Biological and Genomic Properties of a Geminivirus Isolated from Pepper

D. C. Stenger, J. E. Duffus, and B. Villalon

Address of first and second authors: USDA-ARS, 1636 East Alisal St., Salinas, CA, 93905. Address of third author: Texas Agricultural Experiment Station, Weslaco, TX, 78596.

We acknowledge D. M. Bisaro for providing TGMV DNA clones, G. L. Fail for electron microscopy, and G. E. Middleton for technical assistance.

Accepted for publication 18 January 1990 (submitted for electronic processing).

ABSTRACT

Stenger, D. C., Duffus, J. E., and Villalon, B. 1990. Biological and genomic properties of a geminivirus isolated from pepper. Phytopathology 80:704-709.

A geminivirus causing leaf curl and distortion symptoms was isolated from pepper (*Capsicum annuum*) cultivated in Texas. The Texas pepper geminivirus (TPGV) was transmitted persistently by *Bemisia tabaci* and was also transmitted mechanically to species of the solanaceae. Electron microscopy of purified virions revealed typical geminate particles. Extracts from infected plants, but not uninfected plants, contained a putative replicative form (RF) DNA species of 2.6 kb that was double stranded, circular, and supercoiled. Viral RF DNA, linearized by digestion with the restriction enzymes *Eco*RI or *Hin*dIII, was cloned into *Escherichia coli* plasmid pUC 8. Analysis of cloned DNA by Southern hybridization and restriction endonuclease mapping indicates that two distinct species were cloned from RF DNA. One TPGV DNA hybridized with DNA A of tomato golden mosaic virus (TGMV); however, neither TPGV DNA hybridized with TGMV DNA B. Infectivity assays using cloned TPGV DNAs demonstrated that both DNA species were required for systemic infection of test plants. These results indicate that TPGV is a typical whitefly-transmitted, bipartite genome geminivirus not previously known to occur in the United States.

A new disease of pepper (*Capsicum annuum* L.) was observed in Texas during November of 1987. Affected plants displayed systemic leaf curl and distortion symptoms that suggested a viral etiology. Occurrence of the disease was associated with high populations of the whitefly, *Bemisia tabaci* (Genn.), an insect known to transmit geminiviruses (7). These initial observations suggested that a geminivirus may be the causal agent of this disease.

Geminiviruses are a unique group of plant viruses which have a twin-icosahedral particle morphology (2,10,22,32). The genome structure of geminiviruses is also novel, consisting of one or two species of circular, single-stranded DNA ranging in size from 2.5–3.0 kb (1,9,11,13,15,26,27). Geminivirus genomes replicate via a double-stranded, supercoiled replicative form (RF) DNA that is not encapsidated, but which can be isolated from infected plants (27,28,31). Geminivirus RF DNA has been cloned into *Escherichia coli* plasmid vectors (1,19,27), and the cloned genomes are infectious when mechanically inoculated to host plants (12,25,27,29). Thus, geminiviruses are excellent systems with which to investigate plant molecular biology and viral pathogenesis (14,18).

In this paper, we describe experiments that demonstrate the viral etiology of the new pepper disease. We also characterize the biological and genomic properties of the etiological agent that we have given the provisional name Texas pepper geminivirus (TPGV).

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 1990.

MATERIALS AND METHODS

Virus transmission and maintenance. TPGV-infected *C. annuum* 'Jupiter' or 'Jalapeno' were used as source plants for whitefly transmission and host-range experiments. Nonviruliferous *B. tabaci* were reared under conditions described previously (5). Groups of approximately 100 whiteflies given acquisition access periods of 24 hr and inoculation access periods of 24–72 hr were used for insect transmission of TPGV from source plants to test plants. Infected leaves were ground in 3–10 vol of 50 mM potassium phosphate buffer (pH 7.0), containing 0.1% 2-mercaptoethanol and 0.1% Celite to prepare inocula for mechanical transmission experiments.

Virion and RF DNA purification. TPGV virions were purified from systemically infected leaves of mechanically inoculated *Nicotiana benthamiana* Domin by the method of Stein et al (30) and separated from remaining host material by centrifugation in Cs_2SO_4 equilibrium gradients. Purified preparations were examined for virus particles by electron microscopy after staining with uranyl acetate. Infectivity experiments using purified virus inocula were conducted by mechanical transmission or by feeding virus preparations to groups of whiteflies through Parafilm membranes (5). RF DNA was isolated as described by Sunter et al (31) and separated from host and single-stranded DNA by centrifugation in CsCl-ethidium bromide gradients (20).

