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Nucleotide Sequence of Coat Protein Gene of Kyuri Green Mottle Mosaic Virus Isolated from Zucchini

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The coat protein (CP) gene of kyuri green mottle mosaic virus zucchini strain (KGMMV-Z) isolated from zucchini (*Cucurbita pepo*) in Chonju, Korea in 1999 was sequenced by the reverse transcription and polymerase chain reaction with degenerate and generate primers originated from tobamoviruses. The degenerate primers were very effective in amplification of KGMMV-Z CP region. The KGMMV-Z CP gene consisted of 486 nucleotides and had the same nucleotide length compared with those of cucurbit-infecting tobamoviruses. KGMMV-Z CP gene shared 43.8, 44.2, and 44.4% nucleotide sequence similarity with the CP gene of cucumber green mottle mosaic virus watermelon strain (CGMMV-W), CGMMV-KW1, and CGMMV-SH, respectively, whereas three CGMMV strains among themselves showed 98.6-99.6% nucleotide similarity. The deduced amino acids of KGMMV-Z CP gene were 161 amino acid residues with the molecular weight of 17,181 daltons. The first 24 codons of KGMMV-Z CP gene corresponded to the sequences of the N-terminal amino acid of the viral capsid protein. The amino acid sequences of KGMMV-Z CP had 45.3% similarity compared with those of three CGMMV strains. However, the amino acid sequences of CGMMV strains were identical. These results showed that two cucurbit-infecting tobamovirus members, KGMMV-Z and CGMMV were genetically distantly related.

Keywords : CGMMV, coat protein, KGMMV, tobamovirus, zucchini

Cucurbit-infecting tobamoviruses were classified with two tobamovirus members (species), CGMMV and KGMMV (Regenmortel and Meshi, 1995). But there was considerable confusion regarding the identity of tobamoviruses infecting cucurbits, because they had similar pathogenicity in cucurbits. CGMMV was first described as cucumber virus 3 (CV3) and cucumber virus 4 (CV4) in Europe by Ainsworth (1935), and then a number of strains have been described: Indian strain C (Vasudeva et al., 1949), watermelon strain (Komuro et al., 1971), and SH strain (Ugaki et al., 1991). These CGMMV strains have been shown to be very similar, both chemically (Meshi et al., 1983; Nozu et al., 1971) and serologically (Nozu et al., 1971). On the other hand, KGMMV was originally reported as cucumber strain (CGMMV-C) (Inouye et al., 1967) and Yodo strain (Kitani et al., 1970) of CGMMV from cucumbers showing severe fruit distortion. The CGMMV-C also has been referred to as the Japanese CV3 in spite of its CP being chemically distinct from the original CV3 (Tung and Knight, 1972; Van De Walle and Siegel, 1982). The CGMMV-C was taxonomically different from the strains CV3, CV4 and CGMMV-W, shown by the lack of molecular hybridization, and was suggested a new virus name, 'KGMMV' (Francki et al., 1986). KGMMV is presently classified as a member out of 13 tobamovirus members (Regenmortel and Meshi, 1995).

At present, three viruses, cucumber mosaic virus (CMV) (Lee, 1981), watermelon mosaic virus (WMV) (Lee and Lee, 1981), and zucchini yellow mosaic virus (ZYMV) (Kim et al., 1995), have been reported in *Cucurbita* spp. in Korea. A new disease was found in greenhouse-grown zucchini (*Cucurbita pepo*) showing chlorotic mosaic and fruit malformation in Chonju, in 1999, and severely affected economic value of the products. The agent of this viral disease was considered as a KGMMV strain in view of particle morphology, antigenic property, and reaction of test plants (K. W. Lee et al., unpublished data). Here we called the virus isolated from zucchini as KGMMV zucchini strain (KGMMV-Z) due to zucchini being the new natural host of KGMMV. The nucleotide sequence of KGMMV was not known, but several strains of CGMMV were determined nucleotide sequence of genomic RNA (Lee, 1999; Meshi et al., 1983; Ugaki et al., 1991). In this paper, we determined the nucleotide sequences of the causal virus (KGMMV-Z) CP gene using degenerate primers acting on

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tobamoviruses, and then compared it with those of cucurbit-infecting tobamoviruses.

