Complete Nucleotide Sequences of the Infectious Cloned DNAs of Bean Dwarf Mosaic Geminivirus

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ABSTRACT

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Bean dwarf mosaic geminivirus (BDMV) was shown to have a bipartite genome (DNA-A and DNA-B) from restriction map and sequence data comparisons of cloned DNAs. The nucleotide sequences of the infectious clones of DNA-A and DNA-B were determined. Computer analyses showed that the bipartite genome of BDMV resembles that of other whitefly-transmitted geminiviruses. The DNA-A (2,615 nt) and DNA-B (2,575 nt) have little sequence homology other than that within the common region. The nucleotide sequences of the 187-nt common regions of the two DNAs had 96% identity and included a region of 33 nt that can form a stem-loop structure nearly identical to that of other geminiviruses. Nucleotide sequence analysis identified six open reading frames, four on DNA-A and two on DNA-B, involving viral and complementary strand sense transcription. Comparisons of nucleotide and derived amino acid sequences of BDMV with those of other whitefly-transmitted geminiviruses showed that BDMV was most closely related to Abutilon mosaic geminivirus and not closely related to bean golden mosaic geminivirus. BDMV can be distinguished from other bean-infecting geminiviruses by differences in symptoms, host range, reaction of bean germplasm, and sequence identities; thus, BDMV is a distinct bean-infecting geminivirus.

Additional keywords: bean golden mosaic geminivirus, bean-infecting geminivirus, *Phaseolus vulgaris*, whitefly.

The geminiviruses are a group of plant viruses characterized by their distinctive twinned isometric virion morphology (22,41). In 1974, two different diseases, beet curly top and maize streak, were shown to be caused by viruses with a geminate particle that was different from any previously described virus (1,35). Bean-infecting viruses that were whitefly (*Bemisia tabaci* Genn.) transmitted and subsequently shown to be geminiviruses were first studied by S. A. Costa in Brazil in the 1950s (5) and early 1960s (6,7). One of these viruses caused golden mosaic symptoms and another induced severe dwarfing and limited yellow mosaic symptoms. This latter disease was designated bean dwarf mosaic (7). Kitajima and Costa (7) recognized the presence of 20- to 25-nm long isometric particles in *Sida* sp. infected with a whitefly-transmitted virus. Costa (7) suggested that the virus in *Sida* sp. and the bean dwarf mosaic virus were Abutilon mosaic viruses. These bean-infecting geminiviruses, when first described, were causing only minimal losses (4,7); however, by 1972 they had severely reduced bean production in South America (4,33).

Bean golden mosaic geminivirus (BGMV) and bean dwarf mosaic geminivirus (BDMV) can be distinguished by the symptoms they induce in beans (18,33). BDMV is serologically related to BGMV (33), but it does not hybridize under high stringency conditions with the type-specific DNA probes for BGMV from Brazil or Guatemala (16). This indicates that BDMV may be a distinct bean-infecting virus.

Haber et al (19) first showed that whitefly-transmitted geminiviruses contain two distinct, circular, single-stranded DNA components. These two components, which have been designated

DNA-A and DNA-B, are each about 2,600 nt. In each bipartite geminivirus, the nucleotide sequences of the two genome components differ vastly, except for nearly identical segments of about 200 nt called the common region (28). Nucleotide sequence analysis has identified six open reading frames (ORFs), four on DNA-A and two on DNA-B, involving bidirectional transcription (22,41). DNA-A encodes all functions required for replication of the virus (10,21), and it has been proposed that DNA-B encodes functions necessary for systemic infection (11). Both DNA components are required for infection by bean-infecting geminiviruses (15) and by most other whitefly-transmitted geminiviruses (41).

Nucleotide sequence data have been published only for one bean-infecting geminivirus, BGMV from Puerto Rico (25,34). In this article, the genome of BDMV is further characterized and the complete nucleotide sequences of the two components of BDMV are reported.

MATERIALS AND METHODS

Virus isolates and virus propagation. The bean dwarf mosaic geminivirus isolate CO (BDMV-CO) was collected from infected beans in fields near Palmira (Valle), Colombia, in January 1987 and was maintained in beans (*Phaseolus vulgaris* L. 'Topcrop') by sap transmission (33). Inoculated beans were grown in a growth chamber with a 26 C light period (14-h photoperiod, 310 μE) and a 21 C dark period. Leaf curling was evident 7–10 days after inoculation.

