Purification, Host Range, Serology, and Partial Sequencing of Dendrobium Mosaic Potyvirus, A New Member of the Bean Common Mosaic Virus Subgroup

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ABSTRACT

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Dendrobium mosaic potyvirus (DeMV) was successfully transmitted from honohono orchids (*Dendrobium superbum* Reichb. f.) to *Nicotiana benthamiana*. The coat protein of the virus purified from *N. benthamiana* had a molecular weight of 34×10^3 in sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis, and the viral RNA was approximately 9.5 kb in size in denatured agarose gel electrophoresis. With a polyclonal antiserum produced against purified DeMV particles, DeMV was found to be widespread in honohono orchids in Hawaii. Based on serological reactions in indirect enzyme-linked immunosorbent assay with monoclonal antibodies, DeMV is a potyvirus of the bean common mosaic virus (BCMV) subgroup. DeMV-specific cDNA clones were obtained and sequenced. Sequence comparison using the coat protein amino acid sequence and the 3′ untranslated region sequence showed that DeMV shares 88 to 91% and 85 to 95% identity, respectively, with those sequences of BCMV subgroup members. Data from host range studies suggest that DeMV behaves similarly to other potyviruses in the BCMV subgroup such as azuki bean mosaic virus (AzMV) and blackeye cowpea mosaic virus (BICMV). Based on available information, DeMV is considered a member of the BCMV subgroup.

Dendrobium mosaic potyvirus (DeMV) was first described in Hawaii (22) and was further studied in Japan (13,14). The virus induces chlorosis, mosaic, and distortion of leaves, and color-breaking and distortion of flowers in *Dendrobium superbum* Reichb. f. (known in Hawaii as the honohono orchid) (22). Flexuous, rod-shaped particles of DeMV are 750 nm in length (13). The virus is transmitted mechanically and by *Myzus persicae* in a nonpersistent manner (14). Pinwheel inclusions are produced in DeMV-infected orchid tissues (14). This virus has also been reported to infect other *Dendrobium* spp. and *Chenopodium amaranticolor* and *C. quinoa* (13). DeMV is considered to be a member of the genus *Potyvirus* (6).

Because DeMV was not previously purified, it has not been fully characterized biochemically, serologically, or at the molecular level. In this report, we describe the mechanical transmission of DeMV to *Nicotiana benthamiana*, its purification, and its molecular characterization. The coat protein (CP) gene and 3′ untranslated region sequences of DeMV were compared with those of other potyviruses. The results suggest that DeMV is a member of the bean common mosaic virus (BCMV) subgroup.

MATERIALS AND METHODS

Transmission and host range. One gram of DeMV-infected *Dendrobium superbum* orchid leaf tissue that was found to be free of Cymbidium mosaic virus (CyMV) and Odontoglossum ringspot virus (ORSV) was ground in 10 ml of 0.01 M potassium phosphate buffer, pH 7.0, containing 0.01 M sodium sulfite. Test plants were mechanically inoculated with this inoculum and kept in a greenhouse at 25 to 30°C; *Cassia occidentalis*, *Chenopodium amaranticolor*, *C. quinoa*, *Cucumis sativa* (cv. Straight Eight), *Cucurbita maxima* Duchesne, *Datura stramonium, Emilia sonchifolia*, *Nicotiana benthamiana, N. glutinosa, N. occidentalis, N. tabacum, Physalis peruviona*, *Petunia × hybrida*, *Vigna unguiculata* (Ca blackeye #5) plants, and a series of BCMV differential *Phaseolus vulgaris* cultivars (5), were used.

Purified DeMV (3 mg per ml) was diluted in 0.01 M potassium phosphate buffer (pH 7.2) containing 0.01 M sodium sulfite and mechanically inoculated onto DeMV-free *D. superbum* orchid plants. All inoculated plants were kept in the greenhouse and monitored for symptom expression.

