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PARTIAL CHARACTERIZATION AND HOST RANGE OF TOMATO YELLOW LEAF CURL VIRUS IN SOUTH FLORIDA

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Abstract. **Tomato yellow leaf curl virus-Is (TYLCV-Is) is a whitefly-transmitted geminivirus that was first recognized in the Eastern Mediterranean region in 1939 and for the first time in South Florida in late July 1997. To verify identification of TYLCV-Is in Florida, the intergenic region and most of coding regions for both the replication protein (C1) and the pre-coat protein (V2) of an isolate from Florida were cloned, sequenced, and the sequences then compared to those of related geminivirus isolates from other countries in the Genbank database. Phylogenetic analyses indicated that a TYLCV isolate from Florida and all other isolates from the New World were related to one another and to an isolate from Israel, consistent with the hypothesis that they were introduced from the Eastern Mediterranean region. Analysis of the deduced amino acid sequences at the N-terminal region (the first 229 amino acid residues) of the C1 sequence indicated that the TYLCV isolate from Florida is most closely related to a Cuban isolate. Polymerase chain reaction (PCR) was used to survey the host range of TYLCV-Is in South Florida. A total of 1862 plants of more than 76 species in at least 35 families were tested by PCR. Besides tomato (*Lycopersicon esculentum*), the following species were positive in PCR tests: *Acalypha virginica, Amaranthus retroflexus, Begonia* sp., *Macroptilium lathyroides, Sonchus oleraceus* and *Nicotiana tabacum*. Except for *S. olereacus* and *N. tabacum*, these are all newly identified potentially natural hosts for TYLCV.**

Tomato yellow leaf curl virus (TYLCV-Is) was first reported in Israel in 1939 (Cohen and Harpaz, 1964). TYLCV-Is (family Geminiviridae, genus *Begomovirus*) is a whitefly-transmitted geminivirus species with a single genomic component. TYLCV-Is was first identified in Florida in late July 1997 (Polston et al., 1999). TYLCV can be very devastating to tomato production. For instance, two years after the introduction of TYLCV in the Dominican Republic in 1991, approximately 90% of the tomato production was destroyed by the disease (Alvarez et al., 1995; Nakhla et al., 1994). TYLCV causes curling, distortion and chlorosis of leaves, stunting of the plant, and a high rate of flower abscission. Plants infected in the first half of the growing season generally produce few or no fruits. Whiteflies spread the virus very efficiently (Mansour and Al-Musa, 1992).

TYLCV-Is has a broad host range as indicated by studies in Israel (Cohen and Nitzany, 1966) and Jordan (Mansour and Al-Musa, 1992). Altogether, at least 17 host species in nine families have been identified including the following families and species: Apiaceae (*Chaerogphyllum* sp.), Asclepiadaceae (*Cynanchum acutum*), Asteraceae (*Sonchus oleraceae*), Fabaceae (*Lens esculenta* and *Phaseolus vulgaris*), Gentianaceae (*Eustoma grandiflorum*), Malvaceae (*Malva nicaensis* and *M. parviflora*), Nyctaginaceae (*Boerhavia erecta*), Solanaceae (*Datura stramonium, Lycopersicon esculentum, Hyoscyamus desertorum, Nicotiana glutinosa, N. tabacum, Petunia* sp. and *Solanum nigrum*) and Urticaceae (*Urtica* sp.).

Different TYLCV-Is isolates can vary in pathogenicity to tomato. In Israel two strains have been identified: One causes severe symptoms on tomato plants and the other causes milder symptoms (Czosnek and Laterrot, 1997). Host ranges can vary for TYLCV in different geographic locations. For example, *N. tabacum* is not a natural host for TYLCV in Israel (Cohen and Antignus, 1994) but is a natural host for TYLCV in Jordan (Mansour and Al-Muda, 1992), although both isolates are closely related based on homology of nucleotide and deduced amino acid sequences of their genomes. Furthermore, as TYLCV-Is has spread over time, the host range of the virus has become more evident. For example, during the last three decades TYLCV-Is has been found in three new hosts: *P. vulgaris* (Navas-Castillo et al., 1999), *E. grandiflorum* (Cohen et al., 1995) and *Petunia* sp. (Czosnek and Laterrot, 1997).

