Characterization of a New Virus from Garlic (*Allium sativum* L.), Garlic Mite-borne Mosaic Virus

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Abstract

Flexuous, filamentous virus particles with a length of 700-800 nm were detected in garlic (*Allium sativum* L.) plants showing mosaic symptoms. The virus was sap-transmissible from garlic plants to seven out of 25 plant species tested, inducing local lesions on *Chenopodium murale* and *Gomphrena globosa* and a systemic mosaic on garlic. This virus was transmitted by the eriophyid mite, *Aceria tulipae* Keifer, but not by aphids. Two polypeptides with M.W. 30 kDa and 28.5 kDa were detected by SDS-PAGE of the purified virus preparation. The virus exhibited no serological relationships with leek yellow stripe, onion yellow dwarf or garlic latent viruses. No cytoplasmic cylindrical inclusions were observed in infected leaf cells of garlic or *C. murale*. The 3'-terminal nucleotide sequence of 2518 bp was determined. It contained three putative open reading frames (ORFs) for 40 kDa, 28 kDa (a putative coat protein, CP) and 15 kDa polypeptides. The arrangement and amino acid sequences of these ORFs show close similarities with those of shallot virus X and garlic virus (GV)-A, -B, -C and -D. The putative CP of the mite-borne virus has 98% amino acid sequence homology with CP of GV-C, but only 60-67% identity with CPs of four other viruses, indicating the classification of the mite-borne virus and GV-C as the same virus. We propose the name garlic mite-borne mosaic virus (GmbMV) for the virus.

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Key words : garlic, garlic mite-borne mosaic virus, mite-transmitted virus.

INTRODUCTION

A number of virus diseases and viruses of garlic (*Allium sativum*) have been described²⁸⁻³⁰. Garlic latent carlavirus (GLV)¹⁶ and a potyvirus, designated as garlic mosaic potyvirus (GMV)^{1,16,24}, have been reported to infect garlic in Japan. However, van Dijk²⁹ reported that GMV in Japan is possibly a complex of three distinct filamentous viruses-onion yellow dwarf potyvirus (OYDV)^{2,6}, leek yellow stripe potyvirus (LYSV)² and the garlic strain of onion mite-borne latent rymovirus (OMbLV-G)³⁰. We recently reported that LYSV isolated from garlic is closely related to GMV by biological and serological tests³⁴. In addition, Iwai *et al.*¹¹ detected OYDV from Japanese garlic by ELISA tests. However, OMbLV-G has not been detected in garlic in Japan.

Recent reports based on nucleotide sequence analyses revealed the presence of novel filamentous viruses in commercial garlic in Japan²⁷ and shallots in Russia³¹. The novel filamentous virus reported as shallot virus X (SVX)³¹ was serologically distinct from OYDV and shallot latent carlavirus (SLV), and did not produce granular inclusion bodies in infected plant tissues. The complete nucleotide sequence of SVX has also been

reported¹². Four different viral cDNAs derived from purified preparations of garlic plants with mixed infections were designated as garlic virus (GV)-A, -B, -C, and -D²⁷. GV-A, -B, -C and -D were not isolated from the infected garlic plants by single-lesion transfer, and their biological and serological properties are unknown. The 3'-terminal gene organization of SVX, GV-A, -B, -C and -D are similar, but different from those of other classified filamentous viruses, suggesting they form a new virus group.

We found that a mosaic disease of garlic prevalent in Aomori Prefecture, Japan, was easily transmitted by the eriophyid mite (*Aceria tulipae* Keifer), and was associated with flexuous filamentous virus particles³³. In this paper, we report the biological, physico-chemical, serological, cytopathological and molecular characteristics of the mite-borne virus isolated from garlic, which led us to the conclusion that the virus belongs to a new virus group.