Materials and Methods

Virus isolates. KGMMV in this study was isolated from zucchini showing chlorotic mosaic (Fig. 1A) and fruit malformation (Fig. 1B) in Chonju, Korea in 1999. The zucchini seeds planted in the field were imported from China in 1997.

Virus propagation, purification and RNA extraction. KGMMV-Z was passaged through three times of single local lesion transfers on *Datura stramonium* and propagated in *Cucumis sativus* and *Cucurbita pepo*. The virus was purified from leaves of propagation host according to the modified procedure described by Tung and Knight (1972). The viral RNA was isolated from purified virus by the proteinase K method (Maniatis et al., 1982).

Primers. Five degenerate primers and three generate primers were used for amplifying the region of KGMMV-Z CP gene. Five degenerate primers were synthesized acting on all tobamoviruses based on sequence analysis of 10 tobamoviruses, which were CGMMV (Ugaki et al., 1991), SHMV (Meshi et al., 1981), TMV-crucifer (Dorokhov et al., 1993), ORSV-S1 (Chng et al., 1996), PMMoV-S (Alonso et al., 1991), TMV-Rakkyo (Chen et al., 1996), TMGMV (Solis and Garcia-Arenal 1990), TMV-Ob (Ikeda et al., 1993), TMV-OM (Meshi et al., 1982), and ToMV (Ohno et al., 1984). Primers, MPHR1 and MPHR2, CPHR5 and CPHR3, and CCT1 were originated from the movement protein (MP) gene, CP gene and 3'-terminal of tobamoviruses, respectively. PCGMM-N1, PCGMM-N2, and PCGMM-C2 were the specific primers of CGMMV (Lee, 1999). The nucleotide sequence, length, and origin of these primers are listed in Table 1.

cDNA synthesis. First-strand cDNAs synthesis from KGMMV-Z ssRNA were performed by reverse transcription (RT) using CCT1, CPHR3, and PCGMM-C2 as a downstream primers. RT reaction was performed with 20 μl of total reaction volume which contained 25 pmoles downstream primer, 1 μl KGMMV-Z RNA extracted from purified virus, 50 mM Tris-HCl, 30 mM KCl, 8 mM $MgCl_2$, 1 mM dithiothreithol, 2 mM dNTP, 20 units RNase inhibitor, and 9 units AMV reverse transcriptase. The mixture was incubated in a PTC-100™ Programmable Thermal Controller (MJ

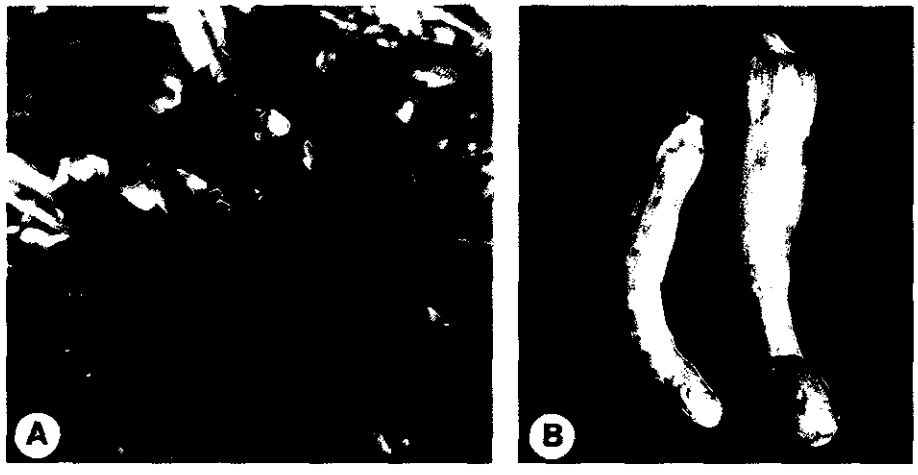

Fig. 1. Symptoms on zucchini plant infected with kyuri green mottle mosaic virus zucchini strain (KGMMV-Z). chlorotic mosaic symptoms on leaf (A) and malformed zucchini fruits (B).