Molecular cloning. TPGV RF DNA was linearized by digestion with *Eco*RI or *Hin*dIII and ligated to plasmid pUC8 previously linearized with the same enzyme. Recombinant plasmids were used to transform *E. coli* strain JM 83 made competent by the calcium-chloride method (20). Transformants containing recombinant plasmids were selected as described by Vieira and Messing (33). Plasmids were purified from broth cultures by a boiling lysis procedure, followed by equilibrium centrifugation in CsCl-ethidium bromide gradients (20). Insert sizes were determined by electrophoresis of plasmids digested with the same restriction enzymes used during the cloning procedure. Recombinant plasmids containing insert DNA of the same size as TPGV RF DNA were confirmed by Southern hybridization using TPGV RF DNA as probe.

Characterization of Cloned DNAs. Restriction endonuclease maps of cloned TPGV-DNA inserts were constructed by single and double digestion of recombinant plasmids (20) using the following enzymes: *Apa*I, *Bam*HI, *Bgl*II, *Cla*I, *Dra*I, *Eco*RI, *Fsp*I, *Hin*dIII, *Kpn*I, *Pst*I, *Nco*I, *Nsi*I, *Sal*I, *Sca*I, *Spe*I, *Sph*I, *Sna*BI, *Ssp*I, *Sst*I, and *Xba*I. The sequence relatedness among TPGV-DNA inserts or plasmid pAT153 containing full-length inserts of tomato golden mosaic virus (TGMV) DNAs A and B (pBH404 and pBH602, respectively; 1,12) was evaluated by Southern hybridization. All hybridizations were conducted at 42 C in 50% formamide using ^{32}P-labeled, nick-translated recombinant plasmids or RF DNA as probes (20). Hybridized blots were washed under conditions of relaxed stringency (1× SSC [0.15 M NaCl, 0.015 M sodium citrate, pH 7.0], 45 C) or under conditions of high stringency (0.2× SSC, 65 C). Autoradiographs were prepared by exposing Kodak X-Omat film at −70 C with an intensifying screen.

Infectivity of cloned DNAs. Tandem head-to-tail dimers of cloned, TPGV-DNA inserts were constructed by cleaving recombinant plasmids with the same restriction endonucleases used for molecular cloning. Fragments were then religated and the resulting plasmids used to transform *E. coli* strain JM 83. Recombinant plasmids containing two copies of insert DNA were identified by agarose gel electrophoresis of native plasmids and the head-to-tail orientation of dimeric inserts confirmed by restriction endonuclease mapping. Infectivity of plasmids containing tandem dimers was determined by mechanical inoculation to test plants alone, or in combination, in 10 mM Tris-Cl, 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.6, with 0.1% Celite.

TABLE 1. Experimental host range of the Texas pepper geminivirus as determined by whitefly transmission

Test plant[a]	Symptoms[b]	Back assay[c]
Capsicum annuum L.		
'California Wonder'	VC,M	+
'Jupiter'	VC,D,St	+
'Jalapeno'	LC,D,VD,St	+
Chenopodium quinoa Willd.	NS	−
Citrullus lanatus (Thunb.) Matsum & Nakai		
'Tom Watson'	NS	−
Cucurbita maxima Duchesne		
'Black Magic'	NS	−
C. melo L.		
'Top Mark'	NS	−
Datura stramonium L.	Mo,VC,St	+
Gomphrena globosa L.	NS	−
Lycopersicon esculentum Mill.		
'#3'	Mo,LC,St	+
'Sunlight'	Mo,LC,St	ND
'743-T'	Mo,LC,St	ND
L. peruvianum (L.) Mill.	Mo,St	+
Malva parviflora L.	NS	−
Nicotiana benthamiana Domin	Mo,D,St,CS	+
N. clevelandii Gray	Mo,VC,LC,St	+
N. rustica L.	Mo	+
N. tabacum L.	Mo	+
Phaseolus vulgaris L.		
'Tendergreen'	NS	−
Physalis wrightii Gray	Mo,D,St	+

[a] Minimum of five test plants per species or cultivar inoculated with Texas pepper geminivirus (TPGV) by groups of 100 *Bemisia tabaci* given a 24-hr acquisition access period on source plants and 24–72 hr-inoculation access period on test plants.

[b] NS = no symptoms, Mo = mosaic, VC = vein clearing, D = distortion, VD = vein distortion, LC = leaf curl, St = stunting, CS = chlorotic spots on leaves inoculated mechanically, M = mild.

[c] Back assay performed by whitefly transmission of TPGV from test plants to cultivar Jalapeno pepper. Results of back assay reported as successful (+), unsuccessful (−), or not determined (ND).

RESULTS

Biological properties. The experimental host range of TPGV was restricted to species of the solanaceae (Table 1). For all susceptible species tested, systemic symptoms developed 2–3 wk postinoculation. 'Jalapeno' pepper, 'Jupiter' bell pepper, and tomato (*Lycopersicon esculentum* Mill.) cultivars infected with TPGV were stunted compared to uninfected controls. Symptoms observed on 'California Wonder' bell pepper were mild and often transitory. No symptoms were observed, and no virus was recovered from plants of seven different species representing five other plant families after inoculation with TPGV.

