Molecular Detection of Fungal and Oomycete Pathogens With a DNA Macroarray

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Background:

Macroarrays are a new tool for fast and accurate detection of plant diseases (1). A macroarray is a detection method consisting of a surface fixed with DNA probes that can hybridize with genomic targets when a perfect match is present. The targets are usually tagged with a radioactive or luminescent dye so that detection of the match can be visualized. The array used in this project consists of 141 species-specific oligonucleotide probes for 32 known and emerging fungal and oomycete pathogens of solanaceous crops. The objective of this project was to confirm the effectiveness of this detection method for diseased plant samples by using optimized parameters of hybridization developed in earlier research with pure pathogen materials. Diseased solanaceous crops (tomato, potato, eggplant, and pepper) collected in 2004-2006 were diagnosed with the DNA macroarray. Both greenhouse tomatoes inoculated with known pathogens and diseased field samples were used in this study.

Materials and Methods:

DNA was first extracted from diseased plant tissue (Fig. 1) with MoBio UltraClean™ Soil DNA Purification Kit (MoBio Laboratories Inc., Carlsbad, CA). The internal transcribed spacer (ITS) region of the ribosomal RNA gene was amplified with universal primers ITS4 and ITS5. The PCR products were first visualized using electrophoresis (Fig. 2A) and then purified using a QIAquick PCR Purification Kit (Quiagen, Madison, WI). The purified PCR products were sequenced at the Biotechnology Resource Center (BRC) at Cornell University (Fig. 2B) and also hybridized with the diagnostic macroarray. Oligonucleotide probes that hybridized with the targets were visualized by exposing Kodak BioMax Light film (Kodak, Rochester, NY) to the luminescent targets and developing the film (Fig. 3).

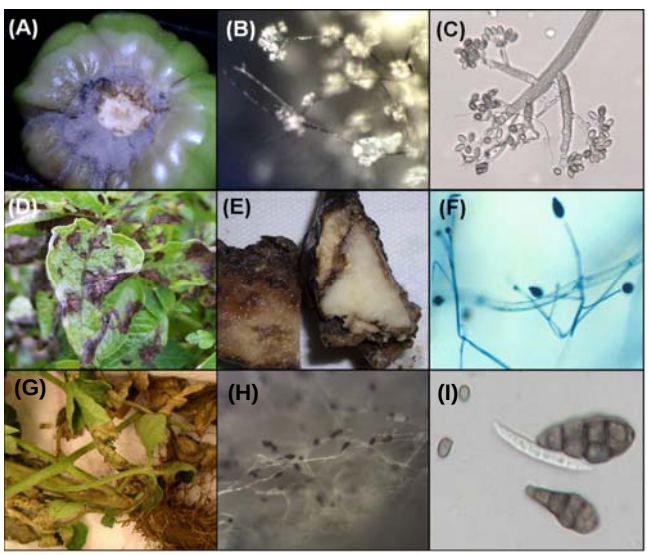

Fig. 1. A) Tomato fruit infected with *Botrytis cinerea.* B) *B. cinerea* pictured under a dissecting microscope (x12). C) *B. cinerea* pictured under a compound microscope (x160). D) Leaf infected with *Phytophthora infestans* E) Potato infected with *P. infestans* F) Sporangia and sporangiophores of *P. infestans* under a compound microscope G) Leaf wilting and vascular discoloration in a tomato H) *Alternaria alternata* from the tomato (G) pictured under a dissecting (x12) microscope I) *Cladosporium sp., A. alternata, and Fusarium sp.* spores obtained from the tomato plant (1G) and pictured under a compound microscope (160x).

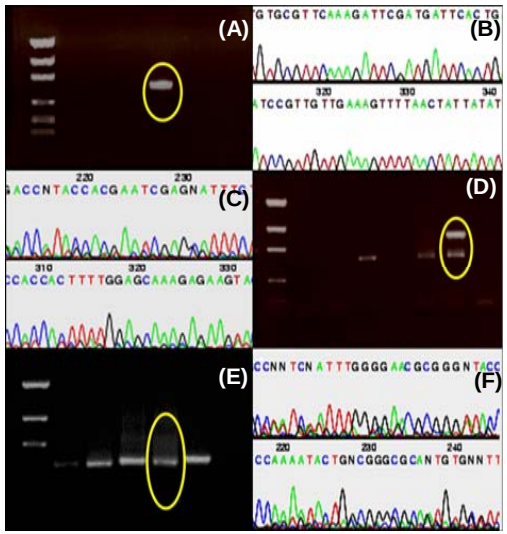

Fig. 2. A) PCR products amplified from extracted DNA from *Botrytis cinerea* (Fig. 1A) and visualized by gel electrophoresis. B) DNA sequence chromatogram of *B. cinerea* C)DNA sequence chromatogram of *Phytopthora infestans*. D) PCR product from *P. infestans* (Fig. 1D) E) PCR product of extracted DNA from diseased tomato plant (Fig. 1G) F) DNA sequence chromatograph of pathogens from the diseased tomato plant in (C).

