

# Preliminary Identification of Begomoviruses Infecting Crops and Weeds in Belize

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## ABSTRACT

Cultivated plants, including pepper, red kidney bean, squash, string bean and tomato, as well as weeds with viral symptoms were collected from five districts in Belize over a three year period with the aim of determining the diversity of the begomoviruses present. Sixty five percent (111 / 171) of the samples screened via DNA hybridization produced signals indicative of begomovirus infection. Subsequent PCR amplifications and nucleotide sequence analyses revealed the presence of four begomoviruses in Belize. *Pepper golden mosaic virus* and *Tomato mottle virus* were found associated with tomato and sweet pepper and the former was also isolated from hot pepper. *Merremia mosaic leaf curl virus* was found infecting hot pepper, sweet pepper and the weed species *Euphorbia heterophylla*. *Euphorbia mosaic virus* was found in hot pepper and *Euphorbia*. This is the first report of the identification of begomoviruses in Belize.

**Keywords:** geminiviruses, Central America, *Pepper golden mosaic virus*, *Merremia mosaic leaf curl virus*, *Tomato mottle virus*, *Euphorbia mosaic virus*

## INTRODUCTION

Belize lies on the eastern, Caribbean coast of Central America and is bordered on the south and west by Guatemala, on the north and northwest by Mexico and by the Caribbean Sea on the east. This 8,867 square miles country depends heavily on agriculture and agro-based industries. Agriculture provides about 71% of the total foreign exchange earnings of the country, and employs almost 30% of the total labour force. Belize produces a variety of vegetables, fruits and root crops for subsistence consumption, commercial, domestic or export markets. The crops produced in Belize include bean, corn, potato, rice, sweet pepper, tomato and cucurbits such as butternut, cucumber, melon, pumpkin and squash (Waight 1999; Gore 2000).

The yields of cultivated plants, particularly vegetables, are often affected by viral diseases transmitted by insect pests. Therefore the control of insect pests is of great significance. In Belize, although farmers use any of a variety of physical, mechanical, cultural, chemical or biological pest control methods, most depend on chemical means of control. However, extension services have been able to convince some tomato farmers to try crop barriers when begomoviruses were suspected to be the cause of severe or total crop losses (O'Doherty 1992).

A change in agricultural practices in Central America occurred in the early 1970's with the beginning of commercial cultivation of cotton and soybean in Argentina, Brazil, El Salvador, Guatemala, Nicaragua and Mexico. Unfortunately, soybean and cotton are both excellent hosts for the growth and reproduction of the whitefly, *Bemisia tabaci*. Therefore monocropping and the extension of the growing season with little or no crop-free time, combined with increased pesticide use, all contributed to the dramatic increase in whitefly populations and the concomitant increase of the associated plant diseases. As a result, whitefly-transmitted geminiviruses (begomoviruses) emerged as major pathogens of food crops in the region.

By the mid 1980's the whitefly had become a serious pest and a wide variety of crops including cotton, cucurbits, lettuce, pepper and tomato were affected by diseases caused by begomoviruses (Galvez and Morales 1989). Over the past few decades, there has been more interest in geminiviruses, especially begomoviruses, as many of the diseases they cause have now reached epidemic proportions. Geminiviruses are significant plant pathogens in areas such as Africa and Australia, southeast Asia, Latin America, the Caribbean, southern Europe and south western United States and has resulted in significant losses annually (Brown 1991; Brown and Bird 1992; Markham *et al.* 1994; Czosnek and Laterrot 1997; Polston and Anderson 1997; Polston *et al.* 1997; Umaharan *et al.* 1998; Faria and Maxwell 1999; Moffat 1999; Rojas *et al.* 2000; Morales and Anderson 2001; Oliviera *et al.* 2001; Varma and Malathi 2003). As a group, the geminiviruses were recognized as distinct from all other groups of viruses in 1977 (Harrison 1977). Since then there has been an increase in the number of geminiviruses identified, particularly the begomoviruses. Currently, *Geminiviridae* form the second largest family of plant viruses (Brown 1991; Brown and Bird 1992; Lazarowitz 1992; Polston and Anderson 1997; Morales and Anderson 2001; Varma and Malathi 2003; Fauquet *et al.* 2008). Central/Latin America is severely affected by begomoviruses, with over five million hectares of agricultural crops in more than twenty countries being affected by more than 30 distinct begomoviruses (Brown 1991; Brown and Bird 1992; Hidayat *et al.* 1993; Polston and Anderson 1997; Umaharan *et al.* 1998; Morales and Anderson 2001; Ala-Poikela *et al.* 2005).