Serial insect transmission experiments indicated that TPGV is transmitted in a persistent fashion (Fig. 1). Groups of *B. tabaci* readily transmitted TPGV to test seedlings during the first 4 days following acquisition. Beyond 4 days, progressively fewer groups of *B. tabaci* transmitted TPGV, and by 11 days postacquisition only 10% of the groups transmitted the virus to test seedlings. Similar results were observed in a second retention experiment in which the virus was transmitted by groups of *B. tabaci* up to 10 days postacquisition (data not shown).

Mechanical transmission of TPGV was erratic and dependent upon the host species used as source and the host species inoculated. Pepper cultivars were successfully infected by mechanical inoculation using pepper or *N. benthamiana* as inocula; however, the transmission rate was generally less than 20%. Although readily infected by TPGV when transmitted by whiteflies, tomato was refractory to infection by mechanical inoculation. In contrast, mechanical transmission of TPGV from *N. benthamiana* to *N. benthamiana* consistently resulted in 80–100% transmission.

Virion properties. Examination of purified virus preparations by electron microscopy revealed typical geminate particles (Fig. 2). Purified virus particles ($30\mu g \cdot ml^{-1}$) were infectious when transmitted mechanically to *N. benthamiana*, with 10 out of 10

and six out of six plants becoming infected in two experiments. *B. tabaci* acquired purified virus ($30\mu g \cdot ml^{-1}$) through membranes and transmitted TPGV to five out of five 'Jalapeno' pepper plants. No transmissions were recorded for groups of nonviruliferous *B. tabaci* given access to buffer lacking TPGV particles.

Analysis of RF DNA. CsCl-ethidium bromide equilibrium-gradient centrifugation of nucleic acid extracted from TPGV-infected *N. benthamiana* resulted in the separation of two DNA-containing zones. The upper, major zone containing host DNA was present in extracts obtained from both infected and uninfected tissue. However, a lower gradient zone that contained DNA of a density similar to that of supercoiled plasmid DNA was found only in extracts from infected plants. Electrophoresis of DNA recovered from the lower zone indicated that the disease-specific DNA species was approximately 2.6 kb in size (Fig. 3). When digested with the restriction endonucleases *Eco*RI or *Hin*dIII, a portion of the RF DNA was linearized, as judged by the appearance of a DNA species with a mobility consistent with a full-length, linear molecule.

Characterization of cloned TPGV DNA. Two distinct classes of recombinant plasmids were recovered from transformed *E. coli*. The plasmids pTPV-E4 and pTPV-H1 were representative of the two classes of recombinants, and they contained 2.6 kb *Eco*RI or *Hin*dIII inserts, respectively (Fig. 4). In a Southern hybridization experiment (Fig. 4), the inserts of pTPV-E4 and pTPV-H1 both hybridized to ^{32}P-labeled TPGV RF DNA. TPGV RF DNA also hybridized to a lesser degree with the TGMV DNA A insert (2588 base pairs) of pBH404, but did not hybridize to the TGMV DNA B insert (2522 base pairs) of pBH602 or to fragments comprised solely of pUC8 or pAT153 sequences.

Restriction endonuclease maps constructed for the inserts of pTPV-E4 and pTPV-H1 were distinct (Fig. 5). Furthermore, the inserts of pTPV-E4 and pTPV-H1 share little sequence homology as judged by Southern hybridization (Fig. 6). Weak hybridization between the two inserts was restricted to the 1.4 kb *Xba*I/*Eco*RI fragment of pTPV-E4 and the 1.3 kb *Apa*I/*Apa*I fragment of pTPV-H1. Only pTPV-E4 hybridized to the TGMV DNA A insert of pBH404 (Fig. 7). Neither pTPV-E4 or pTPV-H1 hybridized to the TGMV DNA B insert of pBH602.

Infectivity experiments using cloned TPGV DNAs resulted in systemic infection only when test plants were inoculated with a mixture of pTPV-E4 and pTPV-H1 tandem dimers (Table 2). Symptoms resulting from infection using cloned DNA inoculum were identical to symptoms observed when the native virus was transmitted by whiteflies or mechanically. No infection of *N. benthamiana* was observed when inocula consisted of only one dimer plasmid. Progeny virus was transmissible by both *B. tabaci* and by mechanical inoculation. Hybridization assays detected both TPGV DNA species in extracts from infected *N. benthamiana* and *C. annuum* which had been inoculated with both dimers (data not shown).

