

Advances and Challenges in Developing Molecular Tools to Detect and Diagnose Potato Diseases

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The ability to rapidly and accurately identify pathogens that cause potato diseases can greatly assist growers' efforts to reduce losses due to disease and costs associated with disease management. Timely identification of pathogens responsible for causing diseases in potato is critical for ensuring that control measures, such as fungicide applications, are appropriately applied to target specific disease causing fungi. Symptoms such as wilting, stunting, and yellowing can be caused not only by pathogens but also by abiotic factors including water availability and quality, nutrient levels, wind, hail, and temperature. Accurate identification of pathogens can reduce costs associated with the erroneous application of chemical controls for symptoms that are the result of these adverse abiotic factors. Unfortunately, many pathogens responsible for diseases of potato cannot rapidly be identified using historically applied microbiological techniques because, in the case of many fungi, it may require weeks before the target organism can be unambiguously identified based on a combination of cell culture and microscopic examination. In addition, several important diseases of potato are caused by organisms that cannot at all be cultured in the absence of a plant host, including all plant viruses, *Spongospora subterranea*, which is a protozoan organism responsible for powdery scab disease, and phytoplasmas, which are responsible for a wide range of potato diseases including purple top, aster yellows and witches' broom. Newer technologies based on the detection of nucleic acid sequences (either RNA or DNA) that are unique to specific pathogens provide great promise for developing methods to accurately and rapidly detect pathogens responsible for a wide range of important potato diseases.

Nucleic Acid Based Detection Assays for Potato Pathogens – The most promising assays for identifying pathogens based on the detection of specific nucleic acid sequences employ the polymerase chain reaction (PCR). In short, PCR involves subjecting a heterogeneous DNA sample, which may consist of host plant DNA, insect DNA and DNA from target and non-target microorganisms, to multiple cycles of varying temperatures in the presence of small DNA sequences that are known to be present in the target pathogen. This results in the exponential amplification of DNA sequences that are diagnostic of a specific pathogen. There are several advantages to PCR assays for detecting plant pathogens as compared to historical methods requiring cell culture and microscopy. PCR assays are rapid, and most pathogens can be unambiguously identified in a single day. PCR assays can be designed so that they are very specific for a target pathogen, and do not require the presence of specialized reproductive spores such as conidia, ascospores, or oospores, which are typically required for correctly identifying specific fungi.

A further advance in PCR assays has been the development of real-time or 'quantitative' PCR technologies. Real-time PCR assays actually allow for the accurate quantification of pathogen levels in infected plants, insect vectors and soil samples. Real-time PCR assays have been developed previously for several pathogens that cause potato diseases, including potato mop top virus and *Rhizoctonia solani*. More recently, a

collaborative effort involving Dr. James Crosslin and Dr. George Vandemark, from the USDA-ARS, Prosser, WA, and Dr. Joseph Munyaneza, from the USDA-ARS, Wapato, WA resulted in the development of a real-time PCR assay for detecting the Columbia Basin Purple Top phytoplasma. This assay can detect and quantify the phytoplasma in infected potato plants, and can also routinely detect the pathogen in single leafhoppers (*Circulifer tenellus*). Besides having direct and immediate impact in allowing for the identification of phytoplasma in symptomatic plants in a single day, the ability to use the assay to detect the presence of the phytoplasma in its insect vector may provide growers with a more accurate method for forecasting disease as opposed to simply relying on leafhopper counts. This assay also can provide growers with information that will assist in the efficient timing of insecticide applications to control leafhoppers, as the mere presence of leafhoppers may not necessitate the application of insecticides if the real-time PCR assay indicates that the leafhoppers do not carry the phytoplasma. Dr. Vandemark has also collaborated with Mexican scientists to develop another real-time PCR assay for a different phytoplasma that is responsible for purple top disease of potatoes in Mexico. Dr. Vandemark has also recently developed a real-time PCR assay for *Spongospora subterranea*, the causal agent of powdery scab. This assay can rapidly detect the presence of the pathogen in symptomatic and asymptomatic plants of both potato and alternate hosts such as nightshade, and also can be used to detect the presence of the pathogen in soil samples. Currently Dr. Vandemark is attempting to develop collaborative effort with plant pathologists to develop real-time PCR assays for detecting and quantifying *Collectotrichum coccodes*, the causal agent of black dot disease, *Phytophthora erythroseptica*, which is responsible for pink rot disease, and *Pythium ultimum*, which causes tuber leak.

