Gentian mosaic virus: A New Species in the Genus *Fabavirus*

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ABSTRACT

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A viral isolate, designated N-1 and obtained from a gentian (*Gentiana scabra*) plant that exhibited mosaic symptoms, was transmitted mechanically to nine plant species in six families. These plants are known as hosts of fabaviruses. The N-1 isolate was composed of isometric particles 30 nm in diameter and included two RNA molecules of approximately 6.0 and 3.6 kb in length, as estimated by agarose gel electrophoresis. The RNAs were encapsidated separately in two of the three types of particle. Each particle contained two distinct proteins with Mr values of 39.3×10^3 and 26.6×10^3 , as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Analysis of complete nucleotide sequences of

the RNAs suggested that each encoded a single large polyprotein, in which putative functional proteins were arranged in a manner similar to those in *Broad bean wilt virus 1* (BBWV-1) and *Broad bean wilt virus 2* (BBWV-2), which are members of the genus *Fabavirus* (family *Comoviridae*). Analysis of the deduced amino acid sequences of the proteins indicated that those of isolate N-1 shared 38 to 66% identity with those of BBWV-1 and BBWV-2 but only 16 to 42% identity with those of a comovirus, *Cowpea mosaic virus*. Phylogenetic analysis, based on the amino acid sequences of RNA polymerase, placed isolate N-1 in a separate lineage from BBWV-1 and BBWV-2. In indirect-enzyme-linked immunosorbent assay, isolate N-1 exhibited distant serological relationship to BBWV-1, BBWV-2, and *Lamium mild mosaic virus*, another fabavirus. Our results suggest that N-1 represents a new species of *Fabavirus*. We propose the name Gentian mosaic virus for this new species.

Viruses in the genus *Fabavirus* (family *Comoviridae*) are transmitted by aphids in a nonpersistent manner and cause diseases in various vegetable and ornamental plants. Fabavirus particles are icosahedral, approximately 30 nm in diameter, and sediment as three components, designated T, M, and B, during density gradient ultra-centrifugation (DGC) (26). The RNA genome consists of two single-stranded molecules of 6.0 to 6.3 kb (RNA-1) and 3.9 to 4.5 kb (RNA-2), which are separately encapsidated in the B and M components, respectively. Each of the three components is composed of two distinct proteins of Mr 40 to 45×10^3 (large coat protein; LCP) and 21 to 27×10^3 (small coat protein; SCP).

Broad bean wilt virus 1 (BBWV-1) and *Broad bean wilt virus 2* (BBWV-2), two distinct species in the genus *Fabavirus*, were differentiated by serology (24). In a previous study, we determined the complete nucleotide sequences of the RNAs of both BBWV-1 and BBWV-2 and reported the genetic relationship between the two viruses (8). Fabavirus RNAs are translated into single polyprotein precursors, from which functional proteins are derived by proteolytic cleavage, as also occurs in comoviruses, which are members of the same family (26). Sequence identity at the amino acid level among isolates of BBWV-2 was 87 to 96% for the polyprotein precursors and 73 to 98% for individual mature proteins

(8). In contrast, amino acid identity between isolates of BBWV-1 and BBWV-2 was only 55 to 61% (polyprotein precursors) and 39 to 67% (mature proteins).

Numerous fabavirus isolates have been obtained from various plants in Japan. Some of the isolates have been defined as BBWV-1 (DDBJ/EMBL/GenBank accession no. AB110537) or BBWV-2 (6,9,11,15). However, most Japanese fabavirus isolates have not been classified. In our current studies of fabaviruses in Japan, we found an isolate with serological reactivities distinct from those of BBWV-1 and BBWV-2, and our observations suggested that we might have found a new species of *Fabavirus*. In this report, we show that the isolate represents a new fabavirus.

