Variability in Geminivirus Isolates Associated with *Phaseolus* spp. in Brazil

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ABSTRACT

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Bean golden mosaic geminivirus (BGMV) is the single most devastating virus of common beans in the tropical and subtropical Americas and the Caribbean Basin. The BGMV from Brazil, named BGMV-BZ, is considered distinct from BGMV-PR isolates from Puerto Rico, Guatemala, and the Dominican Republic because of DNA sequence data, the ability to form pseudorecombinants, and mechanical transmissibility properties. In bean-growing areas of Brazil, samples were collected from beans, lima beans, and the weed *Leonurus sibiricus* displaying typical symptoms of infection by geminiviruses. Viral DNA fragments comprising part of the *rep* gene, the common region, and part of the *cp* gene were

amplified by polymerase chain reaction, cloned, and sequenced. The bean samples had geminivirus with sequences nearly identical to that of BGMV-BZ collected in Goiânia, state of Goiás, in 1986. The sample from lima bean contained a new species of geminivirus that induces symptoms similar to those induced by BGMV-BZ and was named lima bean golden mosaic virus (LBGMV-BR). While all sequences from bean samples clustered with BGMV-BZ, the sequence from the lima bean isolate stood alone. A mixed infection with abutilon mosaic geminivirus was also found in a single sample from the state of São Paulo. DNA sequence comparisons indicate that the virus isolate from *L. sibiricus* represents a new geminivirus species, designated here as leonurus mosaic virus.

Bean golden mosaic of common beans (*Phaseolus vulgaris* L.) was first described in Brazil by Costa (5), who demonstrated that the causal agent was transmissible by the whitefly *Bemisia tabaci*. Initially, the disease caused only minor losses, but, during the 1970s, it became a major constraint for bean production throughout Brazil, Central America, the Caribbean Basin (7,16,21) and, more recently, in Florida (4).

Early studies were conducted on a similar disease of beans from Puerto Rico by Goodman (20), Goodman et al. (21), and Haber et al. (22). They showed that the geminate particles previously observed by Gálvez and Castaño (15) contained circular single-stranded DNA and a bipartite genome. The genomic components of bean golden mosaic virus (BGMV) have been designated DNA-A and DNA-B, possess ~2,600 nucleotides (nt) each, and share a unique region (~200 nt) of nearly identical sequence called the common region (CR), which contains the origin of replication (*ori*) (8,12). The DNA sequence of the complete genomes of BGMV from Brazil (17), Guatemala (8), the Dominican Republic (8), and Puerto Rico (24) (BGMV-BZ, BGMV-GA, BGMV-DR, and BGMV-PR, respectively) have been determined. Major differences in DNA sequence and biological properties separate BGMV into at least two distinct types. Type I is represented by BGMV-BZ, and type II is represented by BGMV-PR, BGMV-GA, and BGMV-DR (8,27).

Even though whitefly-transmitted (WFT) geminiviruses as a group infect a broad range of dicotyledonous hosts, each virus generally has a limited host range. Besides *Phaseolus* spp., BGMV-BZ infects soybeans (*Glycine max* L.), both under natural conditions

and following inoculation by particle acceleration (1,18). A limited search for alternate weed hosts for BGMV-BZ and BGMV-DR has not been successful (19,29).

Genotypic variability among plant viruses is a widely accepted concept. The literature on variability within geminivirus species, however, is scant. Limited sequence variability was found in the hypervariable region (the intergenic region of DNA-B) of the BGMV-DR associated with a small number of bean samples with golden mosaic symptoms from the Dominican Republic (19). Torres-Pacheco et al. (32), utilizing methods of polymerase chain reaction (PCR) and DNA sequencing of begomoviruses in Mexico, found low genomic variability in the viruses studied. On the other hand, Stenger and McMahon (30) characterized considerable variability in beet curly top virus, a curtovirus, using full-length clones and restriction endonuclease analysis.

Efforts to breed BGMV-resistant varieties in Brazil have been only partially successful (3). This has been attributed to the possible existence of variability within the virus. Under field conditions, disease symptoms of naturally infected common beans vary with plant age at the time of infection and with the bean genotype. This has caused confusion in efforts to evaluate the interaction phenotype for the disease (7).

