DNA Analysis Confirms *Macroptilium lathyroides* as Alternative Host of Bean golden yellow mosaic virus

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ABSTRACT

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The leguminous weed *Macroptilium lathyroides* is considered a potential host of the Bean golden yellow mosaic virus (BGYMV; BGMV = Mesoamerican isolates). To determine if *M. lathyroides* could be a host for BGYMV, an infectivity cycle was established between this weed and *Phaseolus vulgaris*. Virus transmission was carried out using the whitefly, *Bemisia argentifolii*, as a vector. Inoculated plants of both species were examined for symptoms such as mosaic, stunting, and leaf distortion. *P. vulgaris* and *M. lathyroides* showed golden yellow mosaic symptoms during all infectivity cycle stages. Symptomatic plants of both species were tested for BGYMV using polymerase chain reaction (PCR) and nucleotide sequence analysis. Two degenerate primers sets were used for PCR to detect viral DNA: PAL1v1978/PAR1c715 and PCRC2/PBL12039. PCR analysis using primers PCRC2/PBL12039 amplified viral DNA for component B from both plant species. Nucleotide sequence analysis revealed a 93% identity between the virus isolated from *M. lathyroides* and the Puerto Rican isolate of BGYMV. These results confirmed that *M. lathyroides* could serve as an alternative host of BGYMV and that an infectivity cycle of BGYMV could possibly occur between *P. vulgaris* and *M. lathyroides* in Puerto Rico.

Additional keywords: DNA nucleotide sequence analysis, fabaceae, geminivirus

Bean golden yellow mosaic virus (BGYMV; BGMV = Mesoamerican isolates) is the most important disease of *Phaseolus vulgaris* (L.) in Central America and the Caribbean (11,12). Geminiviruses (genus: *Begomovirus*) like BGYMV cause devastating crop diseases throughout the tropics and are transmitted by whiteflies of the genus *Bemisia* (Homoptera: Aleyrodidae) (9,11). Disease symptoms include intense yellowing, mosaic, stunting, and distortion of leaves and pods. The term “golden-yellow mosaic”, recently adopted by Fauquet (6), was first used by Bird (2) to describe these symptoms in lima beans (*Phaseolus lunatus*) and common beans.

The role of weeds as reservoirs for BGYMV has been subject to speculation and debate for many years. In 1971,

Gamez (7) observed symptoms in hundreds of bean cultivars and other legumes inoculated with the virus, and suggested that legume weeds might serve as reservoirs of the geminivirus. In 1981, Chagas et al. (3,4) discussed the possibility of various weeds serving as alternative hosts (e.g., a *Canavalia* sp., *Crotalaria* spp., *Macroptilium lathyroides*, and *M. erythroloma*). In 1987, Haber et al. (8), using fingerprint diagnosis, confirmed the presence of distinct sets of restriction fragments that specifically hybridized with BGMV DNA sequences in *M. lathyroides* and *Malvastrum coromandelianum*. None of these early studies, however, established infectivity cycles as proof to corroborate their findings.

Roye et al. (15) characterized geminiviruses infecting *Macroptilium lathyroides* using polymerase chain reaction (PCR), DNA hybridization, and nucleotide sequence analysis. Their results indicate that both crop- and weed-infecting geminiviruses are related, but different, and redefined the geminivirus cluster infecting *M. lathyroides* in Jamaica as new. Idris et al. (10) reported a new geminivirus from *M. lathyroides*, distinct from BGYMV, that caused green-yellow foliar symptoms and stunting, reminiscent of symptoms caused by BGYMV-PR in beans. The authors named the virus *Macroptilium mosaic virus*

(MaMV). More recently, Ramos and Fernandez (13) compared the DNA-A sequence of *M. lathyroides* field samples showing bright yellow mosaic symptoms with various strains of geminiviruses, including BGYMV, Tomato leaf curl virus, and Sida golden mosaic virus. Data revealed a new begomovirus; therefore, they proposed the name *Macroptilium yellow mosaic virus* (MaYMV) for a new virus.

M. lathyroides, or scarlet bean, a common leguminous weed, has been considered to be a potential reservoir for BGYMV. Given the abundance of this weed in and around bean fields, it is important to determine if *M. lathyroides* could be an alternative host for BGYMV in order to develop better management and control strategies under field conditions. To date, no studies have been conducted using an infectivity cycle to determine whether or not this weed is a potential reservoir of BGYMV in Puerto Rico.

