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EMERGENCE OF BEGOMOVIRUSES IN CUBA

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ABSTRACT: **Begomoviruses are plant pathogens that caused important losses to crops of economic interest in tropical and subtropical regions in the last decade. In Cuba, nine begomoviruses have been identified affecting tomato, beans, tobacco, pepper, squash, potato and weeds of different botanical families. The intergenic sequence (IR) and the N-terminal regions of the replication associated protein (*Rep*) of begomoviruses identified in Cuba were analysed using sequence data of gene collections (GeneBank). Statistical programs were used for the alignments, phylogenetic analysis and searching of significant recombination fragments. The detection of possible recombination events was carried out for p=0.05, based on 10000 permutations. Eleven IR recombination event sites between *Macroptilium* Yellow Mosaic Virus (MaYMV) and Tobacco Leaf Rugose Virus (TLRV), and 15 between Tomato Mottle Taino Virus (TMoTV) and *Dicliptera* Yellow Mosaic Virus (DiYMoV) were found. Sixteen recombination event sites between MaYMV and *Sida* Golden Yellow Vein Virus (SiGYVV) were also found in the *Rep* region. *Jatropha* Mosaic Virus (JMV) from *Jatropha gossipifolia* and Tomato Yellow Leaf Curl Virus TYLCV-IL(CU) from tomato and pepper showed base sequences in the alignments that were present in all the polymorphic sites detected and homologous to monomorphic sites. TMoTV and DiYMoV showed 3 and 4 polymorphic sites in the *Rep* N-terminal region, respectively. All these sites constitute evidences of old recombination events destroyed during the evolution, and they may define the characteristics of the begomovirus species identified.**

(Key words: begomoviruses; recombinations; genetic diversity; TYLCV; evolution)

EMERGENCIA DE BEGOMOVIRUS EN CUBA

RESUMEN: **Los begomovirus son patógenos de plantas que en las últimas décadas han causado importantes pérdidas a cultivos de interés económico, en regiones tropicales y subtropicales. En Cuba se han identificado nueve begomovirus afectando cultivos de importancia económica como tomate, fríjol, tabaco, pimiento, calabaza, papa, y malezas de diferentes familias botánicas. En este trabajo se analizaron las secuencias de la región intergénica (RI) y la región N- Terminal de la proteína asociada a la replicación *(Rep)* de los diferentes begomovirus identificados en Cuba, referidas en el banco de datos de genes (Genebank), utilizando programas estadísticos para los alineamientos, análisis filogenéticos y búsqueda de recombinaciones. La detección de posibles eventos de recombinaciones fue realizada para p=0.05, basado en 10000 permutaciones. En la región intergénica se detectaron entre 11 y 15 sitios de recombinaciones, entre el virus del mosaico amarillo del *Macroptilium* (MaYMV) y los virus de la hoja rugosa del tabaco (TLRV), el virus moteado taino del tomate (TMoTV) y el virus del mosaico amarillo de *Dicliptera* (DiYMoV). En la región N-Terminal de la *Rep*., se detectaron además, 16 sitios de recombinaciones entre MaYMV y el virus amarillo dorado de la *Sida* (SiGYVV). Los virus del mosaico de *Jatropha* (JMV) procedente de *Jatropha gossipifolia*, y los aislamientos de TYLCV-IL(CU) de tomate y pimiento, mostraron secuencias que se encuentran en todos los sitios polimórficos detectados y que son homólogas a los sitios monomórficos, de los alineamientos analizados. En la región N-Terminal de la *Rep* del TTMoV y DiYMoV, se detectaron 3 y 4 sitios polimórficos respectivamente, con características similares a los anteriores y constituyen evidencia de recombinaciones pasadas y destruida en la evolución. Todos los sitios identificados en estos aislamientos pueden definir características de las especies de begomovirus identificados.**

(Palabras clave: begomovirus; recombinaciones; diversidad genética; TYLCV; evolución)

INTRODUCTION

The Begomovirus genus, described within the family *Geminiviridae,* is an economically important plant virus genus, characterized by its twinned isometric virions and circular single stranded DNA genomes. Begomoviruses cause serious losses to many important food crops. They are transmitted by the whitefly *Bemisia tabaci* (Gennadius) to dicotyledonous plants.

