Two Newly Described Begomoviruses of *Macroptilium lathyroides* and Common Bean

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Accepted for publication 12 February 2003.

ABSTRACT

Idris, A. M., Hiebert, E., Bird, J., and Brown, J. K. 2003. Two newly described begomoviruses of *Macroptilium lathyroides* and common bean. *Phytopathology* 93:774-783.

Macroptilium lathyroides, a perennial weed in the Caribbean region and Central America, is a host of *Macroptilium yellow mosaic Florida virus* (MaYMFV) and *Macroptilium mosaic Puerto Rico virus* (MaMPRV). The genomes of MaYMFV and MaMPRV were cloned from *M. lathyroides* and/or field-infected bean and the DNA sequences were determined. Cloned A and B components for both viruses were infectious when inoculated to *M. lathyroides* and common bean. Comparison of the DNA sequences for cloned A and B components with well-studied begomovirus indicated that MaMPRV (bean and *M. lathyroides*) and MaYMFV (*M. lathyroides*) are unique, previously undescribed begomoviruses from the Western Hemisphere. Phylogenetic analysis of viral A components indicated that the closest relative of MaYMFV are members of the *Bean golden yellow mosaic virus* (BGYMV) group, at 76 to 78% nucleotide identity, whereas the closest relative for the A component of MaMPRV was *Rhynchosia golden mosaic virus* at 78% nucleotide

identity. In contrast, BGYMV is the closest relative for the B component of both MaYMFV and MaMPRV, with which they share ~68.0 and ~72% identity, respectively. The incongruent taxonomic placement for the bipartite components for MaMPRV indicates that they did not evolve entirely along a common path. MaYMFV and MaMPRV caused distinctive symptoms in bean and *M. lathyroides* and were transmissible by the whitefly vector and by grafting; however, only MaYMFV was mechanically transmissible. The experimental host range for the two viruses was similar and included species within the families Fabaceae and Malvaceae, but only MaYMFV infected *Malva parviflora* and soybean. These results collectively indicate that MaMPRV and MaYMFV are new, previously undescribed species of the BGYMV group, a clade previously known to contain only strains and isolates of BGYMV from the Caribbean region that infect *Phaseolus* spp. Both MaYMFV and MaMPRV may pose an economic threat to bean production in the region.

Additional keywords: Aleyrodidae, *Bemisia tabaci*, Geminiviridae, legume virus, whitefly-transmitted virus.

Macroptilium lathyroides L. Urban is a perennial weed in the family Fabaceae that is common in the Caribbean region and Central America and is also found in southern Florida. *M. lathyroides* plants often exhibit bright or diffuse yellow mosaic patterns on leaves (Fig. 1A and C) that are like those associated with other diseases caused by whitefly (*Bemisia tabaci* (Genn.))-transmitted geminiviruses (2,3,6,7) (genus *Begomovirus*; family Geminiviridae). A suspect begomovirus of *M. lathyroides* in Puerto Rico has been shown to be transmissible to bean, *Phaseolus vulgaris* (L.), by the Sida race of *B. tabaci* (2) and subsequently by the B biotype (19) of *B. tabaci*, a member of this species complex that originated in the Eastern Hemisphere (6,8) that has recently become established in Florida and Puerto Rico (8,33), raising a suspicion that this virus may actually be an important, but previously undetected, bean-infecting virus in the Caribbean region.

Bean golden yellow mosaic virus (BGYMV) has long been thought to be the sole causal virus of the yellow mosaic diseases of bean and *M. lathyroides* in Puerto Rico, owing to similar symptoms observed for this weed and BGYMV-infected bean and their transmissibility by *B. tabaci* (2,3,28). Until recently, all field isolates from bean in the Caribbean region have been identified as sap-transmissible strains or isolates of BGYMV found in Puerto Rico (BGYMV-PR) that infect only *Phaseolus* spp. and possibly *M. lathyroides* but not soybean (26). BGYMV variants are widely

distributed in the Dominican Republic, Florida, Guatemala, Mexico, and Puerto Rico (2,3,5,7,22,23,25,28), locations where *M. lathyroides* is also present. This BGYMV group comprises a clade of closely related strains and isolates in the Caribbean region that share greater than 93% sequence identity (40), are thought to be host-adapted to the family Fabaceae, and possibly, are restricted to the genus *Phaseolus* (26,42).

Other well-studied bean-infecting begomoviruses are common to the Americas but are classified in one of several other Western Hemisphere clades. *Bean dwarf mosaic virus* from Colombia (27) and *Bean golden mosaic virus* (BGMV) from Brazil (25) naturally infect bean and are distinct species in the *Abutilon mosaic virus* from the West Indies and BGYMV clades (41), respectively. *Bean calico mosaic virus* (BCaMV) infects bean in northwestern Mexico, causing green-white and green-yellow foliar discoloration, or calico symptoms, and is a member of the *Squash leaf curl virus* (SLCV) clade (36,40), whose members occur primarily in the south and southwestern United States, western Mexico, and Guatemala (6,9,10,13,15,29,36).

Several other begomoviruses from the Western Hemisphere also infect bean naturally or experimentally, including *Chino del tomate virus* (12,16), *Cotton leaf crumple virus* (11,14), *Cucurbit leaf curl virus* (9), *Tomato leaf curl Sinaloa virus* (34), and SLCV and *Squash mild leaf curl virus* (13,36). These viruses cause severe leaf curling, mild to severe chlorosis, and stunting, in contrast to the golden or bright yellow mosaic phenotype that is typical for Caribbean and Brazilian bean-infecting viruses (2,3,22,25).

A suspect begomovirus previously associated with *M. lathyroides* in Florida (2) was recently shown to infect bean when

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Publication no. P-2003-0508-01R

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experimentally inoculated using the B biotype of *B. tabaci* as the vector or by sap inoculation (46). Although widespread infection of bean in Florida by the *M. lathyroides* virus had not been observed despite an abundance of the whitefly vector there, in 1993, golden mosaic symptoms were observed for the first time in bean fields near Homestead, FL. Because bean plants exhibited golden mosaic symptoms similar to those reported for the *M. lathyroides* virus (28,46), the two isolates were referred to as BGYMV-Florida (BGYMV-FL). Subsequent analysis of partial nucleotide sequences for the bean- and *M. lathyroides*-associated isolates revealed that they were distinct species, leading to the provisional designation of the *M. lathyroides*-associated virus as *Macropitium yellow mosaic Florida virus* (MaYMFV) and the bean-associated virus as BGYMV-FL (5,28,46).

