TECHNICAL SHEET No. 6

Virus Detection: *Cucumber mosaic virus* (CMV)

Method: Capture of plant viruses by GroEL

General
Virus detected: CMV from plants
General methods: Squash blot PCR

Developed by
Name of researcher: Fouad Akad and Hanokh Czosnek, The Hebrew University
Address (Email): akad @agri.huji.ac.il; czosnek @agri.huji.ac.il
Date: December 26, 2005

Goals

Develop a rapid and very sensitive detection of CMV in tissues. This method is novel and not in general use.

Introduction:
See reference cited at end of methods for more details.

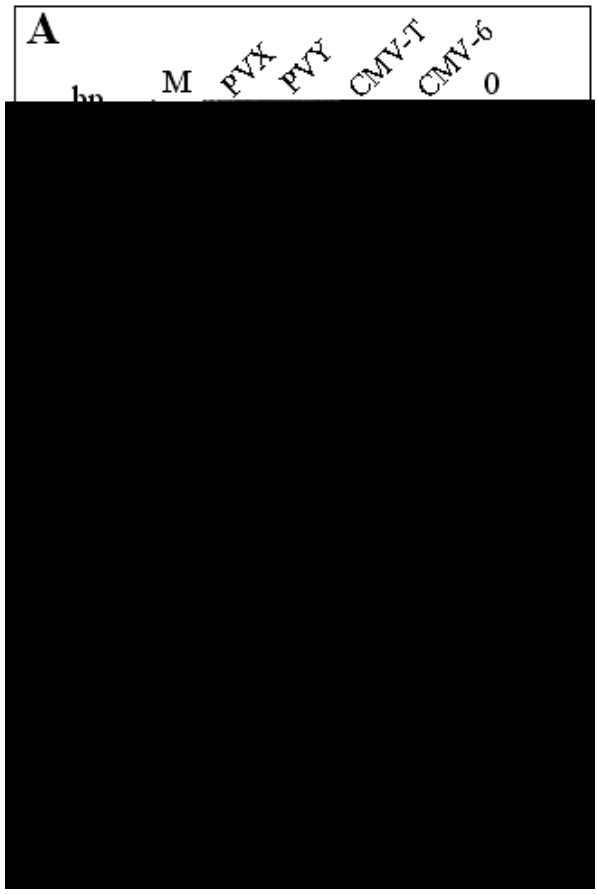
Methods:

1. PCR tubes or 96-well plates were filled with GroEL diluted in ELISA coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$ and 0.02% NaN_3).
2. Incubate for 3 h at 37 ^oC and wash three times with TBST.
3. Add homogenates of infected material to the GroEL coated support, incubate for 18 h at 4 ^oC, and wash three times with TBST.
4. For PCR, reagents were added directly to the tube with primers specific for each virus. The PCR reaction contained: 0.25 µl of the four dNTP 25 mM, 1 µl each virus specific primers (10 pmoles), 2.5 µl Taq buffer (x10) and 1 unit Taq DNA polymerase. The reaction volume was adjusted to 25 µl with ddH_2O and subjected to cycling as follows. One cycle consisting of 3 min at 95 °C, 2 min at 50 °C, 2 min at 72 °C followed by 30 cycles consisting of 1 min at 95 °C, 1 min at 55 °C, 1 min at 72 °C, and terminated by 10 min at 72 °C.
5. The PCR products were subjected to electrophoresis in 1% agarose gel, in Tris-Phosphate- EDTA buffer (TAE) and stained with ethidium bromide (0.5 µg/ml) before photography.
6. For RT-PCR, 13 µl ddH_2O and 1 µl of the virus PCR complementary primer (10 pmoles) were added to the treated tubes. Following 10 min at 70 °C, the tubes were cooled in an ice bath for 10 min and 1µl of each of the four dNTP (25 mM) were added together with 4 µl of reverse transcriptase buffer (5X) and 10 units (1 µl) of AMV reverse transcriptase (Promega). After 1 h at 42 °C, the reaction was stopped by incubating the tube at 90^oC for 10 min; the volume was then adjusted to 50 µl.
   One µl of the reverse transcriptase reaction was used for PCR. Reaction and cycling conditions were as above.

7. The following primers used to detect viruses by PCR were derived from the sequence published in GenBank. For TYLCV (accession No X15656) virion strand (position 61-80) 5'ATACTTGGACACCTAATGGC 3' and complementary strand (position 473-457) 5'AGTCACGGGCCCTTACA 3'. For PVX (accession No AF260641) virion strand (position (5643-5663) 5' CGAAAGATGTCAGCACCAGC 3' and complementary strand (position 6370-6350) 5' GACGTAGTTATGGTGGTGGT 3'. For PVY (accession No M95491) virion strand (position 102-120) 5' TCATCAAACAAACTCTTT 3' and complementary strand (position 937-918) 5' GGAAAGACACCAAAGCGGGA 3'. For CMV (accession No D10538) coat protein (CP) virion strand (position 115-133) 5' GGCATGGCTTTCCAAGGTA 3' and complementary strand (position 470-450) 5' GGAAAGACACCAAAGCGGGA 3'; for CMV movement protein (MP) virion strand (position 120-141) 5' ATGGCTTTCCAAGGTACCATG 3' and complementary strand (position 740-719) 3' TCTGTTGAAAGGCAGTACTAG 5'.

Results

Capture of CMV, but not of PVX and PVY, by GroEL, native and expressed in *E. coli*. (A): Tubes coated with native GroEL (1 μg protein/ml) were incubated with sap of potato leaves infected with PVX or PVY, or with sap of melon leaves infected with two CMV strains (0.1 g in 2 ml). After washing, the RT-PCR mixture was added together with primers specific of the virus CPs. The reaction products were subjected to gel electrophoresis and stained. Thick arrow shows amplified fragment. Thin arrow shows primers. 0: non-infected potato leaves. (B): Extracts of leaves of CMV-infected melon (0.1 g in 2 ml TBST, subsequently diluted 1:25) were incubated in tubes coated with recombinant GroEL (1 μg protein/ml). After washing, the RT-PCR mixture was added together with primers specific of the virus MP. The reaction products were subjected to gel electrophoresis and stained. 0: extract of non-infected melon leaf; P: plasmid containing a full-length clone of CMV RNA3. (C): Detection of PVX and PVY by immunocapture RT-PCR. Tubes were coated with commercial antibodies raised against PVX or PVY (IC), or with GroEL (1 μg protein/ml) and incubated with sap of infected potato leaves. After washing, the RT-PCR mixture was added together with virus-specific primers, the reaction products were subjected to gel electrophoresis and stained. M: molecular weight markers.



Reference

Akad F, Dotan N. and Czosnek H (2004). Trapping of *Tomato yellow leaf curl virus* (TYLCV) and other plant viruses with a GroEL homologue from the whitefly *Bemisia tabaci*. Archives of Virology 149:1481-1497.