TECHNICAL SHEET No. 8

Virus Detection: *Grapevine Leafroll virus 1,3*

Method: IC-RT-PCR

**General**
Virus Detection: *Grapevine Leafroll virus 1,3*
Method: IC-RT-PCR

**Developed by**
Name of researchers: Ghandi Anfoka, Al-Balqa’ Applied University, Al-Salt, Jordan
Address (Email): anfoka@joinnet.com
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**Goal**
Detection of GLRV 1,3 by one step IC-RT-PCR.

**Introduction**
Leaf roll is a vitis disease with a very important economic impact throughout the world. It is characterized by a down rolling of leaves and reddening or yellowing of limbs and its occurrence causes significant reduction in crop quality and quantity. Immunocapture reverse transcription polymerase chain reaction (IC-RT-PCR) is a powerful tool for diagnosis of grapevine viruses.

**Materials and Methods**

**Tissue extraction**
Leaf tissues from grape plants showing disease symptoms were collected from the University research center and kept at 4°C. Using pestle and mortar, 0.5 g of leaf petioles were cut into small pieces and grinded in 2.5 ml extraction buffer. The sap was transferred into an eppendorf tube and centrifuged at 14,000 rpm for 1 min and supernatants were kept on ice.

**IC-RT-PCR**

**Coating with antibodies**
- Polyclonal antibodies, developed by Sediag (France) were diluted using coating buffer (carbonate bicarbonate). Three antibody dilutions were tested (1:1000, 1:500 and 1:250).
- Two hundred microliters of diluted antibody were deposited into PCR tubes and tubes were incubated at 37°C for 4 h.
- Tubes were washed three times with PBS-T and briefly centrifuged to remove the remaining droplets of buffer.
- Two hundred microliters of leaf sap were dispensed into PCR tubes and incubated overnight at 4°C.
- Tubes were washed three times with PBS-T.
- Tubes were incubated at 65°C for 10 min and used for RT-PCR.
**One step RT-PCR**

**Primers**

The primer set of GLRaV 3 designed by Saldarelli and Hadidi (1994) was used:

Downstream primer (C547): 5’- ATT AAC TTG ACG GAT GGC ACG C- 3’.

Upstream primer (H229): 5’-ATA AGC ATT CGG GAT GGA CC -3’.

Length of the predicted product is 340 bp.

**RT-PCR kit**

The access RT-PCR system kit developed by Promega (cat # A1260) was used in this study according to the manufacturer’s instructions.

**RT-PCR mixture:**

- RT mixture per tube consisted of: 26 µl nuclease-free water, 10 µl AMV/Tfl buffer (5X), 5 µl antisense primer (10 µM), 5 µl sense primer (10 µM), 1 µl dNTP (10 mM), 2 µl MgSO$_4$ (25 mM), 0.25 µl AMV reverse transcriptase (20 U/µl) 1 µl Tfl DNA polymerase (5 U/µl). Tubes were vortex and then centrifuged for few seconds.

**PCR cycles**

The PCR cycle previously described by Acheche et al. (1999) was used. It consisted of: 46°C for 60 min, 94°C for 5 min then 30 cycles of 94°C for 30 sec, 56°C for 45 sec, and 72°C for 1 min, and a final step of 72°C for 10 min.

**Agarose gel electrophoresis**

PCR products (8 µl) were electrophoresed on 1% agarose gel and stained with ethidium bromide before gel was photographed under UV illumination. A lambda DNA EcoRI/HindIII digest was used to determine the size of amplified product.

**Results**

Part of the RNA polymerase gene (340 bp) of GLRV 1,3 could be amplified using one step IC-RT-PCR. The expected size of PCR (340 bp) could be only detected in tubes coated with antibodies diluted 1:250 and 1:500 (Fig. 1).

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**Figure 1.** IC-RT-PCR of a grape sample. M: lambda DNA/EcoR1+HindIII marker. 1: tubes coated with antibodies diluted 1:1,000, 2: diluted 1:500, and 3: diluted 1:250.
**Discussion**

This technique is sensitive, saves time and effort and can be easily applied to detect GLRV 1,3 in large number of samples without the need of RNA extraction.

**References**
