TECHNICAL SHEET No. 34

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

Method: Squash Blot Hybridization with Radiolabled Probe

General

Detection from tomato plants and from the whitefly vector
General methods: Squash blot hybridization with radiolabeled probe

Developed by

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Goals

To develop a rapid, sensitive method that allows detection TYLCV in a large number of samples. Easy sampling in field conditions. Samples are stable for several months at ambient temperature.

Introduction

Tomato yellow leaf curl virus, TYLCV, is the name given to a large number of genetically diverse whitefly-transmitted viruses infecting tomato. TYLCV infection reduces yields considerably; losses may reach 100% of the crop. Information about geminivirus can be found on the Web at Gemininet (http://www.danforthcenter.org/iltab/geminiviridae).

Based on sequence comparison, the various TYLCV isolates or different species can be grouped according to a geographically based scheme (Zeidan et al., 1998). 1. From the Middle East (Israel, Egypt, Jordan, Lebanon, Northern Saudi Arabia) (TYLCV) (Navot et al., 1991). 2. From Southwest Europe (Italy) (*Tomato yellow leaf Sardinia virus*, TYLCSV) (Kheyr-Pour et al., 1991). 3. From tropical Africa (Senegal, Tanzania), and 4. From Southeast and East Asia (Thailand, China). TYLCV from the Caribbean Islands and from the Southeast USA originated from the Middle East. All these isolates, except TYLCV from Thailand, have a monopartite genome. This virus is also called *Tomato yellow leaf curl Thailand virus*. Some related whitefly-transmitted viruses infecting tomato are also called *Tomato leaf curl virus*, ToLCV, and have been found in India and Australia. The ToLCV isolates have monopartite genome, except of ToLCV from Northern India. For some of the tomato-infecting begomoviruses there is evidence of recombination (Chatchawankanphanich and Maxwell, 2002).

Materials and Methods

Tomato squashes were done using symptomatic leaves from the field, inoculated in the lab with viruliferous whiteflies or by agroinoculation. The method is based on hybridization method described by Navot and Czosnek (1989).

Squashing and Sampling

Squash tomato leaves (young leaf from the shoot apex, or individual whitefly) on a dry nylon membrane (Qiabrane. Biolabs) using a glass rod, an Ependorf tube or a pen cover. Use an X-ray film matrix in which 5 mm diameter holes have been drilled.

Fixation

This stage is not necessary; however nucleic acids can be fixed by exposing the membrane to UV for 3 min.

Prehybridization

Squash blots are prehybridized using a pre-hybridization solution containing: 12.5 ml 50X SCC, 5 ml Denhardt's solution x 50, 2.5 ml 10% SDS, 3.2 ml NaH_2PO_4, 1.7 ml Na_2HPO_4 and double distilled water up to 50 ml (final pH should be 6.5 to 6.8). Add 0. 7 ml of salmon sperm DNA (1µg/ml) after boiling for 10 min. Keep the mixture for 10 min at 65ºC. Add to the membrane and incubate for at least 3 h at 65 ºC. SSC x 20 is 3M sodium chloride, 0.3 M tri-sodium citrate; Denhardt solution x 50 is 2% bovine serum albumin (BSA), 2% Ficoll 400, 2% Polyvinylpyrolidone.

Preparation of TYLCV- DNA ^{32}P labeled probe

The probe used is a full length clone of TYLCV (plasmid pTYH20.7) as in Navot et al., (1991). Labelling (random priming, Amersham) was done as follows:

1. In a volume of 50 µl add: 5µl of 0.3 µg/µl plasmid, 5 µl of 10x hexanucleotide buffer, and 22µl ddH_2O.
2. Heat for 10 min at 100°C.
3. Keep the mixture in ice for 15 min.
4. Add 10 µl of (0.5mM) of dNTP without dCTP and 3 µl of the Klenow DNA polymerase.
5. Mix. Add 5 µl of the radiolabeled [^{32}P]dCTP. Incubate at 37°C for 3 h.
6. Heat for 10 min at 100°C.

Hybridization and washes

To the pre-hybridization solution, add the boiled labeled probe and incubate for 18 h at 65°C with shaking. The blots are washed with 0.1% SDS and 1x SSC for 20 min, twice. Monitor radioactivity with a Geiger counter. Wrap the membrane in Saran Wrap. Expose the membrane to X-ray film at –80°C for 18 h before development, or expose the membrane to a Phosphor imager screen for 2-3 h.

Results

Figure 1. Squash blot hybridization. Upper panel: squashes; Lower panel: hybridization.

Figure 2. Squash blot hybridization for the screening of resistant plants (e.g. 84-2, 84-10).

Figure 3. Squash blot of whiteflies. Each spot is an individual insect *.
The amount of virus is quantified using standards of cloned TYLCV DNA.

Figure 4. Squashes of organs from infected tomato *

* From Navot N, Ber R and Czosnek H (1989) Rapid detection of *tomato yellow leaf curl virus* in squashes of plants and insect vectors. Phytopathology 79: 562-568.

References:

Chatchawankanphanich, O. and D.P. Maxwell. 2002. *Tomato leaf curl Karnataka virus* from Bangalore, India appears to be a recombinant *Begomovirus*. Phytopathology 92:637-645.

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Navot, N., Pichersky, E., Zeidan, M., Zamir, D., and Czosnek, H. 1991. Tomato yellow leaf curl virus: A whitefly-transmitted geminivirus with a single genomic component. Virology 185:151-161. (GenBank no. X15656)

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