Weeds are important reservoirs for some geminiviruses (Roye *et al.* 1997; Rojas *et al.* 2000; Sanz *et al.* 2000). Begomoviruses infect a variety of weeds in Latin America and the Caribbean Basin (Brown and Bird 1992; Frischmuth *et al.* 1997; Roye *et al.* 1997; Umaharan *et al.* 1998). *Sida golden mosaic Honduras virus* (SiGMHV) was found in the major tomato growing areas of Nicaragua infecting



**Figs. 1-5 Begomoviruses infecting crops and weeds in Belize.** (1) Tomato from Belize showing leaf curling, yellowing and stunting. (2) Bird pepper from Belize showing mild leaf curling and chlorosis. (3) Leguminous weed from Belize showing mottling. (4) Unidentified weed from Belize showing chlorosis. (5) Weed from Belize showing yellow mosaic.

tomato and producing symptoms such as yellow mottling, crinkling, curling and severe stunting (Rojas *et al.* 2000). Since *Sida* spp. are very common weeds of crop fields in Nicaragua and many other countries in Latin America and the Caribbean, this finding is of great significance, showing that weed-infecting begomoviruses do indeed affect crops. Crop-infecting begomoviruses have also been identified in weeds. *Tomato mottle virus* (ToMoV) was found to infect the tropical soda apple (*Solanum varium*) in Florida (McGovern *et al.* 1994). In Trinidad, *Potato yellow mosaic virus* (PYMV) was found in five weed hosts (Umaharan *et al.* 1998). In this paper we provisionally identify for the first time four begomoviruses associated with crops and weeds in Belize. As new and emerging begomoviral diseases and complexes continue to be identified worldwide, studies, such as this one becomes even more useful as it adds to the awareness of the diversity of begomoviruses in the Latin American/Caribbean region. This information may have implications for their control in the region.

## MATERIALS AND METHODS

### Collection of plant samples

The youngest leaves from 171 plants exhibiting symptoms such as leaf curling, yellowing, mottling, mosaic and stunted growth were collected between February 1999 and January 2002 from major vegetable-growing areas in Belize district in the east, Cayo and Orange Walk districts in the west and Corozal district in the north. These plants included tomato (*Lycopersicon esculentum*, **Fig. 1**), hot pepper (*Capsicum chinense*), bird pepper (*C. frutescens*, **Fig. 2**), sweet pepper (*C. annuum*), pumpkin (*Cucurbita pepo*), string bean (*Phaseolus vulgaris* L. var. *vulgaris*), red kidney bean (*P. vulgaris* L.), muskmelon (*Cucumis melo*), squash (*C. moschata*) and watermelon (*Citrullus lanatus*). Weed samples collected included *Euphorbia heterophylla*, *Sida* spp., and several unidentified weeds (**Figs. 3-5**) found within and around the crop fields. Total DNA was extracted from either fresh, frozen (-80°C) or oven dried (60°C) leaf tissue using a modified version of the Dellaporta extraction as described by Rojas *et al.* (1993). Geminiviral DNA was detected via nucleic acid hybridizations with geminiviral probes. Subsequently, PCR amplification and nucleotide sequence analysis were used to identify the begomoviruses present in the plants.

## Preparation of digoxigenin-labeled Geminiviral DNA probes and DNA hybridization

Recombinant plasmids (**Table 1**) containing geminiviral inserts were isolated using the alkaline lysis miniprep procedure (Sambrook *et al.* 1989). The geminiviral inserts were removed by restriction enzyme digestion separated by agarose gel electrophoresis and the desired viral DNA fragment were recovered using QIA quick Gel Extraction Kit, or QIAEX II Agarose Gel Extraction Kit (QIAGEN). The recovered viral DNA fragments were used as templates for making the probes.

The probe labelling and DNA hybridization were done using the Genius II DNA Labelling and Detection Kit (Boehringer Mannheim). The plasmids used to make the probes were used as positive controls and the negative controls were DNA extracted from pepper, red kidney bean and tomato grown in a whitefly-free greenhouse.

