Plant Pathol. J. 23(4) : 251-259 (2007)

The Plant Pathology Journal

Variation in the Pathogenicity of Lily Isolates of *Cucumber mosaic virus*

Jin-A Lee[1], Seung Kook Choi[2], Ju Yeon Yoon[3], Jin Sung Hong[3], Ki Hyun Ryu[3], Sang Yong Lee[4] and Jang Kyung Choi[1]*

[1]*Department of Applied Biology, Kangwon National University, Chunchon 200-701, Korea*
[2]*National Institute of Agricultural Biotechnology, RDA, Suwon 441-707, Korea*
[3]*Division of Environment & Life Sciences, Seoul Women's University, Seoul 139-774, Korea*
[4]*Department of Forest Resources Protection, Kangwon National University, Chunchon 200-701, Korea*

(Received on August 6, 2007; Accepted on October 6, 2007)

Two isolates of *Cucumber mosaic virus* (CMV) originated from lily plants, named Ly2-CMV and Ly8-CMV, were compared with their pathological features in several host plants. Ly2-CMV and Ly8-CMV could induce systemic mosaic symptom in *Nicotiana benthamiana*, but Ly2-CMV could not systemically infect tomato and cucumber plants that have been used for CMV-propagative hosts. While Fny-CMV used as a control infected systemically the same host plants, producing typical CMV symptoms. Ly8-CMV could infect systemically two species of tobacco (*N. tabacum* cv. Xanthi-nc and *N. glutinosa*) and zucchini squash (*Curcubita pepo*), but Ly2 failed systemic infection on these plants. As resulted from tissue-print immunoblot assay, different kinetics of systemic movement between Ly2-CMV and Ly8-CMV were crucial for systemic infection in tobacco (cv. Xanthi-nc). Sequence analysis of full-length genome of two lily isolates showed Ly2 and Ly8 belonged to subgroup IA of CMV. The lily isolates shared overall 98% sequence identity in their genomes. Coat protein, 3a protein, and 2b protein involved in virus movement was highly conserved in genomes of the isolates Ly2 and Ly8. Although there is the low frequency of recombinants and reassortants in natural CMV population, phylogenetic analysis of each viral protein among a number of CMV isolates suggested that genetic variation in a defined population of CMV lily isolates was stochastically produced.

Keywords **:** *Cucumber mosaic virus*, host reaction, sequence analysis, systemic symptom, virus pathogenicity

Genetic variations of plant viruses, including mutation, play an important role in virus evolution in nature. Genetic analysis of plant virus families have been showed various degrees of genetic exchange can occur by recombination of genome regions that are swapped between nucleotide strands, or by reassortment of complete genome segments in viruses with segmented genomes (Domingo et al., 1996, 1997; Roossinck, 1997). These genetic exchange or mutations generated naturally in the replication process of its genomes resulted in novel genetic combinations that could acquire important phenotypic effects (García-Arenal et al., 2001; Gibbs et al., 1997). Such phenotypic effects are associated with host range expansion, with host switches, or with increased pathogenicity, resulting in epidemic of viral disease on crops.

Cucumber mosaic virus (CMV), genus *Cucumovirus*, family *Bromoviridae*, is a positive-sense RNA plant virus with a tripartite genome (Palukaitis and Garicia-Arenal, 2003; Palukaitis et al., 1992). CMV has an extremely broad host range (approximately 1,000 species). By serological data, nucleic acid hybridization, peptide mapping and sequence analysis of the coat proteins (CP) of CMV strains can be divided into three subgroups, designated IA, IB and II (Roossinck et al., 1999). RNAs 1 and 2 encode the nonstructural proteins involved in viral replication. Another open reading frame (ORF), the 2b ORF, that has been studied extensively, is also encoded on RNA 2. RNA 3 encodes a 3a protein (movement protein, MP) and the CP, which is translated from a subgenomic RNA 4.

Numerous strains of CMV have been characterized in aspects of symptomatology and host ranges (Choi et al., 2005; Huppert et al., 2002; Palukaitis and Garcia-Arenal, 2003; Palukaitis et al., 1992; Shintaku and Palukaitis, 1992; Suzuki et al., 1995; Szilassy et al., 1999; Takeshita et al., 2001). One of major pathogenicity determinants in phenotypes of such CMV strains has been implicated in virus movement and symptom severity (Choi et al., 2005; Gal-On et al., 1994; Takeshita et al., 2001; Zhang et al., 1994). Although several isolates of CMV from lily plants has been reported to cause systemic infection in lily plants (Choi et al., 2004; Hagita et al., 1989; Jung et al., 2000; Ryu et al., 2002), most CMV lily isolates seemed to have a narrow host range which they could infect only a small number of host plants, unlike typical CMV isolates. These unique

*Corresponding author.
Phone) +82-33-250-6432, FAX) +82-33-241-1721
E-mail) jkchoi@kangwon.ac.kr

biological properties of lily isolates are speculated that the lily isolates have been evolutionally adapted to lily plants (Jung et al., 2000; Masuta et al., 2002). In this report, we examined pathological properties of two CMV lily isolates on several species of host plants and further analyzed kinetics of systemic infection between Ly2-CMV and Ly8-CMV on tobacco as well. We also determined full-length genome sequences of the two CMV isolates and analyzed multiple alignment and phylogenetic tree with a representative strain of CMV. These results suggested that lily isolates of CMV have consisted of a unique pathological population in nature.

