A Polyacrylamide Gel Electrophoresis Index Method for Avocado Sunblotch

J. G. UTERMOHLEN, Research Assistant, Department of Plant Pathology, and H. D. OHR, Extension Plant Pathologist, Cooperative Extension, University of California, Riverside 92521

ABSTRACT

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An index procedure for avocado sunblotch disease was developed with ribonucleic acid polyacrylamide gel electrophoresis. The gel index was 96% accurate in tests with known healthy and sunblotch-diseased trees (standard biological index). The assay takes 2 days to perform, compared with 3–24 mo for the standard biological index, and uses only 100–200 mg of host tissue.

Avocado sunblotch was first described in 1928 by Coit (1), who attributed the disease to physiologic stress caused by sunburn. In 1931, Horne and Parker (5) showed that the causal agent of the disease was graft-transmissible. In 1962, Wallace and Drake (11) showed that it was seed-transmissible, and in 1979, Desjardins et al (3) demonstrated pollen transmission.

Although the causal agent has not been characterized, Dale and Allen (2), Thomas and Mohamed (10), and Palukaitis et al (8) have proposed that it is a viroid. Although these researchers reported a presumptive viroid RNA-like molecule associated with the disease, none demonstrated infectivity. In 1980, Semancik and Desjardins (9) reported the existence of four additional RNA bands associated with the disease.

The indexing technique reported here uses the 61,000-mol-wt RNA species, detected by Semancik and Desjardins (9) and called ASV_5, as the basis for disease diagnosis. This is the same disease-specific RNA species reported by the other three research groups (2,8,10).

The RNA associated with sunblotch occurs in diseased tissue at such high concentrations that it is easily detected with an RNA polyacrylamide gel indexing method. A similar indexing procedure has already been used to detect potato spindle tuber viroid in potatoes (7).

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MATERIALS AND METHODS

Extraction solutions. Three stock solutions were used to make the extraction buffer. Stock A was 0.2 M Tris, pH 8.9, with 1.5 M LiCl and 7% (w/v) polyvinylpyrrolidone (10,000 mol wt). Stock B was 0.1 M EDTA, and stock C was 5% (w/v) SDS. Stock A was mixed with stocks B and C (4:1:1). Extracted nucleic acids were dissolved and stored frozen in 0.01 M Tris, 0.01 M KCl, and 10^{-4} M MgCl (TKM), pH 7.4.

Extraction procedure. Young succulent avocado leaves 1–2 cm long (100–200 mg) or three to five flowers were placed in a 1.5-ml microsample tube and kept cold (on ice) until frozen in liquid nitrogen. The tissue could be kept at −20 C until extracted but had to be further cooled in liquid nitrogen before extraction began.

The tissue was crushed in the tube with a liquid nitrogen-cooled, tapered bit attached to a variable-speed drill, spinning at half speed, until the sample was completely powdered. (Note that tissue must not defrost during crushing; the sample tube and grinding bit must be periodically reimmersed in liquid nitrogen.)

Neutralized, water-saturated phenol (500 μl), extraction buffer (200 μl), and 2-mercaptoethanol (100 μl) were added to the crushed sample. The tissue was allowed to come to ambient temperature in this solution, and then tissue and solutions were mixed. The sample was agitated for 1 hr at 4 C with a "wrist-

action" shaker, then centrifuged for 1 min at 10,000 rpm in a tabletop centrifuge (Beckman microfuge).

The upper aqueous layer was then removed, placed in another microsample tube, mixed with three volumes of 95% ethanol, and cooled to −20 C for 1 hr. The sample was then removed from cold storage and recentrifuged as described. The ethanol precipitate was saved, and the supernatant was discarded. The precipitate was washed with a solution of 70% ethanol containing 0.1 M sodium acetate. Centrifugation, washing, and centrifugation were repeated in that order. The pellet was saved and allowed to dry either under vacuum for 15 min or at 4 C for 1 hr.

TKM buffer (100 μl) was added to the pellet, which was allowed to dissolve overnight at 4 C. The sample could be used immediately for electrophoretic analysis or stored frozen (−20 C). The RNA gels were a 5% concentration of polyacrylamide as described by Morris and Wright (6) but were used in slab form (0.2 × 12 × 15 cm).

Thawed or fresh extracts were recentrifuged as described, and 30 μl of the extract was mixed with 5 μl of 80% glycerol solution. This mixture was loaded into the sample well. A tRNA-rich extract containing citrus exocortis viroid and bromphenol blue tracking dye at 0.05% concentration in 60% glycerol were used as standards. The samples were subjected to electrophoresis in the refrigerator (4 C) at a constant current of 60 mA for 2–2.5 hr, or until the bromphenol blue tracking dye had moved 7 cm from the bottom of the sample well.

To stain the gel, 30 μl of a 5 mg/ml stock solution of ethidium bromide was added to 200 ml of distilled water. The gel was floated off the glass plates into the stain solution and slowly agitated for 15 min. (Note that ethidium bromide is a strong mutagen and must be used with caution.)

The stained gel was then removed from the staining solution and placed on a short-wave ultraviolet transilluminator (Model C60, Ultraviolet Products, San Gabriel, CA). The gels were photographed with Polaroid 3000 black and white film, type 107.

