A classic yet new method for diagnosis of powdery scab disease

Kiwamu Tanaka* and Joseph B. DeShields
Department of Plant Pathology, Washington State University, Pullman, WA 99164-6430
*For correspondence: kiwamu.tanaka@wsu.edu

Abstract

Potato powdery scab disease has in recent decades insidiously spread in many regions where potatoes are grown, including most potato production areas in Washington state. It is important to forecast this disease during the pre-planting period. On-site diagnosis is important as timely decisions regarding early-stage treatment are often crucial, which can reduce secondary spread of disease in the field. Polymerase chain reaction (PCR) is currently the most sensitive and accurate method for plant pathogen detection, although standard lab-based PCRs and real-time PCRs require expensive laboratory equipment and skilled personnel. In this study, we propose a rapid and simple on-site real-time PCR method comprising of magnetic bead-based nucleic acid extraction, portable real-time PCR, and data analysis done remotely on a laptop computer. The capabilities of a portable real-time PCR were compared with a standard lab-based real-time PCR for the pathogen detection. In conclusion, the method developed in the present study enables a highly sensitive and rapid on-site detection of the powdery scab pathogen in the field.

Importance of on-site diagnosis

Accurate and rapid identification of causative pathogens significantly impacts decisions regarding plant disease management. Soilborne diseases are particularly difficult to diagnose because the soil environment is extremely large, in comparison to plant mass, and complex, making it a challenge to understand all the aspects of soilborne diseases. Moreover, soilborne diseases are sometimes symptomless during early infection stages, and some have long latent periods that result in delayed diagnoses. Many soilborne fungal pathogens have developed survival structures, such as spores or melanized hyphae, known to survive in the soil for many years even in the absence of their hosts. For example, in the case of the powdery scab disease, a causal pathogen *Spongospora subterranea* has infested a field, it remains infectious for many years (Calvert, 1968), that is, management practices, for example, crop rotations, have little impact on this disease. Effective

approaches for soilborne disease management include: (1) avoiding known infested fields; (2) using pathogen-free certified seeds and seedlings; and (3) keeping equipment sanitary. In addition, in-field diagnosis can be a further effective as means for timely decisions regarding early-stage treatments or pre-plant assessments of the fields.

Available methods for molecular detection of pathogens

As shown in Table 1, recent technological advances in the molecular identification of pathogenic agents have increased the efficacy, accuracy, and speed of diagnosis, which have contributed to the detection of pre-symptomatic infections (Boonham et al, 2014).

For on-site diagnosis based on molecular detection, detection power and efficiency are the crucial factors to take into consideration. The lateral-flow assay, e.g., AgriStrip from Bioreba, is the most popular method for on-site pathogen detection because of its simplicity as a one-step assay. However, it occasionally provides ambiguous results if the target pathogen is in low concentrations and can cross react with similar species or genera. Loop-mediated isothermal amplification (LAMP) is also applicable for on-site pathogen detection and is particularly inexpensive due to low-cost reagents, reaction conditions that remain constant, and simple colorimetric visual analysis. However, both of these assays are typically used qualitatively (Hill et al, 2008), becase the specificity is relatively low, which occasionally causes misdetection of off-target microbes such common soil inhabitants. For example, there can be cross reactivity between the serological tests of *Phytophthora* spp. and *Pythium* spp. in the case of potato pathogens (Mohan, 1989), indicating that there are sometimes difficulties detecting the targeted plant pathogens.

Polymerase chain reaction (PCR) offers high specificity, sensitivity, and has quantitative capability in comparison to the aforementioned methods of detection. However, the standard lab-based PCR technology requires expensive laboratory equipment and skilled personnel, which is a major disadvantage in adopting this technology as a detection method for on-site purposes.

Classic yet new methods for molecular detection

In the present study, we propose an on-site diagnostic method using a portable real-time PCR instrument (Genesig q16; Primerdesign Ltd). Real-time PCR technology offers advantages over other methods in terms of quantitative accuracy, sensitivity, and versatility. Because of the recent

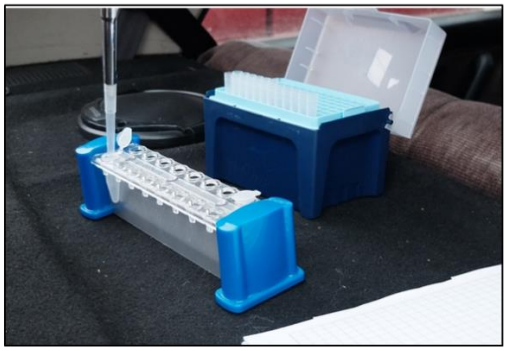
trends of the fast-growing, competitive market, equipment required for PCR technology has continued to develop into a more compact and less expensive (Tomlinson, 2005), while providing high quality data. The method is composed of steps in the following order: magnetic bead-based nucleic acid extraction, portable real-time PCR, and quantitative data analysis that can be done remotely using a laptop computer (Figure 1).

