Complete Nucleotide Sequence of an Infectious Clone of a Mild Isolate of Tomato Yellow Leaf Curl Virus (TYLCV)

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ABSTRACT

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Single-stranded DNA extracted from virions of the originally described tomato yellow leaf curl virus (TYLCV) served as a template for in vitro synthesis of its double-stranded form. Analysis with different restriction enzymes yielded fragments with an overall size of approximately 5.6 kb, twice the suggested virus genome unit size. Based on restriction analysis and hybridization tests, it is suggested that the first described culture of TYLCV consists of two viral DNA populations reflecting some slight sequence differences. Agroinoculation of TYLCV hosts with one of the cloned viral DNA populations resulted in systemic infection, but symptoms on some tomato varieties were significantly milder than those induced by the native virus. Based on sequence data, the genomic organization of the mild infectious clone was found to be the same as described before for another Israeli isolate. However, the nucleotide sequence of its intergenic noncoding region, the putative replicase, and open reading frame C4 have only 78, 87, and 76% homology, respectively, compared with the formerly described Israeli severe TYLCV isolate.

Additional keyword: geminiviruses

The viral nature of tomato yellow leaf curl disease and its association with the whitefly vector *Bemisia tabaci* (Gennadius) was first described in Israel by Cohen and Harpaz in 1964 (5). The virus infects plants from six botanical families, but its major damage is to tomato crops (4). A unique interaction of the virus with *B. tabaci* was termed "periodic acquisition" (5). Later, the virus was isolated, characterized, and assigned as a member of the geminivirus group (7). Tomato yellow leaf curl virus (TYLCV)-like viruses were reported from countries around the Mediterranean Basin, Africa, the Far East, and Australia (1,4,6,10,13,21). In some cases, their nucleotide sequences have been determined (1,6,10,13,19). The nucleotide sequence of a field isolate of a TYLCV-like virus from Israel was published recently (19), and in contrast to other whitefly-transmitted bipartite geminiviruses (17), it possesses a single genomic component (19). Unfortunately, the identification of all the above-mentioned viruses as TYLCV was based essentially on the disease syndrome in tomato rather than on serology, biological characteristics, and interaction with the vector as recommended (11). The current study was initiated to characterize the genome of the originally described TYLCV isolate, which has been maintained in culture in our laboratory for the last 25 yr, and its biological characteristics were studied and described (4).

MATERIALS AND METHODS

Plant growth and virus isolation. TYLCV was propagated in *Datura stramonium* L., and inoculations were carried out using the whitefly vector, *B. tabaci.* The virus was isolated as described by Czosnek et al (7).

Virus DNA extraction. DNA was extracted from partially purified viral preparations by proteinase K–sodium dodecyl sulfate (SDS) treatment, followed by phenol-chloroform extraction (7).

Whitefly maintenance. Colonies were reared on cotton plants (*Gossypium hirsutum* L.) placed in muslin-covered cages. The cages were kept in an insectary greenhouse.

Virus transmission tests. TYLCV was transmitted using adult females of *B. tabaci,* as described previously (5). Membrane acquisition of purified virus preparations by whiteflies was as described previously (4).

Synthesis of TYLCV dsDNA. Synthesis was done using the Amersham kit for "cDNA synthesis system plus" (Amersham, UK). Random hexanucleotide primers initiated the in vitro synthesis using the purified viral ssDNA as a template.

DNA analysis. DNA was electrophoresed in 1% agarose gels in TAE buffer (40 mM TrisCl, 5 mM sodium acetate, and 0.4 mM EDTA) and transferred to nitrocellulose (Schleicher & Schuell, Germany) or nylon RP biotrace membrane (Gelman Sciences, Israel) by Southern transfer (24) or alkaline transfer (20), respectively. When the Southern procedure was employed, DNA was denatured by treating the gels with sodium hydroxide followed by neutralization in 0.5 M TrisCl, pH 7.5, prior to transfer. Hybridizations were performed according to the manufacturer's recommendation (Gelman Sciences, Ann Arbor, MI) in the presence of dextran sulfate and formamide.

Plasmids and cloning. A bluescript plasmid (KS(+), Stratagene, La Jolla, CA) served as a cloning vector. Plasmid DNA was purified from *Escherichia coli* by the alkaline lysis method (3) and further purified by centrifugation to equilibrium on cesium chloride density gradients containing ethidium bromide. The dsDNA form of TYLCV obtained by in vitro synthesis was digested with restriction nucleases and ligated into the multiple cloning site polylinker of the linearized plasmid. DNA manipulations were conducted as described by Maniatis et al (18). *E. coli* JM101 was used as recipient for transformation, and colonies were screened for recombinants by color selection.

