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Occurrence of *Squash yellow mild mottle virus* and *Pepper golden mosaic virus* in Potential New Hosts in Costa Rica

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Leaf samples of *Solanum lycopersicum*, *Capsicum annuum*, *Cucurbita moschata*, *Cucurbita pepo*, *Sechium edule* and *Erythrina* spp. were collected. All samples were positive for begomoviruses using polymerase chain reaction and degenerate primers. A sequence of ~1,100 bp was obtained from the genomic component DNA-A of 14 samples. In addition, one sequence of ~580 bp corresponding to the coat protein (AV1) was obtained from a chayote (*S. edule*) leaf sample. The presence of *Squash yellow mild mottle virus* (SYMMoV) and *Pepper golden mosaic virus* (PepGMV) were confirmed. The host range reported for SYMMoV includes species of the Cucurbitaceae, Caricaceae and Fabaceae families. This report extends the host range of SYMMoV to include the Solanaceae family, and extends the host range of PepGMV to include *C. moschata*, *C. pepo* and the Fabaceae *Erythrina* spp. This is the first report of a begomovirus (PepGMV) infecting chayote in the Western Hemisphere.

Keywords : begomovirus, vegetables, whiteflies

Latin America and the Caribbean Islands possess a great diversity and incidence of begomoviruses (Morales, 2010). Since 1950, weeds of the Euphorbiaceae (Costa and Bennett, 1950) and Malvaceae (Costa and Carvalho, 1960) families have been identified as primary sources of inoculum for the transfer of these viruses to nearby crops. However, it was not until the 1970s that the first important losses in production due to viral infections caused by this group of pathogens were reported in crops such as cotton (*Gossypium hirsutum* and *G. barbadense*) and bean (*Phaseolus vulgaris*) (Morales, 2010).

The genus *Begomovirus* (Geminiviridae) is characterized

by a circular single-stranded DNA genome, encapsidated in twinned icosahedral particles. Begomoviruses of New World have bipartite genomes. The DNA-A component includes several open reading frames (ORFs) related to replication, encapsidation, movement and transcriptional activation of virion sense genes (Harrison, 1985). The DNA-B component has two ORFs implicated in viral systemic movement (Etessami et al., 1988).

The begomoviruses are recognized as infectious agents causing economic losses in crops in tropical and subtropical zones worldwide (Dhaliwal et al., 2010; Navas-Castillo et al., 2011; Rojas et al., 2005). An important group of begomoviruses in America are those infecting cucurbits. Among the most prominent are *Squash leaf curl virus* (SLCV), *Squash mild leaf curl virus* (SMLCV), *Cucurbit leaf crumple virus* (CuLCrV), *Squash yellow mild mottle virus* (SYMMoV) and *Melon chlorotic leaf curl virus* (MCLCuV) which cause economic losses in *Cucurbita moschata*, *C. pepo*, *C. maxima*, *Cucumis melo* and *Citrullus lanatus* (Idris et al., 2008; Karkashian et al., 2002; Morales, 2010). Since these viruses can infect the same plant host species, mixed infections have been found in which rearrangements are formed by the encapsidation of DNA-A of one virus species with DNA-B of a different virus species. The degree of virulence depends on the species that are mixed and on the host plant (Idris et al., 2008).

In the Western Hemisphere, the first reports of begomoviruses in pepper (*Capsicum* spp.) crops were in the United States (Brown et al., 1989; Brown and Poulos, 1990) and Mexico (Leal and Quintero, 1989). Currently, *Pepper golden mosaic virus* (PepGMV) is one of the most important species (Brown et al., 2005) affecting *Capsicum annuum* (bell pepper), with a host range that also includes *Solanum lycopersicum* (tomato) (Nakhla et al., 1994; 2005; Rojas et al., 2000; 2005) and some cucurbits (Ala-Poikela et al., 2005). Other viruses detected in pepper as well as tomato

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include *Pepper huasteco yellow vein virus* (PHYVV) (Morales et al., 2005), *Tomato leaf curl Sinaloa virus* (ToLCSinV) (Brown et al., 1993), *Tomato severe rugose virus* (ToSRV) (Bezerra-Agasie et al., 2006; Nozaki et al., 2006), *Tomato yellow leaf curl virus* (TYLCV) (Ascencio-Ibáñez et al., 1999; Quiñones et al., 2001) and *Potato yellow mosaic virus* (PYMV) (Umaharan et al., 1998).

