Detection and Distribution of Geminiviruses in Mexico and the Southern United States

Irineo Torres-Pacheco, J. Antonio Garzón-Tiznado, Judith K. Brown, Alicia Becerra-Flora, and Rafael F. Rivera-Bustamante

First, second, fourth, and fifth authors: Departamento de Ingeniería Genética, Centro de Investigación y de Estudios Avanzados del IPN, Unidad Irapuato, Apartado Postal 629, Irapuato, Gto. México; and third author: Department of Plant Sciences, University of Arizona, Tucson.

Permanent address of I. Torres-Pacheco and J. A. Garzón-Tiznado: Instituto Nacional de Investigaciones Forestales y Agropecuarias, Apartado Postal 112, Celaya, Gto. México.

This research was partially supported by grants from CONACYT-México (4769-N9406) and ICGEB (CRP/MEX94-03; 95/005) to R. F. Rivera-Bustamante. B. I. Torres-Pacheco received fellowship support from CONACYT.

The sequences reported in this manuscript have been deposited into the GenBank database with the following accession numbers: CdTV B, U57458 and TPV A, U57457.

We thank J. T. Ascencio-Ibañez, B. Jimenez-Moraila, J. Méndez-Lozano, and R. Peña-Ramírez (CINVESTAV), and B. T. Poulos, D. Fletcher, N. P. Goldberg, A. M. Idris, and K. Kiesler (University of Arizona) for their excellent assistance.

Accepted for publication 5 August 1996.

ABSTRACT

Torres-Pacheco, I., Garzón-Tiznado, J. A., Brown, J. K., Becerra-Flora, A., and Rivera-Bustamante, R. F. 1996. Detection and distribution of geminiviruses in Mexico and the southern United States. Phytopathology 86:1186-1192.

Plant samples from important horticultural areas in Mexico and the southern United States were collected during several seasons and analyzed for the presence of geminiviruses by a combination of agarose gel electrophoresis, molecular hybridization, and polymerase chain reaction amplification techniques. A general detection strategy confirmed the presence of geminiviruses in all horticultural areas of Mexico in pepper, tomato, tomatillo (*Physalis ixocarpa*), cucurbits, and tobacco. Specific detection procedures showed that pepper huasteco virus is widely distributed in Mexico; it was found in pepper and tomato samples in both coastal areas, as well as in central Mexico. It was also found in pepper samples from the Rio Grande Valley in southern Texas. Pepper jalapeño virus (PJV) and chino del tomate virus (CdTV) showed a more restricted distribution, although, in all cases, the viruses appeared to become more widely distributed over time. Partial DNA sequences of PJV and CdTV were also obtained. Comparative sequence analysis showed that PJV and the previously described Texas pepper geminivirus are probably strains of the same virus. The name pepper jalapeño virus is, thus, withdrawn to avoid further confusion. Similarly, CdTV showed a very high level of sequence identity with the recently described tomato leaf crumple virus (TLCrV), also suggesting that they both are strains of the same virus.

Geminiviruses are a group of plant viruses with small, twinned, isometric particles and genomes containing one or two single-stranded DNA (ssDNA) molecules. Geminiviruses are classified into three genera according to their host range, insect vector, and genome organization. Members of the genus subgroup III *Geminivirus* of the family Geminiviridae are transmitted by the whitefly *Bemisia tabaci* (Gennadius) and infect dicotyledonous plants. Most members have a bipartite genome divided into two ssDNA molecules (15). During the last decade, several whitefly-associated diseases have caused important losses in several horticultural crops in almost all agricultural areas in Mexico and some southern states of the United States. The symptoms commonly associated with these diseases are foliar distortion (curling, crumpling, rugosity, yellowing, and mosaic patterns), overall stunting of the plants, and dramatic decreases in expected yield. Several geminiviruses have been reported in association with and as causal agents of, separately or in combination, some of these diseases. In some cases, the geminiviruses involved were isolated and characterized. For example, chino del tomate virus (CdTV) was isolated in Sinaloa (5), Texas pepper geminivirus (TPV) was isolated in southern Texas (23), and pepper huasteco virus (PHV) and another uncharacterized virus were isolated in Tamaulipas (13). The latter geminivirus was later partially sequenced and tentatively named pepper jalapeño virus (PJV) (1). In other instances, only a description of the disease and the possible causal agent, separately or in combination, are reported (3,4,6). In addition, there is still a number of diseases whose etiology has never been determined (2).

Corresponding author: R. F. Rivera-Bustamante
E-mail address: rrivera@irapuato.ira.cinvestav.mx

Publication no. P-1996-0904-01R
© 1996 The American Phytopathological Society

In Mexico, the earliest reports on geminivirus diseases came from studies in the northwestern states of Sinaloa and Sonora on tomato, pepper, and bean diseases (12). More recently (late 1980s to the present), similar diseases and symptoms have been observed in many crops in nearly all horticultural areas in the country. In the United States, the presence of squash leaf curly virus (SqLCV) was reported as an economically important problem in the late 1970s (9,11). More recently, whitefly-transmitted geminiviruses reached epidemic proportions in tomato in Florida and in tomato and pepper in the Rio Grande Valley of Texas (2,19,23). Furthermore, there are numerous uncharacterized viruses in crops and weeds that have the potential to cause important economic losses.

Two possible explanations for these new trends in Mexico and the United States sunbelt states are proposed. First, the spread of a group of specific geminiviruses, well established in commercial crops for a long time, from their initial native location (e.g., Sinaloa and Texas/Tamaulipas) to other agricultural areas has been

facilitated by several factors such as the increase in the movement of plantlets between different areas. In the second scenario, each horticultural area harbors its own endemic geminivirus populations that only recently found a way to spread to commercial crops. The introduction of the B biotype of the whitefly vector, variations in environmental conditions that have favored higher vector populations (insecticide resistance, etc.), and the overlapping of crops throughout the year are factors that could have an impact in both cases.

