Requirement of the Common Region of DNA-B and the BL1 Open Reading Frame of Bean Golden Mosaic Geminivirus for Infection of *Phaseolus vulgaris*

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ABSTRACT

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Infectious clones of bean golden mosaic geminivirus (BGMV) can be inoculated onto the economically important host *Phaseolus vulgaris*, causing a reaction indistinguishable from that seen in the field. To elucidate the functions of the BGMV genome in beans, six clones, each with a point mutation, insertion, or deletion in the common region or BL1 open reading frame (ORF) of DNA-B of BGMV (Guatemalan isolate), were constructed. These clones were coinoculated with wild-type DNA-A of BGMV into bean radicles (*P. vulgaris*) by particle acceleration to determine the effect of the mutations on infectivity. Four mutants caused symptoms ranging from very mild mosaic to wild-type mosaic. Two mutants did not cause systemic infection: one that should have produced a truncated BL1 protein and another in which a 74-nucleotide section of the common region, including a putative stem-loop, was removed. We conclude that the BL1 ORF and the area of the DNA-B common region containing the putative stem-loop are required for systemic infection of *P. vulgaris*.

Bean golden mosaic virus (BGMV) is a bipartite, whitefly-transmitted geminivirus with circular, single-stranded DNA components that are approximately 2.6 kb in size (DNA-A and DNA-B) (19). Although BGMV was first described in the early 1960s by A. S. Costa (5), very little has been done to investigate its molecular properties beyond its DNA sequence (19,27). A number of isolates have been sequenced (BGMV-PR from Puerto Rico [19], BGMV-BZ from Brazil [10], BGMV-DR from the Dominican Republic [8], and BGMV-GA from Guatemala [8]), and open reading frames (ORFs) have been identified by computer analysis and compared to those of other bipartite geminiviruses (12,19). Because the positions, relative sizes, and derived amino acid sequences of ORFs of BGMV resemble those of the most intensively studied bipartite geminiviruses, tomato golden mosaic virus (TGMV) and African cassava mosaic virus (ACMV), it is hypothesized that the functions of the ORFs of BGMV are the same as the functions of the corresponding ORFs of TGMV and ACMV. Preliminary data indicate that ORFs AL1 and AR1 of BGMV-GA have functions analogous to the AL1 and AR1 ORFs in TGMV and ACMV (2,16). Detailed studies of other regions have not been performed.

DNA-B has not been studied as intensively as has DNA-A in any of the bipartite geminiviruses. Transcript mapping has confirmed that there are at least two expressed ORFs on DNA-B for TGMV (36) and ACMV (39): one transcribed leftward (BL1) and one rightward (BR1). Both ORFs are necessary for systemic infection to occur, and disruption of either one results in a noninfectious virus (4,7,17). DNA-B cannot replicate without transacting factors from DNA-A (31,37,40), but neither BR1 nor BL1 ORF is required for the replication of either component (17,38). The exact functions of these two ORFs have not been determined. DNA-B is thought to be required for cell-to-cell movement and vascular transport of the virus, since DNA-A is usually unable to spread by itself (31). Recently, the DNA-B region including the BL1 ORF, the left intergenic region, and the common region has been identified as the location of symptom determinants for TGMV (42).

DNA-A and DNA-B contain an important noncoding area called the common region. The nucleotide sequence of each component of an individual bipartite geminivirus is different except for this region of about 200 nucleotides (nt) in which the sequence of the two components is nearly identical. The sequence of the common region is different for each geminivirus (24). The common region has a role in transcription and/or replication of viral DNA (24,43). All common regions, including those of BGMV isolates, have a potential stem-loop with the conserved sequence TAATATTAC (loop) and G-C rich stems. A similar sequence is found in the large intergenic region of monopartite geminiviruses. This stem-loop is essential for replication (26) and may be part of the origin of replication for the viral strand (35).

Because of the lack of information about the function of DNA-B of bipartite geminiviruses and of DNA-B of BGMV in particular, this study was undertaken to determine whether regions of BL1 ORF and the common region of DNA-B of BGMV-GA are necessary for infection of *Phaseolus vulgaris* L. Mutations were introduced into these regions, and their effects on infectivity and symptom expression were observed.

