

Nationwide Detection by RT-PCR of the Cytoplasmic Type of Citrus Leprosis Virus in Brazil

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ABSTRACT. Citrus leprosis virus (CiLV), the causal agent of leprosis disease of citrus, is transmitted by the Tenuipalpidae mite, *Brevipalpus phoenicis*, and the cost for chemical control of the mite and hence leprosis approaches US\$60-100 million/year in the state of São Paulo, Brazil. Two distinct virus particles are associated with leprosis symptoms, cytoplasmic (CiLV-C) and nuclear (CiLV-N) particles, with the former by far the most prevalent. Using RT-PCR with two sets of primers that specifically amplify regions within the putative movement protein and replicase genes of CiLV-C, we detected the virus in a limited number of citrus samples from several municipalities within São Paulo State, Brazil. To expand upon these initial findings, we analyzed 152 symptomatic samples of five species and 21 varieties of citrus from 57 municipalities in nine states, with these samples representing the main citrus producing areas of the country. Symptomatic and asymptomatic tissue from citrus leaves, fruits, and stems was analyzed. RT-PCR analysis of nucleic acid extracts from all except two samples with typical leprosis symptoms yielded bands of the expected sizes as assessed by agarose gel electrophoresis. No bands were amplified from extracts from asymptomatic samples. These results demonstrate the usefulness of RT-PCR as a tool for the detection of CiLV-C isolates from different citrus varieties and geographic regions.

Citrus leprosis virus (CiLV), a tentative member of the family *Rhabdoviridae*, is considered the most economically important virus affecting citrus trees in Brazil, where the chemical control of the vector and associated citrus leprosis disease cost annually US\$60-100 million in the State of São Paulo (4, 5). In recent years, the spread of the disease to other countries in South and Central America, and the worldwide presence of the *Brevipalpus* mite vector, have significantly increased the importance of CiLV as well as the threat it poses to the citrus industries in other countries (2). However, despite its importance, only recently was a specific and accurate reverse transcription polymerase chain reaction (RT-PCR) based tool developed for the diagnosis of the disease, which was done traditionally through assessment of typical symptoms, transmission by the vector and by transmission electron microscopy (TEM) of symp-

tomatic tissues (2). Through TEM, two forms of CiLV particles were recognized in infected citrus tissues: the prevalent cytoplasmic type CiLV-C and the rare, nuclear type CiLV-N (3). The primers used for the diagnosis of leprosis by RT-PCR (2) specifically amplify CiLV-C and do not detect CiLV-N (Freitas-Astúa *et al.*, 1).

One hundred and fifty-two symptomatic leaf, fruit, and branch samples from five species (*Citrus sinensis*, *C. reshni*, *C. reticulata*, *C. deliciosa*, and *C. aurantifolia*) and 21 varieties of citrus, originating from 57 municipalities within the Brazilian states of São Paulo, Minas Gerais, Acre, Tocantins, Paraná, Santa Catarina, Rio Grande do Sul, Rio de Janeiro, and Goiás, were collected for analysis. All leaves, branches, and fruits sampled exhibited typical circular to elliptical chlorotic or necrotic local lesions that could coalesce forming large spots in the infected tissue. RT-PCR was per-

formed using the specific primer pairs Rep and MP, which amplify regions within the putative genes for the CiLV-C replicase and movement proteins, respectively (2). Aliquots of RT-PCR products were analyzed by electrophoresis in 1% agarose gels.

CiLV-C was detected by RT-PCR using Rep and MP primers in 150 out of the 152 samples analyzed. Two samples, one from the State of Acre and another from the State of Rio de Janeiro, produced no product with either primer pair, even though tissues displayed typical leprosis symptoms. Since RT-PCR analyses

were repeated twice for both samples, the lack of product could indicate variation of the virus sequences or problems during RNA extraction.

In conclusion, primers MP and Rep were effective for the detection of CiLV-C isolates from various citrus species and varieties, and from different geographical locations, confirming the efficacy of the test for the diagnosis of leprosis.

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