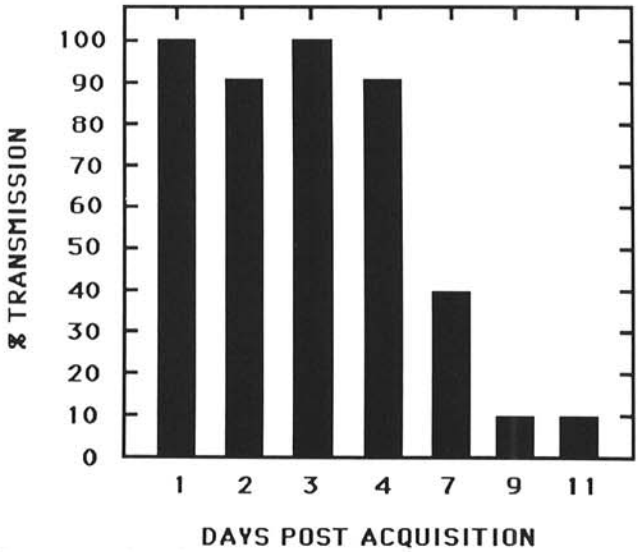

Fig. 1. Retention of Texas pepper geminivirus (TPGV) by groups of *Bemisi tabaci*. Nonviruliferous whiteflies were given a 24-hr acquisition access period on TPGV-infected 'Jalapeno' pepper, then serially transferred as 10 groups of 100 to 'Jalapeno' pepper test plants for 24–72-hr inoculation access periods.

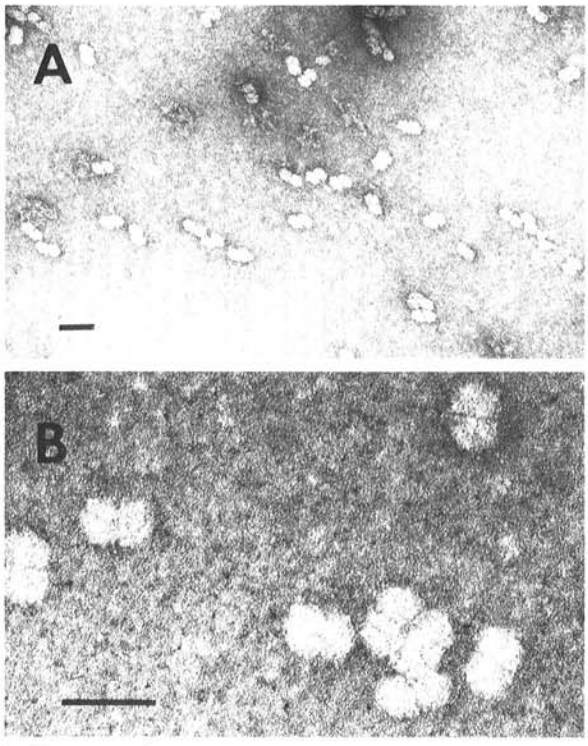

Fig. 2. Electron micrographs (A and B) of purified Texas pepper geminivirus virions stained with uranyl acetate. Bars represent 50 nm.

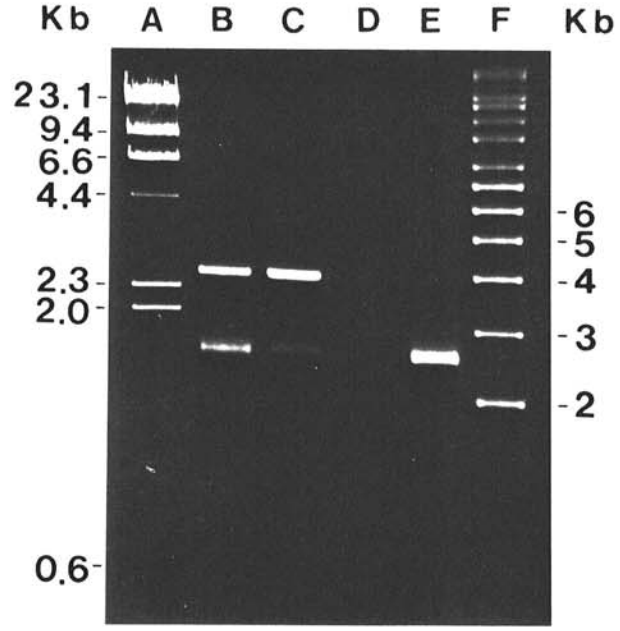

Fig. 3. Electrophoresis of Texas pepper geminivirus (TPGV) replicative form (RF) DNA in a 1% agarose gel. Samples applied to each lane were as follows: **A,** *Hin*dIII-digested λ DNA; **B,** *Eco*RI-digested TPGV RF DNA; **C,** *Hin*dIII-digested TPGV RF DNA; **D,** sample from uninfected plants; **E,** native TPGV RF DNA; and **F,** Bethesda Research Laboratories (Gaithersburg, MD) supercoiled DNA standards. Sizes of DNA standards in kilobases are indicated at the left for *Hin*dIII-digested λ DNA, and at the right for supercoiled DNA standards.

