# RT-PCR for the Simultaneous Detection of Citrus Tristeza and Leprosis Viruses*

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**RESUMO**

**RT-PCR para a detecção simultânea dos vírus da leprose e da tristeza dos citros**

Este trabalho relata a detecção dos dois principais vírus que infetam citros (*Citrus* spp.) no Brasil, o *Citrus leprosis virus* (CiLV) e o *Citrus tristeza virus* (CTV), através de RT-PCR duplex. Os resultados mostram que a técnica eficientemente distinguiu amostras infetadas por apenas um ou pelos dois vírus concomitantemente, confirmando a especificidade dos primers e a viabilidade da detecção simultânea dos vírus em diferentes tecidos vegetais.

The two most important viral diseases affecting citrus (*Citrus* spp.) plants in Brazil are tristeza and leprosis, caused by *Citrus tristeza virus* (CTV), genus *Closterovirus*, family *Closteroviridae* and *Citrus leprosis virus* (CiLV), a tentative member of the *Rhabdoviridae* family. CTV is transmitted by aphids, is systemic in citrus plants, and occurs worldwide. CiLV induces only local lesions in citrus tissues and, even though its vector, the tenuipalpidae mite *Brevipalpus phoenicis* Geijskes, is spread worldwide, the disease is only present in the Americas. Because CiLV virions accumulate in distinct places in infected cells, it has been proposed two types of the virus: the rare, nuclear- and the prevalent cytoplasmic-types (Exper. Appl. Acarol. 30:161. 2003).

The objective of this study was to determine whether or not it was possible to detect CTV and CiLV simultaneously in the same RT-PCR tube, using different plant tissues. Twenty-two doubly infected samples of leaves, fruits, and stems of 'Pera' and 'Natal' sweet orange [*Citrus sinensis* (L.) Osbeck], and 'Mexerica do Rio' and 'Cravo' mandarin (*C. reticulata* Blanco), originated from Brasília, DF, and five municipalities within the states of São Paulo and Minas Gerais, were tested. Total RNA was extracted from typical CiLV symptomatic areas using a protocol described elsewhere (J. Virol. Methods 63:9, 1997), and cDNA strand was done using M-MLV reverse transcriptase and random primers mix (Invitrogen), according to the manufacturer's instructions. Two pairs of primers were used for the PCR, one that amplifies 557 bp within the *p20* gene of CTV (Virus Genes 21:139, 2000), and the other that amplifies a 339 bp region within the putative movement protein (*MP*) gene of CiLV (Plant Dis. 87:1317, 2003). An initial denaturation cycle at 94 °C for 2 min was followed by 32 cycles of denaturation at 94 °C for 30 s, annealing at 53 °C for 30 s, and extension at 72 °C for 40 s. A final 5 min extension was added to the cycle. Aliquots of RT-PCR products were visualized in 1% agarose gels stained with ethidium bromide.

All of the doubly infected samples tested yielded two bands in the gel (Figure 1), corresponding to the expected sizes of the genomic regions of CTV and CiLV. In some cases, the CiLV-associated band was more evident than the CTV-associated one, which can be explained by the fact that RNAs were extracted directly from CiLV lesions and hence, this virus should have higher titer than CTV's in those particular areas. Healthy, virus-free plants obtained by shot-tip grafting did not yield any amplification product after RT-PCR. Plants infected with only one of the viruses resulted in amplification with the primer pair designed only to the homologous virus. These results indicate that it is possible to detect the most important viruses affecting citrus plants in Brazil in a single reaction, and the presence of both viruses in the tissues does not affect their detection by duplex RT-PCR. Since CiLV is localized and CTV is systemic in citrus plants, total RNA should be extracted from leprosis lesions.

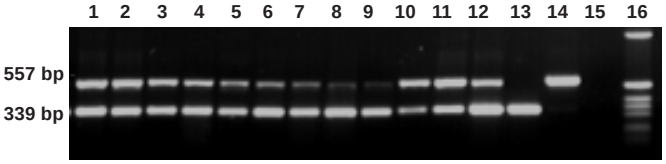


**FIG. 1** - Agarose-gel electrophoresis of duplex RT-PCR products obtained from citrus (*Citrus* spp.) plants doubly infected with *Citrus leprosis virus* (CiLV) and *Citrus tristeza virus* (CTV). Mandarin (*Citrus reticulata*) samples: Lanes 1, 5, 10, 11: Leaves; 2, 4: Fruits. Sweet orange (*C. sinensis*) samples: 3, 7: Fruits; 6, 8, 9: Leaves; 12: Stem; 13: Leaf of *C. sinensis* plant infected with CiLV only; 14: Leaf of *C. sinensis* plant infected with CTV only; 15: Healthy, virus-free *C. sinensis* plant; 16: 1 kb Extension Ladder (Invitrogen).

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