Table 1. Primers used in this study and their nucleotide sequences

Primers	Sequence (5'-3')	Length	Origin
MPHR1[a]	TNGTNKTNWCNGGNSWRTGG	20	MP
MPHR2[a]	CCDYTDDCHBTDGARDTYG	19	MP
CPHR5[a]	GAYACKMGDAATAGRRTMAT	20	CP
CPHR3[a]	ATKAYYCTATTHCKMGTRTC	20	CP
CCT1[a]	TGGGCCSCTACCSGSG	16	3'-terminal
PCGMM-N1[b]	AATCGGAGGTTGGACTCTGCTTCTG	25	MP
PCGMM-N2[b]	CGACTGCTGAGTCGCTTAACGCTGT	25	CP
PCGMM-C2[b]	GAAAACGCGGCTTCAAATGAAGCCC	25	CP

[a] Originated from 10 tobamoviruses, CGMMV (Ugaki et al., 1991), SHMV (Meshi et al., 1981), TMV-crucifer (Dorokhov et al., 1993), ORSV-S1 (Chng et al., 1996), PMMoV-S (Alonso et al., 1991), TMV-Rakkyo (Chen et al., 1996), TMGMV (Solis and Garcia-Arenal, 1990), TMV-Ob (Ikeda et al., 1993), TMV-OM (Meshi et al., 1982), ToMV (Ohno et al., 1984).
[b] Originated from CGMMV strains.

research) at 42°C for 30 min, followed by 95°C for 5 min.

Second-strand cDNAs were synthesized by polymerase chain reaction using MPHR1, MPHR2, CPHR5, PCGMM-N1, and PCGMM-N2 as an upstream primers. For PCR, the 20 μl RT product was transferred to a tube containing 80 μl master mix (25 pmoles upstream primer, 2.5 units Taq DNA polymerase, 10 mM Tris-HCl, 50 mM KCl, 0.4 mM $MgCl_2$). The tubes were heated at 95°C for 2 min followed by 35 reaction cycles of 45 sec at 94°C for melting, 1 min at 50°C for primer annealing, and 1.5 min at 72°C for primer extension. A final step at 72°C for 7 min was carried out prior to holding the samples at 4°C until removal from the thermal controller.

The cDNAs amplified with RT-PCR were purified with QIAquick gel extraction kit (QIAGEN Co.) following manufacturer's instruction.

Nucleotide sequence determination. The cDNA fragments of KGMMV-Z RNA were amplified by using a Taq BigDye™ terminator cycle sequencing kit (Applied Biosystems) and the previously described primers (Table 1). Amplification was performed in the thermal controller and conducted 25 cycles as follows: denaturation at 96°C for 10 sec, annealing at 50°C for 5 sec, extension at 60°C for 4 min. The sequences of the products were analyzed with a model ABI 310 automatic sequencer (Perkin Elmer Cetus).

N-terminal amino acid sequence analysis of KGMMV-Z capsid protein. KGMMV-Z capsid protein was extracted from purified viruses by boiling in sample buffer for 3 min, and the protein was separated on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) which was performed in a 0.75 mm, 12.5% acrylamide-bis gel by the method of Laemmli (1970). After electrophoresis, the protein was electroblotted onto polyvinylidene difluoride membranes. The blots were then stained for 5-10 min with 0.1% Coomassie Brilliant Blue R-250 in distilled water followed by destaining in water. Polypeptide spots that were well resolved were cut out and then subjected to Edman degradation using an ABI model 476A protein sequencer (Applied Biosystems) according to the manufacturer's recommendations.