Materials and Methods

Virus isolates. Two lily isolates were used in this study investigation. Briefly, Ly2-CMV isolated from easter lily (*Lilium longiflorum*) plant was described previously (Jung et al., 2000). Ly8-CMV was originated from a different field-grown *L. longiflorum* that had been shown yellow mosaic symptom at Chunchon, Korea in 2001 (Fig. 1). Fny-CMV was used as a control (Rizzo and Palukaitis, 1990). Each isolate was inoculated onto *N. benthamiana* by mechanical inoculation in 10 mM phosphate buffer (pH 7.0) containing 0.01 M sodium diethyldithiocarbamate. The inoculated tobacco plants were grown in a greenhouse at 20-30°C. For host range test, five or more plants of each species or cultivar of host plants were inoculated mechanically with sap from each CMV isolate, respectively. The inoculum was prepared by grinding Ly2-CMV or Ly8-CMV infected leaves of *N. benthamiana* in a mortar with a pestle in 10 mM phosphate buffer. The inoculated plants were observed symptom development for 4 weeks, and then the plants tested were repeatedly back-inoculated to *N. benthamiana* in order to determine whether the virus was present, or whether there were any changes in pathological properties of isolates Ly2 and Ly8.

RT-PCR analysis. Total RNA was extracted from inoculated and healthy plants, as described previously (Choi et al., 1998). PCR fragment containing the CP gene of CMV was amplified using one-tube RT-PCR mixture kit (Promega) with Cucumovirus-specific primers as described by Choi et al. (1999). Briefly, total RNAs purified from infected plants or mock-inoculated plants were denatured at 70°C for 5 min and were added to the RT-PCR mixture. The conditions of RT-PCR is as follows: one cycle of RT reaction at 42°C for 45 min, 30 cycles of PCR amplification using the programmed steps (94°C, 30 sec; 40°C, 1 min; 72°C, 1 min), and one cycle of final extension at 72°C for 10 min. Ten microliters of the amplified DNA fragments were separated by electrophoresis on a 1.2% agarose gel in 1×TAE buffer and stained with ethidium bromide.

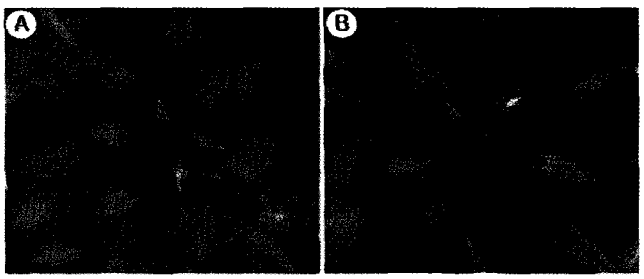


Fig. 1. Mosaic and streak symptoms on easter lily (*Lilium longiflorum*) infected naturally by Ly2-CMV (A) and Ly8-CMV (B).

Tissue-printing immunoblot assay. The inoculated and upper leaves of tobacco plants were pressed onto nitrocellulose membranes (Schleicher and Schuell) using a roller. The membranes were blocked with 1X TBSB [20 mM Tris-HCl (pH 7.5), 150 mM NaCl and 3% BSA] for 30 min. Subsequently, the membrane was incubated with a solution of primary polyclonal antibody against CMV (Agdia) that was diluted 1:2000 in 1X TBSB buffer for 2 hr. Then, the membrane was washed three times with 1X TBSB buffer and incubated with anti-rabbit IgG-alkaline phosphatase (Roche) diluted 1:1000 in 1X TBSB for 60 min. Membranes were further washed three times with 1X TBSB, and then incubated with a solution of NBT/BCIP for color development, according to manufacturer's instruction (Roche).

Cloning of full-length cDNAs and DNA sequencing. The cDNA clones of RNA1, RNA2 and RNA3 of CMV lily isolates were synthesized with proper primer sets specific to viral genomic RNAs using *Taq* DNA polymerase, as described by McGarvey et al. (1995). The information of primer sequences will be available on request. The cDNA inserts were analyzed by digestion with restriction enzymes. To determine nucleotide sequences of cDNAs, recombinant cDNAs of the CMV isolates with restriction enzymes were subcloned into appropriate plasmid vectors digested with the same enzymes. Sequences of full-length cDNA of the isolates were determined by the dideoxynucleotide chain termination method (Sanger et al., 1977) using big-dye terminator kit (version 3.0) and automatic DNA sequencer (Model 377, ABI). Assembled nucleotide sequences and the deduced amino acid sequences were analyzed using the DNASTAR software package (Madison, USA).

Results

Characterization of pathological properties of CMV lily isolates. Ly2-CMV and Ly8-CMV induced similar mosaic

Table 1. Reaction from indicator plants inoculated mechanically with Ly2-CMV, Ly8-CMV and Fny-CMV

Host plants	Symptom[a] (RT-PCR detection)		
	Ly2	Ly8	Fny
Nicotiana benthamiana	SL/M[b]	SL/M	SL/M
N. glutinosa	–/–(–/–)	SL/mM (+/+)[c]	SL/M
N. tabacum cv. Xanthi-nc	NR/– (+/–)	NR/NR (+/+)	SL/M
Lycopersicon esculentum	–/–	–/–	SL/M
Cucumis sativus	–/–	–/–	SL/M
Chenopodium amaranticolor	sL/–	sL/–	L/–
Cucurbita pepo cv. Black Beauty	–/– (–/–)	SL/NR (+/+)	SL/M
Vigna unguiculata	L/–	L/–	L/–

[a]Abbreviations: M, mosaic; NR, necrotic ringspots; L, large size of necrotic local legions; sL, small size of necrotic local legion; SL, symptomless and –, uninfected.
[b]Inoculated leaves/systemic leaves.
[c]Based on RT-PCR analysis, positive reaction (+) and negative reaction (–) were indicated in parenthesis.

