Nucleotide sequence evidence for the occurrence of three distinct whitefly-transmitted, Sida-infecting bipartite geminiviruses in Central America

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The nucleotide sequences of two Sida-infecting geminiviruses from Honduras were determined. The symptoms of both viruses are identical in Sida rhombifolia but different in Nicotiana benthamiana. An additional symptom of one virus was yellow vein clearing on infected N. benthamiana leaves. Both Sida golden mosaic viruses (SiGMV-Ho and SiGMV-Hoyv) have bipartite genomes (DNAs A and B). From the SiGMV-Hoyv-infected S. rhombifolia plant two different DNA B molecules were isolated and cloned. They differ in length by 24 nucleotides [SiGMV-Hoyv B1 (2593 nt) and B2 (2569 nt)] and at eight nucleotide positions. Both proteins encoded by DNA B (BV1 and BC1) are affected by these substitutions. Computer analysis shows that the bipartite genomes resemble those of other whitefly-transmitted geminiviruses. From homology analyses we conclude that both viruses are closely related but distinct. Comparison with a Sida-infecting virus from Costa Rica (SiGMV-Co) showed that the two viruses from Honduras are more similar to each other than either of them are to SiGMV-Co. Exchange of SiGMV-Ho DNA A with SiGMV-Co DNA B while the reciprocal exchange was not infectious in N. benthamiana. SiGMV-Ho DNA A and SiGMV-Co DNA B produced a viable pseudorecombinant virus whereas only pseudorecombination of SiGMV-Co DNA A with SiGMV-Hoyv DNA B2, and not with DNA B1, was infectious in N. benthamiana.

Introduction

Geminiviruses are small plant viruses with circular single-stranded DNA genomes encapsidated in twinned (geminate) particles (Harrison, 1985). Members of this virus family have been divided into three subgroups (Murphy et al., 1995). The majority of group members that infect dicotyledonous plants are whitefly-transmitted and have bipartite genomes (DNAs A and B). DNA A encodes the coat protein (AV1) as well as proteins required for replication (AC1) and gene regulation (AC2 and AC3) (Lazarowitz, 1992; Timmermans et al., 1994). DNA B is essential for disease production but plays no role in DNA replication. The two gene products (BV1 and BC1) encoded by this component are involved in virus spread throughout the plant, development of symptoms and determining the host range of the virus (von Arnim & Stanley, 1992; Ingham & Lazarowitz, 1993; Noueiry et al., 1994; Ingham et al., 1995; Schaffer et al., 1995).

Geminivirus-associated epidemics are currently threatening tomato, bean and pepper production in Central America (e.g. Mexico, Puerto Rico and Costa Rica) and the southern United States (Simone et al., 1990; Hidayat et al., 1993; Torres-Pacheco et al., 1993; Brown et al., 1995). Ornamental weed plants such as Sida rhombifolia have been suggested as natural host reservoirs for geminiviruses from which crop plants might be infected (Gilbertson et al., 1993; Wu et al., 1996; Höfer et al., 1997). Since S. rhombifolia grows all over Latin America and the southern United States we cloned and sequenced genomic components of two bipartite geminiviruses from infected S. rhombifolia, recently collected in Honduras, to determine their relationship to other bipartite geminiviruses from Central America.

Methods

Virus sources and isolation of double-stranded DNA forms. Two SiGMV-Ho-infected S. rhombifolia plants (kindly provided by Peter
Fig. 1. Maps of the SiGMV-Ho and SiGMV-Hoyv genomic A and B components. The solid arrows define the position of the open reading frames (C for complementary and V for virus sense). The CR between DNA A and B is indicated by a shaded box. The positions of selected restriction endonucleases are indicated. The nucleotide differences between SiGMV-Hoyv DNA B₁ and B₂ are indicated and the position numbers are given in parentheses. The two additional restriction sites, \( Sal_{I} \) and \( Eco_{RV} \), in SiGMV-Hoyv DNA B₁ are indicated (underlined). The calculated molecular masses (MW) of ORFs of DNA A and B are shown below the maps.