Sequence analysis. Nucleotide and amino acid sequence were analysed using the software of DNASTAR package (version 1.02, DNASTAR). The nucleotide and amino acid sequence of KGMMV-Z CP gene were compared with the published sequence of CGMMV-W (Meshi et al., 1983), CGMMV-SH (Ugaki et al., 1991), and CGMMV-KW1 (Lee, 1999).

Results

Virus purification. The final suspension contained rod-shaped particles about 300 nm in length with typical helical profiles when the preparation of KGMMV-Z was suspended in 0.02 M phosphate buffer at pH 7.2 and the negatively stained with 2% phosphotungstic acid (Fig. 2).

cDNA synthesis. The cDNA fragments of KGMMV-Z CP region were synthesized from genomic RNA extracted from the purified virus by RT-PCR using degenerate and generate primers. The result was that one or several cDNAs were synthesized according to the property of primer pairs. Ten cDNA products of expected sizes and large quantity among these RT-PCR products were purified (Fig. 3), and then determined for the nucleotide sequence. Seven cDNA fragments (lane 1, 2, 3, 4, 6, 8, and 9 in Fig. 3) of them included the CP gene region of KGMMV-Z, but three cDNA fragments (lane 5, 7, and 9 in Fig. 3) did not. Reaction sites of primers were displayed in Fig. 4. Degenerate primers, MPHR1, CPHR5 and CPHR3, and CCT1 were attached to MP, CP, and 3'-terminal region, respectively. Besides, CCT1 originated from 3'-terminal of tobamoviruses was also attached to CP region, while MPHR2 was not effectively reacted on MP gene. Three generate primers originated from CGMMV, PCGMM-N1, PCGMM-N2, and PCGMM-C2 were also reacted on KGMMV-Z genomic RNA. In conclusion, the degenerate primers origi-

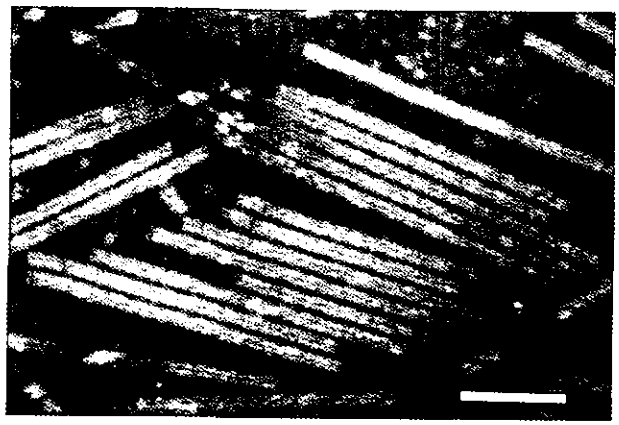

Fig. 2. Electron micrograph of purified preparations of kyuri green mottle mosaic virus zucchini strain (KGMMV-Z) negatively stained with 2% phosphotungstic acid. Bar=100 nm.

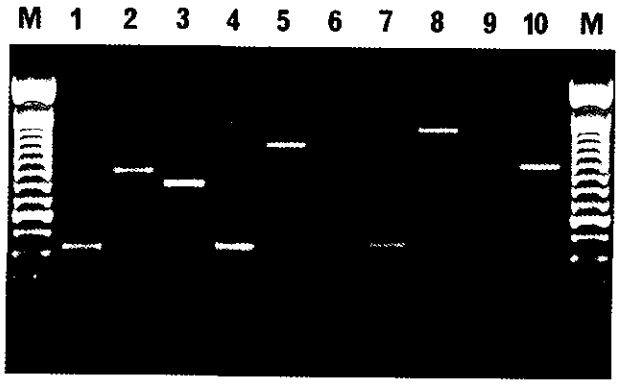

Fig. 3. Purified kyuri green mottle mosaic virus zucchini strain (KGMMV-Z) cDNA fragments synthesized with RT-PCR using degenerate and generate primers. M, size marker (100 bp ladder); lane 1, primers, CCT1 and CPHR5; lane 2, CPHR3 and MPHR1; lane 3 and 8, CCT1 and MPHR1; lane 4 and 5, CCT1 and PCGMM-N1; lane 6 and 7, CCT1 and PCGMM-N2; lane 9 and 10, PCGMM-C2 and PCGMM-N1.

