Spanish Journal of Agricultural Research
16 (2), e1006, 7 pages (2018)
eISSN: 2171-9292
https://doi.org/10.5424/sjar/2018162-11735
Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, O.A, M.P. (INIA)

RESEARCH ARTICLE

OPEN ACCESS

Begomoviruses infecting common beans (*Phaseolus vulgaris* L.) in production areas in Cuba

Lidia Chang-Sidorchuk, Heidy González-Alvarez and Yamila Martínez-Zubiaur
Centro Nacional de Sanidad Agropecuaria (CENSA), San José de las Lajas, Mayabeque, Cuba.

Abstract

Viral diseases caused by begomoviruses are economically important for their depressing impact on common bean production in Cuba. Mayabeque is a Cuban province where this crop is significantly grown and affectations by *Bean golden yellow mosaic virus* (BGYMV) have been detected in the last 30 years. Integrated pest management (IPM) programs in this crop have allowed controlling the disease for a long time. However, in prospections of the last years, an increase of the incidence of various yellowing symptoms typical of begomoviruses has been observed in common bean production areas. DNA was extracted from leaf samples taken from symptomatic plants. Non-radioactive nucleic acid hybridization and a specific PCR assay were used to detect BGYMV, *Common bean severe mosaic virus*, *Common bean mottle virus*, and *Tobacco leaf curl Cuba virus*. Of the 218 bean plants sampled, 89.5 % was positive to BGYMV; the presence of the rest of the begomovirus species was between 3 and 4% (3.08% of CBMoV, 3.08% of TbLCCuV and 4.32% of CBSMV). The viral DNA from some samples was analyzed by rolling circle amplification (RCA), by restriction fragment length polymorphism analysis using restriction enzymes, and by cloning and sequencing of the viral components. The DNA sequences from BGYMV isolates showed 98% of identity with the isolates reported in Cuba in 2003. The infection by *Tobacco leaf curl Cuba virus* (TbLCCuV) was confirmed also in fields in the Cuban western region. This is the first work where the DNA-B of TbLCCuV is identified. These studies will help to strengthen phytosanitary surveillance and management programs implemented in the country to control the whitefly-begomovirus complex in this economically important crop.

Additional keywords: geminivirus; rolling circle amplification; whitefly; identification.

Abbreviations used: BGYMV (*Bean golden yellow mosaic virus*); CBMoV (*Common bean mottle virus*); CBSMV (*Common bean severe mosaic virus*); IPM (integrated pest management); ORFs (Open reading frames); RCA (rolling circle amplification); TbLCCuV (*Tobacco leaf curl Cuba virus*).

Authors' contributions: Study design, interpretation of data, critical revision of the manuscript for important intellectual content: LCS, HGA and YMZ. Performed the experiments: LCS and HGA. Wrote the paper: LCS and YMZ.

Citation: Chang-Sidorchuk, L.; González-Alvarez, H.; Martínez-Zubiaur, Y. (2018). Begomoviruses infecting common beans (*Phaseolus vulgaris* L.) in production areas in Cuba. Spanish Journal of Agricultural Research, Volume 16, Issue 2, e1006. https://doi.org/10.5424/sjar/2018162-11735

Received: 18 May 2017. **Accepted:** 25 Jun 2018.

Funding: The Ministry of Foreign Affairs of Spain; Spanish Agency of International Cooperation for Development (MAEC-AECID) financed the fellowship granted to LCS.

Competing interests: The authors have declared that no competing interests exist.

