Genetic Diversity Among Geminiviruses Associated with the Weed Species *Sida* spp., *Macroptilium lathyroides*, and *Wissadula amplissima* from Jamaica

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ABSTRACT

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Genetic diversity among geminiviruses associated with three common weeds in Jamaica was studied using digoxigenin-labeled geminiviral DNA probes, polymerase chain reaction with degenerate primers for DNA-A and DNA-B, nucleic acid sequencing, and derived amino acid sequences. Geminiviruses with bipartite genomes were found in *Sida* spp., *Macroptilium lathyroides*, and *Wissadula amplissima*. The geminiviruses detected in *Sida* spp. and *M. lathyroides* were nearly identical and were both designated Sida golden mosaic geminivirus (SidGMV-JA), whereas the geminivirus in *W. amplissima* was sufficiently different to be designated Wissadula golden mosaic geminivirus (WGMV). Nucleotide sequence comparisons of the common regions and the N-terminal regions of the AC1 (*rep*) and AV1 ORFs, together with the derived amino acid sequence comparisons of the N-terminal parts of BC1 and BV1 ORFs were used to determine their similarities to other geminiviruses. SidGMV-JA was most similar to potato yellow mosaic geminivirus (PYMV). We propose that these two geminiviruses (SidGMV-JA and PYMV) define a new geminivirus cluster, the potato yellow mosaic virus (PYMV) cluster. WGMV was most similar to members of the Abutilon mosaic virus cluster but is not likely to be included in the Abutilon phylogenetic group because of the divergent sequence of the common region. These results indicate that geminiviruses infecting some weeds in Jamaica are distinct from crop-infecting geminiviruses in Jamaica and define a new geminivirus cluster.

Weeds are important reservoirs for some plant viruses (2,5); however, it is not known if weeds are a significant source of inoculum for any important crop-infecting whitefly-transmitted geminiviruses. During the last decade, total crop failures were associated with geminivirus infection of tomatoes, peppers, beans, and cucurbits in the Western Hemisphere (4; D. P. Maxwell, unpublished data). The introduction into the Western Hemisphere of the B biotype of the whitefly, *Bemisia tabaci*, is partially responsible for these increased losses (4).

The first report of whitefly-transmitted viruses in Jamaica was made in 1975 by Pierre, who reported golden mosaic symptoms on beans and suggested that this disease was the major constraint on bean production on the island (24). This whitefly-transmitted virus was later characterized as bean golden mosaic geminivirus type II, a bipartite geminivirus, and was found in Central America and other

islands of the Caribbean region (20). Recently, the monopartite, whitefly-transmitted tomato yellow leaf curl geminivirus from the Eastern Hemisphere was reported in the Dominican Republic (22) and Jamaica (19; M. E. Werneck, M. E. Roye, M. K. Nakhla, D. P. Maxwell, and W. A. McLaughlin, unpublished). Additionally, a bipartite geminivirus was found infecting both tomatoes and peppers in Jamaica (28).

Preliminary reports indicate that the primary geminiviruses infecting weeds are not the same ones that infect crops (11,25,27), although it has been speculated that a number of common weeds may serve as alternate hosts for crop-infecting geminiviruses. Only limited work has been done to characterize the geminiviruses associated with weeds at the molecular level, whereas several geminiviruses infecting vegetable crops in the Western Hemisphere have been so characterized (see review by Green and Kallo [13]). Rhynchosia mosaic geminivirus, whose natural host is *Rhynchosia minima*, was transmitted to beans, in which it causes typical mosaic, dwarfing, and leaf distortion (9). A *Sida* sp. believed to be an alternate host for bean dwarf mosaic geminivirus (BDMV) proved to be susceptible to this virus (21). Therefore, it is important to determine if weeds are alternate hosts for crop-infecting geminiviruses. These results

may significantly affect the development of strategies for managing the spread of these geminiviruses. In this paper, we report the partial molecular characterization of geminiviruses associated with *Sida* spp., *Macroptilium lathyroides*, and *Wissadula amplissima* from Jamaica to determine if these weeds could be a possible reservoir for crop-infecting geminiviruses.

MATERIALS AND METHODS

Preparation of digoxigenin-labeled geminiviral DNA probes by polymerase chain reaction (PCR). A 1.1-kb DNA-A fragment amplified by PCR from BGMV-JA type II from Jamaica (GenBank no. L34269) was used as the template for synthesizing a geminiviral DNA probe (PBGJA) used at low stringency hybridization (11,20). Digoxigenin-11-dUTP (Dig-11-dUTP) (Boehringer Mannheim Corp., Indianapolis, IN) was incorporated into the DNA during PCR (26) with 30 cycles of 1 min at 94°C, 2 min at 55°C, and 2 min at 72°C and a reaction mixture with concentrations of 0.2 µM for each degenerate primer of the pair PAC1v1978 (viral sense polarity)/PAV1c496 (complementary sense polarity) (26), 1 mM for each of the nucleotides dATP, dCTP, and dGTP; 0.65 mM dTTP; 0.35 mM Dig-11-dUTP; 200 ng of target DNA; and 1.2 units of DNA Taq polymerase (Promega Corp., Madison, WI) in 50 µl. The presence of the expected molecular weight PCR product was confirmed by agarose gel electrophoresis (29). Digoxigenin-labeled probes from DNA-A inserts of recombinant plasmids containing PCR-amplified fragments of viruses from infected *Sida* sp. (pSGJA1) and *W. amplissima* (pWGJA2) were similarly prepared. These probes were hybridized at high stringency (11) to DNA extracted from the original infected plants to confirm that the cloned viral fragments were identical to the geminiviral DNA from the original source plants used for cloning.