MATERIALS AND METHODS

Source of a mite-borne virus. A garlic (*Allium sativum* cv. Fukuchi-howaito) plant showing mosaic symptoms was collected from a field located in Aomori

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Prefecture in 1991. The virus was originally isolated by eriophyid mite transmission from a diseased garlic bulb to virus-free garlic plants. The virus was then transferred from infected garlic plants to *Chenopodium murale* by sap inoculation. After three successive single-lesion passages on *C. murale*, the virus was back-inoculated to virus-free garlic plants.

Mechanical inoculation tests. Infected garlic or *C. murale* leaf tissue was homogenized in 0.05 M borate buffer (BB), pH 8.5, containing 10 mM EDTA, and used to inoculate 25 different species of plants. Six of replicates were used for each species and two different experiments were done. Inoculated plants were maintained in a greenhouse at 18–24°C and symptoms were observed for six weeks after inoculation. Symptomless plants were back-inoculated to *C. murale* by sap inoculation.

Mite and aphid transmission tests. Eggs of eriophyid mites (*A. tulipae*) collected from garlic were transferred onto Welsh onion (*A. fistulosum*) seedlings. After hatching, their non-viruliferous first-instar nymphs were transferred and maintained on Welsh onion seedlings. Mites at different growth stages were allowed an acquisition feeding period of 24 hr on infected garlic leaves and an inoculation access period of 24 hr on virus-free garlic bulbs. Following test periods, the mites were killed by dipping into the insecticide Pirimiphos-methyl. Aphids, *Acyrtosiphon solani* and *Myzus persicae*, were starved for 2 hr, and then allowed an acquisition feeding period of 10–15 min on infected garlic plants and an inoculation access period of 1 hr on virus-free garlic plants. Following test periods, the aphids were killed by spraying the insecticide DDVP. Transmission tests with mites and aphids were done in a greenhouse using five mites or ten aphids per virus-free plant. Inoculated plants were maintained for three months to observe symptoms. Symptomless plants were back-inoculated to *C. murale* by sap inoculation.

Virus purification. Two to three months after inoculation, infected fresh garlic leaves were homogenized in a Waring blender for 5 min with 6 volumes (v/w) of 0.5 M BB, pH 8.5, containing 10 mM EDTA and 0.2% (v/v) thioglycolic acid, and then mixed with 10% (v/v) carbon tetrachloride. After centrifugation of the homogenate at $7000\times g$ for 10 min, 1% (v/v) Triton X-100, 5% (w/v) polyethylene glycol (mol. wt. 6000) and 0.1 M NaCl was added to the aqueous phase. After stirring for 1 hr at 4°C, the suspension was centrifuged at $7000\times g$ for 10 min. The pellet was resuspended in one-fifth the original volume of the extract in a solution of 0.05 M BB, pH 8.3, 0.5 M urea and 10 mM EDTA (suspending buffer), then stirred gently for 1 hr. After two cycles of differential centrifugation ($7000\times g$ for 10 min, and $170,000\times g$ for 1.5 hr), the virus was further purified by equilibrium density gradient centrifugation ($215,000\times g$ for 3.5 hr in Hitachi P65NT rotor) in a CsCl solution, using an initial concentration 36.7% (w/v) in the suspending buffer. The virus zone was collected and

pelleted by centrifugation at $170,000\times g$ for 1.5 hr. The pellet was resuspended in 2 ml of 0.02 M BB, pH 8.0 containing 5 mM EDTA. After a low speed centrifugation ($7000\times g$ for 10 min), supernatant was collected as a purified virus preparation.

Electron microscopy. Leaf extracts and purified virus preparations were observed after negative staining with 1% phosphotungstic acid in distilled water, pH 7.0, by Hitachi electron microscope H-7000. For ultra-thin sectioning, tissue samples of the virus-infected garlic and *C. murale* plants were fixed in 2% glutaraldehyde in 0.1 M phosphate buffer, pH 7.0 (PB), for 4 hr, followed by post-fixation in 1% osmium tetroxide in 0.1 M PB for 3 hr at 4°C. After a dehydration series with 50–100% ethanol, the tissues were embedded in Spurr's resin. Thin sections were double-stained with uranyl acetate and lead citrate.

