New Potato Spindle Tuber Viroid and Tomato Leaf Curl Geminivirus Strains from a Wild *Solanum* sp.

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S. A. A. Behjatnia was supported by a Ph.D. scholarship from the Ministry of Culture and Higher Education of the Islamic Republic of Iran.

The nucleotide sequence data reported in this paper will appear in the GenBank Sequence Database under accession numbers U51893 (TLCV-Au, D1 strain), U51894 (TLCV-Au, D2 strain), and U51895 (PSTVd, Darwin strain).

Accepted for publication 23 April 1996.

ABSTRACT

Behjatnia, S. A. A., Dry, I. B., Krake, L. R., Condé, B. D., Connelly, M. I., Randles, J. W., and Rezaian, M. A. 1996. New potato spindle tuber viroid and tomato leaf curl geminivirus strains from a wild *Solanum* sp. Phytopathology 86:880-886.

Three distinct viruslike agents were isolated from a wild, symptomless *Solanum* sp. suspected of being a natural reservoir of tomato leaf curl geminivirus. Two of these contained circular DNA genomes of approximately 2.7 kb and were identified as geminiviruses. Restriction analysis and partial sequencing of viral DNA indicated that the two geminiviruses are related to, but distinct from, the Australian strain of tomato leaf curl virus. These viruses were not mechanically transmissible; however, a mechanically transmissible disease agent was also present, which caused stunting of infected tomato plants followed by shoot elongation and hairlessness. Analysis of nucleic acids from sap-inoculated tomato plants revealed the presence of a circular RNA, which was isolated and shown to be the causal agent of the transmissible disease. The complete nucleotide sequence of this 356-nucleotide RNA revealed that it was a sequence variant of potato spindle tuber viroid.

Leaf curl is a severe disease of tomato (*Lycopersicon esculentum* Mill.) in the northern parts of Australia (3). The disease agent, Australian tomato leaf curl geminivirus (TLCV-Au), is transmitted by the whitefly *Bemisia tabaci* and contains a single DNA species of 2,766 nucleotides belonging to the subgroup III of geminiviruses (5,14). The genome of TLCV-Au contains six open reading frames (ORFs). Two virion-sense ORFs are arranged in a fashion similar to those of the monopartite, monocot-infecting geminiviruses such as maize streak virus and wheat dwarf virus, whereas the four complementary-sense ORFs resemble those of the DNA A components of the bipartite geminiviruses such as tomato golden mosaic virus and African cassava mosaic virus (5).

In Australia, field infections of TLCV-Au are currently limited to early- to midseason tomato crops in the Northern Territory, where indigenous populations of the whitefly *B. tabaci* occur. The disease has appeared each year since 1970, causing complete crop failure in some very early plantings in coastal areas (B. D. Condé and Connelly, *unpublished*). The continued severity of early-season infections together with bacterial wilt have made tomatoes unprofitable in the Darwin area. Infected tomato plants show varying degrees of stunting, depending on how early they were infected, as well as leaf curl, yellowing, upward leaf rolling, and cessation of fruit production. Tobacco (*Nicotiana tabacum*), eggplant (*Solanum melongena*), datura (*Datura stramonium*), and zinnia (*Zinnia elegans*) are also natural hosts of the virus (3).

In an effort to identify the natural reservoir of TLCV-Au in the Northern Territory, various solanaceous and nonsolanaceous wild plants were tested for the presence of TLCV-Au by either graft- or insect-inoculation of diagnostic hosts. Tomato plants graft-inoculated with scions collected from plants of a wild *Solanum* sp. from Darwin were found to contain TLCV-like viruses but displayed marked symptom differences compared to those caused by the type strain of TLCV-Au (2,5). In this paper, we report the isolation of two new strains of TLCV-Au in mixed infection with a new strain of potato spindle tuber viroid (PSTVd) and analyze their molecular relationships with the type strains of TLCV-Au and PSTVd, respectively.

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Publication no. P-1996-0604-01R

MATERIAL AND METHODS

Disease source and transmission tests. Scions were collected from several plants of the wild *Solanum* weed and grafted individually to cherry tomato (*L. esculentum* var. *cerasiforme*) plants in Darwin. Five of these tomato plants developed delayed symptoms consisting of elongated shoots and a feathery appearance. Scions from two of these symptomatic cherry tomatoes were grafted onto two tomato plants (var. Grosse Lisse) in Adelaide and served as disease sources. These infected tomato plants, referred to as Darwin 1 (D1) and Darwin 2 (D2), were used as inoculum sources to inoculate other plants by grafting, mechanical inoculation, or dodder transmission. For inoculation by grafting, a scion of an infected plant was inserted into a downward cut in the stem of a stock plant, and the two were bound together with a Parafilm strip. Mechanical inoculation was carried out by rubbing sap from infected plants onto leaves of young seedlings dusted with Carborundum.