Detection of geminiviral nucleic acid by dot blot hybridization. *Sida* spp., *M. lathyroides*, and *W. amplissima* with golden mosaic symptoms were collected from five parishes in Jamaica (Table 1). Viral DNA was extracted from either fresh or oven-dried (60°C) leaf samples by a modified Dellaporta extraction method and dissolved in distilled water (6,26). Each DNA extract was spotted onto either a

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Zetabind nylon membrane (CUNO, Inc., Meriden, CT) or a positively charged nylon membrane (Boehringer Mannheim) and baked at 80°C under vacuum for 2 h. Pre-hybridization, hybridization, and immunological detection were completed using the Genius 2 DNA Labeling and Detection Kit (Boehringer Mannheim) according to the manufacturer's instructions. Membranes were prehybridized (1 to 4 h) and hybridized (8 to 12 h) at either low stringency (42°C with 50% formamide) or high stringency (68°C without formamide) in 5× SSC (1× SSC = 0.15 M NaCl, 15 mM sodium citrate, pH 7) containing 0.1% N-lauroylsarcosine, 0.02% sodium dodecyl sulfate (SDS), and 2 to 5% blocking reagent (Boehringer Mannheim). Membranes were washed twice at room temperature with 2× SSC containing 0.1% SDS and twice at either 42°C (low stringency) or 68°C (high stringency) with 0.1× SSC containing 0.1% SDS. After block binding of an antibody conjugate (Boehringer Mannheim) to the hybridized digoxigenin-labeled DNA, hy-

bridization was detected by a purple color reaction initiated at alkaline pH by the addition of 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium.

Amplification of geminiviral DNA by PCR. Viral DNA was amplified using degenerate PCR primer pairs PAC1v1978/PAV1c715 for DNA-A and PBC1v2039/

Fig. 1. Polymerase chain reaction (PCR)-amplified products for (A) DNA-A of geminiviruses from *Wissadula amplissima*, *Macroptilium lathyroides*, and *Sida* sp. and (B) Southern blot DNA hybridizations. (A) Amplification of 1.3-kb fragments with primer pair PAC1v1978/PAV1c715. A 3-μl aliquot of each PCR reaction mixture was analyzed in a 0.7% agarose gel. Left lane = 1-kb ladder size marker. Plant samples are: W, *W. amplissima* sample 27, M, *M. lathyroides* sample 16, W, *W. amplissima* sample 30, M, *M. lathyroides* sample 19, S, *Sida* sp. sample 3, and S, *Sida* sp. sample 9 (Table 1). (B) Southern blot hybridization of the agarose gel in (A) (lanes in the same order). The blot was probed with the BGMV type II from Jamaica geminiviral DNA probe at low stringency.

Table 1. Geographical origin of weed samples in Jamaica used for detection of geminiviral DNA with DNA hybridization methods and polymerase chain reaction

Plant	Sample no.	Origin ^a	Date collected	Symptoms ^b	Hyb I ^c	PCR fragment size (kb)		Hyb II ^e
						DNA-A ^d	DNA-B ^d	
<i>Sida</i> sp.	1	Man	3/93	+	+	1.3	0.5	+
<i>Sida</i> sp.	2	Man	5/93	+	–	ND	ND	ND
<i>Sida</i> sp.	3	St. M	5/93	+	+	1.3	0.5	+
<i>Sida</i> sp.	4	St. M	5/93	+	+	1.3	ND	+
<i>Sida</i> sp.	5	St. T	4/93	+	+	1.3	0.5	+
<i>Sida</i> sp.	6	St. E	6/93	+	+	1.3	0.5	+
<i>Sida</i> sp.	7	Cla	6/93	–	–	–	–	–
<i>Sida</i> sp.	8	St. M	4/93	+	+	1.3	0.5	+
<i>S. urens</i>	9	St. E	6/93	+	+	1.3	0.5	+
<i>S. urens</i>	10	Man	6/93	+	+	1.3	0.5	+
<i>S. urens</i>	11	St. E	3/93	+	+	1.3	0.5	+
<i>S. acuta</i>	12	St. A	3/93	+	–	ND	ND	ND
<i>S. acuta</i>	13	Man	3/93	+	–	ND	ND	ND
<i>S. acuta</i>	14	Man	3/93	+	–	ND	ND	ND
<i>Macroptilium lathyroides</i>	15	St. A	3/93	+	+	1.3	0.5	+
<i>M. lathyroides</i>	16	St. A	3/93	+	+	1.3	0.5	+
<i>M. lathyroides</i>	17	St. T	4/93	+	+	1.3	0.55	–
<i>M. lathyroides</i>	18	St. T	5/93	+	+	1.3	0.5/0.55	+
<i>M. lathyroides</i>	19	St. M	11/94	+	+	1.3	0.5	+
<i>M. lathyroides</i>	20	St. M	4/94	+	+	1.3	0.55	–
<i>M. lathyroides</i>	21	St. E	3/93	+	+	1.3	0.55	–
<i>M. lathyroides</i>	22	St. E	3/93	+	+	1.3	0.55	–
<i>M. lathyroides</i>	23	St. E	3/93	+	+	1.3	0.5	+
<i>M. lathyroides</i>	24	Man	3/93	+	+	1.3	0.5	+
<i>M. lathyroides</i>	25	St. A	10/94	–	–	–	–	–
<i>Wissadula amplissima</i>	26	St. A	10/94	+	+	1.3	0.5	+
<i>W. amplissima</i>	27	St. E	3/94	+	+	1.3	0.5	+
<i>W. amplissima</i>	28	St. E	4/93	+	+	1.3	0.5	+
<i>W. amplissima</i>	29	Man	4/93	+	+	1.3	0.5	+
<i>W. amplissima</i>	30	St. T	6/93	+	+	1.3	0.5	+
<i>W. amplissima</i>	31	St. T	3/93	+	+	1.3	0.5	+
<i>W. amplissima</i>	32	Cla	3/93	+	–	ND	ND	ND
<i>W. amplissima</i>	33	St. E	3/93	–	–	–	–	–

^a Samples were collected in the parishes of: Man = Manchester, St. M = St. Mary, St. T = St. Thomas, St. E = St. Elizabeth, Cla = Clarendon, and St. A = St. Ann.

^b Symptoms: + = golden mosaic, – = no symptoms.

^c Hyb I = hybridization at low stringency with digoxigenin-labeled BGMV-JA type II geminiviral DNA probe (PBGJA). + = hybridization signal detected, – = no hybridization signal detected, ND = not done.