<table>
<thead>
<tr>
<th>ORF</th>
<th>MW (kD)</th>
<th>ORF</th>
<th>MW (kD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC1</td>
<td>41.1</td>
<td>BC1</td>
<td>33.2</td>
</tr>
<tr>
<td>AC2</td>
<td>14.7</td>
<td>BV1</td>
<td>29.4</td>
</tr>
<tr>
<td>AC3</td>
<td>15.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC4</td>
<td>9.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AV1</td>
<td>29.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Markham, John Innes Institute, Norwich, UK) were collected in Honduras (Zamorans Department of Francisco Morazan). Viral double-stranded DNA was isolated either directly from infected \( S. \ rhombifolia \) or from sap-infected \( N. \ benthamiana \) as described previously (Frischmuth & Stanley, 1991).

Cloning and sequencing of SiGMV-Ho genomic components and inoculation of plants. Recombinant DNA techniques were performed as described by Sambrook et al. (1989). Restriction endonucleases and DNA-modifying enzymes were used as recommended by the manufacturers. The sequences of DNAs A and B were determined using either the dideoxynucleotide chain termination method (Pharmacia) with \( \alpha^{25} \)SdATP or by automatic sequence analysis with the Li-Cor system according to the manufacturer’s instructions. The sequence of one infectious clone of each genomic component of the two SiGMV-Ho strains was determined in both orientations from subclones and with manufactured primers (MWG-Biotech).
For production of full-length SiGMV-Ho clones, viral double-stranded DNA was digested with SstI or PstI. Linearized DNA forms were recovered from agarose gels with the Bio-Rad gene clean kit and cloned into the PstI or SstI sites of pBluescript KS (+) (Stratagene). Positive clones were analysed by digestion with SstI to identify genomic components (Fig. 1).

Inoculation of plants and characterization of viral DNA forms. For infectivity tests, 1 µg viral inserts were liberated by digestion with PstI (SiGMV-Ho DNA B and SiGMV-Ho yv DNA A) or SstI (SiGMV-Ho DNA A and SiGMV-Ho yv DNA B), mixed and mechanically inoculated onto N. benthamiana plants as described in Stanley et al. (1990). SiGMV-Ho strains were sap-transmitted from plant to plant as described in Stanley et al. (1990).

The construction of partial repeats of SiGMV-Ho DNA B, SiGMV-Ho yv DNA A and SiGMV-Ho yv DNA B, for agroinoculation will be described elsewhere. The cloning of SiGMV-Co genomic components as well as the constructs used for agroinoculation has been described in Höfer et al. (1997).

For mechanical inoculation of N. benthamiana with SiGMV-Co, the DNA A insert was liberated by PstI and DNA B by EcoRV digestion. N. benthamiana was agroinoculated by stem injection as described by Stanley et al. (1990).

Symptomatic and asymptomatic plants were analysed for the presence of viral genomic components essentially as described in Höfer et al. (1997). Southern blots were hybridized with SiGMV-Co DNA A or DNA B digoxigenin-labelled fragments. These labelled probes cross-hybridize very well with all three Sida-infecting viruses because of their close relationship.

Results

Sap-transmission of SiGMV-Ho and SiGMV-Ho yv from S. rhombifolia to N. benthamiana

Leaves from the two S. rhombifolia plants were used for sap-transmission of SiGMV-Ho to N. benthamiana. The symptoms of the two infected S. rhombifolia were identical, i.e. stunting of the plant, leaf curling and a yellow green mosaic on infected leaves. However, in N. benthamiana the symptoms were different. As well as causing the common symptoms (stunting of the whole plant and leaf curling), one virus caused a fine yellow vein clearing on infected leaves. For distinction, we named this virus SiGMV-Ho yellow vein (SiGMV-Ho yv).