Serology. Purified viruses (0.5 mg) were emulsified with Freund's complete adjuvant (DIFCO Laboratories) (1:1) and intramuscularly injected into a rabbit three times at weekly intervals, followed by two intravenous injections at bi-weekly intervals. The rabbit was bled 2 weeks after the final injection. Antisera to OYDV (supplied by Dr. M. Kameya-Iwaki, Yamaguchi University), LYSV (Dr. T. Maeda, Research Institute for Bioresources, Okayama University), and GLV (Dr. I. Sako, Tottori Fruit, Vegetable and Ornamental Crop Experiment Station) were also used for serological tests. Serological properties were examined by leaf dip serology as described by Langenberg¹⁵⁾, and by double immunodiffusion tests using 0.7% Difco noble agarose gel in 0.1 M PB, pH 7.0, containing 0.5% sodium dodecyl sulfate (SDS) and 0.05% sodium azide.

SDS-PAGE and Western blotting. The molecular weight of the coat protein (CP) from purified viruses was determined by SDS-polyacrylamide gel electrophoresis (PAGE) (12.5% slab gel)¹⁴⁾. After electrophoresis, the gel was stained with 0.1% Coomassie brilliant blue. After proteins were transferred onto nitrocellulose (BIO-RAD Laboratories, Inc.), viral proteins were detected by enzyme-linked immunostaining as described by Shirako and Ehara²⁵⁾ using virus-antiserum diluted with 20 mM Tris-HCl buffer (TBST), (0.15 M NaCl, 0.05% NaN₃, pH 7.5, and 0.02% Tween 20) at 1:4000 and alkaline phosphatase-conjugated anti-rabbit goat immunoglobulin G (Sigma Chemical Company) diluted with TBST at 1:2000.

RNA extraction and cDNA cloning. Viral RNA was extracted from purified viruses using proteinase K and SDS followed by phenol extraction and ethanol precipitation. The molecular weight of viral RNA was estimated by 0.8% agarose gel electrophoresis under denaturing conditions with 2.2 M formaldehyde. cDNA synthesis was carried out with the cDNA Synthesis System Plus kit (Amersham International plc) according to the manufacturer's instructions, using oligo (dT) as a primer. The cDNA was ligated *EcoRV*-cut pBluescript II SK⁺. Competent *Escherichia coli* JM109

cells were transformed with the ligated plasmid.

Nucleotide sequence analysis. Five cDNA clones were examined by southern blot hybridization with a viral RNA probe using the ECL direct nucleic acid labelling and detection system (Amersham International plc). The largest cDNA clone, pGMM88 (2.5 kb), was selected and sequenced by the dideoxynucleotide chain termination method using ABI sequencer 373A. The entire cDNA insert was sequenced in both orientations. Sequences were analyzed by the DNASIS program (Hitachi).

RESULTS

Host range and symptomatology

Based on symptom development, and back-inoculation tests, the virus was transmitted by mechanical inoculation to seven plant species from two families out of the 25 species from eight families tested. Chlorotic local lesions were induced on *C. murale* 2 to 4 weeks after inoculation (Fig. 1B). Mosaic symptoms were produced systemically on garlic (*Allium sativum*). A few local lesions with chlorotic centers and red edges were

produced on inoculated leaves of *Gomphrena globosa*. Local latent infection occurred on *C. amaranticolor* and *C. quinoa*, whereas systemic latent infection occurred in leek (*A. ampeloprasum* var. *porrum* cv. Wander) and *Tetragonia expansa*.

No infection was detected in *Allium cepa*, *A. fistulosum*, *Amaranthus tricolor*, *Beta vulgaris*, *Brassica campestris*, *B. napus*, *Celosia cristata*, *Cucumis sativum* cv. Suoyo, *Cucurbita pepo*, *Datura stramonium*, *Lycopersicon esculentum* cv. Kyoryokubeiju, *Nicotiana clevelandii*, *N. debneyi*, *N. glutinosa*, *N. occidentalis*, *N. rustica*, *N. tabacum* cv. White Burley, *Petunia hybrida*, *Phaseolus vulgaris* cv. Top Crop, *Pisum sativum* cv. Kinusaya, *Vicia faba* cv. Uchikoshiisun, *Vigna sesquipedalis* cv. Kurotanesanjaku.