Clones of components A and B of bean golden mosaic virus (BGMV) in pGEMEX (Promega, Madison, WI) were kindly provided by E. Hiebert, University of Florida, Gainesville.

Preparation of radiolabeled probes. ^{32}P-labeled RNA transcripts of cloned viral DNA were prepared in vitro by T7 or

T3 RNA polymerase according to the manufacturer's instructions (Stratagene).

Sequencing of TYLCV genomic component. DNA sequencing was done by the dideoxy chain termination method described by Sanger et al (23). Sequenase version 2.0 T7 DNA polymerase kit (U.S. Biochemical, Cleveland, OH) served this purpose. The sequencing was carried out on a double-stranded template using the alkaline denaturation procedure (15).

Subclones of two independent clones were obtained by the combined action of *Cla*I and *Acc*I. The resulting four fragments were religated into the *Acc*I or *Acc*I/*Cla*I digested KS(+) bluescript plasmid. The universal T7 and T3 primers served to start the sequencing of each subclone on both of its complementary strands. To obtain sequence data of the internal regions of the subclones, specific oligonucleotides were synthesized (Biotechnology General Ltd., Israel). Special primers were synthesized to verify the identity of the sequence at the junction regions of the subclones with the sequence of the equivalent regions in the full-length clone. Assembly and analysis of the database were done using the following programs: Nucaln, D. J. Lipman and W. J. Wilbur, Mathematical Research Branch, NIADDK, National Institute of Health, Bethesda, MD; Damien, L. L. Domier, Department of Plant Pathology, University of Wisconsin, Madison (*personal communication*); and GCG sequence analysis package (8).

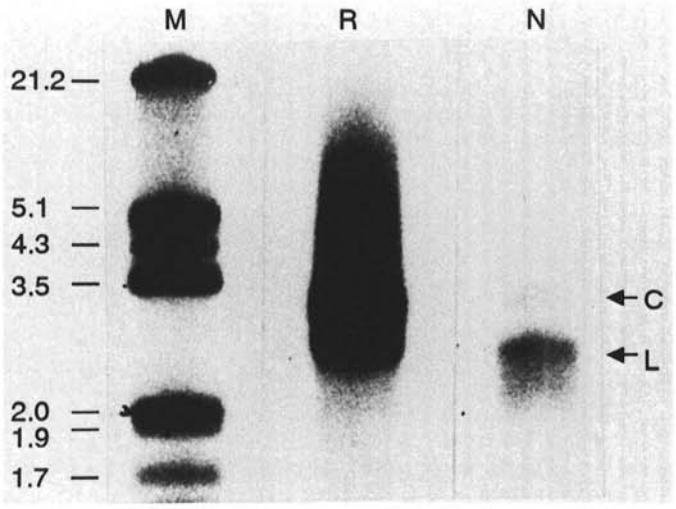

Fig. 1. Autoradiogram of tomato yellow leaf curl virus (TYLCV) dsDNA synthesized in vitro and separated on 1% agarose gel. TYLCV virion ssDNA served as a template in the presence of random primers (R) or in the absence of primers (N). The ^{32}P-labeled reaction products were transferred to an RP Biotrace nylon membrane before exposure to an X-ray film. (L) indicates linear forms of the dsDNA, (C) indicates the circular virus dsDNA, and (M) ^{32}P-labeled Lambda DNA, *Eco*RI and *Hin*dIII digested, was used as a size marker.

Extraction of plant total DNA. DNA was extracted from TYLCV-infected *D. stramonium* plants and from healthy plants as described previously (22).

Restriction analysis of TYLCV replicative form. DNA preparations obtained from TYLCV-infected *D. stramonium* plants were treated with mung bean nuclease (Boehringer GmbH, Mannheim, Germany) to remove the viral ssDNA forms. Preparations were then subjected to restriction enzyme digestion, separated on 1% agarose gel, transferred to a nylon membrane, and probed by a riboprobe obtained from the viral DNA cloned in a KS(+) bluescript plasmid (pTY2.8, pTY1.7, pTY1.1).

Analysis of TYLCV coat protein. Tissue samples from agroinoculated plants were crushed in the presence of liquid nitrogen and stirred with the virus extraction buffer (7) at a ratio of 3:1 (v/w) for 1 h. Preparations were centrifuged for 10 min at 4,000 *g*. Supernatants were removed, mixed, and boiled with gel sample buffer for 5 min and separated on 10% SDS polyacrylamide gels (16). Protein bands were transferred to reinforced nitrocellulose (Schleicher & Schuell) by a semidry electroblotting system according to the manufacturer's instructions (Gelman Sciences, Ann Arbor). Immunostaining of the membranes was carried out as described by Hibi and Saito (12).

