OTHER VIRUSES

Citrus Leprosis Symptoms can be Associated with the Presence of Two Different Viruses: Cytoplasmic and Nuclear, the Former Having a Multipartite RNA Genome

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ABSTRACT. Citrus leprosis has been present in several South American countries for many decades. It's recently reported emergence in Central American countries presents a serious threat to citrus in the Caribbean Basin, Central and North America. Cyto- and nucleo-rhabdovirus-like particles have been associated with citrus leprosis previously by electron microscopy. Screening and sequencing of clones from a cDNA library made from a total RNA extraction from cytoplasmic-leprosis infected tissue has led to the identification of some putative viral sequences. Northern hybridizations have shown consistent association of these putative viral sequences with the cytoplasmic-type leprosis from Panama, but not with the nuclear-type or with healthy citrus. Northern hybridizations using DIG-labeled DNA and RNA (sense and antisense) probes of different clones have shown two different patterns suggesting two RNAs (RNA 1 and RNA 2) associated with cytoplasmic-leprosis infected tissue. Probes of clones from RNA 1 hybridized with two RNAs of approximately 10 kb and 1.5 kb, while probes of clones from RNA 2 hybridized with four RNAs of approximately 4.7, 2.5, 1.6, and 1.0 kb. These results suggest that at least two species of RNAs are associated with leprosis infected tissue where only cytoplasmic type particles may be detected by transmission electron microscopy, and that the cytoplasmic- and the nuclear-type of leprosis are probably caused by two distinct viruses.

Index words. Citrus leprosis virus, hybridization, cytoplasmic, nuclear, *Citrus* spp., Panama.

The GenBank accession number for the nucleotide sequences are DQ388512 (RNA 1), and DQ388513 (RNA 2).

Citrus leprosis is probably the most important virus disease of citrus in Brazil at the present time (33). The Brazilian citrus industry spends over US$100 million annually (about 21% of citrus production cost) on miticides to control the *Brevipalpus* mites that vector the virus (30, 34). In South America, leprosis disease is present in Argentina, Brazil, Paraguay, Uruguay, Bolivia, and Venezuela (6, 9, 10, 16, 24, 34), and in Panama in Central America (14). Leprosis was first reported in Florida in the early 1900s and was a limiting factor in production, then the incidence decreased and the last report of leprosis occurring in Florida was in the 1960s, and it is considered a disease exotic to Florida at the present time (9). There are unconfirmed reports of leprosis from Costa Rica (2), Guatemala (31), Honduras, and Nicaragua (Brlansky, unpublished observations). Symptoms of leprosis-like diseases have been reported from citrus-producing areas of Asia and Africa (16, 23, 32), but none of these reports have been confirmed (28).

In 1972 Kitajima et al. (21) reported the presence of rod-like particles (40-50 nm × 100-110 nm) in the nucleus and cytoplasm of infected cells, commonly associated with nuclear and endoplasmic reticulum (ER) membranes in symptomatic leprosis tissue from Brazil. The cytologi-

cal studies of lesions caused by citrus leprosis disease from Argentina (22) and more recently from Brazil (11) report a different virus morphology, with bacilliform, membrane-associated virus-like particles (50-60 × 100-110 nm) within the cisternae of the ER and with an electron dense, vacuolated viroplasm in the cytoplasm. The latter type of morphology from Brazil (cytoplasmic) appears to be more common than the nuclear-type reported earlier.

Citrus leprosis virus has been tentatively placed as an unassigned rhabdovirus (with no indication of whether the virus is nuclear- or cytoplasmic-type) in the family *Rhabdoviridae*, based on particle morphology and host cytopathology (11, 14, 20, 21, 33, 36). There are two known genera of plant-infecting rhabdoviruses, *Cytorhabdovirus* and *Nucleorhabdovirus*. Cytorhabdoviruses replicate in the cytoplasm of infected cells in association with masses of thread-like viroplasm structure, and virus morphogenesis occurs in association with vesicles of the ER (36). Nucleorhabdoviruses accumulate in the perinuclear space with some particles scattered in the cytoplasm (19). Viral proteins of nucleorhabdoviruses accumulate in the nucleus, and virus morphogenesis occurs at the inner nuclear envelope (36).