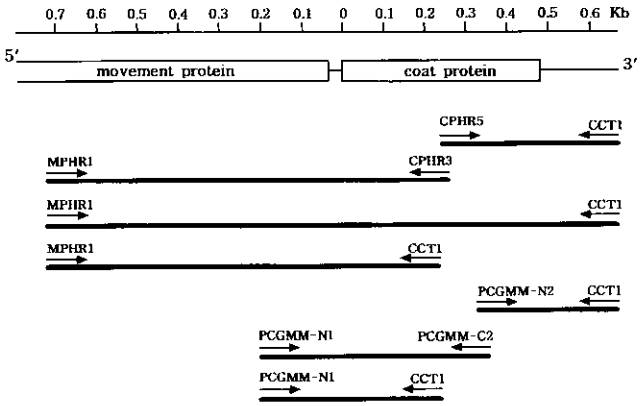

Fig. 4. cDNA fragments used for sequencing the coat protein gene of kyuri green mottle mosaic virus zucchini strain (KGMMV-Z). The numbers on the upper line indicate the distance from the beginning of coat protein gene. Letters and arrows on the bold lines (cDNA fragments) represent the used primers and direction of direct sequencing, respectively.

nated from 10 tobamovirus sequences were very effective in the amplification of KGMMV-Z CP region.

Sequence of KGMMV-Z CP gene. The nucleotide sequences of cDNA fragments were determined and found to be 1,102 nucleotides (Fig. 5). The initiation codon of CP gene was confirmed by N-terminal amino acid sequence analysis of capsid protein. Position 1 to 480 is the part of MP gene, position 481 to 966 is the complete CP gene, and position 967 to 1,102 is the part of 3' non-coding region. The CP cistron is 486 nucleotides encoding a protein of 161 amino acid residues with the molecular weight of 17,181 daltons.

N-terminal amino acid sequence analysis and compositional analysis. Determined N-terminal amino acid sequences of the capsid protein were PYSTSGIRSLPAFSKS-FFPYLELY. The first 24 codon of CP gene and the sequence of the N-terminal amino acid of the capsid protein

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   1 ACGAAGGAACTATCTGCAAGTTCTCAGTCGCCGCATCTGCTCGTGACTTTATGGTTAAGTTAATTCCTAACTATTACGTCACTGCAACCG   90
  91 ATGCTTCATCGAAACCCTGGTCTATTTTCGTTAGAGTGTCTGGTGTGCGTATAAAAGAAGGGTTTTCTCCGCTTACGCTTGAGATCGCTT  180
 181 CTTTAGTAGCTACTACTAACTCTATTCTTAAGAAAGGTCTTCGTGTTAGTGTCTTAGAATCTGTCGTGGGCAGTGATGCTTCTGTCAATT  270
 271 TGGAGTCTGCTTCTGAGAAGGTTCAACCCTTCTTCGATTCAGTTCCTATTACAGCTGCCGTCATTTCTCGCGATAGGTCTTACGTTTCTA  360
 361 AGTCTGGCTTTAATCGTGCTGTACGATCTAAGCCTCCGTCTAAAGGTGGGAAGAAATTTGGCGATTCTGCTGAGAGTTTGTCGGAGGATA  450
 451 GCGCTTCTGAGCTACCCGGTTTATAACAAGATGCCTTACTCTACCAGCGGTATTCGTTCGCTTCCTGCTTTTTCTAAGTCTTTTTTCCCT  540
                  → P Y S T S G I R S L P A F S K S F F P