To distinguish between the two possibilities, samples from plants showing geminivirus-like symptoms collected during the last 10 years were analyzed. The study primarily focused on crops of economic importance such as pepper, tomato, tomatillo (*Physalis ixocarpa* Brot.), squash, and tobacco. In the first part, the aim was to detect and confirm the presence of geminiviruses, thus, providing support for the suspected viral etiology of the diseases observed in the horticultural areas under study. In the second part, the temporal and spatial distributions of the geminiviruses previously reported in Mexico (PHV, CdTV, and PJV) were studied to investigate the possibility of interregional virus introductions. Finally, the relationships between TPV and the tentatively designed PJV, as well as between CdTV and the recently described tomato leaf crumple virus (TLCrV), were analyzed on the basis of recent sequence data.

MATERIALS AND METHODS

Collection of samples. Samples from plants with geminivirus-like symptoms were collected in the past 10 years. In Mexico, the initial sampling was directed towards areas from which geminiviruses had been previously reported (Sinaloa, Sonora, and Tamaulipas). More recent collections were targeted towards areas where relatively new diseases of suspect geminivirus etiology had been reported (Puebla), areas in which geminivirus-like symptoms had been observed recently or for the first time (Chiapas, Nayarit, and Guanajuato), or areas with virus-like diseases associated with infestations of whiteflies (Quintana Roo). Sample collections were focused on the following crops: pepper, tomato, squash, tobacco, tomatillo, and, in some cases, common symptomatic weeds surrounding horticultural fields (mostly members of the families Malvaceae, Solanaceae, and Euphorbiaceae). In the United States sunbelt states, samples were collected for cucurbits of all species and from tomato and pepper.

Virus DNA extraction. Plant nucleic acids containing viral DNA were extracted from infected tissue (0.5 to 1 g) using the procedure reported by Dellaporta et al. (8). The tissue was extracted twice with phenol and once with a mixture of phenol/chloroform/isoamyl alcohol (25:24:1). Nucleic acids in the aqueous phase were precipitated with isopropanol or ethanol and resuspended in sterile, deionized water or in Tris-EDTA (TE) buffer, pH 8.0.

Electrophoretic, Southern, and dot-blot analyses. Samples of nucleic acid extracts (10 μl) were analyzed by agarose gel electrophoresis. Following electrophoresis, nucleic acids were transferred onto Hybond N membranes (Amersham, Buckinghamshire, United Kingdom) for hybridization with DNA probes labeled with ^{32}P-dCTP by random priming. Two type of probes were used: an 800-bp fragment of the PHV coat protein gene as a general probe hybridized under low stringency conditions and a set of specific probes (full-length clones of A or B components from various geminiviruses) hybridized under various stringencies depending on the test. In some cases, a mixture of probes for geminiviral A components was used to increase the likelihood of detecting uncharacterized geminiviruses. All procedures were performed following standard conditions (21).

Design of oligonucleotides (oligos) for polymerase chain reaction (PCR). Several sets of oligos were designed for use in a PCR-based assay. Figure 1 shows the position and orientation of the primers. One set was designed with degenerate oligos (oligos 260 and 261) and was based on a highly conserved region found after alignment of the nucleic acid sequences of coat protein genes from several whitefly-transmitted geminiviruses. The expected product is a full-length component A. Three additional sets were designed to prime the amplification of regions of specific geminiviruses (or very related strains). In the first set, the oligos (425/426) were located in the intergenic region of component A of PJV/TPV. In set 2, the oligos (423/424) were located in the intergenic region of CdTV B. In set 3, the oligos (240/241) flanked the intergenic region of the component A of PHV. Oligo sets 425/426, 423/424, and 260/261 directed the amplification of a fragment of approximately 2.6 kb, whereas oligos 240/241 primed the amplification of a fragment of 350 bp in size. The sequences of the oligos, in orientation 5′-3′ are 240, GGCTTATTTGTAATAAGA-GAGGTGT; 241, GAATTAAAGGTACATGGACCACTT; 260, GGCGTTT^{T}/cGGAAGGTGA; 261, CTA/TACCAAGGCTTGT/CTCG; 423, GGAGTACACCCCTCTTATTA, 424, GCGATTT-TTCTCCCCCTCTA; 425, GGAGTCCCCAAGAGGCTTTA; and 426, ACAATAGAGGGCTGAGATTA.

Viral DNA amplification. Total DNA from plant samples was extracted and used as templates in PCR using an Gene Amp Kit (Perkin-Elmer Cetus, Norwalk, CT) following the manufacturer's recommendations. The final PCR reaction mix (100 μl) contained approximately 1 μg of template DNA, 250 μM of each dNTP, 1 μM of each primer, 10 μl of 10× PCR reaction buffer, 5 mM $MgCl_2$, and 2.5 units of AmpliTaq DNA polymerase (Perkin-Elmer Cetus). PCR was performed in a DNA Thermal Cycler 480 (Perkin-Elmer Cetus) and the cycle parameters used were as follows. For set 3 (PHV intergenic region) the conditions used were 1 min at 94°C, 2 min at 45°C, and 2 min 30 s at 72°C. For the remaining sets, the conditions were 1 min at 94°C, 2 min at 55°C, and 3 min at 72°C. In both cases, the reactions included 30 to 35 cycles and were followed by a final extension step of 7 min at 72°C.