DISCUSSION

Etiology. The development of leaf curl and distortion symptoms accompanied by stunting in pepper experimentally inoculated with purified TPGV virions by whiteflies, or with cloned DNA by mechanical means, demonstrates that TPGV is capable of causing significant disease in the absence of other pathogens. The severe symptoms produced on tomato under experimental conditions suggest that TPGV may also have the potential to cause significant disease in this crop.

Pepper cultivars grown in southern Texas and northeastern Mexico during 1988 were severely affected by a mosaic disease presumed to be of viral etiology (B. Villalon, *unpublished*). Three isolates from Texas that induce mosaic symptoms were transmitted by *B. tabaci* and also mechanically to solanaceous hosts. These mosaic isolates appear to be closely related strains of TPGV, based upon dot-hybridization assays in which both pTPV-E4 and pTPV-H1 probes hybridized to extracts from plants infected with the mosaic isolates (D. C. Stenger and J. E. Duffus, *unpublished*).

Genomic properties. Analysis of recombinant plasmids containing full-length inserts of TPGV DNA indicated that the genome of TPGV consists of two distinct DNA species of similar size. The bipartite nature of TPGV DNA is similar to other whitefly-transmitted geminiviruses, including the following: TGMV (12), cassava latent virus (CLV) (25), bean golden mosaic virus (BGMV) (17), and squash leaf curl virus (SLCV) (18). The

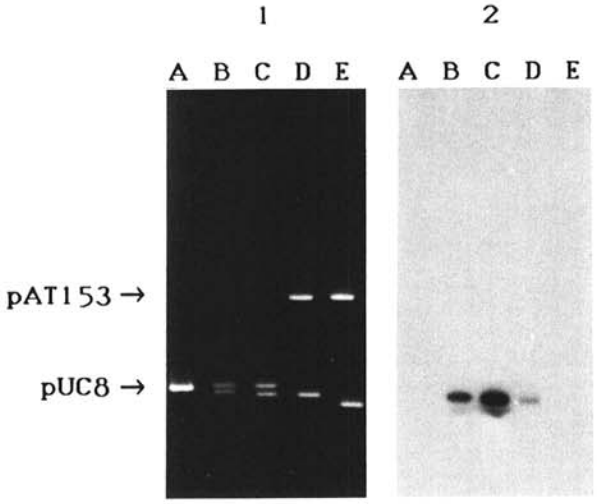

Fig. 4. Electrophoresis of plasmids containing full-length inserts of geminivirus DNA in a 1% agarose gel stained with ethidium bromide (panel 1), or a Southern blot (panel 2) of the same samples probed with ^{32}P-labeled Texas pepper geminivirus (TPGV) replicative form (RF) DNA and washed under conditions of relaxed stringency. Samples applied to each lane were as follows: **A,** *Eco*RI-digested pUC8; **B,** *Eco*RI-digested pTPV-E4; **C,** *Hin*dIII-digested pTPV-H1; **D,** *Eco*RI-digested pBH404; and **E,** *Cla*I-digested pBH602. DNA fragments consisting solely of the cloning vectors pUC8 and pAT153 are indicated at the left.

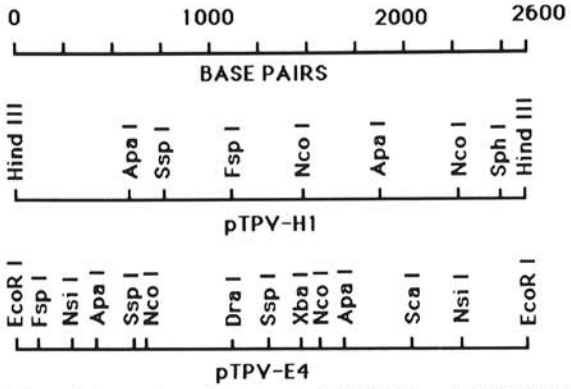

Fig. 5. Restriction endonuclease maps of pTPV-E4 and pTPV-H1 inserts. Endonucleases listed in materials and methods, but not shown in this figure, did not cleave Texas pepper geminivirus DNA inserts.