Most recently, a comparison of sequences was carried out for the coat protein (CP) gene and common region (CR) of the DNA-A component (DNA-A CR) for begomovirus isolates from *M. lathyroides* (M) and bean (B) in Puerto Rico, which were expected to be BGYMV-PR. Surprisingly, comparisons revealed the presence of a previously undescribed begomoviral species in both hosts. The virus, provisionally designated *Macropitium mosaic Puerto Rico virus* (MaMPRV), diverged from BGYMV-PR by 16% with respect to the CP gene and had a unique DNA-A CR sequence (33), collectively, providing support for a distinct viral species (1,6,38,40).

Here, we report the cloning and sequencing of infectious DNA-A and DNA-B components for MaYMFV and MaMPRV, two previously unrecognized begomoviral species associated with yellow

mosaic diseases of bean and *M. lathyroides* in Florida and Puerto Rico, respectively. Phylogenetic analysis of their DNA-A component sequences and those of well-studied begomoviruses indicated that MaYMFV and MaMPRV are the second and third species in the BGYMV clade, which previously contained only BGYMV (40).

MATERIALS AND METHODS

Virus sources and maintenance. For Florida isolates, leaves of MaYMFV-infected *M. lathyroides* exhibiting angular, golden mosaic symptoms, or of bean plants showing golden mosaic symptoms, were collected near Homestead, FL in 1985 and 1990, respectively. Field isolates were established in *M. lathyroides* and common bean (cv. Top Crop) by transplanting symptomatic plants to pots and by serial transfer using sap inoculation, respectively. Cultures were maintained in the greenhouse at the University of Florida, Gainesville (5,28,46). Total nucleic acids were extracted from symptomatic leaves of field samples and greenhouse-maintained stock plants following the method of Sunter et al. (47). Extracts were stored under ethanol at -20 or -80°C until analysis.

Samples were collected in Puerto Rico from *M. lathyroides* plants exhibiting diffuse yellow mosaic or bright yellow mosaic symptoms during 1994, 1995, and 1998. Samples were also collected from symptomatic bean (*P. vulgaris*) plants showing bright yellow mosaic and/or veinal chlorosis during 1989, 1994, 1997, and 1998. To establish virus cultures in the greenhouse, field isolates were transferred to healthy bean or *M. lathyroides* seedlings,

Fig. 1. Typical symptoms of **A**, *Macropitium mosaic Puerto Rico virus* (MaMPRV)-infected *Macroptilium lathyroides* and **B**, bean 'Top Crop', and symptoms of **C**, *Macropitium yellow mosaic Florida virus* (MaYMFV)-infected *M. lathyroides* and **D**, lima bean. Seedlings (three- to four-leaf stage) were biolistically inoculated with an infectious DNA-A and DNA-B component cloned from MaMPRV- or MaYMFV-infected *M. lathyroides*.

using adult *B. tabaci* (B biotype) from a virus-free colony maintained at the University of Puerto Rico, Rio Piedras. Plants that developed symptoms were maintained as virus cultures in a greenhouse at the University of Puerto Rico and were used as source of viruses for transmission studies and subsequent cloning. Plants were protected from possible contamination by covering each with a fine-mesh screen cage. Virus cultures were maintained by serial transmission using *B. tabaci*. Total nucleic acids were extracted from symptomatic leaves (1.0 g) of virus-infected plants using the method of Doyle and Doyle (20).

For host range, transmission, and infectivity studies, test plants were assessed for presence or absence of begomoviral DNA 3 weeks postinoculation (PI). Total nucleic acids were isolated and purified from test plants as described previously (19). Routine virus detection was carried out using polymerase chain reaction (PCR), and universal degenerate PCR primers that amplify a ≈576-bp fragment of the *CP* gene for most, if not all, viruses in the genus *Begomovirus*, making it diagnostic for begomovirus presence (51).

Experimental host range of MaYMFV and MaMPRV/M.

Plants used in experimental host range studies were cultivated crop or weed species common to Florida and Puerto Rico (Table 1). At least four seedlings (two- to three-leaf stage) of each test species were subjected to biolistic inoculation (18) with infectious clones in each experiment and four replicated experiments were carried out for each. Plant species included in the host range study were representative of at least 10 different species in five plant families and included *Arabidopsis thaliana* 'Columbia', bean (*P. vulgaris* 'Red Kidney', 'Tender Pod', and 'Top Crop'), cotton (*Gossypium hirsutum* (L.) 'Delta' and 'Pineland 90'), *Datura stramonium* (L.), lima bean (*P. lanatus* 'Henderson Bush'), *M. lathyroides*, *Malva parviflora* (L.), okra (*Hibiscus esculentum* (L.) 'Clemson Spineless'), pepper (*Capsicum annuum* (L.) 'Anaheim'), pumpkin (*Cucurbita maxima* (Duchnes) 'Big Max'), soy-

bean (*Glycine max* (L.) Merr. 'Bush Shell'), tomato (*Solanum lycopersicon* (L.) Karsten 'Humaya'), and wild morning glory (*Convolvulus arvensis* (L.)). Experimental controls consisted of uninoculated plants and those that were biolistically inoculated with DNA extracted from virus-free *M. lathyroides* (20). Plants were maintained in an environmentally controlled, insect-free growth chamber (27°C, 12 h day/night cycle) and observed periodically for 3 weeks for symptom development.

Mechanical transmission. Virus cultures maintained in *M. lathyroides* and infected with MaYMFV or MaMPRV/M were used for sap transmission experiments. Young leaves were removed from plants infected with MaMPRV/M or MaYMFV and used for mechanical inoculation experiments. Leaves (1 to 2 g fresh weight) were ground (1:4, wt/vol) in 0.2 M potassium phosphate buffer, pH 7.5, containing 0.5% (wt/vol) diatomaceous earth and 1% 2-mercaptoethanol (both from Sigma Chemical, St. Louis). Homogenates were used to inoculate four *M. lathyroides* seedlings (four- to five-leaf stage) in each of three replicated experiments (total of 12 seedlings). For each experiment, several seedlings were mock inoculated with buffer as experimental controls. Inoculated seedlings were maintained in an insect-free greenhouse at ambient temperature and observed periodically for symptom development for 3 weeks. Development of characteristic symptoms 7 to 12 days PI was considered evidence of sap transmission.