Cloning and analysis of cloned products. The PCR products (amplified viral DNA) were cloned directly into the pCR II vector

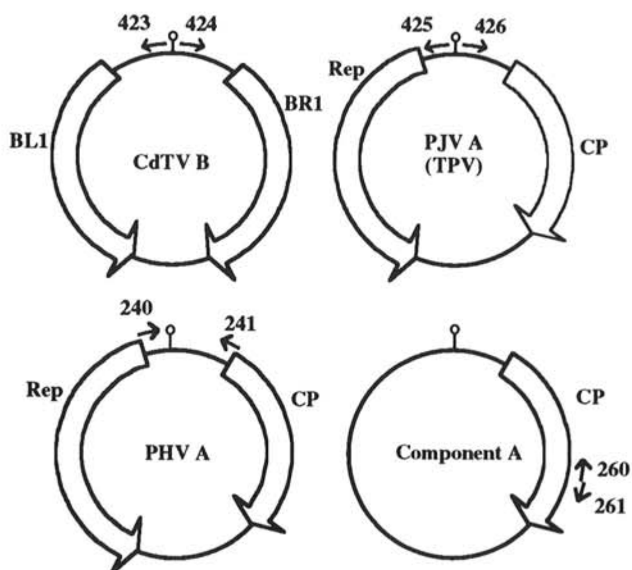

Fig. 1. Position of oligonucleotides (oligos) designed for geminivirus detection. Set 1: Oligos 423 and 424 are located in the common region of chino del tomate virus (CdTV) B. Set 2: Oligos 425 and 426 are located in the common region of pepper jalapeño virus (PJV)/Texas pepper geminivirus (TPV) A. Set 3: Oligos 240 and 241 are located flanking the intergenic region of pepper huasteco virus (PHV) A. Set 4: Oligos 260 and 261 are located in a highly conserved region in the 3′ end on the coat protein gene of most whitefly-transmitted geminiviruses. Sets 1, 2, and 4 prime the amplification of a 2.6-kb fragment; set 3 directs the amplification of a 350-bp fragment.

(Invitrogen Corp., San Diego, CA). After cloning, the fragments were sequenced using the dideoxynucleotide procedure (22).

RESULTS AND DISCUSSION

General detection procedures for geminiviruses. Visualization of viral nucleic acids as a detection procedure has been well documented for DNA and RNA viruses. Several molecular forms of geminiviral DNA often can be visualized after agarose gel electrophoresis of nucleic acid extracts from infected plants (Fig. 2). However, it has also been observed that several factors can affect the visualization of viral DNA (e.g., age of tissue, age of infection, type of plant and extraction procedure, etc.) (13). The detection of viral DNA by molecular hybridization has also been well documented (7,16). In this study, a coat protein gene fragment was used as a general probe, since it is the most conserved gene among dicot-infecting bipartite geminiviruses (17). Similarly, the set of degenerate oligos (260/261) used for PCR amplification was designed based on the coat protein gene.

No single technique by itself was sufficient to guarantee detection of any geminivirus present in a sample, and some of the common variations are shown in Figure 2. Geminivirus DNA was often difficult to visualize by agarose gel electrophoresis analysis of samples from tomato and other hosts, because of its low concentration (Fig. 2A, lanes 2 and 4). In contrast, pepper samples usually showed an intense viral DNA band (Fig. 2A, lane 3). In other species such as tobacco and weed hosts, a high background of plant DNA usually prevented direct visualization of viral DNA (data not shown). In those instances, Southern analysis, PCR amplification, or both were used to confirm the presence of viral DNA (Fig. 2B). The detection of geminiviruses using only DNA-DNA hybridization approaches also presented some problems, because detection requires homology between probe and viral DNA. For example, with some samples, the intensity of the hybridization signal did not correspond with the intensity of the bands observed on the ethidium bromide-stained agarose gel (Fig. 2, lanes 3 and 5). Similarly, samples that yielded a geminivirus DNA band of the appropriate size and produced a strong hybridization signal, separately or in combination, sometimes did not generate the expected product when PCR procedures were used. Thus, to reduce the number of possible false negatives, more than one technique was used to evaluate each sample. The combination

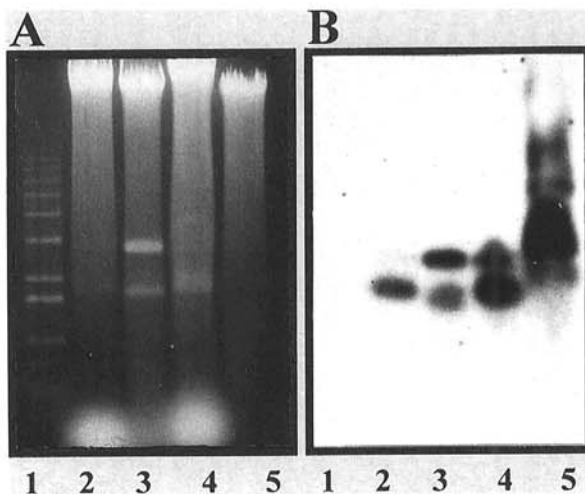

Fig. 2. Agarose gel electrophoresis and Southern hybridization analyses of DNA from selected geminivirus-infected field samples. Field samples from Mexico were selected and analyzed by **A,** agarose gel electrophoresis and **B,** Southern hybridization to demonstrate the variability of the two detection procedures. Lane 1, Molecular weight standard (1 kb; GIBCO BRL, Gaithersburg, MD); lane 2, tomato, San Luis Potosi state; lane 3, pepper, Quintana Roo state; lane 4, tomato, Michoacán; lane 5, pepper huasteco virus-infected pepper as hybridization control.

of techniques used varied and depended primarily on the nature of the specific sample (e.g., species to be tested, age of the plant, etc.).

Occasionally, when the combination of the three detection methods produced ambiguous results (e.g., in some tomatillo samples), a fourth biological test was used. Different host plants were inoculated by particle bombardment with DNA extracts from suspected plants (13). Inoculated plants were observed for symptom development and assayed for the presence of viral DNA using the methods described above.