Begomoviruses identified in Costa Rica to date include PepGMV in pepper (Lotrakul et al., 2000) and tomato (Nakhla et al., 2005), ToLCSinV (Idris et al., 1999; Nakhla et al., 2005) and *Tomato yellow mottle virus* (ToYMoV) (Hilje et al., 1993; Nakhla et al., 1994) in tomato. *Squash yellow mild mottle virus* (SYMMoV) has been detected in *Cucurbita moschata* (squash), *Citrullus lanatus* (watermelon), *Carica papaya* (papaya) (Karkashian et al., 2002) and bean (Karkashian et al., 2011). There are also reports of PepGMV (Hilje et al., 1993), *Bean golden mosaic virus* (BGMV) (Gámez, 1971) and *Calopogonium golden mosaic virus* (CaGMV) (Díaz et al., 2002) in bean.

The Cartago province, one of the most important areas of vegetable production in Costa Rica, has been studied continuously. Research in this region has shown the possibility of an amplified host range for these viruses and the potential emergence of new virus species from the recombination of existing species.

The objectives of this research were to identify and determine the sequence variability of begomoviruses in Cartago in tomato, pepper, squash, *Cucurbita pepo* (zucchini, scaloppini), *Sechium edule* (chayote) and *Erythrina* spp., and to determine phylogenetic relationships between these and previously described sequences.

Materials and Methods

Sample collection and total nucleic acid extraction to obtain begomovirus DNA. A total of 15 leaf samples from tomato, pepper, cucurbits and *Erythrina* spp. were collected in the Cartago province in 2007 and 2008. Samples were stored at -70°C . The protocol described by Dellaporta et al. (1983) was followed to obtain total nucleic acid.

Rolling circle amplification (RCA) and polymerase chain reaction (PCR) for detection of begomoviruses. Total DNA purified from 15 leaf samples were amplified by RCA (Fire and Xu, 1995) using the Illustra™ TempliPhi Amplification Kit (GE Healthcare, UK). RCA products were reamplified by PCR using degenerate primers AV494 and AC1048 (Wyatt and Brown, 1996) for the core of capsid protein gene (AV1) to confirm the presence of begomovirus infection. The final volume of the reactions was 25 μl containing: $1\times$ reaction buffer, 2.5 mM MgCl_2 , 250 μM dNTPs, 0.1 μM each primer, 5U/ μl Dream Taq DNA

polymerase (Fermentas, Hanover, MD) and 2 μl viral DNA amplified by RCA. The PCR program used the conditions defined by Rojas et al. (1993).

PCR for molecular characterization of begomoviruses.

RCA products of 14 samples were reamplified by PCR using degenerate primers PAC1C1960 and PAV1v722 (Zhou et al., 1997), which correspond to a region of ~ 1300 bp of the DNA-A. This region will be referred herein as DNA-A bottom and it includes complete nucleotide sequences of the replication enhancer protein (AC3) and transcriptional activator protein (AC2). Additionally, the amplicon also contains a partial sequence of the replication associated protein (AC1) and coat protein (AV1) genes. PCR reactions were carried out as described previously by Rojas et al. (1993).

Electrophoretic analysis. RCA and PCR products were analyzed by electrophoresis in TopVision 1% agarose gels (Fermentas) in $1\times$ TAE buffer at 100 V for 40 min. The O'GeneRuler™ 100 bp Plus DNA Ladder and the O'RangeRuler 500 bp DNA Ladder (Fermentas) were used as molecular weight markers. The amplicons were stained with ethidium bromide (1 mg/ml) in water, visualized with ultraviolet light (TFX-20.M, Vilber Lourmat), and photographed with a digital camera (Kodak EDAS 290).

Sequencing and phylogenetic analysis. The DNA-A bottom from the 14 previously amplified isolates and the AV1 gene from the Cha157 was sequenced. Sequencing was carried out with a BigDye Terminator 3.1v (Applied Biosystems, Foster City, CA) and the automated sequencer Genetic Analyzer 3130, AB (Applied Biosystems, USA). The BLASTn algorithm (Altschul et al., 1990) was used to compare nucleotide sequences of the amplified PCR products with sequences available in the GenBank database. The sequences of the 15 samples begomovirus-positive samples obtained in this study and those from GenBank that shared the greatest nucleotide identity with our samples, were aligned using the Clustal W algorithm (Thompson et al., 1994). Phylogenetic trees were constructed with the MEGA program version 5 (Kumar et al., 2008), using the nucleotide distances obtained and neighbor-joining phylogenetic analysis (Kimura, 1980). Internal branch robustness was estimated by bootstrap analysis with 1000 replicates.