Organization of SiGMV-Ho and SiGMV-Ho yv genomes

Several PstI and SstI clones of SiGMV-Ho were analysed by SstI digestion. From this analysis, the distinct nature of the PstI and SstI clones was determined. One of the PstI and one of the SstI clones were arbitrarily chosen for further analysis. A mixture of both cloned genomic components was mechanically inoculated onto N. benthamiana and infected plants showed the same symptoms as SiGMV-Ho sap-inoculated N. benthamiana (Table 1).

While all PstI clones of SiGMV-Ho yv were uniform after digestion with SstI, two sets of different molecules were identified after SstI digestion of several SstI clones of SiGMV-Ho yv. Of 14 clones obtained, 12 clones contained two SstI sites and two clones contained three SstI sites (compare Fig. 1). One of each was chosen for further analysis. A mixture of one PstI clone with either of the two SstI clones was inoculated onto N. benthamiana (Table 1). The symptoms were, in both cases, the same as those in SiGMV-Ho yv sap-inoculated N. benthamiana.

The nucleotide sequences of all infectious cloned genomic components were determined. Comparison with known sequences of bipartite geminiviruses identified the SstI clone of SiGMV-Ho as DNA A (the nucleotide sequence is available in the EMBL database under accession no. Y11099) and the PstI clone as DNA B (accession no. Y11098). The PstI clone of SiGMV-Ho yv was identified as DNA A (accession no. Y11099) and the two SstI clones as DNA B (DNA B1 (accession no. Y11100) and B2 (accession no. Y11101)). The sequences were numbered beginning at the first nucleotide of the common region (CR) between DNA A and B. The genomic structure of SiGMV-Ho and SiGMV-Ho yv, deduced from the nucleotide sequences, resembles that of other bipartite geminiviruses (Fig. 1). DNA A codes for five genes and DNA B for two genes (Fig. 1). The sequence of the CR between DNA A and B is almost identical over approximately 150 nucleotides. The CR contains a sequence which is capable of producing a hairpin loop structure (data not shown). This structure is identical to that found in other geminiviruses (Lazarowitz, 1992).

The two SiGMV-Ho yv DNA B molecules differ in eight nucleotides and DNA B2 is 24 nucleotides longer than B1 (Fig. 1). Both proteins encoded by this component are affected by these differences. At position 1488, B2 contains a thymine

Table 1. Pseudorecombination between cloned genomic components of SiGMV-Ho, SiGMV-Ho yv and SiGMV-Co in N. benthamiana

<table>
<thead>
<tr>
<th>Virus</th>
<th>DNA A</th>
<th>DNA B</th>
<th>Infectivity (infected/inoculated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiGMV-Ho</td>
<td>SiGMV-Ho</td>
<td></td>
<td>15/20*</td>
</tr>
<tr>
<td>SiGMV-Ho yv B1</td>
<td>SiGMV-Ho</td>
<td></td>
<td>2/9, 8/9†</td>
</tr>
<tr>
<td>SiGMV-Ho yv B2</td>
<td>SiGMV-Ho</td>
<td></td>
<td>3/9, 4/5</td>
</tr>
<tr>
<td>SiGMV-Ho yv</td>
<td>SiGMV-Co</td>
<td></td>
<td>2/9, 3/9</td>
</tr>
<tr>
<td>SiGMV-Ho yv</td>
<td>SiGMV-Ho yv B1</td>
<td></td>
<td>8/9</td>
</tr>
<tr>
<td>SiGMV-Ho yv</td>
<td>SiGMV-Ho yv B2</td>
<td></td>
<td>8/9</td>
</tr>
<tr>
<td>SiGMV-Ho yv</td>
<td>SiGMV-Ho yv</td>
<td></td>
<td>6/9, 10/10‡</td>
</tr>
<tr>
<td>SiGMV-Ho yv</td>
<td>SiGMV-Co</td>
<td></td>
<td>4/8, 10/10‡</td>
</tr>
<tr>
<td>SiGMV-Co yv</td>
<td>SiGMV-Co</td>
<td></td>
<td>9/9</td>
</tr>
<tr>
<td>SiGMV-Co yv</td>
<td>SiGMV-Ho yv B1</td>
<td></td>
<td>0/9, 0/20, 0/40‡</td>
</tr>
<tr>
<td>SiGMV-Co yv</td>
<td>SiGMV-Ho yv B2</td>
<td></td>
<td>2/9, 3/12</td>
</tr>
<tr>
<td>SiGMV-Co yv</td>
<td>SiGMV-Ho yv</td>
<td></td>
<td>0/9/0, 0/20, 0/20‡</td>
</tr>
</tbody>
</table>

