Complete Nucleotide Sequence of a Nonvector-Transmissible Strain of Abutilon Mosaic Geminivirus in Hawaii

Z. C. Wu, J. S. Hu, J. E. Polston, D. E. Ullman, and E. Hiebert

First and second authors: Department of Plant Pathology, University of Hawaii, Honolulu 96822; third author: University of Florida, GCREC, 5007 60th St. E., Bradenton 34203; fourth author: Department of Entomology, University of California, Davis 95616; and fifth author: Department of Plant Pathology, University of Florida, Gainesville 32611.

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ABSTRACT

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The complete nucleotide sequence of a geminivirus infecting lantern 'ilima (*Abutilon hybridum*) in Hawaii was determined. Sequence analysis showed that this geminivirus was a strain of Abutilon mosaic geminivirus (AbMV-HI). The genome of AbMV-HI consisted of two circular, single-stranded DNA molecules of 2,634 bases (DNA-A) and 2,571 bases (DNA-B). AbMV-HI shared 95 and 91% nucleotide sequence identity with the DNA-A and DNA-B components, respectively, of a West Indian strain of AbMV (AbMV-WI). AbMV-HI also shared 90% identity with the deduced amino acid sequences of the coat proteins of tomato mottle virus, bean dwarf mosaic virus, and potato yellow mosaic virus. Results of transmission experiments showed that AbMV-HI was not transmissible by the sweet potato whitefly *Bemisia tabaci*. The derived amino acid sequences of the coat proteins of two nonwhitefly-transmissible strains of AbMV were compared with those of 19 whitefly-transmissible geminiviruses. Five amino acids in the N-terminal region of the coat proteins were identical for two strains of AbMV, but were different from vector-transmissible geminiviruses. These results suggested that some or all of the five amino acids at the N-terminal end of the coat protein may have been involved in whitefly transmission of bipartite geminiviruses.

Geminiviruses are transmitted by whiteflies or leafhoppers and are characterized by distinct twinned isometric virions and a circular, single-stranded (ss) DNA genome (14,19,20). The family *Geminiviridae* is classified into three genera based upon host ranges, insect vectors, and genome organizations (20). Whitefly-transmitted (WFT) geminiviruses cause serious epidemics in agronomic and horticultural crops and are increasing in prevalence and distribution in subtropical, tropical, and temperate regions of the world (6).

The sweet potato whitefly *Bemisia tabaci* Gennadius biotype B, also known as the silverleaf whitefly *B. argentifolii* Bellows and Perrings (24), causes silverleaf on squash and other disorders on vegetable crops in Hawaii (9). In addition, there is concern that this whitefly may be capable of spreading geminiviruses among crops in Hawaii. Recently, a geminivirus was identified in Hawaii in a widely distributed ornamental plant, lantern 'ilima (*Abutilon hybridum* Hort., commonly known as flowering maple) (16,17). Geminivirus-infected lantern 'ilima plants show foliar crinkling and mottling rather than the classic striking yellow mosaic symptoms of Abutilon mosaic virus (AbMV) in flowering maple. Flowers of lantern 'ilima are important to local industries because they are used to make leis. There is concern that this ornamental plant might be a source of a geminivirus that could infect other ornamental and vegetable crops. The purpose of this study was to characterize this geminivirus with respect to insect transmission, host range, genome organization, and relationship to other geminiviruses.

Corresponding author: J. S. Hu; E-mail address: johnhu@hawaii.edu

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MATERIALS AND METHODS

Virus and insect maintenance. Lantern 'ilima plants with characteristic crinkle mottle symptoms were collected from Nii Nursery, Honolulu, Hawaii, and maintained in screened cages in a greenhouse. Young, infected plants (10 cm tall) propagated by cuttings were used for transmission experiments. Colonies of *B. tabaci* biotype B, which were originally collected from squash (*Cucurbita* sp.) in Hawaii (9), were maintained on cotton (*Gossypium hirsutum* L.) in outdoor cages separated from other plants.

Virus transmission. The following test plants were used in vector and mechanical transmission experiments and kept in a greenhouse at 25 to 30°C: *Abutilon hybridum* Hort. 'Royal Ilima'; *Chenopodium amaranticolor* Coste & A. Reynier; *Chenopodium quinoa* Willd.; *Cucumis sativus* L. 'Straight Eight'; *Cucurbita maxima* Duchesne; *Cucurbita pepo* L. 'Ambassado'; *Euphorbia hirta* L.; *E. pulcherrima* Willd. ex Klotzsch; *Glycine max* (L.) Merr.; *Phaseolus limensis* Macfady 'Henderson Bush'; *P. vulgaris* L. 'Kentucky Wonder'; *Gossypium hirsutum* L.; *Malva parviflora* L.; *Lycopersicon esculentum* Mill. 'UH8222', '38AA-30', and 'Rutgers'; *Datura stramonium* L.; *D. metal* L.; *D. metaloides* L.; *Nicotiana benthamiana* Domin.; *N. clevelandii* A. Gray; *N. glutinosa* L.; *N. rustica* L.; *N. tabacum* L. 'Xanthi', 'Samsoun', and 'Turkish'; *Vigna radiata* (L.) R. Wilczek; *V. sinensis* (Thunb.) Mansfeld; and *Solanum melongena* L. 'Waimanalo Long'. Propagation of lantern 'ilima is exclusively vegetative, and all lantern 'ilima samples tested in the state of Hawaii are infected by a geminivirus (16). Therefore, virus-free lantern 'ilima plants were not available for transmission experiments. A different cultivar of *A. hybridum* 'Royal Ilima' was used. Adult whiteflies were exposed to diseased, young leaves of lantern 'ilima for 1 to 3 days to