^d DNA-A = PCR-amplified DNA fragment (primers PAC1v1978/PAV1c715), DNA-B = PCR-amplified DNA fragment (primers PBC1v2039/PCRC2). Expected sizes are 1.3 kb for DNA-B and between 0.35 and 0.65 kb for DNA-A.

^e Hyb II = hybridization at high stringency with viral insert from pSGJA1 (samples 1 to 25) and pWGJA1 (samples 26 to 33). + = hybridization signal detected, – = no hybridization signal detected, ND = not done.

PCRC2 or PBC1v2039/PBV1c800 (5'-ACGACTTGACAGTTVACMGTCCTTTGAAACG-3') for DNA-B (26; M. B. Bett and D. P. Maxwell, *unpublished data*). PCR reaction conditions were as described by Rojas et al. (26). Amplified DNA fragments were electrophoresed in 0.7% agarose (FMC, Rockland, MN) minigels in 0.5× TBE buffer (45 mM tris-borate, 1 mM EDTA, pH 8.0), visualized with UV light after staining with ethidium bromide, and photographed.

Preparation of [³²P]-labeled geminiviral DNA probe and Southern blot hybridization analysis. A [³²P]-labeled geminiviral DNA probe for Southern blot hybridization (29) was used to confirm the geminiviral origin of PCR-amplified DNA fragments. A 0.6-kb fragment of DNA-B of BGMV-GA type II from Guatemala was amplified by PCR using primer pair PBC1v2039/PCRC2 (26). The 0.6-kb fragment was then labeled with [³²P]dCTP using a random priming kit (Bethesda Research Laboratories, Gaithersburg, MD). PCR with this same primer pair generated 0.5- and/or 0.55-kb fragments from DNA extracted from the weeds in this study. These fragments were purified by agarose gel electrophoresis and then transferred to a Zetabind nylon membrane for hybridization with the probe. Prehybridization and hybridization conditions at low stringency were as described by Gilbertson et al. (11).

Cloning of PCR-amplified DNA fragments. PCR-amplified products were digested with *Pst*I (Promega Corp.), extracted twice with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) and once with chloroform:isoamyl alcohol (24:1), precipitated with 2.5 volumes of absolute ethanol, washed twice with 70% ethanol, and dried in a Speed Vac dryer (Savant Instruments Inc., Farmingdale, NJ). The purified PCR products were then ligated into *Pst*I-digested pBluescript II KS+ vector (Stratagene, La Jolla, CA) and used to transform *Escherichia coli* strain XL1-Blue (Stratagene). Bacterial colonies with recombinant plasmids (beta-galactosidase negative) were selected for further characterization (29).

Nucleotide sequencing and analysis. DNA was sequenced, using [³⁵S]-dATP, with Sequenase version 2 according to the manufacturer's instructions (United States Biochemical, Cleveland, OH) and analyzed using the software of the Genetics Computer Group, Version 7, Madison, WI. Relationships among geminiviruses were examined by comparing nucleotide sequences using the GAP program of this software with a gap weight of 5 and a gap weight length of 0.3. Phylogenetic analyses were done with the computer program Phylogenetic Analysis Using Parsimony version 3.1 (PAUP) developed by D. L. Swofford (Illinois Natural History Survey, Champaign, IL). Optimum trees were obtained by the Heuristic method with the

branch swapping options. One hundred bootstrap replications were performed for each data set to assign branch strengths to topological elements. Geminiviruses were assigned to a cluster if bootstrap-replication analysis placed them in the same branch more than 50% of the time. Searches of the sequences in GenBank for similar viruses were completed with the BLAST program (<http://www.ncbi.nlm.nih.gov/BLAST>).

RESULTS AND DISCUSSION

Detection of geminiviral DNA in field-collected weed samples, with a digoxigenin-labeled geminiviral DNA probe. The geminiviral DNA probe from BGMV-JA DNA-A (PBGJA), labeled with digoxigenin, hybridized at low stringency to DNA extracts of 25 of the 30 weed samples with golden mosaic symptoms: 9 *Sida* spp., 10 *M. lathyroides*, and 6 *W. amplissima*. The probe did not hybridize with DNA from three symptomless plants: a

Sida sp. (sample 7), an *M. lathyroides* (sample 25), and a *W. amplissima* (sample 33). Those samples that had golden mosaic symptoms and did not hybridize with the probe included one *Sida* sp. plant (sample 2), three *Sida acuta* plants (samples 12 to 14), and a *W. amplissima* plant (sample 32) (Table 1). These results could be attributed to a titer of geminivirus below the level of detection or the presence of sufficient differences in the nucleotide sequence between the viral DNA and the probe to prevent hybridization at low stringency. Thus, geminiviruses are associated with these three weed genera.

Detection of geminiviruses by PCR. The PCR primer pair PAC1v1978/PAV1c715 for DNA-A amplified the predicted 1.3-kb fragment from all plant samples that gave hybridization signals with PBGJA probe (Table 1). This fragment is expected to include N-terminal parts of the *rep* and AV1 (*cp*) ORFs and the intergenic region, which contains the common region.

Table 2. Recombinant plasmids with geminiviral DNA inserts from polymerase chain reaction fragments from *Sida* sp., *Macroptilium lathyroides*, and *Wissadula amplissima*

Host	Clone no.	Insert size (kb) ^a	Genome position	Sequenced region (nt) ^b	GenBank no.
<i>Sida</i> sp. (sample 3)	pSGJA1	1.3	<i>rep</i>	2,037-2,590	U67926
			<i>cp</i>	469-716	U68177
	pSGJB1	1.4	CR-A	3-310	U69601
			BC1	2,035-2,273	U69157
<i>M. lathyroides</i> (sample 19)	pMGJA1	1.3	BV1	581-803	U69158
			CR-B	53-186	U69602
	pMGJA1	1.3	<i>rep</i>	1,960-2,228	U69159
			<i>cp</i>	400-695	U69160
<i>W. amplissima</i> (sample 27)	pWGJA2	0.55	CR-A	4-184	U70386
			<i>rep</i>	2,032-2,494	U69281
	pWGJA1	0.75	<i>cp</i>	370-707	U69280
			CR-A	3-176	U69603
	pWGJB1	1.4	BC1	2,029-2,199	U69732
			BV1	566-817	U69733
			CR-B	3-181	U69604

^a Inserts in recombinant plasmids are PCR fragments from primer pair PAC1v1978/PAV1c715 for DNA-A and PBC1v2039/PBV1c800 for DNA-B (26). PCR fragments were digested with *Pst*I restriction endonuclease before cloning into *Pst*I-digested pBluescript KS II (+).