 541 TATTTGGAGTTGTATAATTTATTAATAACAAATCAAGGGGCGGCTCTGCAGACGCAAAATGGTAAAGACATTTTGCGTGAGTCGCTCGTT  630
     Y L E L Y N L L I T N Q G A A L Q T Q N G K D I L R E S L V

 631 GGTTTGCTGTCTTCTGTTGCGTCACCCACTTCACAGTTTCCTTCCGGTGTGTTTTATGTGTGGTCTCGTGAGTCGCGCATTGCTGCTTTG  720
     G L L S S V A S P T S Q F P S G V F Y V W S R E S R I A A L

 721 ATCGATTCTCTCTTCGGTGCTTTGGATTCAAGAAATAGGGCTATTGAAGTTGAAAACCCTTCTAATCCATCGACTGGCGAAGCTTTGAAC  810
     I D S L F G A L D S R N R A I E V E N P S N P S T G E A L N

 811 GCTGTTAAGCGCAATGACGACGCGTCTACAGCCGCTCACAACGACATTCCTCAGATTTTATCTGCTCTGAATGAAGGTGCGGGCGTTTTT  900
     A V K R N D D A S T A A H N D I P Q I L S A L N E G A G V F

 901 GATAGAGCGTCTTTTGAATCTGCTTTTGGTCTCGTGTGGACCGCAGGTTCGTCTACCTCGTCTTGAGGCGTGGGGGCTACGATAAGCCCT  990
     D R A S F E S A F G L V W T A G S S T S S *

 991 AGAGTTTTTCCCTCCTCTATAATCGAAGGGCGTTCTTACGCGGTTTCTACCGAGTCTCTGTCGTGTGACGACATGCTGGCGTAAGTTTGT 1080
1081 ATGGGTAGAGGTGTTCGAATCA 1102
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Fig. 5. The nucleotide sequence of kyuri green mottle mosaic virus zucchini strain (KGMMV-Z) coat protein (CP) gene (position 481 to 966) and the deduced amino acid sequences. Arrow indicates initiation codon. Termination codon is indicated by an asterisk. Regions of the CP for which amino acid sequence data were obtained are underlined.

are underlined in Fig. 5. There is a perfect fit between the predicted and determined amino acid. It can be concluded from the nucleotide and amino acid sequence that the open reading frame encodes the 17,181 daltons capsid protein of KGMMV-Z.

Comparison of CP genes. Multiple alignment of nucleotides and amino acids, including our results and previously reported CP data of CGMMV strains is presented in Fig. 6. All CP gene of cucurbit-infecting tobamoviruses had the same length consisted of 486 nucleotides coding 161 amino acid residues. Comparison of the KGMMV-Z CP with those of CGMMV-W, CGMMV-KW1, and CGMMV-SH, showed 43.8, 44.2, and 44.4% nucleotide similarity, respectively. However, three CGMMV strains among themselves showed 98.6-99.6% nucleotide similarity (Table 2). The amino acid sequence of KGMMV-Z had 45.3% similarity compared with those of three CGMMV strains, however the amino acid sequence of CGMMV strains was identical (Table 2). According to these results, the CP gene of KGMMV-Z is very different from those of CGMMV

(A)

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Majority   ATGGCTTACAATCCGATCACACCTAGCAAACTTATTGCGTTTAGTGCTTCTTATGTTCCC-GTCAGGACTTTACTTAATT
CGMMV-W    ************************************************************-******************  79
CGMMV-SH   ************************************************************-******************  79
CGMMV-KW1  ************************************************************-******************  79
KGMMV-Z    ***C*****TC*A*C*G*GGTAT*C*TTCG***CC***T***TC*AAG****T*T*C**TTA*TT***G**-GTA*****  79