Plant samples from 18 different states were collected and analyzed for the presence of geminiviruses. The map shown in Figure 3 summarizes these results and indicates that geminiviruses have been found in nearly all horticultural areas in Mexico. For example, geminiviruses are now known to be in crops grown in both Pacific and Gulf coasts, in the high central plateau, and in the Yucatán peninsula. Likewise, crop and weed samples collected in the sunbelt states of the western United States (California, Arizona, and Texas) were positive for geminiviruses. In some cases, it was possible to identify the same geminivirus occurring on both the United States and Mexico sides of the international border (e.g., PHV and TPV/PJV). Although pepper-infecting geminiviruses may appear, from this report, to be the most widely distributed geminiviruses among those surveyed, this observation may reflect the widespread distribution of cultivated pepper in Mexico and the United States and, thus, analysis of a higher number of samples for this crop.

For tomatoes, early reports on geminivirus diseases were restricted to the major west coastal areas in the state of Sinaloa. More recently, however, tomato fields with geminivirus-like symptoms were observed in the central areas of Mexico (Guanajuato, Michoacán, and Morelos), as well as in the states of Nayarit and Chiapas (R. Rivera-Bustamante and J. Brown, *unpublished data*). Although the results of this and on-going studies have confirmed a suspected geminiviral etiology of these tomato diseases, the precise identification of most of these geminiviruses remains to be determined.

Specific detection of geminiviruses. In addition to the general detection procedures for geminiviruses, two procedures were used to detect specifically PHV, CdTV, and TPV/PJV. Initially, the three viruses were detected by dot-blot hybridization using specific probes and high stringency conditions. More recently, when the nucleotide sequences were available, several oligo sets were designed to prime the amplification of exclusively one geminivirus (or a very related strain). Figure 1 shows the location of the primers in each genome.

PHV was first isolated from Tamaulipas state (Fig. 3) (13), and this isolate has been characterized at the molecular level (24). More recently, PHV has been detected by dot-blot hybridization and PCR amplification in several distant areas in Mexico (Sinaloa, Tamaulipas, Guanajuato, and Quintana Roo) and in the Rio Grande Valley of Texas, suggesting a wider distribution of this geminivirus than previously suspected. Several PCR products that span the viral intergenic region (IR) were sequenced. Alignment of IR sequences indicated nucleotide sequence identities in the range of 95% or greater, suggesting the existence of PHV strains. In Sinaloa, PHV has been detected in symptomatic tomatoes where it causes stunting and yellowing of the leaves (symptoms are first expressed in the base of the younger leaves). However, PHV was not detected in any sample from Sinaloa for this study before April 1990. The original clones of PHV were obtained from an isolate from Tamaulipas. Although highly infectious when inoculated on pepper and tobacco, the PHV-Tamaulipas clones appeared rather inefficient when inoculated on tomatoes. Several PHV isolates obtained in Sinaloa from tomato are also being fully sequenced to study the nucleotide changes responsible for the observed variations in the host range and symptomatology. Two samples (PV-WB and PV-WC) collected

from pepper in the Rio Grande Valley (near Weslaco), TX, in 1987 to 1988 were also analyzed by DNA sequencing of the targeted IR and were confirmed as PHV variants.

Chino del tomate disease was first observed in tomatoes in the state of Sinaloa in the late 1970s (12). A whitefly-transmitted geminivirus (i.e., CdTV) was shown to be the causal agent of the disease in 1984 (5). A full-length clone of CdTV component B was later obtained and used in routine hybridization assays of samples. With the use of the B component of CdTV as a probe in DNA hybridization assays under high stringency conditions, CdTV has been detected in tomato and pepper plantings throughout the state of Sinaloa nearly every year since 1988. (J. Brown, *unpublished data*). Recently, the complete sequence of component B was determined and used to design specific primers for PCR amplification. Based on both hybridization and PCR data, the presence of CdTV in other horticultural areas (Chiapas, Morelos, and Tamaulipas) has been confirmed. Although it appears that CdTV is not as widely distributed as PHV, this observation may only reflect the distribution of the tomato culture itself.

Recently, a second geminivirus, in addition to PHV, was isolated from peppers affected with the "rizado amarillo" complex disease (13). A clone containing a full-length insert of component A was also obtained and sequenced. This virus was tentatively designated as PJV (1). The PJV A component sequence was used to design primers to establish a specific detection procedure based on PCR amplification. With these specific primers, PJV was detected in Sinaloa (tomato and pepper) and Michoacán (pepper). A weak amplification was also obtained with squash samples from Colima. This pepper-infecting geminivirus was neither as widely distributed as PHV nor localized to a specific region.

Virus relationships and sequence comparisons. In a recent report, Paplomatas et al. (18) reported a new sap-transmissible geminivirus infecting tomatoes isolated from Sinaloa, tentatively called TLCrV. In a previous report from the same group, a partial sequence of TLCrV (then called TGV-MX1) was published (20). When the sequences of CdTV B and TLCrV were compared, some interesting observations were raised. Figure 4 shows a comparison of the nucleotide sequences of CdTV and TLCrV. In Figure 4A, an alignment of BR1 open reading frames from both isolates of TLCrV (B from beans and Nb from *Nicotiana benthamiana*) and the corresponding sequence from CdTV are shown. There is a higher nucleotide sequence identity between CdTV and isolate B of TLCrV (96.5%) than between both isolates of TLCrV (94.2%). A phylogeny obtained by three different computer programs showed that, indeed, there is a closer relationship between TLCrV B and CdTV than between TLCrV B and TLCrV Nb (data not shown). Figure 4B shows the alignment of the common region (CR) of CdTV B and the CR of TLCrV (20). Again, there is a higher sequence identity between CdTV B and TLCrV A (99.4%) than between TLCrV A and either B component (93.2 and 94.4% for B and Nb isolates, respectively). In view of these similarities, two main discrepancies between CdTV and TLCrV reports have to be addressed. Paplomatas et al. (18) reported that the symptoms obtained in TLCrV-infected tomatoes do not correspond to the ones induced by CdTV. However, as they also pointed out, "disease symptoms alone are not suitable for identification or differentiation of geminiviruses," because they vary as result of several factors. In addition, they also showed that although the two DNA B components isolated were almost identical (>90% identity), one induced severe symptoms in *N. benthamiana*, whereas the other induced only a mild reaction. This suggested that, as in the case of SqLCV (14), minor sequence changes among variants can be responsible for differences in symptom expression and host range. As for the sap transmissibility of TLCrV, they also showed that the DNA B component cloned from bean was able to confer sap transmissibility, whereas the component cloned from *N. bentha-*