Purification. Symptomatic leaf tissue of DeMV-infected *N. benthamiana* was used to purify DeMV according to the method of Wang (26). Infected leaves (300 g) were triturated in a blender with 600 ml of 0.25 M potassium phosphate buffer, pH 7.5, containing 0.01 M EDTA and 0.1% sodium sulfite. Then 150 ml each of chloroform and carbon tetrachloride was added slowly with stirring to form an emulsion. The emulsion was then centrifuged at $7,000 \times g$ for 15 min at 4°C. Polyethylene glycol (PEG, molecular weight 8,000) was added to the supernatant to a final concentration of 8 g per 100 ml, stirred for 1 h at 4°C, and centrifuged at $8,600 \times g$ for 20 min at 4°C. Pellets were resuspended in 250 ml of 0.1 M potassium phosphate buffer, pH 7.5, containing 0.01 M EDTA by stirring at 4°C overnight. Then samples were clarified by centrifugation at $5,000 \times g$ for 10 min at 4°C. Precipi-

tation was repeated (5% PEG + 0.3 M NaCl) and resulting pellets were resuspended in 0.05 M potassium phosphate buffer, pH 7.5. The suspension was stirred for 2 h at 4°C and centrifuged at 1,200 × *g* for 15 min at 4°C. This suspension was mixed with CsCl to a final concentration of 0.385 mg per ml, and subjected to isopycnic centrifugation at 275,000 × *g* for 6 h at 6°C in a Beckman VTi 65 rotor (Beckman Co., Wakefield, MA). The virus-containing zone was collected, diluted fivefold with 0.05 M potassium phosphate buffer (pH 7.5), and centrifuged at 10,000 × *g* for 10 min. The resulting supernatant was centrifuged at 220,000 × *g* for 30 min at 4°C in a Beckman 70 Ti rotor (Beckman Co., Wakefield, MA). The virus pellet was resuspended in 500 µl Tris-EDTA (TE) buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0) by stirring overnight at 4°C.

Antiserum production. Purified DeMV preparations were injected weekly into New Zealand white rabbits at multiple sites on the back for 3 consecutive weeks as described (12). The first immunization consisted of 1 ml of purified virus (1 mg) mixed with 1 ml of Freund's complete adjuvant. The two subsequent injections consisted of purified virus (0.5 mg) mixed with equal volume of Freund's incomplete adjuvant. The rabbits were bled 1 week after the third injection and every week thereafter. Immunoglobulin G (IgG) was purified by protein A column chromatography (8). The purified IgG was conjugated to alkaline phosphatase type VII (Sigma, St. Louis, MO) for use in enzyme-linked immunosorbent assay (ELISA) (3).

ELISA. The standard double antibody sandwich (DAS) ELISA was used for detection of DeMV and CyMV (10). Absorbance at 405 nm was measured with a Model 450 Microplate Reader (Bio-Rad Laboratories, Richmond, Calif.) about 60 min after the addition of substrate. Negative controls of sample extraction buffer and extracts of healthy orchid, and positive controls of virus-infected orchids, were included in all ELISA tests. DeMV-infected honohono orchid samples were also tested by indirect ELISA (19) against a panel of monoclonal antibodies, some of which recognize diverse potyviruses (15) and some of which recognize members of the BCMV subgroup (19).

Coat protein and RNA analyses. The molecular weight of the viral CP was estimated by electrophoresis in sodium dodecyl sulfate (SDS)–polyacrylamide gels consisting of a 5% stacking gel and 12% resolving gel (17). Coat protein of purified DeMV was dissociated from its RNA by boiling the virions for 2 min in 0.125 M Tris-HCl, pH 6.8, containing 1% SDS, 5% mercaptoethanol, 1 µg/ml bromphenol blue, and 10% glycerol. Proteins were stained with Coomassie Brilliant Blue R-250 after electrophoresis for 50 min at 180 V in a Bio-Rad mini-gel apparatus (Bio-Rad). Protein molecular weight was estimated by comparing its relative mobility to that of molecular weight standards (Sigma).

DeMV RNA was extracted from purified virions with the SDS-phenol extraction method as described by Palukaitis (23), denatured with glyoxal, and analyzed by electrophoresis in a 1% agarose gel, for 90 min at 60 V. The 0.24 to 9.5 kb RNA Ladder (GibcoBRL Life Technologies, Grand Island, N.Y.) and viral RNAs of cucumber mosaic virus (CMV) were used as size standards. Approximately 1 to 2 µg of RNA was applied to each lane of the gels. RNA was visualized by staining with ethidium bromide (1 µg per ml), and mobility of DeMV RNA was compared with those of standards.

