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Loop-Mediated Isothermal Amplification for the Diagnostic Detection of *Meloidogyne chitwoodi*

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In the Pacific Northwest, the Columbia root-knot nematode (CRKN, *Meloidogyne chitwoodi*) is a plant parasitic nematode that causes significant problems on potatoes. The nematode can infect tubers and cause visual blemishes that significantly affect the market value of potatoes (OEPP/EPPO-Bulletin, 2016). In fact, if just 6% of the potato tubers in a field are infected with CRKN, the entire crop may be a total loss (Ingham et al., 2007). It is critical for a grower to know if CRKNs are present in their fields so that they may implement appropriate management strategies.

Current methods for CRKN identification

Nematodes are assessed by extracting nematodes from soil samples for morphological identification. One common technique for isolating nematodes is to mix the soil samples in water and run the water over a series of sieves, starting with course sieves and moving to finer sieves. Course sieves remove plant debris and large soil particles. The flow through, which contains the nematodes, is collected on a fine mesh sieve. After soil extraction, the collected nematodes are observed under a microscope so that they may be classified (e.g. plant parasitic or free-living nematodes). Nematode identification is typically based on morphological characteristics (de Oliveira et al., 2011; Seesao et al., 2017). One of the important features of plant parasitic nematodes is the presence of the stylet, a needle-like structure on the head of the nematode that is used for feeding. All plant parasitic nematodes possess a stylet. The absence of a stylet would suggest that the nematode is free-living and not a threat to crop plants. Further identification features, such as tail tip shape, can be used to distinguish CRKNs from other root-knot nematodes found in the PNW, including the Northern root-knot nematode *M. hapla* (Nyczepir et al., 1982). The identification of CRKN (and other nematodes) based on morphometrics requires the expertise of an experienced scientist who has been trained in nematode identification.

One possibility to simplify nematode identification is to use molecular identification techniques (Powers and Harris, 1993). For many molecular techniques, DNA is first obtained from the nematodes isolated from the soil samples. The DNA can then be used in identification techniques such as Polymerase Chain Reactions (PCR). During PCR, regions of DNA are specifically amplified, and the resulting DNA fragments are observed after agarose gel electrophoresis by using a specialized imaging system. By using species-specific primers, a diagnostician can perform PCR and determine the species of soil-extracted nematodes. One of the drawbacks to PCR-based diagnostics is that it requires relatively expensive thermocyclers and imaging systems.

LAMP: An easy molecular assay for CRKN identification

To simplify the diagnosis of CRKNs, the Gleason lab has developed a Loop-Mediated Isothermal Amplification (LAMP) assay that can identify these nematodes. Details for the LAMP assay are published in the journal Plant Disease (Zhang and Gleason, 2018).

LAMP assays are similar to PCR in that they rely on small pieces of DNA oligonucleotides, called primers, that bind and amplify specific regions of nematode DNA. However, unlike PCR, LAMP assays are very simple to perform (Figure 1). The nematode DNA, gene-specific primers (i.e. Oligos), and a special amplification enzyme (Bst 3.0 DNA polymerase) are added to a reaction tube and incubated at