and yellow-streak symptoms in easter lily plants which were their natural hosts (Fig. 1). To characterize further pathological properties between the two lily isolates, two isolates purified were mechanically inoculated onto 8 species of indicator plants. For direct comparison of their pathological properties, CMV strain Fny (Fny-CMV) that has been well studied was used as a control. Host range and symptoms of Ly2-CMV and Ly8-CMV were summarized in Table 1. Although the difference of induction time and severity of symptoms was observed somehow, two lily isolates showed systemic infection on *N. benthamiana*, and local infection on cowpea and *C. amaranticolor*, similar to Fny-CMV. However, Ly2 and Ly8 did not infect systemically tomato and cucumber that are natural CMV hosts, compared to Fny. Interestingly, Ly8-CMV induced mild mosaic symptom on un-inoculated upper leaves *of N. glutinosa* and zucchini squash (*Cucurbita pepo*) and ringspot symptom on both inoculated and systemic leaves of *N. tabacum* cv. Xanthi-nc, respectively (Table 1 and Fig. 2). Ly2-CMV showed successful local infections on inoculated leaves of Xanthi-nc, but failed to produce systemic symptom on these plants.

To determine systemic symptom productions in tested plants were caused by Ly8-CMV, Ly8-CMV detection was analyzed by RT-PCR using Cucumovirus-specific primers, as described previously (Choi et al., 1999). Result from RT-PCR analysis clearly demonstrated the detection of genomic RNA of Ly8-CMV in symptom-induced leaf tissues of the tobacco and squash plants (data not shown). As expected, any RT-PCR product from Ly2-CMV was not detected in the systemic leaves of plants inoculated. These results confirmed systemic infection of Ly8-CMV on tobacco and zucchini squash.

To know whether mutations on the genome of Ly8-CMV or of Ly2-CMV was generated, Ly8-inoculum from systemically infected tobacco leaves or Ly2-inoculum from locally infected tobacco leaves was back-inoculated onto *N. benthamiana* as well as several host plants. We observed indistinguishable pathogenicity of Ly8-CMV and Ly2-

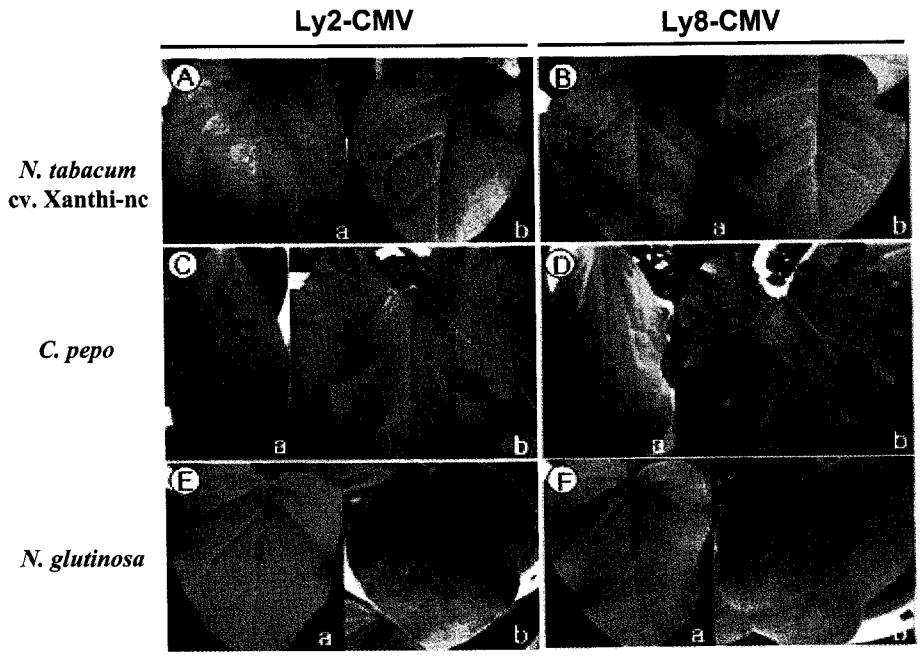


Fig. 2. Symptoms of host plants infected with Ly2-CMV (A, C, E) or Ly8-CMV (B, D, F). Symptom on inoculated leaf (a) and symptom on un-inoculated upper leaf (b) were indicated at the bottom of photos.

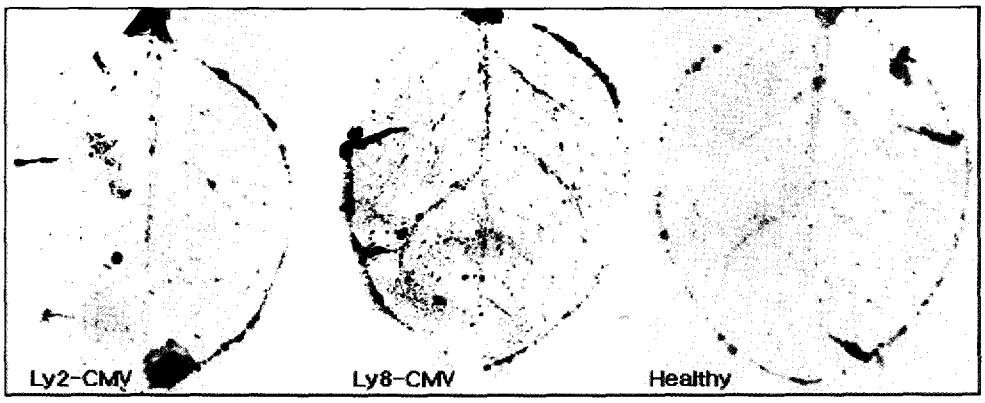


Fig. 3. Leaf-press immunoblot assay for systemic infections of isolates Ly2 and Ly8. Tobacco (cv. Xanthi-nc) was inoculated with Ly2, Ly8 or healthy (buffer). Uninoculated upper leaves of the tobacco were detached and blotted onto nitrocellulose membranes. The membranes were probed with antiserum against CP of CMV and anti-CP antibodies detected using a secondary antibody conjugated to alkaline phosphatase. The purple color foci show the presence of CMV CP.