Transmission of disease via dodder (*Cuscuta campestris*) was used to resolve mixed disease infection. Healthy dodder was established on *Chenopodium quinoa,* and 3 to 5 cm of the distal ends of dodder strands were detached, placed in 1.5-ml eppendorf tubes filled with water, and attached to infected donor plants. Once the dodder was established, 3- to 5-cm ends of the strands

were removed and attached to healthy tomato receptor plants. Following successful vascular connection, as shown by rapid new growth of dodder, the dodder strands were removed. Infected and control plants were maintained in an insect-proof glasshouse under quarantine conditions.

DNA extraction and analysis. Total leaf nucleic acids were extracted as described by Dry et al. (5). The tissues were pulverized in liquid nitrogen, mixed with two volumes of 50 mM Tris-HCl, pH 8, 100 mM NaCl, 10 mm EDTA, 1% (wt/vol) sodium dodecyl sulfate (SDS), and 1% (vol/vol) 2-mercaptoethanol, and extracted four times with an equal volume of phenol: chloroform (4:1) and once with an equal volume of chloroform: isoamylalcohol (24:1). The nucleic acids were precipitated with ethanol, resuspended in TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA) at a rate of 250 µl for each 1 g of tissue extracted, and incubated with ribonuclease A at 50 µg/µl (Boehringer Mannheim, GmbH, Germany) at 37°C for 1 h. DNA samples were subjected to electrophoresis in 1.2% agarose gels in TBE buffer (19) containing ethidium bromide at 0.5 µg/ml and blotted onto a Zeta-Probe nylon membrane (Bio-Rad, Richmond, CA) by a rapid downward transfer system (Schleicher & Schuell, New Hampshire) according to the manufacturer's instructions. The DNA was cross-linked to the membrane using a Stratalinker UV source (Stratagene, La Jolla, CA) and hybridized overnight with a ^{32}P-labeled TLCV-Au probe prepared by random decamer priming of a full-length TLCV-Au type strain DNA-template using a Giga prime labeling kit (Bresatec, Adelaide, South Australia). The membrane was washed twice with 2× SSC (1× SSC = 150 mM NaCl and 15 mM trisodium citrate) and 0.1% SDS, each time for 5 min at room temperature, and twice with 0.1% SSC and 0.1% SDS, each time for 15 min at 65°C. The positions of radioactive bands were determined by autoradiography.

Dot blot analysis was carried out as described by Maule et al. (12). Fifty mg of leaf tissue were ground in liquid nitrogen and extracted in 100 µl of TE buffer. Tissue homogenate was diluted 1:1 with 1 M NaOH and incubated at room temperature for 30 min. After centrifugation at 11,300 × *g* for 10 min, 5 µl of supernatant was spotted onto a Zeta-Probe nylon membrane. The membrane was briefly (20 s) soaked in 2× SSC, exposed to UV light, and subjected to hybridization as outlined above.

Cloning and partial sequencing of geminivirus DNAs. A pair of TLCV-Au-specific oligonucleotide primers, one complementary to residues 150 to 169 (5′-CGTGGACTAAAGGATCCCAC) and the other corresponding to residues 2,339 to 2,353 (5′-CCTGAATGTTCGGAT) of the TLCV-Au type strain (5), was used to prime the amplification of DNA fragments of the TLCV-Au strains. These fragments of approximately 560 bp were blunt-end ligated into the *Eco*RV site of the plasmid Bluescript II SK(+) (Stratagene) and sequenced using the dideoxynucleotide chain termination method (20). Sequence alignment was carried out using NALIGN from the PC/GENE sequence analysis software package (IntelliGenetics Inc., Mountain View, CA). The full-length polymerase chain reaction (PCR) products of the new strains of TLCV-Au were obtained using two specific adjacent oligonucleotide primers, one complementary sense (Pc, 5′-ATTGTCTCCAATTCATTTTG) and another virion sense (Pv, 5′-CAAACTTGCTAAATGAATT), designed from the partial sequence information.

PCR was carried out in a volume of 50 µl containing DNA template extracted from approximately 0.4 mg of leaf tissue, oligonucleotide primers (each at 1 µM), 200 µM each of dCTP, dGTP, dTTP, and dATP, 1.5 mM $MgCl_2$, and 1.25 units of Taq DNA polymerase (Promega, Madison, WI) in the reaction buffer provided by the same source. The mixture was subjected to a 30-cycle PCR program of 1 min at 94°C, 2 min at 55°C, and 3 min at 72°C. The final cycle was followed by a 7-min incubation at 72°C. Full-length PCR products of the TLCV-Au strains were then subjected to restriction enzyme digestions (19) and analysis on 1.2% agarose gels.