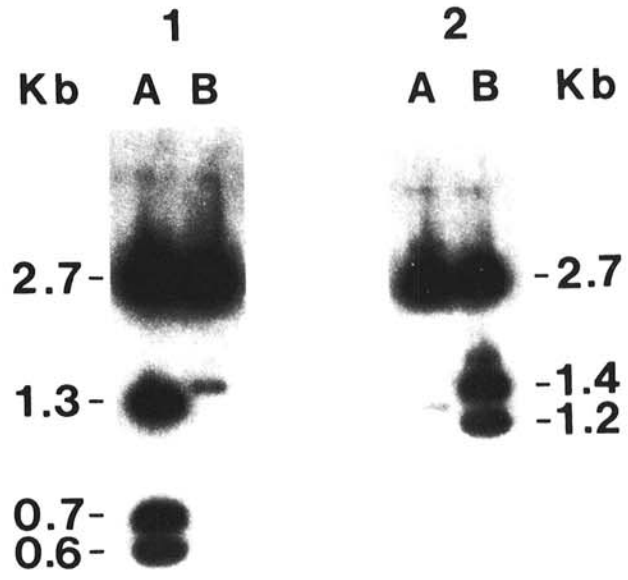

Fig. 6. Sequence relatedness of the inserts of Texas pepper geminivirus (TPGV) plasmids as determined by Southern hybridizations with pTPV-H1 (panel 1) or pTPV-E4 (panel 2) as probes. Samples denoted by letters were as follows: **A,** pTPV-H1 digested with *Hin*dIII and *Apa*I; and **B,** pTPV-E4 digested with *Eco*RI and *Xba*I. Blots were washed under conditions of high stringency. Sizes of DNA fragments in kilobases are indicated at left and right. Arrows denote fragments that hybridized weakly with heterologous probes. The 2.7-kb fragment is comprised solely of pUC8 sequences.

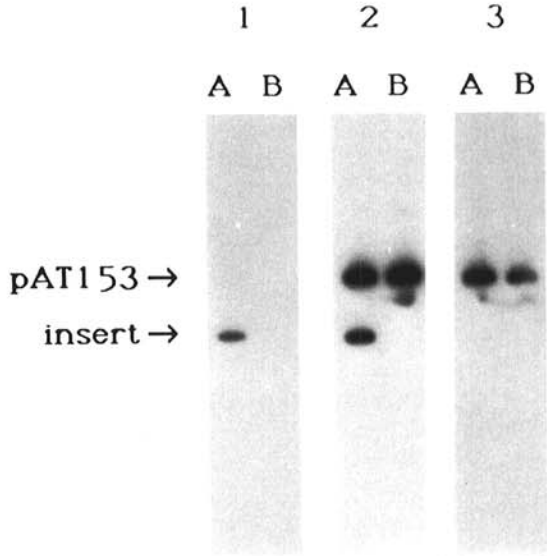

Fig. 7. Sequence relatedness of Texas pepper geminivirus (TPGV) and tomato golden mosaic virus (TGMV) DNAs as determined by Southern hybridizations of *Eco*RI-digested pBH404 (A) or *Cla*I-digested pBH602 (B) probed with ^{32}P-labeled TPGV replicative form (RF) DNA (panel 1), pTPV-E4 (panel 2), or pTPV-H1 (panel 3), and washed under conditions of relaxed stringency. Mobility of TGMV-cloned DNA inserts and linearized pAT153 is indicated at the left.

TABLE 2. Infectivity of Texas pepper geminivirus tandem dimer plasmids

Inoculum[a]	Host[b]	Infectivity[c]
pE4-D1	*Nb*	0/20
pE4-D1	*Nb*	0/10
pH1-D69	*Nb*	0/20
pH1-D69	*Nb*	0/10
pE4-D1 + pH1-D69	*Nb*	20/20
pE4-D1 + pH1-D69	*Nb*	10/10
pE4-D1 + pH1-D69	*Ca* 'California Wonder'	3/18
pE4-D1 + pH1-D69	*Ca* 'Jalapeno'	1/20

[a] Plasmids contained tandem dimers of the inserts from pTPV-E4 (pE4-D1) or pTPV-H1 (pH1-D69). Plants were inoculated with 10 μg DNA per plasmid in 50 μl 10 mM Tris-CL, 1 mM ethylenediaminetetraacetic acid, pH 7.6, containing 0.1% Celite.