CMV on the basis of host responses. Additionally, the analysis of the CP genes from the back-inoculated plants showed no sequence changes (data not shown). Although two lily isolates showed less infectivity than Fny-CMV, the pathogenicity of Ly8-CMV is likely to be higher than that of Ly2-CMV on the basis of infectivity data. These results suggested that pathological diversity had been existed in a population of lily isolates of CMV, affecting that the lily isolates retained a distinct niche in CMV population.

Analysis of systemic infection between Ly2-CMV and Ly8-CMV. As demonstrated above, Ly8-CMV was able to infect systemically tobacco and squash plants, while Ly2-CMV could not.

To determine whether the different infectivity between Ly2-CMV and Ly8-CMV affected rate of local and systemic movements in those plants, we chose tobacco (cv. Xanthi-nc) plants that showed distinguishable systemic symptom. Sap from *N. benthamiana* infected by Ly2 or Ly8 was inoculated onto young leaves of tobacco. Inoculated leaves (5 dpi) or systemic leaves (10dpi) were detached and blotted onto nitrocellulose membrane by physical press, respectively. The membrane blotted with inoculated tobacco leaves showed there were little difference of local movement between Ly8 and Ly2 (data not shown). However, it was clearly shown that systemic spreading of Ly8-CMV was detected on throughout systemic leaves of tobacco along with veins, suggesting that Ly8-CMV was extensively accumulated in systemic leaves of the tobacco. While, accumulation of Ly2-CMV on systemic tobacco leaves was not detectable until 14-20 dpi (Fig. 3 and data not shown). It is coincident with RT-PCR result which confirmed the systemic infection of Ly8-CMV in tobacco. It is possible that host factors involved in a resistance that was specific to prevention of long-distance movement of Ly2-CMV, but not Ly8-CMV. In absence of specific proteins involved in such resistance, it came from virus in itself devoid of the ability of systemic infection.

Sequence analysis of Ly2-CMV and Ly8-CMV. To know whether the alterations of genomic sequences determined the rate of systemic invasion also affected movement of the corresponding viruses, genome sequences of Ly2-CMV and Ly8-CMV were determined by dye-termination protocol, as standard protocol (Sambrook et al., 1989). The determined nucleotide sequences were assembled by using DNASTAR program. The genome sequence of Fny-CMV was used as a control for sequence comparisons. Overall, the sequence identity (approx. 97%) of genome between Ly2 and Ly8 was higher than that of Fny-Ly2 or of Fny-Ly8 (Table 2). The isolates Ly8 and Ly2 belonged to CMV subgroup IA, according to CP analysis (data not shown). There was high sequence identity ranged from 96 to 98% in 1a protein or 2a protein among lily isolates and Fny strain (Fig. 4). Many variation of aa was found in the central region of 1a protein and the N-terminal region of 2a protein. Although we could not find any specific amino acids/sequences conferring unique phenotypes of Ly2-CMV and Ly8-CMV, the amino acids of 3a protein, CP and 2b protein responsible for virus movement and resistance breakage were higher conservation than 1a and 2a proteins (Fig. 4).

Discussion

CMV is one of the commonest plant viruses in many plant species, including pepper, tobacco, cucurbits, ornamentals, vegetables, and legumes, in Korea. In this paper, we characterized biological properties and genome comparison of two lily isolates of CMV. Different isolates of CMV from

Table 2. Sequence comparisons of nucleotides and deduced amino acids between Ly2-CMV and Ly8-CMV or between Ly2-CMV and Fny-CMV[a]

CMV	RNA1 (Homology %)				
	overall	**1a**		5' UTR	3' UTR
		nt	aa		
Ly2	3400	2982	994	95	323
Ly8	3397	2973	991	94	330
	(96.7)	(98.8)	(98.7)	(95.8)	(99.4)
Fny	3357	2982	994	94	281
	(96.4)	(96.6)	(97.2)	(100)	(93.2)

CMV	RNA2 (Homology %)						
	overall	**2a**		**2b**		5' UTR	3' UTR
		nt	aa	nt	aa		
Ly2	3062	2574	858	333	111	83	313
Ly8	3063	2574	858	333	111	83	312
	(97.4)	(99.2)	(98.6)	(97.6)	(99.1)	(98.8)	(98.7)
Fny	3050	2574	858	333	111	85	390
	(94.9)	(95.0)	(96.5)	(94.6)	(94.6)	(91.8)	(93.6)

CMV	RNA3 (Homology %)							
	overall	**3a**		CP		5' UTR	3' UTR	IGR
		nt	aa	nt	aa			
Ly2	2223	840	279	657	219	119	308	299
Ly8	2229	840	279	657	219	119	318	299
	(99.1)	(99.8)	(99.6)	(99.4)	(99.1)	(100)	(98.7)	(100)
Fny	2216	840	279	657	219	119	303	297
	(94.9)	(95.5)	(98.2)	(96.8)	(98.2)	(93.6)	(89.5)	(94.9)