Eriophyid mite and aphid transmission

The virus was easily transmitted from garlic to garlic by second-instar nymphs and adults of *A. tulipae* (Table 1); Virus-infected garlic plants showed mild mosaic symptoms about two months after inoculation. However, none of the 10 garlic plants inoculated with the aphids *A. solani* or *M. persicae* were infected with the virus.

Physical properties

In crude extracts of infected garlic leaves, the virus kept its infectivity to *C. murale* after diluting to 10^{-3} but not to 10^{-4} , after heating for 10 min at 65°C but not at 70°C, and after incubating the crude extracts at 24°C for 3 days but not for 5 days (data not shown).

Electron microscopy

Leaf-dip preparations from infected *C. murale* plants contained flexuous filamentous particles (Fig. 2A). These particles, ranging in length from 700 to 800 nm and averaging 716 nm, were dispersed or clustered in the cytoplasm of mesophyll cells of garlic (Fig. 3A) and *C. murale* (Fig. 3B). No cylindrical inclusions were observed in any cells of infected garlic or *C. murale* plants.

Purification

Yields of purified virus particles ranged from 0.8 to 1.5 mg per 100 g of garlic leaves. Many particles longer than the unit-length, formed by end-to-end aggregation, were observed by electron microscopy (Fig. 2B). The purified preparation showed a UV absorption spectrum characteristic of nucleoprotein with the absorption maximum at 260 nm and the minimum at 246 nm. The ratios of $A_{260/280}$ and $A_{\text{max/min}}$ were 1.22 and 1.11, respectively.

Table 1. Results of transmission tests of garlic mite-borne mosaic virus (GMbMV) by three stages of eriophyid mite (*Aceria tulipae*)

Stages of mite	Number of plants transmitted/ inoculated	Proportions
First-instar nymph	2/40	0.05
Second-instar nymph	23/48	0.48
Adult	41/66	0.62

Fig. 1. Symptoms after mechanical inoculation with GMbMV. (A) Mosaic symptoms on systemically infected garlic cv. Fukuchi-howaito. (B) Chlorotic local lesions on inoculated leaves of *Chenopodium murale*.

Fig. 2. Particles of GMbMV negatively stained with 2% PTA. (A) Particles in sap of *Chenopodium murale*. Bar represents 200 nm. (B) Particles in a purified virus preparation. Bar represents 500 nm. (C) A leaf dip preparation incubated with a 1/50 dilution of antiserum to GMbMV for 15 min before negative staining. Bar represents 200 nm.

Serology

The antiserum to the virus had a titer of 1/512 against the homologous virus preparation in microprecipitin tests. In double immunodiffusion tests, the purified virus preparation reacted with the homologous antiserum, but not with antisera to OYDV, LYSV or GLV (data not shown). After leaf-dip serology, the virus particles were decorated with the homologous antiserum (Fig. 2C), but not with the other antisera.

SDS-PAGE

Using SDS-PAGE of the purified virus preparation (Fig. 4), two major polypeptide bands were detected and estimated to have molecular weights of 30 kDa and 28.5 kDa. Both polypeptides reacted with the virus antiserum during Western blotting (data not shown). The 28.5 kDa component is considered to be a breakdown product derived from the 30 kDa component by proteolytic cleavage during purification, as found for other filamentous viruses^{4,10,12}.

Fig. 3. Ultrathin sections of the infected cells with GMbMV. (A) Fibrous virus particle masses in a leaf cell of garlic cv. Fukuchi-howaito. Bar represents 500 nm. (B) Fibrous virus particle masses in a leaf cell of *Chenopodium murale*. Bar represents 500 nm.