The present study was conducted to obtain greater knowledge of the identity and epidemiology of TYLCV in Florida in order to better manage the disease and prevent its spread.

Materials and Methods

DNA extraction, amplification, cloning and sequencing. A potted tomato (*L. esculentum*) plant exhibiting typical TYLCV symptoms was obtained from a nursery in South Florida in July 1997. Approximately 50 mg of excised leaf tissue were boiled for 5 minutes (min) in 200 µl of 1 × TAE buffer in a 1.5-ml microcentrifuge tube and centrifuged for 2 min. The supernatant was then used as source of DNA template in PCR, and the reaction conducted in 40-µl of reaction mixture (20 mM Tris (pH 8.3), 1.5 mM $MgCl_2$, 25 mM KCl, 0.01% gelatin, 0.05% Tween-20, 100 µM of each dNTPs, 10 pmol of each primer, 2 units of Taq DNA polymerase and 20 µl of DNA template). A PTC-100 PCR machine (M. J. Research) was used and the PCR parameters were 37 cycles of 94°C for 1 min, 50°C for 1 min, 72°C for 3 min and final extension at

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72°C for 7 min. The PCR products were separated by electrophoresis in 0.8% agarose gels in 1 × TAE buffer. The gel was stained with ethidium bromide and visualized by UV light. A pair of general degenerate primers (PAL1v1978 and PAR1c496) (Rojas et al., 1993) was used to amplify a fragment containing most of the replication protein (C1) gene, the intergenic region (IR) and the portions of the coat protein (V1) and pre-coat protein (V2) genes of TYLCV. A 1320 bp-fragment was excised from the gel, purified, cloned into pGEM-T vector and sequenced (Davis et al., 1998).

Comparative sequence analyses. DNA and protein sequences of TYLCV from Florida were used for database searches with the Basic Local Alignment Search Tool (BLAST) program (Altschul et al., 1990), as implemented by the National Center for Biotechnology Information (USA) for searching non-redundant sequences in multiple databases. For phylogenetic analyses, all sequences were obtained from the Genbank database or derived during the present study and deposited in Genbank with accession number AF260331. The nucleotide sequence (634 nt) of the intergenic region, portions of the V1 and V2 genes and the first 229 deduced amino acids sequence of the C1 protein of TYLCV from Florida were aligned and compared with the sequences from the following TYLCV and related geminivirus isolates (geographic origin of each isolate is followed by the Genbank accession number for the nucleotide sequence and then by the Genbank accession number for the amino acid sequence of the C1 gene): Mexico, AF168709 and C1 sequence not available; Dominican Republic, AF024715 and AAD09395; Cuba, AJ223505 and CAA11426; Israel, X15656 and P27259; Australia, S53251 and P36279; Spain, AF071228 and AAC99359; Japan, AB014347 and BAA74446; Portugal, AF105975 and AAD17968; Iran, AJ132711 and CAA10751; Murcia, Z25751 and P38609; Sardinia, X61153 and P27260; Sicily, Z28390 and CAA82237; Almeria, L27708 and AAA47955; and Thailand, AF141922 and AAD39153. Trees were constructed using the Kimura-two-parameter correction for multiple substitutions to generate distances and the Neighbor program to generate phylogenetic inferences from the distances by neighbor-joining procedure as implemented in Phylip Version 3.5 (Felsenstein, 1989).