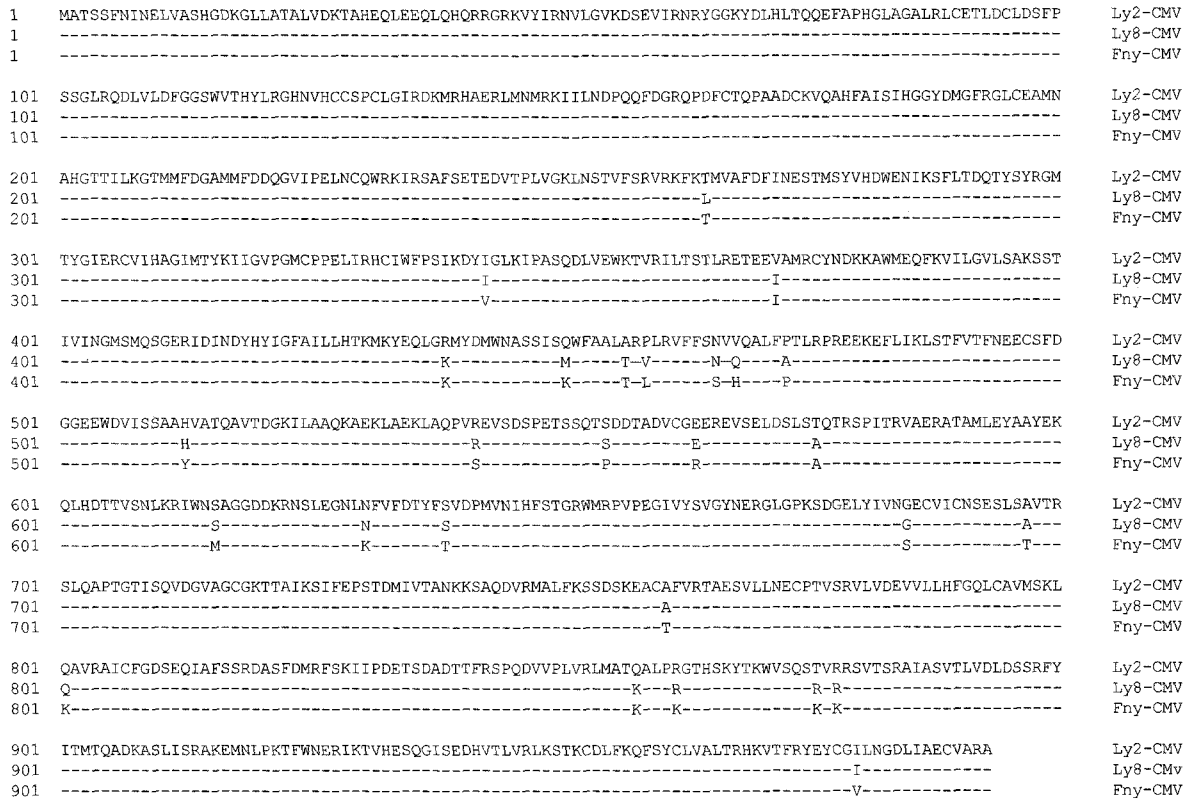
[a]The genomic RNA sequences of Ly2-CMV were deposited to Genbank (AJ296154, AJ535914, and AJ535913). The genome sequence of Ly8-CMV was determined in this study. The genome of Fny-CMV (Genbank accession numbers: D00356, D00355, andD10538) was used for comparison.

lily species have been previously reported and characterized (Chen et al., 2001; Choi et al., 2004; Jung et al., 2000; Masuta et al., 2002; Ryu et al., 2002). In general, most CMV lily isolates showed different host reactions from CMV isolates, which they induced mild systemic symptoms in many indicator plants. Furthermore, CMV lily isolates had a relatively narrow host range, compared to other CMV strains. There were reported that most CMV isolates collected from various plants infected well many species of tobacco and cucurbit plants that have been used for CMV propagation as good hosts (Palukaitis et al., 1992). Although it was reported that CMV isolate Li (Li-CMV) was able to infect tobacco (cv. Xanthi-nc) systemically (Ryu et al., 2002), many CMV lily isolates failed systemic infection, or failed even local infection in many species of tobacco and cucurbit plants. Similarly, pathogenicity of Ly8-CMV was dramatically different from that of Ly2-CMV in tobacco and zucchini squash. We demonstrated that both viruses could infect locally Xanthi-nc plant, but systemic infection of Ly8-CMV was only observed (Table 1 and Fig. 2). Systemic symptom of tobacco induced by Ly8-CMV was distinguished from that of tobacco induced by Fny-CMV (nerotic ringspot vs. mosaic). Furthermore, we clearly showed that Ly2 failed local and systemic infections on *N. glutinosa*, but Ly8 successfully infected the same plants (Table 1 and Fig. 2). Interestingly, Li-CMV which was able to infect Xanthi-nc plant systemically failed even local infection of *N. glutinosa* (Ryu et al., 2002).

Although isolates Ly2 and Ly8 used in this study shared high sequence identity each other or high sequence identity with Fny that is able to infect tobacco. It is plausible that some amino acids of 1a protein and 2a protein are more important for the difference of phenotypes between Ly2 and Ly8 than 2b, 3a protein and CP.