allow whiteflies to acquire the virus. Thirty to fifty whiteflies were transferred to test plant seedlings at the two- to four-true-leaf stage using an aspirator. The plants were covered with transparent plastic cages ventilated with several screened openings. Whiteflies were confined on test plants in a growth chamber with 12-h light-dark cycles and a temperature of 25°C for 5 to 7 days. Plants were then sprayed with Safer insecticidal soap solution (Safer, Inc., Newton, MA), rinsed with water to remove whiteflies, and placed in a greenhouse at 25 to 28°C for 1 month for observation of symptom development. All inoculated plants were tested for virus infection by enzyme-linked immunosorbent assay (ELISA). Five plants were used for each plant species or cultivar; the experiments were repeated once. The Hawaiian colony of *B. tabaci* biotype B was tested in Florida for its geminivirus transmissibility using a whitefly-transmissible bean golden mosaic geminivirus Homestead strain (BGMV-H, 15). Adult whiteflies were exposed to BGMV-H-infected lima beans (*P. lunatus*) for 48 h and then moved to a cage with seven seedlings of *P. vulgaris* 'Topcrop' located in a greenhouse that was isolated from any virus sources.

In mechanical transmission experiments, test plants at the two- to four-true-leaf stage were dusted with Carborundum. Lantern 'ilima samples were ground in one of three extraction buffers using a mortar and pestle. The three different extraction buffers used in this study were buffer A (50 mM potassium phosphate, pH 7.5; 0.1% 2-mercaptoethanol), buffer B (0.2 M potassium phosphate, pH 7.4; 0.1% polyvinylpyrrolidone-40; 0.02 M sodium sulfite), and buffer C (0.1% sodium diethyldithiocarbamate in phosphate-buffered saline). Homogenates were rubbed on cotyledons and/or true leaves of the test plants. Plants were then rinsed with water and kept in a greenhouse at 25 to 28°C for 1 month to allow symptom development. At least five seedlings were used for each plant species or cultivar-buffer combination. Each inoculation was repeated once. Twenty-five 'Royal Ilima' plants were grafted using diseased lantern 'ilima plants as scions.

To determine whether whiteflies could acquire geminivirus particles from infected lantern 'ilima plants, total nucleic acids were extracted from 20 individual whiteflies following an 18-h acquisition access period on diseased lantern 'ilima plants. Virus DNA was detected by polymerase chain reaction (PCR) using primer pairs PAL1v1978 and PAR1c715 (25) according to the method of Navot et al. (21). In addition, 10 potentially viruliferous whiteflies were confined on virus-free zucchini plants for 7 days, after which total nucleic acids were extracted from individual whiteflies and tested by the PCR assay.

ELISA. Triple-antibody-sandwich indirect ELISA was used to detect the geminivirus in transmission experiments. Microtiter plates were coated at a concentration of 1 µg/ml with a polyclonal antiserum 1,110 prepared against a purified geminivirus infecting *Macroptilium lathyroides* in Florida (14). The plates were then incubated overnight at 4°C and washed three times before use (8). The leaf samples (0.1 g) were ground in 2 ml of extraction buffer (8) and 100 µl was added to each well of the plate. After the plates were incubated overnight at 4°C and washed, the monoclonal antibody 3F7, having a broad range of reactivity to WFT geminiviruses (7), was added to wells in an enzyme conjugate buffer (8) at a dilution of 1:2,000 and incubated for 2 h at 37°C. Goat anti-mouse IgG conjugated with alkaline phosphatase (1:2,000 in the enzyme buffer) (Sigma Chemical Co., St. Louis) was then added and incubated at 37°C for 2 h. Finally, substrate (*p*-nitrophenyl phosphate at 1 mg/ml) in 100 µl of substrate buffer was added to each well and incubated for 1 h at room temperature. Absorbance at 405 nm was measured with a Model 450 Microplate Reader (Bio-Rad Laboratories, Richmond, CA). Controls with virus extraction buffers, healthy 'Royal Ilima' samples, and virus-infected lantern 'ilima samples were included in all tests.

Cloning and sequence analysis. Total nucleic acids were extracted from young leaves of infected lantern 'ilima plants according to Hadidi et al. (13). Two sets of broad spectrum, degenerate primers for WFT geminiviruses (25) were used in PCR to prime the amplification of partial fragments of A and B components of the geminivirus infecting lantern 'ilima plants. One set of primers (PAL1v1978 and PAR1c715) directed the amplification of a 1.4-kbp DNA fragment of the A component that included the intergenic region and portions of the replication-associated protein and coat protein genes. A second set of primers (PCRc1 and PBL1v2042) amplified a 600-bp DNA fragment that flanked the BC1 region and the beginning of the common region of the B component (25).

The PCR reaction mixture of 100 µl consisted of 1 µl of total nucleic acid sample, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 200 µM of each dNTP, 2.5 mM $MgCl_2$, 0.2 µM of each primer, and 2.5 units of *Taq* DNA polymerase (Promega Corp., Madison, WI). Viral DNA was amplified by 35 cycles of 1 min at 94°C, 1 min at 46°C, and 3 min at 72°C, followed by a 10-min extension at 72°C. The amplified DNA fragments were electrophoresed in 0.8% (wt/vol) low-melting temperature (LMT) SeaPlaque agarose (FMC BioProducts, Rockland, ME) gels in 1× Tris-acetate-EDTA buffer stained with ethidium bromide and visualized under ultraviolet light. DNA fragments of the expected size were excised from LMT agarose gels and extracted (11). After digestion with restriction enzyme *Pst*I, PCR products were cloned into pBluescript KS$^+$. Virus-specific clones were identified by alkaline miniprep plasmid DNA analysis (26) and sequenced using the dideoxynucleotide chain termination method (27) and the USB Sequenase kit (version 2.0, United States Biochemical Corp., Cleveland, OH) according to manufacturer instructions.