Majority   TTCTAGTTGCTTCACAAGGTACCGCTTTCCAGACTCAAGCGGGAAGAGATTCTTTCCGCGAGTCCCTGTCTGCGTTACCC
CGMMV-W    ************************C************************************************************ 159
CGMMV-SH   ************************************************************************************** 159
CGMMV-KW1  ************************************************************************************** 159
KGMMV-Z    *AT**A*AA*AAAT*****GG*G***C*G*****G***AAT**T*A***CAT***G**T*****G**CGT**GT**G*TG 159

Majority   TCGTCTGTCGTAGATATTAATTCTAGGTTCCCAGATGCGGGTTTTTACGCTTT-CCTCAACGGTCCTGTGTTGAGGCCTA
CGMMV-W    ************************************************************-************************** 238
CGMMV-SH   ************************************************************-************************** 238
CGMMV-KW1  ************************************************************-************************** 238
KGMMV-Z    **T*****T*CGTCACCC*C***ACA***T**TTCC*GT*TG*****T*TG*GGT***GTGA***GC*CA***CT**T*- 238

Majority   TCTTCGTTTCGCTTCTCAGCTCCACGGATACGCGTAATAGGGTCATTGAGGTTGTAGATCCTAGCAATCCTACGACTGCT
CGMMV-W    ************************************************************************************** 318
CGMMV-SH   ************************************************************************************** 318
CGMMV-KW1  ************************************************************************************** 318
KGMMV-Z    *GA***A***T**CT**G*TG*TTT****T*AA*A*******CT*****A****A*A*C***TCT*****AT******GC 318

Majority   GAGTCGCTTAACGCTGTAAAGCGTACTGATGACGCGTCTACAGCCGCTAGGGCTGAGATAGATAATTTAATAGAGTCTAT
CGMMV-W    ***********************************A****G************A********************** 398
CGMMV-SH   ************************************************C************************** 398
CGMMV-KW1  ************************************************************************A***** 398
KGMMV-Z    **AG*TT*G*****G**T*****C*A***C*****************CACAAC**C**TCC*C*GA*TT**TCTG**C* 398

Majority   TTCTAAGGGTTTTGATGTTTATGATAGGGCTTCATTTGAAGCCGCGTTTTCGGTAGTCTGGTCAGAGGCTACCACCTCGA
CGMMV-W    ************************************************************************************** 478
CGMMV-SH   ********************C************************************************************** 478
CGMMV-KW1  ********************C************************************************************** 478
KGMMV-Z    GAA*G*A***GCG*GC****T******A**G**T******T*T**T***GGTC*C**G***A*C*CA*G*T*GT*TA*CT 478

Majority   AAGCTTAG
CGMMV-W    ********  486
CGMMV-SH   ********  486
CGMMV-KW1  ********  486
KGMMV-Z    CGT***GA  486
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(B)

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Majority   MAYNPITPSKLIAFSASYVPVRTLLNFLVASQGTAFQTQAGRDSFRESLSALPSSVVDINSRFPDAGFYAFLNGPVLRPIF
CGMMV-W    ************************************************************************************** 81
CGMMV-SH   ************************************************************************************** 81
CGMMV-KW1  ************************************************************************************** 81
KGMMV-Z    *P*STSGIRS*P***K*FF*YLE*Y*L*ITN**A*L***N*K*IL****VG*L***ASPT*Q**SGV**VWSRESRIAALI 81

Majority   VSLLSSTDTRNRVIEVVDPSNPTTAESLNAVKRTDDASTAARAEIDNLIESISKGFDVYDRASFEAAFSVVWSEATTSKA
CGMMV-W    ************************************************************************************** 161
CGMMV-SH   ************************************************************************************** 161
CGMMV-KW1  ************************************************************************************** 161
KGMMV-Z    D**FGAL*S***A***EN****S*G*A******N*******HND*PQILSALNE*AG*F******S**GL**TAGSSTSS 161
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Fig. 6. Alignment of nucleotide sequences (A) and amino acids (B) of cucurbit-infecting tobamoviruses coat protein. Deletions are noted by hyphens (–), and asterisks (*) that match majority exactly.