Neither variability among the geminiviruses infecting beans in Brazil nor the primary source of inoculum has been previously reported. In this study, we analyzed geminivirus isolates found in samples of bean, lima bean, and the weed *Leonurus sibiricus* L. (family Labiatae) with geminivirus-like symptoms from geographically diverse regions of Brazil in an attempt to detect possible genetic variability in BGMV-BZ. Our findings include a mixed infection of BGMV and abutilon mosaic virus (AbMV) from a single location, the presence of a possible new geminivirus associated with a lima bean sample from the state of Pernambuco, and a previously undescribed geminivirus associated with the common weed *L. sibiricus*. In addition, we found that all of the other samples

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from beans had geminiviruses with sequences nearly identical to that of the BGMV-BZ isolate that was collected from beans in Goiânia, state of Goiás, Brazil, in 1986.

MATERIALS AND METHODS

Collection of plant tissue. Bean (*P. vulgaris*) or lima bean (*P. lunatus*) leaf tissues showing golden mosaic symptoms typical of a geminivirus infection were collected in 1991, 1995, and 1996. Samples were obtained from the main bean-growing states of Brazil. Beans from the February-March planting season were sampled from the following localities in the designated year: Londrina, Paraná (PR), 1991; Wenceslau Bráz, PR, 1995; Campinas, São Paulo (SP), 1991; Taquarituba, SP, 1995; Goiânia, Goiás (GO), 1991 and 1996; Lucas do Rio Verde, Mato Grosso (MT), 1995; Barreiras, Bahia (BA), 1995; and Caruarú, Pernambuco (PE), 1991 (Fig. 1).

In addition, leaves were collected from the common weed *L. sibiricus* in 1992 near Dourados, Mato Grosso do Sul (MS). The plants had yellow symptoms in their upper portions and were heavily colonized by whiteflies. Infected *L. sibiricus* plants were commonly associated with bean fields in the states of PR and MS. Samples from symptomatic plants weakly hybridized to a DNA-A probe of BGMV-BZ (data not shown). Collected leaf tissues were allowed to dry at room temperature pressed between sheets of paper towel for variable lengths of time until utilized for DNA extraction.

DNA extraction, PCR amplification of geminiviral fragments, and cloning procedures. Dry tissue (~1 cm²) was ground in a

sterile mortar with a pestle in 1.0 ml of extraction buffer (100 mM Tris, pH 8.0; 50 mM EDTA, pH 8.0; 500 mM NaCl; and 10 mM 2-mercaptoethanol) (6). Immediately after grinding, 500 µl was transferred to a 1.5-ml microfuge tube, and 33 µl of 20% sodium dodecyl sulfate was added. The tube was vortexed and incubated for 10 min at 65°C. After 160 µl of 5 M potassium acetate, pH 5.5, was added and the solution mixed by vortexing, the tube was centrifuged for 10 min at 10,000 × g. The debris-free supernatant fluid was removed to a new tube and extracted once with an equal volume of phenol-chloroform-isoamyl alcohol (PCI). The DNA was precipitated by adding 0.6 volume of isopropanol and washed twice with 70% ethanol. The pellet was dried and resuspended in 500 µl of sterile distilled water. This DNA extract was stored at -20°C for use in all experiments. Geminiviral DNA was amplified by PCR with the primer pair PAL1v1978/PAR1c496 or PAL1v1978/PAR1c715 (28). These primers amplify part of the *acI* open read frame (ORF) (*rep* gene), which codes for the only viral protein absolutely required for replication; the CR; and part of the *avI* ORF (*cp* gene), which codes for the coat protein (31). This last region is the least-conserved part of the *cp* gene among WFT geminiviruses (26,33). DNA fragments obtained with the primer pair PAL1v1978/PAR1c496 were extracted with PCI, precipitated, digested with *Pst*I, agarose gel-purified, and cloned in pBluescript II KS(+) (pBSII KS(+); Stratagene Inc., La Jolla, CA) previously digested with the same enzyme. Alternatively, DNA fragments amplified with the primer pair pAL1v1978/PAR1c715 were cloned in the vector pCR II (Invitrogen Corp., San Diego, CA).

Fig. 1. Geographical map of Brazil indicating the localities where plant tissue samples were collected. PR = Paraná; SP = São Paulo; MS = Mato Grosso do Sul; MG = Minas Gerais; GO = Goiás; MT = Mato Grosso; BA = Bahia; and, PE = Pernambuco.