MATERIALS AND METHODS

Sequence comparison. All computer manipulations of sequence data were performed with programs from the Genetics Computer Group (6). The GAP program was used to determine the optimal alignments of pairs of derived BL1 amino acid sequences; the gap weight was set at 3.0 and the gap length weight at 0.1. These results were used in the multiple sequence alignment program LINEUP to identify areas of highly conserved sequence. DNA sequences of BGMV-DR (the Dominican Republic isolate; GenBank accession numbers L01635 and L01636 for DNA-A and -B, respectively; J. C. Faria, S. F. Hanson, P. Ahlquist, and D. P. Maxwell, *personal communication*), of BGMV-GA (the Guatemalan isolate; GenBank accession numbers M91604 and M91605 for DNA-A and -B, respectively; J. C. Faria, R. L. Gilbertson,

P. Ahlquist, and D. P. Maxwell, *personal communication*), of BGMV-BZ (the Brazilian isolate) (10), and of bean dwarf mosaic geminivirus (BDMV) (18) were available as were sequences of BGMV-PR (19), TGMV (15), ACMV (34), and squash leaf curl geminivirus (SqLCV) (25).

Construction of mutant clones. All mutant clones of DNA-B were constructed from pGAB1 (11) (Fig. 1), a full-length, double-stranded DNA-B infectious clone of BGMV-GA cloned at the *Bam*HI site in the plasmid pBluescript II KS(+) (pBS+; Stratagene, La Jolla, CA). In some cases, the mutation was made in pGAB2, a full-length DNA-B that had been transferred to the *Bam*HI site of plasmid pSP72 (Promega Corp., Madison, WI). All molecular biology techniques were performed according to Sambrook et al (32), unless stated otherwise. Restriction endonucleases were obtained from Promega and BRL (Bethesda Research Laboratories, Gaithersburg, MD). Insertion mutants were created by digestion with the appropriate restriction endonucleases. The resulting 5′ overhangs were filled in with the Klenow fragment of DNA polymerase I or T4 DNA polymerase (BRL). Blunt ends were ligated with T4 DNA ligase (BRL), and the resulting recombinant plasmids were transformed into *Escherichia coli* strain JM101. The 74-nt deletion mutant (pGABCR3) was created in the common region by digestion of pGAB2 with *Spe*I (nucleotide 107) and *Bss*HII (nucleotide 182) (Fig. 1). The large *Bss*HII-*Spe*I fragment (4,984 nt including pSP72) was separated from the small fragment (74 nt) on a 0.7% SeaKem LE agarose (FMC BioProducts, Rockland, ME) TAE (tris-acetate-EDTA) gel and purified from the gel by a silica matrix (Geneclean, Bio101, La Jolla, CA). The large fragment was processed in the same way as were the insertion mutants. Changes in DNA sequence were confirmed by digestion with the appropriate restriction endonucleases in cases where the mutation created a restriction site or when a large segment of DNA was removed. When a restriction digest could not provide conclusive proof that the mutation was present, the clone was sequenced by the dideoxy chain-termination method (33) with Sequenase (United States Biochemical, Cleveland, OH). The resulting mutant clones are described in Table 1.