[b] *Nb* denotes *Nicotiana benthamiana, Ca* denotes *Capsicum annuum.*

[c] Number of plants infected per number of plants inoculated.

insert of pTPV-E4 represents the TPGV-DNA species analogous to DNA A (or 1) of other bipartite geminiviruses, as determined by hybridization of pTPV-E4 with the TGMV DNA A insert of pBH404. The insert of pTPV-H1 appears to be analogous to DNA B (or 2) of the other bipartite geminiviruses because this DNA species was necessary for systemic infection of test plants inoculated with cloned DNA, and also because of the limited hybridization of specific fragments of pTPV-E4 and pTPV-H1. The weak hybridization that is observed between the inserts of pTPV-E4 and pTPV-H1 likely reflects the presence of a common-region sequence. The common region has been identified in other bipartite geminiviruses as a shared sequence of approximately 200–230 nucleotides that is nearly identical between the two DNA components of a single virus, but distinct between different viruses (18). Within the common region of the geminiviruses examined so far is a nine-nucleotide sequence, TAATATTAC. This invariant sequence fortuitously contains a recognition site for the restriction endonuclease *Ssp*I (AATATT). The restriction map of the pTPV-H1 viral insert contains a single *Ssp*I site, and this site is present within the 1.3-kb *Apa*I fragment that weakly hybridized with pTPV-E4. Thus, the common region of TPGV DNA B can be tentatively mapped to the region of the unique *Ssp*I site of pTPV-H1. In contrast, the viral insert of pTPV-E4 contains two *Ssp*I sites, with both sites mapping within the 1.4-kb *Xba*I/*Eco*RI fragment that specifically hybridized with pTPV-H1. Therefore, the common region of TPGV DNA A could be located at either *Ssp*I site of the pTPV-E4 insert.

The efficiency of infection with cloned geminivirus genomes can be improved by constructing plasmids containing head-to-tail tandem dimers (16,21). From such inocula, unit-length, circular DNAs are produced during infection. Intramolecular recombination or replicational escape (8) have been proposed as models to describe the process. Although the infectivity of excised linear monomers was not tested, infection of test plants using a mixture of tandem dimers indicates that the cloned TPGV DNAs are biologically active.

Relationships with other geminiviruses. The results presented here demonstrate properties that distinguish TPGV from other whitefly-transmitted geminiviruses. Biologically, TPGV resembles TGMV in having a host range restricted to solanaceous species, and in being mechanically transmissible. Although DNA A of TPGV has sequence homology with DNA A of TGMV, the lack of detectable sequence relatedness between DNA B components indicates that TPGV and TGMV are distinct viruses. Similar relationships between the DNA A components of distinct geminiviruses have been described by Roberts et al (24), who observed hybridization of DNA B components only among strains of the same virus. Distinctions between TPGV and other whitefly-transmitted geminiviruses are presently based upon biological properties. Tobacco leaf curl (23), chino del tomate (4), tomato yellow leaf curl (6), and pepper mild tigré (3) viruses share solanaceous host species with TPGV. Unlike TPGV, these viruses have not been reported to be transmitted by mechanical means, and some also infect species outside the solanaceae which are not hosts of TPGV. TPGV is distinct from BGMV (17) by having no host species in common, while the host ranges of CLV (25) and SLCV (18) only partially overlap with TPGV. However, the observed differences in host range and/or mechanical transmissibility between TPGV and other whitefly-transmitted geminiviruses does not provide sufficient information to determine whether TPGV is a unique strain of a previously described geminivirus or a new virus. For this reason, we prefer to use the provisional name Texas pepper geminivirus until the relationships of TPGV with other geminiviruses can be critically examined.