Using this portable real-time PCR method, a soil sample was analyzed to detect a soilborne pathogen, *Spongospora subterranea*, which causes powdery scab disease. The pathogen was chosen as a model in this study because the disease has insidiously spread to many regions where potatoes are grown, including most potato production areas in Washington State (Johnson and Miliczky, 1993). Therefore, it is important to diagnose risk of the disease for pre-plant assessment of fields. The results suggest that the portable PCR method is able to perform accurate and relatively sensitive detection (Table 1).

The on-site detection method we developed can allow the frontline workers in agriculture to make earlier decisions regarding disease management, such as variety selections or rotations, and can quantify a plant pathogen in the sample during a field survey, prior to planting, to avoid potential disease outbreaks.

1. Sample homogenization

2. Magnetic bead-based DNA extraction



3. Portable real-time PCR

4. Data analysis

Figure 1. On-site pathogen detection based on a portable real-time PCR system.

Future prospective

In the present study, we have developed an optimized protocol for on-site molecular detection of the powdery scab pathogen using the portable real-time PCR system by comparing its capabilities with that of a standard lab-based real-time PCR system. We found that the on-site method specifically detects the powdery scab pathogen in the soil sample, while the protocol developed can be easy-to-use for any users with no prior experience in molecular diagnostics.

Conventional methods for pathogen detections are often costly, laborious, inaccurate and time-consuming. The simplicity of the on-site method we developed (Figure 1) allows operators to perform pathogen detection earlier. The promptness and sensitivity of the method can help growers avoid potential secondary infections, further reproduction of the pathogens, and inadvertent spread, thereby preventing further distribution of the pathogens. A diagnosis pipeline including an on-site method might improve the current management of crop diseases that relies heavily on synthetic chemicals and soil fumigations, which are sometimes ineffective (for example, powdery scab disease). In conclusion, the on-site method developed in the present study (Figure 1) enables the accurate and relatively sensitive detection of important soilborne pathogens in the field. This method is applicable to the detection of other pathogens for which primer sequences designed for real-time PCR are available (DeShields et al., 2017).

Table 1. Common molecular methods for pathogen detection

	Portable real-timePCR	Standard lab-based real-time PCR	LAMP	ELISA	Lateral flow (AgriStrip)
Running cost	$0.60 - $8.47 (Genesig)	$0.60 (Bio-Rad)	~$0.75 (Quant-iT PicoGreen)	$0.60 (Bioreba)	$4.74 (Bioreba)
Sensitivity	~50 DNA copies	~10 DNA copies	~10 DNA copies	10 sporosori/mL for *S. subterranea*	10 sporosori/mL for *S. subterranea*
Time expense	90 min	60-240 min	50-90 min	3-24 hrs	10-15 min
Preparation required	• DNA/RNA extraction • Primer design	• DNA/RNA extraction • Primer design	• DNA/RNA extraction • Primer design • DNA staining and imaging (optional)	• Protein extraction	• Not required
Resources required	• Potable real-time thermal cycler	• Standard real-time thermal cycler	• Fluorescent DNA stain • Small incubator	• Washing equipment • Plate reader • Antibodies	• Not required

Acknowledgments

This research was supported by the Washington State Department of Agriculture - Specialty Crop Block Grant Program (grant no. K1764) and the Northwest Potato Research Consortium.

References

Boonham, N., Kreuze, J., et al. (2014) Methods in virus diagnostics: From ELISA to next generation sequencing. *Virus Research* 186: 20–31 doi:10.1016/j.virusres.2013.12.007

Calvert, E. L. (1968) The reaction of potato varieties to potato mop-top virus. *Record of Agricultural Research of the Ministry of Agriculture for Northern Ireland* 17: 31–40

DeShields, J. B., Bomberger, R. A., et al. (2017) On-site diagnosis of soil-borne phytopathogens using a potable real-time PCR detection. *Journal of Visualized Experiments* (in press)

Johnson, D. A., Miliczky, E. R. (1993) Distribution and development of black dot, *Verticillium* wilt, and powdery scab on Russet Burbank potatoes in Washington State. *Plant Disease* 77: 74–9

Hill, J., Beriwal, S., et al. (2008) Loop-Mediated Isothermal Amplification Assay for Rapid Detection of Common Strains of *Escherichia coli*. *Journal of Clinical Microbiology* 46: 2800–4 doi:10.1128/JCM.00152-08

Mohan, S. B. (1989) Cross-reactivity of antiserum raised against *Phytophthora fragariae* with other *Phytophthora* species and its evaluation as a genus-detecting antiserum. *Plant Pathology* 38: 352–63 doi:10.1111/j.1365-3059.1989.tb02154.x

Tomlinson, J. A., Boonham, N., et al. (2005) On-Site DNA Extraction and Real-Time PCR for Detection of *Phytophthora ramorum* in the Field. *Applied and Environmental Microbiology* 71: 6702–10 doi:10.1128/AEM.71.11.6702-6710.2005