Correspondence should be addressed to Yamila Martínez-Zubiaur: yamila@censa.edu.cu

Introduction

The genus *Begomovirus* is the most important and largest in number of species of all genera of the family *Geminiviridae* (Brown *et al.*, 2015). The family *Geminiviridae* includes nine genera established on the basis of the phylogeny, organization of the genome, vector and host range (Varsani *et al.*, 2014, 2017; Zerbini *et al.*, 2017). Begomoviruses have a circular single-stranded DNA genome encapsidated in twinned icosahedral particles and are transmitted by the whitefly *Bemisia tabaci* (Navas-Castillo *et al.*, 2011). Their genomes can be either monopartite or bipartite. The two genomic components of bipartite begomoviruses are designated DNA-A and DNA-B. DNA-A has six open reading frames (ORFs), two in the virion sense (AV1 and AV2) and four in the complementary sense (AC1, AC2 and AC3 and AC4). DNA-B has two ORFs, the virion-sense BV1 and complementary-sense BC1. The AV2 ORF is found in Old World bipartite begomoviruses, but not in New World viruses (Rojas *et al.*, 2005). The two components of bipartite genomes share a common region (CR) of approximately 200 nucleotides that includes the origin of replication (Fondong, 2013). In recent years, begomoviruses have caused significant yield losses in many crops due to their high rates of

incidence and the disease severity caused in cultivated plants in the tropical and subtropical areas in the world. In the Americas, a series of whitefly-transmitted begomoviruses have been reported affecting bean crop. Most of them have been associated with leaf mosaic symptoms and important yield reductions (Morales, 2010).

At the global level, *Bean golden yellow mosaic virus* (BGYMV) is the infection agent of one of the most dangerous diseases affecting common beans in Central America and the Caribbean. It has also been found in the Dominican Republic, Guatemala, El Salvador, Honduras, Nicaragua, and in Florida, USA (Blair *et al.*, 1995; Mather *et al.*, 2003; Morales, 2005).

Common bean (*Phaseolus vulgaris* L.) has a great importance in Cuba because it constitutes one of the basic food for the population. Regarding this crop, diseases with symptoms of golden mosaic have been associated with begomoviruses since the 70s (Blanco *et al.*, 1984). The presence of golden mosaic disease became the main problem of common beans in the country (Blanco & Faure, 1994; Vázquez, 1999). The molecular characterization of the begomovirus responsible for the disease showed it was caused by BGYMV (Echemendía *et al.*, 2001).

The introduction of cultivars with a better behavior against this disease and the implementation of preventive and control measures into an integrated management program allowed reducing productive losses and improving the sustainability of the crop (Echemendía *et al.*, 2010).

Despite of the pest management measures used to decrese the infection rates of BGYMV, an increase of the incidence of various yellowing symptoms typical of the presence of begomoviruses has been observed in prospections carried out in the main bean producing areas in the last three years. Recently, three new species have been reported affecting common beans in different geographic regions in Cuba: *Common bean severe mosaic virus* (CBSMV), *Common bean mottle virus* (CBMoV) in the western region (Chang-Sidorchuk *et al.*, 2017) and *Tobacco leaf curl Cuba virus* (TbLCCuV) in the central region (Leyva *et al.*, 2016).

The objective of the present study was to know the distribution of bipartite begomoviruses species in the main beans production areas in the Cuban western region.

Material and methods

Bean leaves with foliar mottling and bright mosaic symptoms were randomly collected from 218 plants in commercial fields at different localities in south west of Mayabeque province, during the growing season 2013-2016. The localities were Güines, San José de las Lajas, Batabanó, Jaruco, Quivicán and Melena del Sur. Ten to twelve samples were collected in each locality per year. The total DNA was extracted from all samples by the method described by Permingeat *et al.* (1998).

Detection of specific begomoviruses previously identified in Cuban fields

Molecular diagnostic tools such as nucleic acid hybridization and PCR were used to determine the presence of specific begomoviruses detected in common beans in our country.

Specific detection of BGYMV

The samples were analyzed by nucleic acid hybridization with a specific probe to identify the presence of BGYMV and determine its distribution in the localities studied.