MATERIALS AND METHODS

Viral isolates. A fabavirus, designated N-1, obtained from a gentian (*Gentiana scabra*) plant with mosaic symptoms, was provided by I. Fujisawa (National Agricultural Research Center, Ibaraki, Japan). Isolate PV132 of BBWV-1 and isolate PV131 of BBWV-2 were supplied by the American Type Culture Collection (Manassas, VA). *Lamium mild mosaic virus* (LMMV), which is another species in *Fabavirus* (26), was provided by R. G. Milne and M. Turina (Istituto di Virologia Vegetale del CNR, Tolino, Italy). *Chenopodium quinoa* plants were inoculated mechanically with each isolate for propagation.

Host range. Crude sap of *C. quinoa* leaves that had been infected with isolate N-1 was inoculated mechanically to 21 plant

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species in nine families (Table 1). The experiment was conducted twice, and for each test approximately five plants of each species were inoculated and maintained in a greenhouse (16 to 26°C under long day period) and monitored for up to 1 month for symptom development. Inoculated plants were assayed for viral infection by an enzyme-linked immunosorbent assay (ELISA).

Purification of viral particles and physicochemical properties. Most steps of the purification procedure were performed at 4°C. N-1-infected leaves of *C. quinoa* were homogenized with Mcllvaine buffer (0.1 M citric acid and 0.2 M Na₂HPO₄ were mixed to adjust to pH 7.0) that contained 0.01 M MgCl₂, 10% (wt/vol) sucrose, and 0.2% (wt/vol) sodium L-ascorbate. The homogenate was filtered through cotton cloth and clarified by blending for 2 min with 1/5 volume of isoamyl alcohol and subsequent centrifugation at 18,500 × *g* for 17 min. The supernatant was allowed to stand on ice for 10 min and then centrifuged again. The aqueous phase was supplemented with Triton X-100, polyethylene glycol 6000, and NaCl to give final concentrations of 2% (vol/vol), 6% (wt/vol), and 0.1 M, respectively. After stirring for 10 min at room temperature and incubation at 4°C for 20 min, the mixture was centrifuged at 12,096 × *g* for 10 min. The resultant pellet was resuspended in 1/10 volume of Mcllvaine buffer with the additives described previously, and the suspension was centrifuged at 31,000 × *g* for 10 min. The resultant supernatant was centrifuged on a cushion of 20% (wt/vol) sucrose at 225,400 × *g* for 1 h. The resultant pellet was resuspended in Mcllvaine buffer (pH 7.0) that contained 0.01 M MgCl₂. After centrifugation at 6,000 × *g* for 10 min, the supernatant was subjected to DGC on a gradient of 10 to 40% (wt/vol) sucrose in the same solution at 190,400 × *g* for 70 min. The top (T), middle (M), and bottom (B) bands separated by DGC were collected separately and layered individually onto gradients of 40 to 60% (wt/vol) sucrose for a second DGC at 127,000 × *g* for 14 h. Each re-formed band was then collected separately.

Purified viral particles were diluted fivefold with distilled water and visualized by electron microscopy (H-7000; Hitachi, Tokyo) after staining with 2% (wt/vol) uranyl acetate. Viral RNAs were extracted from purified virions using an RNaid Kit (Bio 101, La Jolla, CA) after treatment with 0.1% (wt/vol) proteinase K (Gibco, Rockville, MD). Viral RNAs were separated on a 0.8% (wt/vol) agarose gel prepared with 0.22 M formaldehyde (20). Viral coat proteins (CPs) were separated from the purified virions by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (12) on a 12.5% (wt/vol) polyacrylamide gel. Proteins were stained with 0.2% (wt/vol) Coomassie brilliant blue.