* One representative inoculation experiment.
† Infectivity numbers of arbitrarily selected individual inoculation experiments are separated by commas.
‡ Plants were agroinoculated.
Fig. 2. Comparison of amino acid sequences of DNA B gene products BC1 (A) and BV1 (B) of SiGMV-Ho, SiGMV-Hoyv and SiGMV-Co. Identical amino acids are indicated by a dot and variation by the one letter code of amino acids.

instead of a cytosine resulting in an arginine instead of a glycine in BC1 (Fig. 2A, amino acid position 239). The substitution of thymine by adenine at position 526 in B results in new start codon for the gene encoding BV1. If this start codon is used for translation two amino acids, methionine and isoleucine, would be added to the amino terminus of BV1 (Fig. 2B). All other substitutions and the 24 nucleotide insertion do not affect the composition of BC1 and BV1.

Comparisons between SiGMV-Ho, SiGMV-Hoyv, SiGMV-Co and other bipartite geminiviruses

The similarities between whole DNA A and B sequences of SiGMV-Ho and SiGMV-Hoyv are approximately 90% (DNA A, 90%; DNA B1, 90%; and DNA B2, 89%). Focusing on single ORFs, the amino acid sequence homology ranges from 84-2% for AC1 to 98-6% for BC1 (Table 2). The difference in the amino acid sequence of AC1 is mainly due to one region, amino acid positions 141 to 171 (Fig. 3). The sequence diversity in this region is also responsible for the divergence between AC4 sequences. Apart from these two sequences, the homology is above 90% in all other proteins (Table 2). Noticeable is the high homology observed between BC1 and the considerably lower homology observed between BV1 sequences (Table 2). The amino-terminal amino acid sequence in the BV1 coding region is responsible for this diversity (Fig. 2B). The first 36 amino acids of the BV1 gene of SiGMV-Ho and SiGMV-Hoy show in 11 amino acids while only 10 amino acids differ in the other 222 amino acids (Fig. 2B).

The similarity between the two Sida-infecting viruses from Honduras and SiGMV-Co from Costa Rica is overall lower than that between the Honduras viruses (SiGMV-Co DNA A, 86% similarity to SiGMV-Ho and 83% to SiGMV-Hoyv; SiGMV-Co DNA B, 81% to SiGMV-Ho, 81% to SiGMV-Hoyv B1 and 80% to SiGMV-Hoyv B2). The similarity between amino acid sequences of the DNA A genes is similar to that between SiGMV-Ho and Abutilon mosaic virus (AbMV; Frischmuth et al., 1990), bean dwarf mosaic virus (BDMV; Hidayat et al., 1993) and tomato mottle virus (ToMoV; Abouzid et al., 1992) (Table 2). Slightly higher similarity is observed between the amino acid sequences of the two DNA B proteins in SiGMV-Ho and SiGMV-Hoyv and SiGMV-Co than to other bipartite geminiviruses (Table 2). Noticeable is the difference in the homology of the two DNA B gene products (Table 2). Again, the amino-terminal amino acid sequence in BV1 is responsible for this difference (Fig. 2B). The first 36 amino acids of BV1 of SiGMV-Ho and SiGMV-Co differ in 16 amino acids while only 17 amino acids are different in the 222 residual amino acids (Fig. 2B). A similar distribution of amino acid divergence is observed between SiGMV-Hoyv and SiGMV-Co (Fig. 2B).

