Evidence for a Phytoreovirus Associated with Tobacco Exhibiting Leaf Curl Symptoms in South Africa

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ABSTRACT

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Three forms of tobacco leaf curl (termed classes I, II, and III, based on symptomatology) recently have been described in southern Africa. Numerous attempts to isolate virus particles responsible for a nongeminivirus-induced leaf curl disease (class I) of tobacco in South Africa have been unsuccessful. Recently, 12 dsRNA segments were isolated from tobacco exhibiting class I leaf curl symptoms, suggesting a possible reovirus genome. The objective of our study was to confirm whether the dsRNA segments are associated with a reovirus. Isolation of icosahedral particles with an outer core 60 to 65 nm in diameter and an inner core 40 to 45 nm in diameter was achieved. Twelve distinct nonpolyadenylated dsRNAs were

isolated from purified virions, and the total molecular masses of the dsRNAs ranged from 17.86 to 18.40×10^6 Da in polyacrylamide and agarose gels, respectively. Using hybridization analysis, dsRNAs were identified as non-homologous distinct segments. Comparisons with other known reoviruses revealed a unique banding pattern that was most similar to the wound tumor virus (WTV), the type species of the genus *Phytoreovirus*. Hybridizations of WTV cloned DNA probes (segments S4 and S6 to S9) and dsRNAs from infected tobacco indicated no significant sequence similarity, whereas indirect enzyme-linked immunosorbent assay with a polyclonal antiserum to WTV showed strong positive cross-reactivity to tobacco virions. Our results indicate a virus with features consistent with those of phytoreoviruses. This is the first report of a plant reovirus in tobacco, the first record in Africa, and the second example of a field-isolated dicot phytoreovirus.

Tobacco leaf curl disease was once one of the most destructive diseases in East Africa, Zimbabwe, and South Africa (16) and is currently widespread in India (32). At present in southern Africa, epidemics are sporadic and not as devastating as in the past (31). Specific symptoms in plants exhibiting leaf curl vary widely with respect to type and severity (23). Variable leaf curl symptoms displayed by tobacco worldwide may be due to a complex of viruses or virus strains, but this theory has not been proven. The most characteristic symptoms of tobacco leaf curl are leafy outgrowths (enations) from veins on the lower surface of curled and twisted leaves. Leaves also contain thickened veins and are much reduced in size compared with uninfected leaves (16). In 1978, Osaki and Inouye (23) extracted geminate viral particles associated with tobacco leaf curl in Japan. The tobacco leaf curl virus Osaki and Inouye (24) characterized belongs to the family *Geminiviridae* and occurs mainly in tropical and subtropical regions, but it also has been reported in temperate regions.

Numerous attempts to isolate geminivirus particles from tobacco plants with leaf curl symptoms in South Africa have been unsuccessful, but recently, a geminivirus was confirmed in leaf curl-infected tobacco from Zimbabwe (6,7,26,31). Tobacco plants from South Africa and Zimbabwe exhibiting variable leaf curl symptoms have been grouped in three classes based on symptomatology and common virus particles or viral nucleic acids (25,26). Class I tobacco leaf curl-infected plants have characteristic leafy enations on the underside of leaves. Vein thickenings are clearly visible, and leaves are curled and twisted (Fig. 1). Tumors also have been observed on roots of affected tobacco plants. Twelve dsRNA seg-

ments have been found consistently in class I-infected tobacco plants and are absent from class II- and III-infected tobacco plants. The double-stranded nature of the RNAs was confirmed by ribonuclease digestion under both high- and low-salt conditions (25). Mechanical and seed transmissions of dsRNAs from class I-infected tobacco plants were unsuccessful (25). Class II tobacco symptoms are visible in tobacco plants germinated from seed, and a possible inherited genetic disorder is suspected (26). Class III tobacco symptoms are associated with geminivirus infection (26).

