DNA-based Protocols for Detection and Quantification of Soilborne Pathogens Affecting Almond

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PROJECT SUMMARY

Objectives:

- Develop an affordable macro array for detection of soilborne pathogens.
- Develop and optimize specific quantitative polymerase chain reaction (qPCR) primers and hydrolysis probes for fungi positively and negatively associated with almond replant disease.
- Validate qPCR system for quantification of target fungi in soil and root samples from almond orchards.

Background:

Knowing whether soil borne pests are present and at what levels prior to replanting would help growers better assess when soil pest management is necessary, especially given the regulatory pressures on soil fumigants. Recent efforts to identify the microorganisms associated with Replant Disease (RD) (09-PATH1-Browne) lead to the search of soil testing tools.

Newer DNA-based systems allow organisms to be identified in soils based on their gene sequences rather than through culturing. This project has assessed various DNA-based testing techniques for replant disease microorganism identification.

Discussion:

A prototype macro array was constructed using genomic (i.e., total) DNA purified from various species of *Cylindrocarpon*, *Tricoderma*, *Colletotrichum*, *Verticillium*, *Phytophthora,* and *Pythium*. The array was evaluated for its specificity and sensitivity. While the array was able to detect low levels of organism DNA, it was not sensitive to assay accurately between pathogenic and non-pathogenic species. It was judged that a macro array format would be unlikely to afford the needed specificity and quantification capability.

An alternative approach, quantitative polymerase chain reaction (qPCR), for detection of target almond pathogens was assessed. qPCR can afford rapid, specific, and sensitive detection and quantification of pathogens, however, qPCR allows detection of only one or a few target organisms at a time. The specificity of qPCR reactions is achieved by use of sequence-specific PCR primers and the quantification is achieved by measuring the intensity of fluorescent dye binding to the DNA present. Each qPCR reaction requires testing, optimization, and validation before it is suitable for diagnostic applications.

Testing has started with qPCR primer pairs for *Tricoderma harzianum* (an organism associated with healthy trees in pre-plant fumigated plots in our replant trials) and *Cylindrocarpon* sp. and *Pythium helicoides* (organisms that were associated with RD-affected trees in non-fumigated plots). The testing revealed qPCR primer pairs that were specific to the target fungi, and experiments are in progress to optimize qPCR protocols using hydrolysis probes.

Project Cooperators and Personnel: Leigh Schmidt, USDA/ARS, Dept. of Plant Pathology, UC Davis; Ravi Bhat, Dept. of Plant Pathology, UC Davis.

For More Details, Visit

- 2009-10 Annual Report CD (09-PATH2-Browne/Kluepfel); or on the web (after January 2011) at AlmondBoard.com/ResearchReports
- Related Projects: 09-PATH1-Browne