Jour. Agri. Sci., Tokyo Univ. of Agric., 51 (1), 45-52 (2006)
東京農大農学集報, 51 (1), 45-52 (2006)

Biological Characterization and Complete Nucleotide Sequence of Coat Protein of *Kyuri Green Mottle Mosaic Virus* Isolated From Angled Loofah in Indonesia

By

Budi Setiadi DARYONO*, Susamto SOMOWIYARJO** and Keiko T. NATSUAKI***

(Received November 22, 2005/Accepted March 10, 2006)

Summary : Among many cucurbits grown around Yogyakarta-Indonesia as vegetables, angled loofah (*Luffa acutangula* L.) occupies a minor portion of cultivated area for continuous supply to the local market. During 2-year surveys of viral diseases conducted in Yogyakarta, Indonesia from 2000 to 2001, many angled loofah plants showed mosaic symptom on leaf and fruit malformation. A virus was isolated from symptomatic leaves of angled loofah and shown to be a member of genus *Tobamovirus* based upon viral morphology observed under electron microscopy. Serological analysis revealed that the virus was related to *Kyuri green mottle mosaic virus* (KGMMV). Comparison of its coat protein gene and protein sequences with that of KGMMV-YM, KGMMV-C, and KGMMV-Y indicated that the virus was similar to previously reported KGMMV. By mechanical inoculation, the virus infected 3 families including 15 species, showing mosaic in some cucurbit plants and necrotic local lesions in *Chenopodium amaranticolor* and *C. quinoa*. This virus, however, could not infect *Datura stramonium, Petunia hybrida,* and *Nicotiana glutinosa*. Since no KGMMV on angled loofah have been reported in Indonesia, this is the first report and followed by the report of KGMMV occurrence except on melon (*Cucumis melo* L.) in Indonesia.

Key words : *Tobamovirus*, *Kyuri green mottle mosaic virus*, *Luffa acutangula* L.

Introduction

Angled loofah (*Luffa acutangula* L.) is a member of the family Cucurbitaceae, and it is cultivated and grown widely in tropical and sub-tropical countries. Surveys of viral diseases of Cucurbitaceae plants conducted in Java, Indonesia during 2000 and 2001 showed that angled loofah crops grown in the area were severely affected by a new kind of mosaic disease. The infected plants showed growth retardation, mottle and mosaic on the leaves and water soaked spots on the surface of fruits. Sometimes malformation of fruits was also observed.

Kyuri green mottle mosaic virus (KGMMV) is a member of the genus *Tobamovirus,* a serious disease agent of cucurbit crops and causes significant economical losses in several countries[1] (TAN *et al.,* 2000). This virus was firstly found in Tokushima, Japan where greenhouse-grown cucumber was an important crop[2] (INOUYE *et al.,* 1967) and also reported in South Korea[3] (LEE *et al.,* 2000). Presently, an isolate of KGMMV (KGMMV-YM) has been isolated from melon fields in Klaten, Central Java, Indonesia and characterized symptomatologically, serologically, as well as the genome structures[4] (DARYONO *et al.,* 2005). On the other hand, the mosaic symptoms shown on angled loofah in Sleman, Yogyakarta province, Indonesia was also isolated, but not further identified.

The objectives of this study were to characterize angled loofah-infecting KGMMV in Indonesia bio-

* Laboratory of Tropical Plant Protection, Graduate School of Agriculture, Tokyo Univ. of Agriculture
Present address : Laboratory of Genetics, Faculty of Biology, Gadjah Mada University
** Laboratory of Plant Pathology, Faculty of Agriculture, Gadjah Mada University, Yogyakarta 55281, Indonesia
*** Department of International Agricultural Development, Faculty of International Agriculture and Food Studies, Tokyo University of Agriculture

logically, morphologically and serologically and to complete the sequence of coat protein gene of the isolate.

Materials and Methods

Virus source, maintenance and electron microscopy

Angled loofah leaves showing mosaic mottle and suggestive of virus infection were collected in the traditional fields in Sleman-Yogyakarta, Indonesia and homogenized in 10mM sodium phosphate buffer (pH 7.0) to transmit the viral agent to *Chenopodium quinoa* leaves mechanically.

