Genetic Diversity in Geminiviruses Causing Bean Golden Mosaic Disease: The Nucleotide Sequence of the Infectious Cloned DNA Components of a Brazilian Isolate of Bean Golden Mosaic Geminivirus

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ABSTRACT

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The complete nucleotide (nt) sequence of a Brazilian isolate of bean golden mosaic geminivirus (BGMV-BZ) was determined from the infectious cloned DNA components. The genome of BGMV-BZ was divided between two DNA components, DNA-A (2,617 nt) and DNA-B (2,580 nt), which had little sequence identity except for a 181-nt common region present in both molecules. The genomic organization of BGMV-BZ was similar to that of other bipartite whitefly-transmitted geminiviruses, with six open reading frames (ORFs) encoding putative proteins >10 kDa, four on DNA-A (one in the viral [+] and three in the viral complementary sense [−]) and two on DNA-B (one in the viral and one in the viral complementary sense). All six BGMV-BZ ORFs were analogous to corresponding ORFs in other whitefly-transmitted geminiviruses. The homology between BGMV-BZ sequences and those of a BGMV isolate from Puerto Rico (PR) were relatively low. BGMV-BZ sequences were no more closely related to BGMV-PR than to other distinct geminiviruses from South America, such as tomato golden mosaic geminivirus. Pseudorecombinants made by mixing the infectious cloned DNAs of BGMV-BZ and a BGMV isolate from Guatemala (GA), which was very similar to BGMV-PR, were not infectious. These results support previous observations of differences in sap-transmissibility and bean genotype reactions between BGMV isolates and indicate that at least two distinct types of BGMV exist, designated type I and II.

Additional keywords: Phaseolus vulgaris.

Geminiviruses are a unique group of plant viruses characterized by twin (geminate) isometric virion and a single-stranded, circular DNA genome (21,27,40). Geminiviruses currently are subdivided into three major subgroups (11): 1) those that have a bipartite genome, that are transmitted by whiteflies (*Bemisia tabaci* Gennadius), and that infect dicotyledonous plants; 2) those that have a monopartite genome, that are transmitted by leafhoppers, and that infect monocotyledonous hosts; and 3) those that have a monopartite genome, that are transmitted by leafhoppers, and that infect dicotyledonous plants. The genome of the whitefly-transmitted geminiviruses usually is divided between two DNA components, DNA-A and -B, both of which are required for infectivity (16,19,32,39). However, some tomato yellow leaf curl geminivirus strains, which are whitefly-transmitted, have a single genome component similar to the DNA-A of the bipartite geminiviruses (33).

The complete nucleotide (nt) sequences of the infectious cloned DNAs of bipartite geminiviruses have been determined for Abutilon mosaic virus (AbMV; [13]), African cassava mosaic virus (ACMV; [41]), bean dwarf mosaic virus (BDMV; [23]), bean golden mosaic virus from Puerto Rico (BGMV-PR [24], sequence corrected by A. Howarth, *personal communication*, by the addition of a G between nt 394 and 395), potato yellow mosaic virus (PYMV; [5]), squash leaf curl virus (SqLCV; [28]), tomato mottle virus (ToMoV; [1,18]), and tomato golden mosaic virus (TGMV, 20; corrected in [46]). The DNA-A and -B components from these geminiviruses have little sequence identity except for ~200 nucleotides of noncoding DNA, called the common region, that is almost identical between the two components of any single geminivirus, but that is highly divergent among different geminiviruses.

Bean golden mosaic disease of common bean (*Phaseolus vulgaris* L.) and several other legumes is characterized by a brilliant golden mosaic pattern on infected leaves and by stunted plant growth. The disease is a major constraint on dry bean production in Central and South America and the Caribbean Basin (4,14). BGMV-PR, characterized by Howarth et al (24), was collected from lima bean (*Phaseolus lunatus* L.) in Puerto Rico and was readily sap-transmitted to *P. vulgaris* in which it induces golden mosaic symptoms. In contrast, the geminivirus(es) causing bean golden mosaic in Brazil (BGMV-BZ) are nonsap-transmissible (3,4,10,17). Thus, it has been suggested, based on differences in sap-transmissibility, bean genotype reaction to BGMV isolates, and DNA hybridizations with specific DNA probes for bean-infecting geminiviruses, that genetic variation may exist among geminiviruses causing bean golden mosaic in the Americas and the Caribbean Basin (4,17,31). This research was undertaken to determine the genetic relationship between the geminivirus(es) causing golden mosaic symptoms on beans in Brazil and BGMV-PR.

In this report, we present the complete nucleotide sequence of the infectious cloned DNAs of a nonsap-transmissible isolate of BGMV from Brazil (16,17) and show that genetically distinct viruses are presently designated as BGMV.

MATERIALS AND METHODS

Virus isolate and viral recombinant plasmids. A field isolate (GO 87-1) of BGMV-BZ was collected from beans (*P. vulgaris*)

showing golden mosaic symptoms at Centro Nacional de Pesquisa Arroz-Feijão (CNPAF), Goiânia, Goiás, Brazil, and was maintained in beans by whitefly transmission in a screenhouse (17). Recombinant plasmids with infectious, full-length clones of DNA-A (pBZA1) and DNA-B (pBZB1) of this BGMV-BZ isolate and of DNA-A (pGAA1, GenBank no. M91604) and DNA-B (pGAB1, GenBank no. M91605) of a sap-transmissible BGMV isolate from Guatemala (BGMV-GA) were previously described (16). The BGMV-BZ DNA-A and -B components were cloned at unique *Hin*dIII and *Acc*I sites, respectively, into pBluescript (+)