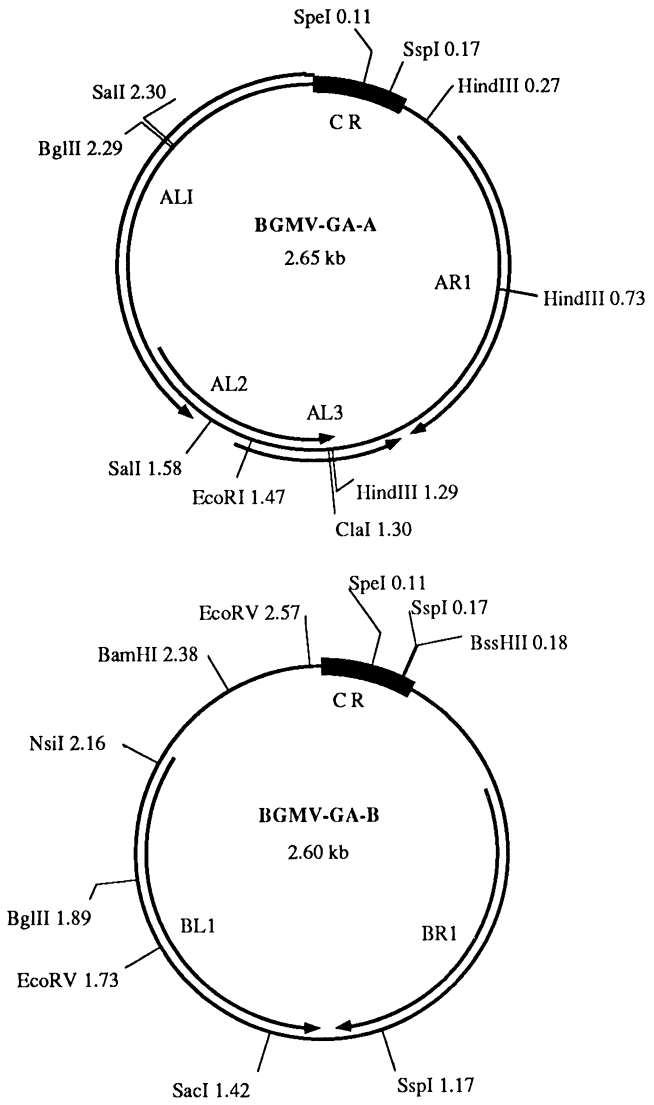

Fig. 1. Genome organization of the Guatemalan isolate of bean golden mosaic virus (BGMV-GA) DNA-A and DNA-B (GenBank accession numbers M91604 and M91605, respectively). Positions of open reading frames: AL1, 6–1, 2,647–1,592; AL2, 1,785–1,267; AL3, 1,520–1,122; AR1, 340–1,125; BL1, 2,187–1,306; and BR1, 503–1,270. Restriction endonuclease sites are followed by nucleotide positions in kilobases from the beginning of the common region (CR). pGAA1, DNA-A cloned in pUC119 at the *Eco*RI site; pGAB1, DNA-B cloned in pBluescript II KS(+) at the *Bam*HI site.

Site-directed mutagenesis. Clones pGABL1-3, pGABL1-4, and pGABL1-5 were created by site-directed mutagenesis (Table 1) (32). The primers used for mutagenesis were cleaned with Sep-Pak C18 cartridges (1): DS6, 5′GAATCTCCCGGATGCAAAACAC (nucleotides 1,445-1,466); DS12, 5′CAACATAGATCTCGAGTACTTTTCCTCG (nucleotides 1,897-1,870); DS8, 5′CTACATGAGAGGTACCGATTG (nucleotides 1,530-1,550). (Underlined nucleotides are changes from wild-type sequence.)

DNA was transformed into *E. coli* strain JM101 and plated onto yeast-tryptone plates containing ampicillin. Isolated plasmids were screened by restriction endonuclease digestion to identify a plasmid with the created or deleted restriction site. The insert DNA with the correct restriction enzyme digestion pattern was sequenced to confirm that the mutation was correct and to check for possible mutations at other sites in the subclone. The full-length clone was rebuilt around the mutated subclone fragment with standard ligation techniques and transformed into *E. coli* strain JM101. Plasmids were then extracted by the alkaline lysis method and checked by restriction endonuclease digestion to confirm correct reassembly and maintenance of the mutation. Plasmid DNA was prepared for plant inoculations by the large-scale alkaline lysis method and purified by centrifugation in CsCl gradients or by polyethylene glycol precipitation.

Plant inoculation by particle acceleration. Mutated clones were inoculated into radicles of bean seeds (*P. vulgaris* 'Topcrop') by particle acceleration (11). Mutant DNA-B clones were coinoculated with a full-length monomer of DNA-A of BGMV-GA from pGAA1 (11). All viral inserts were excised and used as monomers for inoculation. Seed preparation and the particle acceleration procedure were as described by Gilbertson et al (11).

Sap inoculation and detection of viral nucleic acid by squash and dot blot. These procedures are described by Gilbertson et al (13).

Analysis of mutant clones from inoculated plants. Mutant viruses were extracted from systemically infected leaves and subjected to polymerase chain reaction (PCR) amplification. DNA was extracted from a leaf disk (cut out with a sterile #4 cork borer) by a modification of the procedure of Dellaporta described by Gilbertson et al (14).

The amplification of viral DNA by PCR consisted of 30 cycles of 1 min at 94 C, 30 s at 55 C, and 1 min at 72 C. DNA-B fragments were amplified with primers DS16 (5′GGGGATCCCACTCATGTACCGTTGATCG, nucleotides 1,352–1,371) and DS17 (5′GGCTGCAGGGTGCCTATAAACGCAACAGG, nucleotides 1,999–1,979) for BL1 ORF or DS20 (5′CTCGGATCCGGACTAATAACCAAAACGATATCG, nucleotides 2,557–2,580) and DS21 (5′CTCGGTACCCAGGACCAAACTGATTTTCACG, nucleotides 692–671) for the common region. DS20 has a *Bam*HI site on its 5′ end, and DS21 has a *Kpn*I site. The BL1 ORF mutants were all within the 650-bp fragment amplified by primers DS16 and DS17, which have a *Bam*HI site and a *Pst*I site on their 5′ ends, respectively. DNA-A fragments were amplified with primers DS18 (5′CTGTGAGGGTGACGAAGATCG, nucleotides 1,633–1,653) and DS19 (5′CAGGTCAGCACCTTTCCATCC, nucleotides 2,408–2,388).