Host range survey. Plant samples were collected in various locations within Miami-Dade County, Florida. Plants were sampled within or immediately adjacent to tomato plantings with incidences of TYLCV. A rapid procedure for extraction of DNA from multiple plant samples of various species was developed. Disks of leaf tissue, approximately 8 mm in diameter, were cut from leaf lamina using caps of 1.5-ml microcentrifuge tubes as punches. For each extraction, one to four disks were put into a 2.0-ml screw-cap tube containing 1 ml of DNA extraction buffer (3% sodium deoxycholate, 0.1% ß-mercaptoethanol, 100 mM Tris (pH 8.0), 50 mM EDTA and 500 mM NaCl) and two steel slingshot beads (6.35 mm in diameter). The disks were then homogenized for 1.5 min using a Bead-Beater (Biospec Products) apparatus. A modified Dellaporta maize mini-preparation procedure was then used for DNA extraction from the homogenates (Dellaporta et al., 1983). DNA was resuspended in water and stored at -20°C. In most cases, four single leaf disks from four individual plants of the same species were pooled for each DNA extraction.

A pair of degenerate PCR primers based on PAL1v1978 and PAR1c496 were designed to more closely match the target DNA sequence in TYLCV from Florida. These were designated PALv1978N (5'GCCCACATYGTYTTSCCTGT) and PARc496N (5'GGCTTYCKGTACATGGG). These new degenerate primers proved useful for the general detection (annealing at 50°C) of several geminiviruses especially TYLCV, and a size polymorphism among the amplification products differentiated TYLCV from other geminiviruses such as bean golden mosaic virus (BGMV) and tomato mottle virus (ToMV). To verify the detection of TYLCV by PCR with the degenerate primers, a second pair of non-degenerate primers was designed specifically for detection of TYLCV and each were designated TYLCF1 (5'GAATGTTCGGATGGAAATGTG) and TYLCR1 (5'GTGGAAATGATTATATCGCCTG). Under stringent reaction conditions (annealing at 58°C), these primers directed amplification of a diagnostic DNA fragment (776 bp) for TYLCV (Fig. 2C) but not ToMV and most other geminiviruses tested. The identities of PCR fragments were further confirmed with restriction enzyme digestion profiles where necessary.

Results and Discussion

Identification of the TYLCV in Florida. The first TYLCV-symptomatic tomato plant discovered in Florida was found in July 1997 in Collier County in a field planting (Polston et al., 1999). Shortly thereafter, tomato plants with TYLCV symptoms were also found in garden departments of several retail stores in Sarasota and Charlotte Counties. The sources of these plants later were tracked back to two retail plant production facilities in Miami-Dade County in South Florida. Shortly thereafter, we found tomato plants with TYLCV symptoms in the field and greenhouse at the Tropical Research and Education Center in South Florida. To verify the presence of TYLCV in tomato plants in Miami-Dade County, we employed PCR using general degenerate primers (PAL1v1978 and PAR1c496), and the PCR products were further characterized by digestion with two restriction enzymes (*Hinc*II and *Cla*I) (Figs. 1 and 2A). A size polymorphism among the PCR products differentiated that of TYLCV (1320 bp) from those of other geminiviruses (1100 bp) as illustrated

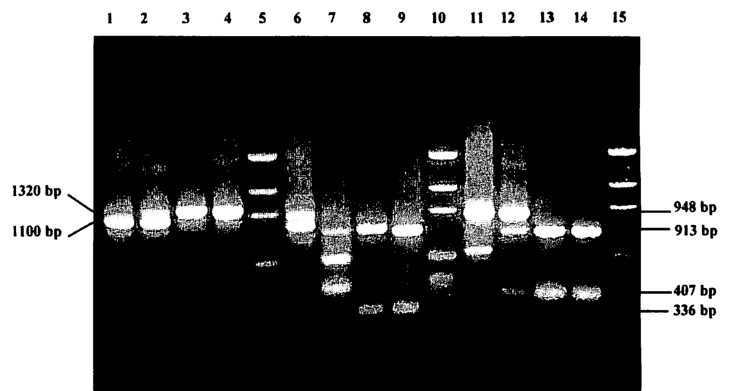

Figure 1. PCR products of TYLCV isolate from Florida generated with the general degenerate primers (PAL1v1978 and PAR1c496) and their restriction patterns with *Cla*I and *Hinc* II digestion. Lane 1, 6 and 11, a field tomato plant infected with another geminivirus; Lanes 2, 7 and 12, a field tomato plant infected with TYLCV and tomato mottle geminivirus; Lanes 3, 8 and 13, a field tomato plant infected with TYLCV; Lanes 4, 9 and 14, a potted tomato plant from a commercial nursery infected with TYLCV; and Lanes 5, 10 and 15: pGEM markers. Lanes 1-4, uncut PCR products; Lanes 6-9, digestion with *Cla*I; Lanes 11-14, digestion with *Hinc*II. The sizes of TYLCV fragments are indicated.

