

Turkish Journal of Agriculture and Forestry

Turkish Journal

of

Agriculture and Forestry

**A New Method for the Detection of Minor Populations of Citrus Tristeza Virus
Strains Infecting Single Citrus Trees**

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Abstract: Fifteen different biologically important citrus tristeza virus (CTV) isolates (B14, B15, B23, B31, B52, B57, T36, D9, D10, D11, D12, D15, D16, D20 and D32) were tested using strain-specific biotin-labeled oligonucleotide probes. Coat protein genes (CPGs) of the samples were amplified by PCR, and the products were hybridized with probes (direct PCR hybridization (DPH)) and sequenced. In the DPH, all samples having probe 0 reactions were infected with CTV. Some isolates giving more than one specific probe reaction showed that they may be infected by mixtures of different CTV strains. In order to investigate this, CPGs from individual bacterial colonies were used as PCR templates and the PCR products were tested. This method developed in this study was called colony PCR hybridization (CPH). It was found that samples such as B57, which were apparently mixtures of strains, were indeed mixtures and other samples, which apparently contained a single strain (B23, D9, D11 and D16), actually contained one or more additional strains not detectable by DPH or other methods. CPH is a sensitive method for the detection and differentiation of CTV strains infecting single citrus trees.

Key Words: CTV, Probes, PCR, hybridization, Colony PCR hybridization.

Turk. J. Agric. For., **29**, (2005), 449-459.

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