The amino acid sequence of the coat protein in SiGMV-Ho and SiGMV-Hoyv are highly homologous and differ in only six positions (data not shown). Comparing the sequence of the coat protein with those of SiGMV-Co and other geminiviruses, tomato leaf crumple virus (TLCrV; Paplomatas et al., 1994) shows an even stronger similarity to SiGMV-Ho than SiGMV-Ho does to SiGMV-Co (Table 2). The main amino acid sequence variations between the two Honduras viruses and SiGMV-Co are located within the amino terminus of the coat protein (data not shown). This part of the coat protein has been described as the variable domain among whitefly-transmitted geminiviruses (Wu et al., 1996).

The amino acid sequence between positions 141 and 171 of AC1 of SiGMV-Ho and SiGMV-Hoyv resembles that of SiGMV-Ho and not that of SiGMV-Hoyv, as shown in the alignment in Fig. 3. The nucleotide sequences of the CRs of SiGMV-Ho and SiGMV-Hoyv show a much higher similarity (88%) than usually
Table 2. Comparison of amino acid sequences of putative genes and the DNA A CR sequence of SiGMV-Ho with those of SiGMV-Ho\textsubscript{yv}, SiGMV-Co and seven other bipartite geminiviruses

Comparisons of the CR sequences were performed with the computer program WDNASIS and of amino acid sequences with PROSIS.

<table>
<thead>
<tr>
<th>Percentage identities to SiGMV-Ho</th>
</tr>
</thead>
<tbody>
<tr>
<td>SiGMV-Ho\textsubscript{yv}</td>
</tr>
<tr>
<td>SiGMV-Co</td>
</tr>
<tr>
<td>AbMV</td>
</tr>
<tr>
<td>BDMV</td>
</tr>
<tr>
<td>ToMoV</td>
</tr>
<tr>
<td>TLCrV\textsubscript{yv}</td>
</tr>
<tr>
<td>PHV</td>
</tr>
<tr>
<td>TGMV</td>
</tr>
<tr>
<td>ACMV</td>
</tr>
</tbody>
</table>

* Sequence sources for comparisons were for SiGMV-Co (Höfer et al., 1997); BDMV (Hidayat et al., 1993), AbMV [Frischmuth et al. (1990), corrected according to S. Frischmuth & S. Lange, personal communication; ToMoV (Abozid et al., 1992), TGMV [Hamilton et al. (1994), corrected according to von Arnim & Stanley (1992)], PHV (Torres-Pacheco et al., 1993) and ACMV (Stanley & Gay, 1983).

† a. Percentage identity within the aligned sequence window; b. amino acid sequence of BC1 of SiGMV-Ho\textsubscript{yv} DNA B\textsubscript{yv} c. amino acid sequence of BC1 of SiGMV-Ho\textsubscript{yv} DNA B\textsubscript{yv} d. amino acid sequence of BV1 of SiGMV-Ho\textsubscript{yv} DNA B\textsubscript{yv} e. amino acid sequence of BV1 of SiGMV-Ho\textsubscript{yv} DNA B\textsubscript{yv} f. Numbers in parentheses are the comparison between SiGMV-Ho\textsubscript{yv} and SiGMV-Co. a. amino acid sequence of BC1 of SiGMV-Ho\textsubscript{yv} DNA B\textsubscript{yv} b. amino acid sequence of BC1 of SiGMV-Ho\textsubscript{yv} DNA B\textsubscript{yv} c. amino acid sequence of BV1 of SiGMV-Ho\textsubscript{yv} DNA B\textsubscript{yv} d. amino acid sequence of BV1 of SiGMV-Ho\textsubscript{yv} DNA B\textsubscript{yv} f. Sequence source for the AV1 and BV1 genes of tomato leaf crumple virus (TLCrV) from Paplomatas et al. (1994). a. amino acid sequence of BV1 of clone pBMX9B (Paplomatas et al., 1994); b. amino acid sequence of BV1 of clone pTMMX4B (Paplomatas et al., 1994).