Plant DNA and negative controls were spotted onto positively charged nylon membranes (Boehringer Mannheim) and cross-linked for 25–50 seconds with ultraviolet light (120,000  $\mu\text{J}/\text{cm}^2$ ) in a Stratalinker, UV Crosslinker1800 (Stratagene Cloning Systems, La Jolla CA, USA). The membranes were pre-hybridized (5 x SSC, 0.1% (w/v) N laurylsarcosine, sodium salt, 0.2% (w/v) SDS, 2% (w/v) blocking reagent), with 50 % formamide (v/v) at 42°C (low stringency) or without formamide at 68°C (high stringency) in a Hybridizer HB-1D (Techne, Cambridge, England). The membranes were rinsed in solution I (2 X SSC, 0.1% SDS) at room temperature (low stringency) or at 65°C (high stringency), followed by rinses in solution II (0.1 x SSC, 0.1% SDS). Detection was achieved by colorimetric or chemiluminescent methods.

## Polymerase Chain Reaction, DNA cloning, sequencing and analysis

DNA extracts from selected plant samples that produced hybridization signals were used in PCR with degenerate and specific primer pairs (**Table 2**) to confirm the presence of geminiviral DNA. Samples used in PCR were selected to include all sample sites as well as a variety of plant types which produced good to very strong hybridization signals. The PCR reaction (25  $\mu\text{l}$ ) contained 0.2  $\mu\text{M}$  of each primer 1 mM each of dATP, dCTP, dGTP and dTTP, 2.5 mM of  $\text{MgCl}_2$ , 0.5 units of *Taq* DNA polymerase and 50 ng total DNA. Template DNA were amplified using 1 min at 94°C, followed by 30 cycles of 1 min at 94°C, 2 min at 55°C, and 2 min at 72°C with a final extension of 5 min at 72°C.

PCR-amplified geminiviral DNA was ligated into the plasmids pGem T or pGem T Easy, (Promega) or TOPOA (Invitrogen Corporation) and used to transform *E. coli* XL1 Blue (Stratagene) competent cells (Ausubel *et al.* 1996). Recombinant colonies were selected by standard molecular biology techniques (Sambrook *et al.* 1989).

Nucleotide sequencing was performed by the Biotechnology Center, University of Wisconsin, Madison, using the Sanger dideoxy chain termination method (Big Dye <sup>®</sup> Terminator Cycle Sequencing Kit, Applied Biosystems, Foster City, CA, USA). Sequences were obtained either directly from PCR products or from recombinant plasmids containing PCR-amplified inserts. Sequences from recombinant plasmids were obtained using the M13 forward and reverse sequencing primers and from PCR products using the appropriate PCR primers.

Initially sequences were analysed using the standard nucleotide-nucleotide comparison of the Basic Local Alignment Search Tool (BLAST) program ([www.ncbi.nlm.nih.gov/BLAST](http://www.ncbi.nlm.nih.gov/BLAST), Altschul *et al.* 1990) which compared the query sequences with all nucleotide sequences in the database. Lasergene software version 3.1 (DNASTar Inc., Madison, WI, USA) was used for further analysis of the sequences.

## RESULTS

### DNA hybridization

Of the 171 plant samples from agricultural regions in Belize screened for begomoviruses, 65% (111/171) and the positive controls produced signals ranging from weak to very

**Table 1** Recombinant plasmids used for making probes.

Clone	Virus	Source	Region of viral genome	Fragment length (Kb)
pTYJA15	TYLCV-[JM]	Wernecke 1995	<i>Rep</i> , <i>TrAP</i> and <i>Ren</i>	1.1
pPYAG5	PYMV	This work	5' <i>Rep</i> , IR and 5' <i>CP</i>	1.3
pToMoBZ18	ToMoV-[Flo]	This work	5' <i>Rep</i> , IR and 5' <i>CP</i>	1.3
pBGJA2	BGYMV-[JM]	Roye 1996	5' <i>Rep</i> , IR and 5' <i>CP</i>	1.3

*CP* = coat protein, IR = intergenic region, Ren = replication enhancement protein, *Rep* = replication protein, *TrAP* = transcriptional activation protein.