The genetic diversity of these viruses has been associated with the occurrence of natural recombination events that play a main role in the emergency of new isolates in the genus. The replication origin (RO) has no encoding sequences with elements associated with the replication process and functional targets of *Rep*., as a conserved nonameric sequence 5´ TAATATTAC 3´ found in all the geminiviruses. This sequence forms a hairpin loop where the replication protein (*Rep*) recognizes specific nick sites to start replication, as well as repetitive and consecutive sequences located at variable distances from the hairpin loop called iterones. These latter constitute the biggest place for recognition of *Rep.* (1, 6).

A Cuban TYLCV-IL(CU) isolate, which shows a high homology percentage to Israel TYLCV isolate, has been identified affecting tomato, pepper, bean and squash crops (9, 11, 12, 13, 20). However, in tomato, as well as in beans, tobacco, pepper and weeds, other bipartite begomoviruses have been identified (4, 5, 7, 8, 13, 14) along with the detection of a mixed infection period between TYLCV-IL(CU) and Tomato Mottle Havana Virus (TMoHV) (8). The molecular analysis of the sequences of all bipartite begomoviruses isolated from different plant hosts in Cuba is herein presented to find out the possible correlation and recombination sites.

MATERIAL AND METHODS

Sequences were aligned using Clustal X programs (version 1.64b) (19). The sequences aligned were analyzed by using Easy Tree, version 1.31 program (3), to build the cladrogram, which was constructed using the neighbour-joining method (3). The alignments were analyzed using GENECONV, version 1.81 program to detect possible conversion gene places. Two sequences from TYLCV-IL(CU) isolates of pepper (17, 18), beans (9) and squash (10) were used.

The bipartite begomovirus sequences used are shown in Table 1. The IR and *Rep* sequences of *Jatropha* Mosaic Virus (JMV), isolated from *Jatropha gossipifolii* in Cuba, were also used, (*Genebank DQ20780***,** 13).

TABLE 1. Bipartite begomovirus sequences used for sequence analysis./ *Secuencias de los begomovirus bipartitos utilizados para los análisis*

Begomovirus	Acronym	Accession number	Ref.
Bean Golden Yellow Mosaic Virus	BGYMV	AJ544531	5
Tomato Mottle Taino Virus	ToMTV	NC001828	13
Tomato Mosaic Habana Virus	ToMHV	Y14874	8
Macroptilium Yellow Mosaic Virus	MaYMV	NC004731	5
Dicliptera Yellow Mottle Virus	DiYMV	AJ549960	5
Tobacco Leaf Rugose Virus	TLRV	TLE488768	4
Sida Golden Yellow Vein Virus	SiGYVV	AJ577395	5

RESULTS AND DISCUSSION

The alignment of the common region sequences allowed confirming the presence of nine begomoviruses infecting different crops and weeds in Cuba. All the TYLCV-IL(CU) isolates from different plant hosts could be observed in one cluster. The presence of bipartite viruses can be observed in different phylogenetic subgroups (Fig. 1), what confirms the differences among them and the presence of eight different tentative viral species according to taxonomy criteria for species demarcation proposed by Fauquet *et al.* (6). These results showed the possibility of the occurrence intra-specific variations. Similar results have been reported in some Latin America countries (2).

A detailed comparison of nucleotide CR sequences among bipartite begomoviruses evidenced the presence in Cuba of a putative *Jatropha* Mosaic Virus species (6) and the possible inclusion at this level of the TLRV isolate identified in tobacco plants (4).

Figure 2 shows variations in the *Rep* sequences of iterons- related-domain (IRD) in TLCV, JMV and SiGYVV according to the ADN/protein polarity model suggested by Argüello and Ruiz (1), and the presence of the iteron group GGGGW. Changes at the consensus motif 1 (AKNYFLTYPQC) in ToMHV and BGMYV isolates can be observed.

