TECHNICAL SHEET No. 38

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

Method: Squash Blot PCR

General

Virus detected: TYLCV from tomato plants and whitefly
Method: Squash blot PCR

Developed by

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Goals

To develop a rapid and very sensitive method for the detection of TYLCV in the tissues of whiteflies and plants. This method allows detection of TYLCV in a large number of samples (leaves and insects) from the field. The nucleic acid is stable up to several weeks 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).

PCR has been a common method for the detection of geminiviruses using degenerative primers (Rojas et al., 1993; Wyatt and Brown, 1996). Discrimination between the different geminiviruses can be done using specific PCR primers.

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 (but not identical to) the one reported in Atzmon et al. (1998):

Sampling

1. Squash tomato leaves (young leaf from the shoot apex, or individual whitefly) on Whatman 3MM paper using a glass rod, an Ependorf tube or a pen cover.
2. Cut out a ~ 1 x 2 mm piece and put it into a PCR tube.
3. Add 0.1 ml 1% Tween-20, and incubate at 65°C for 15 min.
4. Discard the solution and add 0.5 ml 70% ethanol and incubate at room temperature for 10 min.
5. Discard the ethanol and dry the squash at 65°C.

PCR primers

TYLCV (X15656) DNA fragments were amplified using the following pair of primers (0.2 mM each) deduced from the nucleotide (nt) sequence of the genome of TYLCV from Israel (Navot et al., 1991):

Specific primer pairs:

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

2) V781 (nt 781-800, viral strand, 5'CTCACAGAGTGGGTAAGAGG3') and C1256 (nt C1256-1229, complementary strand, 5'TTAATTTGATATTGAATCATAGAAATAG3').

3) V1769 (nt 1769-1790, viral strand, 5'GCGAACAGTGGCTCGTAGAGGG3') and C2120 (nt 2120-2097, complementary strand, 5'CAGGCAAAACAATGTGGGCCAGG3').

PCR

1. Add the PCR reaction mix (with TYLCV-specific primers, 0.2 mM each) to the microfuge tube with the squash blot.
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, 60°C for 1min, 72°C for 1 min; end with an additional cycle of 72°C for 10 min.
3. Subject PCR products to electrophoresis in 1% agarose gel in TAE buffer.

Results

Amplified TYLCV DNA: ~ 400-bp PCR product, primer pair V61 and C473.

M I 0 2 5 7 C

M: molecular weight markers
I: infected plant,
0: non-infected plant,
C: no template.
2, 5, 7: days after squash sampling

References:

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