1999 California Avocado Research Symposium pages 17-18
California Avocado Society and University of California, Riverside

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

Funded Project; Year 1 of 3

J. Allan Dodds
Department of Plant Pathology,
UC Riverside

Cooperating Personnel: D. M. Mathews, J. A. Heick

Benefit to the Industry

Identification of the presence of Avocado Sunblotch Viroid (ASBVd) in new and established varieties as well as certified nursery trees can only strengthen the industry as a whole. New technology is available that will aid in the identification of other viral pathogens and their possible relationship to diseases such as black streak and the problem of using Thomas as a rootstock.

Objectives

1. Improve PCR detection of ASBVd by optimizing system including age and type of tissues used, time of year for sample harvest, and the effect of pooling several samples together.
2. Screen trees for the presence of double-stranded (ds) RNAs which indicate the presence of virus or virus-like pathogens.

Summary

Optimization of PCR detection of ASBVd

We have determined that leaves of a moderate age are optimal for PCR testing. Leaves from a new flush of growth which have not yet hardened off or old leaves which are nearing senescence do not give consistent results. All parts of the leaf are satisfactory; we use a slice of tissue from the middle of the leaves which includes a section of the

midrib. Harvesting of the trees is critical in obtaining a representative sample. We recommend that approximately 20 leaves from around the entire canopy of the tree be collected (5 leaves from each of the 4 compass points, from separate branches works well), which are separated into 2 samples for analysis. The leaves are stacked and a slice of tissue is made, ground in liquid nitrogen, then extracted in buffer and applied to a filter for PCR detection. When testing individual branches of an infected free, not all branches tested positive. Upon retesting, it was concluded that these results were correct, that the entire free was not yet infected with ASBVd. This points out that proper harvesting of tissue is critical is assessing the infection status of the tree.

Avocado fruit itself is also a satisfactory source of tissue and gives a strong PCR result. We have identified several samples in which the fruit was symptomatic for ASBVd and tested positive for it by PCR. However, the free itself repeatedly tested negative for ASBVd. This is a classic example of the fruit becoming infected with ASBVd by pollen transmission. It has been previously reported that this is common and that infection of the free itself does not occur and only those fruit which obtain the viroid from infected pollen are affected. This is important to note because it can be tempting to remove a free with symptomatic fruit, but without testing of leaves from the free itself and confirming that it is infected, that tree may be needlessly destroyed.

For almost a year, monthly collections from symptomless carriers of ASBVd have been made and analyzed by PCR. To date, no decline in the sensitivity of detection has occurred regardless of the time of year. With some pathogens severe heat or cold can affect the titre and therefore detection of the agent. This does not seem to be a problem with ASBVd.

Screening for virus or virus-like pathogens

Three major dsRNA patterns have been previously found in avocado trees. These may occur singly or in apparent mixed infections. We have identified several trees which contain these dsRNAs and are comparing their complexity and distribution to those studied previously. By the end of this first funding year, we anticipate having cDNA clones of some of these dsRNAs which will act as probes for these agents and aid in their detection and characterization. Research includes looking at the association of these dsRNAs with specific varieties and also with diseases which do not have a known pathogen.