Cloning and sequence analysis. First-strand complementary DNA (cDNA) of DeMV-RNA was synthesized by reverse transcription using oligo dT as a primer and AMV reverse transcriptase. Second strand cDNA was then produced by the RNase H/DNA polymerase method (9). The double-stranded cDNA was fractionated by agarose gel electrophoresis, and *Eco*RI-compatible linkers were attached to both termini (21). After digestion with *Eco*RI, the cDNA was cloned into the *Eco*RI site of pBluescript, which had been dephosphorylated (21). Plasmid preparations were made by the alkaline lysis method (21). Plasmids and restriction digests were analyzed by electrophoresis in 1% agarose. Virus-specific clones were sequenced by the dideoxynucleotide chain-termination method (24). Sequence data

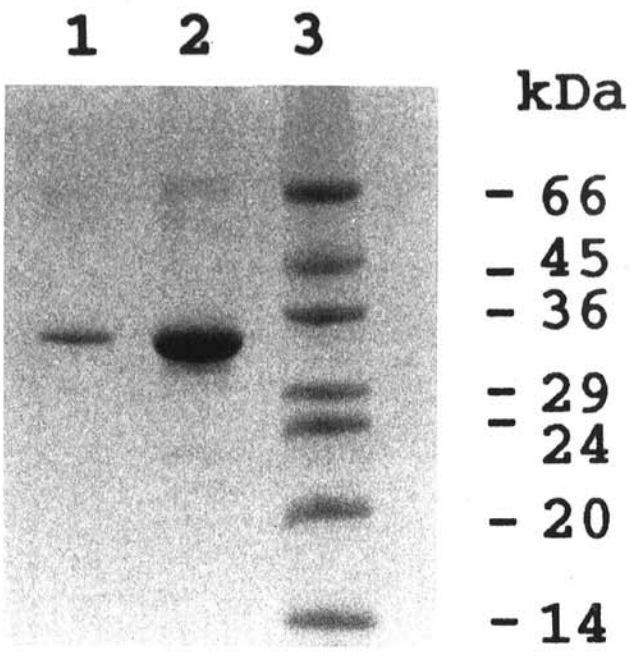

Fig. 1. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis of Dendrobium mosaic potyvirus (DeMV) coat protein. Lane 1, DeMV (0.5 µg); lane 2, DeMV (3 µg); lane 3, protein molecular weight markers: bovine serum albumin (66,000), egg albumin (45,000), glyceraldehyde-3-phosphate dehydrogenase (36,000), carbonic anhydrase (29,000), trypsinogen (24,000), soybean trypsin inhibitor (20,100), and lactalbumin (14,200).

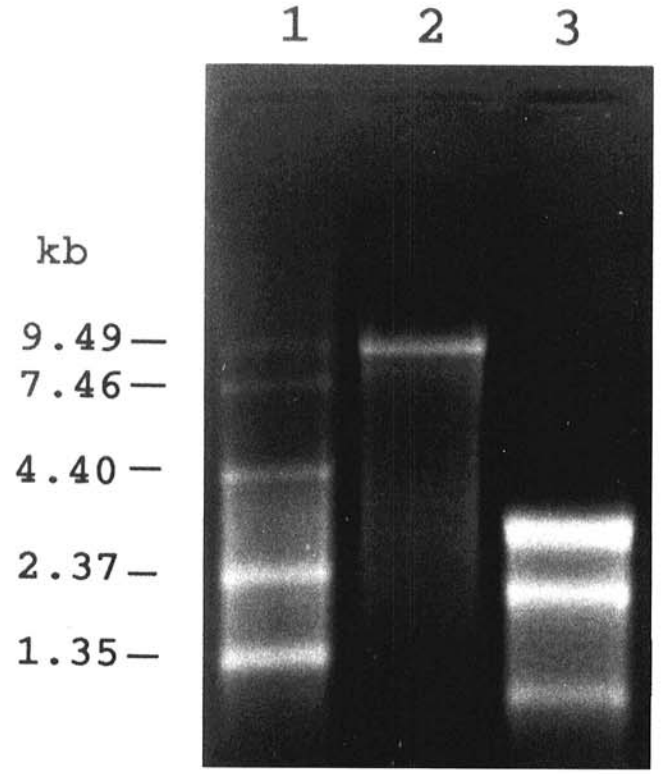

Fig. 2. A 1% agarose gel of virus RNA following denaturation of the samples with glyoxal and DMSO, electrophoresis, and staining with ethidium bromide. Lane 1, RNA size standards; lane 2, Dendrobium mosaic virus (0.5 µg); lane 3, cucumber mosaic virus (2 µg).

were compiled and analyzed using PC/Gene (Release 6.8, Intelligenetics, Inc) and the University of Wisconsin Genetics Computer Group (GCG) computer program, available from GCG, Inc., Madison, Wis. DeMV sequences were compared with those of 40 other potyviruses, whose sequences were available in the literature and/or from GenBank.