Since a pseudorecombinant between Fny-CMV and LK-CMV (designated F1F2LK3-CMV) could move systemically in Xanthi-nc (S.K. Choi, unpublished data) and changed its systemic symptom on zucchini squash (Choi et

1a protein



2a protein

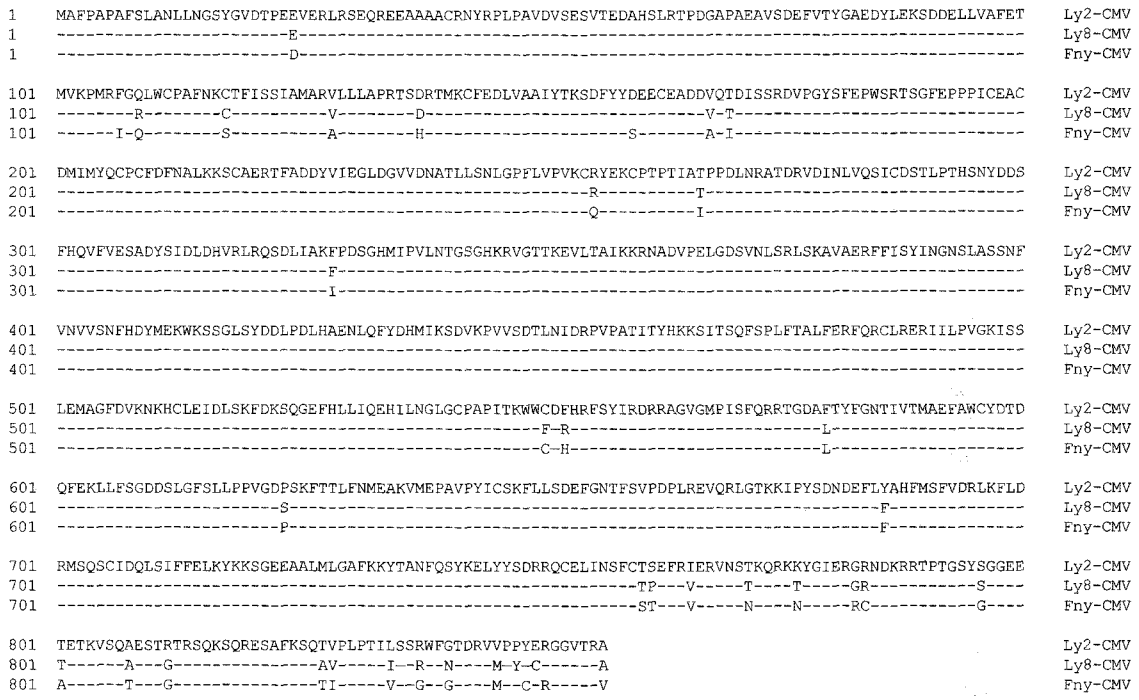


Fig. 4. Multiple alignment analysis of coding proteins among Ly2-CMV, Ly8-CMV and Fny-CMV. The dash bar means that the same amino acids residue. The aa position was indicated on the left side of sequence panel.

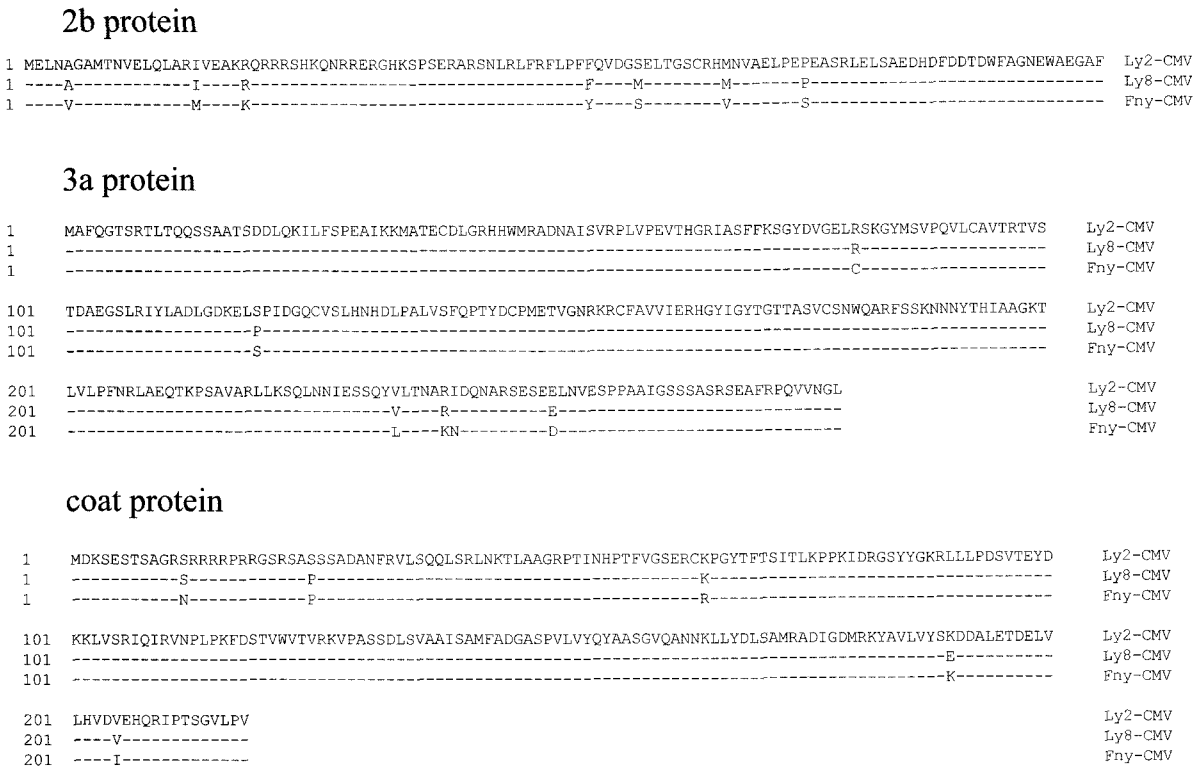


Fig. 4. Continued.

al., 2004). In case of Fny-Sny, additionally, RNA1 controlled induction time of systemic symptoms in zucchini squash (Roossinck and Palukaitis, 1990). In radish and zucchini squash, RNA2 itself or RNA2 and RNA3 was involved in viral systemic spread in radish plant (Choi et al., 2003, 2005; Takeshita et al., 1998). Results from pseudo-recombinant and chimeric viruses between Y-CMV and HL-CMV demonstrated that RNA1 of HL-CMV was a determinant in systemic infection of lily plants (Yamaguchi et al., 2005).