Abutting primers (primer 1: 5′-TGCATCTCTGATGTGACACG-3′; primer 2: 5′-CATTACCTTGCCGAGATGTG-3′) were designed based on the sequence information of the coat protein gene of DNA-A to obtain the full-length DNA of component DNA-A by PCR. Putative full-length PCR fragments were blunt-ended with Klenow fragment, cloned into the *Sma*I site of pBluescript KS$^+$, and transformed into *Escherichia coli*. Clones containing inserts of approximately 2.6 kbp were identified via miniprep plasmid DNA analysis and were selected for further subcloning and DNA sequencing.

Attempts to obtain a full-length fragment of DNA-B component by using the same approach were not successful. Consequently, overlapping PCR products (600, 800, and 1,400 bp) spanning the DNA-B component were made. Primers PCRc1 and PBL1v2042 were used in PCR for the 600-bp fragment; other primers were designed in BC1 and 3′-noncoding regions based on known sequence of the 600-bp DNA fragment (primer 3: 5′-CAGCTAGACTCAGTCGAACC-3′; primer 4: 5′-ATCTTCTGG-GTTGATGGGTA-3′) and designed in BV1 region based on the conserved genome sequence of AbMV, TMoV, and BDMV (primer 5: 5′-GATGTC(T)AGCCCAACGCATAC-3′; primer 6: 5′-AACTGATGAACGCGAGGTGG-3′). The three PCR fragments were blunt-ended with Klenow fragment, cloned into the *Sma*I site of pBluescript KS$^+$, sequenced using the USB Sequenase kit (version 2.0), and autosequenced at Biotechnology-Molecular Biology Instrumentation Facility, University of Hawaii, Honolulu. Both DNA-A and DNA-B components were sequenced completely on both strands. Sequence data of the entire components of DNA-A and DNA-B were analyzed and compared with those of other known bipartite geminiviruses available in GenBank using Genetics Computer Group GCG computer program (University of Wisconsin, Madison).

RESULTS AND DISCUSSION

Genome organization. Nucleotide sequence analysis of the DNA of the geminivirus infecting lantern 'ilima showed that the genome of the virus was bipartite, which is consistent with those

of WFT geminiviruses. The lengths of DNA-A and DNA-B were determined to be 2,634 and 2,571 nucleotides, respectively (Fig. 1). The genome sizes of both components were similar to those of other bipartite geminiviruses (2). Nucleotide 1 in both components corresponded to the first nucleotide of the common region as defined in other bipartite geminiviruses. This common region was 190 nucleotides long with only seven nucleotide differences between DNA-A and DNA-B; the common region sequences shared 93.6% similarity. The DNA-B sequence reported was not derived from a single molecule, and therefore may represent a "consensus" of more than one variant of DNA-B that may have been present.

Nucleotide sequence analysis of the DNA-A and DNA-B components of the geminivirus showed genome organization similar to other bipartite geminiviruses (Fig. 2). A total of seven open reading frames (ORFs) were identified (Fig. 2). DNA-A had five ORFs, AV1 in the viral-sense direction and AC1, AC2, AC3, and AC4 in the complementary-sense direction (Fig. 2). Based upon sequence comparisons with other WFT geminiviruses, ORF AV1 is the coat protein gene, ORFs AC1 and AC3 are proteins involved in virus replication, and ORF AC2 is a protein indirectly required for virus systemic movement (19). An AC4 ORF was present within the N-terminal region of the AC1 ORF. Jupin et al. (18) showed that the protein encoded by ORF AC4 of tomato yellow leaf curl geminivirus (TYLCV), which is a monopartite virus, is involved in virus movement in plants. The role of the AC4 protein of bipartite geminiviruses is unknown. DNA-B had two ORFs, BV1 oriented in the viral-sense direction and BC1 oriented in the complementary-sense direction. The BV1 and BC1 proteins of bean dwarf mosaic virus (BDMV) are involved in virus movement in plants (22).

Sequence comparison of geminiviruses. The DNA-A and DNA-B components of the geminivirus infecting lantern 'ilima shared 95 and 91% nucleotide sequence identity with a West Indian strain of AbMV (AbMV-WI, 12) and 72 to 85% and 66 to 78% nucleotide sequence identity with nine other New World geminiviruses (Table 1). Based upon sequence comparison of 36 geminiviruses, Padidam et al. (23) proposed that any new isolate should be considered a strain of an already described virus if it shares more than 90% sequence identity to a previously characterized virus. The geminivirus infecting lantern 'ilima in Hawaii was, therefore, considered a strain of AbMV (AbMV-HI).

AbMV-HI ORF BC1 shared 93% nucleotide sequence identity with that of AbMV-WI (Table 1), but they shared low amino acid identity (66%). When we compared the nucleotide sequences of AbMV-HI with those of AbMV-WI and other geminiviruses, we found that there were several possible mistakes in the ORF BC1 of AbMV-WI. Therefore, we made specific primers and sequenced the ORF BC1 of an AbMV-WI clone, which was obtained from A. Abouzid at the University of Florida. Based upon our nucleotide sequence data, corrections for ORF BC1 of AbMV-WI were made that resulted in the amino acid identity increasing to 88% with that of AbMV-HI (Table 1). Three T residues at positions 1,633, 1,672, and 1,694 and three C residues at positions 1,653, 1,686, and 1,695 were deleted; one C residue at position 1,535 and two G residues at position 1,798 were added. Positions