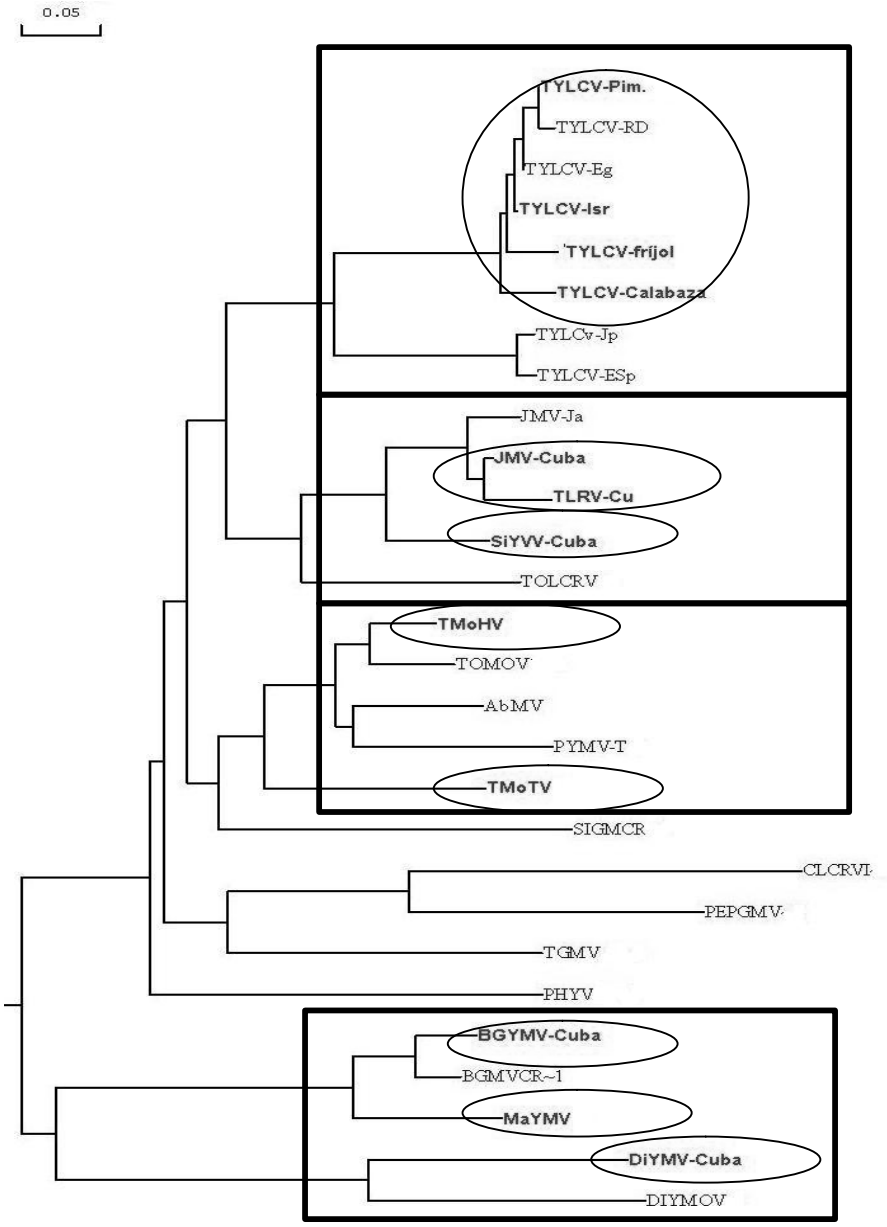

FIGURE 1. Phylogenetic grouping of begomoviruses identified in Cuba (boxes distinguish different subgroup, circles correspond to different viral species of Cuban begomoviruses proposed for Fauquet *et al*. (6)./ *Análisis filogenético de los begomovirus identificados en Cuba (los cuadros distinguen diferentes subgrupos, y los círculos corresponden a la propuesta de diferentes especies de begomovirus cubanos según Fauquet et al. (6).*