Recently, other negative-stranded plant viruses with significant differences from true rhabdoviruses have been described. These include *Orchid fleck virus* (OFV) and *Lettuce big-vein associated virus* (LBVaV), with some sequence similarities to rhabdoviruses (7, 25, 26, 35). However, they differ from rhabdoviruses (single component negative-sense RNA viruses) by having multipartite genomes. In LBVaV, both positive-sense and negative-sense RNAs are encapsidated, but in separate virions. Sasaya et al. (35) recommended a re-evaluation of the taxonomic position of LBVaV in light of these differences. OFV has a bipartite, negative-sense RNA genome. Kondo et al. (25) recommended that OFV and other allied viruses, probably *Brevipalpus* mite-borne viruses, should be placed in a new genus in the family *Rhabdoviridae*, based on particle morphology, genome structure and vector transmission.

Molecular approaches have been employed recently to better understand the leprosis virus genome and develop diagnostic methods (17, 18, 27). Availability of detection methods would be highly useful for quarantine and certification programs, and for developing better disease management strategies. The present study was undertaken to develop molecular information on the cytoplasmic virus associated with citrus leprosis disease.

MATERIALS AND METHODS

Virus samples. Leaves, fruits and twigs from naturally infected sweet orange with typical leprosis lesions were collected from citrus orchards in Boquete and Potrerillos, Chiriqui State, Panama. At each location 15 samples were collected. Small (~2 × 2 mm) pieces from the edge of the leaf lesions, or from leaves collected from trees without symptoms were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2, for 12 h at 4°C, and then washed with 0.1 M phosphate buffer, pH 7.2, with buffer changes at regular intervals for three days. The samples were then post-fixed in 2% osmium tetroxide in 0.1 M phosphate buffer, pH 7.2, dehydrated using an acetone series, then infiltrated and embedded in Spurr's resin as previously described (18). Samples from each tree were examined by transmission electron microscopy to verify the presence of leprosis virus and to determine the location of the virus particles. This information was used to make five tree composite samples for total nucleic acid extractions. Leaves from trees without leprosis symp-

toms collected from Panama and Florida served as controls.

Total nucleic acid extraction and RNA isolation. About 5 g of tissue was powdered in liquid nitrogen and used for extraction of total nucleic acids following the protocol of Morris and Dodds (29) with modifications. Briefly, the powdered tissue was transferred to a 50 ml beaker and the following reagents were added: 7 ml of 2× GPS (0.2 M glycine, 0.1 M Na_2HPO_4, 0.6 M NaCl, pH 9.6); 10 ml of phenol (equilibrated with 0.5 M Tris, pH 8.0); 1 ml of 10% SDS; and 0.1% bentonite. The mixture was shaken for 30 min and then centrifuged at 8,000 *x* g for 20 min. The upper aqueous phase was collected and adjusted to 10 ml with 2× GPS buffer. After adding 0.1 volume of 3.0 M sodium acetate, pH 5.2, and 2.5 volumes of 95% ethanol, the extracts were shipped to Florida. These extractions were stored at -20°C until further processed. For total RNA extractions, an aliquot of about 4 ml of the total nucleic acid extracts was centrifuged at 17,000 g for 20 min at 4°C, and the pellet was resuspended in 100 µl of water and processed with the QIAgen RNeasy Plant Mini Kit (QIAgen, Inc., Valencia, CA) according to manufacturer's instructions.

Construction of cDNA library. Total RNA extracted from leprosis lesions of fruits collected from Potrerillos, Panama, was selected for construction of a cDNA library using the Smart™ cDNA Library Construction Kit (Clontech, Palo Alto, CA) which amplifies mRNAs, following manufacturer's instructions. The Smart IV™ oligonucleotide and the CDS III/3' PCR primers were used to generate first-strand cDNA. The cDNA was amplified employing a high fidelity proofreading long-distance polymerase chain reaction (LD-PCR) kit (Epicentre Tech, Madison, WI) (4, 8) for 25 cycles using the same set of primers. The PCR amplified products were digested with *Sfi* I restriction enzyme to facilitate directional cloning in the phage vector, λ TriplEx2™. The recognition sites for *Sfi* I A and *Sfi* I B were incorporated in Smart IV™ and CDS III/3' primers, respectively. A CHROMA SPIN-400 column was used to size-fractionate the *Sfi* I-digested PCR amplicons; products larger than 500 bp were selected and ligated into *Sfi* I-digested, dephosphorylated bacteriophage λ TriplEx2™ vector (Clontech, Palo Alto, CA). The ligation reactions were packaged using the Gigapack® III Gold-4 packing extract kit (Stratagene, La Jolla, CA), according with the manufacturer's protocol. The packaged bacteriophages were titered by serial dilution, followed by infection of *E. coli* strain XL1-Blue and titering on LB/$MgSO_4$ plates.