Cholorotic local lesions appearing in inoculated *C. quinoa* were used as inoculum source for KGMMV-YL propagation in zucchini (*Cucurbita pepo* L. cv. Diner). *Cucumber green mottle mosaic virus* (CGMMV-U), a gift from Dr. T. NATSUAKI, Utsunomiya University, Japan, and a cucumber isolate of KGMMV (KGMMV-C) were maintained by the same method in zucchini.

Leaf dips of angled loofah and zucchini leaves were stained with 2% phosphotungstic acid (PTA, pH 6.0) for examination under electron microscope (JEM 1000 CX ; JEOL Ltd., Tokyo, Japan) on 200 mesh Collogion-coated copper grids.

Host range determination

To determine the host range of KGMMV-YL, crude sap from leaf samples of the virus, infected zucchini were mechanically inoculated to 15 plant species representing 3 families (Chenopodiaceae, Cucurbitaceae, and Solanaceae) that were pre-dusted with Carborundum (600 mesh). Plants were grown in glass house under continuous illumination (8,000 lux) at 25℃ and observed weekly for symptom development.

Coat protein analysis

KGMMV-YL coat proteins extracted from infected zucchini leaves were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 13.5% SDS-PAGE gels and stained with Coomassie brilliant blue R-50 according to protocols described by SAMBROOK and RUSSEL[5] (2001). Coat proteins separated by SDS-PAGE were transferred onto nitrocellulose (NC) membrane by electro-blotting with a transfer electro blot unit (Bio-Rad, Hercules, USA). Commercial antisera of KGMMV and CGMMV (Agdia Inc., Madison, USA) were used for detection of viral antigens by Western blotting. As the primary antibody, 1 : 1,000 dilution (vol/vol) of commercially available goat anti-rabbit IgG conjugated with alkaline phosphatase (Bio-Rad Laboratories, Hercules, USA) was used to detect antigens and substrates used were nitro blue tetrazolium (NBT) and 5-bromo-4chloro-3-indolylphosphate for substrate BCIP).

Enzyme-linked immunosorbent assay analysis

Serological relationships among KGMMV-YL, KGMMV-C and CGMMV-U were compared by DAS-ELISA as described by CLARK and ADAMS[6] (1977). All samples were tested at the same time in a single assay. The antisera and enzyme conjugated antibodies against KGMMV and CGMMV, respectively were used at 1 : 200 dilutions according to the manufacture instruction (Agdia Inc., Madison, USA). To compare absorbance values among plates directly, a KGMMV or CGMMV infected and a healthy zucchini were included in ELISA analysis. Absorbance at 405 nm was determined with a plate reader (MTP-120 ; Corona Electric, Ibaraki, Japan).

Cloning and analysis of the virus coat protein gene

Viral RNA was extracted from upper leaf of mechanically inoculated zucchini by incubation in 0.3% sodium dodecyl sulfate (SDS) following phenol chloroform extraction[7] (ROSNER *et al.,* 1983). First strand cDNA was synthesized by First-Strand cDNA Synthesis Kit (Amersham Biosciences, Little Chalfant, UK) reverse transcriptase at 37℃ for 1 hour with a primer complementary to the 3′ of the gene on 6336–6356 of KGMMV-YM with accession number AB110084 (5′-ACGCCTCACTTTGAGGAAG TA-′3). This primer and a forward primer on 5864–5885 of KGMMV-YM (5′-GATGTCTTACTCGATCAGTGG-′3) were used to amplify the cDNA product of the first strand synthesis with Takara Ex Taq™ PCR buffer (Takara Biomedicals, Kyoto, Japan) and following by PCR conditions : 30 cycles of 1 minute at 94℃, 1 minute at 50℃ and 1 minute at 72℃. PCR products were fractionated in 1.5% agarose gel, which was then stained in ethidium bromide solution. Bands of the expected size were purified using QIA quick Gel Extraction Kit (QIAGEN, USA) and were cloned into pGEM T-vector (Promega, Wisconsin, USA). Five selected clones were sequenced on an ABI PRISM™ 377 automated sequencer. Nucleotide sequence and its deduced amino acid sequence were analyzed and compared using Mac Vector 6.5 software (Oxford Molecular Ltd., California, USA) and subjected to BLAST searches to identify related sequences available from the DDBJ database. The obtained sequence and 23 available Tobamovirus sequence data were initially aligned with CLUSTAL W[8] (THOMPSON *et al.,* 1994) to assemble the complete KGMMV-YL CP gene, and *Sun hemp mosaic virus* (SHMV) was used as the out group. Phylogenetic trees were obtained from the data by distance method using