**Table 2** Nucleotide sequence of primers used in PCR amplification of geminiviral DNA.

Primers	Sequence (5' to 3')	Reference
<b>Degenerate*</b>		
PAC1v1978	GCACCCACATYGTCTTYCCNGT	Rojas <i>et al.</i> 1993
PAV1c715	GATTTTDA TRTTYTCRTCCATCCA	
AC1048	TTDGARGCATGHGTACATG	Wyatt and Brown 1996
AV494	CYATRTAYAGRAAGCCMAG	
<b>TYLCV specific</b>		
PTYIRv21	AACTGTTGAAATGAATCGGTGTCCC	Nakhla <i>et al.</i> 1993
PTYIRc287	ATATTTGCAAGACAAAAAAGCTGGGACC	

\* nucleotides at degenerate positions are represented by a single letter of the IUPAC ambiguity code: D = A,G,T; H = A,C, T; M = A,C; N = A,C,G,T; R = A,G; W = A,T; Y = C,T

strong when hybridized with the probes. No hybridization signals were observed for the negative controls.

In the *Solanaceae* family, tomato, sweet pepper and hot pepper were screened of which 79% (103/134) produced hybridization signals. These consisted of 77% of the tomato (34/44) from Cayo district and the one sample from the Corozal district. For the sweet pepper samples 78% (35/45) hybridised to the geminiviral probes. Of the nine sweet pepper samples collected from the Belize district, six hybridised to BGYMV probe at low stringency and none hybridised with either PYMV or TYLCV probes at high stringency. Both of the sweet pepper samples from the Corozal district produced no signals with a ToMoV probe at high stringency, but produced moderate signals with the BGYMV probes. Similarly, the only sweet pepper sample from the Orange Walk district was negative with ToMoV and TYLCV probes at high stringency but produced a very weak signal with the BGYMV probe at low stringency. The majority of the sweet pepper samples (33) were collected from the Cayo district and 26 samples produced moderate to good hybridization signals with the BGYMV probes at low stringency. Of note is that among the sweet peppers two samples gave weak signals and one other gave a good signal with the ToMoV probe in high stringency conditions.

Forty-five hot pepper samples (43 hot pepper commonly called scotch bonnet or habanero pepper and two commonly called bird pepper) were collected from farms in the Orange Walk, Cayo and Belize districts of which 74% (32/43) were positive for geminiviral DNA. Five of the 18 scotch bonnet pepper samples collected in the Belize district produced no hybridization signal with any of the geminiviral probes used and the remaining samples hybridised to a BGYMV probe at low stringency and or TYLCV and ToMoV probes at high stringency. Nineteen of the 22 scotch bonnet pepper samples from the Cayo district produced hybridization signals with either ToMoV or TYLCV probe at high stringency. The three samples collected from the Orange Walk district produced good signals with the BGYMV probe. The two bird pepper samples collected from the Belize district hybridized to the BGYMV probe at low stringency and to the ToMoV and TYLCV probes in high stringency conditions.

Of the six red kidney bean samples, four produced hybridization signals with the BGYMV probe at low stringency. Both of the string bean samples produced hybridization signals with BGYMV or TYLCV probes. From the *Cucurbitaceae* family, watermelon, zucchini and muskmelon were collected from the Cayo district and squash was collected from the Belize district. None of the watermelon samples (0/3) were positive for begomovirus when tested with BGYMV, TYLCV and ToMoV probes. The only zucchini sample produced moderate hybridization signals with ToMoV probes. One of the two muskmelon samples was

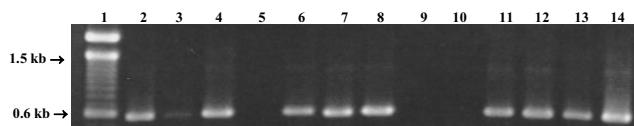
positive for the presence of geminiviral DNA. Two of three squash samples collected hybridised to a BGYMV probe at low stringency.

A total of 19 weed samples were collected from the Cayo and Belize districts. The weeds included 4 samples of *Euphorbia*, five samples of *Sida* and ten unidentified weeds. Twenty-six percent (5/19) of these consisting of one *Euphorbia* (BZ 128) and four unidentified weeds produced weak hybridization signals with a BGYMV probe at low stringency. DNA from select positive samples (to include all sample sites as well as a variety of plant types which produced good to very strong hybridization signals) was used in PCR amplifications to confirm the presence of begomoviral DNA.