LITERATURE CITED

1. Bisaro, D. M., Hamilton, W. D. O., Coutts, R. H. A., and Buck, K. W. 1982. Molecular cloning and characterization of the two DNA components of tomato golden mosaic virus. Nucleic Acids Res. 10:4913-4922.
2. Bock, K. R. 1974. Maize streak virus. No. 133 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst., Assoc. Appl. Biol., Kew, Surry, England.
3. Brown, J. K., Campodonico, O. P., and Nelson, M. R. 1989. A whitefly transmitted geminivirus from peppers with tigré disease. (Abstr.) Plant Dis. 73:610.
4. Brown, J. K., and Nelson, M. R. 1988. Transmission, host range, and virus-vector relationships of chino del tomate virus, a whitefly-transmitted geminivirus from Sinaloa, Mexico. Plant Dis. 72:866-869.
5. Cohen, S., Duffus, J. E., Larson, R. C., Liu, H. Y., and Flock, R. A. 1983. Purification, serology, and vector relationships of squash leaf curl virus, a whitefly-transmitted geminivirus. Phytopathology 73:1669-1673.
6. Czosnek, H., Ber, R., Antignus, Y., Cohen, S., Navot, N., and Zamir, D. 1988. Isolation of tomato yellow leaf curl virus, a geminivirus. Phytopathology 78:508-512.
7. Duffus, J. E. 1987. Whitefly transmission of plant viruses. Pages 73-91 in: Current topics in vector research. Vol. 4. Springer Verlag, New York.
8. Elmer, J. S., Sunter, G., Gardiner, W. E., Brand, L., Browning, C. K., Bisaro, D. M., and Rogers, S. G. 1988. *Agrobacterium*-mediated inoculation of plants with tomato golden mosaic virus DNAs. Plant Mol. Biol. 10:225-234.
9. Goodman, R. M. 1977. Infectious DNA from a whitefly-transmitted virus of *Phaseolus vulgaris*. Nature 266:54-55.
10. Goodman, R. M., Bird, J., and Thongmeearkom, P. 1977. An unusual viruslike particle associated with golden yellow mosaic of beans. Phytopathology 67:37-42.
11. Haber, S. T., Howarth, A. J., and Goodman, R. J. 1983. Restriction map and Southern analysis of the bean golden mosaic virus genome. Virology 129:469-473.
12. Hamilton, W. D. O., Bisaro, D. M., Coutts, R. H. A., and Buck, K. W. 1983. Demonstration of the bipartite nature of the genome of a single-stranded DNA virus by infection with the cloned DNA components. Nucleic Acids Res. 11:7387-7396.
13. Hamilton, W. D. O., Stein, V. E., Coutts, R. H. A., and Buck, K. W. 1984. Complete nucleotide sequence of the infectious cloned DNA components of tomato golden mosaic virus: Potential coding regions and regulatory sequences. EMBO J. 3:2197-2205.
14. Harrison, B. D. 1985. Advances in geminivirus research. Annu. Rev. Phytopathol. 23:55-82.
15. Harrison, B. D., Barker, H., Bock, K. R., Guthrie, E. J., Meredith, G., and Atkinson, M. 1977. Plant viruses with circular single stranded DNA. Nature 270:760-762.
16. Hayes, R. J., Brough, C. L., Prince, V. E., Coutts, R. H. A., and Buck, K. W. 1988. Infection of *Nicotiana benthamiana* with uncut cloned tandem dimers of tomato golden mosaic virus DNA. J. Gen. Virol. 69:209-218.
17. Howarth, A. J., Caton, J., Bossert, M., and Goodman, R. M. 1985. Nucleotide sequence of bean golden mosaic virus and a model for gene regulation in geminiviruses. Proc. Natl. Acad. Sci. U.S.A. 82:3572-3576.
18. Lazarowitz, S. G. 1987. The molecular characterization of geminiviruses. Plant Mol. Biol. Rep. 4:177-192.
19. MacDowell, S. W., Macdonald, H., Hamilton, W. D. O., Coutts, R. H. A., and Buck, K. W. 1985. The nucleotide sequence of cloned wheat dwarf virus DNA. EMBO J. 4:2173-2180.
20. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning, a Laboratory Manual. Cold Spring Harbor Laboratory,

Cold Spring Harbor, NY. 545 pp.
21. Morinaga, T., Ikegami, M., Arai, T., Yazaki, K., and Miura, K. 1988. Infectivity of cloned tandem dimer DNAs of bean golden mosaic virus. J. Gen. Virol. 69:897-902.
22. Mumford, D. L. 1974. Purification of curly top virus. Phytopathology 64:136-139.
23. Osaki, T., and Inouye, T. 1981. Tobacco leaf curl virus. No. 232 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst. Assoc. Appl. Biol., Kew, Surry, England.
24. Roberts, I. M., Robinson, D. J., and Harrison, B. D. 1984. Serological relationships and genome homologies among geminiviruses. J. Gen. Virol. 65:1723-1730.
25. Stanley, J. 1983. Infectivity of the cloned geminivirus genome requires sequences from both DNAs. Nature 305:643-645.
26. Stanley, J., and Gay, M. R. 1983. Nucleotide sequence of cassava latent virus. Nature 301:260-262.
27. Stanley, J., Markham, P., Callis, R. J., and Pinner, M. S. 1986. The nucleotide sequence of an infectious clone of the geminivirus beet curly top virus. EMBO J. 5:1761-1767.
28. Stanley, J., and Townsend, R. 1985. Characterization of DNA forms associated with cassava latent virus infection. Nucleic Acids Res. 13:2189-2206.
29. Stanley, J., Townsend, R., and Curson, S. J. 1985. Pseudo-recombinants between cloned DNAs of two isolates of cassava latent virus. J. Gen. Virol. 66:1055-1061.
30. Stein, V. E., Coutts, R. H. A., and Buck, K. W. 1983. Serological studies on tomato golden mosaic virus, a geminivirus. J. Gen. Virol. 64:2493-2498.
31. Sunter, G., Buck, K. W., and Coutts, R. H. A. 1985. S1-sensitive sites in the supercoiled double-stranded form of tomato golden mosaic virus component B: Identification of regions of potential alternative secondary structure and regulatory function. Nucleic Acids Res. 13:4645-4659.
32. Thomas, J. E., and Bowyer, J. W. 1980. Properties of tobacco yellow dwarf and bean summer death viruses. Phytopathology 70:214-217.
33. Vieira, J., and Messing, J. 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene 19:259-268.