TECHNICAL SHEET No. 20

Virus Detection: *Potato virus Y* (PVY)
(PVY detection in tubers)

Method: Dot Blot Hybridization

General

Virus detected: PVY from potato tubers
General method: RNA extraction from tubers using the PEX method, RNA dot blot hybridization

Developed by
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Date: Oct. 8, 2002

Goals

To extract RNA from large numbers of tuber samples in a short time, using a simple and economic method. This RNA is used for virus detection by RNA dot blot hybridization with a radiolabeled probe.

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^Nstrain is present. The method routinely used for PVY detection is ELISA.

We wanted to develop another method for confirming ELISA or to replace ELISA as the main method for virus detection from potato tubers, in a large-scale potato virus detection scheme. The method is applied for the detection of PVY^N and includes the cloning N-terminus of PVY^N, and development of RNA purification procedures suitable for dot-blot hybridization.

The advantages of this method are:

a) Simplicity and rapid extraction of nucleic acids.
b) No need of RNAase inhibitors, which may increase the costs (the PEX solution by itself is an RNAase inhibitor).
c) The extraction steps are carried out in one tube, minimizing manipulations and allowing the simultaneous handling of a large number of samples at one time.

Materials and Methods

RNA purification from potato tubers using PEX

The procedure was modified from:
Nakahara K, Hataya T, and Uyeda I. (1999) A simple, rapid method of nucleic acid extraction without tissue homogenization for detecting viroids by hybridization and RT-PCR. Journal of Virological Methods 77:47-58.

1. Cut a potato eye (0.05 to 0.1 g) and transfer it to a microfuge tube, freeze in liquid nitrogen; add 500 µl 70% ethanol and let sit for 10 min.
2. Discard the ethanol and add 500 µl of PEX solution; incubated at 65^{o}C for 5 min. (PEX solution: 6.25 mM potassium ethyl xanthogenate, 100 mM Tris- HCl pH 7.5, 700 mM NaCl, 10 mM EDTA pH 8.0).
3. Centrifuge the samples for 6 min at 14,000 rpm and incubate for an additional 15 min at 65^{o}C.
4. Remove the piece of tissue using a toothpick and add 1 ml ethanol and incubate for 30 min at - 80 ^{o}C.
5. Collect nucleic acids by centrifugation for 10 min at 14,000 rpm, and discard ethanol; dry gently under vacuum.
6. Dissolve pellet of nucleic acid with 30 µl ddH_2O, incubate at 65 °C for 15 min and store at -80 °C.

Dot blot hybridization

a) Preparation of membrane

1. Spot 5 µl from each RNA sample onto a nylon-membrane.
2. Cross-link the nucleic acid to the membrane by exposure to U.V. for 3 min.

b) Preparation of ^{32}P labeled probes

1. In a total reaction volume of 50 µl add: 5 µl of 0.3 µg/µl pGEM/PVY, 5 µl of 10 x hexanucleotide buffer, and 22 µl ddH_2O (plasmid pGEM/PVY: contains cloned PVY DNA obtained from PVY TNT leaf tissue supplied with the ELISA kit, cloned by immunocapture RT-PCR using the primers described in the technical sheet allowing to distinguish between the different PVY strains).
2. Heat for 10 min at 100°C, then cool on ice for 15 min.
3. Add 10 µl of dNTPs (0.5 mM each) without dCTP and 3 µl of the Klenow fragment of DNA polymerase I.
4. Add 5 µl of [^{32}P] dCTP and incubate at 37°C for 3 h.
5. Boil for 10 min and immediately put in ice bath.

c) Hybridization

1. Membranes are prehybridized at 42°C with a solution that contains: 12.5 ml 50 x SCC, 5 ml Denhardt solution x 50, 2.5 ml 10% SDS, 3.2 ml NaH_2PO_4 , 1.7 ml Na_2HPO_4 , 0.7 ml boiled salmon sperm DNA (50 µg/ml) and ddH_2O up to 50 ml (final pH should be 6.8). Denhardt solution x 50: 2% bovine serum albumin (BSA), 2%, Ficoll 400, 2% Polyvinylpyrolidone.
2. After 2 hr, add the radiolabeled probe and hybridize for 18 h at 42°C.
3. Wash the blots with 1 x SSC - 0.1% SDS for 20 min at 65 ^{o}C, twice. If necessary, wash again with 0.1 x SSC - 0.1% SDS at 65 oC. SSC x 20 is: 3 M sodium chloride, 0.3 M Tri-sodium citrate.
4. Expose the membrane to X-ray film or to a Phosphor imager.

Results

Figure 1. Four different unrelated potato batches (A, B, C and D) were analyzed. The blots were hybridized with radiolabeled cloned PVY^{N}.