Cloning of the Complete DNA Genomes of Four Bean-Infecting Geminiviruses and Determining Their Infectivity by Electric Discharge Particle Acceleration

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ABSTRACT

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Geminiviruses are small plant viruses that encapsidate a single-stranded circular DNA and form double-stranded DNAs in infected plants. Infection of economically important hosts with cloned DNAs of geminiviruses frequently has been difficult. Four isolates of bipartite bean-infecting geminiviruses (bean golden mosaic geminivirus isolates from Brazil [BGMV-BZ], Guatemala [BGMV-GA], and the Dominican Republic [BGMV-DR]; and bean dwarf mosaic geminivirus from Colombia [BDMV-CO]) were cloned; full-length cloned double-stranded DNA components A and B of each isolate were not infectious when mechanically coinoculated onto bean primary leaves by surface abrasion but were infectious when coinoculated into radicles of beans (*Phaseolus vulgaris*) by electric discharge particle acceleration. This novel method for the introduction of cloned geminiviral DNA into plants was extremely efficient and resulted in symptom expression in as few as 7 days. Moreover, infection of beans by cloned DNAs of BGMV-BZ, which has never been mechanically transmitted as virions or cloned DNAs, indicated that this procedure circumvents plant barriers to mechanical transmission. Three engineered mutants of BGMV-GA component A were constructed and separately introduced into beans with wild-type BGMV-GA component B, and disease phenotypes determined. Soybeans (*Glycine max*) also were infected with cloned DNAs of BGMV-BZ, BGMV-GA, and BDMV-CO, demonstrating a potentially important alternate host for these viruses. Particle acceleration will facilitate genetic analysis of bean-infecting geminiviruses and may allow for the efficient introduction of viral nucleic acids or virions of other viruses into hosts that are refractory to mechanical transmission.

Additional keyword: mutational analysis.

Geminiviruses are a group of plant viruses that possess twinned (geminate) icosahedral virions and a single-stranded DNA genome. They can be subdivided into two groups: those that are transmitted by the tropical whitefly (*Bemisia tabaci* Gennadius), possess a bipartite genome, and infect dicotyledonous plants; and those that are transmitted by various leafhopper species, possess a monopartite genome, and infect monocotyledonous plants (5,15,19). Beet curly top geminivirus, which is leafhopper transmitted and possesses a monopartite genome but infects dicotyledonous plants, may be an evolutionary intermediate between these two groups (33). Two distinct DNA components, A and B, are characteristic of the whitefly-transmitted geminiviruses: bean golden mosaic geminivirus (BGMV; 11), tomato golden mosaic geminivirus (TGMV; 1,12), and African cassava mosaic geminivirus (ACMV; 32). Both DNA components are required for plant infection (13,25,31). The entire DNA sequences of infectious clones of ACMV (31,32), TGMV (14), and BGMV (26; unpublished data) have been determined. Considerable progress in the molecular genetic analysis of TGMV and ACMV was facilitated by the discovery that virions and cloned double-stranded DNAs of these viruses are infectious on the tobacco species, *Nicotiana benthamiana* Domin, after mechanical inoculation (13,31). This host provided a vector-independent model system for studying these viruses. More recently, *Agrobacterium*-mediated infection has been used to more efficiently introduce cloned DNAs of TGMV and ACMV into *Nicotiana* spp. (7,27).

In contrast to TGMV and ACMV, study of the molecular genetics of BGMV has been more difficult, due to the inability of BGMV to infect *N. benthamiana* (9) and to the lack of an efficient procedure for the introduction of cloned BGMV DNAs into plants. Some isolates of BGMV, particularly those from Central America and the Caribbean, are easily mechanically transmitted as virions to beans (23), whereas virions of BGMV isolates from Brazil have not been mechanically transmitted (4,9). Cloned DNAs of a mechanically transmissible isolate from Puerto Rico (BGMV-PR) were infectious when mechanically inoculated onto bean plants (25), but we have been unable to similarly infect bean plants with cloned DNAs of a BGMV isolate from Brazil or an isolate from Guatemala (unpublished data). Thus, other methods of inoculation were needed to advance studies of bean-infecting geminiviruses.