The probe was obtained from a BGYMV clone from the Cuban isolate (Echemendía *et al.*, 2001). The primers called MA2163 (5'-GTAAATATGCGAGTGTCTC-CG-3') and MA2162 (5'-CTGGACTTACACGTGGA-ATGG-3') were designed using the intergenic region (IR) of the viral sequence from the Cuban isolate (Acc. No. AJ544531) obtained from the Genbank. The labelling of the non-radioactive probe was carried out according to the instructions in the reagent kit of PCR DIG Probe Synthesis Kit (Roche). The PCR was performed using a program that consisted in denaturation for 2 min at 94°C, followed by 30 cycles of reaction (1 min at 94ºC, 1 min at 57ºC, 50 s at 72ºC) and 5 min at 72ºC. PCR amplified products were analyzed by electrophoresis on 0.8% agarose gel containing ethidium bromide in 1X TE (10 mM Tris-HCl, EDTA 1 mM, pH 8.0). The PCR fragments obtained were approx. 356 bp. DNA from plants infected with BGYMV was used as positive controls.

All DNA extracts (0.5-1 µg) of each sample were applied in a Hybond N+ membrane and set with UV light (120 mJ/cm^2) using a UV-crosslinker (Biorad). Pre-hybridization was conducted with a solution containing 5X SSC, 0.1% laurilsarcosine, 0.02% SDS and 2% blocking agent. Hybridization was conducted for 18 hours at 65ºC. There were three washings of 15 min each with the washing solution SSC and 0.1% SDS.

Specific detection of CBSMV, CBMoV and TbLCCuV

Species-specific set of primers were designed by using DNA-A sequences (Table 1) of the following

begomovirus: CBSMV (KX011476), CBMoV (KX011473) and TbLCCuV (AM050143). Primer sequences were chosen by multiple alignment analysis of the respective DNA-A sequences using MEGA 6.0 (Tamura *et al.*, 2013). The primer sequences were evaluated for their ability to form homo- and heterodimers as well as hairpins by using Oligo 7 (Rychlik, 2007).

For PCR amplification, mixtures (total volume 25 µl) containing GoTaq Green Master Mix (Promega, Madison, WI, USA) with a final concentration of 1.5 mM $MgCl_2$, 100 µM each dNTPs and forward and reverse primers (Table 1) at 10 pmol each were prepared. Full-length clones of three begomoviruses (Morán *et al*., 2006; Chang-Sidorchuk *et al.*, 2017) served as positive controls for evaluation of the PCR.

PCR was run in a thermocycler (Touch T960) using the following program for primers CBSFw1/CBSRev2 and CBMoFw1/CBMoRev2: an initial denaturation step at 94ºC for 5 min followed by 30 cycles at 94ºC for 30 s, 55ºC for 1 min, 72ºC for 30 s, and a final extension of 72ºC for 7 min. For primers TbLFw1/TbLRev2 the cycling conditions were: 1 cycle at 94ºC for 5 min; 35 cycles at 94ºC for 1 min, 53ºC for 1 min and 72ºC for 1min, followed by a final extension cycle at 72ºC for 10 min. Ten microliters of the amplified products were electrophoresed on a 1% agarose gel containing (10 mg/mL) ethidium bromide for 1 h and visualized under an UV transiluminator.

Genome amplification

The rolling-circle amplification (RCA) on the extracted DNA was carried out using φ29 DNA polymerase (TempliPhi kit, GE Healthcare). Three DNA positive for specific diagnostic (samples 18, 127, 148) were selected from localities with positive samples to new species (Güines, Jaruco, Quivicán) for comparing the molecular characteristic with BGYMV isolated in 2001 (Echemendía *et al.*, 2001). As TbLCCuV had only been reported in the bean crop in the central region of the country (Leyva *et al.*, 2016), a positive sample (sample 8), obtained for specific PCR, was analyzed to confirm its presence in the west of the country and to complete the characterization.

