

TECHNICAL SHEET No. 36

Virus Detection: *Tomato yellow leaf curl virus* (TYLCV) and other begomoviruses

Method: Immunocapture PCR

General

Virus: Geminiviruses detection from whitefly and infected plants

General method: Immunocapture PCR (IC-PCR)

Developed by

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Goals

To develop a sensitive and reliable method for the detection of begomoviruses, based on serology combined with PCR.

Introduction

The coat proteins of whitefly-transmitted geminiviruses (begomoviruses), whether from the New or the Old World, have a high degree of homology in their amino acid sequences. Hence capsids have common serological determinants and a polyclonal antibody raised against one begomovirus may detect the presence of others.

We have used a polyclonal antibody raised against the TYLCV (Israel) coat protein expressed in *E. coli*, a gift from Dr. R. Gilbertson (University of California-Davis). This antibody has a broad-spectrum and is able to detect several TYLCV isolates as well as other begomoviruses such as *African cassava mosaic virus* (ACMV); *Watermelon chlorotic stunt virus* (WmCSV) and *Abutilon mosaic virus* (AbMV).

Materials and Methods

Immunocapture (IC)

1. Add 200 μ l of anti-TYLCV antibody (diluted 1:1000 in coating buffer) to each well of ELISA plate, or to PCR tube. The coating buffer is: for 1 liter, pH 9.6: 1.59 g Na_2CO_3 , 2.93 g NaHCO_3 , 0.2 g NaN_3 .
2. Incubate at 37°C for 3-4 h. Empty the ELISA wells/PCR tube and wash 3 times with TBST. TBST is 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween-20.
3. Homogenize infected leaves (1 g in 20 ml) with extraction buffer I. Alternatively, cut the infected leaf into small pieces and add 0.1 g to 100-200 μ l extraction buffer II into the well or the tube. Alternatively, homogenize 1 to 5 whiteflies with 100-200 μ l extraction buffer II. Extraction buffer I is for 1 liter, pH 9.0: 2.4 g Tris, 8 g NaCl, 0.5 ml Tween 20, 0.2 g KCl, 0.2 g NaN_3 . Extraction buffer II is for 1 liter, pH 9.0: 2.4 g Tris, 20 g PVP, 8 g NaCl, 0.5 ml Tween 20, 0.2 g KCl, 0.2 g NaN_3 .
4. Add 100-200 μ l of the homogenate to the coated ELISA well/PCR tube and incubate for 18 h at 4°C.

5. Empty the ELISA wells/PCR tubes and wash 3 times with TBST.
6. Dry the ELISA wells/PCR tubes, add 10 µl ddH₂O and boil at 100 °C for 5 min.

PCR Primers

TYLCV: V61 (nt 61-80, viral strand, 5' ATACTTGGACACCTAATGG3') and C473 (nt 473-457, complementary strand, 5' AGTCACGGGCCCTTACAA3').

AbMV: AbAV356 (nt 356-379, virion strand, 5' CAAAATGCCTAAGCGCGATCTCCC 3') and AbAC1117 (nt 1117-1096, complementary strand, 5' TTTATTAATTCATGAGCGAATC 3').

PCR Reaction

1. The PCR reaction contains 5 µl from the IC reaction, 0.25 µl 25 mM dNTPs, 1 µl of each primers 1 and 2 (100 pmoles each), 2.5 µl Taq 10 x buffer and 1 unit Taq polymerase; add ddH₂O to a final volume of 25 µl.
2. Cycle: 1 cycle of 95°C for 3 min 55°C for 2 min, 72° C for 2 min; then 30 cycles of 95°C for 1 min, 55°C for 1 min, 72°C for 1 min; end with an additional cycle of 72 °C for 10 min.
3. Subject the reaction products to 1% agarose gel electrophoresis.

Results

The method was applied to the detection of TYLCV in tomato plants and in whiteflies. The same procedure was used to detect another geminivirus present in Israel, AbMV, and ACMV and WmCSV from dry material. The following results are from:

Ghanim M, and Czosnek H (2000) Tomato yellow leaf curl geminivirus (TYLCV-Is) is transmitted among whiteflies (*Bemisia tabaci*) in a sex-related manner. *Journal of Virology* 74: 4738-4745.

Morin S, Ghanim M, Sobol I, and Czosnek H (2000) The GroEL protein of the whitefly *Bemisia tabaci* interacts with the coat protein of transmissible and non-transmissible begomoviruses in the yeast two-hybrid system. *Virology* 276:404-416.

1) Detection of TYLCV in whiteflies and tomato plants

Fig 1. WF* and WF: viruliferous and non-viruliferous whitefly. P*: infected plant. OA: no antibody; OE: no plant or whitefly extract. 1 to 9: samples tested.

2) Detection of AbMV in whiteflies and infected Abutilon plants

Fig 2.

M: molecular weight markers.

W* and W viruliferous and non-viruliferous whiteflies (20 individuals).

P*: infected tomato plant.

C: Cloned AbMV DNA-A.

0A: no antibody; 0: no plant or whitefly extract.

1,2 or a,b: samples tested.