MATERIALS AND METHODS

Plant material. *M. lathyroides* seed were scarified by cutting one end of each seed. *M. lathyroides* and *P. vulgaris* seed were germinated in a moist chamber and planted in Pro-mix using 15-cm-diameter pots (3 seed per pot) and grown in the greenhouse at 27°C and 75% relative humidity.

Virus transmission by whiteflies. Aviruliferous whiteflies were collected from *Glycine max* L. Merr. plants growing in a greenhouse at the University of Puerto Rico–Mayagüez Campus. The primary viral source was taken from the BGYMV-susceptible *P. vulgaris* cv. Top Crop growing and infected once in a greenhouse. Healthy bean plants were maintained in a separate greenhouse. Twelve days after planting, 45 bean seedlings of susceptible cvs. Top Crop or Mayflower were inoculated once with 10 viruliferous whiteflies per plant for 48 h. The whiteflies had fed from bean plants infected with BGYMV, as suggested by Adamés-Mora (1).

Infectivity cycle between *P. vulgaris* and *M. lathyroides*. Twelve-day-old *P. vulgaris* plants were infected once with viruliferous whiteflies under greenhouse conditions. After *P. vulgaris* plants showed symptoms, we allowed virus-free whiteflies to feed on the *P. vulgaris* plants.

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These viruliferous whiteflies were used to transmit the virus from infected bean plants to healthy, 12-day-old *M. lathyroides* plants. After infection, *M. lathyroides* plants were used as a source of inoculum to infect healthy *P. vulgaris* plants, to complete the infectivity cycle between the two plant species. A first set of experiments was conducted with bean cv. Top Crop and a second set using bean cv. Mayflower. Each plant species was inoculated once with viruliferous whiteflies. Negative control consisted of 15 plants of each species inoculated with 10 aviruliferous whiteflies per plant.

PCR. Young leaf tissue samples were collected 15 days after inoculation from *P. vulgaris* and *M. lathyroides* plants for DNA extraction, using the miniprep method described by Dellaporta (5). Degenerate primers designed by Rojas et al. (14) were obtained from the University of Wisconsin–Madison (14). The PCR reaction consisted of 2.5 µl of sample DNA solution (200 ng/ml), 2.5 µl of dNTPs (1 µM), 2.5 µl of Mg²⁺ (25 mM), 2.5 µl of Mg buffer (10×) for a total volume of 25 µl, 0.5 µl of each primer (0.02 µM), and 0.1 µl of *Taq* polymerase (Promega Corp., Madison, WI) in a total volume of 25 µl. Each reaction mixture was covered with 25 to 50 µl of mineral oil to prevent evaporation during the amplification. Viral DNA was amplified using a thermocycler (Gene Amp System 2400; Perkin Elmer Co., Norwalk, CT) by 30 cycles of denaturation, annealing, and extension conditions of 1 min at 94°C, 2 min at 55°C, and 2 min at 72°C, respectively. Amplified DNA was electrophoresed in 0.8% agarose gel for 5 to 7 min at 110 volts, in TBE buffer (45

mM Tris-borate, 1 mM EDTA, pH 8). Gels were viewed with ultraviolet light after staining in 7% ethidium bromide.

DNA cloning and sequencing. The amplified DNA band was excised from a gel previously stained with ethidium bromide and recovered with a glass matrix (Gene Clean; Bio 101, La Jolla, CA). The 600-bp DNA fragment was inserted into plasmid pGEM-T Easy Vector (Promega Corp.). The DNA was cut with the endonuclease *Eco*RI. PCR-amplified DNA with insert was extracted twice with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1; PCI method), then used to transform *Escherichia coli* strain XL1-Blue. Bacterial colonies with recombinant plasmids (β-galactosidase negative) were selected for further characterization. DNA sequences were determined by the dideoxynucleotide chain termination method. DNA sequences were analyzed with the software of the Genetics Computer Group, University of Wisconsin–Madison and BLAST Database.