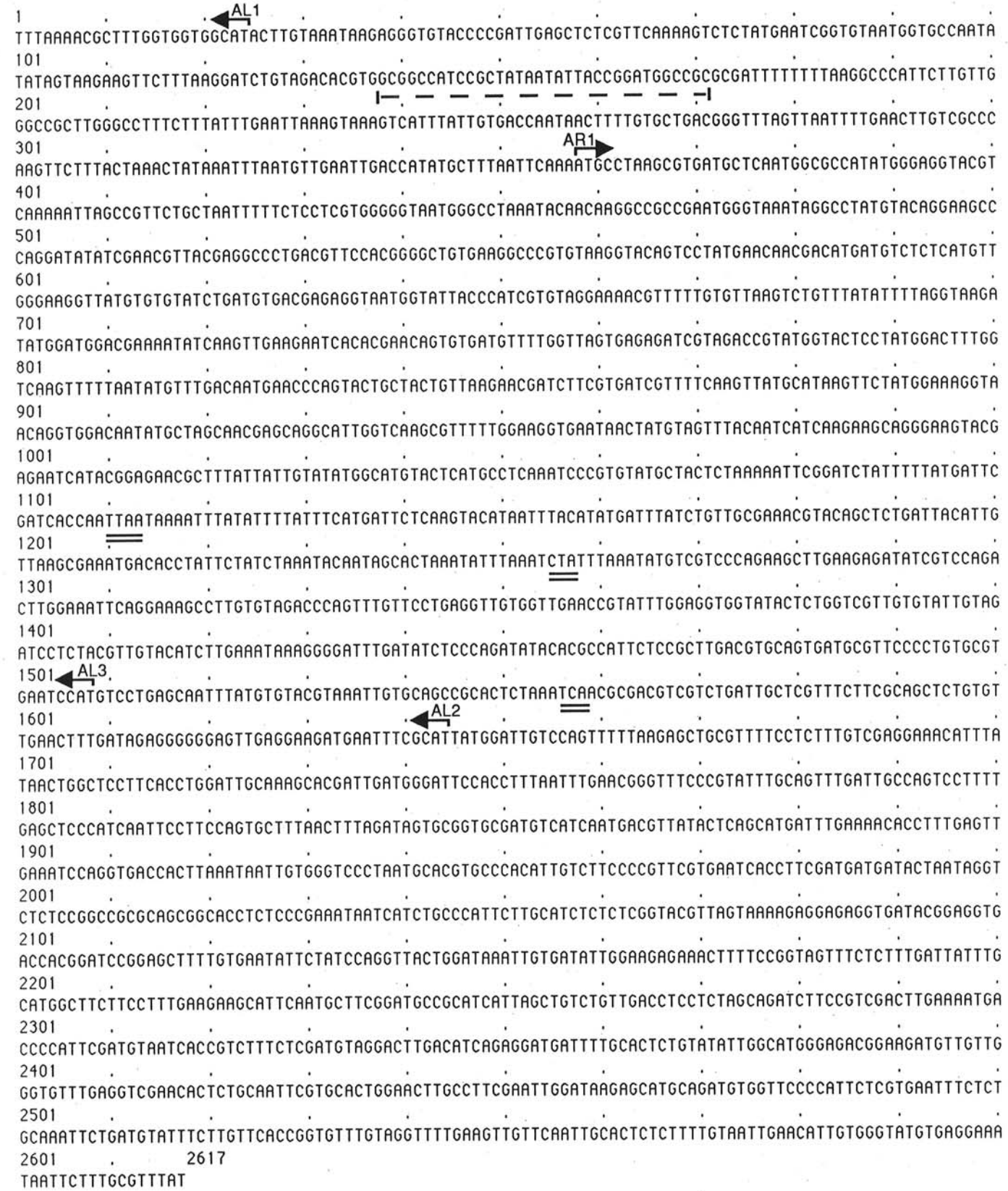

Fig. 1. Nucleotide sequence of the presumed viral sense strand of DNA-A from a bean golden mosaic geminivirus isolate from Brazil. Position 1 is the first nucleotide of the common region, which is shared between DNA-A and -B. The possible stem-loop region conserved in all geminiviruses is located between nt 138 and 171 (underlined with a dashed line). The putative start and stop codons are marked with an arrow and a double underline, respectively. (GenBank no. M88686.)

(Stratagene, La Jolla, CA).

Nucleotide sequencing of BGMV-BZ clones and analysis. Sequencing was accomplished by the dideoxynucleotide chain termination method on single-stranded templates. A library of nested deletions of the inserts in pBZA1 and pBZB1 in each orientation was generated with *Bal*31 exonuclease (30) or exonuclease III (22). The nucleotide sequence of the two full-length clones was determined for both DNA strands. Independent clones of DNA-A and -B, which included the cloning sites for the inserts in pBZA1 and pBZB1, were obtained with *Hin*cII (plasmid = pBZA2) and *Cla*I (plasmid = pBZB2), respectively; sequence determination of these inserts across cloning sites (*Hin*dIII for DNA-A and *Acc*I for DNA-B) showed the inserts in pBZA1 and pBZB1 were full-length clones.

Sequences were assembled and analyzed by programs from the University of Wisconsin Genetics Computer Group (6). Sequence identities between nucleotide and derived amino acid sequences were determined by the GAP (gap weight of 5.0 and gap length