DNA sequencing and analysis. Most of the sequencing was done with the dye terminator sequencing method by the University of Wisconsin Biotechnology Center, Madison. In other cases, sequencing was done by the dideoxynucleotide chain termination method with Sequenase (United States Biochemical Corp., Cleveland), following the manufacturer's instructions. The presence of a conserved *SacI* site in the CR of all inserts was used to prepare two subclones for sequencing the complete clone. Eighteen clones from beans were partially sequenced and 7 clones were completely sequenced to determine possible variation among PCR fragments. Sequences of these clones were compared with the sequence of

BGMV-BZ (GenBank accession no. M88686), obtained from a bean collected at Goiânia (GO, Brazil) in 1986 (17). The clone from *L. sibiricus* was completely sequenced. All sequence manipulations and analyses were done using the software of the Genetics Computer Group, Inc. (Wisconsin package version 9.0, Genetics Computer Group, Madison, WI).

RESULTS

DNA fragments of either 1.1 or 1.3 kilobases (kb) were amplified with PCR primer pair PAL1v1978/PAR1c496 or PAL1v1978/PAR1c715, respectively, and cloned for all samples. More than one independent clone was obtained for each amplified DNA, but, in most cases, only one from each sample was completely sequenced. The others were partially sequenced. All clones obtained were of geminiviral origin.

In all, 20 clones from the bean samples obtained from six states (GO, MT, PR, SP, BA, and MG) were sequenced and nucleotide sequence comparisons made to BGMV-BZ (Table 1). For all the pairwise comparisons for the different regions, the nucleotide identities were greater than 91%, and generally they were greater than 95%. The six completely sequenced geminiviral inserts cloned from beans had nucleotide identities to BGMV-BZ greater than 95%. The CR nucleotide identities for 10 of the clones had 93 to 100% nucleotide identities to BGMV-BZ. These data indicate that there

TABLE 1. Nucleotide identities between bean golden mosaic geminivirus-Brazil and polymerase chain reaction fragments of 20 geminivirus isolates from beans collected in six states of Brazil

Clone ^a	Complete fragment	rep ^b	CR ^c	LIR ^d	cp	Length (bp)
GO91-GYN33	...	...	...	...	97	177
GO91-GYN66	...	...	...	...	94	175
GO91-GYN67	...	98	...	...	...	212
GO91-GYN72	...	...	93	...	...	111
GO96-GYN5	...	...	100	97	96	617
GO96-GYN11	...	...	...	97	96	531
MT96-LRV1	96	98	97	92	95	1,307
PR96-WB1	97	98	99	97	95	1,192
PR96-WB2	...	...	...	...	97	151
PR96-WB3	...	...	...	...	98	229
PR91-L7	...	...	...	...	93	127
PR91-L11	...	100	100	97	97	621
PR91-L17	...	99	100	97	95	660
PR91-L21	...	98	...	...	...	617
SP91-C23	...	...	...	...	96	221
SP91-C73	96	98	95	93	95	1,099
SP96-T1	98	98	100	97	97	1,314
SP96-T4	...	...	...	94	...	265
BA96-B1	96	97	100	97	95	1,174
MG96-U1	97	...	98	92	93	1,156

^a Clones were named after the locality of collection: GO = Goiás, MT = Mato Grosso, PR = Paraná, SP = São Paulo, BA = Bahia, MG = Minas Gerais, GYN = Goiânia, LRV = Lucas do Rio Verde, WB = Wenceslau Brás, L = Londrina, C = Campinas, T = Taquarubeta, B = Barreiras, and U = Unaf.

^b rep = replication-associated protein.

^c CR = common region.

^d LIR = large intergenic region or region between the CR and the coat protein gene (cp).

^e Sequence not available.