Regarding 2a protein, the mutation of amino acid (aa) 631 and 641 of Fny 2a protein allowed host reactions from hypersensitive reaction to systemic mosaic symptom in cowpea (Kim and Palukaitis, 1997). Similarly, previous studies with Pf-CMV had high sequence identity (approx. 98%) with Fny-CMV have demonstrated that the alteration of aa 267 of 2a affected their phenotypes in zucchini squash (Choi et al., 2005). Further delimitation of genomic sequences responsible for pathogenicity determination is to be remained.

In view sides of host plant, tomato and cucumber appeared to have high resistance against isolates Ly2 and Ly8, but not strain Fny. Although we did not have an obvious evident, it is assumed that this resistance was involved in a block in cell-to-cell movement of Ly2-CMV and Ly8-CMV, because any CP genes from tomato and cucumber inoculated were not detected by RT-PCR. As shown similarly the case of extreme resistance for M-CMV (subgroup IA), RNA3 of M-CMV that share high sequence identity (99%) with RNA3 of Fny-CMV failed to infect maize (Ryu et al., 1998). Otherwise, anther possible hypothesis is that genomic RNAs of lily isolates by itself are not able to replicate efficiently in single cell of tomato or cucumber.

Detail explanation for this resistance mechanism specific to CMV lily isolates is to be remained.

According to this resistance concept, we observed that one tobacco species (cv. Xanthi-nc) had resistance specific to Ly2-CMV, but not to Ly8-CMV. Leaf-press blot assay clearly showed the resistance specific to Ly2 is associated with prevention of uploading into phloem tissues (Fig. 3). The CP of CMV plays extensive roles in long-distance movement between different tissue types (Blackman et al., 1998; Taliansky and Garicia-Arenal, 1995). So far, the understanding of systemic movement of viruses on the phloem is limited, both regarding necessity of plant factors and the viral structures that move through sieve tubes (Scholthof, 2005; Waigmann et al., 2004).

Complete nucleotide sequence data would be sufficient to establish relationships between strains. Small changes in nucleotide sequence could give very different phenotypic effects. Substantial variations of CMV lily isolates may occur by exchanges between parts of a multi-partite genome

during viral replication under selection pressure. As a result, the adapted CMV strain has different host ranges that often produce different disease symptoms on some common host (Roossinck, 1997). In these views, systematic study of symptoms for the fitness of several host species or varieties under standard conditions may help considerably delineate strains among large numbers of field isolates of plant viruses, such as CMV, *Tobacco mosaic virus*, *Potato virus Y* and *Alfalfa mosaic virus* (J.K. Choi, unpublished data).

Clearly, data of this study and previous studies show that infections of host plants as well as many other cultivars included in this study, depend on CMV isolates, which in turn indicates biological variation between CMV isolates.

Acknowledgements

This study was supported by a Research Grant (2006) from Kangwon National University.

References

Blackman, M. L., Boevink., P., Santa Cruz, S., Palukaitis, P. and Oparka, K. J. 1998. The movement protein of cucumber mosaic virus traffics into sieve elements in minor veins of *Nicotiana clevelandii*. *Plant Cell* 10:525-537.

Chen, Y. K., Derks, A. F. L. M., Langeveld, S., Goldbach, R. and Prins, M. 2001. High sequence conservation among cucumber mosaic virus isolates from Lily. *Arch. Virol.* 146:1631-1636.

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

Choi, S. K., Choi, J. K. and Ryu, K. H. 2003. Involvement of RNA2 for systemic infection of *Cucumber mosaic virus* isolated from lily on zucchini squash. *Virus Res.* 97:1-6.

Choi, S. K., Ahn, H. I., Kim, M., Choi, J. K. and Ryu, K. H. 2004. Symptom determinant as RNA3 of lily isolates of cucumber mosaic virus on zucchini squash. *Plant Pathol. J.* 20:212-219.

Choi, S. K., Palukatis, P., Min, B. E., Lee, M. Y., Choi, J. K. and Ryu, K. H. 2005. *Cucumber mosaic virus* 2a polymerase and 3a movement proteins independently affect both virus movement and the timing of symptom development in zucchini squash. *J. Gen. Virol.* 86:1213-1222.

Domingo, E., Escarmís, C., Sevilla, N., Moya, A., Elena, S. F., Quer, J., Novella, L. and Holland, J. J. 1996. Basic concepts in RNA virus evolution. *FASEB J.* 10:859-864.

Domingo, E. and Holland, J. J. 1997. RNA virus mutations and fitness for survival. *Annu. Rev. Microbiol.* 51:151-178.

Hagita, T., Kodama, F. and Akai, J. 1989. The virus diseases of lily in Hokkaido. *Ann. Phytopathol. Soc. Japan* 55:1-8.

Huppert, E., Szilassy, D., Salanki, K., Diveki, Z. and Balazs, E. 2002. Heterologous movement protein strongly modifies the infection phenotype of cucumber mosaic virus. *J. Virol.* 76:3554-3557.

