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)

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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, frijol, 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 TMoTV 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 emergence 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 nonamer 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 (ToMHV) (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 cladogram, 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 gossypifolii in Cuba, were also used, (Genbank DQ20780, 13).

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 BGYVV isolates can be observed.

| Table 1. Bipartite begomovirus sequences used for sequence analysis |  |
|-----------------------|------------------------|------------------|--------|
| Begomovirus | Acronym | Accession number | Ref. |
| Bean Golden Yellow Mosaic Virus | BGYMV | AJ544531 | 5 |
| Tomato Mottle Taino Virus | ToMTV | NC001828 | 13 |
| Tomato Mosaic Havana 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 |
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)).

Rep IRD

MaYMV MPPKRFRVSAONYFLYPHC TTGGTGA
DiMYV MPISYITISVANONYFLYPQC TTGGTAG
JMV MPSRVRFRVSAKNYFLYPQC TTGGGCT
TRLV MPSVRFKSKQPKTNFLYPQC TTGGGCT
SiGYVV ---------------MPKTIALSIQTV TTGGGA
TMoHV MPPPKKEVNSKNYFLYPQC TTGGTGT
TMoTV MPTRKGSFSVAKNYFLYPQC TTGGGGA
TYLCV-IL (CU) --MPRLKTYAKNYFLYPNC TCGGTGT
BGYMV MPPQRFVQSNKYFLYPFRC TTGGAGA

ITERON Core
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 DiYMV, 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 DiYMV 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 genius 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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### REFERENCES


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**TABLE 2.** Analysis of statistically significant ($p<0.05$) recombination sites in bipartite begomoviruses present in Cuba. (GO global outer and GI global inner).

<table>
<thead>
<tr>
<th>Fragments detected</th>
<th>Sequence names</th>
<th>$P$ value</th>
<th>Number of polymorphic sites detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>GO</td>
<td>DiYMV</td>
<td>0.0175</td>
<td>4</td>
</tr>
<tr>
<td>GO</td>
<td>TTMoV</td>
<td>0.0451</td>
<td>3</td>
</tr>
<tr>
<td>GI</td>
<td>MaYMV/SiGYVV</td>
<td>0.0180</td>
<td>16</td>
</tr>
<tr>
<td>Common Region</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>DiYMV/MaYMV</td>
<td>0.004</td>
<td>11</td>
</tr>
<tr>
<td>GI</td>
<td>MaYMV/TTMoV</td>
<td>0.0064</td>
<td>15</td>
</tr>
<tr>
<td>GI</td>
<td>MaYMV/TLCV</td>
<td>0.0255</td>
<td>11</td>
</tr>
</tbody>
</table>


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