For a mutation where a restriction site was created, the presence

TABLE 1. Mutations made in DNA-B of bean golden mosaic geminivirus

Plasmid	Region	Mutation[a]	Amino acid codon change	Restriction site changed[b]
pGABCR1	Common region	Filled in 5′ overhang at *Spe*I site (nucleotide 107) 5′-ATACTAGCTAGTAC	...	−*Spe*I
pGABCR3	Common region	Deleted 74 nt between *Spe*I and *Bss*HII sites	...	− *Spe*I − *Bss*HII − *Ssp*I
pGABL1-1[c]	BL1	Filled in 5′ overhang at *Bgl*II site (nucleotide 1,884) 5′-GGAGATCGATCTA	...	− *Bgl*II
pGABL1-3	BL1	Substituted A for G at nucleotide 1,456 and G for C at nucleotide 1,458 (SDM with DS6)	D to H	− *Sma*I
pGABL1-4	BL1	Substituted C for G at both nucleotides 1,884 and 1,882 (SDM with DS12)	H to E	+ *Xho*I
pGABL1-5	BL1	Substituted G for A at nucleotide 1,541 and G for C at nucleotide 1,540 (SDM with DS8)	M to S	+ *Kpn*I

[a] Inserted nucleotides are underlined. Locations for restriction sites are shown in Figure 1. SDM = site-directed mutagenesis.
[b] + = Created restriction site; − = removed restriction site.
[c] Mutation would cause a frameshift and create a truncated protein of 123 amino acids instead of the 296-amino acid wild-type BL1 open reading frame.

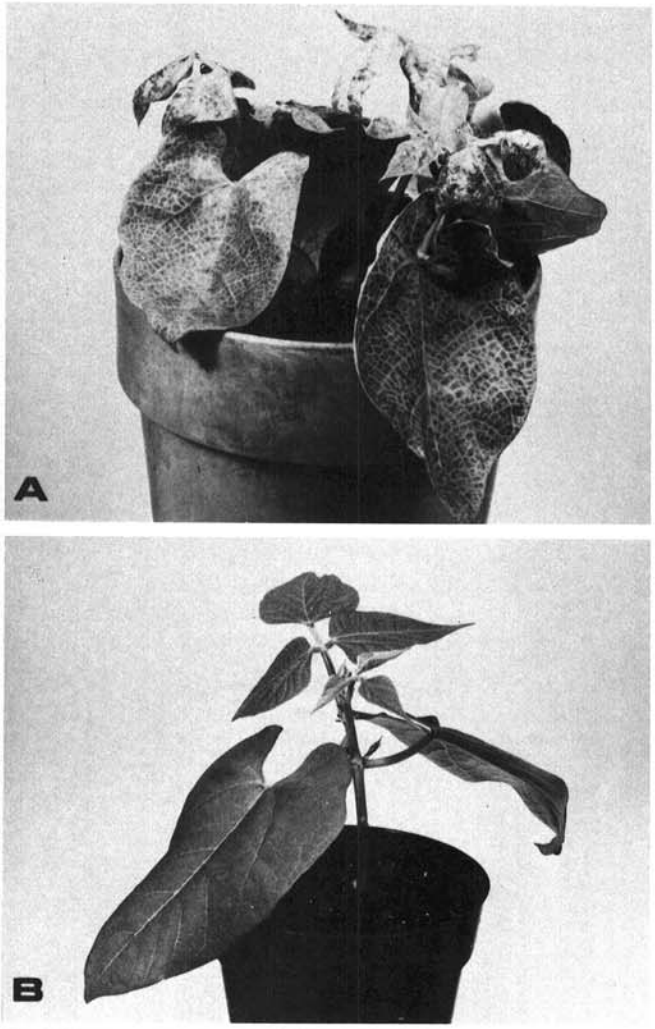

Fig. 2. Bean plants 15 days after radicle inoculation with **A,** excised, linearized monomeric inserts of pGAA1 and pGAB1 (wild-type recombinant plasmids of bean golden mosaic geminivirus) and **B,** gold particles.

of the mutation was verified by digestion with the appropriate restriction endonuclease. For mutants that needed to be sequenced to verify the presence of the mutations, the amplified DNA was purified on a 0.7% SeaKem LE agarose (FMC BioProducts) TAE gel. The DNA was removed from the gel with the Geneclean kit and then precipitated twice with ethanol. The double-stranded DNA was sequenced by the dideoxy chain-termination method (33).

