TECHNICAL SHEET No. 5

Virus Detection: *Cucumber mosaic virus* (CMV)

Method: DAS ELISA

General

Virus detected: *Cucumber mosaic virus* was detected from cucumber, tobacco and watermelon leaves.
Method: DAS ELISA

Developed by

Name of researcher: Hanokh Czosnek, Hebrew University of Jerusalem, Israel
Address (email): czosnek@agri.huji.ac.il
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Goals

To develop a reliable and fast detection system for CMV.

Introduction

CMV is a well studied virus which infects many plant species.

Method

The CMV antibody is a gift from Prof. G. Loebenstein, The Volcani Center Israel

Coating of microtiter plate

1. Dilute the CMV antibody 1:1000 in coating buffer. Coating buffer is for 1 liter (pH 9.6), in ddw: Na_2CO_3 1.59 g, $NaHCO_3$ 2.93 g, NaN_3 0.20 g.
2. Add 100- 200 µl to each well and cover plates tightly.
3. Incubate at 37^{o}C for 4 hr or at 4-6 ^{o}C for 18 h.
4. Wash: Empty the wells and wash 3-4 times with washing buffer, remove any liquid by blotting the plate on paper to wells. 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.

Antigen extraction and binding

1. Homogenize test sample 1:20 in either one of the extraction buffers (both extraction buffer are good). Extraction buffer I: for 1 liter, pH 8.5: Tris-(hydroxymethyl) aminomethane (Tris) 2.40 g, NaCl 8.00 g, Tween 20 0.50 g, KCl 0.20 g, NaN_3 0.20 g. Extraction buffer II: 0.5 M Citrate, 0.1% Thioglycolic acid, 0.1% Triton 100.
2. Add 200 µl per well. Cover plates tightly. Incubate in a moist chamber at 4-6 ^{o}C for 18 h.
3. Wash: 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

1. Conjugate: Dilute anti-CMV alkaline phosphatase conjugate 1:1000 in conjugate buffer or in TBS buffer. Conjugate buffer is, for 1 liter, pH 7.4: Tris-(hydroxymethyl) amino-methane 2.40 g, NaCl 8.00 g, PVP (Polyvinyl-pyrrolidone) MW 24,000 20.00 g, Tween 20 0.50 g, BSA (bovine serum albumin) 2.00 g, $MgCl_2$-6 H_2O 0.20 g, KCl 0.20 g, NaN_3 0.20 g.
2. Add 200 µl per well and cover plates tightly.
3. Incubate at 37°C for 3-5 hr.
4. Wash: Empty the wells and wash 3-4 times with washing buffer, remove any liquid by blotting the plate on paper towels.

Color reaction

1. 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).
2. Add 200 µl per well and incubate at ambient temperature in the dark.
3. Observe reaction and read yellow color development after 30-120 min.
4. Visually and/or read with an ELISA reader at 405 nm.

Results

Black line (upper line): the infected tissue was extracted with extraction buffer I containing uninfected plant tissue. Red line (lower line): the tissue was extracted with buffer II.