Graft transmission. Scions and stocks for graft transmission experiments were obtained from MaYMFV and MaMPRV/M cultures maintained in *M. lathyroides* in either Florida or Puerto Rico, respectively. Scions ≈4 cm long were obtained from symptomatic MaYMFV or MaMPRV/M or from asymptomatic *M. lathyroides* plants as controls. *M. lathyroides* seedlings (five- to six-true-leaf stage) were side-grafted and the graft union was wrapped with Parafilm to optimize cambial contact and prevent desiccation. To further reduce desiccation, grafted plants were

TABLE 1. Experimental host range of *Macropitium lathyroides* and bean-associated begomoviruses determined by biolistic inoculation using total DNA extracted from *Macropitium mosaic Puerto Rico virus*-infected *M. lathyroides* (MaMPRV/M) and *Macropitium yellow mosaic Florida virus*-infected *M. lathyroides* (MaYMFV)

Test species	No. infected/no. inoculated	PCR results	Symptom phenotype
MaMPRV/M			
<i>Arabidopsis thaliana</i>	0/16	–	No symptom
Bean 'Red Kidney'	15/16	+	Mosaic and stunting
Bean 'Tender Pod'	15/16	+	Leaf curl and severe stunting
Bean 'Top Crop'	15/16	+	Leaf curl and severe stunting
Cotton 'Delta' 'Pineland 90'	0/16	–	No symptom
<i>Datura stramonium</i>	0/16	–	No symptom
Eggplant 'Black Beauty'	0/16	–	No symptom
Lima bean 'Henderson Bush'	15/16	+	Bright golden mosaic
<i>Macropitium lathyroides</i>	16/16	+	Diffuse, golden mosaic
<i>Malva parviflora</i>	0/16	–	No symptom
Okra 'Clemson Spineless'	0/16	–	No symptom
Pepper 'Anaheim'	0/16	–	No symptom
Soybean 'Bush Shell'	0/16	–	No symptom
Tomato 'Humaya'	0/16	–	No symptom
Wild morning glory (<i>Convolvulus arvensis</i>)	0/16	–	No symptom
MaYMFV			
<i>Arabidopsis thaliana</i>	0/16	–	No symptom
Bean 'Red Kidney'	16/16	+	Mosaic and stunting
Bean 'Tender Pod'	14/16	+	Leaf curl and severe stunting
Bean 'Top Crop'	16/16	+	Leaf curl and severe stunting
Cotton 'Delta' 'Pineland 90'	0/16	–	No symptom
<i>Datura stramonium</i>	0/16	–	No symptom
Eggplant 'Black Beauty'	0/16	–	No symptom
Lima bean 'Henderson Bush'	15/16	+	Bright golden mosaic
<i>Macropitium lathyroides</i>	16/16	+	Angular, bright golden mosaic
<i>Malva parviflora</i>	6/16	+	Mild leaf curl
Okra 'Clemson Spineless'	0/16	–	No symptom
Pepper 'Anaheim'	0/16	–	No symptom
Soybean 'Bush Shell'	11/16	+	Leaf mottle and crumpling
Tomato 'Humaya'	0/16	...	No symptom
Wild morning glory (<i>Convolvulus arvensis</i>)	0/16	–	No symptom

maintained at 80% relative humidity in a growth chamber at 24°C for 3 days before they were transferred to an insect-free greenhouse. Plants were monitored periodically for symptom development for 3 weeks. Development of characteristic disease symptoms $\approx$ 7 to 14 days PI was considered evidence of a successful graft transmission. The experiment was replicated three times using multiple plants for each grafting experiment to achieve at least six successfully grafted plants for each replicated experiment (minimum of 18 plants).

Cloning and sequencing of MaYMFV and MaMPRV: infectious clones. Total nucleic acids extracted from symptomatic *M. lathyroides* leaves were used as templates to make MaYMFV and MaMPRV (M isolate) DNA-A and DNA-B component-specific probes by PCR-labeling amplicons with digoxigenin. PCR primers (pAV2644, pAC1154), used to amplify the DNA-A component-specific probe, were based on conserved sequences that flank the *CP* gene and yield a 1,100-bp expected size PCR product (33). The B component-specific probes for each virus were amplified using primers BV567 and BC2498 and yielded a 1,931-bp product. Aliquots of total nucleic acid extracts from *M. lathyroides* infected-MaYMFV or -MaMPRV were incubated with selected restriction enzymes. Resultant products of the digestion were analyzed by Southern hybridization using viral DNA-A and DNA-B component-specific probes.

The DNA-A (pMaYMFV-S125) and DNA-B components (pMaYMFV-S11) for MaYMFV were cloned into pGEMEX vector utilizing *SacI* cloning site after digestion of total DNA that had been isolated from infected 'Top Crop' bean as described previously (47). Because digestion with *ClaI* appeared to completely linearize the double-stranded DNA forms of the virus that were detected using DNA-A and DNA-B component probes by Southern hybridization (data not shown), this enzyme was used to clone both MaMPRV components. Total DNA digested with *ClaI* was fractionated in an agarose gel, and linearized viral DNA of expected size was eluted from the gel. Viral DNA was ligated to *ClaI*-digested pGEM7zf+ (Promega, Madison, WI). Replicas of white bacterial colonies were screened separately with DNA-A and DNA-B component probes in Southern hybridization. Three recombinant plasmids containing full-length inserts were identified by Southern hybridization and confirmed by *ClaI* digestion. MaMPRV clones for the DNA-A (pMaMPRV-C10) or DNA-B (pMaMPRV-C16 and pMaMPRV-C17) component were selected and subjected to DNA sequencing bidirectionally.

