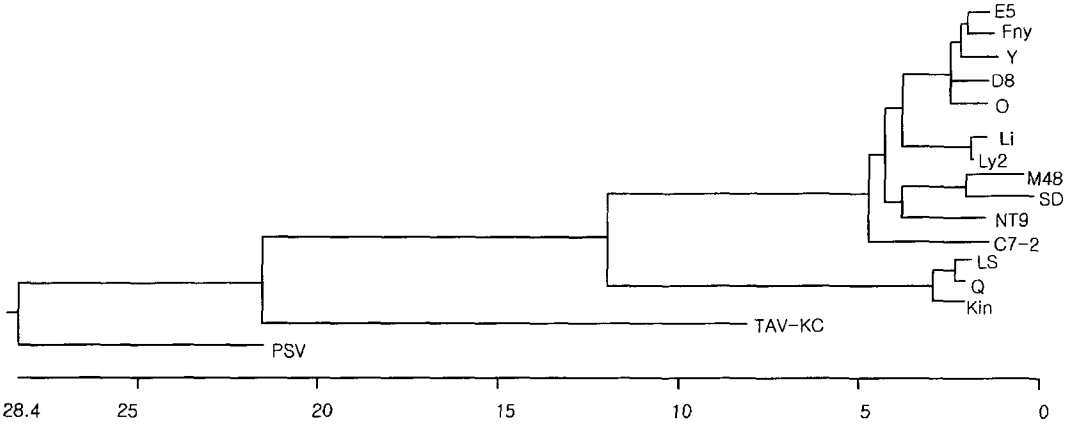

Fig. 7. Relationships of 3a gene sequences between Li-CMV and other CMV strains. The degree of relatedness is indicated by horizontal distance. The dendrogram was constructed from aligned 3a gene sequences using DNASTAR software.

al. (1992). The restriction enzyme digest patterns obtained from other CMV strains known with subgroup I were those expected CMV subgroup I. These results are consistent with the sequence information obtained from CP gene, which allowed differentiation among CMV subgroup.

The purpose of the present research was to determine the general characteristics of Lily isolate of CMV and virus-host interaction in molecular levels. Therefore, it was necessary to construct the full-length cDNA of viral genomic RNAs and to determine their sequences. In this study, the complete nucleotide sequence of RNA3 of Li-CMV was determined using cloned cDNAs. Li-CMV RNA3 was 2,232 nucleotides long. Li-CMV RNA3 possessed two open reading frames like other CMV strains, encoding the 3a protein (281 amino acid) and the coat protein (219 amino acids). This sequence analysis proved that the former biological and serological classifications of the RNA3 molecule of Li-CMV were in subgroup I. The availability of cDNA clones covering the whole Li-CMV RNA3 would allow further research toward understanding the function of RNA3 and the development of pathogen-mediated resistance to CMV.

The nucleotide sequence comparison data among various CMV strains showed that only a very small nucleotide sequence difference existed among strains belonging to the same subgroup (Owen et al., 1990). On the other hand, the two subgroups displayed a much higher divergence, especially in the non-coding regions. Meanwhile, the coding regions were more conserved, particularly at the amino acid sequence level, probably due to the selective pressure of the gene products of the RNA. Phylogenetic analyses of CMV using the coat protein of 53 strains strongly supported the further division of subgroup I into IA and IB (Roossinck et al., 1999). This suggests that the subgroup I strains are evolving more rapidly, hence, rates are not directly comparable. These differences may be reflective of the broader host range including natural original host plants and higher incidence of subgroup I strains compared with subgroup II strains.

Recently, Ly2-CMV was isolated from lily plant (*Lilium longiflorum*) and its properties were characterized (Jung et al., 2000). Li-CMV has a very high homology with Ly2-CMV based on the sequence analysis. Interestingly, the 5' noncoding regions of both Li- and Ly2-CMV from subgroup I were identical. These were isolated from lily plants. Both had some of the main biological, serological and molecular properties characteristic of subgroup I CMV. However, the host range and symptoms of Ly2-CMV and Li-CMV differed in some respects from other subgroup I CMVs. Li-CMV and Ly2-CMV induced systemic mosaic in *Nicotiana benthamiana*. Their biological properties were very similar, compared with that of the host range of other CMV strains.

CMV isolated from ornamental crops of different geographical areas was characterized by comparing the nucleotide sequences of RNA4 and the encoded coat proteins (Chen et al., 2001). Within the ornamental-infecting CMV viruses, both subgroups were represented. CMV isolates of five lilies were identified as belonging to subgroup I. In general, nucleotide sequence homology among the tested subgroup I isolates of the same geographic area was much higher than that between isolates of different areas. In contrast, the nucleotide sequence of isolates from lily plants, i.e., LILY-, LICK-, LISR-, LINB-, LITW-CMV, were highly conserved in spite of the different geographic origins and horticultural varieties (Chen et al., 2001). Hence, comparison of the coat protein nucleotide of Li-, Ly2- and LINB-CMV showed that homology varied from 97.9% to 97.6%. In general, the variation among CMV isolates prevalent in geographic regions as well as those in a specific crop has proven to be rather limited. Interestingly, isolates of lily was very homologous from different regions. One possible reason was the host specificity hypothesis that lily plants are susceptible only to subgroup I CMV isolates. Therefore, further investigations of these lily isolates of CMV are needed to characterize pathogenicity and their evolution.

Acknowledgment

This study was supported by a Basic Research Grant (KRF-2001-015-DP0450) from the Korea Research Foundation.

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