Construction of clones for agroinoculation. Dimers of the 2.8-kb cloned *Cla*I viral fragment were ligated into the *Cla*I site of a bluescript plasmid which was propagated in *E. coli* JM101. The plasmid was cut by *Xho*I, and the 3′ overhang was filled in to produce a blunt end to which *Hin*dIII linkers were ligated. The dimer construct, now having *Hin*dIII sites at each end, was subcloned into the *Hin*dIII site of pBI121 (Clontech, Palo Alto, CA). The recombinant plasmid pBI121 was propagated in *E. coli* HB101 and shuttled into *Agrobacterium tumefaciens* LAB4404 (Clontech) by triparental mating in the presence of the helper plasmid pRK2013 (2).

Agroinoculation tests. Agrobacterium cultures were grown in medium for 48 h at 28 C (2) before being inoculated to healthy test plants at the four- to six-leaf stage. The bacterial preparation was injected into the plant crown with a 2-ml syringe using a 26G1/2 needle.

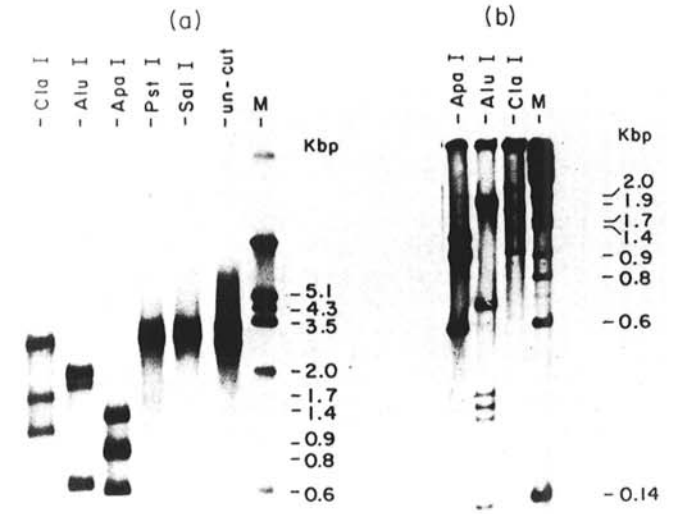

Fig. 2. Restriction analysis of tomato yellow leaf curl virus dsDNA synthesized and ^{32}P-labeled in vitro. Reaction products were separated **A,** on 1% agarose gel or **B,** on 5% polyacrylamide gel and transferred to nitrocellulose membranes, which were exposed to an X-ray film. ^{32}P-labeled *Eco*RI- and *Hin*dIII-digested Lambda DNA served as a marker (M).

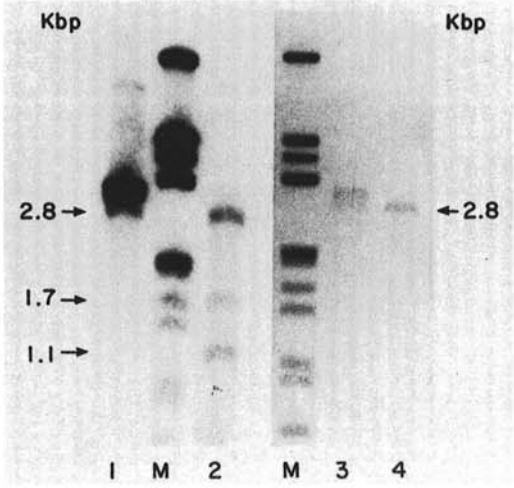

Fig. 3. Restriction analysis of tomato yellow leaf curl virus dsDNA extracted from tissues infected with the native virus. The viral ssDNA form was removed from the preparation by mung bean nuclease. (1) Untreated preparation, (2) *Cla*I-treated preparation, (3) untreated preparation, (4) *Sal*I-treated preparation, (M) *Hin*dIII- and *Eco*RI-restricted Lambda DNA marker.

RESULTS

In vitro synthesis of TYLCV dsDNA. DNA polymerase-directed second-strand synthesis was performed using viral ssDNA as a template. When synthesis was carried out with random synthetic primers, radioactivity accumulated in two bands. The upper band represents the virus circular dsDNA form, while the band with the greater mobility resembles linear viral dsDNA molecules of approximately 2.8 kb (Fig. 1). When a parallel reaction was carried out in the absence of external primers, no appreciable level of radioactivity was accumulated in the circular dsDNA band (Fig. 1).