RESULTS

Comparison of BL1 ORF amino acid sequences of bipartite geminiviruses. The derived amino acid sequences of the BL1 ORF of eight bipartite geminiviruses were compared to find conserved regions of BL1 ORF DNA sequence to be used as possible sites for site-directed mutagenesis of BL1 ORF of BGMV-GA. The geminiviruses compared were BGMV-GA, BGMV-DR, BGMV-PR, BGMV-BZ, TGMV, ACMV, BDMV, and SqLCV. The derived amino acids of the BL1 ORF were highly conserved (>74% identity, >84% similarity) for the pairwise comparisons of the seven geminiviruses from the Western Hemisphere and moderately conserved (46–48% identity, 60–63% similarity) between ACMV and the other seven geminiviruses. The N termini were more similar than were the C termini.

An amino acid sequence with homology to a metal binding domain (3) was present in the consensus sequence resulting from the multiple sequence alignment of derived amino acids for the BL1 ORF. It follows the pattern $C^{50}X_4H^{55}X_{41}C^{97}X_4H^{102}$. Similar sequence motifs have been shown by X-ray crystallography to be involved in metal binding in a diverse group of viral proteins that bind DNA (41). The H^{55} codon was changed to a glutamic acid codon creating mutant pGABL1-4.

Plant inoculation to determine infectivity of mutant clones. Plants inoculated with the wild-type (WT) DNA (pGAA1 and pGAB1) by particle acceleration routinely developed symptoms on primary leaves by the eighth day and on trifoliolate leaves when they emerged (Fig. 2A). Typical symptoms are golden mosaic, stunting, and leaf curl.

Neither of the DNA-B common region mutant clones caused WT symptoms when coinoculated with DNA-A. Six plants inoculated with pGABCR1 (*Spe*I insertion mutant) did not show symptoms for 12–14 days and then produced only a mild mosaic on trifoliolate leaves (Fig. 3A, Table 2). However, one plant developed symptoms similar to those on WT-infected plants and was found to contain WT DNA-B as determined by the presence of the *Spe*I site (nucleotide 107) in the common region. pGABCR3 (74-nt deletion mutant) did not cause systemic infection; inoculated plants were indistinguishable from healthy plants (Fig. 3B); and trifoliolate leaves did not contain viral nucleic acids (Table 2). Squash blot hybridizations at high stringency with pGAB1 as a probe or PCR with primers specific to DNA-B of BGMV-GA confirmed the presence of viral nucleic acids or DNA in symptomatic leaf tissue in all but one plant (Table 2), and no viral nucleic acids were detected in leaves from plants without

symptoms.

Plants coinoculated with BL1 mutants (Table 1) and WT DNA-A exhibited a variety of reactions. pGABL1-1 (frameshift mutant) did not cause systemic infection. The plants had no visible symptoms (Fig. 4A), and viral nucleic acid was not detected in the trifoliolate leaves (Table 2). Plants inoculated with pGABL1-3 (highly conserved D codon changed to H at amino acid position 248) had mosaic-type symptoms that varied in severity from very mild to WT virus-induced symptoms (Fig. 3C). pGABL1-4 (H to E codon change at position 102 in the putative metal-binding motif) caused a very mild mosaic at 11–18 days on trifoliolate leaves but caused no stunting or leaf curl (Fig. 4B). One plant without symptoms was found to have viral DNA in trifoliolate leaves (Table 2). Plants inoculated with pGABL1-5 (M to S codon change at 217 in a region of unconserved amino acid sequence) were indistinguishable from WT viral DNA-inoculated plants (Table 2, Fig. 3D).

Sap transmission of viral DNA from plants inoculated with all mutant clones, including clones that did not cause systemic infection, was attempted. Virions or viral nucleic acids from trifoliolate leaves of plants inoculated with clones pGABCR1, pGABL1-3, and pGABL1-5 were sap transmissible (Table 2), and beans had WT symptoms. Sap transmission of pGABL1-4, a systemically infectious clone, was attempted from two infected plants and was not successful (13 plants inoculated).