RESULTS

Transmission and host range. Both *C. amaranticolor* and *C. quinoa* developed necrotic local lesions 7 days after inoculation with DeMV. Three weeks after inoculation, systemic interveinal chlorosis was evident on new leaves of *N. benthamiana.* When DeMV was transmitted from infected *N. benthamiana* to healthy *N. benthamiana* identical symptoms were produced within 8 days. DeMV systemically infected cowpea (Ca blackeye #5) causing mild mottling symptoms. On Dubble Witte bean, a cultivar universally susceptible to BCMV subgroup members, it caused systemic mosaic, severe leaf distortion, stunting, mottling, and vein clearing. On Bountiful bean, another bean cultivar generally susceptible to BCMV subgroup members, the virus produced necrotic lesions on primary leaves but did not infect systemically. When transferred from infected cowpea to BCMV differentials cultivars (19), DeMV infected Dubble Witte (host group 1) but did not infect cultivars in host groups 2 through 8 (5). This type of behavior (infection of one or more of the generally susceptible cultivars but no infection of cultivars possessing any of the BCMV resistance genes) is similar to that of BCMV strains and other potyviruses in the BCMV subgroup (19).

Purification. On average, 5 mg of purified DeMV virions was obtained from 300 g of *N. benthamiana* leaf tissue. Of the six DeMV-free *D. superbum* orchids mechanically inoculated with purified DeMV virions (3 mg per ml), three developed typical symptoms after 1 month and were ELISA-positive for DeMV.

Coat protein and RNA gel analyses. Molecular weight of the virus CP was approximately 34×10^3 in SDS–polyacrylamide gel electrophoresis (PAGE) analysis (Fig. 1). The virus RNA was approximately 9.5 kb in size, as estimated by denaturing agarose gel electrophoresis (Fig. 2). Both experiments were repeated five times.

TABLE 1. Detection of viruses from *Dendrobium superbum* orchids in enzyme-linked immunosorbent assays (ELISA)[a]

Islands	Location	Symptoms	No. of samples infected/ No. of samples tested	
			DeMV	CyMV
Oahu	Aiea	Yes	20/20	20/20
	Honolulu	Yes	46/46	42/46
		No	0/12	11/12
	Kaneohe	Yes	20/20	20/20
Hawaii	Hilo	Yes	20/20	20/20

[a] Cymbidium mosaic potyvirus (CyMV) and Dendrobium mosaic potyvirus (DeMV) were detected with double antibody sandwich ELISA using specific polyclonal antibodies.

TABLE 2. Reaction of Dendrobium mosaic potyvirus (DeMV) with different monoclonal antibodies to potyviruses[a]

Antibody	Specificity	Absorbance at 405 nm	
		DeMV	Healthy orchid
MAb-PTY 1	Potyvirus group	1.010	0.000
BCMVII 197	Potyvirus group	0.775	0.174
BCMVII 463	BCMV subgroup	0.222	0.000
BCMVI-2	BCMVserotype A	0.000	0.000
BIMVB5E5	BCMV serotype B	0.419	0.050
BYMV-B1	BYMV	0.000	0.000
Buffer	None	0.000	0.000

[a] Indirect enzyme-linked immunosorbent assays were conducted as described previously (19). The absorbance readings are means of four samples 2 h after the substrate was added.

ELISA. High titer, specific polyclonal antiserum to DeMV was produced. *Dendrobium superbum* orchid samples collected from one location on the island of Hawaii and three locations on Oahu were tested for DeMV with DAS-ELISA using anti-DeMV polyclonal antibody. All samples were also tested with DAS-ELISA for CyMV. Of the 118 samples tested, 113 were positive for CyMV, and all 106 symptomatic samples were positive for DeMV. Four samples from symptomatic plants were DeMV-positive but CyMV-negative. None of the asymptomatic plants were DeMV-positive, but 11 of 12 were CyMV-positive (Table 1). Anti-DeMV polyclonal antibody did not react with dasheen mosaic virus, papaya ringspot virus-P, papaya ringspot virus-W, potato virus Y (PVY), watermelon mosaic virus 2, and zucchini yellow mosaic virus in DAS-ELISA. When DeMV was tested using a panel of monoclonal antibodies (15,19), it was detected by two potyvirus-genus-specific monoclonal antibodies (MAb-PTY 1 and II 197), one BCMV-specific monoclonal antibody (II 463), and a monoclonal antibody specific to BCMV serotype B (B5E5). The virus was not detected by a monoclonal antibody specific to BCMV serotype A (I-2) (Table 2).