Molecular cloning of viral dsDNA. Total viral DNA was isolated from BDMV-infected bean leaves by a procedure involving phenol-chloroform extraction and ethanol precipitation (15,31). Nucleic acids were fractionated by horizontal gel electrophoresis in a 0.7% agarose gel in 1× 40 mM tris-acetate and 1 mM ethylenediaminetetraacetic acid (pH 8) buffer. Virus-specific dsDNA was then excised from the gel and recovered by binding to, and elution from, a silica matrix (Gene Clean, Bio 101, La Jolla, CA). Replicative form dsDNA was linearized by digestion with restriction endonucleases *Acc*I, *Bgl*II, or *Bam*HI (Promega, Madison, WI) and ligated to either pBluescript II KS (+) (Stratagene, La Jolla, CA) or pSP72 (Promega) previously linearized with the same enzyme (31). Recombinant plasmids were subsequently transformed into competent cells of *Escherichia coli* strain JM101. Transformants containing recombinant plasmids were selected on 1× YT plates containing ampicillin (0.1 μg/ml) and IPTG/X-Gal and incubated at 37 C overnight. Standard DNA manipulation techniques were followed (31).

Recombinant plasmids with full-length inserts of DNA-A (pBDA1) and DNA-B (pBDB1) were shown previously to be infectious in *P. vulgaris* inoculated by electric discharge particle acceleration (15).

Characterization of cloned DNAs. Plasmids were extracted by the alkaline lysis procedure (31) from *E. coli* grown in 2× YT broth cultures containing ampicillin. Insert sizes were determined by gel electrophoresis of plasmids digested with the appropriate restriction endonucleases. Clones having inserts of the expected size (2.6 kb) were characterized. Restriction endonuclease maps of the viral DNA inserts were prepared using recombinant plasmids, with each viral insert cloned in both orientations. Orientation of inserts was determined by DNA sequencing of the ends of the inserts and by the size of restriction digestion fragments.

Nucleotide sequence determination. Different strategies were used to sequence DNA-A and DNA-B. The DNA-A component was sequenced by subcloning of restriction fragments to make overlapping subclones in pBluescript SK (−) (Stratagene). Nucleotide sequences were determined using double-stranded, supercoiled plasmid DNA with flanking region primers or synthetic oligomers as primers. Potential full-length clones of the DNA-B component were sequenced by first creating libraries of nested deletions using exonuclease III (Erase-a-Base system, Promega). To complete the sequence of DNA-B, synthetic oligomers were used as primers (Biotechnology Center, University of Wisconsin-Madison, and Agracetus, Middleton, WI). Nucleotide sequence was determined by the dideoxy-nucleotide chain termination method using Sequenase (United States Biochemical, Cleveland, OH) according to the manufacturer's instructions. Both DNA strands of each component were sequenced completely to ensure accuracy. The total sequence of the viral DNA was assembled using database management software of the Genetics Computer Group, University of Wisconsin-Madison (9). Relationships among bipartite geminiviruses were examined by comparing nucleotide sequences of different geminiviruses using the GAP program of this same software with a gap weight of 5 and a gap length weight of 0.3.

The nucleotide sequences of BDMV DNA-A (plasmid = pBDA1) and DNA-B (plasmid = pBDB1) have been deposited in GenBank as accession numbers M88179 and M88180, respectively.

Detection of viral nucleic acid by squash and dot blots. Viral nucleic acids were detected in infected plants by nucleic acid squash blot hybridization as described by Gilbertson et al (16). Geminiviral DNA clones, radioisotope-labeled by nick translation with [α-^{32}P] dATP, were used as probes. A mixture of DNA-As and DNA-Bs of three different isolates of bean-infecting geminiviruses (BGMV from Brazil, BGMV from the Dominican Republic, and BDMV) served as a general probe; the DNA-Bs of each isolate were used as type-specific probes.

RESULTS AND DISCUSSION

Isolation and molecular cloning of viral dsDNA replicative form. The DNA extract from BDMV-infected beans contained two predominantly viral DNA bands that comigrated with the linear dsDNA markers of 2.6 and 1.6 kb and hybridized at low stringency with a mixture of DNA-A and DNA-B recombinant plasmids of BGMV from Guatemala (16). This indicated that these bands contained geminiviral DNA, and it was presumed that these bands represented closed circular and open circular dsDNA forms (replicative form [15]). DNA extracted from these bands was digested with *Acc*I, *Bgl*II, or *Bam*HI. A 2.6-kb linear fragment of dsDNA was detected for each of these restriction endonucleases. These linear fragments had the same electrophoretic mobility as full-length, linear molecules of BGMV DNA-A or DNA-B from Guatemala.

After cloning these 2.6-kb fragments, two distinct classes of recombinant plasmids were detected by restriction endonuclease analysis (Fig. 1). The plasmids pBDA1 (*Bgl*II insert), and pBDB1 (*Bam*HI insert) were representative of the two classes of recombinants and were selected for further characterization.

