

## **Approaches to Prevent and Manage Viruses Introduced by Seed**

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With the globalization and widespread exchange of plant materials across the borders, and the ability of seeds to act as a carrier of virus and vectors increases the risk of introduction of disease in to a new geographical region.

The two species of pecluviruses, Peanut clump virus (PCV) and Indian peanut clump virus (IPCV) are currently restricted to the regions of African and Asian continents respectively. APHIS designated them as quarantine pests for peanuts. We initiated a study to develop a robust, and accurate diagnostic assay to detect the presence of PCV and IPCV on peanuts. Due to availability of numerous serotypes, two TaqMan multiplex assays were developed separately for IPCV and PCV for accurate detection. Freeze-dried plant tissues infected with two different IPCV strains (Durgapur (D) and Hyderabad (H)) from India and one PCV strain were collected from Senegal. Specific primers and probes were designed targeting the conserved regions (coat protein-CP; triple gene block-TGB; and RNA dependent RNA polymerase-RdRp) of viral genome. Total RNA was used for RT-PCR to amplify the targeted genes (IPCV H CP, IPCV D CP, IPCV D TGB, IPCV H TGB, PCV CP, PCV RdRP) followed by sequencing for virus confirmation. SYBR-Green-based qRT-PCR was designed and standardized separately for the IPCV D CP, IPCV H CP, IPCV RdRP, PCV CP and PCV RdRP genes along with plant genes (*ADH3* and *ACT11*) as internal controls. Analysis of thermal curves confirmed the specificity of each targeted gene amplification. One-step TaqMan singleplex assays were developed separately for all targeted regions, with the efficiencies and  $R^2$  values within the expected ranges of 90-110% and 0.98-.0.99 respectively. One-step Triplex assay which detects CP, RdRP and Actin genes was developed for PCV. Detection of IPCV D CP, IPCV H CP, IPCV RdRp, Pecluvirus specific RdRp and Peanut Actin gene were successful resulting a five-plex assay for IPCV. As a conclusion, optimized One-Step SYBR Green qRT-PCR and One-step TaqMan based triplex qRT-PCR were developed for an accurate and rapid detection of Pecluviruses to be used for quarantine purposes. The next step is to validated these tools with multiple samples.