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論　文
Articles

Occurrence and Molecular Characterization of *Kyuri green mottle mosaic virus* Isolated from Oriental Melon in Korea

By

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Summary : Mosaic diseases were detected in oriental melons cultivated in Seongju County, Kyungpook Province, Korea. The oriental melons were infected by *Cucumber green mottle mosaic virus* (CGMMV), *Watermelon mosaic virus* 2 (WMV2) and some viruses in the genus *Potyvirus,* according to ELISA detection. Additionally, from one melon, a virus was isolated and identified as *Kyuri green mottle mosaic virus* (KGMMV) by electron microscopy, biological analysis, RT-PCR and sequencing of the movement protein (MP) and coat protein (CP) genes. Results of host reactions of the KGMMV isolate (KGMMV-KOM) were similar to that described for KGMMV-C1 isolated from Japan, while KGMMV-KOM showed latent infection on oriental melon. Although the CP gene of KGMMV-KOM comprised 486 nts, the same as previously reported KGMMV, the MP gene comprised 786 nts, which is shorter than that of other KGMMV (789 nts). Phylogenetic analysis based on the nucleotide sequences of MP and CP genes was also conducted and KGMMV-KOM was found to belong to the major cluster of KGMMV, showing molecularly conservative nature of this species. This is the first report of KGMMV infecting on oriental melon in Korea.

Key words : duplex RT-PCR, Korea, *Kyuri green mottle mosaic virus,* nucleotide sequence, oriental melon

Introduction

Among the family *Cucurbitaceae,* which comprises 118 genera with 825 species[1], cucumber (*Cucumis sativus* L.), watermelon (*Citrullus vulgaris* L.), pumpkin (*Cucurbita* spp.) and melon (*Cucumis melo* L.) are the major cucurbit crops in Korea. Viral diseases of these crops are often the most destructive and difficult to control. More than 50 species of cucurbit-infecting viruses are known, including those in the genus *Tobamovirus*[2]. Eight species such as *Cucumber mosaic virus* (CMV), *Cucumber green mottle mosaic virus* (CGMMV), *Kyuri green mottle mosaic virus* (KGMMV), *Melon necrotic spot virus* (MNSV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus* 2 (WMV2), *Zucchini green mottle mosaic virus* (ZGMMV) and *Zucchini yellow mosaic virus* (ZYMV), have been reported to occur in cucurbits in Korea[3].

Oriental melon (*Cucumis melo* L. var. *makuwa* Makino) is grown virtually year-round in Korea by open field cultivation in the summer and greenhouse cultivation in the cold seasons[4]. It is a popular summer fresh fruit and occupied 6,472 ha in Korea in 2007 according to National Agricultural Products Quality Management Service (http://www.naqs.go.kr/) data. In particular, they are mainly cultivated in Seongju County, Kyungpook Province, Korea. Their production area was estimated to be about 5,530 ha in Seongju County, which represents about 85% of oriental melon cultivation area nationally. CMV, CGMMV, MNSV and WMV2

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have been reported to naturally occur in oriental melons in Korea[5-8].

At present, four cucurbit-infecting tobamoviruses are known ; *Cucumber fruit mottle mosaic virus* (CFMMV), CGMMV, KGMMV and ZGMMV described in the eighth ICTV database[2] and Cucumber mottle virus (CuMoV) was recently reported from cucumber in Japan as a possible new member[9].

CGMMV was first reported in cucumber from UK, and named as Cucumber virus 3 (CV3) and Cucumber virus 4 (CV4)[10]. Since then, CGMMV has caused diseases in many other countries including China, France, Greece, India, Indonesia, Iran, Israel, Japan, Korea, Pakistan, Saudi Arabia and Spain in cucurbits plants. KGMMV was first reported as a CGMMV cucumber strain by INOUE *et al.* (1967)[11] in Japan. Then, CGMMV Yodo strain was isolated from cucumber in Japan in 1969[12]. FRANCKI *et al.* (1986)[13] pointed out that the CGMMV watermelon strain is taxonomically different from the CGMMV cucumber strain, based on their serological and RNA-cDNA hybridization analysis. Consequently, the cucumber strain has been examined and named KGMMV as a distinctive species in the genus *Tobamovirus*. Currently, KGMMV has also been shown to occur in Indonesia and Korea[14-17].

Oriental melons showing mosaic, mottle and leaf distortion were frequently found in Seongju County in 2006. In this study, we characterized an isolate of KGMMV (KGMMV-KOM) from an oriental melon collected in Seongju County, as this is the first report of this virus from oriental melon. We describe the molecular characterization of KGMMV isolates compared with other previously reported cucurbit-infecting tobamoviruses, including CGMMV, from oriental melon in Korea.