Recently, it was reported that nucleic acids could be introduced into plant tissues on microprojectiles accelerated by a ballistic charge (18) or by electric discharge (3,22). Using electric discharge particle acceleration, McCabe et al (22) achieved stable transformation of soybean (*Glycine max* L.) with the β-glucuronidase gene from *Escherichia coli.* In this study, we used electric discharge particle acceleration to demonstrate the infectivity of cloned DNAs of four bean-infecting geminiviruses in beans; clones of three of these viruses also were infectious in soybeans. Additionally, the utility of this inoculation method for mutational studies was demonstrated by phenotypic analysis of BGMV component A.

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MATERIALS AND METHODS

Geminiviral isolates. Three BGMV isolates were used: BGMV-Guatemala (BGMV-GA isolate 0) and BGMV-Dominican Republic (BGMV-DR) were maintained in beans (cv. Topcrop) by sap transmission (9,23); BGMV-Brazil (BGMV-BZ) is not mechanically transmissible via plant sap (9) and was maintained in beans (cv. Topcrop) by whitefly transmission (9). Bean dwarf mosaic geminivirus-Colombia (BDMV-CO) was maintained in beans (cv. Topcrop) by sap transmission (9,24).

Cloning of viral DNAs. Young trifoliolate leaves and buds were collected from infected plants, weighed, and frozen with liquid nitrogen in a mortar. Frozen tissue was ground to a powder and extraction buffer (10 mM Tris-Cl pH 7.5, 10 mM EDTA, and 1% SDS) added (about 1:4, wt/vol ratio). After thorough grinding, the sap was held for 20 min, then centrifuged at 15,000 *g* for 15 min. The supernatant was adjusted to 1 M NaCl, maintained overnight at 40 C, and then centrifuged at 75,000 *g* for 1 h. The supernatant was extracted twice with an equal volume of phenol-chloroform (1:1), once with an equal volume of chloroform, adjusted to 0.3 M sodium acetate, and the nucleic acids precipitated with an equal volume of isopropanol. Nucleic acids were washed with 70% ethanol, dried, and resuspended in TE buffer (10 mM Tris-Cl pH 7.5, 1 mM EDTA).

Nucleic acids from infected plants were fractionated by electrophoresis in 0.7% agarose in Tris-acetate-EDTA buffer (20), virus-specific bands were excised from ethidium bromide stained gels, and nucleic acids recovered using glass matrix (BIO 101, La Jolla, CA) according to the supplier's procedure. These purified fractions, which contained single- and double-stranded viral DNAs (unpublished data), were digested with various restriction enzymes and analyzed by agarose gel electrophoresis and Southern hybridization to identify enzymes that linearized the circular double-stranded DNA components of each virus isolate. Putative full-length clones were obtained by digesting these circular viral DNAs with selected restriction enzymes and cloning into pUC119, pSP72 (Promega Corp., Madison, WI), pBluescript+, or pBluescript II KS+ (Strategene, La Jolla, CA). Clones having inserts of the expected size (~2.6 kb) were characterized by Southern hybridization and by DNA sequencing using the chain termination method (30). Recombinant plasmids containing tandem dimers of the BGMV-GA and BGMV-DR components A and B, respectively, were constructed essentially as described by Hayes et al (16). Large-scale plasmid preparation by the alkaline lysis method (20) was used to produce plasmid DNA for plant inoculations. Plasmid DNAs were used either as undigested molecules or as DNA digests in which full-length viral inserts were excised. Before inoculations, digests were extracted twice with an equal volume of phenol-chloroform, DNA precipitated with ethanol, and resuspended in TE buffer.

