TECHNICAL SHEET No. 16

Virus Detection: *Potato leaf roll virus* (PLRV)

Method: Immunocapture RT-PCR

General
Virus detected: PLRV from potato leaf and potato tubers.
General method was immunocapture RT-PCR

Developed by
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Goals
To develop a sensitive method for PLRV detection based on immunocapture RT-PCR.

Methods
Immunocapture IC

1. Add 200 µl of anti-PLRV antibody (diluted 1:1000 in coating buffer) to each ELISA plate well or to PCR tube and incubate at 37°C for 3 h. We used a commercial antibody (Bioreba).
2. Empty the ELISA well/ PCR tube and wash 3 times with TBST (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween-20).
3. Homogenize 25 mg of plant leaf or tuber with 5 ml of suitable extraction buffer.
4. Add 200 µl of the homogenate to the coated ELISA well/ PCR tube and incubate for 18 h at 4 °C.
5. Empty the ELISA well/ PCR tube and wash 3 times with TBST.
6. Dry the ELISA well/ PCR tube, add 5 to10 µl ddH2O and heat at 70 °C for 15 min.

RT-PCR
cRNA synthesis

1. To 5 µl IC add 8 µl ddH_2O and 1 µl primer C582 (100 pmoles); incubate at 70 °C for 10 min, followed by 30 min in and ice bath.
2. Add 1 µl of each dNTPs (25mM each), 4 µl reverse transcriptase buffer 5X, 1 µl AMV reverse transcriptase (Promega); incubate at 42 °C for 1h.
3. Heat for 10 min at 90 °C; adjust the volume to 50 µl with ddH_2O

PCR
The primers used were as follows (derived from sequence GenBank accession numbers D13954 and D13953):
C582: 5'TATCGTCCATGGGTACGGTCGTGGT3'
R732: 5'TCTAGATCTTTGGGTTTTGCAAAGC3'
Following PCR, a 640 bp band is obtained.

1. The PCR reaction contains 5 µl from the reverse transcriptase reaction, 0.25 µl 25mM dNTPs, 1 µl each primers 1 (100 pmoles each), 2.5 µl Taq 10 x buffer and 1 unit *Taq* polymerase; add ddH_2O to a final volume of 25 µl.

2. Cycling: One cycle: 95 °C for 3 min, 50 °C for 2 min, 72 °C for 2 min. Thirty cycles: 95 °C for 1min, 55 °C for 1min, 72 °C for 1 min. One additional cycle: 72 °C for 10 min.
3. Analysis of reaction products: Subject the reaction products to 1% agarose gel electrophoresis.

Results

The following figure shows the detection of PLRV by IC-RT-PCR and by RT-PCR
For RT-PCR (lanes 1 – 3), RNA was from a *Datura stramonium* infected plant
For IC-RT-PCR (lanes 1' - 3'), samples were from a *Datura stramonium* infected plant.
M: molecular weight marker; P: cloned PLRV DNA; 0: no template.

M 1 2 3 P 1' 2' 3' 0