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ATGGTCAATG AGTACCGATT GACCAAGATT TTTACACTTA TCCCTGGTGT ATCGGTACTC     60
AATATATAGT GAGTACCAAA TGGCATTTTG GTAATAACAT AAAAGTACAT TGCAATTCAA    120
AATTCAAATA AAAAAATCAA ATCATTAAAG CCGGCCATCC GTATAATATT ACCGGAGCTC    180
GGCGCCTTTT TCTTTTATGC TGGTCCCCAC GAGGTTCCAC TGACGTCACT GTCAACCAAT    240
CAAATTGCAT CCTCAAACGT TAGATAGTGT TTATTTGTCT TTATATACTT GGTCCCCAAG    300
TTTTTTGTCT TGCAATATGT GGGACCCACT TCTAAATGAA TTTCCTGAAT CTGTTCACGG    360
ATTTCGTTGT ATGTTAGCTA TTAAATATTT GCAGTCCGTT GAGGAAACTT ACGAGCCCAA    420
TACATTGGGC CACGATTTAA TTAGGGATCT TATATCTGTT GTAAGGGCCC GTGACTATGT    480
CGAAGCGACC AGGCGATATA ATCATTTCCA CGCCCGTCTC GAAGGTTCGC CGAAGGCTGA    540
ACTTCGACAG CCCATACAGC AGCCGTGCTG CTGTCCCCAT TGTCCAAGGC ACAAACAAGC    600
GACGATCATG GACGTACAGG CCCATGTACC GAAAGCCCAG AATATACAGA ATGTATCGAA    660
GCCCTGATGT TCCCCGTGGA TGTGAAGGCC CATGTAAAGT CCAGTCTTAT GAGCAACGGG    720
ATGATATTAA GCATACTGGT ATTGTTCGTT GTGTTAGTGA TGTTACTCGC GGATCTGGAA    780
TTACTCACAG AGTGGGTAAG AGGTTCTGTG TTAAATCGAT ATATTTTTTA GGTAAAGTCT    840
GGATGGATGA AAATATCAAG AAGCAGAATC ACACTAATCA GGTCATGTTC TTCTTGGTCC    900
GTGATAGAAG GCCCTATGGA AACAGCCCAA TGGATTTTGG ACAGGTTTTT AATATGTTCG    960
ATAATGAGCC CAGTACCGCA ACCGTGAAGA ATGATTTGCG GGATAGGTTT CAAGTGATGA   1020
GGAAATTTCA TGCTACAGTT ATTGGTGGGC CCTCTGGAAT GAAGGAACAG GCATTAGTTA   1080
AGAGATTTTT TAAAATTAAC AGTCATGTAA CTTATAATCA TCAGGAGGCA GCCAAGTACG   1140
AGAACCATAC TGAAAACGCC TTGTTATTGT ATATGGCATG TACGCATGCC TCTAATCCAG   1200
TGTATGCAAC TATGAAAATA CGCATCTATT TCTATGATTC AATATCAAAT TAATAAAATT   1260
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CATGATCAAC TGCTCTGATT ACATTGTTAA TGGAAATTAC ACCAAGACTA TCTAAATACT   1380
TAATAACTTC ATATCTAAAT ACTCTTAAGA AACGACCAGT CTGAGGCTGT AATGTCGTCC   1440
AAATTCGGAA GTTGAGAAAA CATTTGTGAA TCCCCATTAC CTTCCTGATG TTGTGGTTGA   1500
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TTATCTTGAA ATATAGGGGA TTGTTTATCT CCCAGATAAA AACGCCATTC TCTGCCTGAG   1620
GAGCAGTGAT GAGTTCCCCT GTGCGTGAAT CCATGATTAC TGCAGTTGAG GTGGAGGTAG   1680
TATGAGCAGC CACAGTCTAG GTCTACACGC TTACGCCTTA TTGGTTTCTT CTTGGCTATC   1740
TTGTGTTGGA CCTTGATTGA TACTTGCGAA CAGTGGCTCG TAGAGGGTGA CGAAGGTTGC   1800
ATTCTTGAGA GCCCAATTTT TCAAGGATAT GTTTTTTTCT TCGTCTAGAT ATTCCCTATA   1860
TGAGGAGGTA GGTCCTGGAT TGCAGAGGAA GATAGTGGGA ATTCCCCCTT TAATTTGAAT   1920
GGGCTTCCCG TACTTTGTGT TGCTTTGCCA GTCCCTCTGG GCCCCCATGA ATTCCTTGAA   1980
GTGCTTTAAA TAATGCGGGT CTACGTCATC AATGACGTTG TACCACGCAT CATTACTGTA   2040
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CCACATTGTT TTGCCTGTTC TGCTATCACC CTCAATGATA ATACTGTTAG GTCTCCATGG   2160
CCGCGCAGCG GAAGACATGA CGTTCTCGGA CACCCATACT TCAAGTTCAT CTGGAACTTG   2220
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GGAAAGTGCT TCTTCGTTTG TTAAGGAGCA ATGAGGATAT GTGAGGAAAT AATTTTTGCA   2760
ATTTATTTGG AAGCGCTTAG GAGGAGCCAT
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Fig. 4. Nucleotide sequence of the mild clone pTY2.8. The sequence represents the virion sense DNA. The first nucleotide at the 5′ end was designated as nucleotide 1. EMBL Data Library accession no. X76319.