Three distinct Sida-infecting geminiviruses

observed between distinct geminiviruses (Table 2). The similarity between the two Honduras viruses and SiGMV-Co is comparable with that between SiGMV-Ho and AbMV, BDMV, ToMoV, TLCrV and pepper huasteco virus (PHV; Torres-Pacheco et al., 1993) (Table 2). In an alignment analysis between the CR sequences of SiGMV-Ho, SiGMV-Ho\textsubscript{yv} and SiGMV-Co, a sequence gap is observed in the region between nucleotide positions 26 and 43 of SiGMV-Co while the rest of the CR shows few differences (Fig. 4). The similarity in the nucleotide sequence of the non-coding region between the CR and the start codon of the gene encoding BC1 is 90% between SiGMV-Ho and SiGMV-Ho\textsubscript{yv} and approximately 60% between the Honduras viruses and SiGMV-Co (60.4% for SiGMV-Ho and 59.7% for SiGMV-Ho\textsubscript{yv}).

Pseudorecombination between SiGMV-Ho, SiGMV-Ho\textsubscript{yv} and SiGMV-Co

Pseudorecombination by exchange of cloned genomic components was observed between SiGMV-Ho and SiGMV-
Ho\text{yv} (Table 1). Also, viable pseudorecombination was observed following exchange of cloned genomic components of SiGMV-Ho DNA A and SiGMV-Co DNA B. However, the reciprocal exchange between SiGMV-Co DNA A and SiGMV-Ho DNA B did not result in viable pseudorecombinant viruses even after utilizing the agroinoculation technique (Table 1). While SiGMV-Ho\text{yv} DNA A and SiGMV-Co DNA B produced an infectious pseudorecombinant virus, only the pseudorecombinant virus produced by SiGMV-Co DNA A and SiGMV-Ho\text{yv} DNA B\text{yv} was infectious in \textit{N. benthamiana} while SiGMV-Co DNA A was not able to form a viable pseudorecombinant with SiGMV-Ho\text{yv} DNA B\text{yv} (Table 1).

**Discussion**

The sequences of two \textit{Sida}-infecting geminiviruses from Honduras (Zamorans Department of Francisco Morazan region) were determined. From sequence analyses we conclude that they are closely related, but distinct, viruses. Sequence comparisons to a \textit{Sida}-infecting geminivirus from Costa Rica showed less homology than observed between the Honduran viruses. If all sequence comparison data are taken together, both Honduras viruses are closely related to bipartite geminiviruses from the New World and less related to African cassava mosaic virus (ACMV; Stanley & Gay, 1983) from Africa (Table 2).

From the sequence data on cassava-infecting geminiviruses, Hong \textit{et al.} (1993) pointed out the problem of considering two viruses, isolated incidentally from the same plant species, as strains of the same virus or distinct viruses. One distinguishing factor could be the host range of such viruses. SiGMV-Co (Höfer \textit{et al.}, 1997) and SiGMV-Ho are able to infect tomato whereas SiGMV-Ho\text{yv} is unable to infect this plant species (unpublished observations). It is difficult to assess the host range of viruses and cloned components because of problems with sap-transmissibility, agroinoculation or whitely-transmission to various plant species. The difference in the host range of two strains of the bipartite geminivirus squash leaf curl virus (Lazarowitz & Lazdins, 1991) was due to a single amino acid exchange in the BR1 (BV1 in SiGMV-Ho) gene (Ingham & Lazarowitz, 1993) indicating the problems of using the host range for virus distinction.