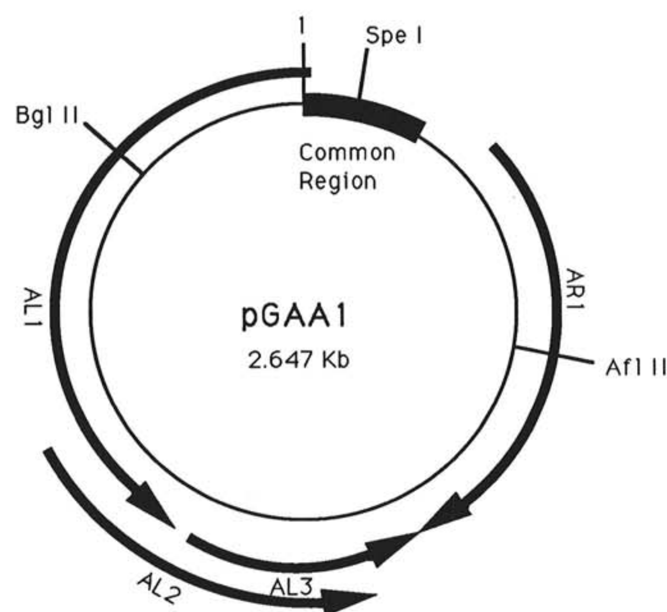

Fig. 1. Restriction map of BGMV-GA DNA component A (for the purposes of this figure, the circularized DNA-A insert of pGAA1 is shown, with nucleotide 1 corresponding to the first nucleotide of the common region) showing the location of restriction sites used to construct mutants used for particle acceleration experiments.

Electric discharge particle acceleration inoculation. Bean (cv. Topcrop) or soybean (cv. Williams) seeds were disinfested by washing with sterile water and soaking in 3% sodium hypochlorite for 5 min. Seeds were washed again with sterile water, placed on moist sterile filter paper in a covered petri dish, and germinated at 28 C in the dark. After 48 h, seeds that had radicles of ~1 cm were placed in a 5-cm-diameter plastic petri dish containing 12% xanthan gum, which immobilized the seeds so that the radicles were exposed for particle acceleration.

Recombinant plasmid DNAs were coated onto gold particles (1- to 3-μm diameter gold particles, Alfa Chemical Co., Ward Hill, MA) by adding 0.1 μg of each recombinant plasmid/mg of gold particles in 25 μM spermidine. The DNA-coated gold then was precipitated by adjusting to 62 μM $CaCl_2$ (3). The supernatant was decanted and the pellet resuspended in ethanol at 1 mg of gold per milliliter of ethanol. Next, 162 μl of the gold-ethanol slurry was pipetted onto an 18-mm square of aluminized Mylar sheet. The particles were allowed to settle, the ethanol was removed, and the sheet air-dried.

Particle acceleration was essentially as described by McCabe et al (21). Briefly, the carrier sheet with the DNA-gold particles was placed on the electric discharge apparatus and a metal retaining screen placed above the sheet. A petri plate containing the germinated seed was placed on the screen and aligned above the carrier sheet. The target chamber was evacuated to 500 mm Hg. A 14-kV discharge from a high voltage capacitor accelerated the gold particles into the exposed radicles. The inoculated seeds were planted in sterile potting mix and grown in a chamber maintained at 26 C for a 14-h light period and 21 C for a 10-h dark period.

Construction of mutations in BGMV-GA component A. Mutations were introduced into BGMV-GA component A (pGAA1, Fig. 1) by digestion of the cloned DNA at the indicated unique restriction site to generate a four-nucleotide (nt) 5′ protruding end, which was filled in using Klenow DNA polymerase, and religated (20). After inoculation, total DNA was extracted from infected plants and component A was amplified by the polymerase chain reaction (PCR) and asymmetric PCR (22) to produce single-stranded DNA for sequencing.