TABLE 2. Nucleotide identities between pPE-A1 cloned from lima beans collected in Caruarú, Pernambuco, and pLS2 from *Leonurus sibiricus* and four other Western Hemisphere, bipartite geminiviruses

Virus ^a	pPE-A1			pLS2		
	rep ^b	CR/LIR	cp	rep	CR/LIR	cp
BGMV-BZ	91	84/62	81	72	64/54	72
AbMV	69	53/51	75	77	52/51	67
SiGMV	72	75/...	81	75	73/...	...
SqLCVA	55	54/49	75	60	60/53	73
LBGMV-BR	...	...	...	68	71/40	65

^a BGMV-BZ = bean golden mosaic virus-Brazil M88686; LBGMV-BR = new geminivirus associate with lima beans in Caruarú, Pernambuco, Brazil U92531; LeMV = leonurus mosaic virus U92532; AbMV = abutilon mosaic virus X15983; SiGMV = sida golden mosaic virus-Jamaica U69601; and SqLCV = squash leaf curl virus M38183.

^b rep = replication-associated protein; CR = common region; LIR = large intergenic region or region between the CR and the coat protein gene (cp).

^c Sequence not available.

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AbMV      TACTCC--ACTTGAGGCTC--CTC.AAACTTGCTCATGTA-ATTGGAGTA-TTGGAGGT-CTTTATATA
SiGMV-CR  TACTCC--AGTTGAGCCCTCTC----AACTTGCTGATTCT-ATTGGAGTA-TTAGAGTATCCATATATA
TYVSV     TGCTCCCGATAGAG--CTCCTTC--AACTTGG-CAATGAATTGGGG--AAAGGGTC-TCAATATATA
BGMV-GA   GTCTCC--AATTGAGG--CTCCTC--AACTCTCGCTATGCAATTGGAG--ACTGGAGTA-CAATATATA
BDMV      TACTCC--AATT--CCCCCTCTC-AAAATATCTCATTTCT-ATTGGAGTA-TTGGAGT-TACTTATATA
SiGMV-JA  TTCCCC--TTAGCTCTCTCGCTCAAAAGT--CTTAAG--AATTGGGGGAAGTGGGGG-AACTTATATA
PYMV      TTCCCCCT-ATTGCTCTGGCTCTC-AAAAT--CTTATG--AATTGGGGGAAGTGGGGG-AACTTATATA
LeMV      TACTCCGGG-TTGAGAGCTCGCTC-ATAAGT-CCTTATG--AATTGGAGTA--TGGAGT-GCAATATATA
TGMV      TACTACCAA-TTGAGGAGGGGCTCCAAAGT--TATATG--AATTGGTAGTA-AGGTAG-CTCTTATATA
BGMV-BZ   TACCCCGA--TTGAGCTCTCGTTC-AAAAGT-CTCTATG--AATCGGTGTAA-TGGTCC--CAATATATA
LBGMV-BR  TACCCCGA--TTGAGCTCTCGCTC-ATAAGT-CCTTATG--AATTGGGGTAA-AGGGGA--CAATATATA

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Fig. 2. Alignment of nucleotide sequences of common region motifs observed in DNA of 11 geminiviruses from the Western Hemisphere. Sequences start two nucleotides upstream of the inverted repeat found in all geminiviruses and end at the TATA box. Sequences were aligned by the LINEUP procedure of the Genetics Computer Group package. The inverted repeat and two forward repeats are boxed; the TATA box is marked with an asterisk. Dashes indicate gaps. AbMV = abutilon mosaic virus X15983; SiGMV-CR = sida golden mosaic virus-Costa Rica X99550; TYVSV = tomato yellow vein streak virus U79998; BGMV-GA = bean golden mosaic virus-Guatemala; BDMV = bean dwarf mosaic virus M88179; SiGMV-JA = sida golden mosaic virus-Jamaica U69601; PYMV = potato yellow mosaic virus D00940; LeMV = leonurus mosaic virus U92532; TGMV = tomato golden mosaic virus K02029; BGMV-BZ = bean golden mosaic virus-Brazil M88686; and LBGMV-BR = lima bean golden mosaic virus-Brazil U92531.

is little divergence in nucleotide sequence between these bean-infecting geminiviruses and BGMV-BZ.

The 1.3-kb DNA fragments cloned from the bean tissue sample from Taquarituba (SP) yielded two types of clones: those with high identities to the BGMV-BZ sequence (pSP96-T1 and pSP96-T4, 98 and 94%, respectively) (Table 1) and one (partial sequence of pSP96-T2) with high nucleotide identity (99%) to AbMV (GenBank accession no. X15983) (14) for nucleotide positions 105 to 712.