Size-screening of the λ phage clones. Isolated phage plaques were randomly picked and incubated in 200 µl of 1× lambda dilution buffer (0.1 M NaCl, 0.01 M $MgSO_4$-$7H_20$, and 0.035 M Tris-HCl pH 7.5), for 4 h at 37°C without shaking. PCR screening of lysed plaque DNAs was done using the Advantage 2 PCR Kit (Clontech, Palo Alto, CA) and "λ TriplEx LD-Insert Screening Amplimer Set" primers (Clontech, Palo Alto, CA). The PCR reaction was conducted as follows: 94°C for 3 min, 30 cycles of 94°C for 30 sec, 50°C for 30°C sec, 72°C for 3 min followed by a final incubation at 72°C for 12 min. The PCR products were analyzed by the E-Gel® 96 High-Throughput Agarose Electrophoresis System (Invitrogen Co., Carlsbad, CA). The phages with DNA inserts larger than 500 bp were selected for sequencing.

Conversion of bacteriophage λ TriplEx2 to plasmid pTriplEx2. Over 300 recombinant phages with inserts larger than 500 bp were selected for conversion from bacteriophage into plasmid according to the manufacturer's protocol (Clontech, Palo Alto, CA). Single colonies from each of the conversions were selected and grown overnight in 96

well plates using LB broth (200 µl/well) with carbenicillin (final concentration of 50 µg/ml) and sequenced at the Genome Sequencing Services Laboratory, University of Florida. Capillary sequencing was done using the Amersham Pharmacia Biotech MegaBACE 1000 and the pTriplEx2 universal forward primer.

Comparison of sequences. Over 300 clones were analyzed using the program BLAST (http://www.ncbi.nlm.nih.gov/BLAST/) (1) and compared to sequences available in the database at both the nucleotide and protein level. All sequences with high similarities to plant sequences (with an Expect value of 1.0 or lower) were discarded. Sequences that did not share similarities with known plant sequences were aligned using SEQUENCER software (Gene Codes Corporation, Ann Arbor, MI).

Northern analysis using DNA probes. Clones carrying putative viral sequences in the vector, pTriplEx2, from two different RNAs (clone AG 1-C-09 with an insert of 673 b for RNA 1 and AG 1-A-1 with an insert of 691 b for RNA 2) were used as templates to synthesize digoxigenin (DIG)-labeled DNA probes by PCR using universal primers according to manufacturer's protocol (Roche Applied Science, Indianapolis, IN). About five µg each of total RNA extracts were electrophoresed on a 1% agarose gel containing 2% formaldehyde in 1× MOPS buffer, pH 7.0 (20 mM MOPS, 5 mM sodium acetate, 2 mM EDTA) at 65 volts for 4 h. The gels were stained with ethidium bromide for visualization of ribososomal RNAs. The electrophoresed RNA was transferred from gels to a positively charged nylon membrane by capillary transfer. The blot was then used for hybridization using 100 ng of DIG-labeled DNA probe in 5 ml of DIG-Easy hybridization buffer at 50°C overnight (Roche Applied Science, Indianapolis, IN). After washing, the membrane was incubated with anti-DIG antibody-alkaline phosphatase conjugate; the probe was visualized by chemiluminescent detection using CDP-Star according to the manufacturer's protocol (Roche Applied Science, Indianapolis, IN).