Fig. 4. SDS-PAGE of purified preparations of GMbMV (lane 2) and zucchini yellow mosaic virus (lane 3). Lane 1 contains protein markers.

Nucleotide sequence

The length of viral RNA was about 10 kb as determined by agarose gel electrophoresis. All the examined

GCCGGGCTGTGTTGTCACCATCACTGGGTCGCGGTACAACTCACTAACTGTCCTTTAGA	60	AACGCTGCCATGACTGACCCGTCACCTCAGGGATTCACACGAGCCACGCTGCGCCAGGGA	1320
GCATATACACGACGTGGTAAAGTCTCTCTCGGAGCGCACATGACCATTTGTAACAACTA	120	N A A M T D P S T Q G F N Q A T S A Q G	31
M T I V T T	6		
└─ 40-kDa			
CTTACGTTGACCAAAACAGAGAACGCATCAATGATTGCGTCAATGCGGCTCGCAACGCGT	180	TCAAGACGGGACTTGTATCCGACAGGTTCACAAACGCTAGCAAGAATCCAGACCA	1380
T Y V D Q T R E R I N D C V N A A R N A	26	S R P G L V S G P G S Q T P S K N P R P	51
TGTGCCAAATATCAATATTTGTCAGCTAGCAATGAGCAGCAAGTGGAAAATACATCCA	240	TCCTCTGCGCAAGGTGGACCGACTGTCACAAATGAATTTGTCGAGTGTAGCTGAGCTG	1440
V C Q N I N I V Q A S I E Q Q V E N T S	46	S S A Q G G P T V T N E L L P S E S E L	71
ATGGCATCTCAATCACTTTCTGCGTGGTGGAGTCAATTTGGCAACAGATTTGGGACTT	300	GAGGCAGTAGAGAATGACGTACATCGAATTCGCTAGCTACACAGAGCACAATTCGCGAA	1500
N G I L N H F S A W C E S I G N Q I G T	66	E A V E N D V T S N S V A T Q S T I R E	91
TACCAACAGTAGTTCGGAGCAGTTAACGAGAGAATCCAACACTTAGAACACACGCTCAGCT	360	ATCCTAGACTTACTTCTGTCACGCAAGCCAAACGCTACGCGAAAAGATCTCTTTCCCTT	1560
L P T V V G A V N E R I Q H L E H T L T	86	I L D L L R A R K P N A T P K D L F S L	111
TGAGAACTGGCCACAGCAGCTGCAACCGCTCCACCGATGAACAGGGTGTCTTCTCAA	420	GCATGGGCTTGCTACCACAATGTTCTCTCAGGTATACCAACTAGCACGAGTGGCGG	1620
L R N W P H D L Q P P P P M N R V L F S	106	A W A C Y H N G S S R Y T N L A T D A P	131
ATGCGGCTTAGCTTTTGAAGCAACTCGAACTTACTCTCACATGTGACCACTCGAT	480	TGTGGAATGTCTCATGACAGCTCAAGACCTTGTGTAGGAATTTTGACCTTACGACAA	1680
N A A L A L E A T R N L L S H V P P T R	126	C G M S H A E L K D L V E E F C T L R Q	151
ATAACCTGCCACAGACTACCTTGCCTGTGATGAACCTACGCGCAATTCATCGCCCTGC	540	TTCTGCGGGTTTATGCGAAACTTGTTCAGTAACTGAGCAGACAGAGAATAAACACCA	1740
Y N L P Q T T L P L D E L Y G Q L H A L	146	F C G F Y A K T C Y V T G R Q S T I R E	171
ATCAGAACTCTTGAATGGTTAACTCACATCGGCAGTGACACTGACAGACTTATGTAG	600	GCAAAATGGCAAGGAAGGATTCAGGATGAGTCAAGAAATTTGCTGCTTTGATTTCTTT	1800
H Q N S L E W L T H I G S D T D R L I V	166	A N W A R K G F Q D E S K F A A F D F F	191