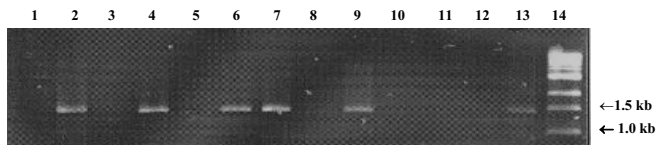
## Polymerase Chain Reaction

Sixty two samples were chosen for further analysis via PCR using degenerate and/or specific begomovirus primers (Table 2). These samples consisted of 19 sweet pepper, 19 tomato, 11 hot pepper, four string bean, two squash, one zucchini and six weeds.

Seven (37%) of the tomato samples, 12 (63%) of the sweet pepper samples, 11 (55%) of the hot peppers and 2 (50%) the string bean samples resulted in PCR products with PAC1v1978/PAV1c715 and the core coat protein primers AC1048/AV494 (Fig. 6). Neither of the two squash samples generated PCR products, but the zucchini sample and four weeds (one of the unidentified weeds and three *Euphorbia* sp.) yielded a 1.3-kb partial DNA-A fragment with the primers PAC1v1978/PAV1c715 (Fig. 7). The TYLCV specific primers generated the expected 0.3-kb product from a sweet pepper (BZ 28) and two tomato samples BZ 37 and BZ 80.



**Fig. 6** Agarose gel of PCR amplifications of core *CP* gene from Belize samples with primers AC1048/AV494. Lane 1= 100 bp (Invitrogen), Lane 2 = BZ 77 (sweet pepper), Lane 3 = BZ 78 (sweet pepper), Lane 4 = BZ 80 (tomato), Lane 5 = BZ 81 (tomato), Lane 6 = BZ 83 (sweet pepper), Lane 7 = BZ 85 (sweet pepper), Lane 8 = BZ 86 (sweet pepper), Lane 9 = BZ 91 (zucchini), Lane 10 = BZ 116 (hot pepper), Lane 11 = BZ 117 (hot pepper), Lane 12 = BZ 126 (hot pepper), Lane 13 = BZ 13 (tomato) 3, Lane 14 = BZ 155 (sweet pepper).



**Fig. 7** Agarose gel of PCR amplifications of partial *CP* and *Rep* genes and *IR* from Belize samples with primers PAC1v1978/PAV1c715. Lane 1 = BZ 112 (weed), Lane 2 = BZ 130 (*Euphorbia* sp), Lane 3 = BZ 161 (weed), Lane 4 = BZ 163 (weed), Lane 5 = BZ 118 (bird pepper), Lane 6 = BZ 124 (hot pepper), Lane 7 = BZ 125 (hot pepper), Lane 8 = BZ 24 (red kidney bean), Lane 9 = BZ 111 (string bean), Lane 10 = BZ 27 (red kidney bean), Lane 11 = BZ 115 (squash), Lane 12 = BZ 127 (squash), Lane 13 = positive control, Lane 14 = 1 kb ladder (New England Bio-Labs).

## Nucleotide sequence analysis

### Core coat protein sequences

Core coat protein nucleotide sequences (Brown *et al.* 2001b) were obtained from one tomato (BZ 58), two hot pepper (BZ 117, 126) and seven sweet pepper plant samples (BZ 7, 34, 77, 83, 85, 86, 155). Multiple alignments and sequence pair distance comparisons indicated that the begomoviruses present in these plants showed high levels of nucleotide sequence identity (94-100%).

BLAST analysis of the ten samples, with lengths ranging from 473 to 520 nucleotides, resulted in nucleotide sequence identity of 95-97% to *Pepper golden mosaic virus* from LaPaz, Mexico (PepGMV-[LaPaz], AY368336).

### Partial DNA-A sequences

Eight partial DNA-A sequences, of approximately 1300 nt, were obtained which represent approximately 600 nt of the 5' end of the replication protein, approximately 400 nt of the 5' end of the coat protein and the entire intergenic region.

Partial DNA-A sequences were obtained from a tomato (BZ 18), two hot pepper (BZ 89, BZ 124), a sweet pepper (BZ 100) a string bean (BZ 111), two *Euphorbia* sp. (BZ 130, BZ131) and an unidentified weed (BZ 163). Accession numbers are given in **Table 3**. Multiple alignments of the partial DNA-A sequences indicated that the sequences represented three distinct begomoviruses. The percentage identity between the sequences revealed that the isolates BZ 89, BZ 130 and BZ 131 were 95-97% similar to each other, but only 46-54% similar to the other partial DNA-A sequences. Whereas, isolates BZ 111, BZ 124 and BZ 163 were 97-98% similar to each other but only 53-55% similar to the other sequences. BZ 100 and BZ 18 shared 98% similarity but only 47-65% with the other sequences.

