TECHNICAL SHEET No. 19

Virus Detection: *Potato virus Y* (PVY)

Method: DAS-ELISA

General

Virus detected: PVY from *Nicotiana glutinosa*, potato leaf and potato tubers.
General method DAS ELISA

Developed by

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Date: October 3, 2002

Goals

To develop a sensitive method for PVY detection based on DAS-ELISA.

Introduction

PVY strains are pest quarantine in Israel, and therefore development of sensitive methods for their detection is pivotal for establishing virus free potato seeds. Israel imports all potato seeds for planting from Europe, where the PVY^N strain is present. The current method routinely used for PVY detection is ELISA.

PVY is a member of the *Potyviridae*, the largest plant virus family. For general information on PVY see the Web Site (life.anu.edu.au/viruses/ICTVdB/57010001.htm). Currently, three main strains of PVY are known. (i) PVY^O (ordinary or common strain) is widely distributed throughout the potato growning areas of the world. This strain induces mild to severe mosaic and can cause stem necrosis in some cultivars. (ii) The PVY^C is the stipple streak strain and produces systemic mosaic in some cultivars and a hypersenstive reaction in other cultivars. In tobacco, it produces the same symptoms as the PVY^O. (iii) PVY^N was recognized in the 1950's as causing severe veinal necrosis in tobacco. It was originally imported from Europe and South America. A new sub-group of PVY^N that causes superficial necrotic ring spots on tubers was first described in 1980. It is called potato tuber necrotic ringspot strain. The tuber symptoms develop during storage (Carnegie and van de Haar, 1999).

A monoclonal antibody assay was reported by Ellis (1996) for distinguishing the PVY^O and PVY^C strains from PVY^N. An ELISA assay is commonly used in the USA for detection of PVY^N. Recently, RT-PCR assays have been developed for detection of PVY^N (Nie and Singh, 2002a and 200b). The existence of recombinants between strains has been suggested (Glais et al., 2002), which makes for difficulties in detection and characterization. There is, currently, considerable efforts being made to better understand the relationship between the different strains of PVY, specifically their genomes and biological properties.

Materials and Methods

Two sources of anti-PVY antibody and conjugates were used: from Bioreba, and from a gift from Prof. G. Loebenstein, The Volcani Center, Israel.

Coating

1. Dilute the PVY 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°C for 4 h or at 4-6ºC 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. 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: For leaves use "General extraction buffer": 20 mM Tris buffer (pH 7.4) containing 137 mM NaCl, 3 mM KCl, 2 % PVP 24 kD, 0.05% Tween 20 and 0.02% sodium azide. For tubers, use "tuber extraction buffer": for 1 liter, dissolve 10 g of egg albumin first in 20 ml using a spatula in a small cup, then make up to 1000 ml with "General extraction buffer" (Prepare freshly before use).
2. Add 200 µl per well. Cover plates tightly. Incubate in a moist chamber at 4-6 °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-PVY 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, $MgCl_2$-$6H_2O$ 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 h.
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.

References

Background on PVY: http://life.anu.edu.au/viruses/ICTVdB/57010001.htm

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