The development of real-time PCR assays that are specific for different isolates of a particular pathogen, as currently are available for phytoplasmas that cause the similar symptoms in potato, may be beneficial for addressing issues associated with meeting phytosanitary requirements for export markets. The availability of multiple real-time assays that can discriminate between isolates would provide exporters the opportunity to unambiguously prove the identity of pathogens detected in their products. This technology could provide evidence for opening new export markets or reducing restrictions on existing export markets by determining if pathogens or pests of phytosanitary concern are already endemic to targeted export markets.

Challenges to Applying PCR-Based Assays for Disease Forecasting - An especially promising application of PCR assays for managing potato diseases is their use as a tool in surveying fields for the presence of specific pathogen prior to planting. Knowledge of pathogen profiles in prospective fields can help growers to select potato cultivars that have resistance to diseases that are likely to be encountered in these fields. For example, a grower may choose not to grow Shepody, which is very susceptible to powdery scab, in a field that is determined to be highly infested with *Spongospora subterranea*. The accurate quantification of pathogen levels in fields prior to planting, especially for pathogens that cannot be cultured such as *Spongospora subterranean*, will require the ability to use real-time PCR assays to quantify pathogen levels from DNA extracted from multiple samples of field soil.

Although the development of a real-time PCR assay to target a specific pathogen is not at all a trivial exercise, as it involves considerable costs and technical expertise, perhaps a greater challenge is the ability to extract DNA from soil samples. Humic acids, which inhibit PCR reactions, typically are co-extracted with DNA from soil, and exhaustive efforts are typically required to remove humic acids from the DNA extract. In addition, most protocols for the extraction of DNA from soil are only suitable for extracting DNA from very small (less than 1 gram) quantities of soil. Commercial kits for extracting DNA from soil can alleviate the problem of co-extraction of inhibitors of PCR, such as humic acids, but the largest available commercial kit can only extract DNA from a 10 g sample of soil, and these kits cost \$15.00 per extraction. Clearly it is economically prohibitive to adequately sample a field for diseases using procedures that cost \$15.00 per 10 g sample of soil.

Dr. Vandemark, in collaboration with Dr. Hal Collins, of the USDA-ARS, Prosser, WA, has been experimenting with developing a more cost-effective method for extracting DNA from soil. Soil was collected from nine irrigated potato fields located in Benton and Walla Walla counties, with three fields being classified as Quincy loam sand and six as Ritzville silt loam, which were further segregated into two variants based on organic matter, clay, silt and sand contents. Multiple soil samples were collected from each field and DNA was extracted from 10 g samples using three different DNA extraction protocols: 1. A commercial DNA extraction kit (Mega-Prep, Bio 101, Inc.); 2. A protocol developed in Mexico for extracting DNA from forest soils (organic matter > 10%), and 3. A protocol developed by Dr. Vandemark. Both the commercial kit and the protocol developed at Prosser were superior to the protocol adapted for extraction of DNA from forest soil. The quality and quantity of DNA extracted using the protocol developed at Prosser was comparable to that realized using the commercial extraction kit. The cost per extraction using the Prosser protocol was approximately \$8-10. In addition, the protocol developed at Prosser allowed for the co-extraction of RNA along DNA from field soil samples. This facilitates the use of the extract for detecting plant viruses, which typically have genomes consisting entirely of RNA. Future research efforts will be devoted to further reducing costs per extraction and increasing throughput of the extraction procedure. Nonetheless, in order for PCR assays to be applicable for surveying of fields for the presence of soilborne pathogens, protocols need to be developed for extracting DNA from larger soil samples so that sufficient soil can be sampled to reflect the total distribution of pathogens in the entire field. Accordingly, Dr. Vandemark has begun to try to develop protocols for extracting DNA from large (> 100g) samples of soil. Recently Dr. Vandemark visited the laboratory of Dr. Nigel Crump, Department of Primary Industries, Victoria State, Australia, where he toured the facility and exchanged information pertaining to protocols developed at Prosser and in Australia for the efficient extraction of DNA from large soil samples.

In summary, considerable efforts have been made by scientists at the USDA-ARS, Prosser, WA to develop a wide range of PCR assays for detecting and quantifying pathogens responsible for serious diseases of potato in the Pacific Northwest. These assays have immediate application for the rapid identification of pathogens that cannot be readily identified using a combination of microbiology and microscopy, such as *Spongospora subterrannea* and phytoplasmas. In addition ongoing research is being conducted, and future research planned, to develop PCR assays for other economically

significant pathogens. Cost effective protocols for extracting DNA from soil samples larger than can be currently handled using available commercial kits are also being developed. These PCR assays and DNA extraction protocols, besides their immediate impact in disease diagnosis, should also in the future provide growers with critical information that will influence timing of chemical control measures, assist in the selection of cultivars with appropriate disease resistance profiles for specific field sites, and help satisfy phytosanitary concerns for export markets.

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