Fig. 3. Distribution of geminiviruses in Mexico and the southern United States. The Mexican states and American border states in which geminiviruses were detected are shown. The following crops were analyzed: pepper (P), tomato (T), tobacco (B), squash (S), and tomatillo (Y). The regions in which positive results were found are marked in the map with the respective letter.

A

```
BR1 TLCrV Nb   MYPFRSKRGT TYVARRSYSR NNVFKRSTIS KRDDGRRRSV NATKPSVEPK MTAQRMHENQ FGPDFVMAHN AALST   75
BR1 CdTV       MYPFRSKRGA TFVARRSYSR NNLFKRSTIS KRDDGRRRSV NATKPNDEPK MTAQRMHENQ FGPDFVMAHN SALET   75
BR1 TLCrV B    MYPFRSKRGA SFVARRSYSR NNLFKRSTIS KRDDGRRRSV NATKPNDEPK MIAQRMHENQ FGPDFVMAHN AALAT   75

BR1 TLCrV Nb   FISFPGLGKT QPNRSRSYIK LKRLRFKGTV KIERVTSDMN MDGSIPKVEG VFSLVVVVDR KPHLGASGSL HTFDE   150
BR1 CdTV       FISFPCLGKT QPNRSRSYIK LKRLRFKGTV KIERVTSDMN MDGSTPKVEG VFSLVVVVDR KPHLGASGSL HTFDE   150
BR1 TLCrV B    FISFPCLGKT QPNRSRSYIK LKRLRFKGTV KIERVMSDMN MDGSTSKVEG VFSLVVVVDR KPHLGASGSL HTFDE   150

BR1 TLCrV Nb   LFGARIHSHG NLSITPSLKD RFYIRHVFKR VLSVEKDSMM VDVEGSTALS NRRFNCWSTF KDIDRDSCNG VYANI   225
BR1 CdTV       LFGARIHSHG NLSIPPSLKD RFYIRHVFKR VLSVEKDSMM VDVEGSTALS NRRFNCWSTF KDLDRDSCNG VYGNI   225
BR1 TLCrV B    LFGARIHSHG NLSITPSLKD RFYIRHVFKR VLSVEKDSMM VDVEGSTALS NRRFNCWSTF KDLDRDSCNG VYGNI   225

BR1 TLCrV Nb   SKNALLVYYC WMSDTMSRAS SFVSFDLDYI G   256     Nb - B:       94. 2 %
BR1 CdTV       SKNALLVYYC WMSDTMSRVS SFVSFDLDYI G   256     Nb - CdTV:    94. 6 %
BR1 TLCrV B    SKNALLVYYC WMSDTMSRAS SFVSFDLDYI G   256     B - CdTV:     96. 5 %
```

B

```
TLCrV Nb B   TAACCGATGG CATTTTTGTA ATAAGAAGGG TGTACTCCGA TTGAGCTCTC AAACTTCTGT GCTATGTTTT   70
TLCrV B  B   TAACCGATGG CATTTTTGTA ATAAGAGGGG TGTACTCCGA TTGAGCTCTC AAACTTCTGT GCTATGTTTT   70
TLCrV    A   TAACCGATGG CATTTTTGTA ATAAGATGGG TGTACTCCGA TTGAGCTCTC AAACTTCTGT GCTATGTTTT   70
CdTV     B   TAACCGATGG CATTTTTGTA ATAAGAGGGG TGTACTCCGA TTGAGCTCTC AAACTTCTGT GCTATGTTTT   70

TLCrV Nb B   GGGGTAAAGG GGACAATATA TACTAGAACT CTCTGTTACG GATTAGCGAC ACGTGGCGGC CATCCGATAT   140
TLCrV B  B   GGGGTAAAGG GGACAATATA TACTAGAAGT CTCTATCGTT GTTTAGCGAC ACGTGGCGGC CATCCGATAT   140
TLCrV    A   GGGGTAAAGG GGACAATATA TACTAGAACT CTTAGTAGCA CTTTAGCGAC ACGTGGCGGC CATCCGATAT   140
CdTV     B   GGGGTAAAGG GGACAATATA TACTAGAACT CTTAGTAGCA CTTTAGCGAC ACGTGGCGGC CATCCGATAT   140
                                                                     GGC CATCCGATAT

TLCrV Nb B   AATATTACCG GATGGCCGCG C   161
TLCrV B  B   AATATTACCG GATGGCCGCG C   161              CdTV B    TLCrV BB    TLCrV NbB
TLCrV    A   AATATTACCG GATGGCCGCG C   161  TLCrV A     99. 4 %   93. 2 %     94. 4 %
CdTV     B   AATATTACCG GATGGCCGCG C   161
             AATATTACCG GATGGCC
```