RESULTS

Viral transmission through whiteflies. Whiteflies effectively transmitted BGYMV between *P. vulgaris* and *M. lathyroides* through an infectivity cycle established at a greenhouse at the University of Puerto Rico–Mayagüez Campus; 100% plants were infected. We observed that *B. argentifolli* preferred young plant tissues and were attracted to both plant species. Viral transmission through its vector is an effective and natural way to spread BGYMV in the field.

Symptoms of BGYMV. *P. vulgaris* cvs. Top Crop and Mayflower showed typical

golden mosaic symptoms on the leaves, whereas *M. lathyroides* showed similar symptoms around leaf veins, 12 to 15 days after inoculation (Fig. 1). Stunting was observed on neither *P. vulgaris* nor *M. lathyroides* plants, although leaf distortion was observed in both species.

PCR. Primers set to detect viral DNA for component A (PCRC2/PBL12039) and viral DNA for component B (PAL1v1978/ PAR1c715) were used successfully before by Rojas et al. (14) to amplify geminiviruses. In our studies, electrophoresis of amplified DNA extracted from infected *M. lathyroides* plants showed intense and clear 600-bp bands, using primers for viral DNA component B (Fig. 2). We chose these products for further PCR experiments because they contained a highly variable region. The DNA product was successfully cloned in an *E. coli* plasmid for further analysis.

Cloning and sequencing. After cloning the viral DNA from *M. lathyroides* plants, we extracted the plasmid pGEM-T Easy Vector and cut it with *Eco*RI, then confirmed viral DNA insertion using electrophoretic techniques (Fig. 3). Two clones were obtained: pII1 and pII3, with a DNA concentration of 347 and 298 ng/ml, respectively. Both DNA clones were further used for DNA nucleotide sequence analysis.

Nucleotide sequence analysis. The viral DNA-B clones obtained from *M. lathyroides* were compared among them and also with sequences available for other geminiviruses using BLAST program and Bio Comp databases. The viral DNA from *M. lathyroides* shared 93, 94, 95, and 96% nucleotide sequence identity with BGYMV

Fig. 1. Golden yellowing mosaic symptoms and leaf distortion observed on leaves of *Macropitium lathyroides* and *Phaseolus vulgaris* plants, 15 days after inoculation with *Bean golden yellow mosaic virus* during an infectivity cycle established under greenhouse conditions.

Fig. 2. Agarose gel electrophoresis of polymerase chain reaction products from DNA of *Bean golden yellow mosaic virus* (BGYMV) obtained from *Macroptilium lathyroides* tissues. Two sets of primers were used to detect component A or B of BGYMV. Ladder size marker = 1.0 kb (lane L); *M. lathyroides* infected with BGYMV was detected with primers for component A (lanes A–H); *M. lathyroides* infected with BGYMV was detected with primers for component B (lanes 1–9).

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1 .....CAGTGGTCGATTTCATACAAC 22
2001 AGGCACCTGTTGCCTATACTCAATTACACAATGATCGATCTTCATACAAC 2050
23 TCGCACTGAATCGTGCTGATAATTGAGACGCTGTGGAAGGGAATTGAAGG 72
2051 TCGCACTGAATCGTGCTGATAGTTGAGACGCTGTGGAAGGGAATTGAAGG 2100
73 ATTATCTCAGTTAGATCATGAGACAATTGATATTCTGCTCTGTGAGACTC 122
2101 ATTATCTCAGTTAGATCATGAGACAATTGATATTCTGCTCTGTGAGACTC 2150
123 TACGTAATTAATGCATTGAGAGATTGCTAACTGAGAATCCATTTAAG 172
2151 TATGTAATTAATGCATTGAGAGATTGCTAACTGAGAATCCATTTAAG 2200
173 AATAAATGGCGCGCAGCGGAATTGCTCAGAGAGATAATGTTCACTCGAC 222
2201 AATAAATGGCGCGCAGCGGAATTGCTCAGAGAGATAAGGTCCACTCAAC 2250
223 TAGTTGATTGTTAACTAAGAACTCTATTTGTGAAACACTCATACAAC 272
2251 TAATGGATAGCTAACAATAAGAACTATATTGTGAAACACTCATACAAC 2300
273 TGATATGCTGACTAGGAGAATGTGAAATAGGTAATTGTGAGATAGATAAG 322
2301 TGATATACTGACAAGGAGAATGTGAAATAGATTATAGTGAGATAGAGAAG 2350
323 CATTGTTGTGACTTATATAGACAGTTGTATGTGATTAAGTTCTTTTGA 372
2351 CATTGTTGTAACCTTATATAGACAGTTGTATGCGATTAAAGCTCTTTTGA 2400
373 AAAAGAACATATTATTTAAGTAATATGTTAGTGTAAAGTATTTGATCA 422
2401 AAAAGAACATATTATTTAAGTAA.....TGATCA 2429
423 TGAATATATATCTTATATATATAT.TTGTATGAATTGGACATGAATACT 471
2430 TGAATATATATCTTATATATATATATTGTATGAATTGGACATGATTATT 2479
472 GTTATACTAAATGTCGTCTGATGATCATTAGTTAATATGAATTATATGGA 521
2480 GTTATACTAAATGTCGTACGATGATCAGAAAGTTAATATGGAATTATATTGT 2529
522 TTTTATGAATTATTTGAAGGTTACTAAGCAAAACGAAGTCGTTTGAAC 571