A

AbMV-HI DNA-A

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   1  CGGTGGCATT TTTGTAATAA GAAGGGGTAC TCTAGATGAG TTACTCTAGT
  51  TGAGGCTCCT CCAAAACCTG CTCATGTAAT TGGAGTATTG GAGTTCTATA
 101  TATACTAGAA CTCTCATTAA CGGATTTGCA ACACGTGGCG GCCATCCGCT
 151  ATAATATTAC CGGATGGCCG CGCGCCCCCC CTGGTGCCGT ACACTCCCGC
 201  GCGCTTTTTC AGCCTTTAAT TTAGAATTAA AGGCAGTCTC TGGCGCTTTG
 251  TCCAATCATA ATGCGCCTGA CGAGTCAATA TAATTTGAAC AACTTGGGCG
 301  CTAAGTTGTT GGGTTGTTTA TAAATGAAAA GCCCATTGGC CCACGAGCTT
 351  TAACCCAAAA TGCCTAGGCG CGATCTCCCA TGGCGATCGA TGCCTGGAAC
 401  TTCCAAGATT AGTCGTAACG CTAATTATTC TCCTCGTGCT CGTATTGGGC
 451  CAAGATCTGA CAAGGCCTCT GAATGGGTGC ACAGTCCCAT GTACAGGAAG
 501  CCCAGGATCT ACCGGACGCT GAGGACGGCC GATGTGCCCA GAGGCTGTGA
 551  AGGGCCTTGT AAGGTCCAGT CGTATGAACA CCGTCACGAC ATCTCACATG
 601  TCGGCAAGGT AATGTGCATC TCTGATGTGA CACGTGGTAA TGGCATTAAC
 651  CAACGTGTGG GTAAGCGTTT CTGTGTTAAG TCTGTGTATA TTTTAGGGAA
 701  GATCTGGATG GACGAGAACA TCAAGCTCAA GAACCACACG AACAGTGTCA
 751  TGTTCTGGTT GGTCAGAGAC CGTAGACCGT TTGGCACGCC CATGGATTTC
 801  GGTCAGGTGT TCAACATGTT CGACAACGAG CCCAGTACTG CCACCGTGAA
 851  GAACGATCTC CGTGATCGCT ACCAGGTCAT GCACAAGTTC TATGGCAAGG
 901  TCACAGGTGG ACAGTATGCC AGCAATGAAC AGTCAATCGT CAAGCGTTTC
 951  TGGAAGGTCA ACAATCATGT GGTCTACAAT CATCAAGAGG CTGGCAATTA
1001  CGAGAATCAC ACGGAGAACG CTCTACTATT GTATATGGCA TGTACTCATG
1051  CCTCTAACCC TGTTTATGCA ACTTTGAAGA TCCGAATCTA TTTCTATGAT
1101  TCGCTCATGA ATTAATAAAA TTTGAATTTT ATTGAATGAT TCTCCAGTAC
1151  ATAATTTACA TACGATCTGT CTGTCGCAAA CTGAACAGCT CTAATTACAT
1201  TGTTAATGGA AATCACGCCT AATTGATCTA AGTACATGTT GACTAAACGC
1251  GTAAATCTAG CTAAATAAGT TGACCCAGAA GCTTTCATCG ATGTCGTCCA
1301  AACTTGGAAG TTCAGGTAAG CTTTGTGGAG ATGCAACGCT CTCCTCAGGT
1351  TGTGGTTGAA CCGTATTTGT ACATGGTATA TTTTCGTTCT GGTGTATAAT
1401  GGTTCTTCTA CTTTGTACAT CCTGAAATAC AGGGGATTTT TTATCCCCCA
1451  GGTATACACG CCATTCTCCG CCTGACGTAC AGTGATGAGT TCCCCTGTGC
1501  GTGAATCCAT GTCCCGTACA GTCTATGTGG AAGTAGATGG AGCAGCCGCA
1551  CTGCAGATCA ATCCTCCGCC GTCTGATTGC CCTCCTCTTG GCCTGCCTGT
1601  GTGCTTTCTT GATAGAGGGG GGATGTGAGG GTGATGAAGA TCGCATTTTT
1651  GCGAGTCCAG TTTTTGAGAC CTGTATTTTC CTCTTTGTCC AGGTACTCTT
1701  TATAGCTGGA ACCCTCACCA GGATTGCAAA GCACGATTGC TGGGATTCCT
1751  CCTTTAATTT GAACTGGCTT GCCGTATTTG CAATTTGACT GCCAGTCTTT
1801  TTGGGACCCC AGCAACTCTT TCCAGTGCTT TAGCTTTAGA TACTGCGGTG
1851  CGACGTCATC AATGACGTTA TACAACACTT CGTTCGAATA GACACGACCA
1901  TTGAAGTCCA GGTGTCCACT GAGATAGTTA TGTGGGCCTA ACGCACGTGC
1951  CCACATCGTC TTCCCTGTCC TTGAATCACC TTCGACTATG AGACTCAATG
2001  GTCTGTGTGG CCGCGCAGCG GCACCACTCC CAAAATAATC ATCCGCCCAC
2051  TCCTGCATCT CCTCCGGGAC GGCCGTGAAA GAGGAGAGTG GAAACGGAGG
2101  AACCCATGGT TCCGGAGCCT TTGCGAATAT TCTTTCGAGA TTGGAGCGGA
2151  TGTTATGATT CTGAAGGACA TAATCTTTTG GCTGTTCCTC CTTCAGAATG
2201  TTTAAGGCAG ATTGAACATC TATTGCATTC AACGCCTTGG CATATGAATC
2251  ATTAGCAGTC TGTTGGCCTC CCCTAGTAGA TCTGCCGTCG ATCTGGAATT
2301  CTCCCCATTC AGCTGTATCT CCGTCCTTGT CGATGTAGGA CTTGACGTCG
2351  GAGCTCGATT TAGCTCCCTG TATGTTTGGA TGGAAATGTG CTGCCCTGGT
2401  TGGGGAGACC AGATCGAAGA ATCTATAATT CGTGCATTGG AATTTGCCTT
2451  CGAACTGTAT GAGCACATGG AGATGAGGCT CCCCATTCTC GTGAAGCTCT
2501  CTACAGATTT CGATGAACTT CTTGTTTACC GGTGTTTCTA GGTTTTGTAA
2551  TTGGGAAAGT GCCACCTCTT TGGCAAGAGA GTACTGGGGA TATGTGAGGA
2601  AATAATTTTT GGCCTGTACT CTAAATTTCT CTGG
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B

