Sensitive Detection and Characterization of Viroid, Virus, and Virus-like Agents of Avocado

Continuing Project: Year 4 of 5

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Benefit to the Industry

In the last 5 years our laboratory has refined the RT-PCR test for ASBVd detection in avocado and succeeded in having this test approved for registration of foundation stock trees by CDFA (Mathews et al., 1997). Several other researchers as well as many growers have had their plants tested by our laboratory which has helped to prevent the distribution of infected sources of plant material as well as allowing confident release of ASBVd negative material. In addition to studying ASBVd and potential sources of the viroid which pose a threat to the industry, we also continue to try and identify other viral or viral-like diseases of avocado which may lead to the ultimate improvement of the crop.

Objectives

A. Test nurse seeds/seedlings for the presence of ASBVd.
B. Clone and sequence viral-like dsRNAs found in avocado.
C. Identify symptomless carrier trees and study transmission and disease progression of ASBVd through seed and grafting.

Summary

In addition to our funded research we continue to test trees for the presence of ASBVd for any researchers or growers that wish to use our service. This year we have tested 242 trees from 2 UCR researchers and 5 commercial growers. Six of those (2.5%) were found to be positive for ASBVd. Many of the ASBVd negative trees were then used as registered sources of budwood. If interested, please contact Deb Mathews or Jim Heick using the addresses/numbers above for collection, pricing and scheduling information

A. Test nurse seeds/seedlings for the presence of ASBVd.

Seeds are purchased in bulk for use as nurse seedlings for grafting of the desired rootstock and scion varieties. These nurse seeds are not screened for ASBVd. If they were harvested from a symptomless carrier tree or were from fruit pollinated with ASBVd infected pollen, these seeds could readily transmit the viroid to the grafted varieties. This year we started a pilot program for ASBVd testing of nurse seeds/seedlings from several sources in California. Bulk seed was not available this summer, so with the greatly appreciated help of Ben Faber and Gary Bender, we were able to test germinated nurse seedlings which had not yet had other varieties grafted onto them. Nurserymen were concerned initially about participating in the testing program due to possible ramifications of finding positive seeds, but once their anonymity was assured during this first round of testing, most agreed to provide access to their plants. Ben and Gary did the collecting and returned the tissue to us using simple code numbers so that they could trace back any positives to the specific nurseries.

Seventy-five trees were tested from each of 4 nurseries (300 seedlings total). Small, single shoot seedlings like these are optimal candidates for pooling of samples so the cost and effort of testing can be reduced. Each set of 75 trees were pooled into 5 groups of 15 (2 lvs/tree) which were then assayed and tested in duplicate by RT-PCR. Healthy and positive controls as well as a 1/15 pooled positive control (1 infected lf/14 healthy lvs) were tested for

each group. Each of the pools were scored as negative for ASBVd, while all controls were as expected (Figure 1.) This Fall/Winter we will be testing additional nurse seeds/seedlings as they become available.

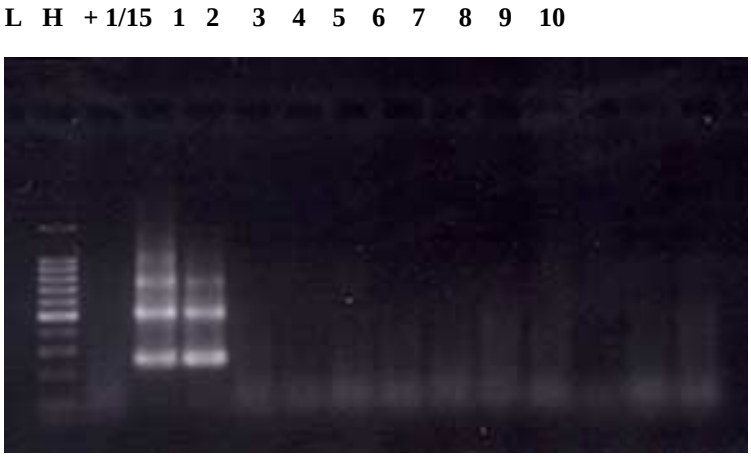

Figure 1. Agarose gel electrophoresis of RT-PCR products from pooled nurse seed samples. Lanes are molecular weight marker ladder (L); healthy (H); positive control (+); pooled 1/15 diluted positive control (1/15); 10 representative pooled samples from a nursery (1-10, all negative).