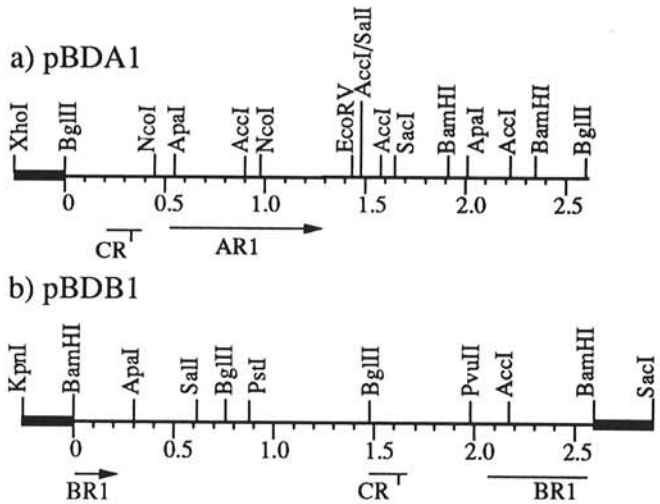

Fig. 1. Restriction endonuclease patterns of recombinant plasmids containing cloned bean dwarf mosaic geminivirus DNA: **A,** pBDA1, and **B,** pBDB1. Thick bar represents polylinker region of plasmid vectors. CR and associated horizontal bar = location of the common region. Vertical bar = approximate location of the conserved stem-loop region. Arrows AR1 and BR1 = locations of open reading frames.

Nucleotide sequence analysis of the cloned viral DNAs. Nucleotide sequences of the terminal portions of the BDMV inserts were compared with the sequence of a BGMV isolate from Puerto Rico (BGMV-PR) (25). These comparisons showed that the insert in pBDA1 had the greatest sequence identity with DNA-A (86%; 2,288–2,430 nt) and the insert in pBDB1 with DNA-B (72%; 973–1,123 nt) of BGMV-PR. Thus, restriction endonuclease maps (Fig. 1) and nucleotide sequence analysis of the cloned DNAs show that the genome of BDMV is bipartite. This is consistent with results for other whitefly-transmitted geminiviruses, including tomato golden mosaic geminivirus (TGMV) (20), squash leaf curl geminivirus (SqLCV) (29), BGMV-PR (19), African cassava mosaic geminivirus (ACMV) (40,42), tomato yellow leaf curl geminivirus isolate from Thailand (TYLCV-T) (38), Abutilon mosaic geminivirus (AbMV) (13), Texas pepper geminivirus (43), and potato yellow mosaic geminivirus (PYMV) (8). Recently, four bean-infecting geminiviruses from the Americas or the Caribbean Basin, including BDMV-CO were shown to require the two DNA components for infectivity (15). In contrast, two whitefly-transmitted strains of TYLCV from the Mediterranean region are monopartite (27,36).

The DNA-A component insert in pBDA1 had 2,615 bp (Fig. 2) and the DNA-B component insert in pBDB1 had 2,575 bp (Fig. 3). The inserts in pBDA1 and pBDB1 were determined to be full-length by sequencing across the restriction sites used for cloning these inserts in independent BDMV DNA-A and DNA-B clones obtained with different restriction enzymes. These genome sizes for DNA-A and DNA-B are similar to those of other bipartite geminiviruses from the western hemisphere (20,25,29) and are slightly smaller than the components for ACMV from Africa (42) and TYLCV from the Mediterranean (27,36).

Relationship of BDMV to other whitefly-transmitted geminiviruses. Nucleotide sequence analysis of the DNA-A and DNA-B components of BDMV showed a genomic organization similar to that of other bipartite geminiviruses (Fig. 4). There were four ORFs on the DNA-A and two ORFs on the DNA-B, which are conserved with respect to size and relative location in genomes of TGMV ([Original sequence [20] was corrected by addition of two nt in BL1 [45]), BGMV-PR (25), and AbMV(13). The BDMV DNA-A genome organization was similar to that of SqLCV DNA-A (29), but the position of ORFs in DNA-B in relation to the common region was different. The size of the intergenic region