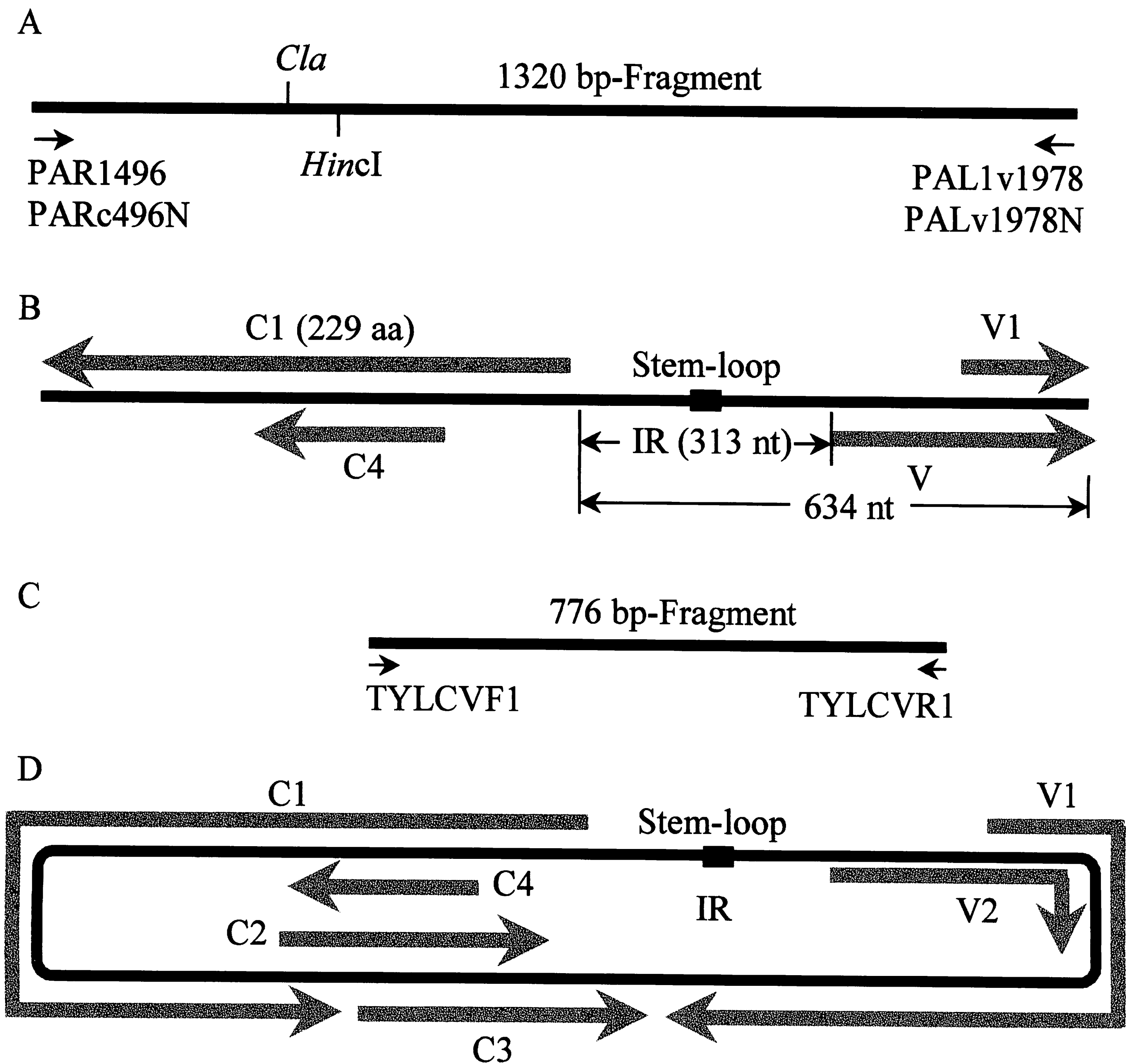

Figure 2. (A) Diagrammatic representation of the 1320 bp-fragment of TYLVC isolate from Florida, (B) open reading frames of the 1320 bp-fragment, (C) the 776 bp-fragment flanked by the TYLCV-specific primers (TYLCVF1 and TYLCVR1) and (D) the genome organization of TYLCV. Open reading frames are shown in both the virus strand sense (+) and the complementary sense (-). The intergenic region, stem-loop, primer locations, and restriction sites of *Cla*I and *Hinc*II are indicated.

in lanes 1-4 of Figure 1. Restriction products also had the expected sizes for TYLCV: 984 and 336 bp-fragments for *Cla*I digestion, and 913 and 407 bp-fragments for *Hinc*II digestion (Figs. 1 and 2A). Mixed infection of tomato plants with both TYLCV and another geminivirus, presumably ToMV, were evidenced by additional 1100-bp fragment in some samples. The identity of the 1320 bp-fragment as that of TYLCV was confirmed by double-strand sequencing, and the sequence was deposited in Genbank with accession number AF260331.

Computer-assisted sequence comparison of TYLCV from Florida with those of other TYLCV isolates and related geminiviruses present in the Genbank database revealed that the 1320 nt sequence contained an intergenic region and movement protein (C4) gene, and portions of the C1, V1 and V2 genes (Fig. 2B and 2D). In the 313 nt intergenic region (Fig. 2B), the DNA sequence of TYLCV from Florida had a 98.7% identity to those of isolates from Mexico and from Dominican Republic, a 98.1% to that of an isolate from Cuba, and 96.2% to that of an isolate from Israel.

Phylogenetic analyses. Figure 3A shows a phylogenetic tree based on the alignment of the nucleotide sequences of the IR, and portions of V1 and V2 genes of 15 TYLCV and related geminvirus isolates. The phylogenetic tree shows that the TYLCV isolate from Florida grouped with other New World