B. Clone and sequence viral-like dsRNAs found in avocado.

We have found that over 85% of all avocado trees contain one or more of 3 known double-stranded (ds) RNA patterns (types 1, 2 and 3). There doesn't appear to be any correlation between the patterns found and variety. No viral particles have been found to be associated with these dsRNAs, so their origin is currently unknown and there isn't any easily recognizable disease syndrome related to their presence. We have spent much of the last year isolating each of the 3 dsRNA types, cloning and sequencing them in order to compare them to other available sequences.

Trees from the Avocado Foundation Block at UC Riverside were used as sources of the dsRNA since several different varieties are available and we have screened them to be negative for ASBVd. A single tree (17-1) was used as the source of type 1, and 2 trees each were used as sources of types 2 (trees 16-6, 18-2) and 3 (trees 15-3, 17-3). Three different methods were used for cloning the dsRNAs, but only one resulted in the production of cDNA clones. Seventy-two clones were selected and screened from each of the dsRNA types (216 total). Clones were sized and 30 of the largest were used to make probes for Northern blot analysis against their source dsRNAs in order to confirm their identity as well as a plant that contains all 3 dsRNA patterns (tree 16-3) which was not used as a cloning source (Figure 2). From the Northern analyses, differences between sources of each dsRNA were observed. One of the clones for dsRNA type 1 (clone 1-5) which hybridized to both the high and low molecular weight (MW) dsRNAs from its source plant 17-1, did not react with the type 1 dsRNA from plant 16-3 (Figure 2, panel A). Another type 1 clone (1-10) did hybridize to both sources of type 1 dsRNA (data not shown). Similar differences in hybridization affinities were seen with clones from the type 3 dsRNAs. These results indicate significant genetic diversity between these dsRNAs. Only 1 confirmed clone was obtained from the type 2 dsRNAs, most clones screened hybridized not to the type 2 dsRNA band, but to the abundant material at the top of the gel which is most likely contaminating host nucleic acid. For the next round of cloning, the type 2 dsRNA will be isolated by gel purification in order to ensure a higher proportion of homologous clones.

After screening, 3 clones from dsRNA type 1, one clone from type 2, and 8 clones from type 3 were sequenced and compared to sequences in the GenBank database. One of the type 1 clones has a significant region of sequence similarity with several plant chloroplast sequences in one segment of its genome. It was previously reported (Cook et al., 1994) that the type 1 dsRNA hybridized to DNA from a non-dsRNA containing avocado plant indicating the dsRNA was from a host plant source. We have confirmed this result and isolated it to chloroplasts. However, neither of the other type 1 clones nor any of the other type 2 or 3 clones show any sequence similarity to any published sequence at the nucleotide or protein level. DsRNAs from other plant species have been proposed to have evolved from defective single-stranded RNA viruses and after co-evolution with their hosts may share some sequence similarity with them (Gibbs et al., 2000; Pfeiffer, 2002). Avocado dsRNA type 1 especially fits this hypothesis. We are continuing to investigate these dsRNAs in order to try and identify their origin and effect, if any, on avocado.