pBDA1

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1
TGGCATTTTTGTAATAAGAGCTGGTACTCCAGTTGAGTTACTCCAATTCCCCCCTCTCAAAACTATCTCATTCTATTGGAGTATTGGAGTTACTTATATA
101
CTAGAACCCTCAATCTGGTTTCGGAACACGTGGCGGCCATCCGTATAATATTACCGGATGGCCGCGCTTCGGAGTACGCTCTCTCTCCCTTTAATTTG
201
AATTAAAGCGCACTGCTTTCGTCTCAGCCAATCATATTGCGCCTGACGAGCTTAGATATTTATAACAACTTGGGCCCTAAGTTGTTGGTTGTACGATATA
301
AATTAAAGGTAACCCGGCCCACTGTCTTTAACTCAAAATGCCTAAGCGCGATGCCCCATGGCGCTCTATGGCGGGAACGACAAAGGTCAGTCGCAATGCC
401
AATTACTCTCCCCGTGGGGGAATTGGGCCAAAGATGACAAGGGCCGCAGAGTGGGTTAACAGGCCCATGTACAGGAAGCCCAGGATCTATCGAACGCTAA
501
GGACGCCTGACGTCCCACGAGGTTGTGAAGGCCCATGTAAGGTGCAGTCTTATGAACAGCGTCACGATATTTCACATGTTGGGAAGGTAATGTGTATCTC
601
TGATGTCACACGTGGTAATGGCATTACTCACCGTGTTGGCAAGCGTTTTTGTGTTAAGTCTGTGTATATTCTAGGAAAGATTTGGATGGATGAGAATATC
701
AAGCTCAAGAACCACACGAACAGTGTTATGTTCTGGTTGGTCAGGGACCGTAGACCGTATGGAACGCCCATGGATTTCGGCCAGTTGTTCAACATGTTTG
801
ACAACGAGCCCAGCACTGCCACGGTTAAGAACGATCTTCGCGATCGTTTTCAAGTTATGCATAAGTTCTATGGGAAAGTCACAGGTGGACAGTATGCGAG
901
CAATGAACAGGCAATCGTCAAGCGTTTTTGGAAGGTCAACAATCATGTGGTTTACAATCATCAAGAGGCTGGCAAGTATGAGAATCATACGGAGAACGCC
1001
TTATTATTGTATATGGCATGTACACATGCCTCTAATTCTGTGTATGCAACTCTGAAAATTCGGATCTATTTTTACGATTCGATCATGAATTAATAAAGTT
1101
TGAATTTTATTGAATGATATTCTATTACATGAGTTACATACGATCTGTCTGTTGCGAATCGAACACCTCTAATGACATTGTTAATGGATATAATTCCTAA
1201
TTGATCTAAATACATAATAACTAAATGTTTAAATCTATTTAAATAAGTCGACCCAGAAGCTGTCGTCGATATCGTCCAGACTTGGAAGTTCAGGAAGGCT
1301
TTGTGGAGATGCAACGCTCTCCTCAGGTTGTGGTTGAACCGTATCTGTACGCTGTATACCCTGCTGTTGGTGTACAACGGTTCCTCTACTCTGTATATCT
1401
TGAAATAGAGGGGATTTTCTATCTCCCAGATATACACGCCATTCTCTGCTTGAAGTGCAGTGATGAGCTCCCCTGTGCGTGAATCCATGTCCCGTACAAC
1501
CTATGTGTATGTAGATGGAGCAACCGCATTCCAGGTCAATGCGGCGTCTCCTGATCGCCCGCCGCTTGGCTTGCCTGTGTGCCTTCTTGATAGAGGGTGG
1601
AGTCGAGAGTGATGAAGATTGCATTCTTCAACGTCCAATTCCTTAGCGCTGTATTTTCCTCTTTGTTTAGGAAATCTTTATAGCTGGCACCCTCACCAGG
1701
ATTGCATAGCACGATTGATGGGATCCCTCCTTTAATTTGAACAGGCTTGCCGTACTTGCAATTTGACTGCCAATTCTTTTGGGCCCCAATCAATTCCTTC
1801
CAGTGCTTTAACTTTAGATAATGCGGTGCGACGTCATCAATGACGTTATACTCCACTTCGTCTGTGAACACTTTGGAATTGAAGTCTAGGTGTCCACTTA
1901
AATAATTATGTGGGCCTAGTGCTCGAGCCCACATCGTCTTCCCTGTTCTCGAATCACCTTCTACTATGAGACTTACTGGTCTTTCTGGCCGCGCAGCGGA
2001
ACCTCTCCCGAAATAATCGTCTGCCCACTCTTGCATCTCGTCTGGAACGTTAGTAGACGAAGAGAGGGGAAACGGAGGAACCCATGGTTCCGGAGCCTTT
2101
TTGAATATGCTGGTGGCGTTAGCGACCAGGTTGTGATGCTGAAGGAAGAAATGTTGCGGTTGTTCTTCCTTTATTATGCGCAGAGCTTGCTCTGCGGATC
2201
CTGCATTTAACGCCTTTGCGTATGTATCGTTAGCAGACTGCTGACCTCCTCTAGCAGATCTGCCGTCGATTTGAAACACTCCCCATTCGACGGTGTCGCC
2301
GTCTTTGTCGATGTAGGACTTGACGTCGGAGCTGGATTTAGCTCCCTGAATGTTCGGATGGAAATGTGCTGACCTGGTTGGGGAAACCAGATCGAAGAAT
2401
CTGTTATTCGTGCATTGGTATTTACCTTCGAACTGGATGAGGACGTGCAGATGAGGTTCCCCATCTTCGTGTAATTCCCTGCAAATCTTGATGAATTTCT
2501
TGTTAACTGGAGTTTTTAGGTTTTGGATTTGGGAAAGTGCTTCCTCTTTAGTAAGAGAGCACTGTGGATATGTGAGGAAATAGTTCCTCGATTGAACTCT
2601
AAATTTCTTAGGCGG
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Fig. 2. Nucleotide sequence of the infectious bean dwarf mosaic geminivirus DNA-A component insert in pBDA1. Nucleotide 1 is the beginning of the 187-nt common region. GenBank accession no. = M88179.