Results

The symptoms observed in plants infected with begomoviruses in this study included yellow mosaic, yellow veins, changes in leaf size and morphology, leaf thickening, thickened vein, and yellow wrinkled leaf margins (Fig. 1).

Fig. 1. Symptoms in (A) *Sechium edule* (chayote), (B) *Erythrina* spp. (poro), (C) *Solanum lycopersicum* (tomato), (D) *Capsicum annuum* (bell pepper), (E) *Cucurbita moschata* (squash) and (F) *Cucurbita pepo* (scallopini), infected with whitefly-transmitted viruses.

Symptomatic leaf samples detected with begomovirus were collected from tomatoes, peppers, cucurbits and *Erythrina* spp. In addition, begomovirus infection was detected in one

asymptomatic zucchini sample.

RCA products of 14 samples reamplified by PCR using primers targeted to the DNA-A bottom of the viral genome yielded products circa 1,300 bp for all samples (Fig. 2). These 14 amplicons and the amplicon corresponding to the coat protein (AV1 gene) from the chayote leaf sample (Cha157) were sequenced. Sequences of ~1,100 bp were obtained from the genomic component DNA-A of the 14 samples and one sequence of ~580 bp corresponding to the AV1 gene was obtained from Cha157 sample.

The presence of SYMMoV or PepGMV was confirmed in those 15 samples. Seven sequences shared high similarity with SYMMoV (Table 1); and the remaining eight sequences were similar to PepGMV (Table 2). Only seven out of the latter eight sequences are shown in Table 2, because only the sequence corresponding to the capsid protein (AV1 gene) was obtained for sample Cha157. Nevertheless, the AV1 gene region is also useful for the preliminary identification of a virus species (Brown et al., 2001a). The nucleotide differences between these 15 isolates and other viral sequences obtained from the NCBI GenBank database were not clustered into particular regions of the DNA-A bottom or AV1 gene sequence.

The sequences similar to SYMMoV were from cucurbit samples and two were from solanaceous hosts (tomato and pepper). The sequence of the DNA-A bottom region (Table 1) shared 96 – 97% similarity with SYMMoV (GenBank, AY064391) and 89 – 90% with MCLCuV-GT (GenBank, AF325497). Four of the PepGMV sequences were from cucurbit samples, three were from pepper and one was from *Erythrina* spp. (Fabaceae). Sequences of the DNA-A bottom region (Table 2) shared 97 – 99% identity with PepGMV-CR (GenBank, AF149227) and 95 – 97% with PepGMV-Ser (GenBank, AY928516). The capsid protein gene sequence (AV1, ~580 bp) from the chayote sample Cha157 shared

Fig. 2. PCR analysis from 14 RCA products performed with the degenerate primers PAC1C1960 and PAV1v722, which correspond to a partial DNA-A sequence. (A) (MW) contains a 100 bp DNA size marker, samples Po3696, Cha4265, Sq229 (Lane 1 – 3), negative control (Lane 4) and positive control a bean sample infected by *Bean golden yellow mosaic virus* BGYMV (Lane 5) (B) (MW) contains a 500 bp DNA size marker, samples Sq142, Sq143, Sq150, Zu162, To166, Ch176, Es182, Sq185, Ch189, Ch201, Ch228, (Lane 1 – 11), negative control (Lane 12) and positive control a bean sample infected by BGYMV (Lane 13).