Gal-On, A., Kaplan, J., Roossinck, M. J. and Palukaitis, P. 1994. The kinetics of infection of zucchini squash by cucumber mosaic virus indicates a function for RNA1 in virus movement. *Virology* 205:280-289.

García-Arenal, F., Fraile, A. and Malpica, J. M. 2001. Variability and genetic structure of plant virus populations. *Annu. Rev. Phytopathol.* 39:157-186.

Gibbs, A. J., Calisher, C. H. and Garcia-Arenal, F. 1995. Molecular basis of virus evolution. Cambridge University Press, UK.

Jung, H. J., Ueda, S., Ryu, K. H., Lee, S. Y. and Choi, J. K. 2000. A novel strain of *Cucumber mosaic virus* isolated from *Lilium longiflorum*. *Plant Pathol. J.* 16:306-311.

Kim, C. H. and Palukaitis, P. 1997. The plant defense response to cucumber mosaic virus in cowpea is elicited by the viral polymerase gene and affects virus accumulation in single cells. *EMBO J.* 16:4060-4068.

Masuta, C., Seshimo, Y., Mukohara, M., Jung, H. J., Ueda, S., Ryu, K. H., and Choi, J. K. 2002. Evolutionary characterization of two lily isolates of *Cucumber mosaic virus* isolated in Japan and Korea. *J. Gen. Plant Pathol.* 68:163-168.

McGarvey, P., Tousignant, M., Geletka, L., Cellini, F. and Kaper, J. M. 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.

Palukaitis, P. and Garcia-Arenal, F. 2003. Cucumoviruses. *Adv. Virus. Res* 62: 241-323.

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

Rizzo, T. M. and Palukaitis, P. 1990. Construction of full-length cDNA clones of cucumber mosaic virus RNAs 1, 2 and 3: generation of infectious RNA transcripts. *Mol. Gen. Genet.* 222:249-256.

Roossinck, M. J. 1997. Mechanisms of plant virus evolution. *Annu. Rev. Phytopathol.* 35:191-209.

Roossinck, M. J. and Palukaitis, P. 1990. Rapid induction and severity of symptoms in zucchini squash (*Cucurbita pepo*) map to RNA1 of cucumber mosaic virus. *Mol. Plant-Microbe Interact.* 3:188-192.

Roossinck, M. J., Zhang, L. and Hellward, K. H. 1999. Rearrangements in the 5' nontranslated region and phylogenetic analyses of cucumber mosaic virus RNA3 indicate radial evolution of three subgroups. *J. Virol.* 73:6752-6758.

Ryu, K. H., Kim, C. H. and Palukaitis, P. 1998. The coat protein of cucumber mosaic virus is a host range determinant for infection of maize. *Mol. Plant-Microbe Interact.* 11:351-357.

Ryu, K. H., Park, W. M. and Choi, J. K. 2002. Characterization and sequence analysis of a lily isolate of *Cucumber mosaic virus* from *Lilium tsingtauense*. *Plant Pathol. J.* 18:85-92.

Sambrook, J., Fritsch, E. F. and Maniatis, T. 1989. Molecular cloning. A laboratory Manual. Cold Spring Harbor, NY: Cold. Spring Harbor Laboratory.

Sanger, F., Niklen, S. and Colson, A. R. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA*

74:5463-5467.

Scholthof, H. B. 2005. Plant virus transport: motions of functional equivalence. *Trends Plant Sci.* 10:376-382.

Shintaku, M. H., Zhang, L. and Palukaitis, P. 1992. A single amino acid substitution in the coat protein of cucumber mosaic virus induces chlorosis in tobacco. *Plant Cell* 4:751-757.

Suzuki, M., Kuwata, S., Masuta, C. and Takanami, Y. 1995. Point mutations in the coat protein of cucumber mosaic virus affect symptom expression and virion accumulation in tobacco. *J. Gen. Virol.* 76:1791-1719.

Szilassy, D., Salanki, K. and Balazs, E. 1999. Stunting induced by cucumber mosaic cucumovirus-infected *Nicotiana glutinosa* is determined by a single amino acid residue in the coat protein. *Mol. Plant-Microbe Interact.* 12:1105-1113.

Taliansky, M. E. and Garcia-Arenal, F. 1995. Role of cucumovirus capsid protein in long-distance movement within the infected plant. *J. Virol.* 69: 916-922.

Takeshita, M., Suzuki, M., Kuwata, S. and Takanami, Y. 1998. Involvement of cucumber mosaic cucumovirus RNA2 and RNA3 in viral systemic spread in radish plant. *Arch. Virol.* 143:1109-1117.

Takeshita, M., Suzuki, M. and Takanami, Y. 2001. Combination of amino acids in the 3a protein and the coat protein of cucumber mosaic virus determines symptom expression and viral spread in bottle gourd. *Arch. Virol.* 146:697-711.

Waigmann, E., Ueki, S., Trutnyeva, K. and Citovsky, V. 2004. The ins and outs of nondestructive cell-to-cell and systemic movement of plant viruses. *Crit. Rev. Plant Sci.* 23:195-250.

Yamaguchi, N., Seshimo, Y. and Masuta, C. 2005. Mapping of the sequence domain for systemic infection in edible lily on the viral genome of *Cucumber mosaic virus*. *J. Gen. Plant Pathol.* 71:373-376.

Zhang, L., Hanada, K. and Palukaitis, P. 1994. Mapping local and systemic symptom determinants of cucumber mosaic cucumovirus in tobacco. *J. Gen. Virol.* 75:3185-3191.