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

Funded Project; Year 2 of 3

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Benefit to the Industry

Avocado sunblotch viroid (ASBV) should be indexed in traditional and new varieties of both rootstocks and scions especially if a safe and effective evaluation of new varieties is to be made. One of the major concerns is that ASBV could be unknowingly distributed in or acquired (through topworking) by newly developed or about to be released varieties, none of which have been adequately tested for their ASBV status. Another is that ASBV could have been introduced into older lines since the time they were last indexed.

Renewed interest in possible graft transmissible agents has also stimulated concerns that other virus-like agents may be in need of a research program, such as the problems encountered with the use of Thomas as a rootstock, and the older as yet unresolved problem of black streak. The question of virus and/or virus-like agents in avocado has been a relatively inactive project for several years. Technology has advanced to the point where a more systematic approach to characterizing agents previously or yet to be detected could be followed.

Objectives

- Pooling samples for efficient detection
- Strain characterization of ASBVd isolates
- Development of probes for detection of viral-like dsRNAs

Summary

Pooling of samples

Our last report detailed the use of sample pooling for PCR testing of ASBVd. As a reminder, we successfully pooled 15 trees into one sample and easily detected the pathogen. Pooling is recommended for small greenhouse trees, budwood, or rootstocks. Pooling is not recommended for field trees because of the now demonstrated erratic distribution of ASBVd within the canopy of any single tree. Symptomatic tissue is not recommended for pooling due to the high probability of being positive which would result in individual testing for the entire pool. Such tissues would be best tested singly to begin with.

We have now implemented pooling into testing trees for Dr. Mary Lu Arpaia. To date we have tested approximately 350 trees in sets of 15. This resulted in a saving of $8300 as compared to single testing every tree. Of the 24 pools, 4 were detected as positive and single testing was then performed on those trees making up each positive pool. Fourteen out of 27 individual trees have been detected as ASBVd positive by this method (one pool has not yet been tested singly). After the individual testing, the final savings realized was still $7200.

An unanticipated result from this pooling was that some of the samples tested were from trees from new budwood that was grafted onto rootstocks which had *not* been tested for ASBVd. Several of the scion samples were positive when tested individually. It is not clear whether the source of the pathogen was the budwood or the rootstock. This raises the issue raised in the "Benefits to the Industry" section, which is that prior to grafting, rootstocks grown from seed should be tested so that time and effort, not to mention valuable budwood, is not wasted. If the rootstocks are screened first for ASBVd, then it could be concluded that any positive trees would be due to infected budwood.

Strain characterization

After PCR is performed the remainder of any positive sample can be used for strain comparison using a gel based "single stranded conformational polymorphism" (SSCP) assay. We continue to analyze all ASBVd positive samples and compare them. Each geographic area seems to have slightly different strains of ASBVd. We are currently comparing isolates that cause symptoms with those that do not and our poster will have the most recent data.

Development of probes for dsRNA detection

There are 3 major types of dsRNA patterns found in avocado trees. A small percentage of trees do not have any of the dsRNA patterns. We now have probes available that can detect each of the three types directly in order to rapidly screen avocado trees for the different patterns. Clones of each type are being prepared for DNA sequencing in order to look for similarities to other known pathogens or sequences from other organisms. The Avocado Foundation Block at UCR has been tested for the presence of these dsRNAs and the majority of them contain one or more of the patterns. Data is still being compared at this writing to correlate certain patterns with specific varieties.