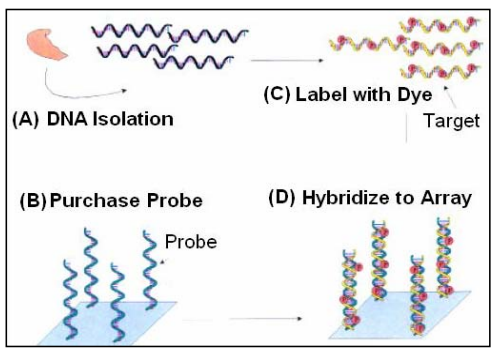

Fig. 3. The process of hybridizing a membrane for Macroarray detection. DNA is isolated from the sample; oligonucleotide probes designed for specific pathogens are fixed to the nylon membrane; the extracted DNA (target) is labeled with a luminescent dye; the labeled targets are hybridized to the probe. (2).

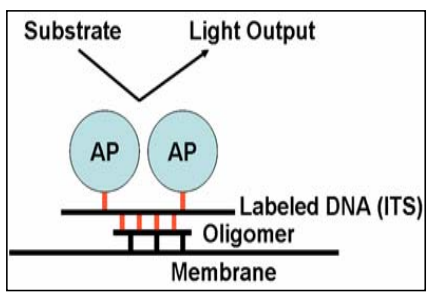

Fig. 4. The labeling and detection mechanism. Thermostable alkaline phosphatase (AP) is crosslinked to the nucleic acid (ITS PCR product). The AP enzyme can then be detected using the chemiluminescent substrate-dioxetane. The substrate emits visible light upon enzyme-catalyzed decomposition, which is visualized by exposing film.

Results:

To date, 12 different fungal pathogen species from 54 samples have been detected by the macroarray method: *Botrytis cinerea* (grey mold), *Pythium aphanidermatum* (root rot), *Fusarium oxysporum* (wilt, crown and root rot), *Colletotrichum acutatum* (anthracnose), *Colletotrichum coccodes, Phytophthora infestans* (late blight), *Fusarium solani* (root rot), *Septoria lycopersici* (leaf spot), *Sclerotinia sclerotiorum* (white rot), *Phoma destructiva* (Phoma rot), *Oidium neolycopersici* (powder mildew), *and Alternaria alternata* (early blight). The macroarray detection method was able to identify the infecting pathogen when DNA sequencing failed. In some cases, multiple pathogens were detected from the same sample while direct sequencing identified only one. DNA sequencing works best when DNA from a single source is present, as samples containing more than one pathogen often received poor or failed results from sequencing. Macroarrays, however, can detect multiple pathogens in a single assay. Our experiment results confirmed that the macroarray was able to detect pathogens in greenhouse and field-grown plants. They clearly showed the power of the diagnostic macroarray technique.

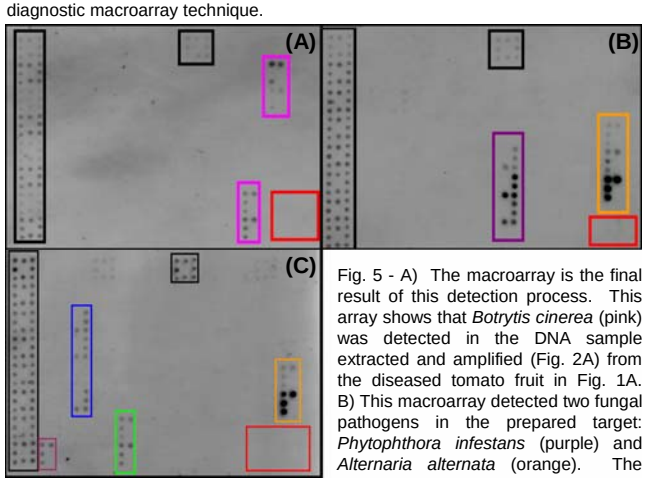

Fig. 5 - A) The macroarray is the final result of this detection process. This array shows that *Botrytis cinerea* (pink) was detected in the DNA sample extracted and amplified (Fig. 2A) from the diseased tomato fruit in Fig. 1A. B) This macroarray detected two fungal pathogens in the prepared target: *Phytophthora infestans* (purple) and *Alternaria alternata* (orange). The target came from DNA extracted from a diseased potato (Fig. 1D). C) This array shows four pathogens found in a tomato plant collected from Ontario County, NY in July 2006: *Fusarium solani* (purple), *Phoma destructiva* (blue), *Fusarium oxysporum* (green), and *Alternaria alternata* (orange). Positive controls are outlined in black; negative controls are outlined in red.

Future Work:

A microarray slide will be created, on which pathogen-specific oligonucleotide probes developed for fungal, viral and bacterial pathogens of solanaceous crops will be spotted. The goal is to enable the detection of all pathogens of solanaceous crops.

Acknowledgements:

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References:

1. Lievens, B., M. Brouwer, A. C. R. C. Vanachter, C. A. Lévesque, B. P. A. Cammue, and B. P. H. J. Thomma. 2003. Design and development of a DNA array for rapid detection and identification of multiple tomato vascular wilt pathogens. FEMS Microbiol. Lett. 223:113-122.

2. Freedman, Willard M., Daniel J. Robertson, and Kent E. Vrana. "Fundamentals of DNA Hybridization Arrays for Gene Expression Analysis." BioTechniques 29 (2000): 3. 22 June 2006 <http://photoscience.la.asu.edu/Photosyn/courses/MCB_576/Freeman_et_al_arrays.pdf>.