Pests and diseases - Viruses

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Sensitive detection of potato virus Y and differentiation of O and N types by reverse transcription loop-mediated isothermal amplification

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Introduction: Reverse transcription loop-mediated nucleic acids amplification (RT-LAMP) is based on an isothermal amplification of the nucleic acids at a constant temperature. Several RT-LAMP assays were developed to detect PVY in potato [1, 2, 3]. Here we describe an assay for sensitive PVY detection, which also facilitates N and O typing.

Material and methods: ***Source of PVY isolates.*** Isolates 12/94, Ny, Wi, and LW were provided by the Młochów Research Center of the Plant Breeding and Acclimatization Institute. Isolates PV-0403, PV-0410, PV-0348, PV-0345, PV-0343 were purchased from German Collection of Microorganisms and Cell Cultures (DSMZ). ***RNA isolation***. *Silica capture*. RNA was purified as previously described [4]. *Magnetic capture*. RNA was captured as in [4] but magnetic particles (Novazym) were used instead of silica. ***RT-LAMP***. The Y4 primer set was designed as previously described [3]. The reaction contained F3/B3 (0.375 µM), FIP/BIP(1.5 µM), loop primers (0.75 µM), 1X Isothermal Master Mix (Novazym), reverse transcriptase (0.25 U, Novazym) and total RNA (0.1 µg). The amplification (65 °C, 30 min) and analysis of annealing temperatures (Ta) were conducted in a Genie II (OptiGene Ltd.). The amplification in a CFX96 Touch™ Real-Time PCR Detection System (BioRad Ltd) was conducted by one step program (30 cycles of 65°C for 60 sec), followed by melting temperature (Tm) analysis.

Results and discussion: PVY detection by Y4 set was compared with RT-LAMP primer sets: Y5 [3], N [2] and AD [1]. The Y4 primed the most rapid detection. The Y4 was also the most sensitive among tested sets. To determine if the Y4 could discriminate between the PVY strains, RNAs from plants infected with European PVY isolates representing strains: PVY^{O}, PVY^{N-Wi}, PVY^{N} and PVY^{NTN} were tested on Genie II. The isolates harboring O and N type of coat protein had a 0.47 °C variation in Ta values. To check if the difference in Ta correlates with a difference in melting temperatures (Tm), strains were tested by RT-LAMP performed on a real-time thermal cycler and the Tm was determined. Strains with N type coat protein had Tm = 84°C while O type strains had Tm = 84.5°C. To validate these results, the RT-LAMP assays was performed on RNAs isolated from potato plants infected with North American PVY^{O}, $PVY^{N:O}$ PVY^{N-NA} and PVY^{NTN} strains. Again, the O type strains had a Tm value of an order of 0.5°C higher (85°C) compared to N types (84.5°C). To simplify RNA capture, in place of the silica, magnetic microspheres were used. This had no negative impact on the RT-LAMP sensitivity.

Conclusion: RT-LAMP assay with Y4 primers facilitated sensitive PVY detection and differentiated the O and N types. The use of magnetic beads to capture RNA simplified the assay in a cost-effective manner.

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