Northern analysis using riboprobes. The purified plasmid, AG 1-C-09 was linearized independently by *Sal* I and *Eco* R I restriction enzymes to use as template for preparing sense (in the same orientation as the messenger RNA) and antisense (complementary to messenger RNA) DIG-labeled RNA probes according to the manufacturer's protocol (Roche Applied Science, Indianapolis, IN). Probe amounts were adjusted for comparable signal strength by hybridizing to the cloned plasmid, AG 1-C-09. About 1 µg each of total RNA extract was electrophoresed and transferred to nylon membrane as described above. The DIG-labeled RNA probe from a 20 µl transcription reaction was mixed in 5 ml of DIG-Easy hybridization buffer. Prehybridization, hybridization and chemiluminescent detection of the probes on the blots were done as described above and according to the manufacturer's protocol (Roche Applied Science, Indianapolis, IN) except for hybridization that was at 65°C overnight.

RESULTS

Leprosis symptoms observed in Panama were essentially the same as those described earlier (28, 33), and were present on leaves, bark and fruits (Fig. 1A-D) of most of the trees in Potrerillos and Boquete. Lesions on leaves were usually round to elliptical, yellowish spots with dark brown centers. In some cases, the dark center was absent and the lesions were larger (Fig. 1B). A sample from fruit with typical symptoms collected from Potrerillos was used to construct the cDNA library. After reverse transcription and LD-PCR, followed by removal of

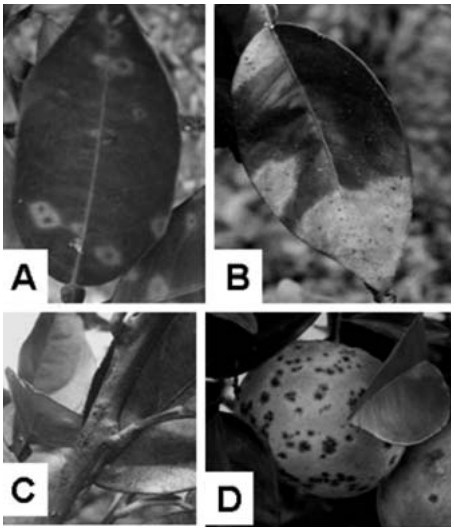

Fig. 1. Leaf, fruit and bark symptoms of citrus leprosis disease from Panama. The leaf lesions appear as concentric spots, with a central dark spot, and are surrounded by a chlorotic halo (A); or without central dark spot and leading to larger lesions (B). Bark lesions begin as small chlorotic concentric rings with a necrotic area in the center (C). The rings enlarge over time until the twig is girdled. Fruit lesions are necrotic with a dark central area (D).

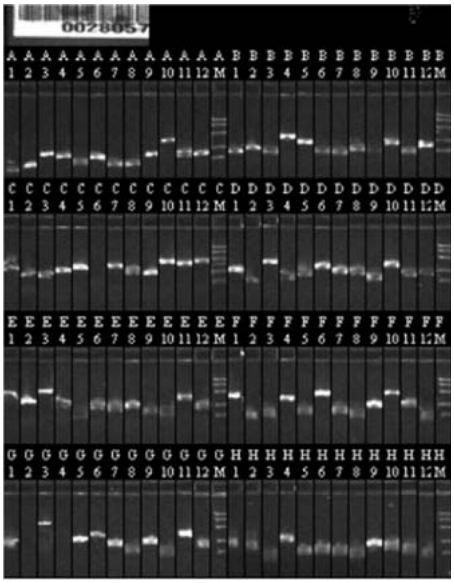

Fig. 2. Rapid size-screening of the recombinant λ TriplEx2 phages using the E-Gel® 96 High-Throughput Agarose Electrophoresis System. A sample gel loaded with 96 PCR products is shown here. Lanes labeled 'M' contain markers of the High Range Quantitative DNA® of sizes 400, 800, 2000, 4000 and 10,000 bp.

the primers by *Sfi* I restriction, the cDNA product was passed through a CHROMA SPIN-400 column, and the fractions were analyzed for size using agarose electrophoresis. Fractions 4-12 were selected for ligation into λ arms since they contained mRNAs larger than 500 bases.

Individual phage plaques were used as templates for PCR screening. An analysis of some PCR amplified products using standard agarose gel electrophoresis showed that the insert sizes varied from a few hundred bp to over 1,000 bp. Further PCR amplifications were done in 96 well plates and analyzed by using the E-Gel® 96 High-Throughput Agarose Electrophoresis System (Fig. 2). About 1,300 phage plaques from the cDNA library were size screened by PCR and over 300 plaques were found to contain inserts larger than 500 bp.