Materials and Methods

Virus isolates and their propagation

Eleven samples of oriental melons showing mosaic were collected in Seongju County, Korea in August and October 2006. All samples were imported from Korea to Japan under plant quarantine permission of Ministry of Agriculture, Forestry and Fisheries, Japan. One isolate (KGMMV-KOM) originated from a dried sample that was collected in August and propagated in zucchini (*Cucurbita pepo* cv. Diner). The other isolates, CGMMV-KM4 and -KM7 were also isolated from oriental melons with mosaic symptoms in Seongju. Although they were isolated as a mixed infection along with some potyviruses, CGMMV-KM4 and -KM7 were identified by electron microscopy, ELISA and RT-PCR and used for further molecular study as reference strains.

Electron microscopy

A drop of sap from leaf samples on a copper grid with carbon coated collodion film was negatively stained with 2% phosphotungstic acid (PTA, pH 6.0). The grid was examined under electron microscope H-7600 (Hitachi, Ltd., Tokyo, Japan).

Serological characterization

Serological characterization was conducted by means of an enzyme-linked immunosorbent assay (ELISA) using antiserum for each virus. Compound direct ELISA kit for CMV and DAS ELISA kit for WMV2 (Agdia, USA) were used according to the manufacturer's instructions. To detect CGMMV and KGMMV, DAS ELISA kits from Japan Plant Protection Association (JPPA) were used. KGMMV-C1 maintained in Tokyo University of Agriculture (TUA) and CGMMV-To-TUA provided by Dr. T. NATSUAKI, Utsunomiya University, Japan, were used as positive controls. For the detection of viruses in the genus *Potyvirus,* the potyvirus group test indirect ELISA kit (Agdia, USA) was used according to manufacturer's instructions. Absorbance at 405nm was recorded using a plate reader (Model 680 Microplate Reader, Bio-Rad, USA). Reactions were considered as positive when absorbance values recorded thirty minutes after incubation with substrate at room temperature were three times greater than those of the corresponding negative controls and higher than 0.1.

Host range analysis

Indicator plants of eight genera in three families, including *Chenopodium amaranticolor, Cucumis sativus, Datura stramonium, Nicotiana tabacum* cv. Samsun, and *Petunia hybrida* were used to determine the host range of KGMMV-KOM. They were mechanically inoculated using inocula from systemically infected zucchini in 0.1 M phosphate buffer (pH 7.0), 1 : 10 (w/v). Symptom development was observed until at least one month after inoculation.

RNA extraction, cDNA synthesis and RT-PCR

Trizol reagent (Invitrogen, USA) was used to extract total RNA from the original dried sample of oriental melon or fresh zucchini leaves inoculated by KGMMV-KOM. Resultant pellets were dissolved in 125μl of RNase-free water. ReverTra Ace -α-® kit (TOYOBO, Japan) was used for the synthesis of cDNA. Second-strand cDNAs were synthesized by polymerase chain reaction using TaKaRa Ex Taq™ (Takara Biomedicals,

Table 1 Primer pairs for cDNA synthesis by RT-PCR/duplex RT-PCR from CGMMV and KGMMV

Target	Primer pairs*	Sequence (5'-3')	Product size (bp)	PCR program
KGMMV MP	KGCP-F (+) KGCP-R (-)	GTCTTACTCAACCAGTGGT TCACTTTGAGGAAGTAGCG	484	95 °C 1' 52 °C 1' 72 °C 1'30" 30 cycles
KGMMV CP	KGMP-F (+) KGMP-R (-)	ATGTCTGTAAGTAGCGTCG GTTATAAACGAGGTGGTTC	790	95 °C 1' 50 °C 1' 72 °C 1'30" 30 cycles
CGMMV MP and CP KGMMV MP and partial CP	CKF (+) CKR (-)	TCGACGATGCAATCCACGAAT AACTAAGCTTTCGAGGTGGTAG	CGMMV 1,368 KGMMV 1,170	95 °C 1' 52 °C 1' 72 °C 1' 30 cycles

* (+) sense primer, (-) antisense primer

Japan). PCR primers were designed for duplex RT-PCR to detect two cucurbit-infecting tobamoviruses, CGMMV and KGMMV, simultaneously. Also, two pairs of specific primers were designed, based on previously reported sequences of KGMMV-C1 (AJ295948) and KGMMV-C (AB015144), to amplify the coat protein (CP) and movement protein (MP) regions of the KGMMV genes. PCR primers and programs (performed using PTC-100® Peltier Thermal Cycler ; MJ Research, INC., USA) are indicated in Table 1. The amplified products were separated on a 2% agarose gel in Tris-Acetate-EDTA (TAE) buffer, stained with ethidium bromide solution and photographed using EDAS 290 (Kodak, Japan) under UV illumination.