Cloning using PCR and abutting primers. Leaves of naturally infected symptomatic bean plants maintained in the greenhouse in Puerto Rico were assessed for begomovirus presence by PCR using primers that direct the amplification of the *CP* gene and its flanking regions ($\approx$ 1.1 kbp) (34). Three 1.1-kbp PCR amplicons were obtained and cloned, and three clones were selected from each ligation reaction and the sequence obtained. Comparison of the *CP* gene sequence with the MaMPRV/M *CP* gene sequence revealed that BGYMV was also present in bean samples collected in 1989, 1994, and 1997; however, MaMPRV was present in one bean sample (PR90) collected in 1997, designated here as MaMPRV/B. To clone full-length A and B components from bean (PR90), abutting primers AV1308 (5'-CTGCAGTGATGGGTTCCCCTG-3') and AC1307 (5'-CTGCAGTTCAAGCAACGAATG-3') and BV30 (5'-CGTACGCCGCATTTGCTGGA-3') and BC29 (5'-CGTACGGACTAAAGAAACGC-3') were designed based on sequences for pMaMPRV-C10 and pMaMPRV-C16. Apparent full-length DNA-A and DNA-B components for MaMPRV/B were cloned into T-Easy vector (Promega). Recombinant plasmid containing (apparent) full-length A or B components were obtained for each.

DNA sequencing. The DNA sequence was obtained for cloned inserts by automated sequencing using an automated ABI 377 sequencer (Perkin-Elmer Applied Biosystems, Foster City, CA) at the Genomics Analysis Technology Core facility, University of

Arizona. Universal primers contained within the polylinker of the plasmid vector were used to obtain the initial sequence for the cloned inserts. The sequence for the remaining portion of each insert was determined using primers synthesized according to the sequence obtained in the first reaction. Design of primers accounted for a minimum overlap of 22 nucleotide (nt). The DNA sequence was determined bidirectionally for two independent clones for two viral A and B components for MaMPRV and MaYMFV. Sequences were compiled using FAKtory 1.41, an online program developed by Biotechnology Computing Facility, University of Arizona, which employs Phred, an algorithm available from the University of Washington (21). A consensus sequence was developed for each component, and locations of viral open reading frames of >10 kDa were predicted using EditSeq (DNASTAR Inc., Madison, WI).

Infectivity of cloned MaYMFV and MaMPRV/M. Plasmids bearing a monomer of MaYMFV DNA-A (pMaYMFV-S125) and MaYMFV DNA-B (pMaYMFV-S11) digested with *SacI*, or MaMPRV DNA-A (pMaMPRV-C10) and MaMPRV DNA-B (pMaMPRV-C16) digested with *ClaI*, to release the viral insert were precipitated onto microprojectiles and used for biolistic inoculation. A mixture containing 0.5 μ g of each cognate pair of viral components was used to inoculate seedlings of *M. lathyroides* and bean as described previously (18), with the exception that a 400 lb/in² rupture disk was used. Six *M. lathyroides* or bean seedlings were inoculated with each virus in three independent experiments. Controls consisted of mock-inoculated seedlings (minimum of two per replicate) for each test plant species with either plasmid DNA (0.5 μ g) or water (20 μ l). Inoculated plants were maintained in a whitefly-free greenhouse and monitored periodically for symptom development for 3 weeks. Test plants were considered positive or negative for infection based on development of characteristic disease symptoms and demonstration of virus presence or absence using PCR. Total nucleic acids were isolated from 0.5 μ g of leaf tissue per test plant at 3 weeks PI, and PCR was carried out using degenerate primers that amplify a diagnostic $\approx$ 576-bp *CP* gene fragment for all begomoviruses as described previously.

Phylogenetic analysis. Viral sequences used for phylogenetic analyses were obtained from GenBank (Table 2) and included sequences for well-studied geminiviruses and MaYMFV, MaMPRV/M, and MaMPRV/B. Sequences were aligned using ClustalX (50) and imported into Sequence Alignment Software (Se-Al version 2.0a10; 1996, A. Rambaut, Department of Zoology, University of Oxford) for manual alignment. Manual nucleotide alignment was carried out so that predicted viral open reading frames (ORFs) (>10 kDa) were maintained in-frame and noncoding regions were generally left unchanged unless a mismatch was obvious owing to a consensus for most sequences. Pairwise nucleotide distances were estimated for full-length DNA-A and DNA-B components using ClustalX (49).

Phylogenetic tree estimation was performed using the maximum likelihood algorithm available in phylogenetic analysis using parsimony (PAUP) version 4.0b10 (48), using *Beet curly top virus* and *African cassava mosaic virus* (ACMV) as the outgroup for the DNA-A and DNA-B component trees, respectively. The choice of substitution model was based on the approach of Huelsenbeck and Crandall (31). Likelihood scores were obtained for a variety of substitution models and likelihood ratio tests were performed with the program Modeltest (43). The general time reversible (GTR) model with gamma-distributed rate heterogeneity across sites (44,52) provided the best representation of substitution model for our data set. This substitution model was used to estimate tree topology and substitution rate under a likelihood framework by the Bayesian method using the MrBayes (32) computer program in order to carry out a statistical evaluation of the tree topology, which is not possible for this number and length of nucleotide sequence when employing maximum likelihood analysis with

bootstrap or jackknife tests. The Bayesian method seeks the most likely trees given the data and the evolutionary model. The posterior probabilities of the phylogeny, branch lengths, base frequencies, and substitution rate parameters are approximated by a Markov chain Monte Carlo (MCMC) process by sampling trees from the posterior distribution of tree-space exploration. A total of 300,000 MCMC steps were run to allow convergence on a solution followed by 100,000 MCMC steps and sampling every 500th tree. These algorithms were based on the >60% majority-rule consensus tree in PAUP with clade probabilities placed at major nodes.

RESULTS

Experimental host range for cloned MaYMFV and MaMPRV genomes. Results of host range studies using biolistic inoculation of apparently full-length cloned genomes for MaYMFV and MaMPRV/M indicated that both viruses systemically infected *M. lathyroides* (bean; 'Red Kidney', 'Top Crop', and 'Tender Pod' and lima bean; 'Henderson Bush'). However, MaYMFV infected soybean and *Malva parviflora* while MaMPRV/M did not. Neither virus infected *A. thaliana*, cotton, *D. stramonium*, eggplant, okra, pepper, tomato, or wild morning glory (*Convolvulus arvensis*). Test species were considered either a virus host or nonhost based on development of systemic symptoms and the presence or absence of the diagnostic viral CP gene fragment, respectively, as determined by PCR (Table 1).