Sequence analysis. One oligo dT-primed cDNA clone with an insert size of 2.1 kbp was selected for sequencing. The C terminal portion of the DeMV polyprotein and the complete 3′ untrans-

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V  G  C  G  E  S  V  H  L  Q /S  G  N  N  N  P  P  E  V  D          20
gtaggatgcggagagtctgtgcatttacaatcaggaaacaacaacccaccagaagtggat       60

A  G  M  E  A  G  K  D  E  K  K  K  S  S  K  G  K  G  P  E          40
gctggtatggaagcaggcaaggatgaaaagaagaaaagcagcaaaggaaaaggccctgaa      120

S  K  E  G  S  G  N  N  S  R  G  A  E  N  S  P  M  R  D  K          60
agcaaggaagggtcaggaaacaatagccgaggagcagaaaactcaccaatgagagacaag      180

D  V  N  A  G  S  K  G  K  V  V  P  R  L  Q  R  I  T  K  R          80
gatgtaaatgctggttccaaagggaaggttgttccccggcttcaaaggatcacaaagagg      240

M  N  L  P  M  V  K  G  N  V  I  L  N  L  D  H  L  L  D  Y         100
atgaatctgcccatggtgaaagggaatgtgatcttaaatttagatcatctgttggattac      300

K  P  E  Q  T  D  L  F  N  T  R  A  T  K  M  Q  F  E  M  W         120
aagccagaacaaactgatcttttcaacacaagagcaacaaagatgcagtttgagatgtgg      360

Y  N  A  V  K  G  E  Y  E  M  D  D  E  H  M  S  I  V  M  N         140
tacaatgctgtgaagggcgagtatgaaatggatgatgaacacatgtcaattgtgatgaat      420

G  F  M  V  W  C  I  D  N  G  T  S  P  D  V  N  G  T  W  V         160
ggctttatggtgtggtgcattgacaatggtacttcaccggatgtgaatggcacttgggtc      480

M  M  D  G  D  E  Q  V  E  Y  P  L  K  P  M  V  E  N  A  K         180
atgatggatggagatgagcaggtggaatacccactcaaaccaatggttgaaaatgcaaaa      540

P  T  L  R  Q  I  M  H  H  F  S  D  A  A  E  A  Y  I  E  M         200
ccaacactccgtcaaattatgcaccacttctcagatgcagctgaagcatacattgagatg      600

R  N  S  E  R  P  F  M  P  R  Y  G  L  L  R  N  L  R  D  K         220
agaaattctgagagaccgtttatgcctagatacggactacttcggaatttgagggacaaa      660

N  L  A  R  Y  A  F  D  F  Y  E  V  T  S  K  T  S  D  R  A         240
aatctagctcgctacgcttttgatttctatgaagtgacatccaaaacatcggatcgagca      720

R  E  A  V  A  Q  M  K  A  S  M  L  S  N  V  T  S  K  L  F         260
agagaagcagtagcacagatgaaggctagcatgcttagcaacgttactagcaagttgttt      780

G  L  D  G  N  V  A  T  T  S  E  N  T  E  R  H  T  A  R  D         280
ggacttgatggtaatgtggcaacaaccagcgagaatactgaaaggcacactgcaagggat      840

V  N  Q  N  M  H  S  L  L  G  M  G  S  P  Q  *                    295
gtcaaccagaacatgcactcacttcttggcatgggttccccgcagtaaagattgggtcaa      900

ccgatcacagttagcatctcgcgtcgctgaataattacatatagtaatcttttatgttct      960

ctttagtttcagtgtggttgcaccacctttgtgttactattgtgatagtgtggttagtcc     1020

accaacatattgtgagtactttatgtttatgagtaagccggaagaaccattgcaatagcg     1080

agggcatgcagaatggttttaccatgcgtcatgaagtagctacggcaatgtttgttgttc     1140

caa                                                              1143
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Fig. 3. The nucleotide sequence of the 3′-terminal regions of Dendrobium mosaic potyvirus and the deduced amino acid sequence. Q/S = the putative cleavage site for the coat protein and is underlined; * = the stop codon. The DAG sequence is underlined. GenBank (EMBL) Data Library accession no. U23564.