Cloning and sequencing

The bands of the expected size were purified using a Wizard® SV Gel and PCR Clean-Up system (Promega, USA). Three μl of purified DNA sample were mixed with 5μl of 2x Rapid ligation buffer, 1μl of 1/10 pGEM T-vector (Promega, USA), and 1μl of T4 DNA ligase and incubated at 4℃ overnight. The ligated products were transformed into competent cells of *Escherichia coli* (GibcoBRI, Life Technologies, USA), and cells were placed on LB plates containing ampicillin and X-Gal. Plasmid DNA was isolated and purified by a LaboPass™ Plasmid Mini kit (Hokkaido System Science, Japan) and analyzed for the presence of insert DNA on a 1% electrophoresis gel. Plasmids with the desired length of insert were selected and sequenced. DNA sequence was obtained by an Applied Biosystems 3130/3130xl genetic analyzer (Applied Biosystems, USA) from at least five independent clones. Nucleotide and amino acid sequences were analyzed using MacVector 6.5 software (Oxford Molecular Ltd., USA). The obtained sequences were initially aligned using Clustal W from DDBJ with default parameters. The strengths of the internal branches of the resulting tree were statistically tested by bootstrap analysis from 1,000 bootstrap replications. Using the neighbor-joining (NJ) algorithm, phylogenetic analysis of KGMMV-KOM was performed to determine the relationship with other cucurbit-infecting member species of the genus *Tobamovirus*.

Results

Epidemiology and biological characterization

Many oriental melons cultivated in Seongju County, Korea, in August and October 2006 showed mosaic and other virus disease-like symptoms. They were surveyed for the relative incidence of CMV, CGMMV, KGMMV, WMV2 and presence of species in the genus *Potyvirus*. Of the four species and one genus tested, CGMMV and viruses belonging to the genus *Potyvirus* were found in ten out of 11 samples as mixed infections. Among those infected by genus *Potyvirus,* seven samples reacted with antisera against WMV2 (As-WMV2). In particular, one sample showing mosaic, mottle and leaf distortion specifically reacted only with As-KGMMV but not with other antisera.

Electron microscopy of this sample revealed it had rod-shaped particles of about 300 nm in length, showing the typical morphology of the genus *Tobamovirus* (data not shown).

By artificial inoculation, this rod-shaped virus, named as KGMMV-KOM, was infectious and caused necrotic or chlorotic local lesions in *Chenopodium amaranticolor, Datura stramonium, Nicotiana tabacum* cv. Samsun and *Petunia hybrida*. KGMMV-KOM systemically infected *Cucumis sativus, Cucurbita pepo* and *Lagenaria siceraria* and developed mosaic symptoms. This result was similar to that described for KGMMV-C 1 isolated from Japan[18]. Yoon *et al.* (2001)[18] and Daryono *et al.* (2005)[14] reported that KGMMV could infect tomato (*Solanum lycopersicum*) ; therefore we inoculated KGMMV-KOM mechanically into ten cultivars of tomato to test its infectivity. By ELISA, KGMMV-KOM was detected to infect systemically

without showing any conspicuous symptoms in six cultivars, but not in four other cultivars (data not shown). In back-inoculation into oriental melon, KGMMV-KOM was systemically infective but showing no symptoms 30 days post inoculation.

Molecular characterization

Single PCR products of 484 bp (encoding CP) and 790 bp (encoding MP) were amplified from KGMMV-KOM. In duplex RT-PCR, which can detect two bands such as the 1,170 bp product encoding complete MP and partial CP genes of KGMMV and/or the 1,368 bp product encoding complete MP and CP genes of CGMMV, KGMMV-KOM was recognized as a distinct band of 1,170 bp when compared with CGMMV-KM4 and -KM7 as reference virus strains (Fig. 1).

Nucleotide sequence alignments and phylogenetic analysis of CP and MP regions of KGMMV-KOM were performed to determine the relationship among KGMMV isolates and also other cucurbit-infecting tobamoviruses. The CP gene of KGMMV-KOM (AB 433895) comprised 486 nucleotides (nts), was the same as KGMMV-C 1 (AJ 295948), KGMMV-C (AB 015144), KGMMV-Y (AB015145) and KGMMV-YM (AB162006). However, the MP gene of KGMMV-KOM (AB433894) comprised 786 nts, which was different from the other four strains of KGMMV that comprised 789 nts. KGMMV-KOM MP shared 99% nucleotide homology with KGMMV-C1 from Japan, 90% with -YM from Indonesia and 86% with -C and -Y, both from Japan. In contrast, KGMMV-KOM CP shared 99% nucleotide homology with KGMMV-C1 and -C, 93% with -YM, and 88% with -Y.