Fig. 4. Comparison of chino del tomate virus (CdTV) and tomato leaf crumple virus (TLCrV) sequences. **A,** Comparison of the amino acid sequences of BR1 proteins predicted from the nucleic acid sequences of CdTV and the two isolates of TLCrV, TLCrV Nb (*Nicotiana benthamiana*) and TLCrV B (bean) (18). The conserved amino acids are boxed. The results of pairwise comparisons of the different BR1 proteins are also shown. **B,** Comparison of the common region of both isolates of TLCrV (18) and CdTV. TLCrV B Nb is the component B isolated in *N. benthamiana*, TLCrV B is the component B isolated in beans, TLCrV A is the component A isolated in beans, and CdTV B is the component B of CdTV. The conserved bases are boxed. The 30-base element (bases 128 to 157) conserved in all dicot-infecting geminiviruses is shown. The results of pairwise comparisons between TLCrV A and the three B components are also shown.

miana was unable to do so. This suggests that sap transmissibility may not be a good parameter to differentiate viruses. Thus, it is not unreasonable to think that the CdTV B component, whose sequence is reported here but was isolated in the early 1980s, is a third variant of the component B of a single virus. Differences in the time of its isolation and method (whiteflies transmission) and in its maintenance (only in tomato) could have influenced the selection of this variant. Nevertheless, the sequence data strongly support the hypothesis of a single virus. Therefore, it is suggested that the original name (CdTV) be kept to avoid confusion.

Recently, a partial sequence of the TPV CR was published (10). A comparison of TPV and PJV CRs demonstrated a close relationship (>95% identity) between these two isolates, suggesting that they should be considered strains of the same virus. For example, the putative stem-loop structure detected for both TPV and PJV was only 28 bases long, whereas most bipartite geminiviruses have a 30-base conserved element. Also, a partial DNA sequence (designated PEMAC1a) from a pepper-infecting geminivirus isolated in Sinaloa was deposited in the GenBank database with accession number L27270. The PEMAC1a sequence represents a partial sequence of a geminivirus AC1 protein. When compared with several AC1 proteins from bipartite geminiviruses, PEMAC1a showed the highest sequence identity with PJV/TPV. Figure 5 shows the sequence of the component A of PJV/TPV and its alignment with the partial sequences of TPV and PEMAC1a. Because of the high number of ambiguities in the PEMAC1a sequence, it was not possible to calculate a reliable percentage of identity at either nucleic acid or peptide levels. Collectively, however, these data indicate that TPV, PJV, and the virus from which PEMAC1a was derived are all probably strains of the same virus and also confirms the presence of PJV/TPV in Sinaloa fields. Therefore, the tentative name of PJV is withdrawn to avoid further confusion. We are in the process of continuing the sequencing of both components of TPV (provided by D. Stenger) to compare the entire genome.

Geminiviruses are now in most horticultural areas in Mexico. The distribution of characterized geminiviruses shows that some (e.g., PHV) may be detected throughout Mexico, regardless of the geographic characteristics of the regions surveyed. CdTV and PJV also occur in separate geographic locations; however, they are not as widely distributed as PHV. There remain a number of uncharacterized geminiviruses infecting various crops. The distribution of geminiviruses in Mexico appears to be dynamic, and the dispersal of a given complex of geminiviruses may change from season to season. In contrast, there appear to be fewer distinct geminiviruses in United States cropping systems, but the diseases they cause persist on a annual basis, because of consistent pressures exerted by the whitefly vector.

