

IMPRINT HYBRIDIZATION FOR DETECTION OF CITRUS VIROIDS.

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Commercial citrus are vulnerable to viroid induced diseases, exocortis and cachexia-xyloporosis. Availability of fast and reliable indexing methods is critical to implement sanitation, quarantine and certification programs, and to identify trees suspected of being infected with viroids.

In the past citrus viroids were detected by biological indexing using Etrog citron 861-S1 and Parson's Special mandarin as indicators. However, it was demonstrated that nucleic acid analysis of inoculated citrons by sequential gel electrophoresis (sPAGE) provided a faster and more reliable method for the routine indexing of all citrus viroids. This method which combines the desirable characteristics of citron as an excellent host for viroids and the sensitivity of nucleic acid analysis was adopted at IVIA for viroid indexing.

Recently, an imprint hybridization method has been designed to simplify the processing of citron samples. Freshly cut stem tissues from inoculated citrons are imprinted by firmly press the cut surface onto positively charged Nylon membranes. The imprinted samples once fixed on the membranes, can be processed immediately or stored. Processing of the imprinted membranes involves: a) preparation of DIG labeled probes (RNA probes synthesized by *in vitro* transcription or DNA probes synthesized by PCR using cloned DNA related to the specific viroid sequences); b) hybridization of the membranes against the DIG-labeled viroid probes; c) detection of DIG-labeled hybrids (using anti-DIG-alkaline phosphatase conjugate); d) visualization of the DIG-alkaline phosphatase (using the chemiluminescence substrate CSPD). Probes for the five viroid groups identified in citrus have been assayed and shown to be highly specific. In addition a single hybridization using a mixture of the five probes allows a fast screening for viroid-free versus viroid containing samples. The overall process is simple and can be easily performed by non-specialized personnel including some nurserymen. Membranes containing positive and negative controls and the chemicals needed (including the mixtures of labeled probes) could be easily commercialized as detection kits.

Efforts are now being devoted to adapt this imprint hybridization method for the detection of citrus viroids directly from commercial varieties.