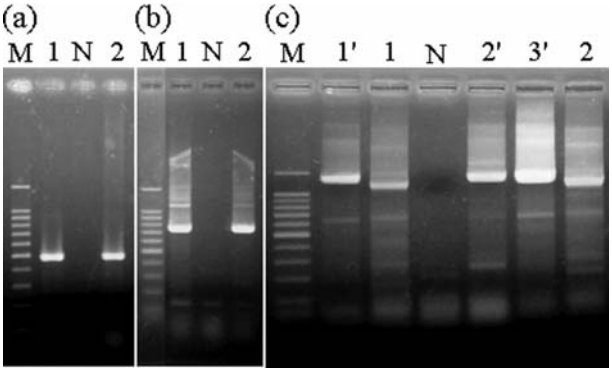

Fig. 1 2% Agarose gel electrophoresis of reverse transcriptase-polymerase chain reaction (RT-PCR) products amplified with KGMMV CP (a) and MP (b) specific primers, respectively. Primers were designed to amplify 484 bp cDNA of KGMMV CP and 790bp cDNA of KGMMV MP. (c) Fragments of 1,368 bp and 1,170 bp, amplified from CGMMV and KGMMV respectively, by Duplex RT-PCR primers for detecting CGMMV and KGMMV simultaneously. (M, 100 bp DNA ladder (Promega, USA) ; N, healthy zucchini ; 1, KGMMV-C1 ; 2, KGMMV-KOM ; 1′, CGMMV-To-TUA ; 2′, CGMMV-KM4 ; 3′, CGMMV-KM7)

A phylogenetic analysis with other isolates of cucurbit-infecting tobamoviruses was conducted (Fig. 2). For this purpose, the regions from the MP to CP genes of CGMMV-KM4 (AB447984), -KM7 (AB447985) and -To-TUA (AB462480) were also sequenced. All three isolates had 795 nts of MP genes and shared 99% homology with that of CGMMV-Y (AJ243353), showing two nucleotides differences. On the other hand, CP genes of CGMMV-KM4 and -KM7, comprising 486 nts, shared 100% homology with CGMMV isolated from Korean watermelon (AF225984). However, CGMMV-To-TUA showed the highest homology (99%) with CGMMV-Liaoning (EF611826) isolated from watermelon in China.

In this study, results from the phylogenetic trees of the MP and CP coding regions of KGMMV-KOM, CGMMV-KM4, and CGMMV-KM7 along with other previously reported isolates confirmed that cucurbit-infecting tobamoviruses constituted two subgroups ; CGMMV and CuMoV in subgroup I, and CFMMV, KGMMV and ZGMMV in subgroup II. KGMMV-KOM belonged to subgroup II, closely resembling other KGMMV strains (Fig. 2).

Discussion

Three cucurbit-infecting tobamoviruses, CGMMV, KGMMV and ZGMMV, have been reported to infect several cucurbit plants in Korea[16,19–20]. CGMMV was first found in watermelon in Korea in 1989[19] and was subsequently detected in cucumber[16,21], oriental melon[16] and melon[22]. KGMMV was isolated from zucchini in the city of Chonju in 1999 and named KGMMV-Z by comparison of its CP gene sequences with other CGMMV isolates[16]. RYU *et al.* (2000)[20] also isolated a virus from zucchini showing mottle, severe mosaic and abnormal fruits symptoms. They suggested that the virus was a new tobamovirus species based upon CP gene sequence analysis and comparison with other tobamoviruses, and designated it as ZGMMV. Hence, CHOI *et al.* (2001)[23] also proposed to re-examine KGMMV-Z[16] and identified it as ZGMMV by the sequence homology and serological characteristics using the multi-RIPA. The comparison of CP gene sequences of KGMMV-Z (AF239212) and ZGMMV (AJ295949) indicates only one nucleotide substitution (a silent muta-