the PAUP 4.0 phylogentic package. The distance matrix was analyzed by algorithms based on Saitou and Nei's neighbor-joining (Saitou and Nei, 1987). The strength of the internal branches from the resulting tree was statistically tested by bootstrap analysis from 1,000 bootstrap replications.

Results

Isolation of KGMMV from angled loofah

Many angled loofah plants cultivated in Sleman, Yogyakarta, Indonesia showed mosaic symptoms on leaves and fruit malformations between May and August 2000–2001. An agent was mechanically transmitted from symptomatic angled loofah leaves to *C. quinoa* and developed a few local lesions. Electron microscopy of leaf dips prepared from loofah leaves revealed rod-shape particles with a length of ~300 nm and ~17 nm in width consistent with those of Tobamoviruses.

Host range determination

Results of an experimental host range for KGMMV-YL were shown in Table 1. No evidence of infection was detected in four species of the family Solanaceae i.e. *Datura stramonium, Petunia hybrida, Nicotiana glutinosa* and *N. benthamiana*. Symptoms were produced on inoculated leaves of nine species in the Cucurbitaceae (*Cucurbita pepo, Cucurbita maxima, Cucurbita hispida, Cucumis melo, Cucumis sativus, Citrullus vulgaris, Lagenaria siceraria, Luffa cylindrical,* and *Momordica charantia*) and two species in the Solanaceae (*N. tabacum* cv. *Samsun and Lycopersicon esculentum*). *Chenopodium amaranticolor* and *C. quinoa* commonly used as indicator hosts for Tobamovirus developed chlorotic local lesions on inoculated leaves. In addition, KGMMV-YL infected eight cultivars of melon (Table 1).

Coat protein analysis and serological relationships

Coat protein of KGMMV-YL purified from infected

Table 1 Comparison of experimental host range between isolates of KGMMV

Family	Species	KGMMV			
		YL	YM[1]	C[2]	Y[3]
Chenopodiaceae	*Chenopodium amaranticolor*	NLL	NLL	0	NLL
	Chenopodium quinoa	NLL	NLL	NT	NT
Cucurbitacaeae	*Cucurbita pepo* cv. Diner	M, D	M, D	NT	NT
	Cucurbita maxima	M, D	M, CLL	NT	NT
	Cucurbita hispida	M, CLL	M	M	M
	Cucumis melo cv. Blewah	M	M, D	NT	NT
	C. melo cv. Sunnet 858	M, D	M, D	NT	NT
	C. melo cv. Andes	M, D	M	NT	NT
	C. melo cv. Prince	M	M, CLL	NT	NT
	C. melo cv. New-melon	M, D	M, CLL	NT	NT
	C. melo cv. Alice	M, D	M, D	NT	NT
	C. melo cv. Katsura-shirouri	M, D	M, CLL	NT	NT
	C. melo cv. Kinmei	M, D	M, D	NT	NT
	C. sativus cv. Mutiara	M	M, D	NT	NT
	C. sativus cv. Aohagauri	M, D	M, D	NT	NT
	Citrullus vulgaris	M, CLL	M	M	M
	Lagenaria siceraria	M, CLL	M	M	M
	Luffa cylindrical	M, CLL	M	M	M
	Momordica charantia	M, D	M, CLL	NT	NT
Solanaceae	*Datura stramonium*	0	M	0	M
	Petunia hybrida	0	M	NLL	NLL
	Nicotiana glutinosa	0	0+	NT	NT
	N. benthamiana	M	M	NT	NT
	N. tabacum cv. *'Samsun'*	0	0+	NLL	0

