## Development of Taqman Assay for the Detection of Groundnut Bud Necrosis Orthotospovirus on Peanut: A Quarantine Pathogen in the USA

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It is extremely important to avoid the entry of exotic pests while allowing the safe exchange of plant propagation material, which is important for genebanks and breeding programs. Groundnut bud necrosis orthotospovirus (GBNV) is a thrips-transmitted virus restricted to southeast Asian countries. The presence of major hosts (peanut, tomato and potato) and the vectors (*Frankliniella schultzei, Scirtothrips dorsalis* and *Thrips palmi*) within the USA make GBNV an important quarantine pathogen. The objective of the current study is to develop sensitive and specific, diagnostic assay to detect GBNV facilitating quarantine and phytosanitary procedures and transfer the work instruction to USDA-APHIS-PPQ. For assay optimization isolates of GBNV collected from ICRISAT, Hydrabad and IARI New Delhi, in India. Specific primers and probe sets were designed to target conserved regions of the nucleocapsid (NP) and movement protein (MP) genes using the GBNV complete NP (54) and MP (47) sequences which were available in NCBI GenBank till April 2019. Plant gene Actin 11 (*act11*) specific primers and a probe were used as internal control. Using conventional RT-PCR the presence of the virus was confirmed in the received samples. The amplification of NP and MP genes were successful using SYBR Green based qRT-PCR. The specificity and qualitative ability of the assays were confirmed using amplification profiles and melting curve analysis. Disassociation curves with a single peak were obtained for the targeted NP and MP regions of GBNV genome and *ACT11*. In singleplex TaqMan assays, the efficiencies observed were 91.6% and 90.6 % for NP and MP, respectively. The assays were sensitive to detect GBNV in a minimum of 0.05ng/µl of total RNA. For all the specific reactions, high fluorescence signals were observed, and no significant non-specific amplification plots were obtained with other orthotospoviruses GRSV, TCSV, and TSWV present within US. As a conclusion, optimized TaqMan based triplex qRT-PCR was developed for detection of GBNV.