Restriction enzyme analysis of TYLCV DNA. ^{32}P-labeled virus dsDNA was synthesized in vitro and digested with restriction enzymes. The viral dsDNA was cleaved once by *Sal*I and *Pst*I, yielding a linear fragment of 2.8 kb (Fig. 2A), which was considered a genome unit length of TYLCV (19). Digestion with *Alu*I resulted in fragments of approximately twice the expected unit genome length (Fig. 2A and B). A similar total was obtained for the fragments produced by *Cla*I digestion (Fig. 2A and B) and *Acc*I (not shown). *Cla*I cleaved the viral DNA into three fragments, the largest corresponding in size to the expected full-length viral DNA (2.8 kb), while the two others were approximately 1.1 and 1.7 kb. *Apa*I digestion yielded three fragments with a total sum size of 2.8 kb (Fig. 2A and B), indicating that the two putative dsDNA populations had some sequence similarities.

*Cla*I digestion profiles of the TYLCV replicative form extracted from plants infected with the native virus were identical to those obtained from the in vitro synthesized dsDNA form (Fig. 3), confirming the authenticity of the latter.

Cloning of TYLCV. Synthesized TYLCV dsDNA was digested by *Cla*I. The digestion products were ligated into a KS(+) bluescript plasmid with insert sizes of 2.8 kb (pTY2.8), 1.7 kb (pTY1.7), and 1.1 kb (pTY1.1).

A riboprobe was produced from clones pTY2.8, pTY1.7, and pTY1.1 and used for cross-hybridization tests. The results of these experiments indicate that clones pTY2.8, pTY1.7, and pTY1.1 represent the 2.8 kb, 1.7 kb, and 1.1 kb fragments of the *Cla*I digested viral dsDNA respectively (data not shown). A riboprobe obtained from the cloned DNA A of BGMV hybridized with the above-mentioned three *Cla*I fragments under low stringency hybridization conditions. No hybridization was observed when a probe of BGMV DNA B was used under the same conditions (data not shown).

Sequence analysis of cloned TYLCV. Sequence data presented in Figure 4 show that the viral DNA (clone pTY2.8) comprises 2,790 nucleotides. The 5′ end of the noncoding intergenic region (IR) was designated as nucleotide 1.

TABLE 1. Homologies in the nucleotide sequence of a mild clone of tomato yellow leaf curl virus (TYLCV) (pTY2.8) and a severe Israeli isolate (TYLCV-I) (19)

ORF	Polarity	TY2.8 Start	TY2.8 Stop	TYLCV-I Start	TYLCV-I Stop	Homology (%)
V1	+	477	1,251	474	1,256	98
V2	+	317	665	314	656	100
C1	−	2,790	1,713	2,787	1,714	87
C2	−	1,802	1,400	1,805	1,400	99
C3	−	1,654	1,259	1,657	1,255	99
C4	−	2,632	2,342	2,636	2,343	76
IR	+	1	316	1	313	78

TABLE 2. Amino acid sequence similarity between putative translation products of a mild tomato yellow leaf curl virus (TYLCV) infectious clone (pTY2.8) and TYLCV isolates from Israel and other geographical regions

Virus[a]	Amino acid identity (%)[b] V1[c]	V2	C1	C2	C3	C4
TYLCV-I	98	100	87	97	97	47
TYLCV-E	98	97	89	97	97	50
TYLCV-Sic	88	82	79	67	69	62
TYLCV-S	86	90	80	68	64	58
TLCV	77	62	81	65	67	64

[a] TYLCV-I, Israeli isolate (19), TYLCV-E, Egyptian isolate (1), TYLCV-Sic, Sicilian isolate (6), TYLCV-S, Sardinian isolate (13), TLCV, Tomato leaf curl virus from Australia (10).

[b] Amino acid identity was calculated using the Nucaln program (K-tuple size 2, Window size 20, Gap penalty 4).

[c] The viral coat protein open-reading frames of the viruses have been defined as V1 for this comparison.