The 1.1-kb DNA fragment from the lima bean tissue collected in Caruarú (PE) had an internal *Pst*I site, whereas BGMV-BZ does not have this restriction endonuclease site. Both fragments were cloned in pBSII KS(+) digested with *Pst*I. From an independent PCR reaction using newly prepared DNA and utilizing the primer pair PAL1v1978/PAR1c715, a 1.3-kb fragment was obtained and cloned in the pCR II vector (clones pPE-A1 to pPE-A5). The complete sequence for pPE-A1 was obtained (GenBank accession no. U92531), as well as sequences for three of the *Pst*I partial clones. The three *Pst*I subclones had 100% nucleotide identities to the sequence of the complete PCR fragment (pPE-A1). This indicated that a single geminivirus was present. The DNA sequence of pPE-A1 showed 81% nucleotide identity to BGMV-BZ in the N-terminus portion of the *cp* gene (Table 2), and the derived amino acid identity was 89%. The CR of this isolate showed typical iteron arrangements for Western Hemisphere geminiviruses (Fig. 2) (2,8) and had 84% nucleotide identity to BGMV-BZ, the highest sequence homology identified in the GenBank, EMBL, DDBJ, and PDB databases using the BLAST program (Genetics Computer Group). The *rep* gene showed the highest identity to BGMV-BZ, with 91% for both the nucleotide and amino acid sequences. The large intergenic region, 3' from the stem-loop motif to the ATG of the *cp* gene, had only 62% identity to BGMV-BZ and even lower identities to other geminiviruses (Table 2) in the database. The other cloned fragments from bean, which were similar to BGMV-

TABLE 3. Replication-associated protein (*rep*) gene and derived amino acid sequence variations between bean golden mosaic virus-Brazil (BGMV-BZ) and the polymerase chain reaction fragments of nine isolates nearly identical to BGMV-BZ

Codon change	Position (AA) ^a	AA change	Nature of change ^b	Frequency ^c	AA in other bean geminiviruses ^d
CCA→CCG	4	P	Silent	1/7	P (4)
CTT→CTC	32	L	Silent	4/7	L (4)
CAA→CAG	33	Q	Silent	1/7	Q (4)
CTC→ATC	78	L→I	Conservative	4/5	L (4)
ACA→ACC	83	T	Silent	1/5	R (4)
ATA→ATT	91	I	Silent	1/5	I (4)
AAA→AAG	106	K	Silent	4/5	K (4)
GCA→GCC	137	A	Silent	4/5	A (4)
TCA→TCC	142	S	Silent	2/5	S (4)
CTA→CTG	154	L	Silent	4/5	Q (4)
GAA→GAG	156	E	Silent	1/5	K (3), Q (1)
TTA→TTG	165	L	Silent	1/6	I (3), L (1)
AGT→AGC	167	S	Silent	1/6	S (3), A (1)
GAT→GAC	170	D	Silent	1/6	E (3), T (1)
CCG→CCA	183	P	Silent	1/6	P (4)
TAT→TTT	184	Y→F	Conservative	5/6	F (4)
TCT→TCC	188	S	Silent	1/6	S (4)
TTT→TTC	189	F	Silent	5/6	F (3), S (1)
ACT→ACC	190	T	Silent	1/6	V (1), I (2), T (1)
GTA→GTT	192	V	Silent	1/6	I (1), V (3)
CCG→CCA	193	P	Silent	1/6	P (4)
AGA→AGC	194	R→S	Nonconservative	4/6	V (3), D (1)
GAG→GAA	195	E	Silent	1/6	Y (3), E (1)
CCG→TCG	212	P→S	Nonconservative	3/4	P (4)
ACG→ACT	226	T	Silent	1/2	T (4)

^a Based on the amino acid (AA) position in the *rep* gene of BGMV-BZ.

^b According to the nature of the amino acid side groups.

^c Sequences with changes/sequences observed.

^d Bean golden mosaic virus from Guatemala, from the Dominican Republic, and from Puerto Rico; and bean dwarf mosaic virus (BDMV). Number in parenthesis is the number of geminiviruses analyzed in which the specific AA is present.

BZ, showed identities varying from 92 to 97% to BGMV-BZ in this sequenced area (Table 1).