ATTTTGGGAGCTTTTCAAGTACAGCGTGTGCAATGATGATGATGACAAACGCACTTAG	660	AACGAGTGTGAGCGACTACGCCCCAAACCTCTGCGCGCATGCGCTTTAAACCTACG	1860
D F G T F S S T A V A N D D R M T T H L	186	N A V S S D S A P N P P G G H R F K P T	211
CAAAATTCGGGATGAGCTGCACACTCTCCGAGACTTACCAAACTTCTCAACAGGCTAC	720	CAAGCAGAAATCTTTGGGCACTCCCTGAACGCGAAAATGTCAATCATCGATCACGCAAG	1920
A N I R D E L H T L R D L P Q L L N R L	206	Q A E I L G H S L N A K M S I E S R K	231
TCAAAACAGCAAGAGCTCCACCTGACAGACTCTCACAGACACAAAAAGAGATTACTGCTC	780	GCTACCAACATGTTTCCACAGAGCTGATGTTATAGCGCAACACAGATTACGAACT	1980
L K Q Q E L H L Q T L T D T Q K E I T A	226	A T N M V S T R A D V I A Q Q Q I H E P	251
TCAAACTCTCATACATATAATTTGACCAATCACAGACCCAGGAGTCCAGTACACCAA	840	CCCAAGCTCTCATGTTAACTTTTGATAAACTGCCCAATATGCAACCTTATAATTTA	2040
L K T L I H H N L T Q S Q T Q E S S T P	246	P K P L M L T F	6
		└─ 15-kDa	
GCTCTAGCCAGAGTGGCGGACAAACACACCGCGCAACCGAGTGAATTTGACCCATACC	900	AATTTACTTTGCTGCTCCACTTCTCAAAACCCACTCTCCCGCTTGAATAGACTGTAC	2100
S S S Q S G A T T H Q P Q P S D L H P Y	266	N L L C C L H F S K P T L P L E L R L Y	26
AAGCGGCGATCCCAAACTCGCTCCGCACTTATGTTACCTTATATACAAATGGGTCAA	960	ATATACAACTTAGCCGCTCAAACTTGTATTTAGGAGCTAAGATGCTCAAAAATAAGCCC	2160
Q A R H P T T R C R T Y G T L I Y N G S	286	I Y N L A A P N L L L G R K M L Q N K P	46
GTTTACACATGCCGATGACATATAGGTAAACCGGCGAGTACAGCCCTACAGCTGCAAG	1020	TTCCAGGCACTTCCAAAGTGTGCTGCCCGCGCAGAGCGAAAGCTTATAACAGATGTTTC	2220
S L H M P M D I L G K P A S T A L Q L Q	306	F Q G T S K C A A R R R A K R Y N R C F	66
TTGACATTAACCTTCGCGGGAGGACCAACGTGACCTACAAATTAGCGGATATGGAG	1080	GACTGTGCGGATATTTGCTAAATATCATGTGTGTAAAGCAATTCGCAACTCTGTCGCACT	2280
V D I K P S P G G T N V T Y K L A D N G	326	D C G Y L L N N H V C K Q P F T R A S	86
CGCTGTTTGAAGCGAGGATATTCACACCAACAAGCTGAATGAAGCTCTAAGTGAGC	1140	ACAAGCTGCTCAATGTCATTCACGAAGGCGCTGCTAAGCTATATGCTGAAGGGCGGTC	2340
A L L L S D G E I F T P H K L N K P L S D	346	T S C F N V I H E G P A K L Y A E G A F	106
CTCTTTCTTATTACATGATAATTTGCTCAAACTTCATCTACAAACAAGCGGCTCGGC	1200	CGACGAGTCTCTTGTGCTGAGCAGCTTACTGAACGACTTGAGTGTGATGAAGCTACAC	2400
A L S L L H D N C S N F I Y N N K G G R	366	R R D S F A E Q L I L N D L E M K L H	126
TTTGTAAGTTGTAGTGTAGTTGAATGAGTGAGGAGCAGCTATCTGACGACTCTGCTG	1260	AATAATTAGGCTTTGCTCAAGCTCCACCGGTTTACAGAGCTCTGGACATGGACAAAG	2460
L C	11	N N	129
		└─ CP (28-kDa)	
		ACACCATGCTGTTGATATTTACTAACTTACCTTGTAAACATTTTGTCAAGGGAC	2518