Cloning of viral genome. RNA-1 of isolate N-1 was cloned by the same procedure used to clone RNA-1 of isolate PV132 (8). After reverse transcription-polymerase chain reaction (RT-PCR) using a 3' rapid amplification of cDNA ends (RACE) system (Gibco) with the degenerate primer designated YGDDNL (8), cDNA was synthesized with the reverse primers N-1-135 (5'-⁴⁹³⁰CACAATCAGCTAGTCTTCTAAAA⁴⁹⁰⁸-3') and N-1-0109L54 (5'-³¹⁰⁰ACCCTCTGTTTGGCATAAG³⁰⁸²-3'). The 5'-terminal region was amplified by the 5'RACE method (2) with nested reverse primers N-1-100L (5'-¹³¹GCTGTTTAAAGAAGGCGT-TTC¹¹²-3') and N-1-20L (5'-⁵⁴ACCGAAAGCTGTTTTAGTA-AC³⁴-3').

For analysis of N-1 RNA-2, total RNA was extracted from N-1-infected *C. quinoa* leaves with an RNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and used as template for amplification of 3'-terminal region of RNA-2. RT-PCR was performed with the degenerate primer IP-1885dU (8) and the 3'RACE system. Amplified cDNA products were separated by electrophoresis on a 1% agarose gel. Putative cDNA products from N-1 RNA-2 purified with a QIAquick Gel Extraction Kit (Qiagen) were cloned into the pGEM-T Easy Vector (Promega, Madison, WI), and three clones were sequenced. RNA-2 was extracted from the purified virions of isolate N-1 using the RNeasy Plant Mini Kit after treat-

ment with 0.1% (wt/vol) proteinase K and used as templates for subsequent cDNA amplification. After single-stranded cDNA was synthesized with the reverse primer N-1-411L (5'-³⁰⁰³ACTTGCTCCCATGATAACC²⁹⁸⁵-3') using Superscript II RNaseH⁻ Reverse Transcriptase (Gibco), double-stranded cDNA was amplified by PCR with the reverse primer N-1-38L (5'-¹⁸⁵⁹CACCAATCACAGCAGAGAA¹⁸⁴⁰-3') and the forward degenerate primer IP-1276dU (5'-¹¹⁸⁹T(A/T)(C/T)CCI(A/G)A(A/G)GTIAA-(C/T)(C/T)TIGA(C/T)AA(C/T)TA¹²¹⁴-3') that was based on the highly conserved amino acid sequence ³³⁶(F/Y)P(E/K)VNLD-NY³⁴⁴ of the N-terminal proteins of two isolates of BBWV-1 (PV132 and PV176) and seven isolates of BBWV-2 (E, IP, L, MB7, PatMMV, PV131, and I-2). Amplified cDNA products were cloned into the pGEM-T Easy Vector, and three clones were sequenced. The 5'-terminal region of RNA-2 was amplified by the 5'RACE method with universal primers (4) and reverse primer N-1-200L (5'-¹⁴³³TAGTCTTCAGCATCTTCATC¹⁴¹⁴-3'). Amplified cDNA products were cloned into the pGEM-T vector (Promega), and five clones were sequenced.

Sequence analysis. Sequence data were compiled and analyzed with GENETYX-MAC programs (Software Development Co., Ltd., Tokyo). The sequence of isolate N-1 was compared with those of all isolates of BBWV-1 and BBWV-2 and a comovirus, *Cowpea mosaic virus* (CPMV), which are available in the DDBJ/EMBL/GenBank databases.

Phylogenetic analysis was performed to determine the relationship of isolate N-1 with fabaviruses, comoviruses, and nepoviruses, which are members of three genera in the family *Comoviridae* (26). A phylogenetic tree was generated from the deduced amino acid sequences of RNA-dependent RNA polymerases (RdRp) by the neighbor-joining algorithm with ClustalX programs (23). The data set was subjected to 100 bootstrap replicates. *Satsuma dwarf virus*, which was tentatively designated as a nepovirus and more recently placed in a putative new family because of its unique genome features (7), was used as the outgroup.

