General

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

Developed by

Name of researcher: Fouad Akad and Hanokh Czosnek, The Hebrew University
Address (Email): akad@agri.huji.ac.il and Czosnek@agri.huji.ac.il

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Goals

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 PVYN 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 know. (i) PVYO (ordinary or common strain) is widely distributed throughout the potato growing areas of the world. This strain induces mild to severe mosaic and can cause stem necrosis in some cultivars. (ii) The PVYC is designed the stipple streak strain and produces systemic mosaic in some cultivars and a hypersensitive reaction in other cultivars. In tobacco, it produces the same symptoms as the PVYO. (iii) PVYN was recognized in the 1950’s as causing serve veinal necrosis in tobacco. I was originally described from Europe and South America. A new sub-group of PVYN 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 PVYO and PVYC strains from PVYN. An ELISA assay is commonly used in the USA for detection of PVYN. Recently, RT-PCR assays have been developed for detection of PVYN (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, consider effort to better understand the relationship between the different strains of PVY, there 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: Na₂CO₃ 1.59 g, NaHCO₃ 2.93 g, NaN₃ 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 4-6 °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 towels. Washing buffer is (for 1 liter, pH 7.4): in ddw, NaCl 8.00 g, KH₂PO₄ 0.20 g, Na₂HPO₄ 1.15 g, KCl 0.20 g, Tween 20 0.50 g, NaN₃ 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, KH₂PO₄ 0.20 g, Na₂HPO₄ 1.15 g, KCl 0.20 g, Tween 20 0.50 g, NaN₃ 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 (Polyvinylpyrrolidone) MW 24,000 20.00 g, Tween 20 0.50 g, BSA (bovine serum albumin) 2.00 g, MgCl₂·6H₂O 0.20 g, KCl 0.20 g, NaN₃ 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, NaN₃ 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**

http://www.unece.org/trade/agr/meetings/ge.06/1999_09_r/pvyntn.doc


Glais, L., M. Tribodet, and C. Kerlan.  2002.  genomic variability in Potato potyvirus Y (PVY): evidence that PVY\textsuperscript{NW} and PVY\textsuperscript{NTN} variants are single to multiple recombinants between PVY\textsuperscript{O} and PVYN isolates.  Arch. Virol. 147:363-378.


Confirmed by

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