Problems determining the status of relationship are also encountered by evaluation of symptoms in infected plant species. The two \textit{Sida}-infecting viruses from Honduras show identical symptoms in infected \textit{S. rhomboifolia} but distinct symptoms in \textit{N. benthamiana}. The two strains of the tomato golden mosaic virus (TGMV; Hamilton \textit{et al.}, 1984), TGMV common strain and TGMV yellow vein strain, showed similar symptom differences in infected \textit{N. benthamiana} (von Arnim & Stanley, 1992). However, this difference is not reflected in the nucleotide sequence differences. The overall nucleotide sequence variation between the DNA B components of the two TGMV strains is 3\% (von Arnim & Stanley, 1992) whereas a 10\% difference is observed between the SiGMV-Ho and SiGMV-Ho\text{yv}.

Another indicator for the status of the relationship between the two bipartite geminiviruses might be whether they can form pseudorecombinants. Although bipartite geminiviruses are closely related, the production of viable pseudorecombinants by reassortment of infectious cloned components is generally limited to strains of a particular virus (Stanley \textit{et al.}, 1985; Lazarowitz, 1991; von Arnim & Stanley, 1992; Frischmuth \textit{et al.}, 1993; Sung & Coutts, 1995). An exception to this, i.e. viable pseudorecombination between distinct geminiviruses, ToMoV and BDMV, has been reported (Gilbertson \textit{et al.}, 1993). The pseudorecombinant virus produced by exchange of genomic components between SiGMV-Ho and SiGMV-Ho\text{yv} was infectious in \textit{N. benthamiana}. Exchange of genomic components between SiGMV-Ho and SiGMV-Co resulted in a viable pseudorecombinant virus only in the case of SiGMV-Ho DNA A and SiGMV-Co DNA B while the reciprocal exchange was not infectious in \textit{N. benthamiana}. In the case of SiGMV-Co and SiGMV-Ho\text{yv}, DNA A of SiGMV-Ho\text{yv} formed a pseudorecombinant virus with SiGMV-Co DNA B while, surprisingly, only DNA B\text{yv} of SiGMV-Ho\text{yv} produced an infectious pseudorecombinant virus with SiGMV-Co and not DNA B\text{yv}. Therefore, it is difficult to draw conclusions about the relationship between the two viruses by their capability to form an infectious pseudorecombinant virus because even two such closely related DNA B molecules of SiGMV-Ho\text{yv} behave differently in their capability to produce viable pseudorecombinant viruses. Hou & Gilbertson (1996) discussed the increased pathogenicity of pseudorecombinant viruses produced by ToMoV and BDMV, suggesting that pseudorecombination is a complex phenomenon that involves interactions among virus- and host-encoded factors as well as viral DNA components, thus indicating the problems of the pseudorecombination phenomenon.

Homology comparisons of nucleotide sequences as well as amino acid sequences of gene products are probably the best way to evaluate the relationship between two viruses. In these analyses, whole sequences of genomic components and amino acid sequences of gene products, as well as special sequence
parts like the CR and the non-coding region between the CR and the BCI gene start codon, should be included to give a detailed result. Problems involved in drawing conclusions about the status of a relationship between two viruses by comparing sequences of single ORFs are well discussed by Padidam et al. (1995) and the authors suggest a taxonomic structure after considering sequence comparisons and biological properties. The use of several aspects, e.g. biological as well as sequence data, is reflected in the virus species definition by the International Committee on Taxonomy of Viruses (ICTV) (Murphy et al., 1995).

From our analyses, we conclude that two distinct Sida-infecting geminiviruses are present in the Zamorans Department of Francisco Morazan in Honduras and, considering SiGMV-Co from Costa Rica, at least three are present in Central America.

SiGMV-Ho clones were held according the Gentechnik Gesetz (licence 76-14/8829.02/Uni.S.01.01-5 and 76-14/8829.02/Uni.S.01.04-2). The work was supported by DFG grants to T.F. (Fr. 1122/1-1 and Fr. 1122/1-2).

References


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