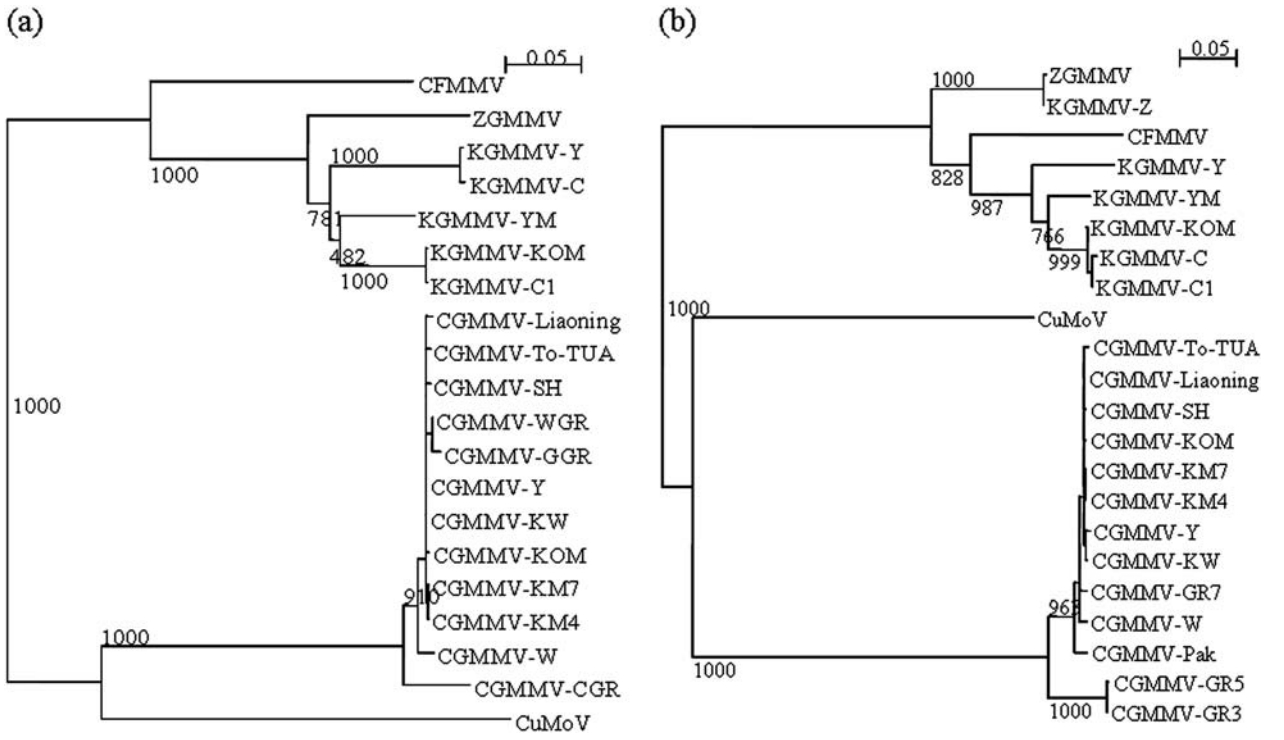

Fig. 2 Phylogenetic relationships of KGMMV-KOM and CGMMV-KM4, -KM7 and the members of cucurbit-infecting tobamoviruses based on nucleotide sequences of the movement protein (MP) gene (a) and coat protein (CP) gene (b). The branching pattern was generated by the neighbor-joining method. (CFMMV, AF321057 ; CuMoV, AB261167 ; CGMMV-CGR*, AY584529 ; CGMMV-GGR*, AY584530 ; CGMMV-GR3**, AJ459421 ; CGMMV-GR5**, AJ459422 ; CGMMV-GR7**, AJ459423 ; CGMMV-KOM, AF 417243 ; CGMMV-KW, AF417242 ; CGMMV-Liaoning, EF611826 ; CGMMV-Pak**, AB127937 ; CGMMV-SH, D12505 ; CGMMV-WGR*, AY584528 ; CGMMV-W, AB015146 ; CGMMV-Y*, AJ243353 ; CGMMV-Y**, AJ 245440 ; KGMMV-C, AB015144 ; KGMMV-C1, AJ295948 ; KGMMV-Y, AB015145 ; KGMMV-YM, AB162006 ; KGMMV-Z**, AF239212 ; ZGMMV, AJ295949) (*used for MP and **for CP analysis only)

tion) of the T and G at position 333 nucleotides. These two isolates have 84% and 85% nucleotide homology based upon CP genes with KGMMV-C1, so-called type strains of KGMMV. Our results also showed that these two viruses formed a distinct cluster from other KGMMV (Fig. 2).

The phylogenetic tree shown in this study also suggests that the known KGMMV isolates in Korea and ZGMMV may come from the same ancestor. Thus to avoid confusion of KGMMV/ZGMMV classification system, future experiments must reexamine KGMMV isolates from Korea with more isolates of KGMMV and ZGMMV in Korea.

In this study, we detected both CGMMV and KGMMV from oriental melons. CGMMV has been reported to infect oriental melons in Korea[16] ; however, there had been no report on the occurrence of KGMMV in oriental melon in Korea and other countries. We characterized the KGMMV isolate from oriental melon (KGMMV-KOM) and compared it with other cucurbit-infecting tobamoviruses on host reaction and the CP and MP genes sequences.

CGMMV and KGMMV are distinguishable on the basis of their different reaction on *D. stramonium* and *C. amaranticolor*. CGMMV produces local lesions on inoculated leaves of *C. amaranticolor,* but not on *D. stramonium,* while KGMMV produces local lesions on inoculated leaves of *D. stramonium,* but not on *C. amaranticolor*[11-12,24-25]. KGMMV-KOM reactions on host plants were similar to those described for KGMMV-C 1[18]. However, unlike other KGMMV, KGMMV-KOM produced local lesions on *C. amaranticolor* as well as *D. stramonium,* and thus the host reaction cannot be used as the sole determinant for distinguishing between CGMMV and KGMMV.