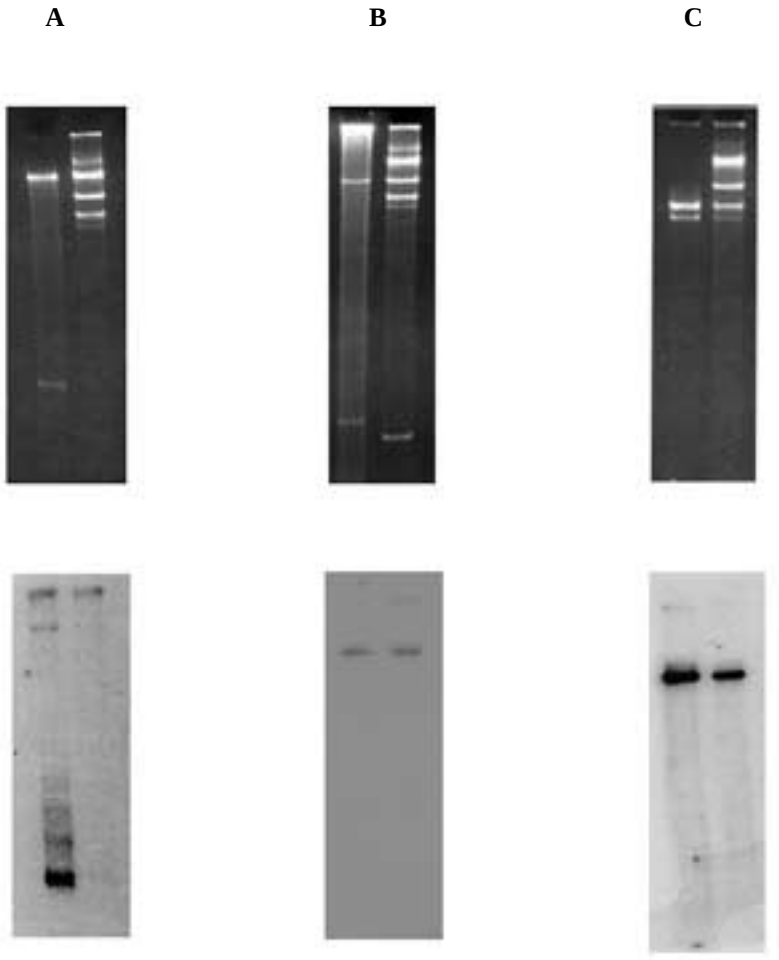

Figure 2. Northern blot analysis of cDNA clones of dsRNAs from avocado. Each upper figure is polyacrylamide gel analysis of dsRNA type 1 (A), type 2 (B), or type 3 (C) and the lower figure is the autoradiograph of the dsRNAs on the gel after probing with a radioactive probe made from a cDNA clone of the corresponding dsRNA.

C. Identify symptomless carrier trees and study transmission and disease progression of ASBVd through seed and grafting.

This year we have tested approximately 25 trees from various locations that fit the profile of a potential symptomless carrier (SC) tree: very little fruit set with abundant tree growth and no visible sunblotch symptoms. Only one of these trees, from P.R. Desjardins- professor emeritus UCR, was found to contain ASBVd. We continue to analyze the 4 SC trees at UCR and have collected and planted approximately 12 seed from 2 of the trees for germination. The fruit flesh from these seeds tested positive for ASBVd. These seedlings will be used for a disease progression study by analyzing when and where on the plant ASBVd can be first detected on: intact SC seedlings, healthy scions grafted onto infected SC seedlings, and healthy seedlings grafted with infected ASBVd tissue. This objective will take at least 1-2 more years for completion.

Conclusions

- Failure to detect ASBVd in the number of samples tested to date does not necessarily indicate there is no problem in the nurse seed industry or that it is not a potential source of the pathogen . We feel that additional random periodic testing is the best way to assess this threat due to the random nature of the nurse seed production industry.
- DsRNAs found in avocado are not uniform genetic elements and appear to exist as strains or variants of 3 identifiable pattern types.
- Symptomless carriers of ASBVd are difficult to identify, but still warrant research since they can act as sources of infected pollen and budwood to unsuspecting growers.

Literature Cited

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