Table 1. Percent nucleotide identity among sequences of the DNA-A bottom of isolates of *Squash yellow mild mottle virus* (SYMMoV)

GenBank Accession number		¹ Sample number	² Sq142	Sq143	Sq185	Sq229	³ Zu162	⁴ To166	⁵ Ch189
AY064391		SYMMoV	97	97	96	97	97	97	97
AF325497		MCLCuV-GT	90	90	89	90	90	90	90
Family	Species	¹ Sample number	² Sq142	Sq143	Sq185	Sq229	³ Zu162	⁴ To166	⁵ Ch189
Cucurbitaceae	<i>Cucurbita moschata</i>	Sq142	⁶ ID	-	-	-	-	-	-
		Sq143	99	ID	-	-	-	-	-
		Sq185	98	98	ID	-	-	-	-
		Sq229	98	99	97	ID	-	-	-
	<i>Cucurbita pepo</i>	Zu162	98	98	97	98	ID	-	-
Solanaceae	<i>Solanum lycopersicum</i>	To166	99	99	98	99	99	ID	-
	<i>Capsicum annuum</i>	Ch189	99	99	98	99	99	100	ID

¹Numbers represent the code for each of the positive samples, ²Sq = Squash, ³Zu = Zucchini, ⁴To = Tomato, ⁵Ch = Pepper, ⁶ID = 100% identity

Table 2. Percent nucleotide identity among sequences of the DNA-A bottom of isolates of *Pepper golden mosaic virus* (PepGMV)

GenBank Accession number		¹ Sample name	² Ch176	Ch201	Ch228	³ Sq150	⁴ Es182	⁵ Cha4265	⁶ Po3696
AF149227		PepGMV-CR	99	99	99	99	97	99	98
AY928516		PepGMV-Ser	97	96	97	97	95	96	97
Family	Crop	¹ Sample name	² Ch176	Ch201	Ch228	³ Sq150	⁴ Es182	⁵ Cha4265	⁶ Po3696
Solanaceae	<i>Capsicum annuum</i>	Ch176	⁷ ID	-	-	-	-	-	-
		Ch201	99	ID	-	-	-	-	-
		Ch228	99	99	ID	-	-	-	-
Cucurbitaceae	<i>Cucurbita moschata</i>	Sq150	99	99	99	ID	-	-	-
	<i>Cucurbita pepo</i>	Es182	97	97	98	98	ID	-	-
	<i>Sechium edule</i>	Cha4265	99	99	99	100	98	ID	-
Fabaceae	<i>Erythrina</i> spp.	Po3696	98	98	99	99	97	99	ID

¹Numbers represent the codes for each of the positive samples, ²Ch = pepper, ³Sq = squash, ⁴Es = scallopini, ⁵Cha = Chayote, ⁶Po = *Erythrina* spp, ⁷ID = 100% identity

98% similarity with PepGMV-CR (GenBank, AF149227) and 95% PepGMV-YV (GenBank, AF063139).

Phylogenetic relationships among sequences corresponding to the DNA-A bottom region are shown in Fig. 2. Isolates from solanaceous plants (Ch189 and To166) and cucurbits (Sq142, Sq143, Sq185, Sq229 and Zu162) formed a well-defined group that was clearly related to the Costa Rican isolate of SYMMoV obtained by Karkashian et al. (2002). Isolates from cucurbits (Sq150, Es182, Cha4265), pepper (Ch176, Ch201, Ch228) and *Erythrina* sp. (Po3696) were grouped with isolates of PepGMV.

Discussion

Symptoms observed in the analyzed samples were similar to those previously described for plants infected with begomovirus (Hull, 2002) (Fig. 1). These viruses have caused negative effects on production in crops such as cotton, bean, tomato, pepper and cucurbits (Rojas et al.,

2005). Moreover, begomoviruses are considered to be agronomically important pathogens in Costa Rica (Hilje et al., 1993) and worldwide (Morales, 2010; Navas-Castillo et al., 2011; Rojas and Gilbertson, 2008). The percent of nucleotide identity among partial sequences of the SYMMoV isolates detected in this study was between 97 and 100% (Table 1), indicating a low variability between them. Further more isolates and research are needed to confirm this observation. The phylogenetic relationships shown in Fig. 3 indicated that the partial sequence of the SYMMoV isolates obtained during this study are more closely related to MCLCuV-GT than to other begomoviruses infecting cucurbits. It has been considered that SYMMoV may be an isolate of MCLCuV (Brown et al., 2005). The sequence similarity for the DNA-A of the two viruses (88.8%) is at the limit 89% defined by Fauquet et al. (2008) to distinguish between isolates of the same species. However, there are differences in symptoms, hosts range and sequence similarity in conserved regions to suggest that further

Fig. 3. Phylogenetic relationships established by neighbor-joining analysis based on partial sequences of DNA-A (~1,100 bp) of begomovirus species shown in Table 3. Horizontal line length is proportional to the number of nucleotide differences at each branch. Bootstrap values for 1,000 replicates were used. *African cassava mosaic virus* (ACMV-[KE]) was used as an outgroup.

information is required to elucidate this case.