ELISA. Antiserum against isolate N-1 was raised in a rabbit immunized with virions (approximately 0.5 mg) by intravenous injection followed by intramuscular injection 1 week later with

TABLE 1. Experimental host range of Gentian mosaic virus and symptoms of infected plants

Family	Species	Symptoms ^a
Aizoaceae	<i>Tetragonia tetragonoides</i>	(CS), NS or RS/—
Amaranthaceae	<i>Gomphrena globosa</i>	(CS)/CS, M, Mal
Asteraceae	<i>Lactuca sativa</i>	—/—
Brassicaceae	<i>Brassica rapa</i> var. <i>glabra</i>	—/—
	<i>Raphanus sativus</i>	—/—
Chenopodiaceae	<i>Beta vulgaris</i> var. <i>vulgaris</i>	(CS), NS/—
	<i>Chenopodium amaranticolor</i>	CS/—
	<i>Chenopodium quinoa</i>	CS/CS, (Mal)
	<i>Spinacia oleracea</i>	(CS), NS, (N)/ (M), N, Mal
Cucurbitaceae	<i>Cucumis sativus</i>	—/—
	<i>Cucurbita moschata</i>	—/—
Fabaceae	<i>Vicia faba</i>	(NS, N)/N
	<i>Vigna unguiculata</i> var. <i>unguiculata</i>	—/—
Gentianaceae	<i>Gentiana scabra</i> var. <i>buergeri</i>	+/M
Solanaceae	<i>Capsicum annuum</i>	—/—
	<i>Datura stramonium</i>	—/—
	<i>Nicotiana benthamiana</i>	—/M
	<i>Nicotiana glutinosa</i>	—/—
	<i>Nicotiana tabacum</i> cv. Bright Yellow	—/—
	<i>Nicotiana tabacum</i> cv. White Burley	—/—
	<i>Petunia × hybrida</i>	—/—
	<i>Physalis floridana</i>	—/—

^a Symptoms on inoculated leaves and on upper uninoculated leaves are denoted to the left and right of the slash (/), respectively. CS, chlorotic spots; NS, necrotic spots; RS, ring spots; —, no infection; M, mosaic; Mal, malformation; N, necrosis; and +, symptomless latent infection. Parentheses indicate occasional appearance.

virions (approximately 0.5 mg) that had been emulsified with an equal volume of Freund's complete adjuvant. One month after the second injection, the rabbit was given two intravenous injections (approximately 0.3 mg of virions per injection) at intervals of 1 week. The rabbit was bled 1 week after the fourth injection. Preparation of antiserum against PV132 was described previously (8), and antiserum against PV131 was prepared by the same procedure as that used to prepare the antiserum against PV132. Antiserum against LMMV was provided by R. G. Milne and M. Turina.

Antisera incubated with extracts of noninoculated leaves of *C. quinoa* were used for the non-precoated indirect-ELISA that was performed as described by Takeuchi et al. (21) with some modifications. Each leaf sample was macerated in phosphate-buffered saline (pH 7.5) and crude sap was subjected to a series of 10-fold dilutions up to 10^{-4} . Diluted samples were placed in duplicate wells.

RESULTS

Host range. Isolate N-1 successfully infected a variety of plant species in six families: Aizoaceae, Amaranthaceae, Chenopodiaceae, Fabaceae, Gentianaceae, and Solanaceae (Table 1). Thus, the N-1 host range resembled those of fabaviruses (14,16,22). However, N-1 only induced localized symptoms on *C. amaranticolor*, which exhibits systemic symptoms upon infection with other fabaviruses. Furthermore, the N-1 isolate produced symptoms only on *Nicotiana benthamiana* among a variety of solanaceous plants that frequently react to fabavirus infection. Gentian plants infected with the N-1 isolate developed mosaic systemic symptoms.

Fig. 1. Electron micrographs of purified particles of Gentian mosaic virus after staining with 2% uranyl acetate. **A**, T component (empty particles); **B**, M component (intact particles); and **C**, B component (intact particles). Bar = 30 nm.