Plant reoviruses (family *Reoviridae*) can be placed in one of three genera, *Phytoreovirus*, *Fijivirus*, or *Oryzavirus*, and include a garlic dwarf reovirus (GDV) (15) that has an uncertain taxonomic position (11). Reoviruses have been isolated from South and Far East Asia, Europe, Australia, and North and South America. Genomes consist of 10 to 12 dsRNA segments, (S1 to S12, in order of slowest to fastest mobility in polyacrylamide gel electrophoresis [PAGE]) and are packaged in inner cores ≈ 40 nm in diameter

Fig. 1. Tobacco leaf exhibiting class I leaf curl symptoms: leafy enations (arrow) and thickened veins.

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and outer protein shells 60 to 80 nm in diameter (5,8,11,13). Plant reoviruses are not efficiently sap (mechanically) transmissible but are transmitted by leafhoppers. Infectivity can be determined only by injecting preparations into virus-free vectors tested for the ability to transmit the virus to susceptible plants (11). Plant reovirus-like symptoms include enations, vein swellings, and dwarfing (11). Wound tumor phyto-reovirus (WTV) is the only plant reovirus known to infect dicotyledonous plants (21,22). Although tobacco has not been recorded as a natural host for plant reoviruses, the dsRNA evidence previously reported suggests the presence of a plant-infecting reovirus in class I-infected tobacco plants (25,26). The objective of our work was to determine whether the dsRNA segments isolated from class I leaf curl-infected tobacco plants represents the genome of a reovirus.

MATERIALS AND METHODS

Virus extractions. Reovirus extractions, using a modification of the method described by Kimura (12), were performed on healthy, symptomless tobacco (control) and class I-infected tobacco plant materials (*Nicotiana tabacum* cvs. TL33 and TL38). Extractions were performed several times with 50 g of frozen (-80°C) leaf material per extraction. A single extraction with 150 g of fresh leaf material also was performed. Field-grown tobacco plants were collected from two cultivation areas in the western and eastern regions of South Africa over four growing seasons and maintained in the greenhouse. After extraction in buffer (0.05 M sodium phosphate buffer, pH 7.8, containing 5 mM Na_2EDTA and 10 mM diethyldithiocarbamate) and clarification with 20% carbon tetrachloride, the supernatant was centrifuged in a JA-14 centrifuge (Beckman Instruments, Fullerton, CA) at 6,000 rpm for 10 min at 4°C . The supernatant was distributed in five centrifuge tubes, placed in a Beckman type 42.1 rotor, and centrifuged through 20% sucrose cushions at 30,000 rpm for 45 min at 4°C in a Beckman L8-55 ultracentrifuge. Pellet was resuspended in 0.05 M sodium phosphate buffer (pH 7.8) (partially purified virus preparation), and the sample was purified further by centrifugation through a sucrose density gradient (10 to 50%) in 0.05 M sodium phosphate buffer (pH 7.8) for 2 h at 27,000 rpm and 4°C (Beckman L8-55 ultracentrifuge and SW28 rotor). Virions recovered from the viral band were resuspended in 0.05 M sodium phosphate buffer (pH 7.8) containing a few crystals of chlorobutanol for preservation and stained with either 2% sodium

phosphotungstate or 2% uranyl acetate for 15 min and viewed under a JEM S-100 electron microscope (Jeol, Tokyo) (9). Virus yields were estimated roughly by spectrophotometry ($A_{260\text{nm}}$, 1-cm light path).

Extraction of dsRNA from virions. One volume of sucrose density gradient-purified virions was incubated in one volume of disassociation buffer (2% sodium dodecyl sulfate [SDS], 2 mM EDTA, and proteinase K at 100 $\mu\text{g}/\text{ml}$) at 50°C for 2 h, and the mixture was extracted as described by Paximadis et al. (25).

Extraction of dsRNA from class I-infected tobacco plants. The method used to extract dsRNA from class I-infected tobacco plants was essentially the method described by Morris and Dodds (20). dsRNA also was extracted from healthy tobacco plants as a control. Detection of possible poly(A) tracts at the 3' ends of dsRNAs was performed using the oligo (dT) trapping method (Amersham International, Little Chalfont, England). As positive controls, mRNAs (cDNA cloning kit, Boehringer GmbH, Mannheim, Germany) were used at 10-, 100-, and 1,000-ng concentrations.