The symptoms described for MCLCuV-GT and SYMMoV are different. MCLCuV-GT produced severe leaf deformation and reduced fruit set in *Cucumis melo* (melon) (Brown et al., 2001b). While, SYMMoV caused no symptoms in the fruit (J. Karkashian, personal communication) and leaf chlorotic mottle are often mild in *Carica papaya* (papaya) and in cucurbits (Karkashian et al., 2002). Additionally, MCLCuV-GT and SYMMoV also differ in their hosts range. The first report of MCLCuV-GT was in melon (Brown et al., 2001b) and there have since been other report in this crop (Jord et al., 2005). MCLCuV-GT experimentally infects *Cucurbita maxima*, *C. pepo*, *Citrullus lanatus*, *Phaseolus vulgaris* (bean), *Nicotiana benthamiana* and *Datura stramonium* (Idris et al., 2008). On the other hand, natural infections of SYMMoV have been detected not only in cucurbits but also in papaya (Karkashian et al., 2002) and more recently in bean (Karkashian et al., 2011). This is an uncommon characteristic for begomoviruses, which tend to have a narrow host range (Rojas et al., 2005). Moreover, MCLCuV-GT has not been found infecting tomato or

pepper even under experimental conditions (Idris et al., 2008), whereas SYMMoV was detected in both solanaceous crops in this study.

Regardless the sequence similarity of 88.8% between SYMMoV and MCLCuV-GT complete DNA-A, their association is less evident when only one region as the capsid protein gene is considered. According to Brown et al. (2011) sequence similarity among the capsid protein genes and the common region of the genomic components of these two viruses was less than 87%. These differences were also observed with partial sequences of the capsid protein gene of two Nicaraguan isolates (GenBank, AJ8442150, AJ8442151) detected in *Cucurbita argyrosperma* (Ala-Poikela et al., 2005). Comparison of a ~260 bp sequence from the Nicaraguan isolates (GenBank, AJ842150, AJ842151) and partial sequences of isolate Sq142 (GenBank, JN408719) obtained in this study showed 94 – 98% nucleotide similarity with SYMMoV (GenBank, AY064391) and only 80 – 83% with MCLCuV-GT (GenBank, AF325497).

More biological data such as the ability to generate infectious reassortants, sap transmissibility and germplasm

Table 3. Sequences of isolates of begomoviruses from this study, and other obtained from GenBank used in phylogenetic analysis

Virus [isolate]	Acronym	Accession number	Sequence
<i>Pepper golden mosaic virus</i> -[Costa Rica] ¹	Ch176	JN592718	DNA-A bottom region
	Ch201	JN592719	
	Ch228	JN592720	
	Sq150	JN592721	
	Es182	JN609290	
	Cha4265	JN609291	
	Po3696	JN609292	
<i>Squash yellow mild mottle virus</i> ¹	Sq142	JN408719	Complete DNA-A
	Sq143	JN408721	
	Sq185	JN573299	
	Sq229	JN592722	
	Zu162	JN592715	
	To166	JN592716	
	Ch189	JN592717	
<i>Pepper golden mosaic virus</i> -[Costa Rica]	PepGMV-CR	AF149227	Complete DNA-A
<i>Pepper golden mosaic virus</i> -[Mild tigre]	PepGMV-mild	EF210556	
<i>Pepper golden mosaic virus</i> -[Serrano]	PepGMV-SER	AY928516	
<i>Pepper golden mosaic virus</i> -[San Luis Potosi]	PepGMV-SPL1	GU564594	
<i>Pepper golden mosaic virus</i> -[Tamaulipas]	PepGMV-Tam	U57457	
<i>Squash yellow mild mottle virus</i>	SYMMoV	AY064391	
<i>Melon chlorotic leaf curl virus</i> -[Guatemala]	MCLCuV-GT	AF325497	
<i>Squash mild leaf curl virus</i> -Imperial Valley	SMLCV-IV	AF421552	
<i>Squash leaf curl virus</i>	SLCV	M38183	
<i>Cucurbit leaf crumple virus</i>	CuLCrV	NC002984	
<i>African cassava mosaic virus</i> -Kenya	ACMV-KE	J02057	

¹Isolates obtained in this study

susceptibility; will help to clarify the relationship of MCLCuV-GT and SYMMoV differences. There are previous examples for parallel evolution of new crop-infecting begomoviruses species in multiple locations, including bean golden mosaic disease (Gilbertson et al., 1993) and African cassava mosaic (Varma and Malathi, 2003). These discrepancies in host range, symptoms and sequence difference in conserved regions, may indicate that these are two related but distinct virus species or that these are two isolates from the same species with divergent evolution (Brown et al., 2011).