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Percent Similarity: 93.011 Percent Identity: 93.011
pIIIB.seq x bgmvbpr.seq

Fig. 3. DNA nucleotide sequence analysis: Comparison between clone pII1 and *Bean golden mosaic virus*-Puerto Rico (BGMV-PR). Complementary nucleotides between clone pII1 and BGMV-PR were paired by a dash. These two clones shared a 93% similarity.

isolates from the Dominican Republic, Guatemala, Puerto Rico, and Puerto Rico–Japan (GenBank accession numbers L01636, M91605, M10080, and D00200), respectively. In spite of the high variability of the amplified noncoding protein region of the DNA-B, sequence analysis showed a high percentage of nucleotide sequence identity of the DNA virus found in *M. lathyroides* with BGYMV.

Confirmation of BGYMV infectivity cycle. To confirm that whiteflies had transmitted BGYMV from *M. lathyroides* to *P. vulgaris*, we amplified the DNA extracted from whitefly-infested *P. vulgaris* cv. Mayflower using primers for component B, to detect BGYMV. The 600-bp band obtained from *P. vulgaris* tissues was consistent with the band observed before for BGYMV from *M. lathyroides*. These products were consistent with PCR products obtained by Rojas (14).

DISCUSSION

Based on our results, we conclude that BGYMV infected both bean and the common legume weed *M. lathyroides*. Symptom expression in *M. lathyroides* was a golden yellow mosaic near the leaf veins. Nucleotide sequence analysis showed a 93% identity with BGYMV. Our findings, using DNA nucleotide sequence analysis, indicate that there is little divergence in the viral nucleotide sequence present in *P. vulgaris* and *M. lathyroides* tissues. These findings differ from Roye et al., who report phylogenetic differences among several weed viruses, including a golden mosaic virus from *M. lathyroides*, using DNA sequencing (15,16). The authors established that the geminiviruses infecting weeds in Jamaica are different from those infecting important crops even though the authors do not specify whether or not *M. lathyroides* was exposed to BGYMV. Roye et al. (15,16) described a partial coat protein gene sequence for *Macroptilium* spp.-infecting geminiviruses from Puerto Rico and that this virus was different from *Macroptilium* spp.-infecting viruses found in Jamaica. They concluded that the majority of the viruses characterized from Jamaica were new viruses that had not been reported previously.

In addition, PCR amplification of viral DNA-B confirmed that the virus infecting both plant species was bipartite, such as BGYMV. The amplified region of component B is considered of high variability because there is no gene coding for proteins in this region. This high variability region among DNA-B of different geminiviruses allows a more specific search when characterizing the virus. We obtained a 600-bp fragment for DNA-B, which is consistent with BGYMV. Fragment sizes can be different among geminiviruses; therefore, this fact is useful for viral characterization (14).

In our study, we provide evidence that *M. lathyroides* might be a reservoir of BGYMV under field conditions in Puerto Rico. Management of *M. lathyroides* growing in and around bean fields could be a useful strategy to avoid the spread of BGYMV. Further research on interactions with BGYMV and other weed geminiviruses with *P. vulgaris* is recommended.

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