Fig. 5. The sequence of the 3'-terminal 2518 nucleotides of GMbMV RNA. Predicted amino acid sequences of the 40 kDa, 28 kDa (a putative coat protein) and 15 kDa proteins are shown.

cDNA clones hybridized specifically with the viral RNA probe (data not shown). The nucleotide sequence of the 3'-terminal 2518 nucleotides of the virus genome was determined from the largest clone pGMM88 (Fig. 5). The sequence has been deposited in the GSDB, DDBJ, EMBL and NCBI database with the accession number D49443. Analysis of the sequence gave three open reading frames (ORFs) in the positive strand (Fig. 5). The arrangement of these three ORFs showed close similarities with those of SVX¹², GV-A, GV-B, GV-C and GV-D²⁷ (Fig. 5). The first ORF coded for an unknown protein with a calculated molecular weight of 40,960 Da (40 kDa).

The second ORF coded for a putative coat protein (CP) with a calculated molecular weight of 27,978 Da (28 kDa). This protein showed high amino acid sequence homology (60–98%) with the CPs of SVX¹², GV-A, GV-B, GV-C and GV-D²⁷ (Table 2). (The sequences of GV-B, GV-C, and GV-D with the DDBJ accession numbers D11158, D11159, and D11160, respectively, have been corrected, Sumi, S. personal comm.) However, it showed only weak homology (28–33%) with the CPs of

nine filamentous viruses; potato virus X²²) and narcissus mosaic potexviruses³⁶), poplar mosaic⁸), potato virus S¹⁷) and GV1 carlaviruses¹⁹), potato virus Y²³) and GV2 potyviruses¹⁹), wheat streak mosaic rymovirus²⁰), and barley yellow mosaic bymovirus¹³) (Table 2).

The third ORF coded for a protein with a calculated molecular weight of 14,775 Da (15 kDa). This 15 kDa protein contained the putative zinc finger domain CX₂CX₇HXC, where X is any amino acid, (at position 2215–2256) like the analogous proteins of other carlaviruses⁷). This protein showed 99% amino acid sequence homology with the 15 kDa protein of GV-C²⁷) (Table 2).

The 3' non-coding region of the mite-borne virus showed high nucleotide sequence homology (81–100%) with the corresponding regions of SVX¹²), GV-A, GV-B, GV-C and GV-D²⁷) (Table 2).

DISCUSSION

A number of filamentous viruses have been reported in garlic and other *Allium* species in many countries. They

Table 2. Percent amino acid or nucleotide sequence homology between 3 ORF products and 3' non-coding regions of GMbMV and other filamentous viruses^{a)}

Viruses	GMbMV			
	40 kDa	CP	15 kDa	3'NCR
GV-A	41 ^{b)}	62	58	82
GV-B	43	67	66	87
GV-C	—	98	99	100
GV-D	—	61	52	81
SVX	40	60	63	76
GV-1	—	31	28	42
GV-2	—	28	—	18
PVS	—	29	27	53

a) The sources of sequence data were: GMbMV (this paper), GV-A, -B, -C and -D²⁷⁾, SVX¹²⁾ GV-1 and -2¹⁹⁾ and PVS¹⁷⁾.

b) The amino acid sequences of the 40 kDa, CP and 15 kDa proteins of GMbMV and the nucleotide sequence of its 3' non-coding region (3' NCR) were compared with those of corresponding proteins or regions of other viruses by the MAXIMUM MATCHING program of DNASIS.