Nucleic acid squash and dot blot hybridization. Viral nucleic acids were detected in infected plants by nucleic acid squash or dot blot hybridization as described by Gilbertson et al (9). Cloned DNAs of appropriate geminiviral isolates were labeled with [α-^{32}P]dATP by nick translation (20) and used as probes.

RESULTS

Viral clones. Full-length clones of DNA components A and B of BGMV-BZ, BGMV-GA, BGMV-DR, and BDMV-CO (Table 1) were constructed as described earlier. The tandem orientation of dimer inserts was confirmed by restriction mapping.

Infection of plants with cloned DNAs. Introduction of excised

TABLE 1. Full-length clones of the DNA components A and B of four bean-infecting geminiviruses

Geminivirus	Component	Vector/cloning site	Plasmid name
BGMV-BZ	DNA-A	pBS(+)/*Hin*dIII	pBZA1
	DNA-B	pBS(+)/*Acc*I	pBZB1
BGMV-GA	DNA-A	pUC119/*Eco*RI	pGAA1
	DNA-B	pKS(+)/*Bam*HI	pGAB1
BGMV-DR	DNA-A	pKS(+)/*Eco*RI	pDRA1
	DNA-B	pKS(+)/*Hin*dIII	pDRB1
BDMV-CO	DNA-A	pSP72/*Bgl*/II	pBDA1
	DNA-B	pKS(+)/*Bam*HI	pBDB1

linear double-stranded DNA components A and B of each geminiviral isolate or dimers of BGMV-GA or BGMV-DR components into the radicle of bean seeds by particle acceleration resulted in symptoms on primary and/or trifoliolate leaves in 7–14 days (Table 2). Beans inoculated with gold particles without DNA, with components A and B in undigested plasmids, with excised individual genome components of BGMV-GA or BGMV-DR, or with linearized pUC119 did not develop symptoms (Table 2). Primary leaves of plants infected with BGMV-GA or BGMV-DR developed striking vein yellowing. In some cases, symptoms initially appeared on one-half of a primary leaf, with delayed and reduced symptoms on the other half. Primary leaves of plants infected with BGMV-BZ were symptomless or developed faint yellow spots or slight vein clearing, whereas those infected with BDMV-CO had vein clearing and were cupped downward and distorted. Trifoliolate leaves of plants infected with the BGMV isolates developed typical golden mosaic symptoms (Fig. 2), often accompanied by reduced and distorted growth; the appearance of symptoms on BGMV-BZ inoculated leaves was typically delayed compared to BGMV-GA or BGMV-DR. As in normal BDMV-CO infections, the first trifoliolate leaves of BDMV-infected plants were dwarfed, cupped downward and distorted, but did not develop golden mosaic symptoms (Fig. 2). Soybeans infected with BGMV-BZ developed a mild golden mosaic with yellow-gold flecks on leaves, and little or no leaf distortion, whereas those infected with BGMV-GA and BDMV-CO were symptomless (Table 3).

Symptomatic bean leaves that were tested for geminiviral nucleic acids by squash or dot blot hybridization with cloned BGMV-GA, BGMV-BZ, and BDMV-CO DNA components A and B as probes were always positive (Table 2, Fig. 3); whereas plants without symptoms were negative (Table 2, Fig. 3). To confirm that bean plants were infected by BGMV-BZ, a BGMV-BZ specific probe was used (9); at high stringency, the probe hybridized with squashes of bean plants infected with BGMV-BZ but not those infected with BGMV-GA, BGMV-DR, or BDMV-CO. Infection of soybeans by BGMV-BZ, BGMV-GA, and BDMV-CO similarly was confirmed using squash blot hybridization with appropriate probes.