Nuclease digestions. Total leaf nucleic acids were extracted from mechanically infected tomato plants as previously described up to and including ethanol precipitation. The nucleic acids were then treated with either DNase-free RNase A at 10 µg/µl (Boehringer Mannheim) in TE buffer and/or with RNase-free DNase (type DN-EP, Sigma, St. Louis, Missouri) at 10 µg/µl in 10 mM Tris-HCl, pH 7.5, containing 10 mM $MgCl_2$. The samples were incubated at 37°C for 1 h and used for mechanical inoculation as described above.

Viroid extraction, purification, and analysis. Viroid RNA was extracted from infected material and purified as described by Rezaian et al. (16). Nucleic acids extracted from 50 g of tissue were purified by chromatography on cellulose CF-11 and enriched for soluble RNA species by salt fractionation (16). RNAs present in the 2 M LiCl soluble fraction were concentrated by ethanol precipitation, and the viroid species was separated from other nucleic acids by two-dimensional polyacrylamide gel electrophoresis (22). The circular viroid RNA band was excised and eluted from the gel (16), recovered by ethanol precipitation, and suspended in 30 µl of TE buffer.

Samples of viroid RNA were subjected to dot blot hybridization as described previously (16) using a PSTVd-specific probe synthesized from a full-length PSTVd cDNA clone (from RH Symons, Adelaide University, South Australia), as described above. Purified circular viroid RNA (3 µl) was mixed with 3 volumes of 10× SSC containing 20% formaldehyde. The mixture was heated at 65°C for 15 min, then at 90°C for 2 min, and applied to the Zeta-Probe membrane. The RNA was cross-linked to the membrane by exposure to UV light for 1 min. Hybridization, washing, and autoradiography were carried out as described above.

Viroid cDNA synthesis, cloning, and sequencing. Two different sets of oligonucleotide primers were used for reverse transcription and amplification of the viroid template. The first set, P1 complementary to residues 256 to 273 (5′-GTTTCC-ACCGGGTAGTAG) and P2 containing residues 274 to 295 (5′-AACTGAAGCTCCCGAGAACCGC) of PSTVd, was selected from published PSTVd sequence (8). The second set of overlapping primers was designed to a conserved *Bam*HI site that is also present in PSTVd and had two non-PSTVd nucleotide residues at their 5′-ends. These primers, P3 complementary to residues 68 to 92 (5′-CCGGATCCCTGAAGCGCTCCTCCGAGC, *Bam*HI site underlined) and P4 containing residues 87 to 110 (5′-TCGGATCCCC-GGGGAAACCTGGAGCG) of PSTVd were used to confirm the sequences of regions selected for the first set of primers.

Purified circular RNA (3 µl) was mixed with 2 µl of either P1 or P3 at a concentration of 10 µM, heated to 95°C for 3 min, and quickly cooled on ice. Reverse transcription of viroid RNA was carried out in a 15-µl reaction containing the template and primer, 50 mM Tris-HCl (pH 8.3 at room temperature), 10 mM $MgCl_2$, 25 mM KCl, 500 µM each of dCTP, dGTP, dTTP, and dATP, 1 mM DTT, and 4 units of AMV reverse transcriptase (Promega). The mixture was incubated at 42°C for 15 min. PCR was carried out in a 50-µl reaction containing 5 µl of cDNA reaction, and P1 and P2 or P3 and P4 (each at 1 µM), using the conditions described above.

PCR fragments were cloned into a T-tailed Bluescript II SK(+) plasmid prepared as described by Marchuk et al. (11). Two independent full-length PSTVd clones were sequenced in both directions using the dideoxynucleotide chain termination method (20). Multiple alignment of viroid sequences was carried out using the program PILEUP (Genetics Computer Group, University of Wisconsin).

RESULTS

Detection of TLCV-Au-like DNAs. Symptoms of the two infected source tomato plants, D1 and D2, differed from each

other. D1 displayed leaf curl, yellowing, and upward leaf rolling, suggesting it contained a TLCV-Au-like geminivirus. However, D2 showed bunched growth with distorted leaves in initial growth, and spindly and hairless shoots and leaf narrowing at a late stage. Dot blot analysis using a TLCV-Au probe gave a positive signal with the D1 tomato but not with the D2 tomato plant (data not shown). A more sensitive analysis for the presence of TLCV-Au-like species was carried out using PCR with two TLCV-Au-specific primers designed to direct the amplification of a fragment composed of the TLCV-Au intergenic region and the 5′ end of the C1 ORF (5). PCR fragments corresponding to the expected 562-bp DNA product of tomato plant infected with the TLCV-Au type strain were obtained with extracts from both D1 and D2 plants (Fig. 1). However, the level of amplified DNA from the D2 plant extract was significantly lower than that obtained with extracts from D1 and the wild-type TLCV-Au-infected plants. This observation was consistent with the lack of a positive signal with the D2 plant extract in the dot blot assay. These experiments showed that the D1 and D2 tomato plants contained TLCV-Au-like DNA.