lated region are shown in Figure 3. The dipeptide QS located at amino acid position 10 has been selected as a putative cleavage site for the CP of DeMV because cleavage of potyviral polyprotein has been shown to take place mainly between the dipeptide sequences Q/A, Q/S, or Q/G. The conserved upstream residue motif (VHLQ) also helped to identify the putative protease cleavage site. Cleavage at the selected dipeptide generates a predicted CP 34.2 kDa in size, which correlates well with the size of CP determined by SDS-PAGE (34 kDa), as shown in this study. Alignment of the deduced DeMV CP sequence with the CP sequence of other potyviruses further supports this conclusion (data not shown).

Computer-aided comparisons of the CP and 3′ untranslated region sequences of DeMV with those of 40 other potyviruses suggested that DeMV belongs to the BCMV subgroup (data not shown). Table 3 compares the CP amino acid and 3′ untranslated region sequence identities between DeMV and each of 10 different potyviruses. DeMV shares 88 to 91% CP amino acid and 85 to 95% 3′ untranslated region sequence identities, respectively, with members of the BCMV subgroup. In particular, DeMV shares approximately 91% CP amino acid sequence identity with peanut stripe virus–370 (PStV-370) and BCMV-US1, and more than 93% 3′ untranslated region sequence identity with PStV-370, BICMV, and BCMV-US1. In contrast, it shares only 56 to 81% CP amino acid and 33 to 60% 3′ untranslated region sequence identities, respectively, with other potyviruses. Sequence comparison data also showed that two BCMV serotype A strains (NL3 and NL8) are different from DeMV and other BCMV subgroup members (Table 3).

DISCUSSION

Although DeMV has been studied for more than 40 years, it has only been partially characterized because of the lack of systemically susceptible herbaceous plants suitable for virus propagation and purification. Inouye (13,14) tested 45 plant species and found none to be systemic hosts of DeMV. The successful transmission of DeMV to *N. benthamiana* and purification of DeMV from *N. benthamiana* in this study has allowed the further characterization of this virus.

In a previous survey using the monoclonal antibody against a shared epitope of potyviruses (MAb-PTY 1) (15), potyviruses were detected from *D. superbum* orchids, but not from any other orchids tested in Hawaii (11). In this study, DeMV was found to be widespread in *D. superbum* orchids using anti-DeMV specific antibodies. Based on results from inoculation and association of the virus with the disease, DeMV was shown to be the causal agent of this disease. Because these orchids are propagated vegetatively, DeMV appears to be spread during propagation. It is important, therefore, to index all propagation stocks before large numbers of plants are produced. Because DeMV is mechanically transmissible, we recommend that cutting tools should be cleaned with 1% NaOH, which effectively inactivates CyMV and ORSV (11). Provided DeMV encodes a biologically active helper component, it is expected to be aphid-transmissible, because it does have the DAG motif in the N terminal portion of the CP typical for aphid-transmissible potyviruses (1).