isolates in a distinct cluster closely related to an isolate from Israel and separated from others. Based on the chronology of the first appearance of TYLCV in the different geographic areas, this indicates that isolates from the New World originated from the Eastern Mediterranean region and are members of the TYLCV-Is species. This agrees with the conclusion of others (Nakhla et al., 1994; Polston et al., 1999).

Figure 3B shows the phylogenetic tree obtained with the N-terminal 229 amino acids of C1 protein sequences for 14 TYLCV and related geminivirus isolates. The same general patterns were observed as in Fig. 3A. Again, the Israel group isolates are well separated from other isolates. Within the Israel group, the Caribbean isolates appear to be slightly more related to one another than to the Israel isolate.

Host range survey. A total of 1862 plants of more than 76 species in at least 35 families were sampled within Miami-Dade County and primarily in eight of 14 commercial tomato production areas where tomato plants were affected by TYLCV. Most locations were sampled periodically throughout the winter of 1998-1999. A total of 482 DNA extracts, consisting of pooled leaf samples from one to four plants collected at the same location on the same date and grouped according to appearance, were processed and tested by PCR (Table 1). Besides tomato (*L. esculentum*), the PCR tests were positive with both degenerate (1320 bp product) and non-degenerate (776 bp product) primer pairs for some samples of the following plant species: *Amaranthus retroflexus* (Amaranthaceae), *Acalypha virginica* (Euphorbiaceae), *Begonia* sp. (Begoniaceae), *Macroptilium lathyroides* (Fabaceae), *Nicotiana tabacum* (Solanaceae) and *Sonchus oleraceus* (Asteraceae). All were identified in at least two locations by two or more separate PCR assays (Table 1). Except for the samples of *Begonia* sp. from commercial nursery operations, all plants that tested