Over 300 sequences were individually analyzed by the NCBI BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/). Sequences without similarities to plant sequences were selected for further analysis. Using the SEQUENCER computer program, two major contigs were identified. Contig 1 consisted of 19 overlapping clones of 1,115 bases containing a putative open reading frame (ORF) of 263 amino acids. Six clones contained the 1,115 bases, and the rest of the clones had only partial sequences. This contig will be referred to as RNA 1. Contig 2 contained 34 overlapping clones of 982 bases and contained a putative ORF of 214 amino acids. The 982 bases were contained in nine clones while the other clones had only partial sequences. Contig 2 will be referred to as RNA 2. Sequences of both contigs probably code for novel proteins since they did not show

similarity with any known viral or other sequences in BLAST analysis.

A 673 bp DIG-labeled DNA probe was synthesized based on the putative viral sequence from RNA 1. This probe hybridized specifically with total RNA extracts from symptomatic leprosis samples collected from Potrerillos, but not with those from Boquete or with healthy controls (Fig. 3). The probe hybridized with two major RNAs of approximately 10 kb and 1.5 kb in total RNA extracts from leprosis lesions from leaves, fruits and twigs collected from Potrerillos (Fig. 3, lanes 1-3, 9 & 10). RNA extracts from symptomless tissue from around the lesions of leaves, fruits and twigs contained only the smaller RNA of 1.5 kb (Fig 3, lanes 4-6) which hybridized with the DIG-labeled probe from RNA 1. However, a larger RNA was visible in lanes 4-6 after longer exposures (data not shown). This probe hybridized with the PCR fragments from plasmids used as positive control on the Northern blots, as expected.

A different pattern was observed when the 691 bp DIG-labeled DNA probe derived from putative leprosis sequence from RNA 2 was used in hybridization assays (Fig. 4). Samples from leprosis-infected tissue collected from Potrerillos which contained only cytoplasmic virus particles showed hybridization with RNAs of approximately 4.7, 2.5, 1.6, and 1.0 kb (Fig. 4, lanes 1 and 2), but not with healthy tissue (Fig. 4, lane 5). Tissue from non-lesion areas of symptomatic leaves showed no hybridization at 5 min exposure (Fig. 4, lane 3), but after an expo-

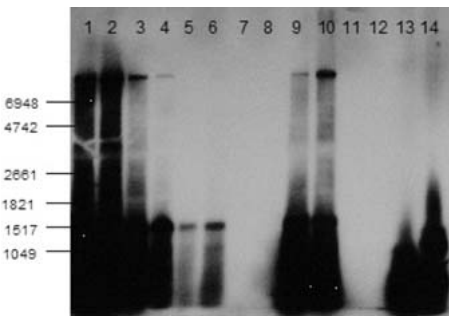

Fig. 3. Northern blot of total RNA extracts from citrus tissue using the DIG-labeled DNA probe for putative cytoplasmic citrus leprosis virus sequence of RNA 1. Total RNA extracts include samples collected from Potrerillos (lanes 1-6, 9-11), and Boquete (lanes 7, 8, 12), both located in Panama. Lesion tissue from leaves (lanes 1, 7 & 9), fruit (lanes 2, 8 & 10) and twigs (lane 3) as well as nonsymptomatic areas of infected leaves (lane 4), fruit (lane 5) and twigs (lane 6) were used. Leaves from healthy trees (lanes 11 & 12) were used as negative controls. PCR products from plasmids with two different size inserts (254 bp in lane 13 and 414 bp in lane 14) were used as positive controls.