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1
TTTAAAACGCTTTGGTGATGGCATACTCGTAAATAAGAGGGTGTACCCCGATTGAGCTCTCGTTCAAAAGTCTCTATGAATCGGTGTAATGGTGCCAATA
101
TATAGTATGAAGTTCTTTAAGGATCTGGAGACACGTGGCGGCCATCCGTTATAATATTACCGGATGGCCGCGCGATTTTTTCCCCTCTGAAATCCTGGCC
201
GTTTATTTCAGTTCCGCAAAACTAGTTGAGCGTGTTTTTGAAATCCGTTGTCCATGTGTTGACACGTGTTCCATACAGGGGCTTATTTGTCATATTAGGT
301
GATGACCAACTTTAGTTTTGCACGTGGCGCGTTTTACAATGTTGCATTTAATTGGAAATTTTTATCTAACGGCTATATTTGATTCAAATTTTGAATCATG
401
TGAGAGTTGTCAGAAATTGGCTTTATATTTCTGACATTGTACTATATATTGAGATGGACCAATTAAATGCCTTGTTTCGTGTCTATTTAAATATTGTTGT
501
AGATATATTGTGTATAAATTGTGTTCATTTTTATTAAGGAGACATGTGTATTTGAAAATGTATTTTTCGCGAAACAAACGTGGTGCCACTACTACTTCGA
601
GACGAAGCTATTCAAGGTATCCCTTGTCTAGGCGTTCGTATAGTGTGAATAGAATCAATGGCAAACGTGGATCGAATATTCATAGAAAGGCCCATGATGA
701
TGGTAAAATGTTAGCCCAACGTTTACATGAGAATCAGTTTGGGCCTGAATTTGTCATGGCCCATAATTCAGCGATATCAACGTTTATTAATTTCCCTAGT
801
CTTGGTAAGACTGAACCGAATCGATCAAAGAGCTTTATTAAGTTAAAACGTTTACGTTTTAAGGGTACTGTTAAAATTGAACGTGTTTTGAATATGGATG
901
GTTCAACTCCTAAGATTGAAGGCGTATTTTCCATGGTCCTTGTTGTTGACCGAAAACCTCACTTGGGATCATCTGGATGTCTCCATACATTTGATGAATT
1001
GTTCGGTGCAAGGATTCATAGTCACGGTAATTTAGCCATTACTGCCACATTGAAAGAGCGATTCTATATACGTCATGTTTATAAGCGTGTGCTTTCAGTT
1101
GAGAAGGATAGCATGATGGTCGACGTCGAAGGAAGCACATACTTATCTAGTAGGCGTTTTAATTGTTGGGCTACATTTAAAGATGATGAACGTGACTCTT
1201
GTAATGGCGTTTATGCTAACATAAGCAAGAATGCTCTTTTAGTCTATTATTGTTGGATGTCGGATGCTATGTCCAAGGCATCTACGTTTGTATCATTTGA
1301
TCTTGATTATGTTGGATGAATAATAGTAAGTTTCAAATAAGCCCTGTATATTTCCTGTTTATAGTTGCTTAAAATATTTATAAAATATTTTATTTCAAC
1401
GACTTTGGTTGAGCAGGAGTACAATTACTGTTAATACATTCTTGGGCCGCAGTTTTAACCAATTCGTTTAATTGGGCCATTGAAAGGGTGATACTTGACT
1501
CGGTTCTTCGCGCTCCAACTATAGATGCGGAATCTCCAGGGTCTAGAATACTTGTGTCCAATCTATGAAGTTGTCTATATGGATGTACTGCGTTGTCTAA
1601
TTCAGAGTCCGCATCTGAAGTACCGGTTCCTATTGTACTTCTTGTTGCCCATGTCTCACCGGGTTTTAATGTAATTGGGCTGTGAAGCCCATATCGAGAT
1701
GTGGATGCGGACTTTATTAATTTCCTCTCCCATTTCCCGTAACCAACGTGGGAGAAATCCACATCTTTCTCAGTGAATTGTGTAGATAATATTTTCACGG
1801
TTGGGGGCTTGAAAGGAATATCCACCGAATGTTTCGCAGTGGATATCTTCAGTTTCCCCTTAAATTTTGCGAAGTGTGTTCGTTGATGAACATTTGTGTC
1901
GCTAACTCTGTAATACAATTTCCAGGGAATTGGGTCTTTAAGGGAGAAGAATGAAGATGAAAAAAAGTGGAGATCTATGTTACACCTTATGGGATATGTC
2001
CATGATGCTTGTAATGACTCATTATCCGTCATTCTTTTGTCGTGAATCTCCACAATCACAGTTCCTGTTGCGTTGATAGGAACTTGTTGCCTGTATTCAA
2101
TGACGCAATGATCTATCTTCATACAGCTTCGACTTAATCTGGCGCTTATTTGAGCTGTTGTTGAAGGAAATTGTAATACTATCTCATTCAAGTCATGAGA
2201
CAGTTGATATTCATCACGGTTAGATTCTACATAATTGAACGCTGTTGGTGGATTAACTAATTGAGACTCCATTATCAACTGAATATAAGAATGAATGGCC
2301
GCGCAGCGCACTCGGTTCGGGAGTTTATGATGCAGAGACTATAAAAGAGCTGTTTTCTTGCTTAAGAACAATTTGACAGAAAGGAAGGAAGAGAAGTTGT
2401
TATATATGATAATGGGCGTTCCCCAATGTTTAAATAGACAAGTTTATTTTCGTACAACTCTGTAAAGTAATCAATGATTATGTATGTTCAAGTTGGACAA
2501
GTAATCATTTTTTACTTTATTAAATGGTGTTTATTTAGAGTAAACTTATGTGTATCTGTGTGTATAAGTTTGTGTTAAAG
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BR1

BL1

.2580

Fig. 2. Nucleotide sequence of the presumed viral sense strand of DNA-B from a bean golden mosaic geminivirus isolate from Brazil. Position 1 is for the first nucleotide of the common region. The possible stem-loop of the common region is underlined with a dashed line, and putative start and stop codons are marked with an arrow and a double line, respectively. (GenBank no. M88687.)

weight of 0.3) and BESTFIT programs.

Infectivity of pseudorecombinants between the DNA components of BGMV-BZ and those of BGMV-GA. Pseudorecombinants between the infectious DNA-A and -B clones of BGMV-BZ and -GA were formed by mixing the excised DNA-A monomer of BGMV-BZ from pBZA1 or of BGMV-GA from pGAA1 with the excised DNA-B monomer of BGMV-BZ from pBZB1 or of BGMV-GA from pGAB1. Viral DNAs were inoculated into *P. vulgaris* (cv. Topcrop) by electric discharge particle acceleration as previously described (16). Beans were grown in plant growth chambers, and characteristic golden mosaic symptoms developed within 10–14 days. Infections were confirmed by squash blot hybridization methods with ^{32}P-labeled viral probes for BGMV-BZ and -GA (17).

RESULTS

Nucleotide sequences of infectious cloned BGMV-BZ DNA components. The infectious inserts of DNA-A in pBZA1 and DNA-B in pBZB1 of BGMV-BZ (16) were sequenced. DNA-A had 2,617 bp (Fig. 1; 26% A, 19% C, 23% G, and 32% T), whereas DNA-B had 2,580 bp (Fig. 2; 28% A, 16% C, 21% G, and 35% T). The nucleotide sequences of BGMV-BZ DNA-A and -B had little sequence identity, except for a 181-nt region that was 97% identical and was the characteristic common region of the bipartite geminiviruses (27). The 5′ end of the common region was designated nt 1 (20,41).