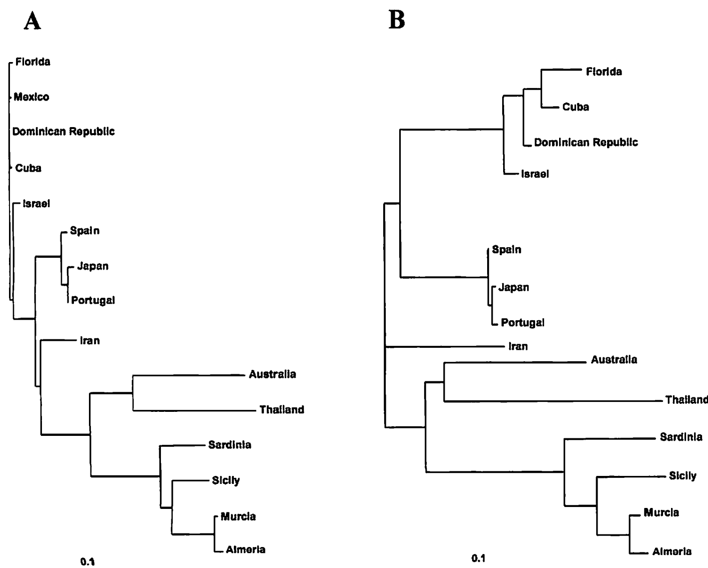

Figure 3. (A) Phylogenetic tree obtained from the alignment of nucleotide sequences (the 634 nt including the intergenic region, and portions of the V1 and V2 genes) of 15 TYLCV and related geminivirus isolates, and (B) phylogenetic tree obtained from the deduced amino acid sequences of replication protein (C1) (the 229 aa started from the N-terminus) of 14 TYLCV and related geminivirus isolates. Distances between isolates are expressed as the sum of the horizontal distances between terminal taxa and the common ancestral node joining a pair of isolates and are based on the estimated number of the nucleotide substitutions per sequence position. Note that the tree is unrooted.

Table 1. Host range survey of TYLCV in South Florida.

Family and species	No. of locations	No. of plants	No. of assays[a]	No. of positive[b]
Acanthaceae				
Barlaria cristata	1	4	1	0
Blechum brownei	3	16	4	0
Amaranthaceae				
Amaranthus retroflexus	6	69	18	2 (2)
Anacardiaceae				
Schinus terebenthifolius	1	4	1	0
Apocynaceae				
Catharanthus rosea	1	4	1	0
Araceae				
Syngonium sp.	1	4	1	0
Asclepiadaceae				
Asclepias sp.	3	9	3	0
Asteraceae				
Ambrosia artemisiifolia	4	64	16	0
Bidens alba var. radicans	7	89	23	0
Conyza annus	1	4	1	0
Emilia fosbergii	1	1	1	0
Erechitites hierachifolia	1	4	1	0
Flaveria trineria	1	1	1	0
Parthenium hysterophorus	6	85	22	0
Pluchea camphorata	1	4	1	0
Sonchus oleraceus	6	126	34	7 (2)
Balsaminaceae				
Impatiens sp.	1	4	1	0
Begoniaceae				
Begonia sp.	2	12	6	2 (2)
Brassicaceae				
Brassica olearea var. capitata	1	1	1	0
Lepidium virginicum	5	48	12	0
Caricaceae				
Carica papaya	1	1	1	0
Chenopodiaceae				
Chenopodium alba	2	25	5	0
Convolvulaceae				
Dichondra repens	1	4	1	0
Ipomoea coccinia	1	4	1	0
Cucurbitaceae				
Momordica charantia var. abbreviata	3	20	5	0
Euphorbiaceae				
Acalypha virginica	7	90	26	5 (2)
Chamaesyce sp.	1	1	1	0
Euphorbia cyathaphora	7	68	19	0
Euphorbia cymasancese	1	4	1	0
Euphorbia hirta	4	28	7	0
Euphorbia nutans	6	44	11	0
Euphorbia sp.	2	12	3	0
Euphorbia triloba	3	12	3	0
Phyllanthus amarus	6	37	10	0
Ricinus communis	2	8	2	0
Fabaceae				
Cassia sp.	1	4	1	0
Cajanus cajanus	1	3	2	0
Desmodium sp.	5	24	4	0
Indigofera sp.	4	61	16	0

