TECHNICAL SHEET No. 12

Virus Detection: *Grapevine virus A* (GVA)

Methods: RNA dot blot hybridization

General

Virus detected: GVA from grapevine leaves, petioles, and stems.
General Methods are RNA dot blot hybridization.

Developed by

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Goals

To develop sensitive methods for GVA detection in grapevines.

Introduction

See other technical sheets on *Grapevine virus A* for information about this virus and references.

Methods

RNA purification

1. Cut a leaf or stem grape tissue and transfer it to a microfuge tube, freeze in liquid nitrogen, and add 500 µl 70% ethanol 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 (12,000 x g) 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, discard ethanol and dry gently under vacuum.
6. Dissolve the pellet of nucleic acid with 30 µl ddH_2O, incubate at 65 °C for 15 min and store at –80 °C.

Dotting RNA on 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.

Preparation of ^{32}P labeled probes by Random priming method (kit from Amersham)

1. In a total reaction volume of 50 µl add: 5µl of 0.3 µg/µl plasmid pGEM/GVA, 5µl 10 x hexanucleotide buffer, and 22 µl ddH_2O.
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 (3000 Ci/mmole) and incubate at 37 °C for 3 h.
5. Boil for 10 min and immediately put in ice bath.

Hybridization

1. Membranes are prehybridized at 42 °C with a solution that contains, for 50 ml: 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.
2. After 2 h, add the radiolabeled probe and hybridize for 18 h at 42 °C.
3. Wash the blots with 1xSSC - 0.1% SDS for 20 min at 65^{o}C, twice. If background is dirty, wash with 0.1 x SSC - 0.1% SDS at 65 ^{o}C.
4. Expose the membrane to X-ray film or to a Phosphor imager.

Results

The same samples are compared using dot blot hybridization and northern blot hybridization.