Potential coding regions. The virion and complementary sense sequences of DNA-A and -B were analyzed in all six frames for open reading frames (ORFs) encoding proteins >10 kDa (Table 1). DNA-A had four ORFs (Fig. 3), one in the rightward or viral sense direction (AR1) and three in the leftward or viral complementary sense direction (AL1, AL2, and AL3). AL1, AL2, and AL3 were in different reading frames; the 3′ end of AL1 overlapped the 5′ end of AL2, and AL2 overlapped all but the 3′ end of AL3. AR1 and AL3 overlapped in opposite directions in an AT-rich region and shared 4 nt at their respective C-termini, which contained the translational stop codons. This overlap of the AL3 and AR1 ORFs also is found in other bipartite geminiviruses (13,20,28). An AL4 ORF (nt 2,227–2,464) encoding a 9.5-kDa protein was present within the N-terminus of the AL1 ORF; however, genetic analyses of the AL4 ORFs of ACMV (8) and TGMV (7) indicated that this ORF had no role in infectivity or symptom development. On DNA-B (Fig. 3), two nonoverlapping ORFs were identified; one in the rightward direction (BR1) and one in the leftward direction (BL1). A 71-nt intergenic region was located between the 3′ ends of these ORFs.

Noncoding and possible control regions. One of the most striking features of the bipartite geminiviruses is the common region, which is highly conserved between the two components of any single geminivirus, but which is quite divergent among different geminiviruses (21,27,37). It is believed that this region contains regulatory elements and is the origin of DNA replication (27,29). The common region of BGMV-BZ was 181-nt long, as compared to the 205-nt common region of BGMV-PR (24). A 20-nt direct repeat sequence in BGMV-PR was not present in the common region of BGMV-BZ nor in the other bipartite geminiviruses (37). Interestingly, when the common regions of BGMV-PR and -BZ were aligned (Fig. 4), the sum of the nucleotides in the two gaps inserted into the BGMV-BZ common region was 30 nt, which was the difference between the total length of BGMV-BZ DNA-A (2,617 bp) and BGMV-PR DNA-A (2,647 bp) (24). As in all sequenced geminiviruses, the common region of BGMV-BZ contained a potential stem-loop structure (nt 138–171), which was involved in viral DNA replication (29,43). Furthermore, within the BGMV-BZ stem-loop region was the loop sequence TAATATTAC (nt 152–160), which resembled the DNA nicking site for the bacteriophage ΦX174 gene A protein (36), also found in all sequenced geminiviruses (37). Outside the conserved stem-loop sequence, there was significant divergence among the common-region sequences of whitefly-transmitted geminiviruses (Fig. 4; [37]), except for the start codon of the AL1 ORF and a TATA-box motif.

TABLE 1. Potential open reading frames (ORFs) identified for bean golden mosaic virus (BGMV-BZ) DNA-A and -B

ORF[a]	Polarity[b]	Nt coordinates		No. of amino acids	Protein MW (Da)
		Start[c]	Stop[d]		
AR1	v	358	1,113	251	29,128
AL1	vc	24	1,556	361	41,023
AL2	vc	1,644	1,255	129	14,558
AL3	vc	1,508	1,110	132	15,824
BR1	v	558	1,319	253	28,969
BL1	vc	2,272	1,391	293	33,031

[a]ORF coding for proteins >10 kDa. A = DNA-A; B = DNA-B; R = rightward transcription; L = leftward transcription; number = number of ORF.
[b]v = viral sense DNA strand; vc = viral complementary sense DNA strand.
[c]Includes the first nucleotide (nt) of the start codon for each ORF.
[d]Includes the last nt of the stop codon for each ORF.

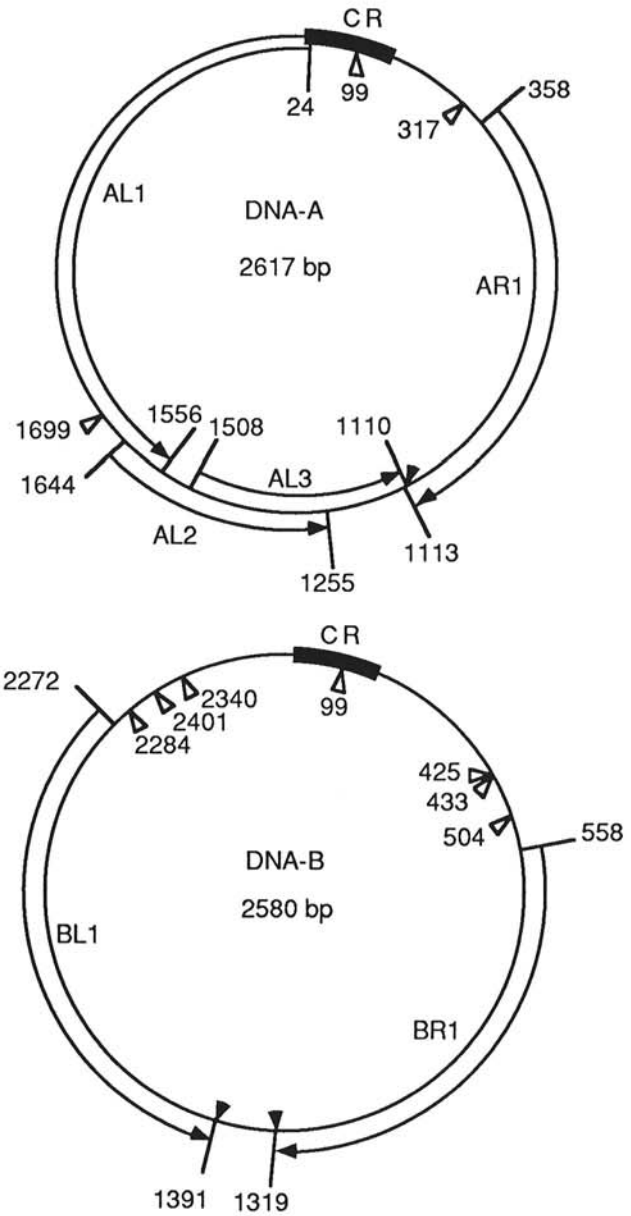

Fig. 3. Potential coding regions identified in DNA-A and -B from a bean golden mosaic geminivirus isolate from Brazil. Arrows indicate the position and orientation of open reading frames with the potential to encode proteins >10 kDa. The position of possible TATA-box and polyadenylation (AATAAA) sequences are shown by open and solid triangles, respectively.