We also confirmed that KGMMV-KOM has infectivity to tomato. Tan *et al.* (2005)[26] mentioned that the host range and virulence of a virus are usually among its most malleable characters. Although KGMMV-KOM has so far shown no or mild symptoms on tomato, the infectivity to tomato and other *Solanaceae* plants of KGMMV isolates should be ex-

amined to learn the process of adaptation of plant viruses upon transfer between plants in different families. Transmissibility of KGMMV-KOM through tomato seeds must also be determined. For symptom development by back inoculation to oriental melon, we need to inoculate KGMMV-KOM into more varieties of oriental melons and observe them for a longer period under natural conditions.

A number of cucurbit-infecting tobamoviruses have been fully sequenced, such as 6,424 (CGMMV-SH), 6,423 (CGMMV-W), 6,515 (KGMMV-Y), 6,514 (KGMMV-C1), 6,512 (KGMMV-YM), 6,562 (CFMMV), 6,513 (ZGMMV) and 6,485 (CuMoV) nts[14, 18, 27–31]. Comparing the full genome sequences of CGMMV-SH and CGMMV-W, UGAKI *et al.* (1991)[30] suggested that the three amino acid substitutions found in the MP genes might be responsible for adaptation to different host species. CGMMV, reported widely in the world, does not present any obvious bio- and geographical diversity, mainly due to the stability of the nucleotide sequence. This study showed that the phylogenetic trees of CGMMV, including Korean isolates based upon CP or MP regions, indicated no remarkable molecular diversity among them except for Greek isolates CGMMV-CGR (AY 584529), -GR3 (AJ459421), and -GR5 (AJ459422). As for KGMMV, they are more diverse than CGMMV, although identities among KGMMV isolates are comparatively high (>85% in MP regions and >87% in CP regions).

ANTIGNUS *et al.* (2001)[27] and YOON *et al.* (2002)[31] suggested that cucurbit-infecting tobamoviruses should be separated into two subgroups based on comparisons of sequences and phylogenetic analysis ; subgroup I comprising CGMMV and subgroup II comprising CFMMV, KGMMV and ZGMMV. KGMMV-KOM belongs to subgroup II and CGMMV from Korean oriental melons belongs to subgroup I. Recently, CuMoV was reported as a new species of cucurbit-infecting tobamoviruses from Japan[9]. They proposed that cucurbit-infecting tobamoviruses subgroups are better designated as three sub-subgroups. Consequently, CGMMV is in sub-subgroup I, CFMMV, KGMMV and ZGMMV are in sub-subgroup II and CuMoV is in sub-subgroup III[9,28]. According to this classification, KGMMV-KOM is also classified as sub-subgroup II.

The study of cucurbit-infecting tobamoviruses is necessary for early detection of their occurrence in the field, healthy seed production and plant quarantine, as more cucurbit crops are grown and traded internationally. Tobamoviruses, due to their possible transmissibility by foliage contact, soil contamination and through seeds, must be checked more carefully to prevent prohibited virus diffusion to international as well as domestic regions. Indeed, it is thought that the major cause of the CGMMV outbreak that occurred on watermelons in Korea in 1998 was transmission through virus-contaminated bottle gourd seeds imported as watermelon rootstock from China[32]. Also, in Japan, the occurrence of CGMMV on watermelons in 1968 might have occurred by virus-contaminated pumpkin seeds imported from India[25]. Although the occurrence of KGMMV is still limited to Korea, Japan and Indonesia at present and they have low genetic diversity, cucurbit crops in Asia are prone to KGMMV invasion and dispersion. Monitoring of genetic diversity of cucurbit-infecting tobamoviruses is thus necessary to detect their emergence and diffusion in each country. There might also be new cucurbit-infecting tobamoviruses, such as CuMoV. Constant and careful observation and study on KGMMV and other cucurbit-infecting tobamoviruses are therefore necessary.