Potyviruses are numerous and diverse (5). They infect many wild and cultivated plant species, often causing devastating diseases. Previously, it has been difficult to classify potyviruses, but now they can be differentiated more accurately based upon molecular properties (2,16,28). In a comparison of tobacco etch virus, tobacco vein mottling virus, PVY, and three strains of plum pox virus, Ward et al. (28) showed that strains of the same potyvirus exhibit high nucleotide sequence identity (≥90%), while distinctly different viruses have significantly lower sequence identity (25 to 65%) between equivalent gene products. Additional studies indicated that strains of a potyvirus share ≥83% nucleotide sequence identity whereas different potyviruses only have 39 to 53% sequence identity in the 3′ untranslated region (7). Wang et al. (27) compared the CP sequence of vanilla necrosis virus (VNV) with that of other potyviruses and found that the CP gene of VNV shared 97% amino acid sequence identity with that of WMV-2, leading to the conclusion that VNV is a strain of WMV-2. In this study, our results show that the levels of amino acid sequence identity in the CP gene and the nucleotide sequence identity in the 3′ untranslated region between DeMV and other BCMV subgroup members were 88 to 91% and 85 to 95%, respectively. It is clear that DeMV belongs to the BCMV subgroup, which contains AzMV, BCMV, BICMV, cowpea aphid-borne mosaic virus (CABMV), and PStV (19). It has been difficult to precisely identify these five potyviruses in the BCMV subgroup (19). Previous data suggests that AzMV, BICMV, and PStV share many taxonomically significant biological and serological properties with serotype B strains of BCMV (18,19,25). McKern et al. (18), using high-pressure liquid chromatography peptide profiles of CP digests, suggested that AzMV, BICMV, and PStV are all strains of the same potyvirus. Recently, based on CP and 3′ untranslated region sequences, it was proposed to consider AzMV and BICMV as strains of BCMV (4,16). Our sequence comparison data (Table 3) suggest that DeMV, BCMV-US1, PStV-370, and BICMV might be considered strains of BCMV serotype B. Further studies are needed to clarify the relatedness of BCMV subgroup members. Based on sequence comparisons in this study, DeMV is distinct from two BCMV serotype A strains (NL3 and NL8), which were proposed to be a different potyvirus: bean

TABLE 3. Coat protein amino acid and 3′ untranslated region sequence identities between Dendrobium mosaic potyvirus (DeMV) and other potyviruses[a]

Viruses	DeMV	PStV-B	PStV-370	BICMV	BCMV-NL1	BCMV-US1	BCMV-NL3	BCMV-NL8	WMV2	ZYMV	BYMV
DeMV		84.7	94.9	93.7	88.5	92.5	60.0	54.7	58.2	35.5	32.9
PStV-B	90.2		84.3	84.9	79.3	76.0	56.3	57.2	55.2	32.5	36.7
PStV-370	91.6	91.2		96.8	91.6	96.1	58.0	52.7	56.8	38.3	31.2
BICMV	89.1	88.4	89.6		92.0	95.6	58.4	53.9	58.8	37.3	34.1
BCMV-NL1	87.7	86.3	87.5	88.5		94.8	55.9	44.0	59.2	37.1	33.8
BCMV-US1	90.5	89.1	90.9	92.0	94.1		57.6	53.9	60.0	36.6	31.1
BCMV-NL3	81.2	81.2	82.3	82.7	83.1	78.9		95.8	62.6	38.7	32.4
BCMV-NL8	79.8	79.3	82.4	82.8	82.0	78.2	79.3		56.4	34.5	30.5
WMV2	71.5	72.2	73.7	74.4	72.6	71.2	73.3	77.3		30.9	29.2
ZYMV	67.7	67.0	70.6	73.5	71.7	69.9	72.3	75.0	74.7		34.1
BYMV	55.7	57.1	57.5	56.8	50.4	57.9	57.9	58.1	57.9	57.1	

[a] Protein amino acid and nucleotide sequences compared using PAUGN and NALIGN (PC/Gene). The sources of data sequence were from the following GenBank accession numbers: bean common mosaic virus (BCMV) strain NL1 (S66251 and S66275); BCMV-NL3 (S66274 and S66280); BCMV-NL8 (25); BCMV-US1 (L12740); bean yellow mosaic virus (BYMV; D00490); blackeye cowpea mosaic virus (BICMV) strain W (S66280 and S66253); peanut stripe virus (PStV) blotch strain (PStV-B, X63559); PStV-370 (Z21700); watermelon mosaic virus 2 (WMV2-US; D13913); zucchini yellow mosaic virus (ZYMV-F; D13914); and /or literature references. Comparisons above the diagonal refer to 3' untranslated region sequences; numbers below the diagonal are from coat protein amino acid sequence comparisons.

common mosaic necrosis virus BCNMV (16,20,25). Results from this study also show that DeMV is serologically related to the BCMV serotype B but not to serotype A strains. In addition, biological data from host range studies suggest that DeMV behaves similarly to other potyviruses in the BCMV subgroup such as AzMV and BICMV (19). The serological, biological, and molecular biological data obtained in this study, in conjunction with the results obtained previously by Inouye on virus morphology, particle length, inclusion bodies, and vector transmission (13,14), suggest that DeMV is indeed a potyvirus that belongs in the BCMV subgroup.

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