Fig. 2. Electrophoresis on a 0.8% (wt/vol) denaturing agarose gel of viral RNAs extracted from the T (lane 2), M (lane 3), and B (lane 4) components of Gentian mosaic virus. Lane 1, Single-stranded RNA markers (Gibco, Rockville, MD).

Physicochemical properties of purified viral particles. Purified particles of isolate N-1 were separated as three components, corresponding to T, M, and B (26), and collected separately from the three bands that formed during DGC. Electron microscopy revealed that each of the three components contained isometric particles of approximately 30 nm in diameter. The T component consisted of empty particles, while the M and B components included mostly intact particles (Fig. 1). Electrophoresis on an agarose gel under denaturing conditions revealed that the T component contained no RNA, while the M and B components contained an RNA of 3.57 kb (RNA-2) and an RNA of 5.99 kb (RNA-1), respectively (Fig. 2). Each of the three components contained two CPs, corresponding to LCP and SCP (26), with Mr values of 39.3×10^3 and 26.6×10^3 , respectively, as determined by SDS-PAGE (Fig. 3).

Characterization of the genome. The complete nucleotide sequence of RNA-1 from isolate N-1 consisted of 5,836 nucleotides (accession no. AB084452). The RNA appeared to contain a single, long open reading frame (ORF) with an initiation codon at positions 175 through 177 and a termination codon (UGA) at positions 5734 through 5736. The 5' nontranslated region (NTR) of the molecule contained the repeated motif AAACAGCUUUC (18) from positions 23 to 33, 41 to 51, 85 to 95, and 125 to 135,

Fig. 3. Fractionation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 12.5% (wt/vol) gel of coat proteins from the T (lane 2), M (lane 3), and B (lane 4) components of Gentian mosaic virus, after staining with Coomassie brilliant blue. Lane 1, molecular mass markers (Amersham, Little Chalfont, UK). LCP, large coat protein; and SCP, small coat protein.

Fig. 4. Genome organization of Gentian mosaic virus. Short horizontal lines and large rectangles represent noncoding sequences and long open reading frames, respectively. Vertical lines through the long rectangles indicate putative sites of polyprotein cleavage. Calculated values of Mr and positions of mature proteins are indicated. Co-pro, cofactor required for the proteinase; Hel, putative helicase; VPg, genome-linked protein; Pro, proteinase; RdRp, RNA-dependent RNA polymerase; MP, movement protein; LCP, large coat protein; and SCP, small coat protein. Conserved motifs in each protein are indicated by shading.

and the 3'NTR was followed by a poly(A) tail, as do RNA-1 and RNA-2 of BBWV-1 and BBWV-2 (8).

The complete nucleotide sequence of RNA-2 from isolate N-1 consisted of 3,372 nucleotides (accession no. AB084453) and contained a single long ORF, which initiated at positions 184 through 186 and terminated with a UGA codon at positions 3244 through 3246. The 5'NTR contained the same repeated motif as that found in RNA-1 at positions 22 through 32, 61 through 71, and 91 through 101, and the 3'NTR was followed by a poly(A) tail.

The 5'NTRs of RNA-1 and RNA-2 of N-1 were only 60% identical to one another. In contrast, high identity was apparent when we compared only the 35 nucleotides at each 5' end, in which 33 nucleotides were identical. This conserved sequence resembled that conserved among RNAs of BBWV-1 and BBWV-2 (8). Moreover, the first 23 nucleotides of RNA-1 and the first 21 nucleotides of RNA-2 may form a stem/loop structure similar to hairpin I, which was reported to be important for replication of the comovirus, CPMV (3). The 5' nucleotides of the RNAs of BBWV-1 and BBWV-2 also may form a similar structure (8).