Agarose gel electrophoresis. dsRNAs from virions and infected tobacco leaves were separated and analyzed directly on 1% agarose gels stained with ethidium bromide (0.5 $\mu\text{g}/\text{ml}$ final concentration) (30). Segments were separated at 60 to 100 V with a E321 power supply (Consort nv, Belgium) at room temperature for 1 to 4 h. Purified dsRNAs from tobacco were coelectrophoresed on agarose gels with dsRNA from other known plant-infecting reoviruses as comparisons. Known viruses included maize rough dwarf fijivirus (MRDV); GDV (15); rice dwarf phyto-reovirus (RDV); and rice black-streaked dwarf fijivirus (RBSDV) (T. Omura, National Agriculture Research Centre, Ibaraki, Japan). The size of each dsRNA segment from infected tobacco was estimated with *Pst*I-digested lambda dsDNA fragments; dsRNA segment sizes of other known plant reoviruses were used as standards (3).

SDS-PAGE. Ten percent polyacrylamide gels (14) were used to compare dsRNA from class I-infected tobacco plants with dsRNA from MRDV, GDV, rice ragged stunt reovirus (RRSV), RBSDV, and WTV. Gels were run at 30 mA for 30 to 34 h using Laemmli's (14) buffer system and stained with 6 mM silver nitrate for 30 min. The sizes (base pairs) and total molecular masses (daltons) of dsRNA

Fig. 2A. Agarose gel (1%) of dsRNAs isolated from class I-infected tobacco. Lane 1, *Pst*I-digested lambda dsDNA molecular markers, sizes (base pairs) of markers are indicated to the left; lanes 2 through 5, dsRNAs isolated from leaf curl-infected tobacco. **B.** Agarose gel (1%) of dsRNAs isolated from class I-infected tobacco (lane 2) for comparison with dsRNAs from other plant-infecting reoviruses: lane 3, maize rough dwarf fijivirus; lane 4, garlic dwarf reovirus; lane 5, rice dwarf reovirus; and lane 6, rice black-streaked dwarf fijivirus. Lane 1, *Pst*I-digested lambda dsDNA molecular weight markers. Twelve dsRNA segments (sizes are provided in Table 1) in infected tobacco (lane 2) show a different pattern compared with other reoviruses. **C.** Agarose gel (1%) of dsRNAs extracted from purified virus particles isolated from infected tobacco; there are 10 segments, but bands 4 and 5 and 6 and 7 (arrows) are doublets.

Fig. 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel (10%) used to compare dsRNAs isolated from class I leaf curl-infected tobacco (lane 1) with dsRNAs from other reoviruses: lane 2, wound tumor virus; lane 3, maize rough dwarf fijivirus; lane 4, rice black-streaked dwarf fijivirus; lane 5, rice ragged stunt reovirus; and lane 6, garlic dwarf reovirus. dsRNA segments (lane 1, arrows, S1 through S12; sizes are provided in Table 1) from tobacco have unique banding patterns compared with other reoviruses (lanes 2 through 6).

segments were estimated using the sizes of dsRNAs of other plant-infecting reoviruses as standards.

RNA hybridizations. The four largest tobacco dsRNA segments (S1 to S4) were extracted individually from low melting temperature agarose gels and labeled using the enhanced chemiluminescence labeling and detection system as outlined in the Amersham instruction booklet. Dot blots were probed with four labeled RNAs. Dot blots were prepared by spotting 1 μ l (50 ng) of heat-denatured dsRNA on Hybond N (Amersham) membranes. dsRNA was fixed on membranes by exposing to UV light for 5 min. S1 to S4 dsRNAs were spotted separately, whereas S5 to S12 dsRNAs were pooled as a single sample and spotted on membranes. Total RNA extractions from class I-infected and healthy tobacco were used as positive and negative controls, respectively.