Analysis of mutant clones from infected plants. Retention of mutations in BL1 ORF or the common region in infected plants was evaluated. PCR fragments from these regions of DNA-B were obtained from DNA extracted from systemically infected leaves, and the presence of these mutations was confirmed by restriction enzyme cleavage or by sequencing. Since mutations in pGABL1-4 and pGABL1-5 each created a restriction site, their presence could be detected by digestion with the appropriate

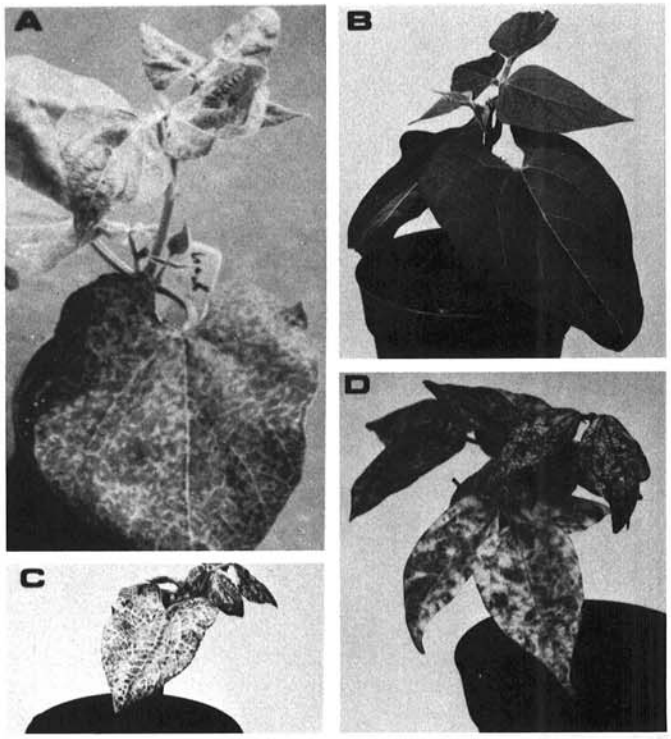

Fig. 3. Bean plants 15–24 days after radicle inoculation with excised, linearized monomeric inserts of **A,** pGAA1 and pGABCR1; **B,** pGAA1 and pGABCR3; **C,** pGAA1 and pGABL1-3; and **D,** pGAA1 and pGABL1-5.

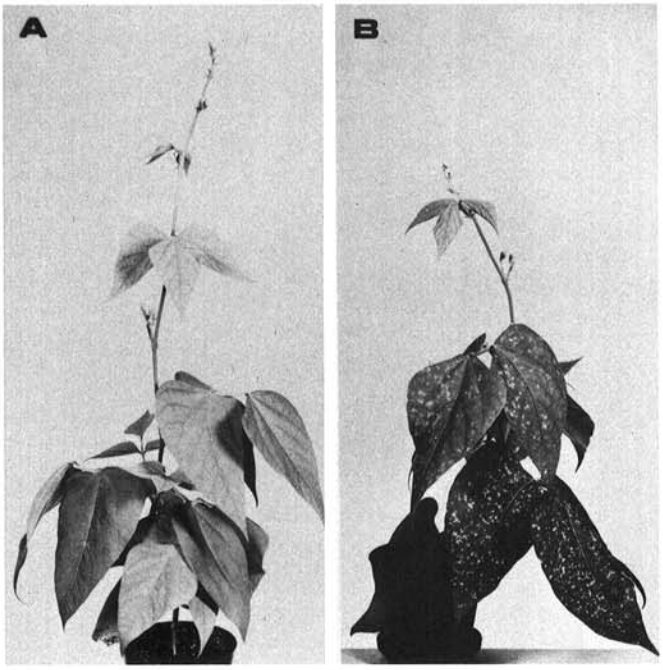

Fig. 4. Bean plants 24 days after radicle inoculation with excised, linearized monomeric inserts of **A,** pGAA1 and pGABL1-1 and **B,** pGAA1 and pGABL1-4.

TABLE 2. Inoculations of plants with wild-type and mutant DNA-B clones of bean golden mosaic geminivirus (BGMV)

Treatment[a]	Number of experiments	Symptoms/total[b]	Symptom description[c]	Viral DNA present[d]	Sap transmissible
pGAA1 + pGAB1	4	15/24	WT	8/12[e]	+
No DNA	4	0/24	N	0/12	−
pGAA1 + pGABCR1	2	7/11	M, D	6/11	+
pGAA1 + pGABCR3	3	0/18	N	0/18	−
pGAA1 + pGABL1-1	2	0/10	N	0/10	−
pGAA1 + pGABL1-3	2	5/12	M, WT	5/12	+
pGAA1 + pGABL1-4	3	3/23	M, D	4/23	−
pGAA1 + pGABL1-5	2	8/12	WT	8/12	+