RCA products were digested with *Hpa*II, *Bam*HI, *Eco*RI, *Hind*III, *Nco*I, *Nhe*I, *Sac*I and *Pst*I. The restriction fragments of approximately 2.6 kbp, obtained with the single cutting enzymes *Pst*I, *Nco*I, *EcoR*I and *Hind*III were selected. These fragments were cloned using pGem-T-Easy and pBluescrip SK II (+) previously digested with the same restriction enzyme. The plasmids with the cloned viral fragments were purified according to Serghini *et al.* (1989) and the putative viral sequences present in the clones selected were completely sequenced (Macrogen Inc., Seoul, South Korea).

Sequence analysis

Sequences obtained were initially assembled with the program SeqMan (DNAStar, Inc., Madison, WI, USA) and analyzed using the Blastn algorithm for preliminary species assignment according to the recommendations of the *Geminiviridae* Study Group of the International Committee on Taxonomy of Viruses. Percentage identities with the most closely related begomoviruses were calculated with the program SDT (Species Demarcation Tool) version 1.0 (Muhire *et al.*, 2014). Genomic sequences were aligned with the MUSCLE module implemented in MEGA 6.0 (Tamura *et al.*, 2013). Phylogenetic trees were inferred by the neighbour-joining and maximum likelihood methods. A phylogeny test was conducted by the bootstrap method (1000 replicates).

Results and discussion

Of all the common bean plants collected in this study, 145 were positive to BGYMV when they were analyzed by hybridization. The fields from Batabanó and Quivicán localities showed the highest infection values, 100% and 91% respectively, whereas the occurrence in the fields of San José de las Lajas displayed the lowest percentage (8.8%). The negative signal in the samples analyzed suggests the presence of other plant pathogens, such as phytoplasmas, that cause symptoms of yellowing in common bean plants (Zamora *et al.*, 2012).

Table 1. List of primers used for amplification of begomoviruses in PCR.

Primer name	Sequence	Virus species	Product size (bp)	Tm (°C)
TbLFw1	5′CCTGCCTGTGTGCTATC 3′	TbLCCuV	1196	53
TbLRev2	5′GTGAGTGTACGGCACCAG 3′			
CBSFw1	5′GCT TAA CGA ACG TAA CTG G 3′	CBSMV	424	55
CBSRev2	5′CAC TGG CTC AAT GTA CGA GG 3′			
CBMoFw1	5′GTACACCAAATGAAAATGCG 3′	CBMoV	520	55
CBMoRev2	5′GGTACATCGGGAGTCCTG 3′			

The PCR showed that between 3% and 4% of the samples corresponded to new begomovirus species detected in Cuba (Leyva *et al.*, 2016; Chang-Sidorchuk *et al.*, 2017). Five samples were positive to CBMoV in Güines, seven samples to CBSMV in Güines and Jaruco, and five samples to TbLCCuV in Güines and Quivicán, making evident the low distribution of the new begomovirus species. Of all the plants sampled, 162 (74.3%) showed infection by some of the analyzed begomoviruses. BGYMV was the predominating virus, being present in 89.5% of the samples.

The restriction analysis with *Hpa*II enzyme of the RCA products of the four samples showed distinct restriction patterns. But, based on the sequencing, it was established that samples 18, 127 and 148 were infected with BGYMV despite the differences found in the restriction patterns. Differences in the restriction patterns of these samples could be due to nucleotide changes in the sequences of the viral components A and B of this species. The enzyme *Hpa*II recognizes a cut sequence of four nucleotides (GGCC) and mutating a nucleotide in any of the isolates affects the restriction patterns. However, as it was then demonstrated with the complete sequencing, these changes were not superior to the species demarcation criteria established for begomoviruses (Brown *et al.*, 2015).

The sequenced fragment of the sample 18 (1490 bp) (KY886918) included a partial fragment of the genes BV1 and BC1 belonging to the component DNA-B of BGYMV, with an identity of 99% with the Cuban isolate KU145406.