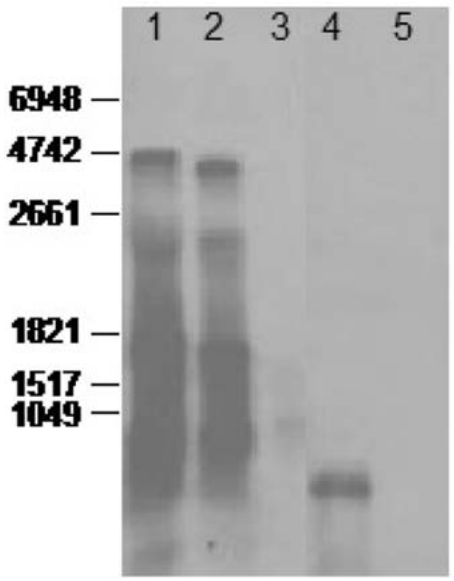

Fig. 4. Northern blot of the total RNA extracted from citrus from Potrerillos, Panama, using a DIG-labeled DNA probe for putative cytoplasmic form of leprosis virus sequence of RNA 2. Total RNA extracted from leprosis lesions from symptomatic tissue of leaves (lane 1) and fruit (lane 2); and from nonsymptomatic area of leaves with lesions (lane 3) were used. Total RNA extracts from the leaves of a visually healthy tree (lane 5) was used as a negative control. PCR amplified products of the plasmid AG 1-A-1 using universal primers (lane 4) was used as positive control.

sure time of 30 min it showed a banding pattern similar to lanes 1 and 2 (data not shown).

Further analysis of the total RNA extracts from leprosis infected tissue were done using both sense and antisense DIG-labeled riboprobes transcribed from RNA 1. Three RNA extracts from symptomatic leprosis tissues from Potrerillos, one from Boquete and one from visually healthy tissue were used in the analysis. Hybridization using the negative-sense probe (Fig. 5, panel A) showed the same pattern obtained by hybridization with DIG-labeled DNA probe at 10 min exposure, but the positive-sense probe (Fig. 5, panel B) required over 50 min of exposure to show approximately the similar intensity as that of negative sense probe. In both RNA blots, two bands were observed; one of about 10 kb, and other of about 1.5 kb.

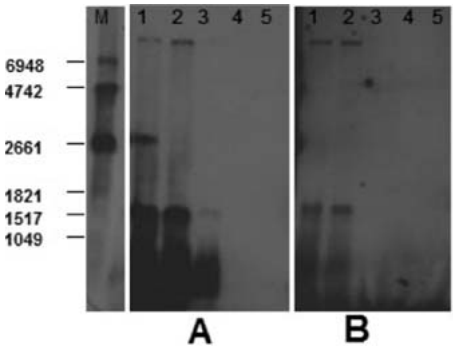

Fig. 5. Northern blots of total RNA extracts from leprosis infected tissue from Panama using negative-sense (panel A) and positive-sense (panel B) riboprobes of putative cytoplasmic form of leprosis virus sequence of RNA 1. Total RNA extractions from citrus tissues collected from Potrerillos (lanes 1 to 3 and 5) and Boquete (lane 4) were used. Leprosis lesion tissue from leaves (lanes 1, 4), fruits (lane 2) and the nonsymptomatic area of leaves with lesions (lane 3) were used. Total RNA extracts from leaves of visually healthy citrus trees were used as negative control (lane 5). Lane M represents 100 ng of RNA molecular weight marker.

DISCUSSION

Samples with typical leprosis symptoms on leaves, fruits and twigs were collected from two different locations in western Panama. Samples from these two areas were used in an earlier study for transmission electron microscopic (TEM) analysis which reported cytoplasmic virus-like particles in samples from Potrerillos and nuclear virus-like particles in samples from Boquete (14). TEM analysis of the samples used in the present study showed the presence of cytoplasmic type of virus particles in samples collected from Potrerillos and nuclear type of virus particles in samples from Boquete (data not shown).

Leprosis infected tissue from Brazil has been extensively studied by TEM (11, 14, 20, 21, 22, 33). Based on particle morphology in 1972 (21), leprosis virus was considered as a putative member of the family *Rhabdoviridae* in the order *Mononegavirales* with nucleorhabdovirus-like particles which were incompletely enveloped. Later studies associated cytoplasmic bacilliform-like virus particles with leprosis (33). Some authors have suggested that the same virus may be capable of existing in both the cytoplasm and nucleus depending on different developmental stages (11). Others hypothesized that the leprosis symptom may be caused by two entirely different viruses, one present in the nucleus and the other in the cytoplasm (10, 20).