The positions of potential regulatory sequences conforming to the consensus TATA box (TATAA/T; [2]) and the polyadenylation signal AATAAA (35) in the BGMV-BZ genome were similar to those reported for other whitefly-transmitted geminiviruses (Fig. 3; [13,20]). All BGMV-BZ ORFs had one or more possible TATA boxes within 100 nt of the putative start codon, except for the AL3 and BL1 ORFs, the latter of which had a TATA box 135-nt upstream from the start codon (Fig. 3). One TATA box was identified in BGMV-BZ for the AL2 and AL3 ORFs at nt 1,699; this TATA box was 55- and 191-nt upstream from the start codons for the AL2 and AL3 ORFs, respectively. In TGMV, a similar TATA box was present (20). Northern blot analysis and low-resolution S_1 mapping of virus-specific poly(A) RNA from ACMV-infected *Nicotiana benthamiana* Domin. indicated that a single 700-nt transcript might encode the AL2 or AL3 gene products (45), whereas a similar study with TGMV-infected *N. benthamiana* identified two transcripts of different sizes that transversed both AL2 and AL3 ORFs (44). Two complementary sense transcripts corresponding to AL2 and AL3 ORFs of AbMV DNA-A also were detected by Frischmuth et al (12). In these studies, there was no evidence of the RNA processing that has been demonstrated for the complementary sense transcript of leafhopper-transmitted, monopartite geminiviruses (38).

High-resolution mapping of the AR1 ORF (coat-protein gene) transcript from TGMV (34,44) and ACMV (45) showed that the TGMV AR1 transcript had a 7- or 8-nt untranslated leader sequence, whereas that of ACMV was ~160 nt. In BGMV-BZ, as in TGMV, putative promoter sequences (nt 317–320) and a putative translation initiation codon (nt 358–360) were conserved

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          1                                                    50
TGMV-BZ   .......... .GATGCGATG GCATTTTTGT AATTAAGAGG CTTACTA...
BGMV-BZ   TTTAAAACGC TTTGGTGGTG GCATACTTGT AAATAAGAGG GTGTACC...
BGMV-GA   .......... ........TG GCATATTTGT AAATATGCGA GTGTCTCCAA
BGMV-PR   .......... ........TG GCATATTTGT AAATATGCGA GTGTCTCCAA
  CONS    .......... ........TG GCATatTTGT AAaTA.G.G. gTgtctc...

          51                                                   100
TGMV-BZ   .......... ........CC AATTGAGGAG GGGCTCCAAA AGTTATATGA
BGMV-BZ   .......... ........CC GATTGAGCTC TCGTTCAAAA GTCTCTATGA
BGMV-GA   ATGAGTTTGC GAGTGTCTCC AATTGAGGCT CCTCAAACTC TCGCTATGCA
BGMV-PR   ATGAGTTTGC GAGTGTCTCC AATTGAGGCT CCTCAAACTC TCGCTATGCA
  CONS    .......... ........CC aATTGAGgct cc.c..a... tcg.t....A

          101                                                  150
TGMV-BZ   ATTGGTAGTA AGGTAGCTCT TATATATT.A GAAGTTCCTA AGGGG.....
BGMV-BZ   ATCGGT.GTA ATGGTGCCAA TATATAGTAA GAAGTTCTTT AAGGATCTGT
BGMV-GA   ATTGGA.GAC TGGAGTACAA TATATACTAG TACCCTCAAT CTCGTGAATT
BGMV-PR   ATTGGA.GAC TGGAGTACAA TATATACTAG AACCCTCAAT CTCTTGAATT
  CONS    ATtGG..G.. .gGag..caa TATATAcTa. gA...TCa.t .t.gtgaatt

          151                                                  200
TGMV-BZ   .......... ..CACGTGGC GGCCATCCG. TTTAATATTA CCGGATGGCC
BGMV-BZ   AG........ .ACACGTGGC GGCCATCCGC TATAATATTA CCGGATGGCC
BGMV-GA   ATCAGATTCA CACACGTGGC GGCCATCCGA TATAATATTA CCGGATGGCC
BGMV-PR   ATCACATCCA TACACGTGGC GGCCATCCGA TATAATATTA CCGGATGGCC
  CONS    at........ .aCACGTGGC GGCCATCCGa TaTAATATTA CCGGATGGCC