[a]Leaf samples from one to four plants were pooled for each assay. Each pooled sample represented a single plant species from the same location, sampling date and apparent state of health.
[b]Number of samples in PCR analyses that tested positive with both pairs of the new degenerate primer pair (PARc496N and PALv1978N) and the TYLCV-specific primer pair (TYLCVF1 and TYLCVR1), and matched expected restriction patterns when digested with restriction enzymes. Numbers in parentheses are the numbers of locations where the positives were sampled.

Table 1. (Continued) Host range survey of TYLCV in South Florida.

Family and species	No. of locations	No. of plants	No. of assays[a]	No. of positive[b]
Macroptilium lathyroides	7	77	26	2 (2)
Medicago sativa	3	20	5	0
Melilotus niger	2	12	3	0
Phaseolus vulgaria	1	4	1	0
Rhynchosia minima	3	21	6	0
Geraniceae				
Geranium carolinianum	2	8	2	0
Hypericaceae				
Hypericum perforatum	1	4	1	0
Lauraceae				
Persea americana	2	13	3	0
Malvaceae				
Abelmoschus esculentum	1	4	1	0
Hibiscus rosa-sinensis	1	4	1	0
Sida sp.	7	233	32	0
Moraceae				
Ficus sp.	1	12	3	0
Oleaceae				
Jasminum sp.	2	16	4	0
Oxalidaceae				
Averrhoa carambola	1	4	1	0
Oxalis corniculata	4	17	5	0
Papaveraceae				
Argemone mexicana	3	16	4	0
Passifloraceae				
Passiflora incarnata	1	4	1	0
Poaceae				
Saccharum officinarum	1	4	1	0
Sorghum bicolor	1	4	1	0
Unknown grass species	2	20	5	0
Polygonaceae				
Antigonon leptopus	1	4	1	0
Portulacaceae				
Portulaca oleracea	5	52	13	0
Rosaceae				
Eriobotrya japonica	1	4	1	0
Rosa sp.	1	4	1	0
Rubiaceae				
Borreria sp.	3	25	7	1
Richardia sp.	1	4	1	0
Sapotaceae				
Manilkara zapota	1	4	1	0
Solanaceae				
Capsicum annuum	1	1	1	0
Datura stramonium	1	4	1	0
Lycopersicon esculentum	8	57	17	12 (6)
Nicotiana tabacum	2	10	10	4 (2)
Solanum americana	3	18	6	0
Solanum melangona	1	8	2	0
Urticaceae				
Pilea microphylla	1	8	2	0
Verbenaceae				
Lantana camara	6	52	13	0
Vitaceae				
Cissus sicyoides	1	4	1	0
Parthenocissus quinquefolia	2	8	2	0

[a]Leaf samples from one to four plants were pooled for each assay. Each pooled sample represented a single plant species from the same location, sampling date and apparent state of health.
[b]Number of samples in PCR analyses that tested positive with both pairs of the new degenerate primer pair (PARc496N and PALv1978N) and the TYLCV-specific primer pair (TYLCVF1 and TYLCVR1), and matched expected restriction patterns when digested with restriction enzymes. Numbers in parentheses are the numbers of locations where the positives were sampled.

Table 1. (Continued) Host range survey of TYLCV in South Florida.