Mutants. Three separate mutants of BGMV-GA component A (pGAA1, Fig. 1) were constructed by filling in 5′ protruding ends resulting from cleavage of the viral DNA with selected restriction enzymes. The nucleotide sequence of pGAA1 has been determined (unpublished data). An insertion mutation was introduced in ORF AR1 (homologous to the coat protein gene of TGMV; 17) at the *Afl*II site (Fig. 1) at nt 736 by an incomplete fill-in (CTTAAG to CTTATTAAG) to produce pGAAR1, and a frameshift mutation was introduced in ORF AL1 (homologous to TGMV AL1 ORF, which is necessary for viral DNA replication; 2,6) at the *Bgl*II site (Fig. 1) at nt 2288 (AGATCT to AGATCGATCT) to produce pGAAL1. A fill-in mutation in the common region, which is the ~200-nucleotide region that is highly conserved between the two components of any geminivirus transmitted by whitefly (15, Fig. 1), was introduced at the *Spe*I site (Fig. 1) at nt 107 (ACTAGT to ACTAGCTAGT) to produce pGACR1. These mutations were confirmed by DNA sequence analysis. Each component A mutant was introduced into beans with wild-type BGMV-GA component B (pGAB1). No symptoms developed and no viral nucleic acids were detected in leaves of beans inoculated with the AL1 mutant. Delayed and attenuated symptoms were observed on beans inoculated with the common region and coat protein mutants. Trifoliolate leaves of beans infected with the coat protein mutant eventually developed typical

TABLE 2. Infection of *Phaseolus vulgaris* with cloned double-stranded DNAs of four bean-infecting geminiviruses mediated by electric discharge particle acceleration

Inoculum[a]	Number of experiments	Number with symptoms/ number inoculated[b]
No treatment	3	0/12
Gold particles	8	0/36
pUC119 linearized	1	0/5
BGMV-GA excised (A)	3	0/15
BGMV-GA excised (B)	3	0/17
BGMV-GA undigested (A+B)	3	0/17
BGMV-GA excised (A+B)	10	35/43
BGMV-GA dimers (A+B)	3	8/14
BGMV-DR excised (A)	3	0/18
BGMV-DR excised (B)	3	0/15
BGMV-DR undigested (A+B)	3	0/17
BGMV-DR excised (A+B)	7	21/26
BGMV-DR dimers (A+B)	3	15/16
BGMV-BZ excised (A+B)	8	44/54
BDMV-CO excised (A+B)	8	32/38

[a] "Linearized" indicates that a circular plasmid was digested at a unique restriction site, resulting in a single fragment; "excised" indicates that the plasmid was digested with the restriction enzyme used to insert the viral DNA (Table 1), resulting in the excision of the double-stranded form of the indicated component(s); "dimers" indicates that for each of the indicated DNA components, recombinant plasmids containing tandem or direct repeats (16) of these molecules were used; "undigested" indicates that the indicated recombinant plasmids were used as undigested molecules.

[b] Beans were evaluated for symptoms on trifoliolate leaves 14 days after inoculation by particle acceleration. Symptoms on leaves included golden mosaic and leaf distortion for BGMV isolates and stunting and distortion for BDMV.