[a] pGAA1 and pGAB1 are wild-type recombinant plasmids; common region mutants: pGABCR1 has a 4-nucleotide (nt) insert at the *Spe*I site; pGABCR3 has nucleotides 108–182 deleted; mutations in BL1: pGABL1-1 has a 4-nt insert at the *Bgl*II site; pGABL1-3 has nucleotide 1,456 changed from G to A and nucleotide 1,458 from C to G; pGABL1-4 has nucleotides 1,882 and 1,884 changed from G to C; pGABL1-5 has nucleotide 1,540 changed from C to G and nucleotide 1,541 from A to G. Plants were inoculated with excised, linearized viral inserts (11).

[b] Number of plants with symptoms per total number of plants inoculated.

[c] WT = wild type; N = none; M = mild mosaic; and D = delayed.

[d] Number of plants with viral nucleic acid in trifoliolate leaves per total number of plants tested; viral DNA-B was detected by hybridization with radioactive probe specific to DNA-B of the Guatemalan strain of BGMV (BGMV-GA) or by polymerase chain reaction with primers specific to DNA-B of BGMV-GA (primers DS16 and DS17).

[e] Six plants from one experiment (two with symptoms) and two plants with symptoms for each of three experiments were probed. Only plants with symptoms hybridized with the viral probe.

restriction endonuclease. The *Xho*I site in the viral DNA from the four plants infected with pGABL1-4 was still present, and the *Kpn*I site was retained in DNA from the eight plants infected with pGABL1-5. The *Spe*I site (nucleotide 107) was present in viral fragments from two plants, one of which had WT symptoms, for the common region insertion mutant (pGABCR1). A combined sample from three other symptomatic plants also contained WT DNA-B as determined by the presence of an appropriate size fragment obtained by *Spe*I cleavage. These three reversions to WT occurred in three separate inoculation experiments. Mutants in which a restriction site was destroyed were checked for retention of the mutation by sequencing the amplified DNA. Amplified DNA samples from five plants inoculated with pGABL1-3, one of which had WT symptoms, were sequenced, and all five samples retained the original mutation. Approximately 50 nt on either side of the mutation were sequenced, and no secondary mutations were found.

DISCUSSION

Very little is known about the genome functions of BGMV. Because its genome structure is very similar to TGMV and ACMV, it is assumed that the ORFs perform the same or similar functions. It is known that the AL1 ORF is required for infectivity of BGMV (16) and that DNA-A or DNA-B alone do not cause systemic infection in beans (11).

The common region of TGMV, and specifically a putative stem-loop, is involved in replication, possibly as part of a replication origin (29,35) or, alternatively, as a cleavage site (21). Similarity of this stem-loop sequence to a stem-loop that is the origin of second-strand DNA synthesis in ϕX174 and to the gene A protein cleavage site of ϕX174 has been noted by Lazarowitz (24). This region of sequence is nearly identical in TGMV and BGMV. The results from infectivity tests of one DNA-B mutant (pGABCR3) indicate that the common region of DNA-B is required for infectivity. Removal of a 74-nt section containing the putative stem-loop resulted in a clone that was not systemically infectious. This deleted fragment corresponds to the section of SqLCV common region that is recognized by the AL1 protein and was found to be necessary for replication of ssDNA and dsDNA (26). Another common region mutant, pGABCR1, which had a 4-nt insertion at the *Spe*I site (nucleotide 107), delayed systemic infection and caused a mild mosaic in some plants but caused a WT reaction in others. A WT reaction was obtained upon sap transmission from pGABCR1-infected plants. A mutation made in this *Spe*I site (nucleotide 107) in the common region of DNA-A of BGMV-GA by Gilbertson et al (11) caused delayed and attenuated symptoms. They proposed that the mutation might disrupt replication of the virus or transcription of AL1 ORF.