The 1.3-kb DNA fragment from the weed *L. sibiricus* was also cloned in the pCR II vector and completely sequenced (pLS2, GenBank accession no. U92532). A BLAST search in the GenBank, EMBL, DDBJ, and PDB databases found no closely related geminivirus. The highest nucleotide identity was 77% to AbMV for the *rep* gene. The nucleotide identity of 72% to BGMV-BZ for the *cp* gene of pLS2 (first 154 nt) clearly indicates that this virus is not BGMV-BZ (Table 2). Sequence variations between BGMV-BZ and the PCR fragments for the *rep* and *cp* genes and their derived amino acids are presented in Tables 3 and 4.

The alignment of the 154 nt from the least-conserved part of the *cp* gene for selected geminiviruses and representatives of the cloned fragments from bean that were similar to BGMV-BZ was used to construct a cladogram for these isolates and viruses (Fig. 3).

TABLE 4. Coat protein (*cp*) gene and derived amino acid sequence variations between bean golden mosaic virus-Brazil (BGMV-BZ) and the polymerase chain reaction fragments of the 16 isolates nearly identical to BGMV-BZ

Codon change	Position (AA) ^a	AA change	Nature of change ^b	Frequency ^c	AA in other bean geminiviruses ^d
CCT→CCC	2	P	Silent	8/14	P (4)
GCT→GCG	6	A	Silent	1/14	A (4)
CAT→CAC	10	H	Silent	14/14	M (2), N (1), S (1)
ATG→GTG	11	M→V	Conservative	1/14	M (3), N (1)
GGA→GCA	12	G→A	Nonconservative	12/14	A (4)
TCT→TCG	20	S	Silent	2/14	S (3), N (1)
GCT→TCT	21	A→S	Conservative	2/14	G (3), A (1)
TTT→TTC	23	F	Silent	2/13	Y (4)
GGT→GGA	28	G	Silent	2/13	G (4)
AAA→AGA	32	K→R	Conservative	2/13	K (4)
TAC→TAT	33	Y	Silent	2/13	S (3), M (1)
AAG→AGG	35	K→R	Conservative	1/13	K (3), R (1)
GCC→GCT	37	A	Silent	4/13	N (3), A (1)
GTA→GTC	40	V	Silent	1/12	V (4)
GTA→GTG	40	V	Silent	1/12	V (4)
GTA→GTT	40	V	Silent	10/12	V (4)
AAT→AAA	41	N→L	Nonconservative	2/13	N (4)
AAT→AAC	41	N	Silent	8/13	N (4)
CCT→CCA	43	P	Silent	1/13	P (4)
CCT→CCC	43	P	Silent	1/13	P (4)
CCT→CCG	43	P	Silent	1/13	P (4)
TAC→TAT	45	Y	Silent	3/13	Y (4)
AGG→AGA	46	R	Silent	2/11	R (4)
TAT→TAC	51	Y	Silent	1/9	Y (4)
ACG→ACT	53	T	Silent	1/9	M (3), T (1)
TTA→ATA	54	L→I	Conservative	1/9	Y (3), L (1)
TTA→GTA	54	L→V	Conservative	1/9	Y (3), L (1)
CGA→AGA	55	R	Silent	7/9	K (2), R (2)
GGC→AGC	56	G→S	Nonconservative	3/9	S (3), T (1)
CCT→CCC	57	P	Silent	2/9	P (4)
GAC→GAT	58	D	Silent	8/9	D (4)
CGG→CCG	61	R→P	Nonconservative	1/9	K (3), R (1)
GAA→GTA	64	E→V	Nonconservative	1/9	E (4)
TGT→TGC ^e	67	C	Silent	1/8	C (4)
GTA→GTT	69	V	Silent	1/8	V (4)
TCC→TCA	71	S	Silent	6/8	S (4)
TCC→TCT	71	S	Silent	1/8	S (4)
GTC→GTG	78	V	Silent	1/8	I (4)
TCT→TCA	79	S	Silent	1/8	S (4)
TCT→TCC	79	S	Silent	6/8	S (4)
AAG→AAA	83	K	Silent	2/8	K (4)
TCT→TCC	88	S	Silent	1/8	S (4)
GTG→GTA	90	V	Silent	1/8	V (2), I (2)
ACG→ACA	91	T	Silent	7/8	T (4)

^a Based on the amino acid (AA) position of the BGMV-BZ *cp* gene.