Mechanical transmission of wild-type MaYMFV and MaMPRV. Characteristic systemic symptoms were observed in 17 of 48 *M. lathyroides* seedlings 8 days PI with MaYMFV. Dis-

ease symptoms did not develop in any of the buffer-inoculated control plants. None of the 48 *M. lathyroides* seedlings inoculated with MaMPRV/M extracts developed disease symptoms, and mock-inoculated controls were asymptomatic for the duration of the experiment. The expected size, diagnostic CP gene fragment (≈576 bp), as determined by PCR, was obtained for all test plants that developed systemic symptoms, whereas the diagnostic amplicon was not detected for any of the asymptomatic plants (data not shown; Table 1).

Graft transmission of wild-type MaYMFV and MaMPRV. Side grafting was successful for *M. lathyroides* for both viruses examined, as was evident by development of leaves from all grafted scions (data not shown). Graft-mediated viral transmission was obtained for 18 of 18 plants for MaMPRV/B and MaMPRV/M, and MaYMFV, based on the development of characteristic disease symptoms. The experimental control, which showed no symptoms, included plants to which virus-free scions were grafted to achieve mock inoculation and nongrafted scions of virus-free plants. Presence or absence of the diagnostic begomoviral CP gene fragment in inoculated plants and mock-inoculated controls was corroborated with development of symptoms using PCR. The expected size product (≈576 bp) was obtained for plants in which typical virus symptoms developed, whereas no PCR product was obtained for plants that did not develop disease symptoms (data not shown).

Cloning and sequence of MaMPRV/M and MaYMFV. The nucleotide sequence was obtained for the MaYMFV, cloned from *M. lathyroides* from Florida DNA-A (2,642 nt) and DNA-B (2,605 nt) components, and sequences were deposited as GenBank Accessions nos. AY044135 and AY044136, respectively.

TABLE 2. Geminiviruses used in comparative sequence analyses with their respective GenBank accession numbers

Geminivirus	Acronym	GenBank Accession No.	
		A component	B component
<i>Abutilon mosaic virus</i> -West Indies	AbMV	X15983	X15984
<i>African cassava mosaic virus</i> -Kenya	ACMV-KE	J02057	J02058
<i>African cassava mosaic virus</i> -Nigeria	ACMV-NG	X17097	X17096
<i>Ageratum yellow vein virus</i>	AYVV	X74516	...
<i>Bean calico mosaic virus</i>	BCaMV	AF110189	FA110190
<i>Bean dwarf mosaic virus</i>	BDMV	M88179	M88180
<i>Bean golden mosaic virus</i> -Brazil	BGMV	M88686	M88687
<i>Bean golden yellow mosaic virus</i> -Dominican Republic	BGYMV-DO	L01635	L01636
<i>Bean golden yellow mosaic virus</i> -Guatemala	BGYMV-GT	M91604	M91605
<i>Bean golden yellow mosaic virus</i> -Mexico	BGYMV-MX	AF173555	AF173666
<i>Bean golden yellow mosaic virus</i> -Puerto Rico	BGYMV-PR	M10070	U65530
<i>Beet curly top virus</i>	BCTV	M24597	...
<i>Cabbage leaf curl virus</i>	CaLCV	U65529	U65530
<i>Chino del tomate virus</i>	CdTV	AF226664	U57458
<i>Indian cassava mosaic virus</i>	ICMV	Z24758	Z24759
<i>Macroptilium yellow mosaic Cuba virus</i>	MaYMCV	AJ344452	...
<i>Mung bean yellow mosaic virus</i>	MYMV	D14703	...
<i>Pepper huasteco yellow vein virus</i>	PHYVV	X70418	X70419
<i>Pepper golden mosaic virus</i> -Tamaulipas	PepGMV-TAM	U57457	AF49227
<i>Potato yellow mosaic virus</i>	PYMV	NC001934	NC001935
<i>Rhynchosia golden mosaic virus</i>	RhGMV	AF239671	...
<i>Sida golden mosaic Costa Rica virus</i>	SiGMCRV	X99550	X99551
<i>Sida golden mosaic Honduras virus</i>	SiGMHV	Y11099	AJ250731
<i>Sida golden mosaic Honduras virus</i> -yellow vein	SiGMHV-YV	Y11097	...
<i>Sida golden mosaic Florida virus</i>	SiGMFV	U77964	U77963
<i>Squash leaf curl virus</i>	SLCV	M38183	M38182
<i>Tomato golden mosaic virus</i>	TGMV	K02029	K02030
<i>Tomato leaf curl New Delhi virus</i>	ToLCNDV	U15015	U15017
<i>Tomato leaf curl Taiwan virus</i>	ToLCTWV	U88692	...
<i>Tomato leaf curl virus</i> -Australia	ToLCV	S53251	...
<i>Tomato mosaic Havana virus</i> -Quivican	ToMHV-Qui	Y14874	Y14875
<i>Tomato mottle Taino virus</i>	ToMoTV	AF012300	...
<i>Tomato mottle virus</i>	ToMoV	L14460	L14461
<i>Tomato rugose mosaic virus</i>	ToRMV	NC002555	AF291706
<i>Tomato yellow leaf curl Sardinia virus</i>	TYLCSV	X61153	...
<i>Tomato yellow leaf curl Sardinia virus</i> -Sicily	TYLCSV-Sic	Z28390	...
<i>Tomato yellow leaf curl Thailand virus</i>	TYLCTHV	M59838	...
<i>Tomato yellow leaf curl virus</i> -Israel	TYLCV	X15656	...

The MaMPRV/B DNA-A and DNA-B components cloned from bean from Puerto Rico were 2,615 and 2,571 nt, respectively, and were deposited as GenBank Accessions nos. AF449192 and AF449193, respectively. The nucleotide sequences for MaMPRV/M DNA-A (2,615 nt) and DNA-B (2,571 nt) components cloned from *M. lathyroides* from Puerto Rico were obtained and deposited as GenBank Accessions nos. AY044133 and AY044134, respectively.

