Detection of potato viruses by reverse transcription loop-mediated isothermal amplification

Krzysztof Treder, Mateusz Mielczarek, Bogumila Zacharzewska
Plant Breeding and Acclimatization Institute - National Research Institute, Poland

Loop-mediated isothermal amplification assay (LAMP) facilitates fast and sensitive detection of DNA and RNA targets. The speed of detection of RNA can be improved by the addition of reverse transcriptase to form an RT-LAMP assay. RT-LAMP was reported as a favorable method of detection of many RNA viruses with a sensitivity similar to, or higher than, the real-time RT-PCR. The advantage of RT-LAMP is also its speed. When the assay is performed in the real-time fluorescent detection mode, the target virus can be detected in 5-30 minutes since the amplification commence. Furthermore, polymerases used in the LAMP reaction are less sensitive to amplification inhibitors than polymerases used in PCR reaction. Consequently, the RT-LAMP can be performed on crude tissue extracts or crude RNA preparations, saving the time and cost the assay. Availability of many color-changing approaches for detection of the LAMP amplicons facilitates the development of RT-LAMP variants with visual detection as a point-of-care test. Here, we present a development and optimization of RT-LAMP assays to detect the most important potato viruses including potato virus Y (PVY), potato virus M (PVM), potato leafroll virus (PLRV), potato virus S (PVS) and potato virus X (PVX). The poster will present different RT-LAMP assay procedures, dedicated to quantitative, real-time detection in the laboratory as well as for fast end-point colorimetric detection in the field.