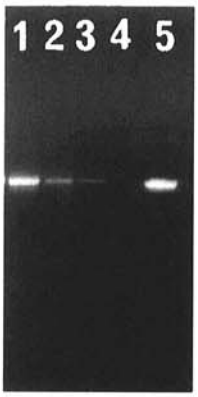

Fig.1. Amplification of Australian tomato leaf curl geminivirus (TLCV-Au) DNA by polymerase chain reaction (PCR). Lanes 1 and 2, D1 extract diluted 10 and 100 times, respectively; lanes 3 and 4, D2 extract diluted 10 and 100 times, respectively; lane 5, TLCV-Au type strain extract diluted 10 times. Nucleic acids were extracted and resuspended as described in Materials and Methods.

Isolation of the mechanically transmissible agent. To resolve the symptom differences between D1 and D2 tomato plants, we examined mechanical disease transmission from both plants. Tomato plants inoculated mechanically from both sources produced disease symptoms that were similar and consisted of a zone of stunted growth with shorter, distorted leaves that emerged 7 to 10 days after inoculation (Fig. 2A). The next phase of symptom development occurred 2 to 3 months after inoculation and produced a zone of elongated spindly shoots, smaller leaves, and hairless stems (Fig. 2B and C). Necrotic streaking of the stem, vein necrosis of leaflets, and small seedless fruit (Fig. 2D) were also characteristic symptoms. Dot blot and PCR analyses using TLCV-Au-specific probe and primers, respectively, did not detect any TLCV-Au-like DNA species in these plants, indicating that these symptoms were not due to TLCV-Au. Seventeen datura plants mechanically inoculated with sap of D1 and D2 tomato plants did not produce symptoms; however, back-inoculation of inoculated datura plants onto healthy tomato plants produced typical symptoms of the mechanically transmissible agent, showing that datura is a symptomless host of this agent. *Nicotiana tabacum, Solanum melongena, S. torvum, S. mauritianum,* and *Capsicum annuum* were also symptomless hosts of the mechanically transmissible agent. These experiments indicated that both D1 and D2 tomato plants contained a TLCV-Au-like virus together with a mechanically transmissible agent.