^b Based on the amino acid side groups.

^c Sequences with changes/sequences observed.

^d Bean golden mosaic virus from Guatemala, from the Dominican Republic, and from Puerto Rico; and bean dwarf mosaic virus. Number in parenthesis is the number of geminiviruses analyzed in which the specific AA is present.

^e Possible Zn finger motif.

DISCUSSION

PCR-amplified, geminiviral DNA-A fragments were obtained and cloned for the nine bean samples with golden mosaic symptoms and from the weed *L. sibiricus*. The bean samples represented the major bean-production areas of Brazil as well as the main edaphoclimatic regions. The low divergence ($\leq 7\%$) of the *cp* gene nucleotide sequence from that of BGMV-BZ for 20 clones from six states (Table 1) is less than the 10% that is recognized to classify an isolate as a new geminivirus (26). Only the isolate pPE-A1 (Table 2), from lima beans collected in Caruarú (PR), can be considered a new geminivirus.

A BLAST search of the GenBank database found the highest identity for the 365 nt available in the N-terminus of the pPE-A1 *cp* gene to be 81% with BGMV-BZ. This level of sequence identity clearly distinguishes this new virus from BGMV-BZ (Table 2). Because of this lack of high sequence identity with known geminiviruses, we propose the name LBGMV-BR to differentiate it from BGMV-BZ, which causes similar golden mosaic symptoms on beans. The distribution of this virus should be further studied and, because resistance to one bean-infecting geminivirus may not provide resistance to another (10), the resistance of bean germ plasm to LBGMV-BR needs to be evaluated.

Common region. The sequence of the CR has provided information on nucleotide motifs that are characteristic of phylogenetic clusters (8,13) and predictive of the ability to form recombinants between components of distinct geminiviruses (2,8). The specific putative *rep* protein-binding motif (GGNGN3-5GGNG, with the first and last Ns being G, T, or A but not C, GGTGTAATGGTG in BGMV-BZ) (8) for nucleotide positions 83 to 95 in BGMV-BZ was conserved in all 10 clones analyzed (data not shown), but not

in LBGMV-BR, which had the homologous motif GGGG (Fig. 2). This motif is also found in potato yellow mosaic geminivirus (PYMV) and sida golden mosaic geminivirus from Jamaica (SiGMV-Jamaica). In addition, the inverted motif to this direct iteron (2) is also present in BGMV-BZ, PYMV, and SiGMV-Jamaica. It is possible that pseudorecombinants could be formed among these geminiviruses because they are between tomato mottle geminivirus and bean dwarf mosaic geminivirus (23).

***rep* gene.** In nine clones from beans (Table 1), we analyzed sequences coding for a total of 1,269 amino acids of the *rep* gene (Table 3) and found 54 nt changes. Of these, 16 changed nucleotides resulted in 9 conservative and 7 nonconservative amino acid substitutions. The other 38 changes in nucleotides resulted in silent changes (Table 3).

All amino acid changes in the *rep* gene, both conservative and nonconservative, are unlikely to be due to PCR artifacts, because they were present at the same position in independently obtained clones. The amino acid residues in conserved motifs, such as the ones in the putative DNA-nicking motif F¹⁵L¹⁶T¹⁷Y¹⁸-C²¹ and Y¹⁰³ (8,25), were absolutely conserved. The 1.26% overall rate of change (16 amino acids out of 1,269 sequenced) indicates that these BGMV-BZ isolates are part of a single virus population.

***cp* gene.** For the N-terminal 66 amino acids of the *cp* gene of 16 clones, sequence analysis revealed 109 nt changes: 27 of which resulted in codon changes (8 conservative and 19 nonconservative amino acid changes) and 82 that were silent changes (Table 4). The 8 conservative and 19 nonconservative changes represent approximately 1.0 and 2.4% changes, respectively, out of the total of 790 amino acids examined in the *cp* gene. Since the nonconservative amino acid changes were 2.4 times the conservative amino acid changes, it is proposed that there are not rigid constraints on the amino