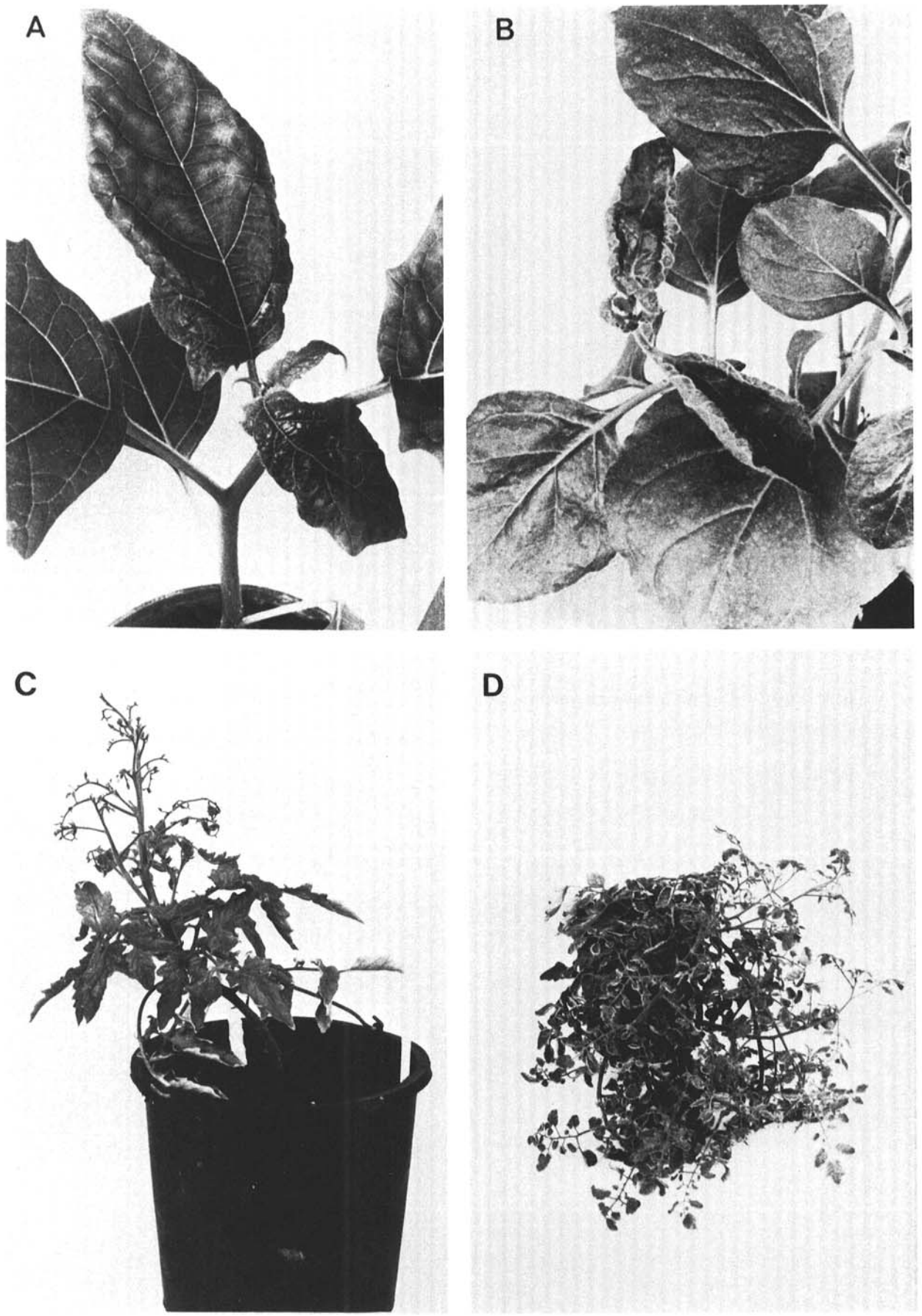

Fig. 5. Tomato yellow leaf curl virus symptoms on different host plants: Agroinoculation of **A,** *Datura stramonium* and **B,** *Nicotiana benthamiana* with the cloned DNA (pTY2.8). **C,** Tomato plants infected with the native virus by whitefly inoculation. **D,** Agroinoculation of *Lycopersicon esculentum* with the cloned DNA (pTY2.8).

The genomic organization of this clone was found to be the same as for another Israeli isolate of TYLCV (19). The genomic IR, open-reading frame (ORF) C1 (encoding the putative replicase), and ORF C4 have only 78, 87, and 76% identity, respectively, compared with a formerly described Israeli isolate of TYLCV (19). A 99–100% homology was found for the other ORFs of these isolates (Table 1).

The amino acid similarity between ORFs of clone pTY2.8 and the equivalent putative ORFs from other described TYLCV isolates is shown in Table 2. A high degree of similarity was found in all compared isolates for ORF V1 encoding the viral coat protein except TLCV. However, significant differences are shown for the ORFs located on the complementary strand (C1, C2, C3, and C4) (Table 2).