The spatial and temporal distribution of the characterized geminiviruses (PHV, CdTV, and PJV/TPV) suggest that some geminiviruses may have been spread from their initial points of origin (Sinaloa for CdTV and Tamaulipas/Texas for PHV and PJV/TPV) to new agricultural areas. However, the recognition that there are a large number of uncharacterized geminiviruses throughout the region adds credence to the possibility that certain locations have exclusively endemic populations of geminiviruses. It is possible

```
PJV A     ACATTGTAAACGAAAGGATTTTGGTGGTAGTGGCATTTTTGCAAATATGGTGTAGGACTCCAGCAGA-GTCCCTCAACTTC-TGTCATATGTTGTGGAGT
TPV                                        CATTTTTGCAAATATGATATAGGACTCCAGCAGATGTCCCTCAACTTCCTGTCATAT-ATGTGGAGT

PJV A     CCTGGAGTCCCTTATATACATAAAG-CCTCTTGGGGACTCCAAGGGCAAAAGCGGCCATCCTATAATATTACCGGATGGCCGCGATTTTTTGGAGTCTTT
TPV       CCTGGAGTCCCTTATATACATAAAGCCCTCTTGGGGACTCCAAGGGCAAAAGCGGCCATCCTATAATATTACTGGATGGCCG

PJV A     AGGGGACCACTCACAATAGAGGGCTGAGATTAAATAGACAGAGTGAGGGGACCACCAATTATTTGATTTTGACTGACCAATCACATCCCGCCTGTCAAGT

PJV A     CTAGATATTTGTGCCTTGGTGACCAAGGTGTGGGCCTATAAATGCACGTGCATCTGCATTTATCGCTTTAATTCAAAATGGTTAAGCGGGATGCCCCATG

PJV A     GCGTTTAATGGCGGGGACCTCTAAGGTTTCCGGCTCTGCGAATTATTCACGTGGCACGGGTATGGGGCCTAAATTCGATAAGGCCGCTGCTTGGGTTAAC

PJV A     AGGCCCATGTACAGGAAGCCCAGTATATATCGTACGTATAGAAGCCCAGATGTGCCTAGASSATGTGAAGGGCCATGTAAGGTCCAGTCCTTCGAGCAGC

PJV A     GGCATGATATCTCGCATGTTGGCAAGGTCATGTGCATTTCTGACGTGACACGTGGTAATGGTATTACCCATCGTGTAGGCAAGCGTTTCTGCGTCAAGTC

PJV A     TGCATACGTTCTGGGTAAGATATGGATGGACGAGAATATCAAGCTCAAGAACCACACCAACAGCGTCATGTTCTGGCTCGTGAGAGATAGGAGACCATAT

PJV A     GGTACCCCTATGGACTTTGGTCAGGTGTTCAACATGTATGACAACGAGCCCAGTACAGCTACTGTGAAGAACGATTTGCGTGATCGTTTCCAAGTCATGC

PJV A     ACAGGTTCTCGGCTAAGGTCACAGGTGGACAGTATGCCAGCAACGAGCAGGCATTGGTTAGGCGTTTCTGGAAGGTGAACAACTACGTCGTGTACAACCA

PJV A     TCAGGAAGCTGGCAAATACGAGAATCATACTGAGAACGCCCTGCTATTGTATATGGCATGTACACATGCCTCTAACCCTGTGTACGCGACATTGAAAATT

PJV A     CGAATCTATTTTTATGATTCGATAACAAATTAATAAAGTTTGCATTTTATTTCATGATTCTCAAGTACATAATTTACATAGGATTTGTCCGTCGCAAAAC

PJV A     GAACAGCTCTGATTACATTGTTTATGCCTATGGCCCCTAGCGTATGTAAATACAACATGACGCGAAATTTGAATCTACTTAAATATGTCGTCCCAGAAGC

PJV A     TGTCATCGAAGTCGTCCAGACTTGGAAATGGAGGAATGCCTTGTGGAGAGCTAATGCTCGACGTAGGTTGTGGTTGAACCGGACTACGATGTGGTATATC

PJV A     CTGGTTCTGGTGAACAACGGGTCCTCCACGTGCTGTATCCTGAAAAATAGGGGATTTGGAACCTCCCAAATAAAAACGGAATTCTCTGCCTGATGCACAG

PJV A     TGATGCTCTCCCCTGTGCGTGAATCCATTATTAGCGCAGTTGATGTGGAGAAAGATAGAGCAGCCGCAGTTTAGGTCTATGCGTCGTCTGCGAATAGGTC

PJV A     GCTTCTTAGCTATCCTGTGCTGTGCTTTTATAGAGGGGAGCGTGGAGGATGACGAATTGAGCATTTTTTAATGTCCACGCTCTTAATTCTGCATTTTCCT

PJV A     CTTTGTTGAGGAAAGCTATATAGCTGCTACCTTCCCCTGGATTGCACAGCACGATTGATGGTATCCCGCCTTTAATTTGAACTGGCTTTCCGTATTTACA

PEMAC1a                                                 TCANCTCNTAGGATAATGGGGCGTGATATCATCANTGACGTTATACTCCACAT
PJV A     GTTGGACTGCCAGTCTTTTTGGCCCCAATAAGCTCTTTCCAGTGTTTCATCTT-TA-GATATTGGGGCGTGATATCATCAATGACGTTATACTCCACAT

PEMAC1a   CGTTTGAATAGACTTTTGAATTGAAATCAAGGTGACCGCTCAAATAATTATGTGGGCCTAATGNACGTGCCCACATTGTCTTGCCGCTTCGCGAATCACC
PJV A     AGTTTGAATAGACCTTTGCATTGAAATCAAGATGACCGCTCAAATAATTATGTGGGCCTAGTGCACGTGCCCACATTGTCTTGCCGCATCGCGAATCACC

PEMAC1a   CTCAATGACAATACTANTCGGNCTATCTGGCCGNGCAGCGGAACTCCGACCAAAATANTCAGCANCCCANTTTNGCATATCCTCAGGAACGTTATTTACC
PJV A     CTCAATGACAATACTAATCGGTCTATCTGGCCGCGCA-C--AACTCCGACCAAAATATTCAGCAACCCAATGTTGCATATCCTCAGGAACGTTGTTAAAC

PEMAC1a   GAGGAAATTGGATAAGGAGGAGCCCANGGCTTAGGAGGAG
PJV A     GAGGATAATGGATAAGGAGGAGTCCATGGCATAGGAGGAGTTGTAAATAGGCGCTCGATGTTAGCCTTTATGTTATGATAACTAACAATGAACGTTTTCG

PJV A     GATCACCAGCTTTGATTATGTCGAGCGCCTCCCCCGCACTTGCAGCATTTACAGCGTTATGGTAGACGTCGTCTTTATTTGCTTTTGACCCCCCGGAAAC

PJV A     TTTGTACTGCCCAGATTCACAATAATCACCATCTTTTGTTATGTAATTTTTAACGGCATTTGCGTCCTTGGATGCCTGAACGTTTGGATGAAACTGGGCA

PJV A     GATCTCCGGGGGTGAGTAATGTCGAAGAACCGGGCATCTTTGATGTTGCATTTTCCAGTCAACTGGATTAGACAGTGCAAGTGCGGGAACCCATCGGAAT

PJV A     GCTCCTCTCGTGATACCCGGAGATAGATGGGTTTTACCACTGCCCATTTTAAATTACGGAGCATTTCGAGAGCTTCATCCTTTGGGATATCGCATTGTGG

PJV A     ATATGTTAAGAAAATGTTTTT
```

Fig. 5. Comparison of pepper jalapeño virus (PJV) and Texas pepper geminivirus (TPV). The sequence of the component A of PJV was aligned with a TPV partial sequence (common region) (10) and a partial sequence of a pepper-infecting geminivirus (PEMAC1a) isolated in Sinaloa state by D. Maxwell and deposited in the GenBank. The region underlined corresponds to the stem-loop element conserved in all dicot-infecting geminiviruses. In this case (PJV/TPV), the element is only 28 bases long. PEMAC1a is a partial sequence of the Rep (AC1) gene.

that these endemic populations may also become displaced by other endemic or introduced viruses (e.g., PHV) in the event that they acquire some advantages in particular agroecosystems.