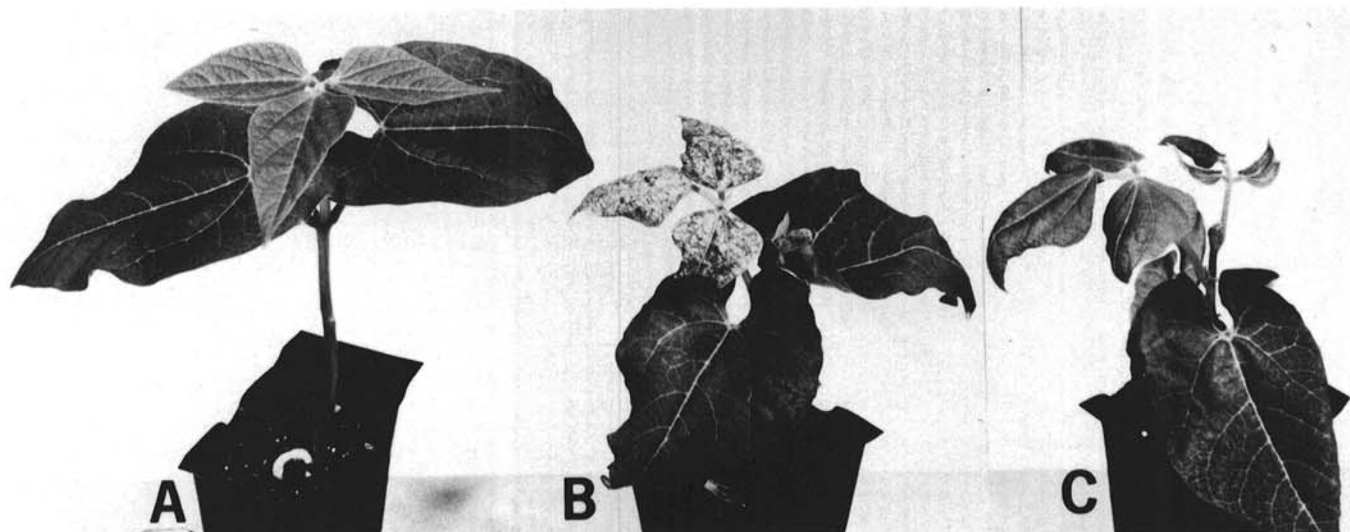

Fig. 2. Bean plants 10 days after inoculation by electric discharge particle acceleration with **A,** gold particles alone, or with the cloned double-stranded DNA components A and B of **B,** BGMV-BZ, or **C,** BDMV-CO.

golden mosaic symptoms, whereas those infected with the common region mutant remained attenuated. To confirm that infections were caused by the mutants, the regions containing the mutations were amplified by PCR using primers that annealed at the 5′ and 3′ ends of the common region or ORF AR1, then single-stranded DNA was produced by asymmetric PCR (21) and sequenced. In each case, the presence of the introduced mutation in component A was confirmed.

Transmission by mechanical inoculation of plant sap. BGMV-GA, BGMV-DR, and BDMV-CO were readily mechanically transmitted (> 95% inoculated plants were infected) from bean plants inoculated by particle acceleration by grinding young infected trifoliolate leaves in 0.1 M potassium phosphate to produce a sap inoculum (presumably containing virions) and inoculating primary leaves of 7- to 10-day-old bean plants (cv. Topcrop) with sap as described by Morales and Niessen (23). The symptoms and time of their appearance (~7 days after inoculation) were identical to those observed with standard sap transmission of these isolates to this bean cultivar. BGMV-BZ was also sap transmitted using this procedure; however, only 11% (17/129) of inoculated plants developed symptoms, and symptoms were delayed (~14–21 days after inoculation) and initially attenuated. Under high stringency conditions, the BGMV-BZ probe hybridized only to squash blots of the BGMV-BZ infected plants, confirming that BGMV-BZ had been sap transmitted. The common region and coat protein mutants were readily transmitted by mechanical inoculation of plant sap.