Table 2. Percentage nucleotide and amino acid sequence similarities between coat protein genes of four cucurbit-infecting tobamoviruses[a]

	1	2	3	4		
1	*	43.8	44.4	44.2	1	KGMMV-Z
2	45.3	*	98.6	98.6	2	CGMMV-W
3	45.3	100.0	*	99.6	3	CGMMV-SH
4	45.3	100.0	100.0	*	4	CGMMV-KW1
	1	2	3	4		

[a] Percent similarities were calculated by the DNASTAR MEGALIGN program. Similarities are presented for a coat protein nucleotide sequence (above diagonal) and for a coat protein amino acid sequence (below diagonal).

strains.

Discussion

Presently, CGMMV, CMV, WMV, and ZYMV mainly have occurred on cucurbit crops in Korea (The Korean Society of Plant Pathology, 1998). Especially, CGMMV has caused severe damages in commercial watermelon production, since first reported in 1990 (Lee, 1990). Considering the new disease of zucchini, KGMMV-Z has not been discovered in Korea till recently, and the virus particles were detected from zucchini seeds imported from China (K. W. Lee, unpublished data), the casual agent, KGMMV-Z seems to be introduced into the country through the imported seeds. For the above reasons, this viral disease of zucchini would not be completely established yet, so the virus could be controlled by means of the integrated management such as the supply of virus-free seeds, roguing the infected plants, removal of plant remains in the soil, and phytosanitary treatment of the agricultural tools.

Five degenerate primers (Table 1), which were originated from highly homologous regions by nucleotide sequence comparison of 10 tobamovirus members (species) were very effective for the amplification of KGMMV CP gene (Fig. 3, 4). The primers could be also used for direct sequencing of cDNA fragments synthesized by RT-PCR. Therefore we could rapidly determine the sequence of KGMMV CP region. These primers may also work on the other tobamoviruses which have no sequence information because they were designed to attach MP gene (MPHR1 and MPHR2), CP gene (CPHR5 and CPHR3), and 3'-terminal (CCT1) of tobamoviruses.

Since CGMMV was first described by Ainsworth (1935), several strains of CGMMV including CGMMV-W (Komuro et al., 1971) have been reported in cucurbits. The CP gene and/or the complete nucleotide sequence of CGMMV-W (Meshi et al., 1983), CGMMV-SH (Ugaki et al., 1991), and CGMMV-KW1 (Lee, 1999) were known. The CP gene of three strains of CGMMV was composed of 486 nucleotides without exceptions, and the sequence of amino acid was identical (Fig. 6). KGMMV was first described as CGMMV cucumber strain (Inouye et al., 1967), and then renamed by Francki (1986) based on serological and molecular hybridization analyses. And now it is a member of tobamoviruses (Regenmortel and Meshi, 1995). Until recently, the complete nucleotide sequence of KGMMV has not been known, but only the sequence of CP gene was first revealed by Lee et al. (1999). The nucleotide sequence similarities of KGMMV-Z CP gene ranged from 43.8 to 44.4% compared with those of CGMMV strains, which supports the result of Francki's molecular hybridization (1986). It is interesting that the CP gene of two cucurbit-infecting tobamoviruses, KGMMV and CGMMV, consists of 486 nucleotides although the sequence homology between the two tobamovirus CP genes is very low.

Previously described two KGMMV isolates, KGMMV-C and KGMMV-Y were isolated from cucumber (Brunt et al., 1997), but KGMMV-Z in this study was detected from zucchini which is a new natural host of KGMMV. The host range of KGMMV-Z is very similar to that of the other strains of KGMMV, but KGMMV-Z differs in host reactions of *Spinacia oberaces* and *Gomphrena globosa* (H. S. Choi, unpublished data). Further researches on biological, serological, and molecular relationships among three KGMMV strains will be needed in detail.

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