^b Nucleotide positions represent the nucleotide numbers for the homologous regions of bean golden mosaic virus from Guatemala (7).

Table 3. Percent nucleotide sequence identities between the common region (CR) and the *rep*, *cp*, BC1, and BV1 (open reading frames) of geminiviruses from *Wissadula amplissima* and *Sida* sp. and the respective regions of seven other geminiviruses representing the major phylogenetic clusters for the Western Hemisphere geminiviruses

Geminiviruses ^a	CR	<i>rep</i>	<i>cp</i>	BC1	BV1
BGMV-GA	76/65 ^b	74/69	74/80	81/80	67/67
ToMoV	80/79	78/74	86/84	86/85	75/76
TGMV	71/73	76/76	80/79	79/80	61/67
BGMV-BZ	73/77	68/74	79/78	76/76	59/67
PYMV	67/86	75/85	84/80	84/86	59/63
PHV	63/61	76/70	74/76	84/80	61/66
SqLCV-E	57/53	60/55	78/78	77/76	51/55

^a BGMV-GA = bean golden mosaic virus from Guatemala type II; ToMoV = tomato mottle virus; TGMV = tomato golden mosaic virus; BGMV-BZ = BGMV from Brazil type I; PYMV = potato yellow mosaic virus; PHV = pepper huasteco virus; SqLCV-E = squash leaf curl virus extended host range.

^b Numerator = percent nucleotide identity for pairwise comparisons of sequence of the geminivirus from *W. amplissima* with the other geminiviruses. Denominator = percent nucleotide identity for pairwise comparisons of the sequence of the geminivirus from *Sida* sp. with the other geminiviruses.

The DNA-A probe (PBGJA) hybridized to all of the six selected 1.3-kb fragments: *Sida* sp. (samples 3 and 9), *M. lathyroides* (samples 16 and 19), and *W. amplissima* (samples 27 and 30). Therefore, these fragments are confirmed geminiviral DNA (Fig. 1). The DNA-B primer pair, PBL1v2039/PCRC2, designed to amplify a 0.3- to 0.6-kb fragment that includes the N-terminal part of BC1 and the intergenic region between the common region and BC1 ORF, amplified 0.5- and/or 0.55-kb fragments from all three plant genera, which gave hybridization signals with the PBGJA probe. Two fragment sizes obtained with primer pair PBC1v2039/

PCRC2 for DNA-B indicated that *M. lathyroides* samples were probably infected with one or more geminiviruses, and that sample 18 had a mixed infection as indicated by the amplification of the two different PCR fragment sizes. A second DNA-B primer pair, PBC1v2039/PBV1c800, amplified the expected 1.4-kb fragment from one representative of each of the three plant genera (samples 3, 19, and 27). The 1.4-kb fragment is expected to include parts of the BC1 and BV1 ORFs, plus the intergenic region that includes the common region. The [³²P]-labeled geminiviral probe from DNA-B of BG MV-GA hybridized at low stringency to selected PCR-amplified 0.5-

kb fragments from *Sida* sp. (sample 3), *M. lathyroides* (sample 19), and *W. amplissima* (sample 27). These results indicate that these three weeds are infected with bipartite geminiviruses.

The 1.3-kb DNA-A fragments generated by PCR were cloned, and recombinant plasmids were obtained for *Sida* sp. (sample 3, pSGJA1) and *M. lathyroides* (sample 19, pMGJA1). Two fragments (0.75- and 0.55-kb) were obtained when the 1.3-kb PCR-amplified fragment from *W. amplissima* (sample 27) was digested with *Pst*I for cloning. The 0.75- and 0.55-kb fragments were cloned as pWGJA1 and pWGJA2, respectively. Similarly, the pu-