          201                                                  246
TGMV-BZ   GCGCGATCGT CACCCGACCC GCTTCCGCAA ATTACGCCGC ATTGTCG
BGMV-BZ   GCGCGATTTT TT
BGMV-GA   GCCCGCGCCC CTTTATTTCC GTAC
BGMV-PR   GCCCGCGCCC CTTTATATCC GTAC
  CONS    GC.CG..cc. ctttatatCC Gtac
```

Fig. 4. Alignment of the nucleotide sequences of the common regions of tomato golden mosaic geminivirus and bean golden mosaic geminivirus isolates from Brazil (BGMV-BZ), Guatemala (BGMV-GA) (GenBank no. M91604), and Puerto Rico (BGMV-PR). CONS denotes the consensus sequence with conserved nucleotides as uppercase letters, predominant nucleotides as lowercase letters, and positions with no consensus nucleotides as a period (.). Gaps were inserted to achieve maximum alignment of nucleotides. The nearly perfect direct repeat for BGMV-PR and -GA (type II isolates of BGMV) is located between nt 46 and 55 and between nt 67 and 77, as numbered above the alignment. The conserved TATA box is between nt 123 and 126. The stem-loop region is between nt 169 and 202.

in the same position relative to the AR1 ORF. Thus, transcription of the BGMV-BZ AR1 ORF may be similar to that of TGMV.

Putative polyadenylation signals (the AATAAA motif) were located within AT-rich regions at or near the 3′ ends of AR1 and AL3 ORFs and BR1 and BL1 ORFs (Fig. 3). These AT-rich regions were highly conserved in DNA-A and -B of the whitefly-transmitted geminiviruses (12). BGMV-BZ ORFs, AL1, AL2, and AL3, may share a single polyadenylation signal located just inside the 3′ end of ORF AL3 (nt 1,126–1,131). This appears to be the case for TGMV (44) and AbMV (12). The proposed polyadenylation signal for the BL1 ORF of BGMV-BZ included the TAA termination codon. Similarly, a possible polyadenylation signal for the BR1 ORF of BGMV-BZ also included the A nucleotide of the termination codon, as did that of AbMV (12).

Sequence comparisons among whitefly-transmitted geminiviruses. The nucleotide and derived amino acid sequences of the BGMV-BZ AR1, AL1, and BR1 ORFs were compared to those of bipartite, whitefly-transmitted geminiviruses AbMV, ACMV, BDMV, BGMV-PR, PYMV, SqLCV, ToMoV, and TGMV. Previous phylogenetic studies of geminiviruses with the sequences for the AR1 and AL1 ORFs (25) demonstrated the clear divergence of the monopartite, leafhopper-transmitted, monocotyledon-infecting geminiviruses and the bipartite, whitefly-transmitted, dicotyledon-infecting geminiviruses, as well as the separation of the latter group into western hemisphere and eastern hemisphere subgroups. The results of the nucleotide and amino acid comparisons showed that BGMV-BZ was most similar to the bipartite whitefly-transmitted geminiviruses from the western hemisphere (Table 2). The AR1 ORF, which encoded the coat-protein gene in TGMV (26) and ACMV (45), had the most conserved nucleotide and amino acid sequences among the whitefly-transmitted geminiviruses. The derived amino acid sequence of the BGMV-BZ AR1 ORF was more than 94% similar to those of the bipartite geminiviruses from the western hemisphere (AbMV, BDMV, BGMV-PR, PYMV, SqLCV, ToMoV, and TGMV), and more than 80% similar to that of ACMV, which was from the eastern hemisphere (Table 2). Similar trends were evident for the sequence comparisons of the AL1 ORF and the most divergent ORF, BR1. These comparisons also clearly showed that BGMV-BZ diverged surprisingly from BGMV-PR, which caused almost identical disease symptoms in beans. The BGMV-BZ sequences were no more related to those of BGMV-PR than to those of distinct geminiviruses from South America, such as TGMV and PYMV (Table 2). For example, the derived amino acid sequence of the BGMV-BZ AL1 ORF was 81% similar to that of BGMV-PR versus 87% similar to those of TGMV and PYMV. Additionally, the common-

TABLE 2. Percent nucleotide identities and derived amino acid similarities between the AR1, AL1, and BR1 ORFs (open reading frames) of bean golden mosaic virus (BGMV-BZ) and the respective ORFs of other bipartite whitefly-transmitted geminiviruses

	AR1		AL1		BR1	
Geminivirus[a]	nt[b]	aa[c]	nt	aa	nt	aa
BGMV-PR	82	95	71	81	72	82
BDMV	83	95	75	85	73	83
ToMoV	81	95	75	85	70	81
TGMV	80	95	75	87	73	81
PYMV	81	96	74	87	67	76
AbMV[d]	80	94	73	84	69	82
SqLCV	82	97	67	74	67	78
ACMV	64	80	64	74	43	48

[a] BRMV-PR = bean golden mosaic virus from Puerto Rico; BDMV = bean dwarf mosaic virus; ToMoV = tomato mottle virus; TGMV = tomato golden mosaic virus; PYMV = potato yellow mosaic virus; AbMV = Abutilon mosaic virus; SqLCV = squash leaf curl virus; and ACMV = African cassava mosaic virus.

[b] Percent nucleotide (nt) identity.

[c] Percent derived amino acid (aa) similarity.

[d] AR1, AL1, and BL1 sequences of AbMV (13) were amended (18,23) based on results from alignments with corresponding sequences of other geminiviruses.

region sequence of BGMV-BZ was 66, 58, 58, 57, 56, 56, and 52% identical to those of TGMV, PYMV, ToMoV, BGMV-PR, AbMV, SqLCV, and BDMV, respectively. These common-region comparisons also indicated that BGMV-BZ was not more closely related to BGMV-PR than to the other geminiviruses with western hemisphere origins.

Pseudorecombinants between cloned DNAs of BGMV-BZ and -GA. Pseudorecombinants of the infectious cloned DNAs of BGMV-BZ and -GA, which had a >95% sequence identity to BGMV-PR in DNA-A (9), were made in all combinations, and beans were inoculated by electric discharge particle acceleration. Systemically infected beans were obtained only with the homologous combinations of DNA-A and -B components and not with either of the two heterologous mixtures of the DNA components of BGMV-BZ and -GA (Table 3).

DISCUSSION