1: KGMMV-YM (Daryono *et al*., 2005); 2: KGMMV-C (Tan *et al*., 2000 and Yoon *et al*.[10], 2001); 3: KGMMV-Y (Tan *et al*., 2000 and Yoon et al.[10], 2001). M: Mosaic; NLL: Necrotic local lesions; D: Leaf deformation; CLL: Chlorotic local lesions; 0: No symptom; NT: Not tested; 0+: No symptom but infection was confirmed by RT-PCR.

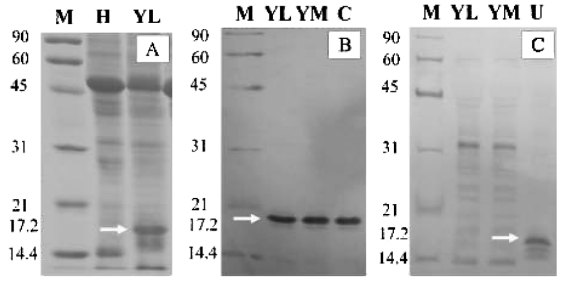

Fig. 1 SDS-PAGE of KGMMV-YL viral coat protein (A) and Western blot analysis Immuno-probed NC membrane with KGMMV (B), and CGMMV (C) CP antibodies. H : Healthy ; YL : KGMMV-YL ; YM : KGMMV-YM ; C : KGMMV-C ; U : CGMMV-U. M : Protein standard markers (BIO-RAD). Arrows indicate approx. 17.2 kDa coat protein for KGMMV-YL.

leaf samples were analyzed by SDS-PAGE (Fig. 1 A). In Western blot analysis, antiserum against KGMMV (As-KGMMV) reacted with the KGMMV-YL, KGMMV-YM, and KGMMV-C and of their coat protein molecular weight was ~17.2 kDa (Fig. 1 B). On the other hand, antiserum against CGMMV (As-CGMMV) reacted strongly with CGMMV-U, but not at all with KGMMV-YL or KGMMV-YM (Fig. 1C).

DAS-ELISA analysis confirmed the match of serological cross-reaction between KGMMV-YL and KGMMV-C. The commercial antiserum against KGMMV reacted strongly with KGMMV-YL, KGMMV-YM, and KGMMV-C but not at all with CGMMV-U indicating their specificity to KGMMV. In contrast, the commercial antiserum against CGMMV reacted strongly with CGMMV-U and reacted weakly with KGMMV-YL and KGMMV-YM (Table 2), but not with KGMMV-C. Furthermore, RT-PCR results showed a specific DNA band of coat protein gene of KGMMV-YL, KGMMV-YM, and KGMMV-C around 492 base pair (Fig. 2).

Table 2 Serological relationships among *Kyuri green mottle mosaic virus* angled loofah strain (KGMMV-YL), KGMMV melon strain (KGMMV-YM), a strain of KGMMV (KGMMV-C), and *Cucumber green mottle mosaic virus* (CGMMV-U) determined by double-antibody enzyme-linked immunosorbent assay (DAS-ELISA)

Antigen	Polyclonal antiserum against[1]	
	KGMMV	CGMMV
KGMMV-YL	+++++	++
KGMMV-YM	++++	+
KGMMV-C	++++	-
CGMMV-U	-	+++++

[1] Serological reactivity measured by DAS-ELISA as the absorbance (A) at 405 nm):
-: A<0.05; +, 0.05<A<0.1; ++, 0.1<A<0.5; +++, 0.5<A<1.0; ++++, 1.0<A<1.5; and +++++, A>1.5