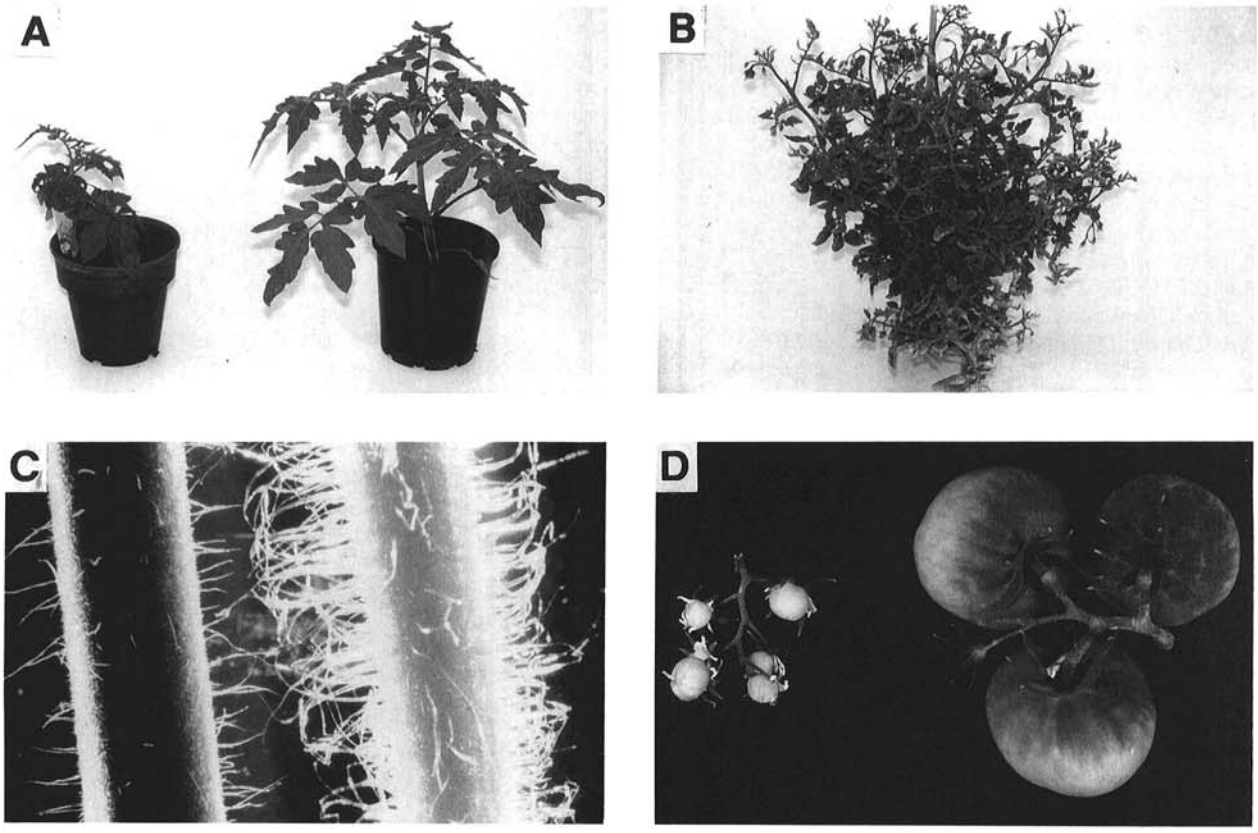

Fig. 2. Symptoms caused by the mechanically transmissible agent in tomato plants. **A,** Strong epinasty of leaves and leaflets and bunched growth 7 to 10 days after inoculation (left) compared with healthy plant (right). **B,** Spindly shoots and small leaves 2 to 3 months after inoculation. **C,** Hairless stem of infected plant (left) compared with healthy stem (right). **D,** Small fruit produced by infected plant (left) compared with normal fruit of healthy plant (right).

Separating the TLCV-Au-like viruses from the mechanically transmissible agent. Dodder transmission was used to resolve the disease complex. A group of 10 tomato plants was inoculated with D2 tomato plant by dodder. Two of these plants developed symptoms characteristic of TLCV-Au, another two plants displayed symptoms characteristic of the mechanically transmissible agent, and the other six plants remained symptomless. Both receptor plants showing TLCV-Au-like symptoms lacked the mechanically transmissible agent, as the extracts of these plants failed to produce any symptoms when mechanically inoculated onto 11 healthy tomato plants and observed for 30 days. However, all eight healthy tomato plants mechanically inoculated with the sap of two receptor plants with symptoms associated with the mechanically transmissible agent showed characteristic symptoms of this agent after 30 days. This experiment showed that dodder separated TLCV-Au-like virus from the mechanically transmissible agent, which coexisted in the D2 plant. Although the titer of the geminivirus in the original D2 tomato plant was very low, the virus could be readily detected by dot blot hybridization in tomato plants infected via dodder transmission. This observation raises the question that there may be an interaction between the TLCV-Au-like viruses and the mechanically transmissible agent. To test this possibility, tomato plants were coinoculated with the mechanically transmissible agent as well as with TLCV-Au-like virus recovered from the D2 tomato plant. Both agents were detectable in inoculated tomato plants after 30 days, indicating that there was no inhibitory interaction between them during that period. The low level of geminivirus DNA in the original D2 plant may relate to the age of infection or the presence of an unidentified viral component.

Molecular characteristics of the TLCV-Au strains. Southern blot analysis of DNA from plants infected with the new TLCV-Au strains indicated the presence of both single- and double-stranded DNA replicative forms indistinguishable in size from those of the TLCV-Au type strain (5). A segment of their viral genomes corresponding to a 562-bp fragment of the TLCV-Au type strain encompassing the intergenic region and 5′ end of C1 ORF was amplified by PCR and sequenced. D1 and D2 strains were found to have 84% identity to the TLCV-Au type strain across this region, while D1 and D2 strains showed 97% sequence identity to each other in this region (Fig. 3). To produce full-length PCR DNA of the new strains, two adjacent primers were designed from the partial nucleotide sequence of new strains in such a manner that they discriminated between the TLCV-Au type strain. Full-length PCR DNA of the new strains were approximately 2.7 kb in size, in agreement with the genome size of the TLCV-Au type strain. Restriction enzyme analysis of full-length PCR products (Table 1) demonstrated that there were nucleotide sequence differences between TLCV-Au type and new strains outside the region sequenced.

