SIMULTANEOUS DETECTION OF POME FRUIT TREE VIRUSES BY TRIPLEX QUANTITATIVE RT-PCR

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Introduction

Apple mosaic virus (ApMV), *Apple stem pitting virus* (ASPV) and *Apple stem grooving virus* (ASGV) are among the most important pome fruit viruses, freedom from which is a prerequisite in fruit tree certification programs. The development of quantitative real time PCR (qPCR), using novel chemistries and instrumentation platforms, led to improved rapidity, sensitivity, reproducibility and reduced risk of carry-over contaminations. These characteristics often make it the method of choice in routine diagnostics. The possibility of multiplexing, allowing simultaneous detection of different targets in a sample, makes qPCR even more appealing to diagnosticians and epidemiologists (1, 2). In this project, a single tube multiplex RT-qPCR assay was developed to detect simultaneously three pome fruit viruses, so as to minimize the time and labor required for diagnosis while being highly sensitive and specific.

Materials and Methods

Total RNA extraction was performed using CTAB (3). Primers and TaqMan MGB probes were designed in this study using appropriate software tools (Primer Express© Software v3.0.1, Multiple Primer Analyzer). Optimization was carried out for $MgCl_2$ concentration, duration of the RT step, as well as nucleic acids, primers and probe concentrations. Nucleic acid standards were prepared by cloning qPCR target regions for each pathogen to plasmid vector pCR®II-TOPO® TA followed by synthesis of RNA transcripts using standard procedures.

Results and Discussion

The viruses were simultaneously detected in 10-fold serial dilutions of total RNA from a naturally triple-infected apple tree into RNA of virus-free tested pear, up to dilution 10^{-4} for ApMV and ASPV, and 10^{-3} for ASGV. The newly developed RT-qPCR assay was at least a hundred times more sensitive than conventional single RT-PCRs tested in the same transcript and natural infected RNA dilutions. Simultaneous detection of the three targets was achieved in composite samples at least up to the ratio of 1:150 triple-infected to healthy tissue, demonstrating that the developed assay has the potential to be used for rapid and massive virus screening in the frame of certification schemes and surveys.

References

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2. Malandraki et al., 2015. *Journal of Virological Methods* 213: 12-17.
3. Gambino et al., 2008. *Phytochemical Analysis* 19: 520-525.