The present study was initiated assuming that leprosis was caused by a rhabdo-like virus. The genomes of rhabdoviruses (36) and some other plant viruses of similar morphology, including OFV (7, 25, 26) and LBVaV (35), are negative-stranded and have related sequences. The genome size of rhabdoviruses ranges between 11 to 13 kb. Both OFV and LBVaV have bipartite genomes while the plant rhabdoviruses have monopartite genomes. None of these

genomes are polyadenylated, but all of them produce subgenomic RNAs which are polyadenylated at the 3' end and capped at the 5' end (3, 5, 15, 25, 26, 35).

Since it is presently difficult to obtain purified virus (12) and because leprosis is exotic to North America, total nucleic acids were extracted from leprosis lesions and later used for extraction of the total RNA. The total RNA extract was used to construct a cDNA library using an oligo dT primer to anchor on the 3' end of mRNAs. Even though we could not target the viral genome of putative rhabdoviruses by using this approach, mRNA sequences, including viral mRNAs, were likely targets since they should be abundant in these tissues. Since most mRNAs of rhabdoviruses (other than those corresponding to the L gene) are between 700 to 1,700 bases (13), an effort was made in this study to select cDNAs larger than about 500 bases in size.

Analysis of the sequences of over 300 clones showed that about 53 sequences did not show significant homology to known plant sequences. By alignment, those sequences formed two contigs, referred to as RNA 1 and RNA 2. Hybridization patterns using probes specific for RNAs 1 and 2 with total RNA extracts from symptomatic leprosis tissues indicated RNAs 1 and 2 are present only in total RNA extracts from leprosis-infected tissue that contains only cytoplasmic virus particles. Indeed positive hybridization using leprosis-specific probes was obtained only from samples collected from Potrerillos which contained cytoplasmic virus particles, but not from samples collected from Boquete which contained predominantly nuclear-type virus particles, as detected by TEM analysis (18). Hence, RNAs 1 and 2 appear to be associated with the presence of the cytoplasmic-type virus particles. The results clearly suggest that there are two distinct viruses, the cytoplasmic-type and the nuclear-type, associated with leprosis disease that may be causing similar symptoms, and the occurrence of these two virus types have been seen using electron microscopy (11, 14, 21, 22).

The probe for RNA 1 hybridized with two RNAs of about 10 kb and 1.5 kb indicating that at least two ORFs may be present in RNA 1. The probe for RNA 2 hybridized with RNAs of 4.7, 2.5, 1.6 and 1.0 kb indicating that at least four different ORFs are present in RNA 2. The hybridization patterns with probes containing sequences of RNAs 1 and 2 suggest that the virus is at least bipartite with a gene expression strategy consisting of 3' co-terminal sub-genomic RNAs. Hybridization with positive- and negative-sense DIG-labeled RNA probes of RNA 1 showed the presence of both positive- and negative-sense RNAs of both genomic RNA and subgenomic RNAs in infected tissues. Negative-sense RNA appeared to be present in lesser amounts than the positive-sense RNA. The predominance of plus strands of RNA in infected cells excludes association of the RNAs with rhabdoviruses, which have negative-stranded genomes and generate monocistronic mRNAs. LBVaV contains both positive- and negative-stranded RNAs encapsidated in separate virions (35), but the presence of negative-stranded subgenomic RNAs has not been reported. The above results strongly suggest that a bipartite (or multipartite) positive-sense RNA virus is associated with the cytoplasmic leprosis disease.

The sequences of contigs 1 and 2 (RNA 1 and 2, respectively) have been obtained (GenBank accession numbers DQ388512 and DQ388513 for RNA 1 and 2, respectively). RNA 1 codes for a putative 28.9 kDa protein of 263 amino acids, with no significant homology to any known sequence. RNA 2 codes a putative 23.5 kDa protein of 214 amino acids, without significant homology to any known sequence. The lack of signifi-

cant homology with any of the known viral sequences available in the database suggests that the cytoplasmic leprosis associated virus may belong to a new genus of plant viruses.

The data presented here further confirms previous observations that the symptoms commonly associated with leprosis disease may be associated with at least two distinct viruses, one in which virions accumulate in the cytoplasm; and the other where virions are found mainly in the nucleus. The nuclear-type leprosis has been reported less often (14, 18, 20, 34). Even though the molecular methods developed here would facilitate rapid detection of the most prevalent type of virions that occur in the cytoplasm, there is a definite need for molecular characterization of the nuclear type of leprosis virus.

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