It is unlikely that a mutation in the common region of DNA-B would affect transcription unless the transcripts for the DNA-B ORFs begin in the common region or the promoter sequences for BR1 or BL1 ORF are located in this region. The transcripts of DNA-B of BGMV have not been mapped. BR1 ORF transcripts in TGMV start approximately 200 nt downstream from the common region, but one of the three BL1 ORF transcripts starts in the common region (36). Similar transcripts have been found for Abutilon mosaic virus (9). If BGMV DNA-B has similar transcripts, the BL1 ORF transcript could be affected by the mutation at the *Spe*I site (nucleotide 107). This mutant appears to have reverted to WT in two plants and in at least one other plant (combined sample from three other plants) as indicated by the presence of the *Spe*I site (nucleotide 107) in viral DNA-B recovered from infected plants. Since these reversions occurred in three separate inoculations, it is not likely that the presence of WT DNA-B was the result of contamination during inoculation. This would explain the WT symptoms seen on some radicle-inoculated plants and in the sap transmission tests. Reversion could have occurred by recombination with the DNA-A, since the common region of both DNAs is nearly identical. This is an interesting reversion, because Gilbertson et al (11) did not observe any reversions in the *Spe*I mutant (nucleotide 107) of DNA-A of BGMV-GA. Thus, this area may have a different function in DNA-B than in DNA-A.

From this evidence, we concluded that some part of the 74-nt section between the *Spe*I (nucleotide 107) and *Bss*HII (nucleotide 182) sites in the common region of DNA-B contains a domain(s) or maintains the proper secondary structure required for infectivity. Revington et al (29) also found that insertion of nucleotides on the 5′ side of the putative stem-loop of TGMV DNA-B reduced replication of DNA-B and caused attenuated symptoms, whereas insertions downstream from the stem-loop had no effect on replication or symptom development, but insertion of an *Xho*I linker (8 nt) in the *Ssp*I site within the loop of the putative stem-loop prevented replication of DNA-B.

The three BL1 ORF mutant clones each with single codon changes were all capable of causing systemic infection. The pGABL1-3 clone (D^{248} to H^{248}) caused a slight attenuation of symptoms in some plants and produced WT symptoms in other plants. The nucleotide substitution in pGABL1-3 was in a region of highly conserved amino acid sequence near the C terminus of the protein. This amino acid substitution does not change the predicted secondary structure of the protein. The variation in symptoms suggests that the mutation might have been repaired in some cases. However, viral DNA was sequenced from five infected plants, including one with WT symptoms, and the mutation was present. It is possible that in some instances the original mutation was complemented by a secondary mutation that would change symptom expression. The pGABL1-4 mutant (H^{102} to E^{102}) caused very attenuated symptoms, and one plant had a symptomless infection. This virus mutant has not been sap transmitted. The mutation should have resulted in substitution of glutamic acid for histidine in a hydrophilic region of the protein. This mutation causes the predicted protein secondary structure in that area to change from a β sheet to an α helix. This mutation was in a conserved amino acid of a possible metal-binding site (3), and these metal-binding sites are found in proteins that are known to bind DNA (41). The pGABL1-5 (M^{217} to S^{217}) mutant-inoculated plants gave WT symptoms. This mutation should have caused a change from methionine to serine in an unconserved area of the amino acid sequence. Although there are differences in the reactivity and structure of these two amino acids and they cause a change in predicted protein secondary structure (α helix to a turn), apparently this region has no important function, or other regions of the genome can compensate for the effects of this mutation.

Mutant pGABL1-1 should have resulted in a frameshift and a truncated BL1 protein of only 123 amino acids (WT BL1 = 296 amino acids) with 22 amino acids between the frameshift and the termination codon. This mutant failed to cause systemic infection. Therefore, the BL1 ORF of BGMV-GA is required for systemic infection and the 5′ 300 nucleotides are not sufficient to code for a functional BL1 product. BL1 ORF is needed for systemic infection but not for replication of DNA in TGMV and ACMV (4,7,17,38).

The requirement of DNA-B for systemic infection with BGMV is contrary to the results of recent experiments with ACMV and TYLCV, which indicate that DNA-A of ACMV and the Thailand isolate of TYLCV are weakly infectious without DNA-B (23,30). The Israel and Sardinia isolates of TYLCV may not have a DNA-B (22,28). Systemic infection with only DNA-A of BGMV has not been possible in beans (11). This contradiction may be explained in terms of evolution. ACMV and TYLCV may have evolved along one evolutionary line for Eastern Hemisphere geminiviruses and BGMV along another evolutionary line (20). Further study of ACMV and TYLCV may reveal areas of DNA-A that assume the functions of DNA-B of other bipartite geminiviruses.

These experiments show that the BL1 ORF and the common region of DNA-B of BGMV-GA are required for systemic infection of *P. vulgaris*. This is consistent with results of similar experiments performed in *Nicotiana* species with other bipartite geminiviruses. Further analysis of BGMV-GA with replication assays and transcript mapping will be necessary to determine how replication and transcription are performed in *P. vulgaris*.

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