AbMV-HI DNA-B

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   1  CGGTGGCGTT TTTGTAATAA GAAGGGGTAC TCCAGATGAG TTACTCTAGT
  51  TGAGGCTCGT CAAAACCTGC TCATGTAATT GGAGTATTGG AGTTCTACAT
 101  ATACTAGAAC TCTCATTAAC GGATTTGCAA CACGTGGCGG CCATCCGCTA
 151  TAATATTACC GGATGGCCGC GCGCCCCCCC CCTGGTGCCG CTTTTTCAAT
 201  CGCCCCCCCC TGGTGCTGTG CTCAAGCGCG CCTCCCATTG GTGCGGGTCC
 251  CTCACGGTCC TCTTTTTCGG TGGCCTTTAA GTTGAATTAA AGGTTCATAC
 301  TTTCTCGCGC GCGTGCTTTA TATTTTGAAT TATTGTCGCG CGATGACCGA
 351  CTATGGGCCA TTGTACCTGG TGAAGGACGT GTCCATAAAT GGACCTTGCT
 401  ACTGAGTCTA TTTGCTATTA ATGTTGGCCC ATCTACCTAT ATATTGGGTT
 451  GGATGGGTTT GACTCANCAA TCCACGACGT TTTTCATCCT TGGTTAATCG
 501  TTGTTGTTNA TCTAATATTA TCATCTGATA ATGTACTTGT TTAGGAATAA
 551  ACGTGGATCC TACTTTAATC AACGTCGACA GTATTCACGC AATCATGTGT
 601  GGAAACGGCC AACTGCTGGG AAGAGACATG ACTGGAAGCG TCGACCTTCA
 651  CATACCAGCA AGCCCAACGA CGAGCCCAAG ATGTCCGCCC AACGCATACA
 701  TGAGAATCAG TATGGGCCAG ATTTTGTAAT GGCCCAGAAC TCGGCCACCT
 751  CGTCGTTCAT CAGTTATCCT GACTTGGGTA GGTCCGAGCC CAACCGAACC
 801  AGGTCGTATA TCAGATTGAA GCAACTACGC TTCAAAGGGA CGGTGAAGAT
 851  TGAACAGGTT CAATCGCCTA TGAACATGGA CGGATCTACC CCCAAAGTGG
 901  AAGGAGTGTT CTCCCTTGTC ATTGTTGTGG ATCGTAAACC ACACCTTGGT
 951  CCCACTGGTT GTCTGCATAC ATTTGACGAG CTGTTTGGTG CTAGGATCCA
1001  CAGTCATGGT AACCTCAGCG TAATTCCCTC GTTGAAAGAT CGCCATTACA
1051  TCCGCCACGT GTGCAAACGT GTGTTATCTG TGGAGAAGGA TACGCTGATG
1101  GTAGACGTGG AAGGATCCAT ACCTCCCTCT AACAGGCGTA TTAATTGTTG
1151  GGCCACGTTT AAGGACGTGG ATCGTGAGTC ATGCAAGGGT GTTTATGATA
1201  ACATAAGCAA GAACGCCCTG TTAGTCTATT ACTGCTGGAT GTCGGATACG
1251  CCTGCTAAGG CATCCACTTT TGTATCGTTT GACCTTGACT ACATTGGTTA
1301  AGTGAATAAA TAAGTAATTT AAAGTTGATC ATTTTATTTG TACAGGCAAA
1351  ACATACAATT ATTTCAATGA TTTGGCTTGA GAAGCCTGAC AGTTATTATC
1401  GATACATTCT TGGACCGTTG TCCTGACTAA TTCGTTCAGC TGGCCCATTG
1451  ACATTGTGAT ATTGGACTCC GCTCTCTGGG CCCCCACAAT CGAAGCAGAA
1501  TCCCCTGGGT CTAGAACGCT GGCCCCCAGC CTGTTTAGGT GTCTGTATGG
1551  ATGGGGCTCG TTCTCCACAT CTGATTGCGC ATTGGACTGG GCTGTCCCTA
1601  TGTCGCTTCT GGAAGCCCAT GACTCACCAG GCCTTAGCTC AATTGGGCCT
1651  CGTATTCCTA GCCTGGACAT GGACTCGCAT CTGATGGGCT TCCTCTCCCA
1701  TTTCCCATAA TCCACATGGG AAAAGTCCAC GTCTTTTTCG GTGAACTGTT
1751  TCGACGGTAT CCTTACTGTC GGTGGCCGGA ACGGGATGTC GACCGAATGT
1801  TTCGCCGTGG ACAATTTCAG TTTCCCTTTG AACTTGGCGA AGTGGGTCCG
1851  CTGATGCACG TTTGTATCGC AGACTCTGTA GTATAATTTC CATGGAATGG
1901  GGTCCTTCAG CGAGAAGAAC GAAGCCGAGA AATAGTGGAG ATCTATGTTG
1951  CGTCTGATCG GAAAAGTCCA CGTGGCCTGT AAAGACTCGT TGTCCGTCAT
2001  TCTCTTTGTCG TGTATCTCCA CAACCACCGT CCCGGAGGCG TTGATTGGTA
2051  CTTGCTGTCT GTATTCAATT ACGCAGTGAT CGATCTTCAT GCAGGTTCGA
2101  CTGAGTCTAG CTGTCAACTG AGACGCCGTG GACGGAAATT GCAGTATTAT
2151  CTCAGTTAGG TCATGTGAAA GTTGATATTC GTCACGGTGT GACTCGATGT
2201  ATCTGAAGGC GTTCGGAGGA ATTACTAACT GAGAATCCAT TTGAAGAAGT
2251  ACGGCCGCGC AGCAATTGGA ACTGATTGCT GCTGTTGAAC AGTTAAGAAT
2301  ATGAACAGTC AGTGATGAAC AATCACTGTT GATCAGATAT GTAGTGAAGC
2351  TCTTCTCTTt GAGAAaGTCA GATGTCTCTG AGCTAAAACT GACCTGATTG
2401  ATGCGGAGTA ACTGGTGAAG AGTCGAGTTG TAGAAAAGAA AAGAGTTTTG
2451  ATGGAGAATT TGAGAGAGAT CTGGAAATTT AAGAGTTTGT GTATGAACCC
2501  AGATCTTCTG GGTTGATGGG TATTTAAATT GGAAAGTGTT CTTCTACTTC
2551  TGAGAGAAGC TATGTGTTAA A
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Fig. 1. Nucleotide sequence of the viral-sense strand of both components **A,** DNA-A and **B,** DNA-B of Abutilon mosaic virus Hawaiian strain (AbMV-HI).