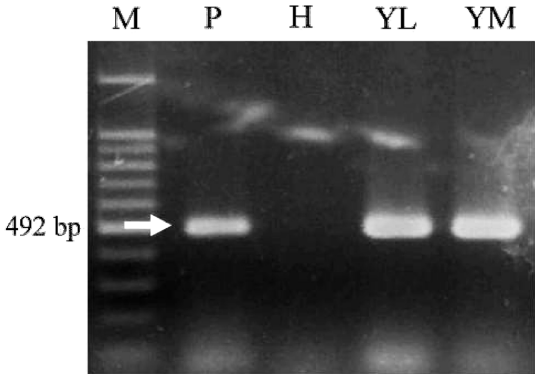

Fig. 2 Comparison among the RT-PCR products of coat protein genes of KGMMV strains by agarose gel electrophoresis. P : positive control (KGMMV-C) ; H : healthy zucchini ; YL : KGMMV-YL ; YM : KGMMV-YM. M is DNA marker (Promega Inc., Wisconsin, USA). Arrow indicates the 492 bp of KGMMV-YL CP gene.

CP sequence analysis

The nucleotide sequence of the putative KGMMV-YL CP gene was determined from several overlapping cDNAs clones. The CP amino acid sequence was deduced from this sequence (Genebank accession no. AB182577). The CP gene encoded a protein consist of 161 amino acids with a predicted translation product of ~17.2 kDa, consistent with the size of SDS-PAGE. The only sequence producing significant alignments from initial BLAST query was tobamovirus coat proteins including many strains of KGMMV and CGMMV. The CP of KGMMV-YL was composed of 486 nucleotides and 161 amino acid residues, as same as CP of KGMMV-YM and KGMMV-C, however two amino acids shorter than KGMMV-Y. Coat protein of KGMMV-YL shared 96%, 91%, and 82% amino acid identities with KGMMV-YM, KGMMV-C, and KGMMV-Y, respectively while with ZGMMV and CFMMV shared 72 and 75%. However, KGMMV-YL amino acid identities of CP were only 31 to 44% identical at deduced amino acids to all others tobamovirus amino acid identities of the CP (Table 3). Result of phylogenetic tree for CP gene analyses displayed KGMMV-YL presents in clusters of cucurbits infecting tobamovirus (Fig. 3) together with KGMMV-YM, KGMMV-C, KGMMV-Y, ZGMMV, CFMMV, and

strains of CGMMV.

Discussion

An Indonesian isolate of angled loofah infecting Tobamovirus (KGMMV-YL) was originally detected and isolated from a field of angled loofah in Sleman, Yogyakarta, Indonesia, and it appears to be a new isolate of KGMMV based on several criteria. The virion morphology of KGMMV-YL is consistent with those of tobamoviruses. Furthermore, the experimental host range of KGMMV-YL was similar but not identical to KGMMV-YM, however distinguishable from KGMMV-C and KGMMV-Y (Table 1). KGMMV-YL and KGMMV-YM induced necrotic local lesions on *Chenopodium amaranticolor,* whereas KGMMV-C did not induce (Table 1). KGMMV-YL and KGMMV-C also differed drastically in their effects on *Datura stramonium.* KGMMV-YL could not infect in this host, whereas KGMMV-C produced necrotic local lesions (Table 1). The difference in the response of *Chenopodium amaranticolor* and *Datura stramonium* to KGMMV-C indicates that these strains are not identical in host reactions although they shared their natural host preference.

The CP of KGMMV-YL was similar in size to those of other strains of KGMMV and CGMMV, however antiserum against KGMMV-YL did not cross-react with CGMMV in Western blots (Fig. 1), providing evidence for a distant relationship between the viruses.

Alignment of KGMMV-YL CP with CP sequences of previously reported tobamovirus species shares 31 to 96% amino acid identity and 41 to 97% nucleotide identity. Based upon CP sequence, KGMMV-YL is most similar to KGMMV-YM, sharing 96 and 97% of amino acid and nucleotide identity, respectively and it was found that only 6 amino acids were different between KGMMV-YL and KGMMV-YM. KGMMV-YL shares 44 to 96% amino acid identity and 52 to 97% nucleotide identity with the CPs and CP of cucurbit infecting tobamovirus species. However, it shares 31 to 41% amino acid identity and 42 to 50% nucleotide identity with the CPs and CP gene of other recognized tobamovirus species.