were classified mainly into two aphid-borne virus groups-LYSV^{2,18,21,34)} and OYDV^{2,6,11,29)}, belonging to the genus *Potyvirus*; and carnation latent virus⁵⁾, GLV¹⁶⁾, garlic common latent virus²⁸⁾ and SLV^{3,28)}, belonging to *Carlavirus*. The virus examined in this study showed no serological relationship to OYDV, LYSV or GLV. The virus was easily transmitted by the eriophyid mite, but not by aphids. No cytoplasmic inclusion body commonly observed in the family *Potyviridae*⁹⁾ was detectable in the mite-borne virus-infected plants. Thus, the mite-borne virus differs clearly from potyviruses and carlaviruses.

The mite-borne virus resembles the garlic strain of OMbLV-G³⁰⁾ in host range, vector relationships and particle morphology. However, they differ in their cytopathological characteristics. The garlic strain of OMbLV induces cylindrical inclusions in the cytoplasm of infected leaf cells³⁰⁾, whereas the mite-borne virus does not. The mite-borne virus is related to SVX, which also does not induce inclusion bodies in its shallot plant host³¹⁾.

The CP of the mite-borne virus migrates during SDS-PAGE as a 30 kDa protein, although its molecular weight was calculated as 28 kDa from its deduced amino acid sequence. The same results have been reported for the CP of SVX¹²⁾. The ORF5-28 kDa protein of SVX migrated as a 32–36 kDa, perhaps because of its high hydrophilicity as indicated by its amino acid sequence.

The 3'-terminal gene organization of the mite-borne virus is quite similar to those of SVX and GV-A, -B, -C and -D. Their genome organizations are apparently different from those of filamentous viruses, potyviruses^{26,32)} and rymoviruses^{20,35)}, carlaviruses^{7,8,17)} and potexviruses^{22,36)}, demonstrating that a new virus group needs to be established to accommodate them.

The amino acid sequences of the CP and 15 kDa proteins of the mite-borne virus and GV-C showed 98% and 100% homology, respectively, and the entire nucleotide sequences of their 3' non-coding regions are 100% identical. Thus, they should be classified as the same virus.

We propose the name 'garlic mite-borne mosaic virus (GMbMV)' for the virus characterized in this study. Further investigations are need to clarify its relationships to SVX, GV-A, -B, and -D.

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和 文 摘 要

山下一夫・酒井淳一・花田 薫：ニンニクから分離された新ウイルス、ニンニクダニ伝染モザイクウイルス

モザイク症状を呈するニンニク (*Allium sativum* L.) から長さ 700~800 nm のひも状ウイルスが分離された。本ウイルスはニンニクおよびリーキに全身感染したほか、*Chenopodium murale*, *C. quinoa* およびセンニチコウなどに局部感染した。本ウイルスはチュウリップサビダニで伝搬されたが、アブラムシでは伝搬されなかった。純化ウイルスから、SDS-PAGE により 30 kDa および 28.5 kDa のタンパク質が検出された。本ウイルスはリーキエローストライプウイルス、ネギ萎縮ウイルスおよびニンニク潜在ウイルスの各抗血清には反応せず、ニンニクおよび *C. murale* の感染細胞内には細胞質封入体は観察されなかった。本ウイルスゲノム RNA の 3' 末端から 2518 塩基の配列を決定した。この領域は、40 kDa タンパク質、外被タンパク質と推定される 28 kDa タンパク質および 15 kDa タンパク質をコードすると思われた。これらの遺伝子の配置および翻訳産物のアミノ酸配列は、未分類の shallot virus X および garlic virus (GV) -A, -B, -C, -D と類似していた。28 kDa タンパク質のアミノ酸配列の相同性は、GV-C とは 98%、他の 4 種ウイルスとは 60~67% であったことから、本ウイルスと GV-C は同種のウイルスであると考えられた。本研究により性状が明らかになったウイルスを、ニンニクダニ伝染モザイクウイルス (garlic mite-borne mosaic virus: GMbMV) と命名するよう提案したい。