between BL1 and the common region is considerably smaller in SqLCV than in BGMV-PR, BDMV, AbMV, and TGMV.

Nucleotide sequence comparisons indicated that BDMV is distinct from other whitefly-transmitted geminiviruses, including BGMV-PR, which is the other bean-infecting geminivirus that has been sequenced. Nucleotide sequence identities for the pairwise comparisons of the most conserved ORF of DNA-A (AR1) and DNA-B (BL1) among AbMV, BDMV, BGMV-PR, SqLCV, and TGMV (Table 1) indicated that BDMV is most similar to AbMV (86 and 86% identities for AR1 and BL1, respectively) and least similar to SqLCV (81 and 72% identities for AR1 and BL1, respectively). Comparisons of common region nucleotide sequences among these five geminiviruses further support the conclusion that BDMV is a distinct geminivirus and most similar to AbMV (Table 1).

In each bipartite geminivirus, the nucleotide sequences of the DNA-A and DNA-B components are different, except for a highly conserved ~200-nt sequence referred to as the common region (25,28). Since the common region sequence is a characteristic feature for each bipartite geminivirus (28), common region comparisons may be indicators of the relatedness of bipartite geminiviruses (30,39,40). The BDMV common region, determined from comparison of DNA-A and DNA-B, is 187 nt long and 96% identical. By contrast, the BDMV common region sequence showed 79% identity with that of AbMV and less than 70% with four other geminiviruses (Table 1). The BDMV common region contains a stretch of 33 nt that can form a stem-loop structure (28) consisting of a stem of nine GC and two AT pairs and a loop of 11 nt including the invariant sequence TAATATTAC. This stem-loop region is a likely site for the origin of DNA replication (44). A direct repeat of TACTC is located at nt positions 25–29 and 39–43 in BDMV. This same repeat is present in the closely related virus, AbMV, at nt 28–32 and 42–46. The common region of BGMV-PR does not contain the direct repeat of TACTC, but does have a longer direct repeat of TGCGAGTGTCTCCAA at nt positions 18–32 and 40–54. Whether these direct repeats have functional significance remains to be investigated.

Pairwise comparisons of the derived amino acid sequences for the six ORFs in DNA-A and DNA-B of the two bean-infecting viruses, BDMV and BGMV-PR, were made (Table 2). The percent amino acid identities for these pairwise comparisons of AR1, AL1, AL2, AL3, BR1, and BL1 were 87, 80, 65, 71, 71, and 83%, respectively. Thus, as with the nucleotide sequence com-