DNA/RNA hybridizations. Investigation of possible nucleotide sequence homology between the genomes of WTV and the virus isolated from tobacco was undertaken by slot-blot hybridization analysis. Cloned DNAs from WTV segments S4 and S6 to S9 (1,33) were used as probes (provided by N. Suzuki, University of Maryland Biotechnology Institute, College Park). Viral inserts were excised from pGEM plasmid vectors and electrophoresed on 1% agarose gels, and viral bands were extracted with a Biotech Sephaglas BandPrep kit (Pharmacia LKB Biotechnologies, Piscataway, NJ). DNA probes were digoxigenin (DIG)-labeled, and hybridization and detection were performed with a DIG DNA-labeling and -detection kit (Boehringer). Twelve dsRNAs from WTV and infected tobacco were denatured before they were applied to Hybond N membranes. RDV and MRDV dsRNAs also were used for comparisons, and positive hybridization controls of denatured WTV S4 and S6 to S9 DNA also were included. Several hybridization temperatures, buffers, and washing solution stringencies were tested. The final hybridization buffer used was the standard buffer from the Boehringer DIG kit. Final prehybridizations were performed at 55°C for 6 h, and hybridizations were performed overnight at 55°C. Slot blots were washed in 2 $\times$ SSC (1 $\times$ SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate, pH 7.0) and 0.1% SDS at 55°C for 30 min, followed by two washes in 0.5 $\times$ SSC and 0.1% SDS at room temperature.

Enzyme-linked immunosorbent assay. Antiserum to MRDV was used in indirect plate enzyme-linked immunosorbent assay (ELISA) (Clark and Adams [4], modified according to the Agdia [Elkhart, IN] instruction manual) to test for possible serological cross-reactivity with the partially purified tobacco virus preparation (described above). Sap extracts from healthy tobacco material were used as negative controls, while partially purified virions of MRDV (unknown concentration) were used as positive controls. Because the concentrations of the tobacco virus and MRDV antigens were unknown, various antigen dilutions were used to optimize ELISA tests. Antigens were used at a final dilution of 1:1,000. MRDV primary antibody (titer 1:256) was optimized at a dilution of 1:1,000. Goat anti-rabbit alkaline phosphatase (Sigma Chemical Co., St. Louis) was used as the secondary antibody at a dilution of 1:10,000. Confidence intervals (CI: 95%) were calculated using the mean for healthy tobacco values $\pm$ 2 times the standard deviation. Any significant differences in absorbance values for the samples tested was determined using the 95% CI of values for healthy tobacco. Values greater than the 95% CI implied cross-reactivity to the primary antibody tested and were recorded as positive. Any value that was within or below the 95% CI was recorded as negative.

Immunoslot blots. Tobacco virions purified by sucrose density gradients ($\approx$ 1 mg/50 g of leaf tissue) were tested for cross-reactivity with antiserum to WTV (ATCC PVAS 235 [American Type Culture Collection, Manassas, VA]) in slot blots, using the amplified alkaline phosphatase goat anti-rabbit immunoblot assay (Bio-Rad Laboratories, Hercules, CA). WTV-infected and healthy clover roots (provided by B. Hillman, Rutgers University, New Brunswick, NJ) were used as positive and negative controls, respectively. Root tissue was ground in the same buffer used for virion extractions, spun briefly at 10,000 rpm to remove heavy debris, and a few grains of Na azide

were added to prevent microbiological deterioration. Healthy tobacco leaf sap extracts also were included as negative controls. Healthy tobacco plant and clover root extracts and tobacco virions were tested at several dilutions, ranging from undiluted to 1:2,000, and a final dilution of 1:100 was selected for best results. The WTV antibody (titer 1:640 to 1:1,280) was used at a dilution of 1:1,000.