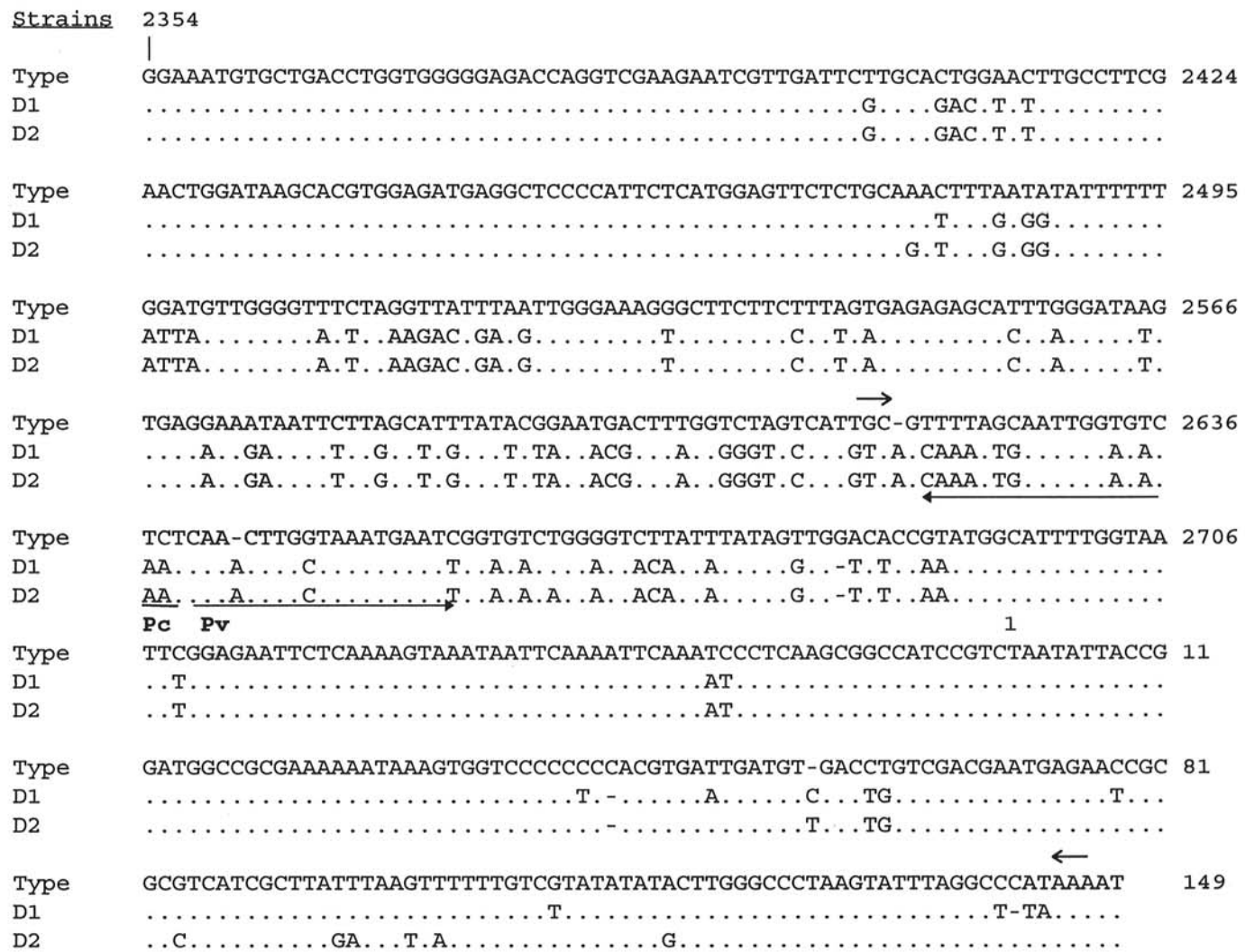

Fig. 3. Sequence alignment of the DNA fragments of the Australian tomato leaf curl geminivirus (TLCV-Au) strains composed of the intergenic region and 5′ end of C1 open reading frame (ORF). Numbering of the residues is as in Dry et al. (5), where residue 1 is the first nucleotide of the TAATATTAC sequence, which is conserved in all geminiviruses. The short arrows (→) span the intergenic region. The position of the primers Pv and Pc described in Materials and Methods are underlined by the long arrows. Dots indicate that the residues are the same as the nucleotides in the top sequence.

The mechanically infectious agent is a viroid. Several attempts were made to isolate virus-specific DNA or dsRNA from the mechanically transmissible viral agent, but these attempts were unsuccessful. Total nucleic acid extracts from infected plants were found to be fully infectious when mechanically inoculated onto healthy tomato plants. The infectious nucleic acid extract was treated with RNase and/or DNase and then used for mechanical inoculation of healthy plants. All five tomato plants inoculated with DNase-treated nucleic acid showed typical disease symptoms. However, none of the five tomato plants inoculated with nucleic acids treated with RNase or RNase + DNase displayed any symptoms. This observation indicated that the mechanically infectious agent has an RNA genome.