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References

1) JEFFREY, C., 1990. Systematics of the Cucurbitaceae : an overview. In : BATES, D.M., ROBINSON, R.W., JEFFREY, C., editors. Biology and utilization of the Cucurbitaceae. New York, USA : Cornell University Press. pp 3–9.
2) FAUQUET, C.M., MAYO, M.A., MANILOFF, J., DESSELBERGER, U. and BALL, L.A., 2005. Virus Taxonomy : Eighth Report of the International Committee on Taxonomy of Viruses. USA and UK. Elsevier Academic Press.
3) The Korean Society of Plant Pathology. 2004. List of Plant Diseases in Korea (Fourth edition). Korea (in Korean).
4) CHUNG, H.D., 2007. Oriental melon (Cucumis melo L. var. Makuwa, Cucurbitaceae). In : LEE, J.M., CHOI, G.W., JANICK, J., editors. Horticulture in Korea. Korean Society for Horticultural Science, Suwon, Korea : CIR Communication Co., Ltd. pp 52–55.
5) CHUNG, B.J., PARK, H.C. and LEE, S.H., 1975. Studies on the host range of Cucumber mosaic virus in Korea. *Korean J Plant Prot* **14** : 185–192 (in Korean).
6) KIM, S.M., LEE, J.M., YIM, K.O., OH, M.H., PARK, J.W. and KIM, K.H., 2003. Nucleotide sequences of two Korean isolates of Cucumber green mottle mosaic virus. *Mol Cells* **16** : 407–412.
7) CHOI, G.S., KIM, J.H. and KIM, J.S., 2003. Characterization of Melon necrotic spot virus isolated from muskmelon. *Plant Pathol J* **19** : 123–127.
8) LEE, S.H. and LEE, K.W., 1981. Incidence of Watermelon mosaic virus in cucurbits. *Korean J Plant Prot* **20** : 191–195.
9) KUBOTA, K., ORITA, H., SAKAI, J., OKUDA, M., TANAKA, Y.,

Hanada, K., Imamura, Y. and Iwanami, T., 2006. Molecular and serological characterization of a new species of cucurbit-infecting Tobamovirus. *Jpn J Phytopathol* **72** : 279–280 (abstract).

10) Ainsworth, G.C., 1935. Mosaic disease of cucumber. *Annu Appl Biol* **22** : 55–67.

11) Inoue, T., Inoue, N., Asatani, M. and Mitsuhata, K., 1967. Studies on Cucumber green mottle mosaic virus in Japan. *Nogaku Kenkyu* **51** : 175–186 (in Japanese).

12) Kitani, K., Kiso, A. and Shigematsu, Y., 1970. Studies on a new virus disease of cucumber (Cucumis sativus L. var. F1 Kurume-Otiai-H type) discovered in Yodo. *Proc Assoc Plant Prot Shikoku* **5** : 59–66 (in Japanese).

13) Francki, R.I.B., Hu, J. and Palukaitis, P., 1986. Taxonomy of cucurbit-infecting tobamoviruses as determined by serological and molecular hybridization analyses. *Intervirology* **26** : 156–163.

14) Daryono, B.S., Somowiyarjo, S. and Natsuaki, K.T., 2005. Biological and molecular characterization of melon-infecting Kyuri green mottle mosaic virus in Indonesia. *J Phytopathol* **153** : 588–595.

15) Cheon, J.U., Lee, S.H., Choi, H.S., Kwon, S.H., Ko, S.J. and Lee, K.W., 2000. A new strain of Kyuri green mottle mosaic virus isolated from cucumber : biological properties and nucleotide sequence of coat protein gene. *Plant Pathol J* **16** : 358 (abstract).

16) Lee, S.H., Lee, Y.K., Park, J.W., Choi, H.S., Kim, Y.T., Cheon, J.K. and Lee, K.W., 2000. Nucleotide sequence of coat protein gene of Kyuri green mottle mosaic virus isolated from zucchini. *Plant Pathol J* **16** : 118–124.

17) Lee, S.H., Cheon, J.U., Choi, H.S., Kwon, S.H., Ko, S.J. and Lee, K.W., 2001. A new strain of Kyuri green mottle mosaic virus isolated from cucumber. *Plant Pathol J* **17** : 388 (abstract).

18) Yoon, J.Y., Min, B.E., Choi, J.K. and Ryu, K.H., 2001. Completion of sequence and generation of highly infectious transcripts to cucurbits from full-length cDNA clone of Kyuri green mottle mosaic virus. *Arch Virol* **146** : 2085–2096.

19) Lee, K.W., Lee, B.C., Park, H.C. and Lee, Y.S., 1990. Occurrence of Cucumber green mottle mosaic virus disease of watermelon in Korea. *Korean J Plant Pathol* **6** : 250–255 (in Korean).

20) Ryu, K.H., Min, B.E., Choi, G.S., Choi, S.H., Kwon, S.B., Noh, G.M., Yoon, J.Y., Choi, Y.M., Jang, S.H., Lee, G.P., Cho, K.H. and Park, W.M., 2000. Zucchini green mottle mosaic virus is a new tobamovirus ; comparison of its coat protein gene with that of Kyuri green mottle mosaic virus. *Arch Virol* **145** : 2325–2333.

