

TECHNICAL SHEET No. 31

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

Method: Direct Elisa

General

Virus detected: TYLCV from tomato leaf.

General method: Direct ELISA

Developed by

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Goals

TYLCV detection based on direct ELISA

Introduction

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, 2) from Southwest Europe (Italy, 3) from tropical Africa (Senegal, Tanzania, 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).

The coat proteins of the tomato begomoviruses 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. L. Gilbertson, University of California-Davis.

Materials and Methods

Antigen extraction and coating

1. Homogenize test sample 1:20 in coating buffer. Coating buffer is (1 liter, pH 9.6), in ddw: Na₂CO₃ 1.59 g, NaHCO₃ 2.93 g, NaN₃ 0.20 g.
2. Add 100-200 µl per well. Cover plates tightly. Incubate at 4-6°C overnight.

- Alternatively:* cut tomato leaf and/or stem and place them in ELISA well containing 100-200 μ l coating buffer. Incubate at 4-6°C overnight.
- Empty the wells and wash 3-4 times with washing buffer, remove any liquid by blotting the plate on paper towels. Washing buffer is (for 1 liter, pH 7.4): in ddw, NaCl 8.00 g, KH_2PO_4 0.20 g, Na_2HPO_4 1.15 g, KCl 0.20 g, Tween 20 0.50 g, NaN_3 0.20 g.

Conjugate

- Dilute anti-TYLCV antibody (e.g. 1: 1000 in conjugate buffer or TBS buffer) and add 200 μ l per well. Conjugate buffer is for 1 liter, pH 7.4: Tris-(hydroxy-methyl) aminomethane 2.40 g, NaCl 8.00 g, PVP (Polyvinyl-pyrrolidone) MW 24,000 20.00 g, Tween 200.50 g, BSA (bovine serum albumin) 2.00 g, $\text{MgCl}_2 \cdot 6 \text{H}_2\text{O}$ 0.20 g, KCl 0.20 g, NaN_3 0.20 g.
- Cover the plates tightly and incubate at 37°C for 3-5 h or 18 h at 4 to 6°C.
- Empty the wells and wash 3-4 times with washing buffer, remove any liquid by blotting the plate on paper towels.
- Conjugate: Dilute anti-rabbit alkaline phosphatase conjugate 1:1000 in conjugate buffer or in TBS buffer, and add 200 μ l per well.
- Cover the plates tightly and incubate at 37 °C for 3-5 h.
- Wash 3-4 times with washing buffer, and then remove any liquid by blotting the plate on paper towels. It is important to remove all liquid by blotting the plate on paper towels.

Color reaction

- Dissolve p-nitrophenyl phosphate to 1 mg/ml in substrate buffer. Substrate buffer is for 1 liter (pH 9.8): in ddw, diethanolamine 97.00 ml, NaN_3 0.20 g (the substrate buffer available as 5x concentrate, Art. No. 110130).
- Add 200 μ l per well and incubate at ambient temperature in the dark.
- Observe reaction and read yellow color development after 30-120 min.
- Visually and/or read with an ELISA reader at 405 nm.

Results

Effect of homogenization, virus and antibody dilutions, on the detection of TYLCV by direct ELISA



Discussion

This antiserum against TYLCV (Israel) should be tested against other begomoviruses.

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.
- Navas-Castillo, J., Sánchez-Campos, Noris, E., Louro, D., Accotto, G. P., and Moriones, E. 2000. Natural recombination between Tomato yellow leaf curl virus-Is and Tomato leaf curl virus. *J. Gen. Virol.* 81:2797-2801.
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