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NEW SENSITIVE ASSAY FOR THE DETECTION OF POTATO LEAFROLL VIRUS BY CONVENTIONAL AND REAL-TIME REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION

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Introduction: Potato leafroll virus (PLRV) is one of the most important viral pathogens infecting potato. The virus is phloem limited and its titer in infected plants is low. Thus, the pathogen detection is difficult. Here we report on a development of new RT-PCR and real-time RT-PCR assays based on the same primers for very sensitive PLRV detection.

Material and methods: **RT-PCR**. Total RNA was purified as previously described [3]. RT reactions were carried out with the Reverse Verte KIT (Novazym). cDNA was amplified using Singh primers [2] and designed pairs: L2, L4, L5, L6. PCR was performed using a 25 µl reaction mixture: 2.5 µl of cDNA, 2 mM MgCl2, 0.2 mM dNTPs containing dATP, dCTP, dGTP and dUTP, 0.4 µM of each forward and reverse primer, 0.5 U Uracil DNA Glycosylase (Bioline), 1xPolymerase Green Buffer and 1.25 U Go-TaqHotStart Polymerase (Promega). The temperature profile followed: initial denaturation at 95°C for 3 min, 35 cycles at 94°C for 10 sec., annealing at 60°C for 20 sec., extension at 72°C for 30 sec. and a final extension at 72°C for 5 minutes. **Real-time PCR** was carried out on a CFX96 Touch™ Real-Time PCR Detection System (BioRad Ltd.) using AmpliQ Real-Time PCR OptiProbe kit or AmpiQ Real-Time PCR SybrGreen kit (Novazym). Final reactions contained 2.5 µl of cDNA, 5 µl of respective AmpiQ Real-Time PCR kit, 0,15 µl of 20 µM stock of forward and reverse primers and 0,15 µl of 10 µM probe in probe-based assays, which was supplemented by nuclease-free water in dye-based assays. All reactions were supplemented with nuclease-free water up to 10 µl. Thermal cycling conditions were 10 min at 95°C followed by 60 cycles of 10 s at 95°C and 20 s at 60°C.

Results and discussion: Primer pair designed for PLRV detection by Singh et al. [2] become one of the most intensively used in PLRV-related works. Thus, we determined if any of designed primers can facilitate more sensitive detection than that set. For L4 primers amplification product was observed up to 1 pg of RNA. Primer set designed by [2], L6, and L5 facilitated PLRV detection up to 100 fg of RNA. L2 set was the most sensitive (10 fg). The sensitivity of PLRV detection using L2 primers was further tested by conventional RT-PCR and real-time RT-PCR. Total RNAs were isolated from sap dilutions and amplified by conventional RT-PCR based on L2 primers and by real-time RT-PCR. The real-time RT-PCR was performed with different sets of primers including (i) the L2 primers in the presence of SybrGreen (SG), (ii) the PLRV-specific primers designed by Singh et al. [2] in the presence of SG and (iii) the PLRV specific primers and TaqMan probe (TM) designed by Boonham et al. [1]. Conventional RT-PCR amplified specific product up to $2x10^6$-fold diluted sap. The SG dye and L2 primers based real-time RT-PCR facilitated detection of PLRV in sap diluted up to $2x10^6$-fold. The SG real-time RT-PCR with primers according to Singh et al. [2] and TM real-time RT-PCR with primers and probe according to Boonham et al. [1] detected PLRV in samples diluted up to $5x10^5$ fold.

Conclusion: Among tested primers, L2 set facilitated the most sensitive PLRV detection both by RT-PCR and by real-time RT-PCR. The sensitivity of conventional RT-PCR and real-time RT-PCR was the same when both assays utilized L2 primers.

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