TECHNICAL SHEET No. 15

Virus Detection: *Potato leaf roll virus* STRAINS

Method: RT-PCR

General

Virus detected: PLRV strains from potato samples
Method: RT-PCR

Developed By

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Goals

To develop a method for rapid detection of PLRV strains.

Materials and Methods

Nucleic acid extraction

Sample leaves were ground (1/5, w/v) in PBS-Tween buffer (8 g of NaCl; 0.2 g of KH_2PO_4; 2.9 g of Na_2HPO_4 ($12H_2O$) and 0.5 ml of Tween 20 per liter) containing 2% (w/v) polyvinylpyrrolidone K25 and 20 mM DIECA. After centrifugation at 13,000 rpm for 10 min, 200 µl of supernatant were transferred to a microfuge tube, mixed with SDS to 1% (w/v) final concentration and incubated at 55°C for 15 min. One hundred microliters of 3 M potassium acetate were added, the mixture vigorously vortexed and incubated on ice for 5 min. After centrifugation (5 min, 13,000 rpm, 4°C), the supernatant was adjusted to 4.2 M NaI. Five microliters of a suspension of silica particles (Sigma) were added, carefully mixed by low speed vortexing and the mixture incubated at room temperature for 5 min. After a brief centrifugation (1 min, 5,000 rpm at room temperature) the supernatant was discarded and the pellet gently resuspended in 500 µl of washing buffer (20 mM Tris-HCl pH 7.5; 1 mM EDTA; 100 mM NaCl; 50% (v/v) ethanol). The centrifugation was repeated and the pellets of silica particles similarly washed twice more before being resuspended in 400 µl sterilized water. After incubation at 55°C for 5 min and centrifugation at 13,000 rpm for 2 min, 300 µl of the supernatant were transferred to a new microfuge tube. Total nucleic acid extracts were then used directly for RT-PCR amplification or stored at –20°C until used.

RT-PCR Amplification of a part of the coat protein region

a- Reverse Transcription

- 13 µl of total RNA
- 1 µl dNTP (10 mM each)
- 1 µl DTT (0.1 M)
- 4 µl transcriptase buffer (5X)
- 0.5 µl antisense primer (25 µM)
- 0.1 µl of MMLV Reverse Transcriptase (200 U/µl)
- H_2O 20 µl

The reaction is incubated for one hour at 37°C.

b-Amplification PCR

- 2.5 µl of Reverse Transcription reaction (see above)
- 2.5 µl of 10X *Taq* DNA polymerase buffer without $MgCl_2$
- 1 µl MgCl2 (25 mM)
- 0.5 µl dNTP (10 mM each)
- 0.75 µl sens primer (10 µM)
- 0.75 µl antisens primer (10 µM)
- 1 µl de *Taq* DNA polymerase (1 U/µl)
- H_2O 25 µl

PCR Program: 35 cycles of 5 min 94°C, 1 min 94°C, 1 min 50°C, 1 min 72°C, 10 min 72°C

Sequence of primers

Sense primer: 5' CGC GCT AAC AGA GTT CAG CC 3'
Antisense primer: 5' GCA ATG GGG GTC CAA CTC AT 3'

Results and Discussion

Using this primer pair, RT-PCR allows amplification of a fragment of 336-bp fragment corresponding to part of the coat protein gene for PLRV. This technique is reliable and allows for a very rapid detection of most PLRV strains spreading in different potato varieties.