	1				50
BGMV-DR	CATATTTGTA	AA.TATGCGA	GTG.TCTTCC	GAATGGGTTT	GCGAGTGTCT
BGMV-GA	CATATTTGTA	AA.TATGCGA	GTG.TCTCCA	AATGAGTTTG	CGAGTG.TCT
AbMV	CATTTTATGTA	A..TAAGAAG	GGGTACTCTG	GATGAGTTA.	CT
BDMV	CATTTTTGTA	A..TAAGAGC	TGGTACTCCA	GTTGAGTTA.	CT
ToMoV	CATTTTTGTA	A..TAAGAAG	AGGTACTCCA	GATGAGTT..	ACT
TGMV	CATTTTTGTA	A..TTAAGAG	G.....		...CTTACTA
BGMV-BZ	CATACTTGTA	AA.TAAGAGG	GTG.....		TAC
PYMV	CATTTTTGTA	AA.TATGAAT	GTTC.TCCCA	ATAT.....	GTTCC
SidGMV-JA	CATTTTTGTA	AA.TATATGA	T.....		GTTCCC
MacGMV-JA	CATTTTTGTA	A..TATGAGG	AT.....		GTTCC
WGMV	CATTTTTGTA	A..TAAGAGG	GGTGTCACCA	ATTGAG.ATG	AGGGGTGTCA
MacGMV-DR	CATACTTGTA	AA.TAAGAGC	TT.....		GTCCA
TLCrV	CATTTTTGTA	A..TAAGATG	GGTA.....		CT
PHV	CATATTTGTA	A..TAAGAGA	GGTGA....		CA
SqLCV-E	CATATTTCTG	AAATATGCAT	CG.....		GGACA
BCMoV	ATATTTGTA	AA.TATGAGC	CA.....		GGACa
SqLCV-R	CATATTTGGT	AAATATGAAC	CG.....		GGACA
Cons	ATttttTgta	Aa.Tatgagg	gg.....		tc.
	51				100
BGMV-DR	CCAATT..GA	GG...CTCCT	CAAACCTCTCG	CATTCAAT..	TGGAGA..CT
BGMV-GA	CCAATT..GA	GG...CTCCT	CAAACCTCTCG	CTATGCAAT.	TGGAGA..CT
AbMV	CCAATT..GA	GG...CTCCT	CAAACTTGC	TCATGTAAT.	TGGAGT.ATT
BDMV	CCAATT....	.CCCCCTCT	CAAACTATC	TCATTCTAT.	TGGAGT.ATT
ToMoV	CCATTT..GA	G...CCTTCT	CAAACCTGCT	CATTCAAT..	TGGAGTAT.T
TGMV	CCAATT..GA	GGAGGGGCTC	CAAAAGTTAT	ATGAAT....	TGGTGA.TAA
BGMV-BZ	CCCGATT.GA	G.CTCTCGTT	CAAAAGTCTC	TATGAATC..	.GGTGA.AT
PYMV	CCTATTGCT	CTGG...CTCT	CAAACTCTT	ATGAAT....	TGGGGGAACT
SidGMV-JA	CCCTTAG...CTCTCTCGCT	CAAAAGTCTT	AAGAAT....	TGGGGGAACT	
MacGMV-JA	CCAATAG...CTCTGGCTCT	CAAACTTCCT	ATGAAT....	TGGGGGAACT	
WGMV	CCAGTT..GA	GG...CTCTCT	CAAACTTGCT	CAAGCAAT..	TGGTG...ACT
MacGMV-DR	CCACTC..GA	G...CTCCTCT	CAAACTGTCT	TATACGTTT.	TGGTGA..CT
TLCrV	CCGATT..GA	G....CTCT	CAAACTTCTG	TGCTATGTTT	TGGGGTAA.A
PHV	CCGATTG.GA	G...CTCTT	TAACCTGGGC	TTATGTATC	.GGTGTAT.T
SqLCV-E	CCAG....GA	GGTGTCCTCT	CAACTTCTCT	ATATTGC...	TGGTGTC.CT
BCMoV	CCAGGgGGGA	G..CTCTCTC	CAAAACCTAT	TGTTTT....	TGGTGTC.CT
SqLCV-R	CCAGGGG.GA	G..CTCTCTC	TAAACCTAT	TATTGC....	TGGTGTC.CT
Cons	CCaatt..ga	g....c.cct	cAAactttct	ttttat....	tGGtgtt...ct

Fig. 2. continued on next page

Fig. 2. Nucleotide sequence alignment of the common region of 14 whitefly-transmitted geminiviruses from the Western Hemisphere. Sequences start at the initiation codon of the *rep* ORF (CAT, viral sense polarity) and terminate at the 3' end of the stem-loop motif. The TATA box and stem-loop motif are marked with an asterisk (*). The repeat motifs downstream of the start of the common region, the repeat motifs upstream of the TATA box, and the conserved motif of the SqLCV cluster are all underlined. Gaps were inserted to provide maximum identity among the viruses. BGMV-DR = bean golden mosaic geminivirus from Guatemala (GenBank accession no. M91604), BGMV-GA = bean golden mosaic geminivirus from the Dominican Republic (L01635), AbMV = Abutilon mosaic geminivirus from West Indies (X15983), BDMV = bean dwarf mosaic virus from Colombia (M88179), ToMoV = tomato mottle virus from Florida, TGMV = tomato golden mosaic geminivirus from Brazil (K02029), BGMV-BZ = bean golden mosaic geminivirus from Brazil (M88686), PYMV = potato yellow mosaic geminivirus from Venezuela (D00940), SidGMV-JA = Sida golden mosaic virus from Jamaica (U69601), MacGMV-JA = Macroptilium golden mosaic virus from Jamaica isolate 2 (U73511), WGMV = Wissadula golden mosaic virus from Jamaica ((U69603), MacGMV-DR = Macroptilium golden mosaic virus from the Dominican Republic (25), TLCrV = tomato leaf crumple virus from Mexico (L34748), PHV = pepper huasteco virus from Mexico (X70418), SqLCV-E = squash leaf curl geminivirus from California, with extended host range (M38183), BCMoV = bean calico mosaic geminivirus (L22757), SqLCV-R = squash leaf curl geminivirus from California, with restricted host range (16), and Cons = consensus sequence.

tative DNA-B fragments generated by PCR with primer pair PBC1v2039/PBV1c800 were cloned, and recombinant plasmids with 1.4-kb *Pst*I inserts were obtained for *Sida* sp. (sample 3, pSGJB1) and *W. amplissima* (sample 27, pWGJB1). Nucleotide sequences for the cloned parts of *rep*, *cp*, BC1, and BV1 ORFs, and the common regions of DNA-A and DNA-B, were obtained for each insert from these recombinant plasmids (Table 2). The sequences of the ends of each insert were as expected for the predicted annealing position of the PCR primers (26). The *Pst*I site in the 1.3-kb DNA-A fragment of *W. amplissima* was near the start of the *rep* ORF.

Hybridization of the viral inserts of pSGJA1 and pWGJA1 to weed samples.