1,570 to 1,575 (GAGCTC) were the *Sac*I digestion site, which is the cloning site of the DNA-B of AbMV-WI (12). Six nucleotides (CTCGTT) that interrupted the *Sac*I digestion site between GAG and CTC were likely omitted during cloning, based on the alignments of nucleotide sequences of WFT geminiviruses. An A at position 360 of AbMV-WI DNA-A was changed to a G as suggested by Coutts et al. (10).

AbMV-HI was closely related to tomato mottle virus (TMoV), BDMV, and potato yellow mosaic virus (PYMV). It shared 90% identity with the derived amino acid sequences of the coat proteins of TMoV, BDMV, and PYMV (Table 1). In Florida, TMoV was found to be closely related to a geminivirus in *Sida* spp. and it has been proposed that the *Sida* geminivirus might be the origin of TMoV (1). If AbMV-HI were to become vector transmissible, tomato, potato, and beans are potential crop hosts.

Transmission. None of the test plants from 27 species or cultivars used in whitefly or mechanical transmission experiments showed any symptoms of virus infection. These plants tested negative for geminivirus infection by ELISA. ELISA absorbance readings (A_{405}) after 1-h incubation of AbMV-HI-infected lantern 'ilima samples ranged from 0.5 to 1.0, whereas all readings of test plants were below 0.1. Adult whiteflies of the same colony from Hawaii transmitted BGMV-H in Florida. Two of seven seedlings exposed to viruliferous whiteflies developed symptoms 12 days after the inoculation feeding. In grafting experiments, one 'Royal Ilima' plant was successfully grafted with lantern 'ilima, became

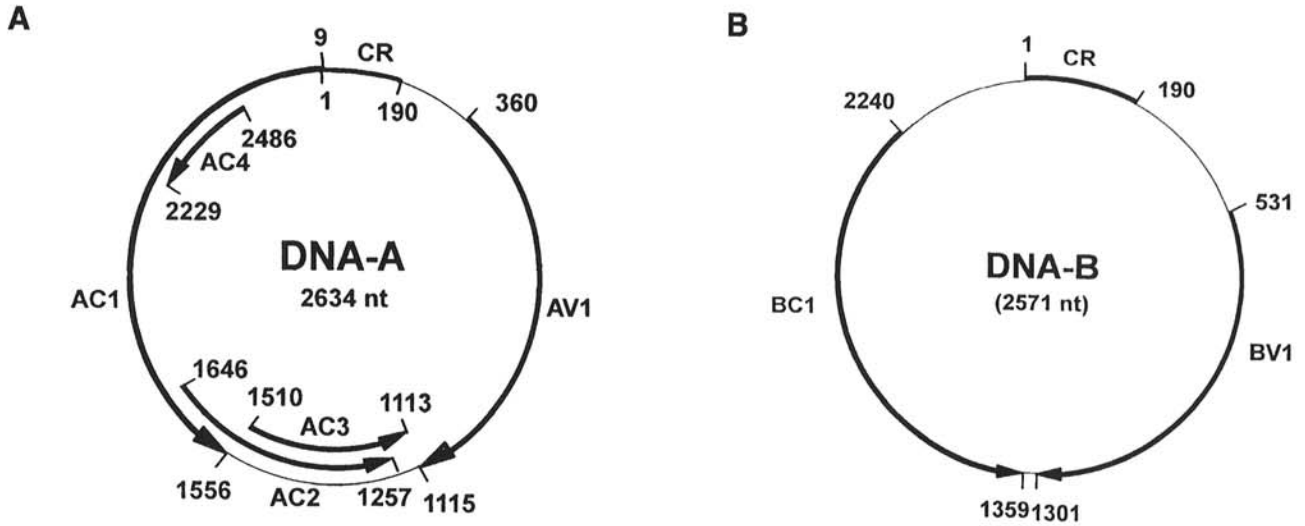

Fig. 2. Genome organization of Abutilon mosaic virus Hawaiian strain (AbMV-HI) **A,** DNA-A and **B,** DNA-B components. Open reading frames (ORFs) are designated A or B (DNA-A or DNA-B component, respectively); V or C (virus-sense or complementary-sense, respectively); and 1, 2, 3, or 4 (position of ORF in relationship to the common region [CR]). The nucleotide positions of the beginning and end of each ORF and the CR are indicated. Nucleotide 1 is the beginning of the CR.