The 3'NTRs of RNA-1 and RNA-2 of N-1 were 67% identical, with relatively conserved sequence in the last 83 nucleotides, in which 68 nucleotides were identical. The consensus sequence TAGT or TATGT, which plays a role in the termination of tran-

scription of some genes and in the polyadenylation of their transcripts in yeast (27), was found 97 to 93 and 80 to 77 nucleotides upstream of the poly(A) tail in RNA-1 and 80 to 77 and 14 to 10 nucleotides upstream of the poly(A) tail in RNA-2, as also is the case for BBWV-1 and BBWV-2 (8). In contrast, the polyadenylation signal for most eukaryotic mRNAs, AAUAAA (17), was found 66 to 61 nucleotides upstream of the poly(A) tail in both RNA-1 and RNA-2 of N-1. The signal sequence was found in the 3'NTR of BBWV-1 and BBWV-2 RNA-2, with an adjacent conserved sequence of 45 nucleotides. However, both molecules of N-1 have the signal sequence without the adjacent conserved sequence.

The RNA-1 of N-1 encoded a polypeptide of 1,853 amino acids with a calculated Mr of 206,708. Putative sites of proteolytic cleavage were identified at the Q/F, Q/S, Q/A, and Q/S dipeptides at positions 323/324, 914/915, 940/941, and 1149/1150, respectively, in the polypeptide (Fig. 4), on the basis of a comparison with the corresponding sites in the polypeptide of CPMV. These sites in the polypeptide of N-1 had a glycine residue at the -2 position, resembling those in polypeptides encoded by RNA-1 and RNA-2 of isolates of BBWV-1 and BBWV-2, with the exception of the second site (914/915), in which alanine was found before the dipeptide, as occurs in the RNA-1-encoded protein of isolate

TABLE 2. Nucleotide and amino acid sequence identity of RNA-1 between Gentian mosaic virus and select viruses of the family *Comoviridae*

Genus	Species	Isolate	Accession no.	Sequence identity (%)							
				Nucleotide ^a		Amino acid ^b					
				5'NTR	3'NTR	Poly	Co-pro	Hel	VPg	Pro	RdRp
<i>Fabavirus</i>	<i>Broad bean wilt virus 1</i>	PV132	AB084450	56	50	59	42	64	65	53	64
		B935	AF149425	59	55	59	39	64	54	53	66
	<i>Broad bean wilt virus 2</i>	IA	AB051386	57	57	59	39	63	54	54	66
		IP	AB023484	58	55	59	39	63	46	56	65
		Korea	AF144234	— ^c	55	—	—	64	54	54	66
		MB7	AB013615	57	54	58	38	63	59	55	66
		ME	AF225953	58	56	58	39	63	46	55	65
		PatMMV	AB050782	57	54	59	38	63	54	55	66
<i>Comovirus</i>	<i>Cowpea mosaic virus</i>		X00206	50	47	33	20	31	29	31	42

^a NTR, nontranslated region.

^b Poly, polypeptide precursor; Co-pro, cofactor required for the proteinase; Hel, putative helicase; VPg, genome-linked protein; Pro, proteinase; and RdRp, RNA-dependent RNA polymerase.

^c Sequence data are not available.

TABLE 3. Nucleotide and amino acid sequence identity of RNA-2 between Gentian mosaic virus and select viruses of the family *Comoviridae*

Genus	Species	Isolate	Accession no.	Sequence identity (%)					
				Nucleotide ^a		Amino acid ^b			
				5'NTR	3'NTR	Poly	MP	LCP	SCP
<i>Fabavirus</i>	<i>Broad bean wilt virus 1</i>	Delphinium	AB110537	— ^c	—	—	—	63	62
		PV132	AB084451	53	48	58	51	64	62
		PV176	AB018703	—	45	—	—	64	59
		Singapore	AF225955	53	45	58	50	64	59
	<i>Broad bean wilt virus 2</i>	B935	AJ132844	56	48	55	48	61	58
		E	AB018699	—	48	—	—	62	59
		IA	AB032403	58	46	55	48	62	59
		IP	AB018698	62	47	55	49	62	57
		Korea K	AF104335	—	46	—	—	61	58
		L	AB018700	—	46	—	—	61	59
		MB7	AB013616	60	49	55	49	61	59
		ME	AF225954	60	42	55	48	61	58
		PatMMV	AB011007	58	46	54	49	56	60
		PV131	U65985	—	—	—	—	61	—
		P158	AF228423	56	49	55	47	62	59
		South Korea	AF316363	—	—	—	—	—	60
		1-2	AB018701	—	45	—	47	61	58
			X00729	50	47	20	21	20	16
<i>Comovirus</i>	<i>Cowpea mosaic virus</i>								