Searches of the GenBank using the BLAST programme revealed that the three distinct begomoviruses (**Table 3**) were *Tomato mottle virus* (ToMoV), *Euphorbia mosaic virus* (EuMV) and *Merremia mosaic leaf curl virus* (MeMV). The nucleotide sequence of the tomato (BZ 18, 1339 nt) and

the sweet pepper (BZ 100, 1391 nt) were about 97% similar to Tomato mottle virus-[United States of America:Florida:1989] (ToMoV-[US:Flo:89], L14460) over the length of the sequences, and BZ 89 (1348 nt), BZ 130 (1373 nt) and BZ 131 (1358) were 97-98% similar over the length of the sequences to *Euphorbia mosaic virus-A* [Mexico:Yucatan:2004] (EuMV-A[MX:Yuc:2004], DQ318937). BLAST searches of the representative of the third "group", BZ 111 (1291 nt), BZ124 (1330 nt) and BZ 163 (1277 nt) showed that they were 96% similar to *Merremia mosaic virus-Puerto Rico* [Puerto Rico] (MeMV-PR[PR], AF068636). This data suggests that these isolates are likely to be MeMV-PR[PR]. Pairwise sequence comparisons of the Belize sequences with the sequence they were most similar to using DNASTar gave results similar to the blast searches.

The amplification of the expected size fragments with the TYLCV-specific primers suggests that this virus infects tomato in Belize. Although no sequence data was generated based on hybridization signals and or PCR products obtained, pumpkin, zucchini, bird pepper and red kidney beans from Belize were likely infected with begomoviruses.

## DISCUSSION

Symptoms caused by whitefly-transmitted geminivirus were first reported from Brazil by Costa (1965). These symptoms are now known to be caused by *Bean golden mosaic virus* (BGMV, Gilbertson *et al.* 1993). In fact, Central America and the Caribbean Basin have been reported as the probable geographic origin of a number of begomoviruses such as *Abutilon mosaic virus* (AbMV), *Euphorbia mosaic virus* (EuMV), and *Macroptilium golden mosaic virus* (MaGMV, Brown 2003). In Belize, there was one report that confirmed the presence of begomoviruses in beans and hot pepper using ELISA (Gibson and Kenyon 1995). The study did not identify the begomovirus(es), but only concluded that they were present. This finding was not unexpected as Belize shares a border with Mexico and Guatemala, both of which have documented evidence of numerous begomoviruses (Faria *et al.* 1994; Brown *et al.* 2001a; Ascencio-Ibáñez *et al.* 2002; Garzón-Tiznado *et al.* 2002). This report adds to previously available information by not only confirming the presence of begomoviruses in Belize but also in determining their probable identity.

Plant samples were collected from four of the six districts in the northern, western and central regions of the country. At the time of the collections, there were three major crops in the field, tomato, hot pepper and sweet pepper. Due to the current realization that weed-infecting begomoviruses may infect crops and that weeds may serve as a reservoir for crop-infecting geminiviruses, random symptomatic weeds found in or around the crop fields were also collected (Roye *et al.* 1997; Ascencio-Ibáñez *et al.* 2002; Bracero *et al.* 2003; Idris *et al.* 2003; Rampersad and Umaharan 2003).

The four begomoviruses present in the samples collected from Belize were ToMoV, PepGMV, MeMV, EuMV. Of these, ToMoV was found infecting tomato and sweet pepper, both from Cayo. This was confirmed by a multiple

