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Findings

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In this study, *Pogostemon cablin* (patchouli) leaf samples, collected from different regions of India, were subjected to RT-PCR based screening for detection of virus infection. Patchouli leaf samples collected from Assam, Karnataka and Palampur were found to be infected with *Peanut stripe virus* (PStV) and *Broad bean wilt virus 2* (BBWV-2). The PStV CP gene from Assam, Karnataka and Palampur isolates showed highest identity (94-96%) with Taiwan, Vietnam and Thailand isolates. Likewise, the BBWV2 CP gene from Assam, Karnataka and Palampur isolates showed highest sequence identity with isolates from China and Germany, 91% at nucleotide and 97% at amino acid level. The CP gene sequences of the detected PStV and BBWV-2 isolates were determined and submitted to the NCBI GenBank database.

A duplex RT-PCR assay was developed for the first time for simultaneous detection of PStV and BBWV-2 infecting patchouli plants. Furthermore, one-step colorimetric RT-LAMP assays were developed for the first time to detect PStV and BBWV-2 with 100 % specificity and were found to be 100 fold more sensitive than their conventional RT-PCR assays. It simplifies the detection procedure, enhances sensitivity, and improves field practises. A closed-tube colorimetric RT-LAMP assay with pre-reaction HNB indicator dye was also developed to detect the PStV as well as BBWV-2. The same results were achieved with colorimetric endpoint. These assays require no additional step for the confirmation of results, thus avoiding the use of toxic chemicals and sophisticated laboratory equipments. The colorimetric RT-LAMP assays of this study proved to be reliable, highly specific for point of care (POC) detection of PStV and BBWV-2.

This research, hence successfully shows the development of duplex RT-PCR and RT-LAMP assays for detection of PStV and BBWV-2 viruses, thereby simplifying the procedure and granting their broad spectrum on field application.