^a NTR, nontranslated region.

^b Poly, polypeptide precursor; MP, movement protein; LCP, large coat protein; and SCP, small coat protein.

^c Sequence data are not available.

PV132 of BBWV-1 (8). The putative motifs specific for proteinase cofactor (19), helicase (10), genome-linked protein (18), proteinase (10), and RdRp (10) all were present in the polyprotein encoded by RNA-1 at positions 183 to 256, 483 to 591, 915 to 940, 974 to 1127, and 1428 to 1540 (Fig. 4), supporting the prediction that RNA-1 of N-1 encodes functional proteins in a similar manner to the corresponding RNAs of BBWV-1, BBWV-2 (8), and a comovirus, CPMV (26).

The RNA-2 of N-1 encoded a polyprotein of 1,020 amino acids with a calculated Mr of 114,650. Comparison with a polyprotein encoded by RNA-2 from isolate IP of BBWV-2, which contains two CPs in the C-terminal region (8), suggested putative sites of cleavage at the Q/A dipeptides at positions 421/422 and 823/824 in the polyprotein of N-1 (Fig. 4). These sites have a glycine residue at the -2 position. The putative motif specific for movement protein (1) was found in the N-terminal region at positions 197 through 329, as is the case for BBWV-1, BBWV-2 (8), and CPMV (26).

Amino acid sequence identity between N-1 and BBWV-1 ranged from 58 to 59% for the polyprotein precursor and from 42 to 65% for individual mature proteins (Tables 2 and 3). The corresponding values for N-1 and BBWV-2 were from 54 to 59% (polyprotein) and from 38 to 66% (mature proteins).

In a phylogenetic analysis based on amino acid sequences of the RdRp, isolate N-1 grouped with members of the genus *Fabavirus* (Fig. 5). In the fabavirus group, isolate N-1 diverged from a node basal to that shared by BBWV-1 and BBWV-2.

Serology. Isolate N-1 was detected only weakly, or not at all, by indirect-ELISAs performed with antisera raised against PV132 (BBWV-1), PV131 (BBWV-2), and LMMV (Table 4). As with the other fabavirus antisera, the antiserum against N-1 reacted

strongly with the homologous antigen and weakly or not at all with heterologous antigens from different species in *Fabavirus*.

DISCUSSION

Physiochemical properties of N-1 virions were similar to those of both fabaviruses and comoviruses. However, biological properties of N-1 were more similar to fabaviruses than to comoviruses. Comoviruses tend to have narrow host ranges (25). In contrast, N-1 was easily transmitted via infected sap to plants in six families, with a host range similar (but not identical) to those of fabaviruses.

The genome organization of isolate N-1 was similar to that of BBWV-1, BBWV-2 (8), and a comovirus, CPMV (26). The low amino acid sequence identity of proteins encoded by the RNAs of N-1 to those of CPMV (16 to 42%; Tables 2 and 3) suggests that N-1 is not a comovirus. In addition to BBWV-1 and BBWV-2, LMMV and *Patchouli mild mosaic virus* (PatMMV) have been

TABLE 4. Serological relationships among Gentian mosaic virus and three species in the genus *Fabavirus*, as determined by indirect enzyme-linked immunosorbent assays (ELISA)

Antisera against		Results of ELISA ^a			
Species	Isolate	N-1	PV132	PV131	LMMV
Gentian mosaic virus	N-1	+++	+	–	–
<i>Broad bean wilt virus 1</i>	PV132	+	+++	–	–
<i>Broad bean wilt virus 2</i>	PV131	–	+	+++	–
<i>Lamium mild mosaic virus</i>		–	–	–	+++

^a Values for 100-fold diluted sample of leaf sap are shown. Absorbance at 415 nm: +++, more than 2.0; +, 0.3 to 0.6; and –, less than 0.3.