pBDB1

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1
TGGCATTTTTGTAATAAGAGATGGTACTCCAGTTGAGTTACTCCAATTCCCTCCCCTCAAAACTATCTCATTCTATTGGAGTATTGGAGTTACTTAAATA
101
CTAGAACCCTCAATCTGGTTTCGGAACACGTGGCGGTCATCCGTATAATATTACCGGATGGCCGCGCTTCGGAGTACGCTCTCTCCTCTCCCCTGGTG
201
CGTTCCTGGTCCCCTGCCACCTGCCACTCTCCTAGTGGATGGTCGCTCGTCTTTTCCTGCGAGTTGTGGGCCGTAGTTTGAATGATTAATCTTTAATTTA
301
AATTAAAGATGACTTTTACATGTCGCGCGATCTCATTTGAATCTTGAATAATTGTCTCGCGGTTCATGACTACGGCCCACTGTACTATATAATGGACGTG
401
GCTGATTTTAGACCATGCTGCTATGTCTATTTATTCAATTTGAACAACCATTTTCTATAAATGAGGACGGACCATACATTCCATTCAAGCTGACTCAGCT
501
GTCAACGACGTGAAAATATCTATTTGTATTGGTATTATCGCCTAATAATGTATGGTTTGCGGAATAAACGTGGTTCATCGTTCAGCCATCGCCGATTTTA
601
TTCACGTAGCAGTTTTTTAAATCGCTTGTCCGCTAATAAGCGTCATGATGGCAAACGTCGAGCTATGAATCCTAGTAAGCCCATTGACGAGCCCAAGATG
701
TCAGCCCAACGCATACATGAGAACCAGTATGGGCCTGAATTTGTAATGGCCCATAATTCAGCCATTTCTACGTTTATCAGCTACCCCAGCAAGGGCAAGA
801
TGGAACCCAACCGATCGAGGTCCTATATTAAGTTGAAACGACTTCGTTTCAAAGGGACTGTCAAGATTGATCGTGTTCAACCAGATATGAACATTGACGG
901
TTCTGCCCCAAAAGTGGAAGGAGTGTTCTCTCTGGTGGTTGTTGTGGATCGTAAACCCCACTTGGGTGCGTCTGGATGCCTGCATACATTCGACGAGCTG
1001
TTCGGTGCAAGGATCCATAGCCATGGTAATCTCAGCATAACACCCTCTTTGAAAGACCGATTCTACATAAGACACGTGTTCAAACGTGTATTGTCCGTGG
1101
AGAAGGATACGATGATGGTTGACGTGGAAGGATCTACATCGCTCTCTAACAGGCGATATAATTGTTGGTCCACTTTTAAGGATCTTGACCATGAGTCATG
1201
CAAGGGTGTTTATGACAACATCAGCAAGAACGCCCTCCTAGTATATTACTGTTGGATGTCAGATACTATGTCAAAGGCATCTACTTTTGTATCGTTTGAC
1301
CTTGATTATATCGGTTGATTAATGATAATTGTAATAAAAAGCTATTATTGAACTTTCAATTCCTCAACAAAGAAATTATTGCAACGATTTGGGCTGATAA
1401
GCCTTACAGTTACTATTTATACACTCCTGGACAGTGTTTTTCACTAGCTCGTTTAATTGCCCCATCGACATAGTAATGTTGGATTCCGCTCTCTGGGCCC
1501
CTACAATTGAGGCAGACTCCCCTGGGTCTAAGACGCTTGTTCCAAGCCTGCTGAGATGCCTATATGGATGCATTGCGTTTTCCACCTCTGAGTCGGCATC
1601
GGAGTTGCTGAGCCCAATTGTACTCCGTGAAGCCCATGATTCACCCGGCTTGATCTCTATTGGGCCTGGTAGTCCAATCCTTGACATGGATGCGCATCTT
1701
ATGGGTTTCCTTTCCCATCTTCCGTAGTCGACATGTGAAAAGTCGACATCTTTATCTGTGAACTGTTTCGACAGGATCTTTACTGTCGGTGCCCGGAAGG
1801
GTATATCCACGGAGTGTTTCGCCGTCGATAGTTTCAGTTTCCCTTTGAACTTGGCGAAGTGGGTCCTCTGATGAACATTCGTATCGCAAACCCTATAATA
1901
GAGTTTCCATGGAATTGGGTCTTTCAACGAGAAGAACGAAGCCGAGAAATAATGTAGATCTATGTTGCATCTGATTGGAAATGTCCAGGAAGCCTGCAGC
2001
GATTCGTTGTCTGTCATTCGCTTGTCGTGGATCTCGACAATGACCGACCCTGTCGCGTTAATCGGCACTTGTTGCCTGTACTCTATGACGCAGTGGTCGA
2101
TCTTCATGCAGCTACGGCTCAGCCTAGCTGTTAACTGCGACGCCGTGGAAGGGAATTGCAGAATTATCTCAGTGAGGTCATGAGAAAGTTGATATTCGTC
2201
CCGATGGGACTCTATGTAGTTAAATGCGTTGGGAGGATTGACCAATTGAGAATCCATATAAAGAATAATGGCCGCGCAGCGGAACCGATTGCTGAAGTTG
2301
AACTGGTGAAGAAGAATTTAGGGCTGTAATTGAAGAACAAATGATGAACTGTTCTTGAATGTGGAGAGGGTTTCTGGGAAACTCAGAAAGTTTGTAAAGA
2401
AATTGATGAACAGTTGTTGAACTTCTGCTGAATATGAGATGTTTTTTGAGAAAGAGTAGAAAGCTGAAGAGGAATTACTTGTTTATACTCTGCTAGATCT
2501
GTTAGGGTTTATATAGAAGGTTAGAATCTGTGTTTATAGTTGAGAGCTTCCACGAGAAGTCTATAATAGAAGAGAA
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Fig. 3. Nucleotide sequence of the infectious bean dwarf mosaic geminivirus DNA-B component insert in pBDB1. Nucleotide 1 is the beginning of the 187-nt common region. GenBank accession no. = M88180.

parisons, the most highly conserved amino acid sequences between BDMV and BGMV-PR were those of AR1 and BL1.

