1998 California Avocado Research Symposium pages 7-10
California Avocado Society and University of California, Riverside

Detection of Avocado Sunblotch Viroid by PCR

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Benefit to the Industry

The ability to biologically index avocado trees for the presence of avocado sunblotch viroid (ASBVd) no longer exists at UCR due to retirement of key personnel. We were approached last year to develop the polymerase chain reaction (PCR) for the detection of ASBVd. Using previously published methods with modifications developed in our laboratory, we have produced an assay which enables indexing of materials within several days of harvest as opposed to 2-4 years for biological testing. The PCR test costs approximately $25.00 per tree which includes two replications of each sample, the minimum we recommend at this time for confident diagnosis. The short time period now required for ASBVd diagnosis by PCR along with the relatively low cost of the assay will allow avocado growers and researchers to identify which trees harbor the viroid and eliminate them from cultivation. Our results are being reviewed by CDFA and approval of the use of PCR for ASBVd detection is pending.

Objectives

The first objective was to test the ability of the PCR test to detect ASBVd in trees known by previous biological indexing to be infected with the viroid, both those which had characteristic sunblotch symptoms and those known to be non-symptomatic carriers, while giving a negative result for uninfected trees. The second objective was to retest previously indexed ASBVd negative trees in the UCR variety collection by PCR to ensure that the level of false positives, sometimes a criticism in PCR testing, was non-existent or minimal. Finally, we tested grove trees from South Coast Field Station, including one with ASBVd symptoms, and applied PCR to an actual field situation to check for feasibility of collection, sample storage and processing, and PCR analysis.

Summary

In trees that were known to be infected with ASBVd from prior biological indexing done several years ago, whether showing symptoms or not, PCR yielded a strong positive reaction, (a 247 bp fragment visualized on an agarose gel) in *5* out of 5 samples tested, while known negative trees were all scored as negative by PCR. In one instance where gel analysis of PCR scored 1/5 replicates of a "Todd" variety sample as negative, subsequent dot-spot analysis confirmed the sample as a weak positive (Figure 1). Fifty-two trees representing approximately 40 different combinations of rootstock and scion varieties grown in the UCR variety collection at AgOps were tested by PCR. These trees were also indexed several years ago as negative for ASBVd. All of the trees were scored as negative, in duplicate, for ASBVd by PCR. All positive controls included in the tests were scored as positive. We are confident that if

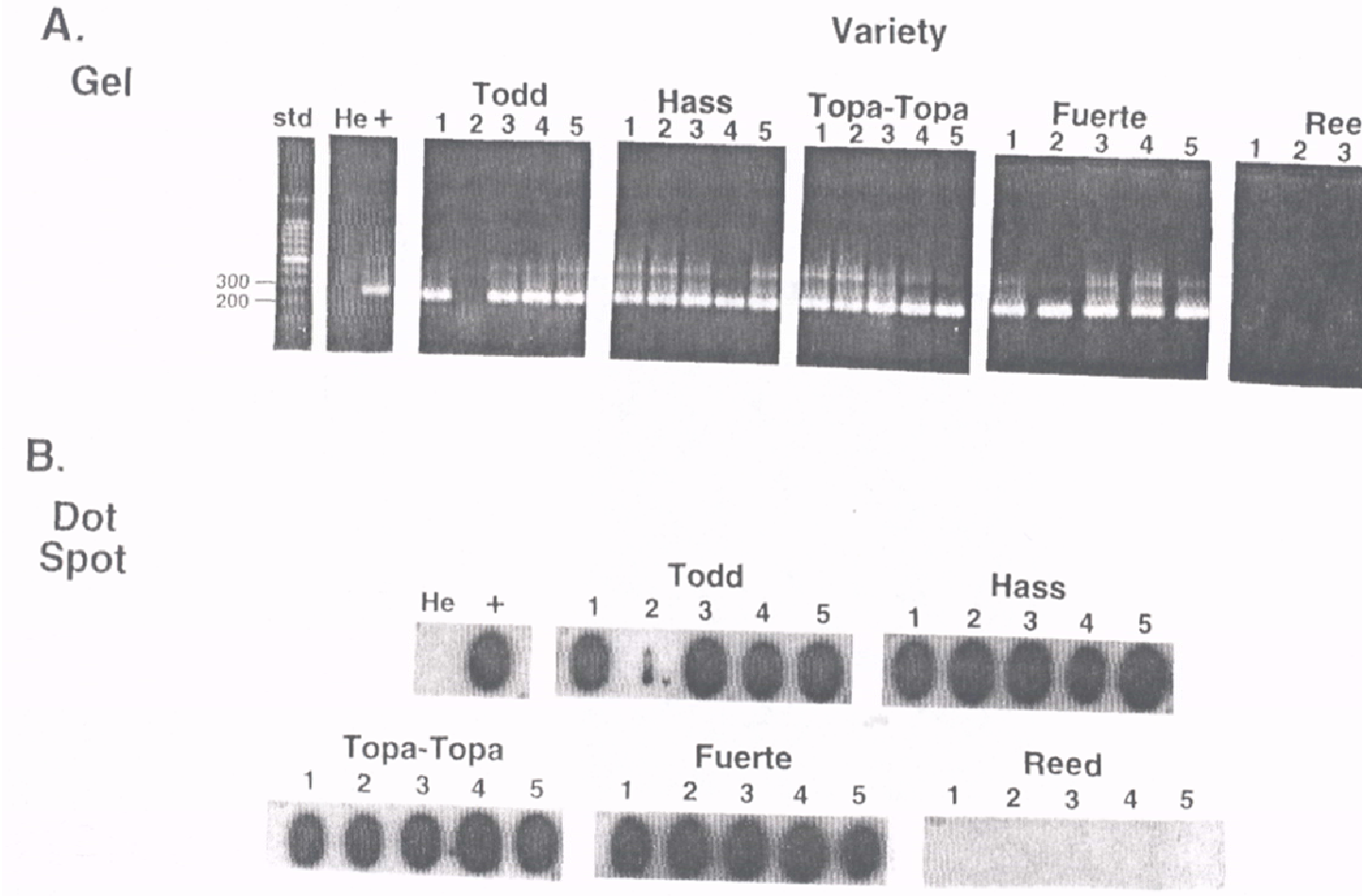
samples are handled and processed with normal due care, the incidence of false positives is minimal based on these results where the opportunity for contamination was present. We initially tested 12 trees from SCFS (samples provided by Dr. Mary Lu Arpaia's lab), one which had yellow streaks on the leaves and fruit. The 11 non-symptomatic trees were scored as negative for ASBVd, while the one with symptoms was scored as a strong positive (Figure 2).