Family and species	No. of locations	No. of plants	No. of assays[a]	No. of positive[b]
Vitis sp.			2	
Unknown Dicotyledon species	9	90	26	0

[a]Leaf samples from one to four plants were pooled for each assay. Each pooled sample represented a single plant species from the same location, sampling date and apparent state of health.
[b]Number of samples in PCR analyses that tested positive with both pairs of the new degenerate primer pair (PARc496N and PALv1978N) and the TYLCV-specific primer pair (TYLCVF1 and TYLCVR1), and matched expected restriction patterns when digested with restriction enzymes. Numbers in parentheses are the numbers of locations where the positives were sampled.

positive for TYLCV were collected late in the winter and within several meters of tomato plants with TYLCV symptoms. Except for *S. oleraceus* and *N. tabacum*, these are all newly identified potentially natural hosts for TYLCV.

Except for tomato, none of the other hosts of TYLCV consistently exhibited characteristic disease symptoms. Various leaf symptoms including intervienal chlorosis, mottling, and crinkling, were observed in some, but not all, plants of *Ac. virginica, Am. retroflexus, M. lanthyroides*, and *N. tabacum* that tested positive in PCR. However, none of the symptoms observed in any of these plants were confirmed experimentally to be associated with infection by TYLCV. Additionally, some plants were apparently infected with other geminivirus, as evidenced by the amplification of an 1100 bp DNA product with the degenerate primers but no products with the non-degenerate primers (data not shown). The 1100 bp DNA product was often associated with plants exhibiting mosaic symptoms such as bean, *Sida* sp., and *M. lathyroides.*

The results of the host survey can only be considered to be preliminary. Further experimentation would be necessary to definitely conclude that those plant species for which positive PCR results were obtained were in fact infected with TYLCV. The positive results simply indicate that the virus was present on or within the plants. Furthermore, negative results might have been obtained in some cases due to low virus titers or the presence of PCR inhibitors in extracts. Nevertheless, the approach used to identify possible natural weed hosts of TYLCV was deemed appropriate, considering the difficulty associated with finding natural hosts among numerous plant species that may or may not be infected and, if infected, may not exhibit symptoms.

The results of the host range survey in Florida support the findings in other geographical locations that TYLCV has a relatively wide host range which is not confined to closely related plant species when compared with that of most other geminiviruses. In Florida, the potential hosts of TYLCV included seven species in six families (Table 1). In Jordan, four species (*D. stramonium, L. esculentum, N. tabacum* and *N. glutinosa*) in one family were found (Mansour and Al-Musa, 1992). In Israel, six species (*C. acutum, E. grandiflorum, L. esculentum, M. parviflora, Petunia* sp. and *P. valgaris*) in five families were found (Cohen and Nitzany, 1966; Cohen et al., 1995; Czosnek and Laterrot, 1997). In the Dominican Republic, two species (*D. stramonium* and *L. esculentum*) in one family were found (Nakhla et al., 1994).

However, despite the apparent wide host range of TYLCV in Florida, none of the species identified as possible hosts oth-

er than tomato itself appeared to be important from an epidemiological point of view. In general, plants testing positive for TYLCV were found late in the tomato production season and in close proximity to tomato plantings with a high incidence of TYLCV. Those plants of the same species when sampled at other times of the year, when the incidence of TYLCV was low, were generally negative for the virus.

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TOMATO YELLOW LEAF CURL VIRUS RESISTANT TOMATO VARIETY TRIALS

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Abstract. **Six of the most promising tomato yellow leaf curl virus (TYLCV) resistant hybrids currently available were evaluated in trials conducted in the fall, winter and spring of 1999-2000. In the Palmetto/Ruskin production area, two observational trials were planted on commercial farms in Ruskin and Bradenton, and one trial was conducted for harvest at the Gulf Coast Research and Education Center in Bradenton. In Palm Beach County, one trial was conducted for harvest on a commercial farm in Boynton Beach. An additional trial was conducted at the Southwest Florida Research and Education Center in Immokalee. At all locations, six TYLCV-resistant varieties were compared to at least 2 standard varieties. Virus pressure was light at the 4 sites in Manatee and Palm Beach**

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