Because Howarth and Vandemark (26) had used AR1 amino acid alignments to construct phylogenetic trees for monopartite and bipartite geminiviruses, the derived amino acid sequence of AR1 of BDMV was compared with those of the AR1 for AbMV, BGMV-PR, PYMV, SqLCV, and TGMV. All five identities were greater than 86% (Table 1). The lowest identity (87%) was for BDMV and BGMV-PR. Again, this indicated that BDMV should be considered a different virus from BGMV-PR. This high conservation of the derived amino acid sequence of the coat protein among whitefly-transmitted geminiviruses explains why serological studies with polyclonal antisera (37) showed that whitefly-transmitted geminiviruses are very closely interrelated, yet distinct from, leafhopper-transmitted geminiviruses, which also have very similar amino acid sequence homologies for their coat proteins (26). Briddon et al (2) showed that the coat protein of BCTV, a leafhopper-transmitted geminivirus, determines its vector specificity, and it is likely that the coat proteins of whitefly-transmitted geminiviruses similarly determine their vector specificities (26).

From these nucleotide and amino acid comparisons, it is evident that BDMV is most closely related to the AbMV isolate from the West Indies. Nucleotide sequence identities for comparisons of the common region and ORFs were higher between BDMV and AbMV than among four other western hemisphere, whitefly-transmitted geminiviruses (Table 1). The importance of understanding the relatedness of geminiviruses has recently been shown by the creation of infectious pseudorecombinants (heterologous mixtures of DNA-A and DNA-B) from strains of BGMV (12). Gilbertson et al (17) showed that BDMV-CO is closely related to tomato mottle geminivirus from Florida, and subsequently, that a pseudorecombinant between these two distinct geminiviruses would infect the common host, *N. benthamiana* (24). Pseudorecombination between distinct geminiviruses may be an important way in which new geminiviruses evolve. Futhermore, the inability to form pseudorecombinants between the components of TGMV and SqLCV (30), bipartite geminiviruses with a low common region sequence identity (<70%), suggests that the com-

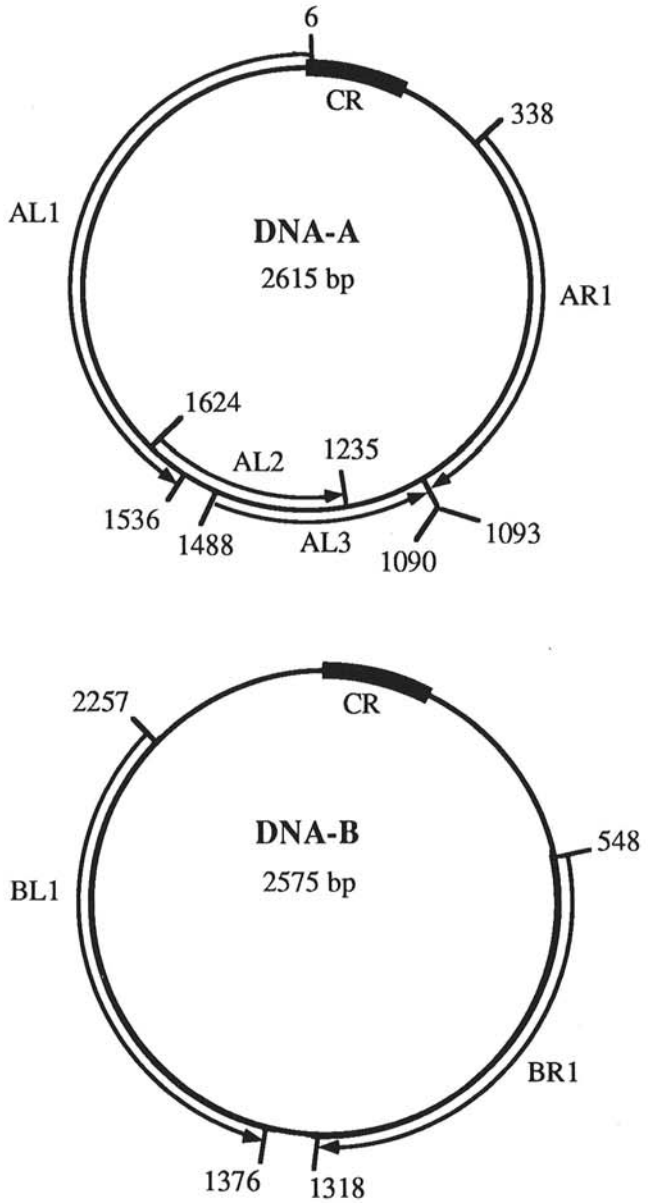

Fig. 4. Genome organization of bean dwarf mosaic geminivirus DNA-A and DNA-B. Open reading frames (ORFs) are designated A or B (DNA-A or DNA-B component, respectively), R or L (rightward or leftward transcription, respectively), and 1, 2, or 3 (position of ORF in relationship to common region). The nucleotide position of the beginning and end of each ORF is indicated. The common region (CR) is represented by the wide bar. Nucleotide 1 is the beginning of the common region.