To determine the distribution of the geminiviruses cloned from *Sida* sp. and *W. amplissima*, digoxigenin-labeled DNA-A viral inserts of pSGJA1 and pWGJA1 were used as probes at high stringency in dot blot hybridization to the weed samples that gave hybridization signals with the geminiviral DNA probe from pBGJA. The probe from pSGJA1 was used with the samples of *Sida* spp. and *M. lathyroides*, and gave strong hybridization signals from all the *Sida* spp. samples. Also, this probe gave strong hybridization signals with 60% of the *M. lathyroides* samples (Table 1),

and these were the samples that gave the 0.5-kb PCR-amplified fragment with the primer pair PBC1v2039/PCRC2. All six *W. amplissima* samples hybridized strongly with the probe from pWGJA1. The probe from pSGJA1 did not hybridize with DNA from the four symptomless weed samples or with pWGJA1. Likewise, the probe from pWGJA1 did not hybridize with the DNA from the four symptomless weed samples or with pSGJA1. This indicates that these two viruses, the one from *Sida* spp. and the one from *W. amplissima*, are widely distributed on the island and that the geminivirus from *Sida* spp. is also present in some *M. lathyroides* samples. Also,

Fig. 2. continued from previous page

	101				150
BGMV-DR	GGAGTA..CA	ATATATACTA	GAACCCCTCAA	TCTCGTGAAT	TACGAGATTCT
BGMV-GA	GGAGTA..CA	ATATATACTA	GTACCCTCAA	TCTCGTGAAT	TATCAGATTCT
AbMV	GGAGGT..CT	TTATATACTA	GAACCTCTCAT	TAACGGATTT	G.....
BDMV	GGAGTT..AC	TTATATACTA	GAACCCCTCAA	TCTGGTTTCG	G.....
ToMoV	AGAGTAACTT	ATATATA..A	GAACCCCTCTA	TAGAACTATT	AATCTGGTTCT
TGMV	GGTAG..CTC	TTATATATTA	GAAGTTCCTA	AGGGG.....	
BGMV-BZ	GGTG...CCA	ATATATAGTA	AGAAGTTCTT	TAAGGATCTG	TAG.....
PYMV	GGGGG..AAC	TTATATAGTA	GAAGTTCCTA	AAGGCAGAT.	
SidGMV-JA	GGGGG..AAC	TTATATAGTA	GAAGTTCCTT	AAAGGCTAAT	
MacGMV-JA	GGGGG..AAC	TTATATACTA	GAAGCTCCTA	AGGTAAAAC.	AA.....
WGMV	GGTGT..ACA	ATTTATACTA	GAACCCCTCAA	TAGAACTTTC	AATCGtGTTCT
MacGMV-DR	GGTGT....A	TAATATACTA	GAACGCTCTA	TAGAACTTCT	AATCTCATTC
TLCrV	GGGGA...CA	ATATATACTA	CAACTCTTAG	TAGCACTTTA	GCG.....
PHV	GGTAG..CCA	ATATATAGTA	TATGGGAGTT	ATCTAGGATC	TTCGT.....
SqLCV-E	GGTGT..CCT	ATATATACCT	CAAGACACAT	AAAGCCTCTA	GGGGACACCA
BCMoV	GGTGT..CCC	ATATATACTA	CAAGTCTCTT	TAGGCCTCTT	TAGGACACCA
SqLCV-R	GGTGT..CCC	ATTTATACAA	CTCTCT....	GGG	GAGGACACCA
Cons	ggtgt...ccc	ataTATActa	gaaccctcta	tagg.ctatt	tatca.a..c

	151				200
BGMV-DR	ACACACGTGG	..CGGCCATC	CGATATAAATA	TTACCGGATG	GCCGCGCGCG
BGMV-GA	ACACACGTGG	..CGGCCATC	CGATATAAATA	TTACCGGATG	GCCGCCCCGCG
AbMV	CAACACGTGG	..CGGCCATC	CGCTATAAATA	TTACCGGATG	GCCGCGCG
BDMV	..AACACGTGG	..CGGCCATC	CG..TATAATA	TTACCGGATG	GCCGCG
ToMoV	ATACACGTGG	..CGGCCATC	CGATATAAATA	TTACCGGATG	GCCGCG
TGMV	...CACGTGG	..CGGCCATC	CG..TTTAATA	TTACCGGATG	GCCGCG
BGMV-BZ	..ACACGTGG	..CGGCCATC	CGCTATAAATA	TTACCGGATG	GCCGCG
PYMV	CAACACGTG.	..CGGCCATC	CGTTATAAATA	TTACCGGATG	GCCGCG
SidGMV-JA	CAACACGTGT	..CGGCCATC	CGTTATAAATA	TTACCGGATG	GCCGCGCG
MacGMV-JA	.CACACGTGG	..CGGCCATC	CGCTATAAATA	TTACCGGATG	GCCGCGCG
WGMV	GCACACGTGG	..CGGCCATC	CGCTATAAATA	TTACCGGATG	GCC
MacGMV-DR	GCACACGTGG	..CGGCCATC	CGATATAAATA	TTACCGGATG	GCCGCG
TLCrV	..ACACGTGG	..CGGCCATC	CGATATAAATA	TTACCGGATG	GCCGCG
PHV	..ACACGTGA	G..GGCCATC	CGTTATAAATA	TTACCGGATG	GCCG
SqLCV-E	AGGGGCAAAA	T..CGGCCATC	CGCAATAAATA	TTACCGGATG	GCCGCCCCG
BCMoV	TGGGCAAAAT	..CGxCCATC	CGAAATAAATA	TTACCGGATG	GCCGCG
SqLCV-R	GGGGCAAAAT	..CGGCCATC	CGCAATAAATA	TTACCGGATG	GCCGCG
Cons	acacacgtgg	..cGgCCATC	CGataTAATA	TTACCGGATG	GCCGcGCGG
		*****	*****	*****	*****

the geminivirus in *M. lathyroides*, which gave a 0.55-kb PCR fragment with primer pair PBC1v2039/PCRc2, needs to be identified.

Sequence analysis. The nucleotide sequences of parts of the common region, and the *rep*, *cp*, BC1, and BV1 ORFs of cloned geminiviruses from *Sida* sp., *M. lathyroides*, and *W. amplissima* are listed in Table 2.