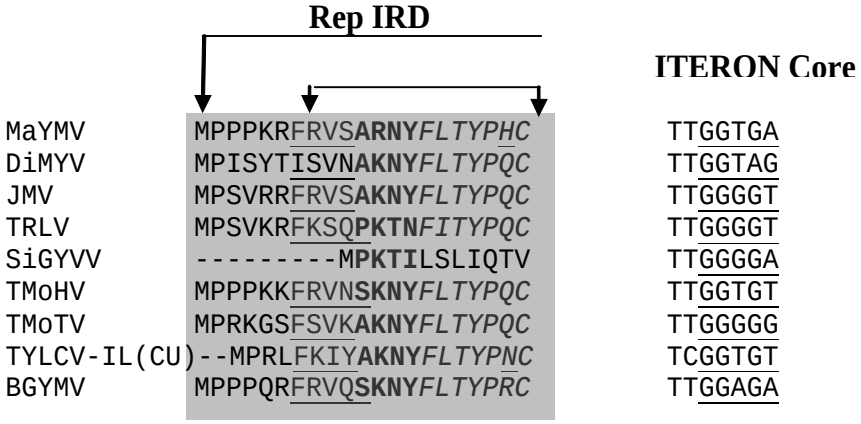

FIGURE 2. Variations in the ***Rep*** iteron-related-domain (***Rep***-IRD) in Cuban begomovirus isolates./ *Variaciones de los dominios relacionados a iterones en la **Rep** (**Rep**-IRD) en los aislamientos de begomovirus cubanos.*

TABLE 2. Analysis of statistically significant ($p<0.05$) recombination sites in bipartite begomoviruses present in Cuba. (**GO** *global outer* and **GI** *global inner).*/ *Análisis estadístico ($p<0.05$) de los sitios de recombinaciones ente los aislamientos bipartitos presentes en Cuba (**GO**, sitios de recombinaciones pasadas, **GI** sitios de recombinaciones internas)*

N-terminal	*Rep* Region		
Fragments detected	Sequence names	*P* value	Number of polymorphic sites detected
GO	DiYMV	0.0175	4
GO	TTMoV	0.0451	3
GI	MaYMV/SiGYVV	0.0180	16
Common	Region		
GI	DiYMoV/MaYMV	0.004	11
GI	MaYMV/TTMoV	00064	15
GI	MaYMV/TLCV	0.0255	11

Although DiYMV and SiGYVV possess similar iteron sequences that describe isolate relationships, they have changes in the *Rep*-IRD sequences. DiYMV keeps the aminoacid (aa) sequence of the motif 1, with total change or a substitution for FX1L*X3 domain. In the case of SiGYVV, it has a complete deletion of the *Rep*-IRD sequence including motif 1. These changes may be associated with the host range and could indicate either a possible late isolate recombinant or that the isolates do not have a wide natural replication capacity.

The detection of possible recombination events for p=0.05 can be seen in Table 2, based on 10000 permutations. For TTMoV and DiYMoV, 3 and 4 global outer statistically significant fragments for ***p*** values of 0.0175 and 0.0451 in N-terminal *Rep* region were detected.

These are evidences of past conversion events that have been destroyed by later mutations or gene conversions. Global inner (GI) statistically significant fragments between MaYMV and SiGYVV for *p=0.018* at 16 polymorphic sites in the alignment were also detected in this region, and two sequences differed at 65 sites for all of them.

In the CR region, range 11 and 15 GI statistically significant fragments were detected among MaYMV, TLRV, TMoTV and DiYMoV for ***p*** values between 0.0040-0.0255. In all the sequences, 25 to 32 different sites were identified. In all the cases, the lengths of the recombinant fragments varied from 5 to 35 nucleotides for the N-terminal *Rep* region and 93-98 for CR.

The probability of recombination between JMV, TMoHV and TYLCV isolates coincides with previous reports for CR region between begomoviruses from the New and Old World, from comparisons of complete genome sequences of different genus of *Geminiviridae* family (6). Particularly, the gene conversion sites between TYLCV-IL(CU) and TMoHV, constitute an evidence of possible effects of mixed infection (by these viruses) being detected in the Cuban tomato production since 1998 (11, 12).

Phylogenetic analyses, detection of gene conversion sites and changes in *Rep*-IRD are evidences of a wide begomovirus diversity in our country, which can rebound in the appearance of new more stable species capable of infecting economic important crops just as it has occurred in tobacco, tomato, potato, beans and pepper. However, other less viable isolates with sequence mutations that interfere the viral replication are likely to be identified as it was observed for SiGYVV.

Recombination is not a rare phenomenon among begomoviruses, where an additional variation factor does not produce prospective effects on virus pathogenesis, contributing to their evolution (15, 16, 19, 21).On the other hand, knowledge of TYLCV sequence variants and of new begomoviruses present in certain geographical regions is essential for the establishment of efficient control programs; regarding strategies of plant breeding they have been considered as the best approach for the control of begomoviruses.

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