Infectivity of viral clones. Infectivity assays in which bean seedlings were inoculated with the cloned DNA-A and DNA-B components for MaYMFV indicated that 66.7% (12/18) of plants developed symptoms characteristic of this virus compared with only 50% infectivity (9/18) for MaYMFV-inoculated *M. lathyroides* seedlings. *M. lathyroides* and bean seedlings inoculated with cloned MaMPRV DNA-A and DNA-B components for the two *M. lathyroides* isolates also developed disease symptoms indistinguishable from those observed in the respective field-infected plant species (Fig. 1A and C; symptoms are shown for the *M. lathyroides* isolate only). Infectivity in bean for the cloned MaMPRV/M DNA-A and DNA-B components was 83.3% (15/18) compared with 44.4% (8/18) for MaMPRV/M-inoculated *M. lathyroides* plants.

Genome organization. The genome organization for MaMPRV/B and MaMPRV/M and MaYMFV was typical of other bipartite begomoviruses in that six ORFs of characteristic size and position were identified (predicted) in their respective viral and complementary orientations (Fig. 2). Assessment of viral component sequences revealed a CR of 149 nt for MaYMFV A and B components, which shared ≈97% for the cognate viral A and B components, and one of 126 nt for MaMPRV/B/M at 94% shared identity for cognate components. The CR for all six components

contained the nonanucleotide sequence TAATATT↓AC that is present in the stem loop for all geminiviruses and constitutes the viral origin of rolling circle replication (↓ denotes the nick site between T/A [35]). Nucleotide numbering of viral sequences follows the geminivirus convention in which the ↓A at the nick site in the origin of replication is designated nucleotide 1. Other expected features of the viral CRs included a TATA-box and the directly repeated, or iterated sequences (1) involved in binding of viral replication (Rep)-associated protein (AC1 ORF).

For MaYMFV A and B components, the predicted Rep binding site (37), GGTGTAAAGGGGT, was identical but atypical in that the 3' repeat was different from its 5' counterpart, and therefore suggesting either a single site mutation or recombination with a distinct, but as yet unknown, begomovirus, presumably containing a GGGGT as a portion of the Rep binding element. Also, the Rep binding site for the A and B components for the two MaMPRV isolates was identical and consisted of an imperfect, directly repeated motif, separated by a CT spacer, GGTGACTGGTGT (Table 3). The latter motif is identical to the predicted Rep binding site for *Wissadula golden mosaic virus* (WGMV) (WGU69603, WGU69604), but is distinct from DNA-A and DNA-B components for strains or isolates of BGYMV, which share an identical motif (39; Table 3).

Sequence analysis for MaYMFV and MaMPRV. Phylogenetic analysis for the DNA-A (Fig. 3A) and DNA-B (Fig. 3B) components of MaYMFV (*M. lathyroides* isolate) and MaMPRV/B and MaMPRV/M with other well-studied viruses indicated that these two newly described viral species, from the Western Hemisphere, grouped with other begomoviruses also known to have originated from the Western Hemisphere, form a clade separate from begomoviruses from the Eastern Hemisphere (Fig. 3A and B).

Fig. 2. Genome maps of *Macrotipilium yellow mosaic Florida virus* (MaYMFV) and *Macrotipilium mosaic Puerto Rico virus* (MaMPRV). Nucleotide numbering follows the geminivirus convention. The location and orientation of open reading frames (ORFs) in either A, DNA-A or B, DNA-B are shown as V for viral strand and C for complementary strand. Numbers refer to the start and stop coordinates for each ORF.

Distance analysis (Neighbor-Joining) for MaYMFV DNA-A component indicated it shared 74 to 78% nucleotide identity with its closest relatives, which includes all of the previously described isolates and strains of BGYMV (Table 4). This result suggests that, based on the <90% species rule (38), MaYMFV is a new previously undescribed species.

The nucleotide sequence for MaMPRV isolates from *M. lathyroides* (MaMPRV/M) and bean (MaMPRV/B) were 97.7% (A component) and 98.3% (B component) identical, suggesting they are simply two isolates of the same viral species and hence MaMPRV/M was used in most of the nucleotide analyses. Distance analyses (Neighbor-Joining) for the DNA-A component for MaMPRV/B and MaMPRV/M indicated that their closest relatives were the same and included BGYMV from Dominican Republic (BGYMV-DO), and *Pepper huasteco yellow vein virus* (PHYVV)

from Mexico (Fig. 3A) at 73 and 76% nucleotide identity, respectively (Table 4). This result indicated that MaMPRV was also a new previously unidentified begomoviral species (38).

A comparison of nucleotide distances for the DNA-B components of MaYMFV indicated its closest relatives were other isolates and strains of BGYMV from the Caribbean region at 67 to 68% nucleotide identity (Table 4).

Similarly, though surprisingly, the DNA-B component for extant BGYMV isolates and strains were also the closest relatives for the MaMPRV/B and MaMPRV/M DNA-B components at ≈68 to 72% nucleotide identity (Table 4; data not shown for MaMPRV/B).

The lack of concordant taxonomic placement for MaMPRV DNA-A and DNA-B components suggested that portions of the extant genome may have evolved along different paths, perhaps

TABLE 3. The predicted replication-associated protein binding element was identified, based on the occurrence as a directly repeated sequence (underlined) in the common region for *Macropitium mosaic Puerto Rico virus* (MaMPRV) and *Macropitium yellow mosaic Florida virus* (MaYMFV), and compared with their closest relatives

Begomovirus	DNA-A	DNA-B
<i>Bean calico mosaic virus</i>	<u>GGTGTCTCTGGTGT</u>	<u>GGTGTCTCTGGTGT</u>
<i>Bean dwarf mosaic virus</i>	<u>GGAGTATTGGAGT</u>	<u>GGAGTATTGGAGT</u>
<i>Bean golden mosaic virus-Brazil</i>	<u>GGTGTAAATGGTG</u>	<u>GGTGTAAATGGTG</u>
<i>Bean golden yellow mosaic virus-Puerto Rico</i>	<u>GGAGACTGGAG</u>	<u>GGAGACTGGAG</u>
MaMPRV/M	<u>GGTGACTGGTGT</u>	<u>GGTGACTGGTGT</u>
<i>Macropitium yellow mosaic Cuba virus</i>	<u>GGTGATAAGTGTA</u>	...
MaYMFV	<u>GGTGTAAGGGGT</u>	<u>GGTGTAAGGGGT</u>
<i>Tomato golden mosaic virus</i>	<u>GGTAGTAAGGTAG</u>	<u>GGTAGTAAGGTAG</u>
<i>Pepper huasteco yellow vein virus</i>	<u>GGTGTATTGGTAG</u>	<u>GGTGTATTGGTAG</u>
<i>Wissadula golden mosaic virus</i>	<u>GGTGACTGGTGT</u>	<u>GGTGACTGGTGT</u>