21) Ko, S.J., Lee, Y.H., Cha, K.H., Lee, S.H., Choi, H.S., Choi, Y. S., Lim, G.C. and Kim, K.H., 2006. Incidence and distribution of virus diseases on cucumber in Jeonnam Province during 1999–2002. *Plant pathol J* **22** : 147–151.

22) Cho, J.D., Lee, S.H., Chung, B.N., Kim, J.S. and Kim, T.S., 2006. Cucumber green mottle mosaic virus (CGMMV) reported newly on melon in Korea. *Plant Pathol J* **22** : 426 (abstract).

23) Choi, G.S., Kim, J.H., Chung, B.N., Kim, H.R. and Choi, Y.M., 2001. Simultaneous detection of three tobamoviruses in cucurbits by rapid immunofilter paper assay. *Plant Pathol J* **17** : 106–109.

24) Komuro, Y., Tochihara, H., Fukatsu, R., Nagai, Y. and Yoneyama, S., 1971. Cucumber green mottle mosaic virus (watermelon strain) in watermelon and its bearing on deterioration of watermelon fruit known as 'Konnyaku' disease. *Ann Phytopath Soc Japan* **37** : 34–42 (in Japanese).

25) Tochihara, H. and Komuro, Y., 1974. Infectivity test and serological relationships among various isolates of Cucumber green mottle mosaic virus ; some deduction of the invasion route of the virus into Japan. *Ann Phytopath Soc Japan* **40** : 52–58 (in Japanese).

26) Tan, Z., Gibbs, A.J., Tomitaka, Y., Sánchez, F., Ponz, F. and Ohshima, K., 2005. Mutations in Turnip mosaic virus genomes that have adapted to Raphanus sativus. *J Gen Virol* **86** : 501–510.

27) Antignus, Y., Wang, Y., Pearlsman, M., Lachman, O., Lavi, N. and Gal-On, A., 2001. Biological and molecular characterization of a new cucurbit-infecting Tobamovirus. *Phytopathology* **91** : 565–571.

28) Orita, H., Sakai, J., Kubota, K., Okuda, M., Tanaka, Y., Hanada, K., Imamura, Y., Nishiguchi, M., Karasev, A.V., Miyata, S. and Iwanami, T., 2007. Molecular and serological characterization of Cucumber mottle virus, a new cucurbit-infecting tobamo-like virus. *Plant Dis* **91** : 1574–1578.

29) Tan, S.H., Nishiguchi, M., Murata, M. and Motoyoshi, F., 2000. The genome structure of Kyuri green mottle mosaic tobamovirus and its comparison with that of cucumber green mottle mosaic tobamovirus. *Arch Virol* **145** : 1067–1079.

30) Ugaki, M., Tomiyama, M., Kakutani, T., Hidaka, S., Kiguchi, T., Nagata, R., Sato, T., Motoyoshi, F. and Nishiguchi, M., 1991. The complete nucleotide sequence of Cucumber green mottle mosaic virus (SH strain) genomic RNA. *J Gen Virol* **72** : 1487–1495.

31) Yoon, J.Y., Min, B.E., Choi, J.K. and Ryu, K.H., 2002. Genome structure and production of biologically active in vitro transcripts of cucurbit-infecting Zucchini green mottle mosaic virus. *Phytopathology* **92** : 156–163.

32) Choi, G.S., 2001. Occurrence of two tobamovirus diseases in cucurbits and control measures in Korea. *Plant Pathol J* **17** : 243–248.

韓国のマクワウリにおけるキュウリ緑斑モザイクウイルスの発生とその特性

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要約：韓国慶尚北道星州郡で採集したモザイク症状を示すマクワウリ（*Cucumis melo* L. var. *makuwa* Makino）に，スイカ緑斑モザイクウイルス（*Cucumber green mottle mosaic virus,* CGMMV），カボチャモザイクウイルス（*Watermelon mosaic virus* 2, WMV2）を含む複数の *Potyvirus* 属ウイルスの単独または重複感染が ELISA 法で認められ，さらにモザイク症状を呈していた試料一つからはキュウリ緑斑モザイクウイルス（*Kyuri green mottle mosaic virus,* KGMMV）が検出された。本分離株（KGMMV-KOM）の宿主範囲は日本で分離された KGMMV-C1 と類似し，接種試験でマクワウリに潜在感染した。外被タンパク質（CP）のコード領域は 486 塩基で既報の KGMMV と同様だったが，移行タンパク質（MP）のコード領域は 786 塩基で他の KGMMV（789 塩基）より短かった。KGMMV-KOM の MP と CP は，既報の KGMMV の MP と CP のコード領域の塩基配列と高い相同性を示した。韓国のマクワウリにおける KGMMV の発生報告は本報が最初のものである。

キーワード：duplex RT-PCR, Korea, *Kyuri green mottle mosaic virus,* nucleotide sequence, oriental melon

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