Analysis of purified nucleic acid by two-dimensional gel electrophoresis and silver staining revealed the presence of a circular nucleic acid migrating more slowly than the plant linear RNAs, typical of a viroid (22). This RNA, which was absent in healthy tomato plants (Fig. 4), was isolated from the gel and found to be infectious, producing the same symptoms in tomato as seen in Figure 2. These symptoms resembled those of PSTVd in tomato described by Diener and Raymer (4). Dot blot hybridization of the gel-purified RNA with a PSTVd probe under high stringency conditions produced a positive reaction, suggesting that the mechanically transmissible agent is a PSTVd-like viroid. The size of the viroid was slightly smaller than that of the citrus exocortis viroid (371 nucleotide residues), compared by electrophoresis in polyacrylamide gel under denaturing condition (results not shown).

Primers designed from published PSTVd sequence (P1 + P2) (8) were used in RT-PCR reactions to prime the amplification of viroid sequences. A PCR product was obtained and cloned. Two of the clones were partially sequenced, and a second set of

TABLE 1. Some restriction endonuclease sites in DNA of Australian tomato leaf curl geminivirus (TLCV-Au) strains

Restriction endonuclease[a]	TLCV-Au type strain	TLCV-Au D1 strain	TLCV-Au D2 strain
*Bam*HI	1	1	2
*Hin*dIII	–	–	–
*Kpn*I	1	1	1
*Pst*I	–	–	1
*Sac*I	1	–	1
*Sca*I	–	1	2
*Xba*I	1	–	–
*Xho*I	1	1	–

[a] All the restriction sites except for *Pst*I site are outside the sequence shown in Figure 3.

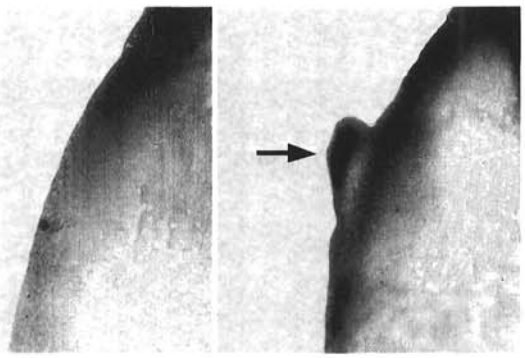

Fig. 4. Analysis of nucleic acids from tomato plants by 2-D gel electrophoresis. Purified nucleic acids from mechanically inoculated (right) and from healthy plants (left) were analyzed and stained with silver as described in Materials and Methods. Direction of nondenaturing electrophoresis in the first dimension was from right to left and in the second denaturing dimension was from top to bottom. The circular RNA band in infected tissue is shown by an arrow.

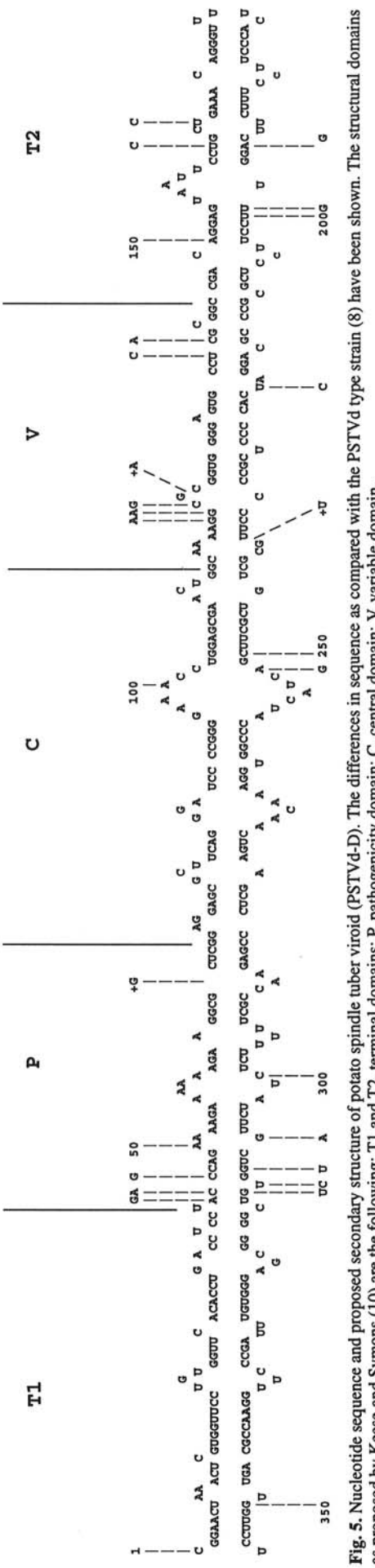

Fig. 5. Nucleotide sequence and proposed secondary structure of potato spindle tuber viroid (PSTVd-D). The differences in sequence as compared with the PSTVd type strain (8) have been shown. The structural domains as proposed by Keese and Symons (10) are the following: T1 and T2, terminal domains; P, pathogenicity domain; C, central domain; V, variable domain.