The complete nucleotide sequence of the viral DNA components of the samples 127 and 148 were obtained, which had a respective length of 2644 (KX185517) and 2645 (KX185518) nucleotides. Full-length DNA-A comparison using SDT program showed that both isolates had 98% similarity between them and with the BGYMV (AJ544531) previously described in Cuba (Echemendía *et al.*, 2001). Using both methods, similar results were obtained after the two different phylogenetic analyses. These isolates were grouped into the same clade with the rest of the BGYMV isolates described in Florida, Puerto Rico, Mexico, and Guatemala (Fig. 1).

The analysis with Blastn of the viral sequences obtained from the two clones derived from the sample 8 showed that these sequences corresponded to the DNA-A (2609 nt) and DNA-B (2567 nt) genomic components of a bipartite begomovirus. BLASTn search and analysis with the SDT program showed that DNA-A (KX011471) from the sample 8 exhibited its highest nucleotide sequence identity (97.8%) with the DNA-A of TbLCCuV (AM050143), a begomovirus described in Cuba infecting tobacco (Morán *et al.*, 2006) and recently found infecting common bean in Sancti Spiritus province (Leyva *et al.*, 2016).

BLASTn search and analysis with the SDT program showed that DNA-B (GQ355487) from the sample 8 exhibited its highest nucleotide sequence identity (82%) with the DNA-B of Wissadula golden mosaic virus (GQ355487), which corresponded to the phylogenetic relationships observed in Figure 1. Both components had a common viral region of 203 bp with a 98.5% identity and four copies of identical iterons (two inverted), indicating that both viral components found in the sample 8 were a cognate pair. This is the first work where the DNA-B of tobacco leaf curl Cuba virus (TbLCCuV) is identified; it had not been previously reported in this species.

The presence of TbLCCuV in common beans confirms the adaptive capacity of this species to infect more than one host. This may be an epidemiological threat to other crops of economic importance in the country and suggests the need for further epidemiological studies to ascertain the impact on the production of the crops.

These results show that the dramatic increase of incidence and severity of begomoviruses symptoms on common beans is mostly associated with the presence of BGYMV with high molecular stability respect to the isolate reported in 2001. But in addition, other factors influence, such as the begomovirus species TbLCCuV, CBSMV, CBMoV, and the presence of *B. tabaci* MEAM1 (Muniz *et al.*, 2011). The wide distribution of begomoviruses in Mayabeque province is largely due to the presence of *B. tabaci* (Middle East Asia Minor 1), which feeds on a wide range of plants, making it more likely to acquire and transmit a high number of begomoviruses to new potential hosts (Muniz *et al.*, 2011). These results emphasize the need to continue the viral diagnosis and surveillance in order to design proper management strategies.

The incidence of BGYMV decreased during the end of 90´s and first years of the 2000's due to the introduction of resistant cultivars accompanied by management and prevention programs (Echemendía *et al.*, 2007). However, symptoms similar to those caused by begomoviruses have recently been observed to increase in common beans production areas, possibly influenced by the limited access to new cultivars and increases in seed diversity introduced by farmers, given by the need to increase food, ignoring the plant breeding programs for resistance to begomoviruses (de la Fé-Montenegro, 2016).

This study indicates that the infection with begomoviruses is a problem in beans production and suggests the occurrence of multiplex begomovirus species, making possible viral recombination, which