The common region sequences of the inserts of the DNA-A and DNA-B from *Sida* sp. (sample 3) and *W. amplissima* (sample 27) were compared to determine if these plants were infected with a bipartite geminivirus. The common regions for the putative geminivirus cloned from *Sida* sp. (pSGJA1, pSGJB1) for DNA-A and DNA-B, respectively, were greater than 99% identical, as were the common regions for the DNA-A and DNA-B inserts from *W. amplissima*. Therefore, both the *Sida* sp. and *W. amplissima* samples have a bipartite geminivirus associated with them. A comparison of the DNA-A common regions of pSGJA1 and pWGJA1 gave 61% nucleotide (nt) identity. Nucleotide comparisons of the entire common region and the sequenced parts of *rep* and *cp* ORFs of the viral inserts of pSGJA1 and pMGJA3 were greater than 97% identical, which indicates that these two plant species are infected with the same virus.

The nucleotide sequences of the cloned viral inserts were compared with those of 7 bipartite, whitefly-transmitted geminiviruses representing the major phylogenetic clusters for the Western Hemisphere.

These include tomato mottle geminivirus (ToMoV; GenBank no. L14460, L14461) from Florida, BGMV-GA type II from Guatemala (GenBank no. M91604, M91605), BGMV-BZ type I from Brazil (GenBank no. M88686, M88687), pepper huasteco geminivirus (PHV; GenBank no. X70418, X70419) from Mexico, potato yellow mosaic geminivirus (PYMV; GenBank no. D00940, D00941) from Venezuela, squash leaf curl geminivirus with an extended host range (SqLCV-E; GenBank no. M38132, M38133) from California, and tomato golden mosaic geminivirus (TGMV; GenBank no. K02029, K02030) from Brazil. Nucleotide sequences of the *rep* ORF of pWGJA2 from *W. amplissima* showed the highest nt identity (78%) to ToMoV and less than 77% identity to the six crop-infecting geminiviruses from the Western Hemisphere. Similar comparisons for the geminivirus from *Sida* sp. showed 85% identity to homologous regions of PYMV and less than 77% to the other six viruses (Table 3). The common region, *cp*, and BC1 sequence pairwise comparisons gave similar trends.

Comparison of these sequences for the geminiviruses from *Sida* sp. and *W. amplissima* from Jamaica with sequences of geminiviruses from other weed samples collected in the Western Hemisphere indicated that these geminivirus from Jamaica were not previously reported. Pairwise comparison of the common region sequences of the geminivirus from *Sida* sp. and the one from *W. amplissima* with those of Macroptilium golden mosaic geminivirus

from the Dominican Republic (MacGMV-DR), Sida golden mosaic geminivirus from Costa Rica (SidGMV-CR), and Calopogonium golden mosaic geminivirus from Costa Rica (25) had nucleotide identities of $\leq 80\%$. Thus, different geminiviruses infect these weeds in different regions of the Caribbean Basin and Central America, and none of these weed-infecting geminiviruses have been detected in a crop plant (3,25).

Common region analysis. The organization of the common region is important in determining phylogenetic relationships among geminiviruses (1,7). Common region alignment for the DNA-A for the three geminivirus isolates from this study and nine geminiviruses that represent the major phylogenetic clusters for Western Hemisphere geminiviruses were compared (Fig. 2). A repeat motif of 14 nucleotides GAGGGGTGTACCA was present at approximately 15 nucleotides 3' of the start of the common region for pWGJA1. Smaller repeats in this part of the common region were present in other viruses, including ToMoV and BDMV (TACTCCA), AbMV (TACTC), and PYMV (ATGTTC). This repeat motif was not present in pSGJA1 and pMGJA1. Another repeat motif is found immediately 5' of the TATA box for *rep* ORF (7), which is the likely target for the binding of the homologous Rep protein (8). The motif for pSGJA1 and pMGJA1 is TGGGGGAAC (viral sense polarity) and corresponds to the repeat in PYMV and is distinctly different from those for other geminiviruses (7). This motif for pWGJA1 is TGGTGAC (viral sense polarity) and appears to be unique. This repeat motif for each geminivirus is also present on the complementary strand about 33 nt 3' of the TATA box for *rep* ORF, as originally discussed by Argüello-Astorga et al. (1). In the Western Hemisphere geminiviruses, a CACGTG motif is located immediately 5' of the stem-loop motif, except for the viruses in the SqLCV cluster, which have a GGGGCAA motif at this position (7). These motifs are not present in members of the Eastern Hemisphere whitefly-transmitted geminiviruses (7). The CACGTG motif is similar to the well-defined transcriptional *cis*-regulated elements in plants (1). The conserved stem-loop motif, which is essential for DNA replication, was present in all three geminivirus isolates from Jamaica (7,17), and only slight differences were present. The loop sequence, TAATATTAC, which contains the nicking site essential for rolling circle replication (15), was conserved in the stem-loop regions of these Jamaican isolates.

Phylogenetic analysis. The phylogenetic analyses indicate that the two geminiviruses associated with *Sida* spp. and *W. amplissima* from Jamaica are phylogenetically related to Western Hemisphere geminiviruses (7) and that they are two distinct new viruses.

Fig. 3. Cladogram showing the interrelationship among 17 Western Hemisphere bipartite geminiviruses based on alignment of nucleotide sequence of the common region (Fig. 2). Sequences were analyzed by the PAUP program using Heuristic tree construction and branch strength was tested by construction of 100 trees using bootstrap with branch swapping. The percentages of trees with a given branch are below the horizontal lines. Vertical distances are arbitrary and horizontal distances are proportional to the number of nucleotide differences between branch nodes. BGMV-GA = bean golden mosaic geminivirus from Guatemala (M91604), BGMV-BZ = bean golden mosaic geminivirus from Brazil (M88686), BGMV-DR = bean golden mosaic geminivirus from the Dominican Republic (L01635), AbMV = Abutilon mosaic geminivirus from West Indies (X15983), BDMV = bean dwarf mosaic virus from Colombia (M88179), ToMoV = tomato mottle virus from Florida, TGMV = tomato golden mosaic geminivirus from Brazil (K02029), PYMV = potato yellow mosaic geminivirus from Venezuela (D00940), SidGMV-JA = Sida golden mosaic virus from Jamaica (U69601), WGMV = Wissadula golden mosaic virus from Jamaica (U69603), MacGMV-DR = Macroptilium golden mosaic virus from the Dominican Republic (25), MacGMV-JA = Macroptilium golden mosaic geminivirus from Jamaica (isolate 2, U73511), TLCrV = tomato leaf crumple virus from Mexico (L34748), PHV = pepper huasteco virus from Mexico (X70418), SqLCV-E = squash leaf curl geminivirus from California, with extended host range (M38183), BCMoV = bean calico mosaic geminivirus (L22757), SqLCV-R = squash leaf curl geminivirus from California, with restricted host range (16).