primers (P3 + P4) was designed from the partial sequence. These primers were used to obtain a full-length PCR product in which the sequences at the priming sites were specific to our viroid isolate. The PCR product was cloned, and two independent full-length clones were fully sequenced. Analysis of the data confirmed that it is indeed a sequence variant of PSTVd consisting of 356 nucleotides, three nucleotides shorter than the PSTVd type strain (8). The predicted rodlike secondary structure of the first clone of PSTVd from Darwin (PSYVd-D) is shown in Figure 5. Comparison of the primary structure of the two PSTVd-D clones showed three nucleotide differences. The U residue at position 28 and two A residues at positions 252 and 346 of the first clone were replaced by A, G, and U residues, respectively, in the second clone. These changes do not result in considerable changes to the predicted secondary structure of the PSTVd-D. Compared to the PSTVd type strain, the first clone of PSTVd-D has three nucleotide deletions and 18 nucleotide exchanges (Fig. 5), with an overall sequence similarity of 95% with the type strain. The sequence of PSTVd-D differs from those of 11 published PSTVd sequences (currently in the GenBank database) and is most similar to the Naaldwijk strain of PSTVd from the Netherlands (98.5% similarity) (15). PSTVd-D and the Naaldwijk strain form a cluster separate from all other strains when compared by PILEUP program.

DISCUSSION

Partial nucleotide sequencing and restriction enzyme analysis of two geminiviral DNAs, each derived from an individual wild *Solanum* sp. plant, showed the presence of two DNA species that were more similar to each other than to the TLCV-Au type strain from Australia. Each new strain of TLCV-Au showed 84% sequence identity with the TLCV-Au type strain in the intergenic region and 5′ end of C1 ORF. This is a highly variable region in whitefly-transmitted geminivirus genomes (14), and it is likely that a higher overall sequence identity exists between the complete genomes of the new strains and the type strain.

Despite a high degree of sequence identity, geminivirus isolates often show different biological properties (1,13). In the present study, we observed that infection of tomato plants with the TLCV-Au type strain or the TLCV-Au D2 strain was always associated with severe symptoms, whereas the TLCV-Au D1 strain induced relatively mild symptoms. Phenotypic and genetic analyses have revealed a surprising degree of variability among beet curly top geminivirus (BCTV) strains. Choi and Stenger (2) demonstrated that *cis*- and *trans*-acting DNA replication factors of two strains of BCTV are not functionally interchangeable. They raised the question as to whether the two BCTV strains they studied should be considered distinct viruses or distinct types of the same virus. Similarly, Gilbertson et al. (7) and Faria et al. (6) designated certain strains of bean golden mosaic virus as type I or type II categories. It will be interesting to determine the complete genome structure of the new TLCV-Au strains and to examine whether viral functions are interchangeable between these strains.

Sequence analysis of the viroid recovered from the wild *Solanum* sp. revealed that it was a variant of PSTVd. It consists of 356 nucleotides, the same size as the Naaldwijk strain of PSTVd from the Netherlands (15), which infects tomato and pepino plants (*Solanum muricatum*). PSTVd-D, therefore, differs in size from the commonly occurring 359-base PSTVd strains. As with other strains of PSTVd (15,21), most nucleotide variations in PSTVd-D occur in the pathogenicity (P) and variable (V) domains (10), and the variations in the P domain are in the left part of this domain. Variations in the left part of the P domain have been linked to the symptom severity induced by naturally occurring sequence variants of PSTVd (21).

Weeds have been considered to be important reservoirs of Indian TLCV in nature (17). Our work shows that wild *Solanum* plants may act as reservoirs for PSTVd-D and two new strains of TLCV-Au. The recent arrival of the "B" biotype of *B. tabaci,* an efficient geminivirus vector, in Australia (9) and its rapid spread is considered a risk to the tomato industry, particularly in Queensland where currently about half of the Australian tomato crops are grown. The "B" biotype of *B. tabaci* has a very wide host range and may transmit the new TLCV-Au strains from weeds, including *Solanum* sp., to tomato and other host crops. It will be interesting to test the transmissibility of PSTVd-D from *Solanum* plants coinfected with geminiviruses, in view of the recent report that aphids transmit PSTVd from potato coinfected with potato leaf roll luteovirus (18).

PSTVd has not been recorded in Australia for approximately 10 years and is a quarantinable disease. So far, there has been no evidence of field infection of tomato by PSTVd. We have no evidence to indicate PSTVd-D transmission by whitefly, but the presence of the new biotype of *B. tabaci* in the same geographical region necessitates disease monitoring for the potential spread of geminiviruses to tomato and other crops.

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