Our results show that the genomic organization of BGMV-BZ is similar to that of other bipartite whitefly-transmitted geminiviruses. However, several lines of evidence indicate that BGMV-BZ differs significantly from these geminiviruses, including other isolates designated BGMV from Puerto Rico (BGMV-PR), Guatemala (BGMV-GA), and the Dominican Republic (BGMV-DR), which are closely related to each other (nucleotide identity >95% for DNA-A [9,15]) but less related to BGMV-BZ (~75% nucleotide identity). These results support previous evidence indicating that differences (e.g., sap-transmissibility, bean genotype reaction, and symptomatology in soybeans) exist among BGMV isolates (10,14,16,17). First, based on DNA and derived amino acid sequence comparisons (Table 2) and on phylogenetic analyses of the AL1 ORF (25,37) and the common region (Fig. 4; [15,37]), BGMV-BZ is phylogenetically closer to TGMV from Brazil than to the Central American/Caribbean BGMV isolates. A second line of evidence showing the distinctiveness of BGMV-BZ and the Central American/Caribbean BGMV isolates is our inability to create infectious pseudorecombinants between BGMV-BZ and -GA. This is in contrast to the ability to create infectious pseudorecombinants between BGMV-GA and -DR (9). Stanley (40) recently suggested that the ability to generate infectious pseudorecombinants between the DNA components of the bipartite geminiviruses might differentiate geminivirus strains from distinct geminiviruses. This suggestion was based on previous reports of infectious pseudorecombinants being limited to closely related isolates of ACMV (42) and TGMV (46). Infectious pseudorecombinants could not be created by exchanging DNA components of the distinct geminiviruses TGMV and SqLCV (29); however, more recently, infectious pseudorecombinants have been created by exchanging the DNA components of the distinct geminiviruses BDMV and ToMoV (18). Thus, although the formation of infectious pseudorecombinants may not be the sole criterion for distinguishing geminivirus strains from distinct geminiviruses, the inability to form infectious pseudorecombinants between BGMV-BZ and -GA is further evidence that these are genetically distinct viruses.

TABLE 3. Infection of *Phaseolus vulgaris* with cloned DNAs of bean golden mosaic virus (BGMV-BZ) (BZ) and a BGMV isolated from Guatemala (GA) and pseudorecombinants between the components of these two geminiviruses

DNA-A	DNA-B	No. of plants with symptoms/no. inoculated[a]
GP[b]	GP	0/12
GA	GA	7/9
BZ	BZ	10/12
GA	BZ	0/15
BZ	GA	0/16

[a] Plant radicles were inoculated with electric discharge particle acceleration. Only leaves from plants with symptoms hybridized with a general geminivirus probe (17). Summary of results of three separate experiments.
[b] GP = gold particles only.

In recognition of the distinct differences between BGMV-BZ and the Central American/Caribbean BGMV isolates, we propose that these viruses represent distinct types of BGMV: type I for BGMV-BZ (because the disease was first described in Brazil [3]), and type II for BGMV-PR (24), BGMV-GA (9), and BGMV-DR (9), with the implication that these types may occur beyond their presumed geographical origins and that strains may exist within the type groups. This nomenclature retains the same name for geminiviruses that cause similar disease symptoms in beans, but recognizes the significant differences beyond the strain level that exist between these BGMV isolates. It is important to distinguish between type I and II BGMV isolates because bean germplasm may exhibit different levels of resistance to these BGMV types.

It is interesting to speculate on the evolution of type I and II BGMV isolates. We propose, as previously suggested by Costa (4), that BGMV type I and II isolates evolved from indigenous geminiviruses present in native plants in South America and the Central America/Caribbean Basin, respectively. Parallel evolution of these BGMV types from indigenous geminiviruses is consistent with the absence of significant long-distance geographic movement of BGMV (4) and the independent development of BGMV epidemics in geographically distant areas (14). According to this proposal, BGMV-BZ and TGMV diverged from a common ancestor indigenous to South America, which would explain why BGMV-BZ is phylogenetically related more to TGMV than to type II BGMV isolates.

Future studies on BGMV should recognize that at least two distinct BGMV types exist, and that additional differences in biological properties of these types may be detected. Also, these BGMV types are distinct from BDMV, another whitefly-transmitted, bean-infecting geminivirus (23). These results may have important implications in efforts to breed BGMV-resistant beans for different geographical locations.

LITERATURE CITED

1. Abouzid, A. M., Polston, J. E., and Hiebert, E. 1992. The nucleotide sequence of tomato mottle virus, a new geminivirus isolated from tomatoes in Florida. J. Gen. Virol. 73:3225-3229.
2. Breathnach, R., and Chambon, P. 1981. Organization and expression of eukaryotic split genes coding for proteins. Annu. Rev. Biochem. 50:349-383.
3. Costa, A. S. 1965. Three whitefly-transmitted virus diseases of beans in São Paulo, Brazil. FAO Plant Prot. Bull. 13:121-130.
4. Costa, A. S. 1975. Increase in the populational density of *Bemisia tabaci*, a threat of widespread virus infection of legume crops in Brazil. Pages 27-49 in: Tropical Diseases of Legumes. J. Bird and K. Maramorosch, eds. Academic Press, Inc., New York, 171 pp.
5. Coutts, R. H. A., Coffin, R. S., Roberts, E. J. F., and Hamilton, W. D. O. 1991. The nucleotide sequence of the infectious cloned DNA components of potato yellow mosaic virus. J. Gen. Virol. 72:1515-1520.
6. Devereux, J., Haeberli, P., and Smithies, O. 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. 12:387-395.
7. Elmer, J. S., Brand, L., Sunter, G., Gardiner, W. E., Bisaro, D. M., and Rogers, S. G. 1988. Genetic analysis of the tomato golden mosaic virus. II. The product of the AL1 coding sequence is required for replication. Nucleic Acids Res. 16:7043-7060.
8. Etessami, P., Saunders, K., Watts, J., and Stanley, J. 1991. Mutational analysis of complementary-sense genes of African cassava mosaic virus DNA A. J. Gen. Virol. 72:1005-1012.
9. Faria, J. C., Gilbertson, R. L., Morales, F. J., Russell, D. R., Ahlquist, P. G., Hanson, S. F., and Maxwell, D. P. 1990. Sequence of infectious clones of two mechanically transmissible isolates of bean golden mosaic geminivirus. (Abstr.) Phytopathology 80:983.
10. Figueira, A. R. 1980. Estudos realizados com o vírus do mosaico dourando do feijoeiro do Brasil, visando sua transmissão por métodos mecânicos. Tese de Mestrado. UNICAMP. Campinas, S. P., Brazil. 60 pp.
11. Francki, R. I. B., Fauquet, C. M., Knudson, D. L., and Brown, F., eds. 1991. Classification and nomenclature of viruses. 5th Rep. ICTV. Arch. Virol. Suppl. 2. Springer Verlag, New York. 450 pp.
12. Frischmuth, S., Frischmuth, T., and Jeske, H. 1991. Transcript mapping of Abutilon mosaic virus, a geminivirus. Virology 185:596-