In the areas where only the purported endemic geminivirus populations are thought to exist, their widespread detection in crops indicates that they have likely moved recently from natural weed hosts to monocultured crops. The reason for the recent emergence of indigenous geminiviruses as serious pathogens in cultivated crops is probably twofold. First, local whitefly vector populations appear to have increased in numbers because of increased monoculture efforts in subtropical locales, as well as the possible development of resistance to insecticides; and second, in some areas where solanaceous crops are abundant and the B biotype has become established, it is clear that the viruses are being assisted in their distribution by the introduced B biotype whitefly vector that is well-adapted to solanaceous hosts.

LITERATURE CITED

1. Argüello-Astorga, G. R., Guevara-González, R. G., Herrera-Estrella, L. R., and Rivera-Bustamante, R. F. 1994. Geminivirus replication origins have a group-specific organization of iterative elements: A model for replication. Virology 203:90-100.
2. Brown, J. K., and Bird, J. 1992. Whitefly-transmitted geminiviruses and associated disorders in the Americas and the Caribbean Basin. Plant Dis. 76:220-225.
3. Brown, J. K., Campodonico, O. P., and Nelson, M. R. 1989. A whitefly-transmitted geminivirus from peppers with tigré disease. Plant Dis. 73:610.
4. Brown, J. K., Chapman, M. A., and Nelson, M. R. 1990. Bean calico mosaic, a new disease of common bean caused by a whitefly-transmitted geminivirus. Plant Dis. 74:81.
5. Brown, J. K., and Nelson, M. R. 1988. Transmission, host range, and virus-vector relationships in chino del tomate virus, a whitefly-transmitted geminivirus from Sinaloa, Mexico. Plant Dis. 72:866-869.
6. Brown, J. K., and Poulos, B. T. 1990. Serrano golden mosaic virus: A newly identified whitefly-transmitted geminivirus of peppers and tomato in the United States and Mexico. Plant Dis. 74:720.
7. Crespi, S., Accotto, G. P., Caciagli, P., and Gronenborn, B. 1991. Use of digoxigenin-labelled probes for detection and host-range studies of tomato yellow leaf curl geminivirus. Res. Virol. 142:283-288.
8. Dellaporta, S. L., Woods, J., and Hicks, J. B. 1983. A plant minipreparation. Version II. Plant Mol. Biol. Rep. 1:19-21.
9. Dodds, J. A., Lee, J. G., Nameth, S. T., and Laemmlen, F. F. 1984. Aphid- and whitefly-transmitted cucurbit viruses in Imperial County, California. Phytopathology 74:221-225.
10. Faria, J. C., Gilbertson, R. L., Hanson, S. F., Morales, F. J., Ahlquist, P., Loniello, A. O., and Maxwell, D. P. 1994. Bean golden mosaic geminivirus type II isolates from the Dominican Republic and Guatemala: Nucleotide sequences, infectious pseudorecombinants, and phylogenetic relationships. Phytopathology 84:321-329.

11. Flock, R. A., and Mayhew, D. E. 1981. Squash leaf curl, a new disease of cucurbits in California. Plant Dis. 65:75-76.
12. Gallegos, H. M. L. 1978. Enchinamiento del tomate (chino disease of tomato). Page 119 in: Enfermedades de cultivos en el estado de Sinaloa. Secretaria de Agricultura y Recursos Hidraulicos, Sinaloa, Mexico.
13. Garzón-Tiznado, J. A., Torres-Pacheco, I., Ascencio-Ibañez, J. T., Herrera-Estrella, L., and Rivera-Bustamante, R. F. 1993. Inoculation of peppers with infectious clones of a new geminivirus by a biolistic procedure. Phytopathology 83:514-521.
14. Lazarowitz, S. G. 1991. Molecular characterization of two bipartite geminiviruses causing squash leaf curl disease: Role of viral replication and movement functions in determining host range. Virology 180:70-80.
15. Murphy, F. A., Fauquet, C. M., Bishop, D. H. L., Ghabrial, S. A., Jarvis, A. W., Martelli, G. P., Mayo, M. A., and Summers, M. D. 1995. Classification and nomenclature of viruses: Sixth Report of the International Committee on Taxonomy of Viruses. Arch. Virol. Suppl. 10. Springer-Verlag, New York.
16. Navot, N., Ber, R., and Czosnek, H. 1989. Rapid detection of tomato yellow leaf curl virus in squashes of plants and insect vectors. Phytopathology 79:562-568.
17. Padidam, M., Beachy, R. N., and Fauquet, C. M. 1995. Classification and identification of geminiviruses using sequence comparisons. J. Gen. Virol. 76:249-262.
18. Paplomatas, E. J., Patel, V. P., Hou, Y.-M., Noueiry, A. O., and Gilbertson, R. L. 1994. Molecular characterization of a new sap-transmissible bipartite genome geminivirus infecting tomatoes in Mexico. Phytopathology 84:1215-1224.
19. Polston, J. E., Hiebert, E., McGovern, R. J., Stansly, P. A., and Schuster, D. J. 1993. Host range of tomato mottle virus, a new geminivirus infecting tomato in Florida. Plant Dis. 77:1181-1184.
20. Rojas, M. R., Gilbertson, R. L., Russell, D. R., and Maxwell, D. P. 1993. Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. Plant Dis. 77:340-347.
21. Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular Cloning. A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
22. Sanger, F., Nicklen, S., and Coulson, A. R. 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A. 74:5463-5467.
23. Stenger, D. C., Duffus, J. E., and Villalon, B. 1990. Biological and genomic properties of a geminivirus isolated from pepper. Phytopathology 80:704-709.
24. Torres-Pacheco, I., Garzón-Tiznado, J. A., Herrera-Estrella, L., and Rivera-Bustamante, R. F. 1993. Complete nucleotide sequence of pepper huasteco virus: Analysis and comparison with bipartite geminiviruses. J. Gen. Virol. 74:2225-2231.