RESULTS

dsRNA species. Twelve dsRNA segments were isolated from class I-infected tobacco plants and designated as segments S1 to S12, from largest to smallest band (Fig. 2A and B; Fig. 3). Segments S4 and S5 appeared as a single band (Fig. 2A) but separated after prolonged electrophoresis (Figs. 2B and 3). The approximate sizes (base pairs) and relative molecular masses (daltons) of each dsRNA segment are shown in Table 1. The relative mobilities of the dsRNAs varied slightly with electrophoretic systems, and the total molecular masses of the dsRNAs were calculated as 17.86 and 18.40 $\times$ 10⁶ Da for SDS polyacrylamide and agarose gels, respectively. The dsRNAs did not bind to the oligo (dT)+ paper in the trapping experiment, indicating the dsRNAs were not polyadenylated (data not shown). Positive-control mRNAs bound successfully to the oligo (dT)+ paper.

Comparison of dsRNA segments isolated from class I-infected tobacco plants with dsRNA of other plant reoviruses. Comparisons of dsRNAs from class I-infected tobacco plants with dsRNA of WTV, RDV, MRDV, RBSDV, RRSV, and GDV are depicted in Figures 2B and 3. Agarose gels (Fig. 2B) were run without WTV and RRSV, and SDS polyacrylamide gels (Fig. 3) were run without RDV. The pattern observed for the 12 dsRNAs isolated from class I-infected tobacco plants on 1% agarose gel differed when compared with patterns observed for fijiviruses MRDV and RBSDV and RRSV (an oryzavirus), but the dsRNAs did show certain similarities to dsRNA of RDV, a rice phyto-reovirus with 12 dsRNA segments.

The first eight segments (S1 to S8) of dsRNA isolated from infected tobacco were similar in size to the first eight segments of RDV dsRNA in agarose gels, whereas the remaining four segments (S9 to S12) differed between the two viruses (Fig. 2B). To obtain improved separation of the 12 dsRNA segments isolated from infected tobacco plants, a 30-cm SDS polyacrylamide gel was run (Fig. 3). All doublets separated well (Fig. 3, lane 1), and the banding pattern was compared with those of WTV, RDV, MRDV, RBSDV, RRSV, and GDV. The banding pattern of dsRNA isolated from class I-infected tobacco differed significantly from MRDV, RBSDV, RRSV, and GDV but showed some similarities to WTV. Size distribution of segments S4, S7, S11, and S12 of class I-infected tobacco (Fig. 3, lane 1) and WTV (Fig. 3, lane 2) were similar, whereas segments S1 through S3, S5, S6, and S8 through S10 were dif-

TABLE 1. Estimated numbers of base pairs and relative molecular masses of dsRNAs isolated from tobacco exhibiting class I leaf curl symptoms

dsRNA segments	Approximate no. of base pairs		Relative molecular masses ($\times$ 10 ⁶ Da)	
	Agarose gel	SDS-PAGE ^a	Agarose gel	SDS-PAGE
S1	4,786	4,570	3.25	3.10
S2	3,845	3,913	2.61	2.66
S3	3,467	3,342	2.36	2.27
S4	2,735	2,600	1.86	1.77
S5	2,691	2,470	1.83	1.68
S6	1,778	1,718	1.21	1.17
S7	1,745	1,704	1.19	1.16
S8	1,468	1,442	1.00	0.98
S9	1,291	1,334	0.88	0.90
S10	1,230	1,225	0.84	0.83
S11	1,169	1,138	0.79	0.77
S12	860	844	0.58	0.57
Total size	27,065	26,300	18.40	17.86

^a Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

ferent. S1 through S3 of tobacco appeared to be larger than the corresponding WTV segments. Minor bands (electropherotypes) were observed with segments S3, S7, S8, and S12 (Fig. 3, lane 1), indicating the possibility of heterogeneity in the mobility of individual segments (Fig. 3).