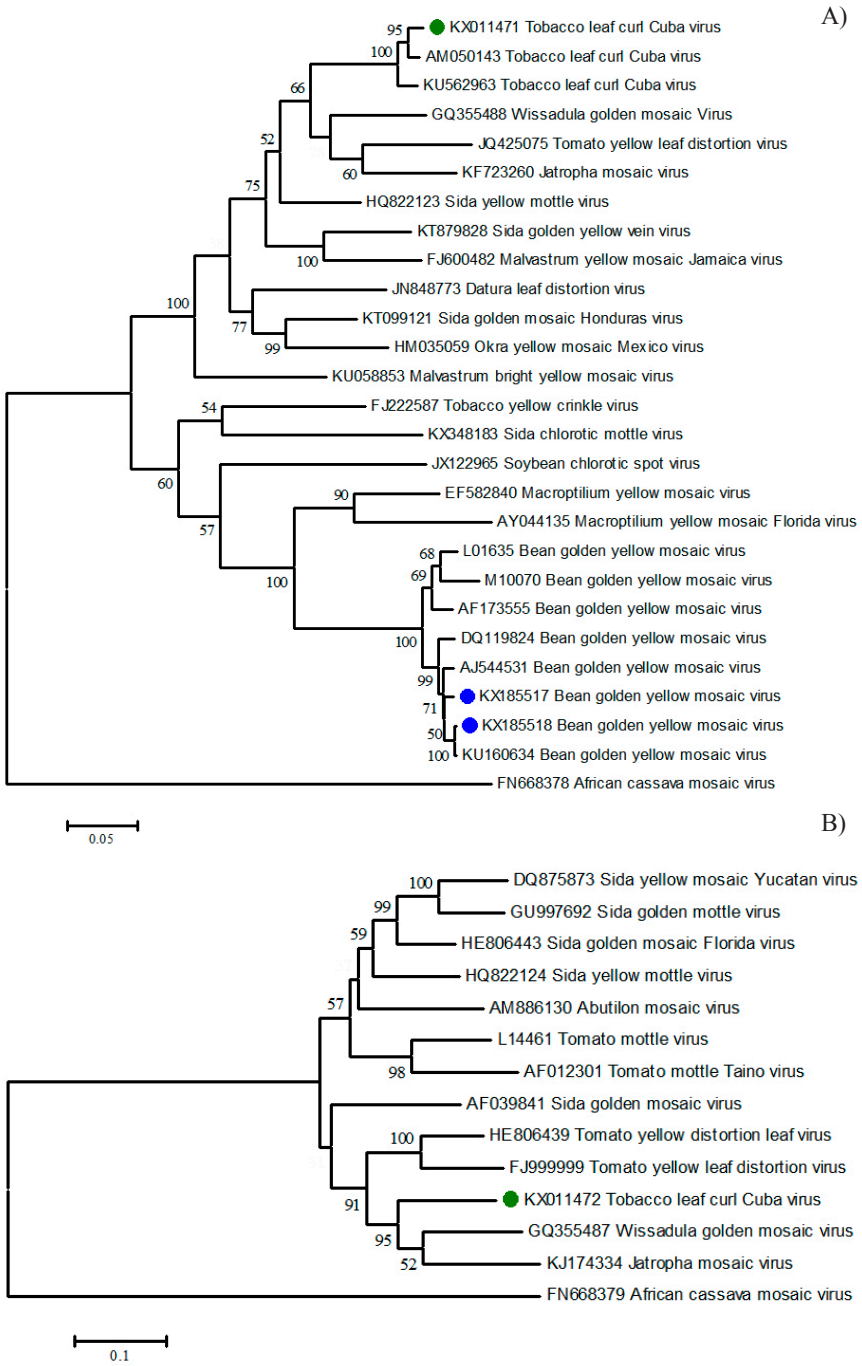

Figure 1. Phylogenetic trees based on an alignment of the complete nucleotide sequences of DNA-A of ToLCCuV and BGYMV (A) and DNA-B of ToLCCuV (B) isolate obtained in this work (highlight) with the genomes of a selected begomovirus obtained from the GenBank. The trees were generated using the maximum likelihood algorithm in MEGA 6. The numbers at nodes represent percentage bootstrap values (1000 replicates). ACMV was used as outgroup.

needs to be taken seriously in crop management strategies and guide the participatory breeding programs implemented in the country to return to the introduction of sources of resistance to begomoviruses and in particular to BGYMV.

Acknowledgements

Thanks are due to Dr. Eduardo Sistachs and MSc. Leyanis Aguilar for the critical review and translation into English of the manuscript.