The low-molecular-weight band, ASV_5, was detected by its intensity and position. ASV_5 RNA should be at least as intense as 7S ribosomal RNA, and its position should be 65% of the distance traveled by the 4S tRNA band from the bottom of the sample well.

RESULTS AND DISCUSSION

Examples of gels with samples from tissue collected from field-, lathhouse-, or greenhouse-grown avocado trees are shown in Figure 1. ASV_5 low-molecular-weight RNA was detected only in extracts from known infected trees (Table 1). The test accurately detected sunblotch trees in 96% of samples (23 of 24) from trees previously diagnosed by biological methods. The one incorrect diagnosis made by gel electrophoresis was a sample from a tree that was diagnosed visually as having sunblotch but that subsequently recovered from the symptoms.

The use of RNA gel electrophoresis as an indexing method has already been demonstrated in culling seed potatoes infected with spindle tuber viroid in a clean-stock program (7). We believe that this method can also be applied to indexing for sunblotch in avocado. The method currently used requires 2 yr for certification in California; the RNA detection method would reduce this time to 2 days and would rapidly identify many trees that are carriers of the disease.

Since the observations of Desjardins et al (3,4), it has been recognized that

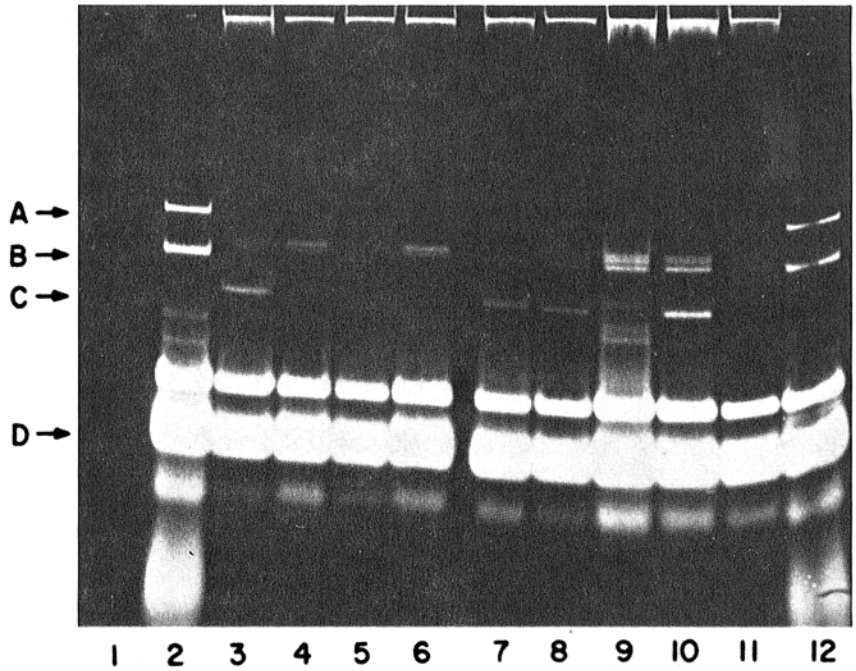

Fig. 1. Five-percent polyacrylamide gels of RNA extracts from 100-mg fresh-weight samples of avocado tissue. Columns 2 and 12 are RNA extracts from citrus exocortis viroid (CEV)-infected *Gynura aurantiaca*. Columns 3, 7, 8, and 10 are RNA extracts from trees indexed as positive for avocado sunblotch viroid. Columns 4–6, 9, and 11 are RNA extracts from healthy avocado trees. Arrows A–D indicate CEV-RNA, 7S host RNA, ASV_5 RNA, and 4S host tRNA, respectively.

Table 1. Biological and RNA polyacrylamide gel electrophoresis indexes for the diagnosis of avocado sunblotch disease in shoot tissues of avocado cultivars

Cultivar	Location	Tissue	Biological index (sunblotch symptoms)	Gel index
Hass	Field	Leaf	+	+
Hass	Field	Leaf	−	−
Hass	Field	Leaf	+	−[a]
Hass	Field	Leaf	−	−
Zutano	Greenhouse	Leaf	+	+
Fuerte	Greenhouse	Leaf	+	+
Hass	Lathhouse	Leaf	−	−
Topa-Topa	Lathhouse	Leaf	−	−
Reed	Lathhouse	Leaf	+	+
Fuerte	Field	Leaf	+	+
Topa-Topa	Field	Leaf	−	−
Zutano	Field	Leaf	+	+
Duke	Field	Leaf	−	−
Duke	Field	Leaf	+	+
Fuerte	Field	Leaf	−	−
Topa-Topa	Field	Leaf	+	+
Fuerte	Field	Flower	+	+
Duke	Field	Flower	+	+
Topa-Topa	Field	Flower	−	−
Huntalas	Greenhouse	Leaf	+	+
Zutano	Field	Flower	+	+
Duke	Field	Flower	−	−
Huntalas	Lathhouse	Leaf	+	+
Fuerte	Field	Flower	−	−

[a] The RNA extraction and gel analyses were performed with tissue collected after the tree had recovered from sunblotch symptoms.

avocado sunblotch disease may be able to spread through an orchard, by pollen or mechanical means, from one infected tree. This possibility can be reduced if orchards are indexed to detect and remove diseased trees.

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