The percent of nucleotide identity among partial sequences of PepGMV from this study was high (97 – 100%, Table 2), in concordance with a previous study with PepGMV isolates from North America (Brown et al., 2005). It should be noted that the partial sequence of sample Es182 showed lower similarity (97% and 95%) with the PepGMV-CR (GenBank, AF149227) and the PepGMV-Ser (GenBank, AY928516) than the other isolates of this study; however it is clearly an isolate of the same virus (Table 2). A close phylogenetic relationship (Fig. 3) was showed between

samples in this study and the original Costa Rican PepGMV isolate obtained by Lotrakul et al. (2000) from *Capsicum frutescens* and *C. chinense*. There was also an association with other previously reported isolates of PepGMV from different geographic regions (Brown et al., 2005).

Diseases caused by PepGMV were previously reported in Costa Rica in pepper (Lotrakul et al., 2000) and tomato (Nakhla et al., 2005). Additionally, this virus has been detected in *Cucurbita argyrosperma* in Nicaragua (Ala-Poikela et al., 2005) as well as in cucurbits in this study. However, this is the first report of a begomovirus (PepGMV) in *Sechium edule* (chayote) in the Western Hemisphere. Prior to the current study, *Chayote mosaic tymovirus* (ChMV; Bernal et al., 2000; Hord et al., 1997) was the only known virus infecting chayote in Costa Rica. Although yield losses associated with PepGMV in chayote in the country are not reported yet; a more detailed study of the incidence and distribution of the virus in chayote plantations is required to establish the potential threat of this begomovirus. This is specially important when Costa Rica is one of the main countries producing and exporting chayote to the United

States, Canada, Central America and the Caribbean (Cadena-Iñiguez et al., 2007).

Indigenous begomoviruses in other regions of the world apparently move between cucurbits and solanaceous crops. This is the case of *Tomato leaf curl virus*-New Delhi (ToLCV-NDe) in India (Mandal et al., 2004; Phaneendra et al., 2012; Sohrab et al., 2003; 2013), *Squash leaf curl Philippines virus* (SLCPHV) in Taiwan (Tsai et al., 2011) and *Tomato leaf curl Palampur virus* (ToLCPMV) reported in Iran (Heydarnejad et al., 2012). Probably PepGMV occurrence in chayote report in this study is also another example of this phenomenon. Additionally, the *Chayote yellow mosaic virus* (ChaYMV, GenBank AJ223191) detected in chayote plantations in the southeast of India and Nigeria, emerged as a potential new begomovirus specie.

The detection of PepGMV in bean (Hilje et al., 1993) and *Erythrina* spp. suggest the ability of this virus to infect also Fabaceae species. Although *Erythrina* spp. is not a crop for human consumption, it is used as a living fence (Muschler and Bonnemann, 1997) and in agroecosystems using a silvopastoral system (Morton, 1994). Close association of *Erythrina* spp. with whitefly infested vegetable crops may facilitate the transmission of the virus to this species when crops are eliminated. *Erythrina* trees may also serve as hosts for the virus in between cropping seasons and reservoirs for infection of new fields.

The results discussed herein may be an example of the adaptation of begomoviruses to new hosts and environments. Further studies are needed to confirm this hypothesis. The emergence of new viral strains and species due to the adaptation of existing viruses is a worldwide process that occurs constantly over time (Anderson et al., 2004; Fargette et al., 2006; Jones, 2009; Rojas and Gilbertson, 2008; Navas-Castillo et al., 2011). Therefore, it is recommended to perform periodical molecular characterization of begomoviruses, in order to determine the relationships between preexisting begomoviruses species and their hosts, and to identify arising new isolates/species. A clear understanding of the factors determining the evolution of these viruses is of importance in order to avoid future economic losses and to avoid their dispersal to other regions and crops.

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