References

Blair M, Bassett M, Abouzid A, Hiebert E, Polston J, McMillan Jr R, Graves W, Lamberts M, 1995. Occurrence of bean golden mosaic virus in Florida. Plant Dis 79 (5): 529-533. https://doi.org/10.1094/PD-79-0529

Blanco N, Lastres N, Bencomo I, 1984. Incidencia de las enfermedades virosas del frijol en Cuba. Ciencias de la Agricultura [Cuba] 19: 21-22.

Blanco N, Faure B, 1994. Situación actual del mosaico dorado del frijol en el Caribe, Cuba. In: El mosaico dorado del frijol, avances de investigación, pp: 82-89. CIAT, Cali, Colombia.

Brown JK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JC, Fiallo-Olivé E, Briddon RW, Hernández-Zepeda C, Idris A, 2015. Revision of *Begomovirus* taxonomy based on pairwise sequence comparisons. Arch Virol 160 (6): 1593-1619. https://doi.org/10.1007/s00705-015-2398-y

Chang-Sidorchuk L, González-Alvarez H, Navas-Castillo J, Fiallo-Olivé E, Martínez-Zubiaur Y, 2017. Complete genome sequences of two novel bipartite begomoviruses infecting common bean in Cuba. Arch Virol 162 (5): 1431-1433. https://doi.org/10.1007/s00705-016-3209-9

de la Fé Montenegro C F, Lamz Piedra A, Cárdenas Travieso R. M, Hernández Pérez J, 2016. Respuesta agronómica de cultivares de frijol común (*Phaseolus vulgaris* L.) de reciente introducción en Cuba. Cultivos Tropicales 37 (2): 102-107.

Echemendía A, Ramos P, Peral R, Fuentes A, González G, Sanpedro J, Morales F, 2001. Cuban isolate of Bean golden yellow mosaic virus is a member of the Mesoamerican BGYMV Group. Plant Dis 85 (9): 1030. https://doi.org/10.1094/PDIS.2001.85.9.1030C

Echemendía A, Ramos González P, Peral Pérez R, Porras González Á, González Arias G A, 2007. Selección de genotipos de frijol común (*Phaseolus vulgaris* L.) resistentes al virus del mosaico dorado amarillo del frijol (BGYMV) por hibridación de ácidos nucleicos. Fitosanidad 11 (4): 3-11.

Echemendía A, Ramos González PL, Villarreal N, Martínez AK, González Arias G, Morales FJ, 2010. Caracterización del virus del mosaico amarillo dorado del frijol en Cuba. Fitosanidad 14 (1): 11-17.

Fondong VN, 2013. Geminivirus protein structure and function. Mol Plant Pathol 14: 635-649. https://doi.org/10.1111/mpp.12032

Leyva RM, Quiñones M, Acosta KI, Piñol B, Xavier CD, Zerbini FM, 2016. First report of Tobacco leaf curl Cuba virus infecting common bean in Cuba. New Dis Rep 33: 17. https://doi.org/10.5197/j.2044-0588.2016.033.017

Mather D, Bernsten R, Rosas J, Ruano AV, Escoto D, 2003. The economic impact of bean disease resistance research in Honduras. Agr Econ 29 (3): 343-352. https://doi.org/10.1111/j.1574-0862.2003.tb00170.x

Morales F, 2005. Whiteflies as vectors of viruses in legume and vegetable mixed cropping systems in the tropical lowlands of Central America, Mexico and the Caribbean. In: Whitefly and whitefly-borne viruses in the tropics: building a knowledge base for global action; Anderson PK, Morales FJ (eds.), pp: 173-176. CIAT, Cali, Colombia.

Morales FJ, 2010. Distribution and dissemination of begomoviruses in Latin America and the Caribbean. In: *Bemisia* bionomics and management of global pest; Stansly PA & Naranjo SE (eds), pp: 283-318. Springer, Berlin.