Nucleic acid hybridizations. Hybridizations of each of the end-labeled S1 to S4 dsRNA probes to the corresponding S1 to S4 segments (positive control) occurred, but there were no cross-hybridizations between the four dsRNA species, nor was there any cross-hybridization of the S5 to S12 dsRNA segments with the S1 to S4 probes (data not shown). No hybridization occurred between DIG-labeled WTV S4 and S6 to S9 fragments or any dsRNAs from tobacco (data not shown). Positive signals were obtained with WTV total dsRNA and homologous S4 and S6 to S9 DNA probes. Positive hybridizations also occurred with the respective S4 and S6 to S9 cloned DNA controls. No hybridizations took place with RDV or MRDV (negative controls) and WTV probes.

Virus extractions. Virions extracted from all infected tobacco material over 4 years were identical in shape and size. Electron microscopy of virus particles and negative staining revealed icosahedral particles that had an outer core ≈ 60 to 65 nm in diameter and appeared to have an inner smooth core 40 to 45 nm in diameter but lacking spikes (Fig. 4A and B). The virions appeared more angular than spherical in sodium phosphotungstate-negative stain at neutral pH, which does not occur with fijiiviruses.

Virus yields from all sources of tobacco were similar for extractions from frozen material and were low and roughly estimated to be 1 to 2 mg/50 g of leaf material. However, the yield for extraction from fresh material appeared to be approximately fivefold higher compared with frozen material.

Twelve segments of dsRNA that corresponded with those extracted from infected tobacco plants were successfully isolated from purified virions (Fig. 2C). Segments S4 and S5 and S6 and S7 appeared as single bands (Fig. 2C).

Fig. 4A and B. Electron micrographs of icosahedral particles (arrows in **A**) (inner core diameter 40–45 nm; outer diameter 60–65 nm; arrow in **B**) isolated from class I leaf curl-infected tobacco. **A**, Bar = 100 nm; **B**, bar = 50 nm.

ELISA. Antiserum to MRDV, a member of the genus *Fijivirus*, was used in ELISA. A 95% CI for each ELISA value was determined, and values were recorded as either positive or negative. The values obtained with MRDV antiserum for class I-infected tobacco material were not significantly different from negative healthy controls (data not shown). Positive binding of MRDV antibodies to the MRDV positive control was observed.

Immunoslot blots. WTV extracts from infected clover roots (positive control) and sucrose gradient density-purified tobacco virions showed strong serological cross-reactivity with antiserum to WTV (Fig. 5A and B, slots 1 through 3). No reaction was detected with healthy clover extracts (Fig. 5A, slots 4 through 6) and healthy tobacco extracts (Fig. 5B, slots 4 through 6). A positive reaction was observed with the primary antibody control (Fig. 5A and B, slots 7).

DISCUSSION

Virus particles resembling phyto-reoviruses were consistently isolated from leaf curl-infected tobacco from the eastern and western tobacco-growing regions of South Africa. Initial extractions from tobacco during the first few years of our research were aimed at isolating geminiviruses, because the literature reported that leaf curl was caused by whitefly-transmitted geminiviruses (16,23). Only after enough evidence was available to reveal the absence of a geminivirus in class I-infected tobacco, were reovirus extractions attempted. Consequently, the majority of subsequent reovirus extractions were performed on stored frozen (-80°C) leaf material, which was the only material available until recently, when an extraction was performed on fresh leaves. Virus yields generally were low from leaf tissue but improved when fresh material, ≈ 20 μg (frozen) to 100 μg (fresh) per g fresh weight, was used. Lack of available plant tissue and low virus yields did not allow comprehensive protein characterization, although some preliminary PAGE and Western blots were achieved and further work is being done.