Fig 3. Cladogram showing relationships among the 10 polymerase chain reaction clones from beans with golden mosaic symptoms, the lima bean, the geminivirus from the weed *Leonurus sibiricus*, and nine selected whitefly-transmitted geminiviruses, based on alignment of nucleotide sequences for the first 154 nucleotides of the *cp* gene. Sequences were analyzed with Phylogenetic Analysis Using Parsimony, version 3.1, developed by D. L. Swofford (Illinois Natural History Survey, Champaign), using heuristic tree construction. Branch strength was tested by constructing 100 trees by the bootstrap method. The number of nucleotide changes are noted on top of the horizontal lines, and the percentage of trees with a given branch are below the horizontal lines. Vertical distances are arbitrary and horizontal distances are in proportion to the number of nucleotide differences between branch nodes: tomato golden mosaic virus (TGMV); tomato yellow vein streak virus (TYVSV) from Brazil; bean golden mosaic virus (BGMV-DR, BGMV-B, and LBGMV-BR); potato yellow mosaic virus (PYMV); bean dwarf mosaic virus (BDMV); abutilon mosaic virus (AbMV); leonurus mosaic virus (LeMV); and cloned fragments from bean, which were similar to BGMV-BZ (SP91-C73, SP96-T1, PR91-L17, PR96-WB1, BA96-B1, GO91-GYN66, GO96-GYN12, MG96-U1, and MT96-LRV1).

acids associated with this region. This is consistent with the report by Padidam et al. (26) that the N-terminus region of the *cp* gene is the most variable region of the coat protein for the WFT geminiviruses. When compared with the variability of the nucleotides for the *rep* gene, that of the *cp* gene N-terminus for the same clones was considerably greater (2.7 times).

The presence of a mixed geminiviral infection was observed in only one case in the current study, even though clones from the same or different PCR amplification rounds were analyzed for all samples utilizing degenerate primers. The bean sample from Taquarituba (SP) clearly had a mixed infection, because two of the three clones obtained yielded typical BGMV-BZ sequences and one yielded AbMV sequence. The implications from this finding include the possibility that double infections could cause more severe disease and facilitate recombination between geminiviruses. If AbMV were to be found more frequently in beans, it could lead to even greater complications in the already difficult task of breeding for disease resistance to geminiviruses.

A BLAST search of the GenBank database for 154 nt of the N-terminus *cp* gene of pLS2 from *L. sibiricus* gave the highest identity of 77% with AbMV. Because of this lack of high sequence identity with other geminiviruses, we propose the name *leonurus* mosaic virus (LeMV) for this geminivirus. LeMV was not able to infect common beans in experiments conducted by Flores and Silberschmidt (11). The component B, albeit completely uncharacterized, is likely to be present. Also, we were unable to infect *L. sibiricus* seedlings by exposure to a BGMV-BZ viruliferous whitefly population for a period of 1 week in a greenhouse (J. C. Faria; *unpublished data*). Thus, it is unlikely that this common weed is a source of inoculum or a host for BGMV-BZ.

Phylogenetic analysis for the first 151 *cp* nt confirms the clustering of all of the isolates from beans with BGMV-BZ, except SP96-T2, which is identical to AbMV. LBGMBV-BR clustered closer to BGMV-BZ than to BGMV-DR and BGMV-GA. This analysis also placed LeMV in a cluster by itself, while the recently described tomato yellow vein streak virus (9) clustered with tomato golden mosaic virus.

The PCR primers used in the current research were those developed by Rojas et al. (28) rather than those described by Wyatt and Brown (33), because the former result in DNA fragments that provide opportunities to examine a broader region of the DNA-A sequences of the WFT geminiviruses including the *rep* gene, origin of replication, and the N-terminus of the *cp* gene, which is the least-conserved region of the coat protein.

The sequenced isolate of BGMV from Brazil (GenBank accessions nos. M88686 and M88687) was collected in the 1986 bean-growing season from commercial fields in Goiânia (GO, Brazil) (18). This work provides evidence that the sequenced BGMV-BZ isolate is representative of BGMV in Brazil. The general lack of variability in the viral fragments cloned from the bean samples collected in seven states of Brazil, representing the main bean-producing areas of the country, indicates that BGMV from these regions may be considered a single population.

The reason for the lack of variability is not revealed by the data. However, it is suggested that it could simply be due to the lack of highly resistant bean cultivars that would create selection pressure toward more aggressive isolates. Programs breeding for disease resistance, using either the traditional genetic methods or genetic engineering approaches with pathogen-derived sequences, would benefit greatly from this lack of genetic variability.

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