Fig. 5. Phylogenetic relationships among Gentian mosaic virus (GeMV) and members of the genus *Fabavirus*, *Comovirus*, and *Nepovirus* in the family *Comoviridae*. The phylogenetic tree was generated from the deduced amino acid sequences of RNA-dependent RNA polymerases by the neighbor-joining algorithm after bootstrapping for 100 replicates. The branch lengths are proportional to genetic distances. Bootstrap values greater than 70% are indicated at the branch points. The viruses included in this analysis are as follows, with accession numbers of deduced amino acid sequences in parentheses: isolate PV132 (BAD00183) of *Broad bean wilt virus 1* (BBWV-1); isolates Korea (AAD38152), MB7 (BAA34928), IA (BAB18312), PatMMV (BAB83045), B935 (AAD39217), IP (BAB40439), and ME (AAK27841) of *Broad bean wilt virus 2* (BBWV-2); SqMV, *Squash mosaic virus* (BAB62139); CPSMV, *Cowpea severe mosaic virus* (number for nucleotide sequence: M83830); BPMV, *Bean pod mottle virus* (AAD09629); CPMV, *Cowpea mosaic virus* (CAA25029); RCMV, *Red clover mottle virus* (CAA46104); ToRSV, *Tomato ringspot virus* (AAA78254); BRV, *Blackcurrant reversion virus* (AAL36026); CNSV, *Cycas necrotic stunt virus* (BAB89369); GCMV, *Grapevine chrome mosaic virus* (CAA33405); TBRV, *Tomato black ring virus* (AAN72830); and BRSV, *Beet ringspot virus* (BAA00234). *Satsuma dwarf virus* (SDV; BAA76746) was used as the outgroup.

classified as species of *Fabavirus* (26). However, recent sequence analysis of PatMMV suggested that it might be synonymous to BBWV-2 (5,6). No sequence data are available for LMMV. The proteins of N-1 exhibited limited identity to those of BBWV-1 (42 to 65%) and to those of BBWV-2 (38 to 66%). The extent of such identity, which was similar to that between proteins of BBWV-1 and BBWV-2 (8), and the results of phylogenetic analysis of the RdRp clearly indicate that isolate N-1 should be a viral species distinct from BBWV-1 and BBWV-2.

One criterion of viral taxonomy is serological relationships. The serological reactivities among N-1, BBWV-1, BBWV-2, and LMMV, as measured by the absorbance in ELISA, clearly showed that only homologous combinations of antigen and the antiserum showed strong reactions. The reactions were very weak or negligible among heterologous combinations. These results suggest that N-1 and LMMV are distinct viruses, and also demonstrate that N-1 should be classified as a new species in the genus *Fabavirus*. We propose the name Gentian mosaic virus (GeMV) for this new species.

In our recent studies of fabaviruses in Japan, we revealed that BBWV-2 was prevalent in Japan. GeMV has been detected only in gentian plants in limited areas in the field. However, experimental data indicate that N-1 can infect several commercial crops, such as spinach (*Spinacia oleracea*), that has been reported to suffer serious damage upon infection by fabaviruses (13). N-1 also was able to infect broad bean (*Vicia faba*), a common host of fabaviruses in China and Japan. Thus, it is possible that GeMV also may occur in the hosts. The antiserum against GeMV prepared in this study will help us to identify the new virus more rapidly in the field.

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