Fig. 3. Phylogenetic trees showing predicted relationships for A, DNA-A or B, DNA-B component for *Macropitium mosaic Puerto Rico virus* (MaMPRV) and *Macropitium yellow mosaic Florida virus* (MaYMFV) and well-studied geminiviruses. Trees were estimated using the maximum likelihood framework and Bayesian method available in MrBayes (32). A total of 300,000 Markov chain Monte Carlo (MCMC) steps were run to allow convergence on a solution followed by 100,000 MCMC steps, sampling every 500th tree. MrBayes trees were estimated and used to generate a majority-rule consensus tree in phylogenetic analysis using parsimony (PAUP), giving support values, or clade probabilities, at each major node for the cladogram.

until quite recently, when they encountered one another in a shared host and underwent reassortment and/or recombination with ancestors from the present-day begomovirus clades that contain PHYVV and BGYMV.

DISCUSSION

The genomes for two previously uncharacterized bean-infecting begomoviruses from the Caribbean region, MaMPRV and MaYMFV, have been cloned and completely characterized. Full-length infectious clones of the DNA-A and DNA-B component for MaMPRV/M and MaYMFV were each cloned from symptomatic *M. lathyroides*. MaMPRV clones experimentally infected bean and *M. lathyroides*, whereas MaYMFV infected bean, *M. lathyroides*, *Malva parviflora*, and soybean. Analysis of their genome sequences revealed that they are distinct begomoviral species originating from the Western Hemisphere. MaMPRV was first identified in symptomatic bean plants in Puerto Rico in 1999 (33), making it only the second begomovirus known to infect bean naturally in Puerto Rico. MaYMFV occurs in bean fields in southern Florida (5,46) and is the third completely characterized bean-infecting virus in the region, although it has not been found widely there. Roye et al. (45) reported a partial genome sequence for *Macroptilium golden mosaic virus* (MGMV) from Jamaica, isolates MGMV-JM1 (GenBank Accession no. AF098940) and MGMV-JM2 (GenBank Accession no. AF098939), and based on evidence for less than 90% shared nucleotide identity with BGYMV, they concluded that this virus does not infect bean. However, such a conclusion remains speculative until infectivity experiments are carried out. MGMV-JM1 and MGMV-JM2 also share less than 90% nucleotide identity with MaMPRV and MaYMFV, indicating that MGMV likely constitutes a unique species. A second partially characterized begomovirus, *Macroptilium yellow mosaic Cuba virus* (GenBank Accession no. AJ344452), has likewise been reported from *M. lathyroides* in the Caribbean region, but only minimal sequence data are available, precluding a definitive comparison.

Phylogenetically, MaMPRV and MaYMFV are the only completely characterized begomoviral species to have been identified from *M. lathyroides* or bean in the Caribbean region that are distinct from other strains and isolates of BGYMV, which until recent reports (2,33,45,46) (GenBank Accession no. AJ344452), was thought to constitute the only Western Hemisphere bean-infecting begomovirus in the Caribbean region. These two viral species can be distinguished from one another in infected *M. lathyroides* in that MaMPRV causes bright yellow mosaic symptoms, whereas an angular golden mosaic is associated with MaYMFV (Table 1; Fig. 1A and C). However, in bean, symptoms for MaMPRV and MaYMFV (Fig. 1B and D) are nearly identical and are also difficult to distinguish from those caused by

BGYMV, which occurs throughout the Caribbean region (2,3,7, 17,22,23,26,28,33,46) and BGMV from Brazil, two bean-infecting begomoviruses that have been the subject of bean breeding programs in the Caribbean and South America, respectively, for many years. Previous studies indicated that *M. lathyroides* was a host of BGYMV in Puerto Rico (26), and it was this knowledge that resulted in the misidentification of the *M. lathyroides* begomovirus in Florida as BGYMV (5,28,46). However, two distinct begomoviruses, BGYMV from bean and MaYMFV from *M. lathyroides*, are now known to occur in Florida.

Results of experimental host range studies for MaYMFV and MaMPRV (Table 1) revealed that the two viruses have moderate and overlapping host ranges that include species within the families Fabaceae and Malvaceae and that only MaYMFV infects soybean, an Eastern Hemisphere host, and *Malva parviflora* (Table 1). Also, results indicated that both MaMPRV and MaYMFV are transmissible to bean and their natural weed host, *M. lathyroides*, by the B biotype of *B. tabaci* and by grafting, but only MaYMFV is mechanically transmissible, indicating differences in their experimental transmissibility. Ability to mechanically transmit MaYMFV, but not BGYMV, could be useful for quickly distinguishing these viruses in field samples, and also to permit the isolation of MaYMFV from a mixture with BGYMV.

For bipartite begomoviruses, phylogenetic analysis did not predict a congruent phylogeny for the cognate DNA-A and DNA-B components of MaMPRV (Fig. 3A and B), a phenomenon which has also been observed for BCaMV (15), *Cabbage leaf curl virus* (29), and PHYVV (50). Prior to sequence analysis for the complete genome, MaMPRV might have been expected to reside in the BGYMV clade, as does MaYMFV, owing to their association with *P. vulgaris* and an uncultivated, indigenous legume species, both hosts in the family Fabaceae. However, surprisingly, the DNA-A component for MaMPRV clustered with the PHYVV clade, whereas its DNA-B component was most closely related to BGYMV (Fig. 3A and B). However, the subsequent placement of the MaMPRV DNA-A component in the same clade as PHYVV, a Western Hemisphere begomovirus with predicted evolutionary links to begomoviruses from the Eastern Hemisphere, provides more substantial evidence for an extant bridge between Eastern and Western Hemispheres. Specifically, PHYVV has been reported to share amino acid sequence similarity in the AC1 ORF (Rep-associated protein) for the bipartite ACMV from Africa and the monopartite viruses, *Tomato yellow leaf curl virus* (TYLCV) from Israel and *Tomato yellow leaf curl Sardinia virus* (TYLCSV) from Sardinia (50). Interestingly, MaMPRV shares 65 to 69% amino acid similarity in the AC1 ORF for at least two isolates from ACMV (Kenya and Nigeria), *Hollyhock leaf crumple virus* from Egypt (35), PHYVV, TYLCV, and TYLCSV. Two other Western Hemisphere begomoviruses, *Rhynchosia golden mosaic virus* and WGMV, are provisional members of the PHYVV clade