Agroinoculation assays with TYLCV cloned DNA. All our efforts to transmit TYLCV particles or the naked viral DNA by mechanical inoculation failed; therefore agroinoculation was used. Head-to-tail dimers of clone pTY2.8 were mobilized into *A. tumefaciens* and agroinoculated to different TYLCV host plants. Agroinfection in all of the following test plants was successful: *Nicotiana glutinosa* L., *Nicotiana benthamiana* Domin., *D. stramonium, Petunia* × *hybrida* Hort. Vilm.-Andr., and *Lycopersicon esculentum* Mill. No symptoms were observed in plants inoculated with *A. tumefaciens* containing binary vectors without the viral genome. TYLCV symptoms were observed in all tested host plants 15–25 days after inoculation (Fig. 5A and B). However, when infected tomato plants were left for a longer period, they seemed to enter a recovery stage, resulting in significantly milder symptoms (Fig. 5D). Plants infected with the native virus from our culture were degenerated in their apex, and their growth was inhibited (Fig. 5C). To confirm that this phenomenon is characteristic to the virus and not associated with the inoculation technique, whiteflies were introduced to agroinoculated plants for an acquisition access and then transferred to healthy plants for inoculation access. Whitefly inoculation led to mild symptoms similar to those obtained by the agroinoculation procedure. The presence of the viral nucleic acid in the agroinoculated plants was demonstrated by dot blot hybridization tests or by Southern blots of plant total DNA using a riboprobe derived from clone pTY2.8 (Fig. 6A and B). The presence of TYLCV coat protein subunits was confirmed by western blots (Fig. 6C). Moreover, we were able to demonstrate the presence of typical gemini particles in purified preparations obtained from agroinoculated plants (Fig. 6D). These particles were acquired and transmitted by whiteflies from purified preparations by the membrane feeding technique. *B. tabaci* was also able to transmit the virus from agroinoculated plants to tomato and *D. stramonium,* which subsequently exhibited TYLCV symptoms. To rule out the possibility of casual contamination as a cause of infection in the agroinoculation tests, DNA was extracted from virions obtained from plants infected by whitefly-mediated inoculation of the cloned and the native TYLCV. dsDNA was synthesized from both preparations, treated with *Cla*I, separated on 1% agarose gel, transferred to a membrane, and hybridized with a riboprobe produced from pTY2.8. As shown in Figure 7, the viral DNA originating from plants infected with the cloned virus yielded only a single *Cla*I fragment of 2.8 kb, while a profile with three fragments was obtained from virion DNA originating from plants infected with the native virus from our culture.

DISCUSSION

In vitro DNA polymerase-directed second-strand synthesis of TYLCV DNA has shown that TYLCV does not possess a self-priming capability (Fig. 1). This is in contrast to the monopartite leafhopper-transmitted geminiviruses, in which the encapsidated ssDNA is hydrogen-bonded to an oligonucleotide approximately 80 nucleotides in length, serving as primers for the synthesis of the virus complementary DNA strands (9,17).