TABLE 1. Nucleotide and derived amino acid sequence identities for AR1 and BL1 open reading frames, and nucleotide sequence identities for the common region for pairwise comparisons between bean dwarf mosaic geminivirus and five bipartite geminiviruses

	Percent identities		
Geminiviruses	Common region[a] (nt)	AR1 (nt/aa[b])	BL1 (nt/aa)
BDMV × AbMV[c]	79	86/92	86/98
BDMV × TGMV	61	80/90	75/82
BDMV × BGMV-PR	68	79/87	76/83
BDMV × SqLCV	53	81/90	72/77
BDMV × PYMV	62	85/94	78/87

[a] 187-nt common region of BDMV was compared to those of the other five geminiviruses using the GAP program of GCG with parameters of gap weight = 5 and gap length weight = 0.3.

[b] nt = nucleotide, aa = derived amino acid.

[c] BDMV = bean dwarf mosaic geminivirus. AbMV = Abutilon mosaic geminivirus (Original sequence [15] modified by substitution of a G at nt 360. This resulted in a start codon for the AR1 ORF identical to that for BDMV [39], and only the amino terminal 456 nt and 152 derived amino acids of BL1 were used in the pairwise comparison because of suspected sequencing errors starting between nt 1798 and 1799 which caused a frameshift. The identity for amino acids 153–293 was 35%). TGMV = tomato golden mosaic geminivirus [20] original sequence corrected in von Arnim and Stanley [45]; BGMV-PR = bean golden mosaic geminivirus from Puerto Rico [25] sequence corrected by inserting a G between nt 394 and 395 in DNA-A ([A. Howarth, *personal communication*]). SqLCV = squash leaf curl geminivirus (29). PYMV = potato yellow mosaic geminivirus (8). The nt and aa identities were obtained from comparisons of the nt or aa for the total ORF except for the specific case of BL1 of AbMV.

TABLE 2. Nucleotide and derived amino acid sequence identities between open reading frames of bean dwarf mosaic geminivirus and bean golden mosaic geminivirus

	Percent identity to BGMV-PR[b]	
ORF[a]	Nucleic acid	Amino acid
AR1	79	87
AL1	76	80
AL2	73	65
AL3	77	71
BR1	70	71
BL1	76	83

[a] Open reading frame (ORF); See Figure 4 for position of ORFs in DNA-A and DNA-B.

[b] Bean golden mosaic geminivirus from Puerto Rico (25; sequence corrected by inserting a G between nt 394 and 395 in DNA-A [A. Howarth, *personal communication*]).

mon region sequence homology may be an important indicator of relatedness among geminiviruses (24,30,39,41).

Reaction of bean germplasm to BDMV. Major differences exist in the disease reaction of bean germplasm to different bean-infecting geminiviruses. More than 10,000 bean germplasm lines have been evaluated under field and laboratory conditions, but none are immune to BGMV (14,32). On the other hand, some bean cultivars of middle-American type, such as Pinto 114, Sierra, and Belneb, are resistant to BDMV, whereas some bean cultivars of the Andean type, such as Topcrop, California Dark Red Kidney, and Improved Tendergreen, are susceptible to it (23,33). These differences in reaction of bean germplasm to BDMV and BGMV support our conclusion that these two viruses are distinct.

In summary, our results and those of others (16,33) clearly demonstrate that BDMV-CO is a distinct bean-infecting geminivirus. BDMV-CO can be distinguished from other bean-infecting geminiviruses, such as BGMV-PR (25), BGMV-BZ (15), and bean calico mosaic geminivirus (3) (A. O. Loniello, R. T. Martínez, M. R. Rojas, R. L. Gilbertson, J. B. Brown, and D. P. Maxwell, *personal communication*) by differences in nucleotide sequence, organization of the common region (e.g., presence of direct repeats), symptoms, host range, and reaction of bean germplasm. Also, the BDMV-CO isolate can be sap-transmitted to beans and, therefore, may be different from the nonsap-transmissible geminivirus isolates causing dwarfing of beans in Argentina (33). Thus, for the development of control strategies, it is necessary to consider BDMV-CO a separate bean-infecting geminivirus. Moreover, because of the availability of sequenced, infectious clones (pBDA1 and pBDB1) of BDMV-CO and the biological properties distinguishing BDMV-CO from the golden mosaic-inducing bean-infecting geminiviruses (15,33), future research can be conducted to identify viral domains associated with biological properties such as symptom expression and host-range determinants.

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