We would like to note that while the PCR test is quite promising and trees that test positive are most definitely infected with ASBVd, trees that test negative could still harbor an undetectable, low litre of the viroid. This of course is the problem with any screening assay, that limits of detection always are at the mercy of current technology. We believe however that the PCR test is a practical and valid method, and is available now for the detection of ASBVd. Our laboratory is willing to test for ASBVd in nursery or field trees. For details and cost call Deb Mathews or Jim Heick at (909) 787-3864.

We wish to acknowledge the financial support from Mr. Hank Brokaw for funds received to conduct the initial experiments in this project. Further research is needed to determine levels of sensitivity, ability to pool several samples so that only pools which screen as positive need to be tested on an individual basis, optimal sample collection methods, best and worst times of year for harvesting tissue, etc. Without funding from other sources we will not be able to conduct such experiments.

Figure 1. PCR analysis of avocado trees. Panel A: Agarose gel electrophoresis of PCR products. A positive result is scored by the presence of a 247 bp DNA fragment. Panel B: Dot spot hybridization of PCR products with an ASBVd-specific radioactive probe. Samples for both assays included previously biologically indexed healthy (He) and ASBVd positive trees (+) used as controls, five replications from single trees of Todd', 'Hass', 'Topa-Topa', and 'Fuerte' varieties previously biologically indexed as ASBVd positive, non-symptomatic carriers, and five replications from a 'Reed' avocado tree adjacent to the other four trees.

Figure 2. Agarose gel electrophoresis of PCR products from trees at South Coast Field Station. Samples are: DNA molecular weight marker (std), positive control (+), negative control (He), and samples designated as 1-12, each from a single grove tree. Note that sample 10 is scored as positive for ASBVd, and that tissue from this sample had yellow streaks on the leaves.



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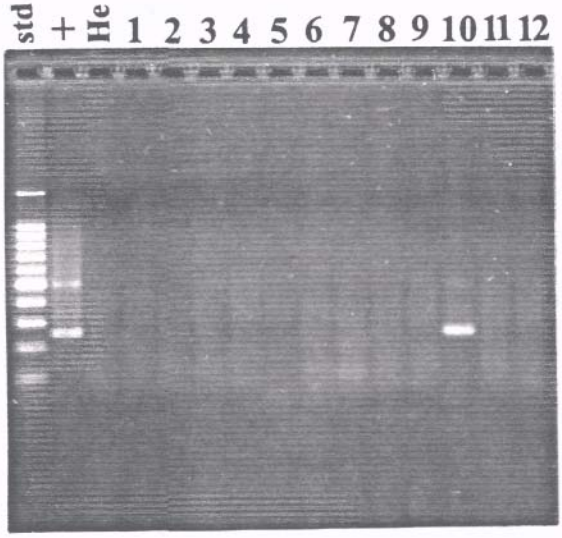

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