DISCUSSION

By exploiting viral double-stranded DNAs isolated from infected plants, the bipartite genomes of four bean-infecting geminiviruses were cloned. These cloned DNAs were introduced into beans and soybeans by electric discharge particle acceleration and shown to be biologically active. This method was extremely efficient and reliable for all four isolates of bean-infecting geminiviruses; over 80% of plants inoculated with excised components A and B were infected. Particle acceleration is a novel method for delivery of geminiviral DNA into plants, and it has several attractive features. The procedure is rapid, requires relatively small quantities of DNA, and is an alternative method to *Agrobacterium*-mediated infection (2,7,27). By introducing viral DNAs into the radicle of germinating bean seed, symptomatic plants can be obtained 8–9 days after initiating seed germination. Particle acceleration of viral DNAs into plants apparently circumvents barriers to mechanical transmission, because our strenuous efforts to infect beans by mechanically coinoculating cloned DNAs of BGMV-GA or BGMV-BZ onto bean primary leaves by abrasion with Carborundum were unsuccessful (unpublished data). Furthermore, cloned DNAs of BGMV-BZ, which has previously not been mechanically transmitted as virions or cloned DNA, were infectious when beans and soybeans were inoculated by particle acceleration. These results indicate that particle acceleration may be a way to deliver cloned DNAs of other geminiviruses into hosts that are difficult to infect by mechanical transmission, e.g., TGMV into tomato or ACMV into cassava, allowing study of these geminiviruses in economically important hosts. Additionally, particle acceleration might mediate infection of monocotyledonous plants with cloned DNA of a monopartite leafhopper-transmitted geminivirus such as maize streak geminivirus (MSV), which has never been mechanically transmitted as virions or cloned DNA (10). Cloned DNA of MSV and other geminiviruses that infect monocotyledonous plants has been shown to be infectious by *Agrobacterium*-mediated infection (10). Inoculation by particle acceleration also seems likely to have far broader applications than for geminiviruses only; it also may be useful for introducing virions, RNAs, or cDNA clones of RNA viruses, particularly those that are phloem limited and difficult or impossible to mechanically transmit, e.g., luteoviruses (29), into germinating seeds, plant organs, and/or protoplasts.

At least two features of particle acceleration are likely to contribute to its efficiency in mediating infection by cloned geminiviral DNAs. First, individual gold beads can carry both components of the viral genome and introduce them into cells; and, as the beads move through the tissue, it is likely that numerous cells are exposed to the DNA. Second, the viral DNA is introduced into tissues that support viral replication and spread. For the geminiviruses, this presumably would be phloem tissue (15), but we cannot rule out the possibility of infections occurring in other tissues, particularly since viral DNA is introduced into tissues at such an early developmental stage. Symptom development on only one primary leaf or half of a primary leaf may reflect introduction of viral DNAs into a cell or group of cells that are precursors of vascular tissues. A similar phenomenon also was observed in soybeans transformed with the β-glucuronidase gene by particle acceleration (22).

The infection of soybeans by these geminiviruses may have important implications in disease epidemiology. Soybeans are widely cultivated in Brazil and other regions where BGMV and BDMV-CO occur, and they are an excellent host for the whitefly vector of these viruses (4). Thus, soybeans may be an important alternate host for these viruses.

TABLE 3. Infection of *Glycine max* with cloned double-stranded DNAs of three bean-infecting geminiviruses mediated by electric discharge particle acceleration

Inoculum	Number of experiments	Number with symptoms/number inoculated[a]	NASH (+/−)[b]
Gold particles	3	0/15	0/15
BGMV-GA excised (A+B)[c]	3	0/13	4/9
BGMV-BZ excised (A+B)	3	7/12	7/5
BDMV-CO excised (A+B)	3	0/10	5/5

[a] Soybeans were evaluated for symptoms 21 days after inoculation by particle acceleration. Symptoms included a light green mosaic pattern and yellow flecking for soybeans inoculated with BGMV-BZ; all other soybeans showed no symptoms.

[b] Nucleic acid squash hybridization (NASH) of 21-day-old trifoliolate leaves was used to detect viral nucleic acids. A mixture of ^{32}P-labeled DNA components A and B of BGMV-BZ, BGMV-GA, and BDMV was used as a probe. +/− = number of plants that hybridized with probe/number of plants that did not hybridize with probe.

[c] "Excised" indicates that the plasmids were digested with the restriction enzyme used to insert the viral DNA (Table 1), resulting in the excision of the double-stranded form of the indicated component(s).