TABLE 4. Comparison of pairwise nucleotide identity (ClustalX) for the DNA-A and DNA-B component of *Macroptilium mosaic Puerto Rico virus* (MaMPRV/M), *Macroptilium yellow mosaic Florida virus* (MaYMFV), and select well-studied begomoviruses^a

Virus	MaMPRV/M		MaYMFV	
	DNA-A	DNA-B	DNA-A	DNA-B
<i>Bean calico mosaic virus</i>	64.8	64.7	69.3	60.6
<i>Bean dwarf mosaic virus</i>	71.8	64.4	74.3	63.7
<i>Bean golden mosaic virus-Brazil</i>	69.6	66.5	73.8	62.8
<i>Bean golden yellow mosaic virus-Mexico</i>	70.3	72.0	78.0	68.2
<i>Bean golden yellow mosaic virus-Dominican Republic</i>	73.1	71.2	77.5	68.5
<i>Bean golden yellow mosaic virus-Puerto Rico</i>	72.9	68.1	76.4	67.3
<i>Bean golden yellow mosaic virus-Guatemala</i>	73.1	70.1	77.2	68.1
MaMPRV/M	—	—	70.6	67.5
MaYMFV	70.6	67.5	—	—
<i>Pepper huasteco yellow vein virus</i>	75.9	63.6	70.0	61.4
<i>Rhynchosia golden mosaic virus</i>	77.5	NA	72.6	NA

^a Numbers are the percent identity between the relevant components. NA = not applicable.

based on comparison of partial DNA sequences, making their precise evolutionary relationship with MaMPRV and PHYVV undetermined at this time (Fig. 3A).

The incongruent evolutionary history of the DNA-A and DNA-B components for MaMPRV, yet conservation of replication specificity element (iteron) (1,39) for these cognate components, suggests that MaMPRV arose from both intermolecular recombination and reassortment processes (4,29,30,53). The closest extant relative for the MaMPRV DNA-A component is WGMV (PHYVV clade), whereas BGYMV (BGYMV clade) is the closest relative of MaMPRV DNA-B, and MaMPRV DNA-A and DNA-B components share an identical replication specificity element. It can be hypothesized that the entire DNA-A component, including the iteron, was inherited from a WGMV-like parent, whereas the iteron for DNA-B was donated by a WGMV-like parent, and the remainder of the recombinant DNA-B component was acquired from a BGYMV-like parent. We found no theoretical evidence for recombination (data not shown) in the MaMPRV genome, except for the intriguing evidence residing in the replication specificity element sequence (Table 3), which points to a possible junction at or near which recombination may have occurred. Although the particular plant species in which the mixed infection occurred is not known, it seems likely that a legume species may have been involved, owing to the restriction of extant BGYMV isolates to bean and *M. lathyroides*. Recent evidence that demonstrates a role for the DNA-B component in symptom development, movement, and tissue tropism (29,42) makes plausible the notion that the restricted host range phenotype of MaMPRV is conferred by the BGYMV-like parent. In contrast, MaYMFV appears to have evolved congruently, because both DNA-A and DNA-B components clustered in the BGYMV clade. It is interesting to note that this virus has not been particularly problematic as a pathogen of bean or in other crops in the region, given that the host range of MaYMFV infects species in at least three plant families (Table 1).

A search of the GenBank database for sequences most closely related to MaMPRV or MaYMFV revealed two begomovirus-like sequences, accessions U81301 and U81304, thought to have been integrated in the *Nicotiana tabacum* (L.) genome. The two sequences, ATTGGTGACTGGTGACAATATATACTA, are identical to and span the Rep binding motif (underlined), the TATA box (italics) (39), and nucleotides flanking the CR (CTA) of MaMPRV. This observation suggests that the *N. tabacum* sequence may have its origin in a MaMPRV/WGMV-like begomovirus. This hypothesis is supported by several lines of evidence. First, many extant begomoviruses are capable of infecting tobacco (2,3,7). Second, *N. tabacum* has its center of diversification in South America (24), and third, tobacco has been cultivated for some time in Central America, Mexico, and the Caribbean region, in and nearby the same locations known to harbor extant begomoviruses (24).

MaYMFV and MaMPRV are newly characterized begomoviruses that are indigenous to Florida and Puerto Rico, respectively. Their recent detection in bean in both locations may be accounted for by one or several factors. First, they may have simply experienced more frequent exposure to bean and possibly other hosts in the region, owing to interactions with the highly polyphagous and fecund B biotype vector (19) that was recently introduced from the Eastern Hemisphere and became established in the region (8). The B biotype whitefly vector differs from the Sida race, the *B. tabaci* that predominated as the vector of begomoviruses in Puerto Rico until about 1989 (6–8,19), and possibly in Florida until about the same time when the B type became established there (2,3,6,8), in that it has a more moderate host range and is less fecund. Increased intensification of monoculture crop production has also occurred throughout Puerto Rico and in certain locations in Florida (6,7), that together with changes in selection pressure imposed by a biologically distinct whitefly vector, may have accelerated the natural diversification of certain begomoviruses including MaYMFV and MaMPRV.

ACKNOWLEDGMENTS

This research (E. Hiebert) was supported by the Florida Agricultural Experiment Station and a grant from the U.S. Department of Agriculture-Tropical/Subtropical Agriculture Research Program. The manuscript was approved for publication as Florida Agriculture Experiment Station Journal Series R-09444. Studies in the Arizona and Puerto Rico laboratories were supported by a grant from the Puerto Rico Economic Development Fund.

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