TECHNICAL SHEET No. 8

Virus Detection: *Grapevine Leafroll virus 1,3*

Method: IC-RT-PCR

General

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Developed by

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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 4ºC.
- 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/TfI 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 TfI 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 *Eco*RI/*Hin*dIII 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).

Figure 1. IC-RT-PCR of a grape sample. M: lambda DNA/*Eco*R1+*Hin*dIII 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 easly applied to detect GLRV 1,3 in large number of samples without the need of RNA extraction.

References

Acheche, H., Fattouch, S., M'Hirsi, S., Marzouki, N., and Marrakchi, M. (1999). Use of optimised PCR methods for the detection of GLRaV3: a closterovirus associated with grapevine leafroll in Tunisian grapevine plants. Plant Molecular Biology Reporter 17:31-42.

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