The relationships between KGMMV-YL and other

Table 3 Homology of nucleotides sequence and amino acids identities of CP gene between KGMMV-YL and other Tobamoviruses

Virus*	GenBank Accession no.	Homology		
		Nucleotides (%)	Amino acid (%)	Number of total amino acid
KGMMV-YM	AB110084	97	96	161
KGMMV-C	AB015144	91	91	161
KGMMV-C1	AJ295948	91	91	161
KGMMV-Y	AB015145	87	82	161
ZGMMV	AJ252188	79	72	161
CFMMV	AF321057	77	75	162
CGMMV-SH	D12505	53	44	161
CGMMV-W	AB015146	52	44	161
CGMMV-KOM	AF417243	53	44	161
CGMMV-KW	AF417242	52	44	161
CGMMV-Y	AJ245440	52	44	161
CGMMV-NS	AJ243831	52	44	161
CGMMV-India	AY309021	53	44	161
CGMMV-GR7	AJ459423	52	43	161
ToMV	X02144	42	31	159
TMV-Fujian	AF395127	47	32	159
PMMV-S	M81413	44	32	157
TMGMV	M34077	45	36	159
YoMV	U30944	41	31	157
ORSV	AF033848	43	34	158
ObPV	L11665	47	33	161
FrMV	AF165884	44	34	174
SHMV	J02413	50	41	163

*KGMMV; *Kyuri green mottle mosaic virus*, ZYMMV; *Zucchini yellow mottle mosaic virus,* CFMMV; *Cucumber fruit mottle mosaic virus,* CGMMV; *Cucumber green mottle mosaic virus,* ToMV; *Tomato mosaic virus,* TMV; *Tobacco mosaic virus,* PMMV; *Pepper mild mottle virus,* TMGMV; T*omato mild green mosaic virus,* YoMV; *Youcai mosaic virus* (=*Ribgrass mosaic virus*) , ORSV; *Odontoglossum ring spot virus,* ObPV; *Obuda pepper virus,* FrMV ; *Frangipani mosaic virus*, SHMV; *Sunn hemp mosaic virus*.