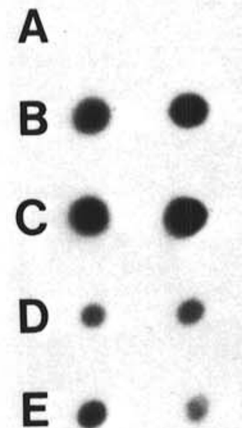

Fig. 3. Autoradiograph of a nucleic acid dot blot of bean leaf tissue from each of two plants inoculated by electric discharge particle acceleration with **A,** gold particles alone, or with the cloned DNA components A and B of **B,** BGMV-BZ, **C,** BGMV-GA, **D,** BGMV-DR, and **E,** BDMV-CO. A mixture of ^{32}P-labeled DNA components A and B of BGMV-BZ, BGMV-GA, and BDMV-CO was used as a probe.

A surprising result was the first successful transmission of BGMV-BZ by mechanical inoculation using plant sap. Successful sap transmission of BGMV-BZ may be related to a difference between inocula derived from plants infected by particle acceleration versus whitefly transmission, and/or the specific conditions used for plant growth in this study. Alternatively, a variant of the BGMV-BZ population may have been selected. Regardless, the low efficiency of transmission and delayed and atypical symptoms observed on these beans confirms the poor mechanical transmissibility of BGMV-BZ relative to the readily mechanically transmissible isolates (BGMV-GA, BGMV-DR, and BDMV-CO). Furthermore, because beans infected with BGMV-BZ mediated by particle acceleration had symptomless primary leaves and symptoms were delayed on the first trifoliolate leaves, BGMV-BZ may spread less efficiently in bean primary leaves than the mechanically transmissible isolates. This may explain the poor transmissibility of BGMV-BZ by mechanical inoculation of primary leaves with plant sap.

Particle acceleration should greatly facilitate the genetic analysis of geminiviruses. We purposely designed three mutants whose phenotypes might be predicted from previous genetic analysis of TGMV in *N. benthamiana* (2,6,8,28). The finding that the BGMV-GA coat protein mutant initially produced delayed and attenuated symptoms but that typical golden mosaic symptoms eventually developed is consistent with previous results suggesting that the coat protein is a pathogenicity factor (8). The insertion mutation in pGAAR1, which is predicted to introduce an isoleucine residue into the coat protein, is not expected to drastically alter the coat protein because the neutral nonpolar isoleucine is inserted among a series of other neutral or basic amino acid residues. The fact that typical symptoms did develop with this coat protein mutant supports this hypothesis, and indicates that a basic viral function such as spread or replication was initially impaired, but not sufficiently to prevent typical symptom development. Furthermore, because the coat protein mutant was readily transmitted via plant sap, it is probable that the mutant coat protein packaged the viral DNA.

The common region of the geminivirus genome is thought to contain the viral replication origin. A potential stem-loop structure in the common region, which is conserved among all geminiviruses sequenced to date, possesses sequence motifs similar to those in simian virus 40 and polyomavirus replication origins (28). In TGMV, DNA component B mutants with the common region deleted, inverted, or with an 8-base-pair (bp) insertion in the stem-loop sequence, were not replicated by wild-type DNA component A in *N. benthamiana* plants or leaf disks. However, a mutant having a 3-bp insertion at a *Sau*3A site located 30 nucleotides upstream from the stem-loop sequence in DNA component B was replicated at lower levels than wild-type DNA-B, and plants infected with this mutant had attenuated symptoms (28). Similarly, the 4-bp insertion at the *Spe*I site in the BGMV-GA DNA component A common region was 40 nucleotides upstream from the stem-loop structure, and plants infected with this mutant had attenuated symptoms. These results demonstrate that this part of the BGMV-GA common region is involved in an important viral function, possibly DNA replication (28). The mechanism by which interference with this function occurred could involve interference with transcription of the AL1 ORF and/or with the interaction of the AL1-encoded replication factor with the common region.

The entire genomes of four bean-infecting geminiviruses have been cloned and their infectivity demonstrated using a particle acceleration inoculation procedure. This will allow detailed genetic analyses of these viruses, which could lead to new molecular approaches for disease control.

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