TABLE 1. Nucleotide and derived amino acid sequence identities (%) between Abutilon mosaic virus Hawaiian strain (AbMV-HI) and 10 other New World geminiviruses[a]

	DNA-A						DNA-B		
Viruses	Total	CR[b]	AV1[c]	AC1	AC2	AC3	Total	BV1	BC1
AbMV-WI	95	95	96	95	94	94	91	94	93
			95	*92*	*91*	*89*		*94*	*88*
TMoV	85	84	86	86	90	87	77	78	83
			90	*87*	*83*	*83*		*76*	*82*
BDMV	83	83	87	80	85	85	75	78	80
			90	*80*	*81*	*78*		*79*	*87*
PYMV	81	72	84	79	83	85	74	70	77
			90	*76*	*80*	*81*		*70*	*84*
TGMV	76	78	80	74	70	81	70	67	77
			85	*73*	*70*	*77*		*65*	*85*
BGMV-Ga	76	80	78	76	75	77	70	69	74
			85	*79*	*68*	*73*		*67*	*82*
BGMV-Dr	76	79	77	77	75	77	71	69	74
			84	*80*	*67*	*71*		*69*	*82*
BGMV-Bz	76	75	80	74	78	80	68	68	73
			88	*73*	*69*	*74*		*69*	*76*
PHV	72	68	76	72	65	69	69	66	72
			84	*68*	*58*	*62*		*64*	*78*
SqLCV	72	74	80	66	73	77	74	67	72
			85	*60*	*64*	*73*		*60*	*77*

[a] The GenBank sources of data sequence were from the following accession numbers: Abutilon mosaic virus West Indian strain (AbMV-WI, X15983 and X15984), tomato mottle virus (TMoV, L14460 and L14461), bean dwarf mosaic virus (BDMV, M88179 and M88180), potato yellow mosaic virus (PYMV, D00940 and D00941), tomato golden mosaic virus (TGMV, K02029 and K02030), bean golden mosaic virus Guatemala isolate (BGMV-Ga, M91604 and M91605), BGMV Dominican Republic isolate (BGMV-Dr, L01635 and L01636), BGMV Brazil isolate, (BGMV-Bz, M88686 and M88687), pepper huasteco virus (PHV, X70418 and X70419), and squash leaf curl virus (SqLCV, M38182 and M38183). Comparisons in italic refer to derived amino acid sequences.

[b] CR denotes the common region.

[c] V and C denote virion-sense and complementary-sense open reading frames, respectively, as shown in Figure 2.

infected by the geminivirus, and developed mottling symptoms. Thus, we concluded that AbMV-HI was not transmissible by whiteflies. Recently, Bedford et al. (4) also showed that a United Kingdom strain of AbMV (AbMV-UK) is not whitefly transmissible using 18 different populations of *B. tabaci* that included seven populations of the B biotype. Because propagation of lantern 'ilima is exclusively vegetative and all lantern 'ilima samples tested are infected by the virus (16), it is clear that vector transmission of the virus in lantern 'ilima is no longer a trait for which selection pressure is present.

PCR assays were used to determine if the whitefly could acquire and retain AbMV-HI. After virus-free, winged, adult whiteflies were exposed to AbMV-HI-infected lantern 'ilima plants for 18 h, a 1.4-kbp DNA fragment was amplified by PCR using primers PAR1c715 and PAL1v1978 from seven of twenty individual whiteflies (data not shown). A DNA fragment of the same size was also amplified from three of ten AbMV-exposed whiteflies after they were kept on virus-free zucchini plants for 7 days (data not shown). The results suggested that whiteflies had the ability to acquire AbMV-HI from diseased lantern 'ilima plants within an 18-h acquisition access period and could retain the virus for at least 7 days after acquisition. The acquisition access period (3 days) and inoculation access period (5 to 7 days) in our vector transmission experiments should have resulted in transmission, if