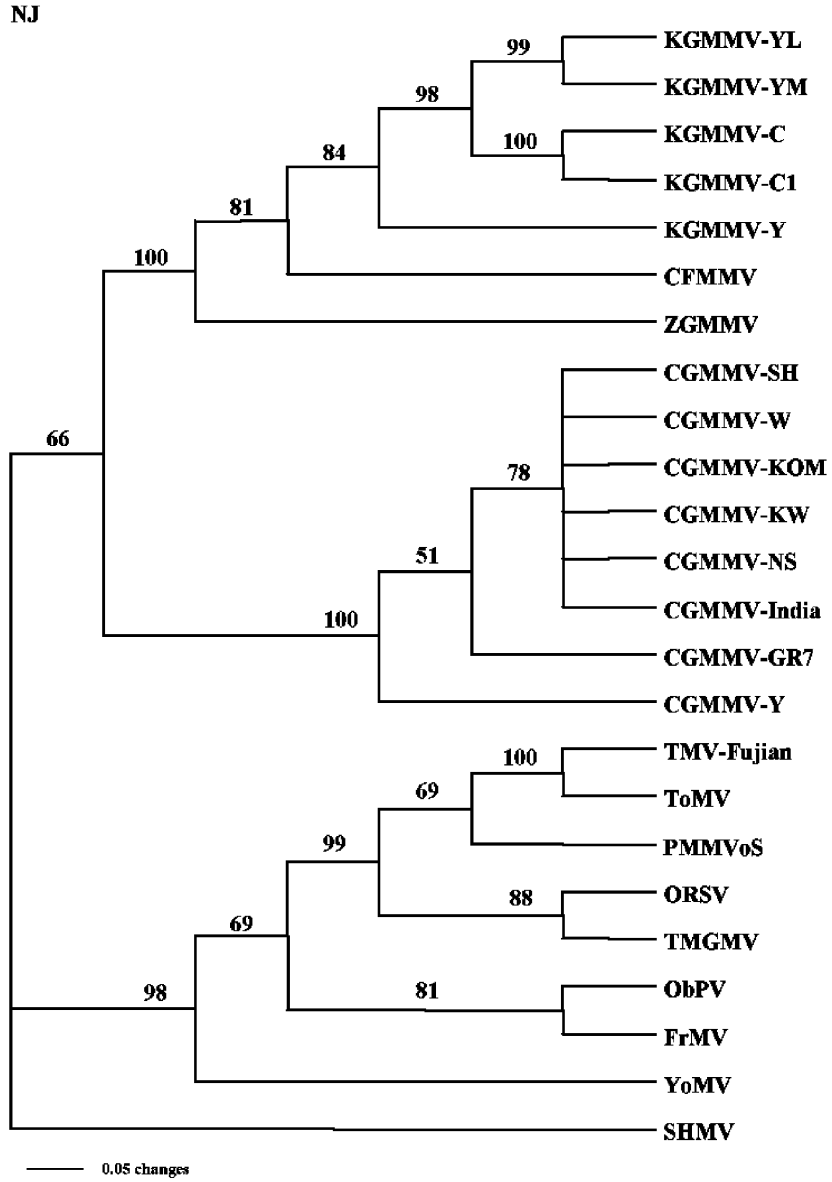

Fig. 3 Comparison of the coat protein (CP) amino sequences of *Kyuri green mottle mosaic virus* angled loofah isolate (KGMMV-YL) and the recognized species in the genus Tobamovirus. The neighbor-joining tree was generated with PAUP 4.1 using *Sun-hemp mosaic virus* (SHMV) as an out-group. Viruses included (listed with abbreviation and GenBank accession number for nucleotide sequence) are : *Kyuri green mottle mosaic virus* (KGMMV-C, AB015144), (KGMMV-Y, AB 015145), (KGMMV-YM, AB110084), (KGMMV-C1, AJ295948), (KGMMV-YL, AB182577), *Zucchini green mottle mosaic virus* (ZGMMV, AJ295949), *Cucumber fruit mottle mosaic virus* (CFMMV, AF321057), *Cucumber green mottle mosaic virus* (CGMMV-SH, D12505), (CGMMV-W, AB015146), (CGMMV-KOM, AF417243), (CGMMV-KW, AF417242), (CGMMV-NS, AJ243831), (CGMMV-India, AY309021), (CGMMV-GR7, AJ459423), (CGMMV-Y, AJ245440), *Tobacco mosaic virus* Fujian strain (TMV-Fujian, AF395127), *Tomato mosaic virus* (ToMV, X02144), *Pepper mild mottle virus* S strain (PMMoV-S, M 81413), *Youcai mosaic virus* (YoMV, U30944), *Odontoglossum ringspot virus* (ORSV, AF033848), *Tobacco mild green mosaic virus* (TMGMV, M34077), *Obuda pepper virus* (ObPV, L11665), *Frangipani mosaic virus* (FrMV, AF165884), *Sunn-hemp mosaic virus* (SHMV, J02413).

recognized tobamovirus species based on the amino acid sequences of the CPs gene with phylogenetic tree analysis resulted in a closer relationship of KGMMV-YL and KGMMV-YM than to CGMMV as to other tobamovirus species. KGMMV-YL and KGMMV-YM may have a common ancestor which could infect some cucurbit plants and then diversified or differentiated its coat protein gene and host range separately.

Since KGMMV is very stable and is mechanically transmissible during pruning or harvesting, or through infected seeds, it is essential to survey the occurrence of cucurbit-infecting tobamoviruses in Asia and produce commercially available resistant cucurbits. For protection, it is also important to sow virus free seeds and use virus free irrigation water and soil.

Acknowledgements

The authors thank Mr. Toshirou KAWANO, Japan Plant Protection Association, Tsukuba-Japan and Dr. T. NATSUAKI for providing samples of KGMMV. Thanks are also extended to Mr. TRIJOKO, Faculty of Agriculture, Shizuoka University, Japan for sampling assistance.

Literature cited

1) 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.