An alignment of approximately 200 nucleotides of the common region (Fig. 2) of DNA-A was used to construct a cladogram (Fig. 3) of 17 whitefly-transmitted geminiviruses from the Western Hemisphere, including three new geminiviruses cloned from plant species sampled in Jamaica. The cladogram placed the Western Hemisphere geminiviruses in previously described phylogenetic groups or clusters including the BGMV type II, AbMV, and SqLCV clusters (7,23). The geminivirus associated with *Sida* spp. and a second geminivirus infecting *M. lathyroides* (GenBank no. U75277) (28) was phylogenetically related to PYMV. Common region analysis showed that these two geminiviruses had identical conserved motifs in the common region (Fig. 2). In the common region cladogram, WGMV was not placed in a designated phylogenetic cluster (Fig. 2). A second cladogram generated from a 450 nucleotide alignment of the N-terminus of the *rep* ORF of 14 whitefly-transmitted geminiviruses from the Western Hemisphere, two monopartite tomato-infecting geminiviruses from the Eastern Hemisphere, and the two new geminiviruses from this study (Fig. 4) gave similar results to the common region generated cladogram.

Faria et al. (7) proposed eight phylogenetic clusters of 15 geminiviruses from the Western Hemisphere and 9 geminiviruses from the Eastern Hemisphere based on the sequence analysis of the common region, *cp*, *rep*, BV1, and BC1 ORFs. Several motifs of the common region are indicative of these clusters and include the repeat motif near the start of the common region, the repeat motif immediately upstream of the TATA box, and the GGGGCAA motif

of the SqLCV cluster a few nucleotides upstream of the stem-loop motif (7). The infectivity of pseudorecombinants was also an indicator of geminiviruses belonging to the same cluster. Only geminiviruses in a similar cluster formed infectious pseudorecombinants, e.g., BGMV-GA and BGMV-DR (7), ToMoV and BDMV (12), and SqLCV-E and SqLCV-R (16); whereas geminiviruses from different clusters have not produced infectious pseudorecombinants, e.g., BGMV-GA and BGMV-BZ (10), and TGMV and SqLCV (18).

Sequence database searches of GenBank with the BLAST program revealed that the viruses from *Sida* sp. and *W. amplissima* have not been previously reported. Thus, we propose that the virus associated with *Sida* sp. (pSGJA1 and pSGJB1) be designated *Sida* golden mosaic virus (SidGMV-JA) from Jamaica and that it be considered a member of the PYMV cluster because of the high degree of identity of repetitive motifs in the common region, and nucleotide and amino acid similarities of the sequenced parts of *rep*, *cp*, BC1, and BV1 ORFs. This same virus was also present in six of 10 samples of *M. lathyroides*. SidGMV-JA is distinct from the SidGMV-CR, as determined by percent nucleotide identity (62%) for the common region. The virus associated with *W. amplissima* (pWGJA1, pWGJA2, and pWGJB1) is given the name *Wissadula* golden mosaic virus (WGMV) from Jamaica. Although it is similar to members of the AbMV cluster, it is not likely to be included in that phylogenetic group because of the different repeat motif (TGGTGAC) immediately upstream of the TATA box in the common region and the 14 nucleotide repeat near the 5' end of

the common region that is not present in the AbMV cluster.

In conclusion, we propose that SidGMV-JA be included as a new member of the PYMV cluster, and that WGMV be tentatively considered a member of a new geminivirus cluster. Neither of these viruses is associated with beans (20), tomatoes, or peppers in Jamaica (28). The common region of the bipartite geminivirus from tomatoes and peppers in Jamaica has 76 and 70% nucleotide identity to SidGMV and WGMV, respectively (M. E. Roye, M. E. Werneck, W. A. McLaughlin, M. K. Nakhla, and D. P. Maxwell, *unpublished data*). Thus, the importance of weeds as alternative hosts for crop-infecting geminiviruses in Jamaica will need further investigation. From our results, it is evident that *Sida* spp., *M. lathyroides*, and *W. amplissima* are not reservoirs for geminiviruses important on vegetable crops in Jamaica.

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Fig. 4. Cladogram showing the interrelationships among 16 geminiviruses from the Western Hemisphere based on alignment of 450 nucleotides of the N-terminal region of *rep* ORF. Sequences were analyzed as in Figure 3. MacGMV-JA = *Macropitulum* golden mosaic geminivirus from Jamaica, isolate 2 (U69159), TYLCV-Sar = tomato yellow leaf curl geminivirus from Sardinia (X61153), and TYLCV-Ipr = tomato yellow leaf curl geminivirus from Israel (X15656). BGMV-DR = bean golden mosaic geminivirus from the Dominican Republic (L01635), BGMV-GA = bean golden mosaic geminivirus from Guatemala (M91604), BGMV-BZ = bean golden mosaic geminivirus from Brazil (M88686), AbMV = Abutilon mosaic geminivirus from West Indies (X15983), BDMV = bean dwarf mosaic virus from Colombia (M88179), ToMoV = tomato mottle virus from Florida, TGMV = tomato golden mosaic geminivirus from Brazil (K02029), PYMV = potato yellow mosaic geminivirus from Venezuela (D00940), SidGMV-JA = *Sida* golden mosaic virus from Jamaica (U69601), WGMV = *Wissadula* golden mosaic virus from Jamaica (U69603), PHV = pepper huasteco virus from Mexico (X70418), SqLCV-E = squash leaf curl geminivirus from California, with extended host range (M38183), BCMoV = bean calico mosaic geminivirus (L22757). The nucleotide sequence for the *rep* ORF of AbMV was adjusted as discussed previously (14).

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