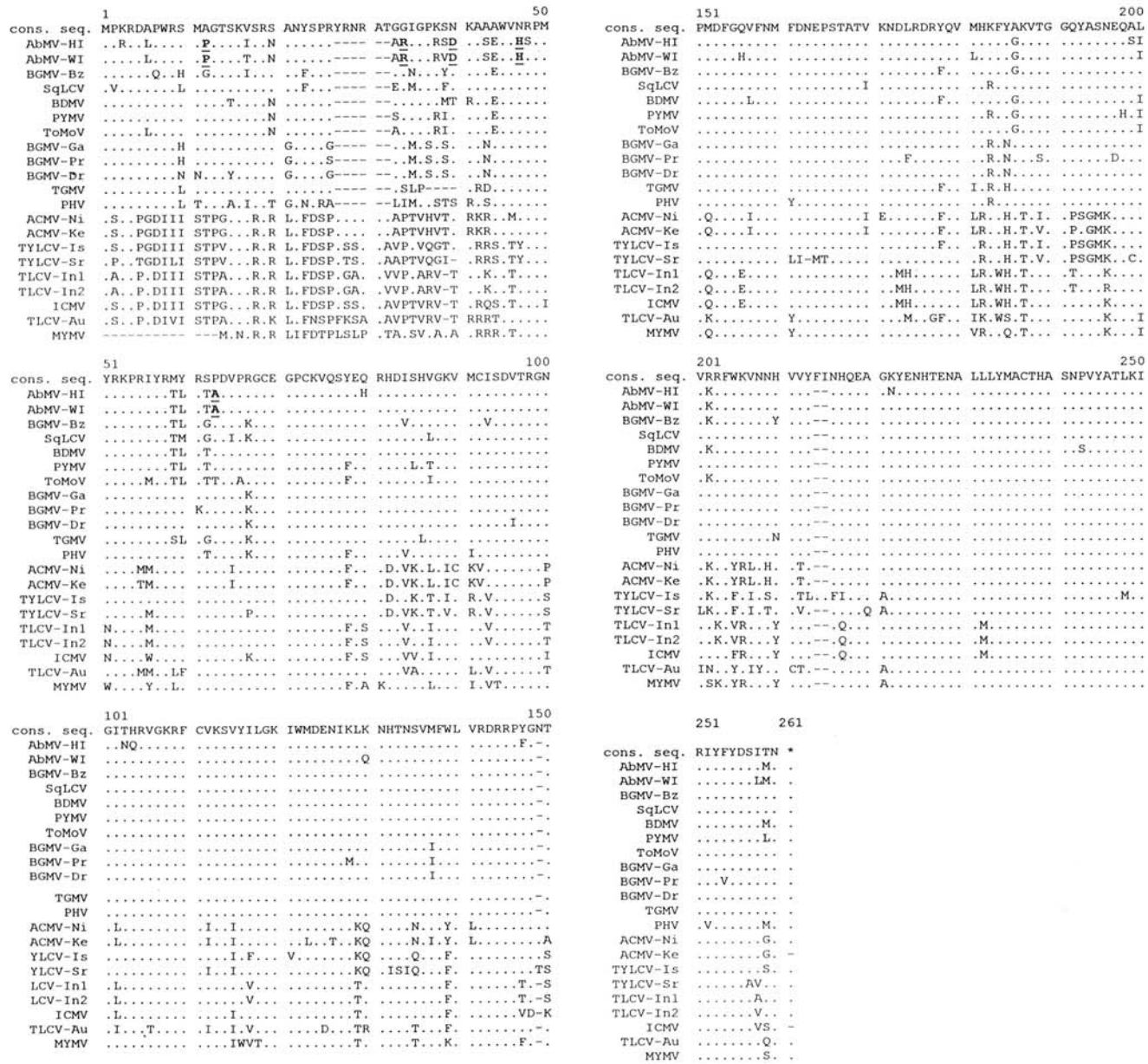

Fig. 3. A comparison of the amino acid residues of the coat proteins of 19 whitefly-transmissible geminiviruses and two nonvector-transmissible strains of Abutilon mosaic geminivirus (AbMV). Dots indicate amino acids that are the same as consensus sequence. The gap in amino acid residues is shown as a dash. The amino acids different between vector-transmissible and nonvector-transmissible geminiviruses are printed bold and underlined. Sequence of bean golden mosaic virus Puerto Rican isolate (BGMV-Pr) DNA-A was changed as described by Padidam et al. (23). Amino acid sequence of tomato mottle virus (TMoV) coat protein was changed as described by Cancino et al. (7). The GenBank sources of data sequence were from the following accession numbers: AbMV-HI (Hawaiian strain, this paper), AbMV-WI (West Indian strain, X15983), BGMV-Bz (Brazil isolate, M88686), squash leaf curl virus (SqLCV, M38182), bean dwarf mosaic virus (BDMV, M88179), potato yellow mosaic virus (PYMV, D00940), TMoV (L14460), BGMV-Ga (Guatemala isolate, M91604), BGMV-Dr (Dominican Republic isolate, L01635), BGMV-Pr (M10070), tomato golden mosaic virus (TGMV, K02029), pepper huasteco virus (PHV, X70418), African cassava mosaic virus Nigerian isolate (ACMV-Ni, X17095), ACMV Kenyan isolate (ACMV-Ke, J02057), tomato yellow leaf curl virus Israeli isolate (TYLCV-Is, X15656), tomato leaf curl virus Indian isolate 1 (TLCV-In1, U15015), TLCV Indian isolate 2 (TLCV-In2, U15016), TLCV Australian isolate (TLCV-Au, S53251), Indian cassava mosaic virus (ICMV, Z24758), and mungbean yellow mosaic virus (MYMV, D14703).

AbMV-HI were vector transmissible. It is possible that changes in the coat protein of AbMV-HI affected its vector transmissibility. Our sequence comparison data supported this hypothesis.

Comparison of coat protein sequences. Our vector transmission experiments showed that AbMV-HI was a nonwhitefly-transmissible strain of AbMV. We speculated that changes had occurred in AbMV during the decades-long vegetative propagation of the infected ornamental plants so that the virus could no longer be transmitted by the whitefly vector. The coat protein of geminiviruses plays a pivotal role in virus transmission by the whitefly vector (3,5). Replacement of the coat protein gene of African cassava mosaic virus with that of beet curly top virus, a leafhopper-transmitted virus, demonstrated that the coat protein is responsible for insect transmissibility (5). Azzam et al. (3) constructed three mutants of bean golden mosaic geminivirus Guatamala isolate (BGMV-GA) by deleting the N-terminal portions or inverting a restriction fragment in the coat protein gene and showed that none of the mutants were transmissible by whiteflies even though they were still infectious in plants. Their results demonstrate that a functional coat protein is important in geminivirus transmission by the whitefly vector. We compared the coat protein amino acid sequences of two nonvector-transmissible strains of AbMV with those of 19 vector-transmissible geminiviruses and found that five amino acids in the N-terminal region of the coat protein were identical in the two AbMV strains but differed between AbMV and other geminiviruses (Fig. 3). This comparison suggested that some or all of the five amino acids at the N-terminal end of the coat protein might be involved in whitefly transmission of bipartite geminiviruses. We were aware, however, that many of the WFT geminivirus sequences used in the comparison were from clones; in many cases, progenies of the cloned viral genomes had not been tested for whitefly transmission. Future mutagenesis experiments will address the question of the role, if any, of these specific amino acids in whitefly transmission. Bedford et al. (4) found that two other geminiviruses, honeysuckle yellow vein mosaic virus and pseudoranthemum yellow vein virus, were not transmissible by whiteflies. It will be interesting to compare the N-terminal coat protein sequence of these two nonvector-transmissible geminiviruses with that of AbMV-HI and AbMV-WI.

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