604.
13. Frischmuth, T., Zimmat G., and Jeske, H. 1990. The nucleotide sequence of Abutilon mosaic virus reveals prokaryotic as well as eukaryotic features. Virology 178:461-468.
14. Gálvez, G. E., and Morales, F. J. 1989. Whitefly-transmitted viruses. Pages 379-390 in: Bean Production in the Tropics. H. F. Schwartz and M. A. Pastor-Corrales, eds. Cent. Int. Agric. Trop., Cali, Colombia. 654 pp.
15. Gilbertson, R. L., Faria, J. C., Hanson, S. F., Hendrastuti, E., Ahlquist, P. G., Morales, F. J., and Maxwell, D. P. 1990. Relationships among bean-infecting geminiviruses. Annu. Rep. Bean Improv. Coop. 33:72-73.
16. Gilbertson, R. L., Faria, J. C., Hanson, S. F., Morales, F. J., Ahlquist, P., Maxwell, D. P., and Russell, D. R. 1991. Cloning of the complete DNA genomes of four bean-infecting geminiviruses and determining their infectivity by electric discharge particle acceleration. Phytopathology 81:980-985.
17. Gilbertson, R. L., Hidayat, S. H., Martinez, R. T., Leong, S. A., Faria, J. C., Morales, F., and Maxwell, D. P. 1991. Differentiation of bean-infecting geminiviruses by nucleic acid hybridization probes and aspects of bean golden mosaic in Brazil. Plant Dis. 75:336-342.
18. Gilbertson, R. L., Hidayat, S. H., Paplomatas, E. J., Rojas, M. R., Hou, Y.-M., and Maxwell, D. P. 1993. Pseudorecombination between the infectious cloned DNA components of tomato mottle and bean dwarf mosaic geminiviruses. J. Gen. Virol. 74:23-31.
19. Hamilton, W. D. O., Bisaro, D. M., Coutts, R. H. A., and Buck, K. W. 1983. Demonstration of the bipartite nature of the genome of a single-stranded DNA plant virus by infection with the cloned DNA components. Nucleic Acids Res. 11:7387-7396.
20. Hamilton, W. D. O., Stein, V. E., Coutts, R. H. A., and Buck, K. W. 1984. Complete nucleotide sequence of the cloned infectious DNA components of tomato golden mosaic virus: Potential coding regions and regulatory sequences. EMBO J. 3:2197-2205.
21. Harrison, B. D. 1985. Advances in geminivirus research. Annu. Rev. Phytopathol. 23:55-82.
22. Henikoff, S. 1984. Unidirectional digestion with exonuclease III creates targeted breakpoints for DNA sequencing. Gene 28:351-359.
23. Hidayat, S. H., Gilbertson, R. L., Hanson, S. F., Morales, F. J., Ahlquist, P., Russell, D. R., and Maxwell, D. P. 1993. Complete nucleotide sequences of the infectious cloned DNAs of bean dwarf mosaic geminivirus. Phytopathology 83:181-187.
24. Howarth, A. J., Caton, J., Bossert, M., and Goodman, R. M. 1985. Nucleotide sequence of bean golden mosaic virus and a model for gene regulation in geminiviruses. Proc. Natl. Acad. Sci. USA 82:3572-3576.
25. Howarth, A. J., and Vandemark, G. J. 1989. Phylogeny of geminiviruses. J. Gen. Virol. 70:2717-2727.
26. Kallender, H., Petty, I. T. D., Stein, V. E., Panico, M., Blench, I. P., Entienne, A. T., Morris, H. R., Coutts, R. H. A., and Buck, K. W. 1988. Identification of the coat protein gene of tomato golden mosaic virus. J. Gen. Virol. 69:1351-1357.
27. Lazarowitz, S. G. 1992. Geminiviruses: Genome structure and gene function. Crit. Rev. Plant Sci. 1:327-349.
28. Lazarowitz, S. G., and Lazdins, I. B. 1991. Infectivity and complete nucleotide sequence of the cloned genomic components of a bipartite squash leaf curl geminivirus with a broad host range phenotype. Virology 180:58-69.
29. Lazarowitz, S. G., Wu, L. C., Rogers, S. G., and Elmer, J. S. 1992. Sequence-specific interaction with the viral AL1 protein identifies a geminivirus DNA replication origin. Plant Cell 4:799-809.
30. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 545 pp.
31. Morales, F. J., and Niessen, A. I. 1988. Comparative responses of selected *Phaseolus vulgaris* germ plasm inoculated artificially and naturally with bean golden mosaic virus. Plant Dis. 72:1020-1023.
32. Morinaga, T., Ikegami, M., Shimotohno, K., and Miura, K. 1987. Total nucleotide sequences of the infectious cloned DNAs of bean golden mosaic virus. Microbiol. Immunol. 31:147-154.
33. Navot, N., Pichersky, R., Zeidan, M., Zamir, D., and Czosnek, H. 1991. Tomato yellow leaf curl virus: A whitefly-transmitted geminivirus with a single genomic component. Virology 185:151-161.
34. Petty, I. T. D., Coutts, R. H. A., and Buck, K. W. 1988. Transcriptional mapping of the coat protein gene of tomato golden mosaic virus. J. Gen. Virol. 69:1359-1365.
35. Proudfoot, N. J., and Brownlee, G. G. 1976. 3′ non-coding region sequences in eukaryotic messenger RNA. Nature (London) 263:211-214.
36. Rogers, S. G., Bisaro, D. M., Horsch, R. B., Fraley, R. T., Hoffmann, N. L., Brand, L., Elmer, J. S., and Lloyd, A. M. 1986. Tomato golden mosaic virus A component DNA replicates autonomously in transgenic plants. Cell 45:593-600.
37. Rojas, M. R. 1992. Detection and characterization of whitefly-transmitted geminiviruses by the use of polymerase chain reaction. M. S. thesis. Univ. Wis., Madison. 92 pp.
38. Schalk, H.-J., Matzeit, V., Schiller, B., and Gronenborn, B. 1989. Wheat dwarf virus, a geminivirus of graminaceous plants needs splicing for replication. EMBO J. 8:359-364.
39. Stanley, J. 1983. Infectivity of the cloned geminivirus genome requires sequences from both DNAs. Nature (London) 305:643-645.
40. Stanley, J. 1991. The molecular determinants of geminivirus pathogenesis. Sem. Virol. 2:139-150.
41. Stanley, J., and Gay, M. 1983. Nucleotide sequence of cassava latent virus DNA. Nature (London) 301:260-262.
42. Stanley, J., Townsend, R., and Curson, S. J. 1985. Pseudorecombinants between cloned DNAs of two isolates of cassava latent virus. J. Gen. Virol. 66:1055-1061.
43. Stenger, D. C., Revington, G. N., Stevenson, M. C., and Bisaro, D. M. 1991. Replicational release of geminivirus genomes from tandemly repeated copies: Evidence for rolling-circle replication of a plant viral DNA. Proc. Natl. Acad. Sci. USA 88:8029-8033.
44. Sunter, G., Gardiner, W. E., and Bisaro, D. M. 1989. Identification of tomato golden mosaic virus-specific RNAs in infected plants. Virology 170:243-250.
45. Townsend, R., Stanley, J., Curson, S. J., and Short, M. N. 1985. Major polyadenylated transcripts of cassava latent virus and location of the gene encoding coat protein. EMBO J. 4:33-37.
46. Von Arnim, A., and Stanley, J. 1992. Determinants of tomato golden mosaic virus symptom development located on DNA-B. Virology 186:286-293.