**Table 3** Summary of begomoviruses identified from Belize.

Sample No.	Plant	District collected [date]	Symptoms	PCR			Virus*	Accession number
				a	b	c		
BZ18	Tomato	Cayo [6/1998]	No record	+			ToMoV	EF028241
BZ89	Hot pepper	Cayo [6/2000]	Leaf curling, mottling	+			EuMV	EU709519
BZ100	Sweet pepper	Cayo [8/2001]	Yellowing, mottling	+			ToMoV	EU709520
BZ111	String bean	Cayo [8/2001]	Mosaic	+	-	-	MeMV	EU709521
BZ124	Hot pepper	Belize [8/2001]	Mild mottling	+	-	-	MeMV	EU709522
BZ130	Euphorbia	Pine Ridge [8/2001]	Mild mottling	+	+	-	EuMV	EU709523
BZ131	Euphorbia	Pine Ridge [8/2001]	Mild mottling	+			EuMV	EU709524
BZ163	Unidentified weed	Bomba [8/2001]	Mild mottling	+	+	-	MeMV	EU709525

+ PCR product obtained, - no PCR product obtained

a = PAC1v1978/PAV1c715; b = AC1048/AV494; c = PTYIRc287/PTYIRv21

\* The virus to which the isolate has most sequence similarity



alignment of the two isolates (approximately 1300 nt) with the corresponding region of ToMoV, which resulted in sequence identities of 97% between the viruses, which confirmed the identity of these isolates. ToMoV was first reported to infect tomato in Florida in 1993 (Polston *et al.* 1993) but, to date, this is the first report of it infecting sweet pepper.

PepGMV was found in sweet pepper and tomato from Cayo, as well as sweet and hot pepper from the Belize district. The district of Cayo is in the western region of the country and shares a border with Guatemala, and there is also movement of plants between that region of the country and Mexico where PepGMV was first reported as the causative agent of an epidemic, which occurred between 1989 and 1993. Other pepper-infecting begomoviruses were also isolated in Central America, the Caribbean, Mexico and south western United States and it was recently determined that these viruses form part of a complex of viruses infecting pepper. PepGMV was found to be synonymous with the former Texas pepper virus (TPV) and Serrano golden mosaic virus (SGMV). Besides Pepper golden mosaic virus-United States of America [Mexico:Tamaulipas] (PepGMV-US[MX:Tam], U57457), this complex include PepGMV isolates from Costa Rica (PepGMV-CR[CR], AF149227) as well other PepGMV segregated based on their symptomology, mosaic (PepGMV-US[US:Mos:87, AY928512] and distortion (PepGMV-US[US:Dis:87], AY928514). It has been suggested that PepGMV and Cabbage leaf curl virus-[United States of America:Florida:1996] (CaLCuV-[US:Fla:96], U65529) share common ancestry with squash viruses, which have broader host ranges than other begomoviruses (Brown *et al.* 2005). Owing to the fact that members of the squash cluster of begomoviruses generally have a wide host range, it is therefore reasonable to speculate that PepGMV may pose a threat to a variety of species.

MeMV was found infecting both hot pepper and *Euphorbia* from the Cayo district. Of note was that this virus was found infecting hot pepper and sweet pepper as well as a weed, suggesting that it has more than one host. The nucleotide sequences of the isolates vary from those of the reference begomovirus in the intergenic region by about 20 nt upstream of the stem loop, where MeMV-PR[PR] were similar but BZ 163 has approximately 20 additional nucleotides, BZ 124, 15 nucleotides and BZ 111, eight nucleotides. The isolates were 94-97% similar to MeMV-PR[PR]. Based on current guidelines for the species distinction, MeMV-PR[PR] and the BZ 111 group are likely the same species of begomovirus. This identification is made only with the level of confidence that can be afforded to partial sequences, as it is generally accepted that recombination in begomoviruses is occurring rapidly to produce a wide variety of recombinant begomoviruses (Padidam *et al.* 1999; Chatchawankanphanich and Maxwell 2002; Monci *et al.* 2002). Any recombination events that may have occurred outside of the region sequenced is not accounted for in this analysis.

The begomovirus found in samples BZ 89 (hot pepper), BZ 130 (*Euphorbia*) and BZ 131 (*Euphorbia*) was EuMV. This virus was initially isolated in 2004 from *Euphorbia heterophylla* in Mexico and was also detected in Puerto Rico and Jamaica (Brown *et al.* 2001b; Collins and Roye 2007; Hernández-Zepeda *et al.* 2007). EuMV-A[MX:Yuc:04] was able to infect pepper (*C. annuum*), red kidney bean and tomato (Hernández-Zepeda *et al.* 2007), showing that this virus infects crops as well as weeds in the Caribbean and Central America. It is now shown for the first time that at least four distinct begomoviruses are infecting a variety of crops and weeds in Belize.

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