2) INOUE, T., INOUE, N., ASATANI, M. and MITSUHATA, K., 1967. Studies on cucumber green mottle mosaic virus in Japan (*in Japanese*). *Nogaku Kenkyu,* **51**, 175–186.

3) LEE, S.H., LEE, Y.G., PARK, J.W., CHEON, J.U., LEE, K.W. and CHOI, Y.C., 2000. Nucleotide sequence of coat protein gene of Kyuri green mottle mosaic virus (KGMMV) isolated from zucchini (*Cucurbita pepo*) in Korea. *Plant Pathol. J.,* **16** (2), 118–124.

4) 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. Phytopathology,* **153** (10), 588–595.

5) SAMBROOK, J. and RUSSELL, D.W., 2001. Molecular cloning : A laboratory manual. 3rd ed. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY.

6) CLARK, M.F. and ADAMS, A.N., 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *J. Gen. Virol.,* **34**, 475–483.

7) ROSNER, A., BAR-JOSEPH, M., MOSKOVITZ, M. and MEVARECH,. M., 1983. Diagnosis of specific viral RNA sequences in plant extracts by hibridization with a polynucleotide kinase-mediated, ^{32}P-labeled, double-stranded RNA probe. *Phytopathology,* **167**, 653–656.

8) THOMPSON, J.D., HIGGINS, D.G. and GIBSON, T.J., 1994. CLUWTAL W : Improving the sensitivity of progressive multiple sequence alignment through sequencing weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res.,* **22**, 4673–4680.

9) SAITOU, N. and NEI, M., 1987. The neighbor-joining method : A new method for reconstructing phylogenetic trees. *Mol Biol Evol.* **4**, 406–425.

10) YOON, J.Y., MIN, B.E., CHOI, S.H. and RYU, K.H., 2001. Completion of nucleotide 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.

インドネシアのトカドヘチマら分離されたキュウリ緑斑モザイクウイルス（*Kyuri Green mottle virus*）の生物学的性状および外被タンパク質遺伝子の塩基配列の解明

Budi Setiadi DARYONO*,# ・ Susamto SOMOWIYARJO** ・ Keiko T. NATSUAKI***

（平成17年11月22日受付/平成18年3月10日受理）

要約：インドネシアジョクジャカルタ市近辺で栽培されている多くのウリ科作物の中でも，トカドヘチマ（*Luffa acutangula* L.）は地元市場への供給を目的として，狭い面積ではあるが常時栽培されている。ジョクジャカルタ市近辺で2000年から2001年にかけて実施したウイルス病の調査において，多くのトカドヘチマが葉のモザイクや葉や果実の奇形という症状を呈しているのが見出された。これらの病葉よりウイルスを分離したところ，まずその粒子の形態から *Tobamovirus* 属ウイルスであると考えられた。また，血清学的には *Kyuri green mottle mosaic virus*（KGMMV，キュウリ緑斑モザイクウイルス）と近縁関係があることが示された。さらに，同分離株の外被タンパク質をコードする遺伝子についてその塩基配列を明らかにして，KGMMV-YM，KGMMV-C，およびKGMMV-Yなど既報のKGMMV分離株比較をしたところ，KGMMVとの高い類似性が認められた。汁液接種では，3科15種の植物に感染性があったが，いくつかのウリ科植物ではモザイク症状を示し，*Chenopodium amaranticolor* および *C. quinoa.* ではえそ斑点を生じた。しかし，同ウイルスはダチュラ（*Datura stramonium*），ペチュニア，*N. glutinosa* などには感染しなかった。インドネシアのトカドヘチマでは今までKGMMVの発生は報告されていなかったが，メロンでの発生が知られているため，KGMMVの発生する植物として2例目と考えられる。

キーワード：*Tobamovirus* 属，キュウリ緑斑モザイクウイルス，*Luffa acutangula* L.

* 東京農業大学大学院農学研究科国際農業開発学専攻　現所属：インドネシアガジャマダ大学生物学部遺伝学研究室
** インドネシアガジャマダ大学農学部植物病理学研究室
*** 東京農業大学国際食料情報学部国際農業開発学科
# 連絡用 e-mail：bs—daryono@mail.ugm.ac.id