The sizes of dsRNAs isolated from class I-infected tobacco plants and virions purified from infected plants were estimated to be similar in range but larger in total size (17.86 to 18.40 kDa) (Table 1) compared with reported sizes for WTV and other phyto-reovirus dsRNAs (15 to 17 kDa) (17). Depending on the electrophoretic system used, the total molecular mass of the WTV genome has been estimated at 15.06 (27), 16.02 to 16.67 (28), and 16.04 to 16.67 kDa (29). However, to our knowledge, segments S1 through S3 have not been sequenced for WTV, and based on the available sequence sizes of the smaller dsRNAs, it is probable that the total size estimations in the literature are inaccurate and should be larger. The phyto-reovirus dsRNA sizes reported in the literature are collectively smaller than the total molecular masses of the 10 dsRNA segments of fijiiviruses (18 to 20 kDa) (19). Furthermore, the size distribution of the dsRNAs, the lack of 3' poly(A) tracts, and the angular shape of the virus particles from tobacco also were inconsistent with the presence of fiji-, oryza-, or GDV-like reoviruses

Fig. 5. Immunoslot blot assay of tobacco virions cross-reacting with antiserum to wound tumor virus (WTV) (1:1,000). **A**, Slots 1 through 3: WTV; slots 4 through 6: healthy clover. **B**, Slots 1 through 3: tobacco virus; slots 4 through 6: healthy tobacco; **A** and **B**, slot 7: primary antibody (1:1,000) control; slot 8: biotinylated polyacrylamide gel electrophoresis marker control. Slot 1: undiluted sample; slot 2: 1:10 sample dilution; slot 3: 1:20 sample dilution.

(11,22). Variations in electrophoretic mobilities of dsRNA segments from infected tobacco were noted. Electropherotypes have been reported for several plant-infecting reoviruses, for example MRDV (18).

A previous study demonstrated that segments S1 through S8 of WTV and RDV exhibited a very similar pattern after coelectrophoresis through agarose gels (29), which was consistent with results obtained in our study, in which we compared the dsRNA segments of RDV and WTV (27,28) to the dsRNA segments isolated from class I-infected tobacco plant material. The banding pattern of dsRNAs isolated from infected tobacco was unique but most similar to the banding pattern of the phyto-reovirus WTV (27,29). The first eight segments of RDV, WTV, and dsRNA isolated from class I-infected tobacco exhibited similar electrophoretic mobilities through agarose and SDS polyacrylamide gels, respectively (Figs. 2B and 3). The differences in dsRNA pattern and total genome size between WTV and the tobacco virion suggest the virus isolated from class I-infected tobacco plants may be a new phyto-reovirus; this theory is supported further by the lack of cross-hybridization between S4 and S6 to S9 of WTV and dsRNAs from infected tobacco. Similarities between particles isolated from class I-infected tobacco and those from intact wild-type WTV (29) were observed, and a serological relationship was established between WTV and the tobacco virus proteins by immunoslot-blot ELISA. To date, no serological relationships have been demonstrated between the only fully characterized dicot phyto-reovirus and other phyto-reoviruses. However, a serological relationship has been established between RDV and rice gall dwarf reovirus, which are monocot phyto-reoviruses (8,11,22). Serological results do not support the presence of a fijivirus in class I-infected tobacco plants, because no antigen binding was observed with MRDV antiserum.

In summary, particle morphology, size, and pattern of dsRNAs and serological data demonstrated that dsRNAs isolated from class I-infected tobacco represent the genome of a member of the genus *Phytoreovirus*. In addition, symptoms of leaf enations, vein swellings, and root tumors in tobacco showed a distinct similarity to symptoms reported for the only dicot-infecting phyto-reovirus, WTV (2,11). The tobacco virus appears to be serologically related to WTV, but lack of significant sequence homology and differences in dsRNAs between WTV and the tobacco virus suggest a distinct phyto-reovirus. Cloning and molecular characterizations are required to establish the identity of the tobacco virus and its relationship to other phyto-reoviruses. The virus isolated from class I-infected tobacco in South Africa is the first report of a phyto-reovirus in tobacco, the first record in Africa, and the second example of a field-isolated phyto-reovirus; the first field isolation was performed in 1991 (10). We propose the name tobacco leaf enation virus (TLEV) for our virus isolate. The etiology of the disease in tobacco needs to be elucidated, and the vector must be identified in further studies.

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