Restriction analysis of TYLCV DNA derived from our culture

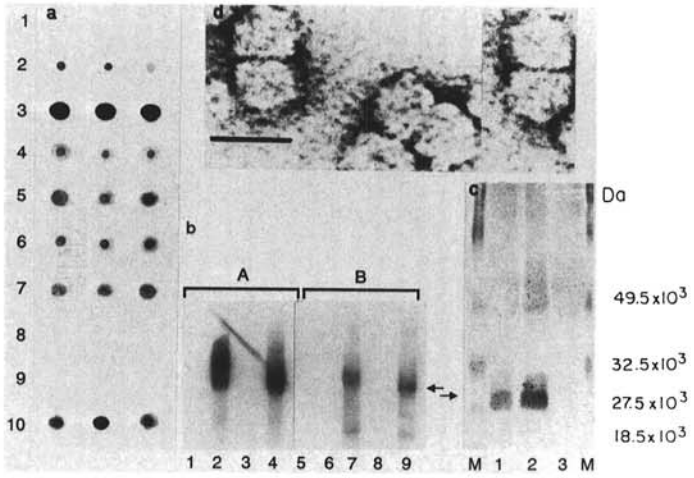

Fig. 6. Evidence for tomato yellow leaf curl virus (TYLCV) infection in plants agroinoculated with TYLCV-cloned DNA (pTY2.8): (a) Dot blot analysis of crude sap from agroinoculated plants: 1–2, *Nicotiana benthamiana*; 3–5, *Nicotiana glutinosa*; 6–7, tomato plants; 8–9, healthy tomato plants; 10, whitefly-inoculated plants. (b) Southern blot analysis of TYLCV-infected *Datura stramonium* plants: (A) Total DNA from plants infected by the native virus, separated on 1% agarose gel and probed with a riboprobe derived from clone pTY2.8 20. (1) DNase treated, (2) RNase treated, (3) S1 nuclease treated, (4) untreated, and (5) healthy plant. (B) Total DNA from plants agroinoculated with pTY2.8. DNA was separated and probed as in A. (6) DNase treated, (7) RNase treated, (8) S1 nuclease treated, and (9) untreated. (c) Western blot analysis of TYLCV coat protein subunits in TYLCV-infected *D. stramonium*: (1) agroinoculated with the infectious clone, (2) infected with the native virus, and (3) healthy. (M) Prestained sodium dodecyl sulfate-polyacrylamide gel electrophoresis molecular weight standards. (d) Micrograph of TYLCV particles purified from agroinoculated *N. glutinosa* plants. Preparation was stained in 2% uranyl acetate and analyzed by Jeol JEM-100XII electron microscope operating at 80 kV.

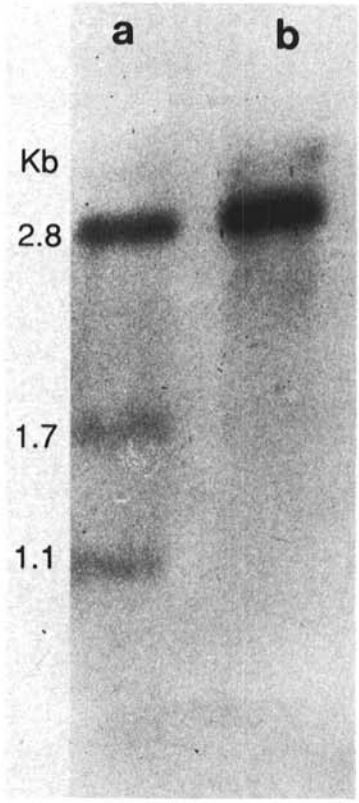

Fig. 7. *Cla*I digestion of in vitro synthesized tomato yellow leaf curl virus (TYLCV) dsDNA. Cleaved TYLCV dsDNA synthesized on an ssDNA template extracted from native virions (lane 1). Cleaved dsDNA synthesized on an ssDNA template, extracted from virions that were isolated from plants agroinoculated with the cloned DNA (lane 2).

clearly showed the presence of two genomic DNA populations: one carrying two *Cla*I sites, and the second, a single site for this enzyme. This is also supported by the restriction profiles of the viral DNA treated with *Alu*I and *Acc*I (Fig. 2). The presence of two viral DNA populations in the inoculum was always associated with severe infections, in contrast to the relatively mild symptoms induced by pTY2.8 representing one of these populations (Fig. 5). An attenuated infection caused by inoculation with component A of bicomponent geminiviruses was reported earlier (14,21). The dimorphism of TYLCV DNA derived from our culture is correlated with symptom severity and may indicate the need for a B component for normal severe symptoms. However, the strong cross-hybridization between the two DNA populations suggests that they share a high sequence homology, in contrast to the nonhomologous A and B components of the bipartite geminiviruses (17). A conceivable explanation for this phenomenon is that the two viral genomic DNA populations represent a mixture of two TYLCV strains differing in symptom severity. An additional support for this possibility is the ability of both DNA populations to hybridize with component A of BGMV, thus suggesting that both are carriers of the virus coat protein gene. These results confirm former reports on the monopartite nature of other whitefly-borne TYLCV-like viruses (10,13,19), which differ from the previously described whitefly-transmitted geminiviruses with two distinct genomic components (17).

The nucleotide sequence of pTY2.8 is almost identical in ORFs V1 (the putative coat protein gene), V2, C2, and C3 to the formerly described severe Israeli TYLCV isolate (Table 1) (19). However, marked differences exist in the sequence of the IR, C1 (the putative replicase gene), and C4 ORFs of these clones. Comparison of the deduced amino acids of the putative ORFs of pTY2.8 with those of other sequenced isolates of TYLCV shows a high similarity with TYLCV-I (19) and TYLCV-E (1). A much lower degree of similarity was found with TYLCV-Sic (6), TYLCV-S (13), and TLCV (10) (Table 2). A high degree of similarity in the viral coat protein of the compared viruses (Table 2) strongly implies a similar mode of insect transmission.

However, sequence comparison alone may not be a sufficient criterion for establishing a relationship among the different TYLCV-like viruses. The nature of these relations needs to be further established by comparative biological characterization.

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