Morán Y, Ramos P, Domínguez M, Fuentes A, Sánchez Y, Crespo J, 2006. Tobacco leaf curl Cuba virus, a new begomovirus infecting tobacco (*Nicotiana tabacum*) in Cuba. Plant Pathol 55 (4): 570. https://doi.org/10.1111/j.1365-3059.2006.01368.x

Muhire BM, Varsani A, Martin DP, 2014. SDT: a virus classification tool based on pairwise sequence alignment and identity calculation. PLoS One 9: e108277. https://doi.org/10.1371/journal.pone.0108277

Muniz Y, Granier M, Caruth C, Umaharan P, Marchal C, Pavis C, Wicker E, Martínez Y, Peterschmitt M, 2011. Extensive settlement of the invasive MEAM1 population of *Bemisia tabaci* (*Hemiptera: Aleyrodidae*) in the Caribbean and rare detection of indigenous populations. Environ Entomol 40 (5): 989-998. https://doi.org/10.1603/EN11129

Navas-Castillo J, Fiallo-Olivé E, Sánchez-Campos S, 2011. Emerging virus diseases transmitted by whiteflies. Annu Rev Phytopathol 49: 219-248. https://doi.org/10.1146/annurev-phyto-072910-095235

Permingeat HR, Romagnoli MV, Vallejos RH, 1998. A simple method for isolating high yield and quality DNA from cotton (*Gossypium hirsutum* L.) leaves. Plant Mol Biol Rep 16: 1-6. https://doi.org/10.1023/A:1007466522028

Rojas MR, Hagen C, Lucas WJ, Gilbertson RL, 2005. Exploiting chinks in the plant's armor: Evolution and emergence of geminiviruses. Annu Rev Phytopathol 43: 361-394. https://doi.org/10.1146/annurev.phyto.43.040204.135939

Rychlik W, 2007. OLIGO 7 Primer Analysis Software. In: PCR Primer Design. Methods in Molecular Biology; Yuryev A. (eds), vol 402. Humana Press. https://doi.org/10.1007/978-1-59745-528-2_2

Serghini M, Ritzenthaler C, Pinck L, 1989. A rapid and efficient 'miniprep' for isolation of plasmid DNA. Nucleic Acids Res 17 (9): 3604. https://doi.org/10.1093/nar/17.9.3604

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S, 2013. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30: 2725-2729. https://doi.org/10.1093/molbev/mst197

Varsani A, Navas-Castillo J, Moriones E, Hernández-Zepeda C, Idris A, Brown JK, Zerbini FM, Martin DP, 2014. Establishment of three new genera in the family *Geminiviridae*: *Becurtovirus, Eragrovirus* and *Turncurtovirus*. Arch Virol 159 (8): 2193-2203. https://doi.org/10.1007/s00705-014-2050-2

Varsani A, Roumagnac P, Fuchs M, Navas-Castillo J, Moriones E, Idris A, Briddon RW, Rivera-Bustamante R, Zerbini FM, Martin DP, 2017. *Capulavirus* and *Grablovirus*: two new genera in the family *Geminiviridae*. Arch Virol 162 (6): 1819-1831. https://doi.org/10.1007/s00705-017-3268-6

Vázquez L, 1999. Caracterización del polimorfismo de la mosca blanca *Bemisia argentifolii* Bellows and Perring (*Homoptera: Aleyrodidae*). Fitosanidad 3 (2): 21-23.

Zamora L, Acosta K, Martínez Y, 2012. First report of '*Candidatus* Phytoplasma asteris' (16SrI group) affecting common bean in Cuba. New Dis Rep 25: 4. https://doi.org/10.5197/j.2044-0588.2012.025.004

Zerbini FM, Briddon RW, Idris A, Martin DP, Moriones E, Navas-Castillo J, Rivera-Bustamante R, Roumagnac P, Varsani A, 2017. ICTV Virus Taxonomy Profile: *Geminiviridae*. J Gen Virol 98 (2): 131-133. https://doi.org/10.1099/jgv.0.000738