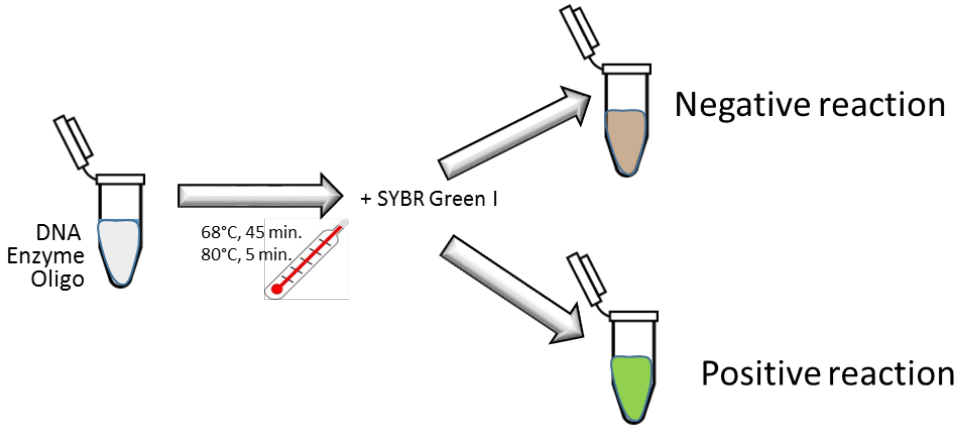

Figure 1. Schematic overview of LAMP assay

one temperature (68º C, 155º F) for 45 minutes. In other words, the reactions can be performed at a single temperature in a short amount of time, and as a result, it removes the need for specialized PCR machines. In addition, LAMP products can be easily visualized using DNA-binding dyes that visibly change color. This eliminates the need for expensive imaging systems (Tomita et al., 2008).

The Gleason lab designed LAMP primers to specifically amplify DNA from CRKNs. To prove that the assay works, we performed the LAMP assays on DNA isolated from CRKNs. When the reactions were completed, we added the DNA binding dye SYBR Green I to the tubes. The reactions positive for CRKNs turned bright green (Figure 2A). We also tested four other common root-knot nematode species (*M. incognita, M. javanica, M. hapla* and *M. arenaria*) in the LAMP assay with our specific CRKN primers. After the LAMP reaction, we added the SYBR Green I dye, but the reaction mixtures did not turn green, indicating negative reactions (Figure 2A). In our LAMP assay, we can determine that CRKNs are present if the reaction mixtures turn green.

There are two races of CRKN that are endemic to the PNW (Race 1 and Race 2). These races are nearly indistinguishable under the microscope, but they differ in their host range. Race 1 is more common than Race 2 in the PNW. In addition, there was a new pathotype of CRKN called Race 1 Roza. This pathotype is likely derived from a Race 1 progenitor, and it reproduces on the resistant, wild potato relative *Solanum bulbocastanum (*Mojtahedi et al 2007). We wanted to know if our CRKN LAMP assay could detect Race 1, Race 2, and Race 1 Roza. All three isolates gave positive LAMP results;

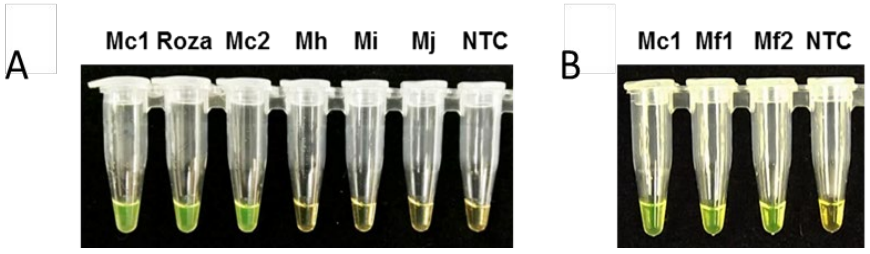

Figure 2. **A.** LAMP assay to detect CRKN,but not other common species of root-knot nematodes: (Mc1 = race 1), Roza (pathotype Roza), Mc2 (Race 2). Other root-knot nematodes: Mh (*M. hapla*), Mi (*M. incognita*), Mj (*M. javanica*), and NTC (no-template control). **B.** The LAMP assay cannot distinguish CRKN (Mc1) from its close relative *M. fallax* (Mf1, Mf2).

the reaction mixtures turned bright green. Thus, the LAMP assay is able to recognize all three CRKN races/pathotypes (Figure 2A).

To further test the specificity of the LAMP primers, we tested DNA from a root-knot nematode that is closely related to CRKN called *M. fallax* (Holterman et al., 2009; van Megen et al., 2009). We discovered that the LAMP assay gave positive results when *M. fallax* was present as the template. We cannot distinguish between CRKN and *M. fallax* because both give positive reaction in the LAMP assay with our CRKN primers (Figure 2B). However, we do not think that this should be problem for growers in the PNW because *M. fallax* is a nematode found in Europe and not present in fields in this region. Therefore, for PNW isolated nematodes, a positive LAMP assay with our CRKN primers would indicate the presence of CRKN.

Next, we compared the sensitivity of our LAMP assay with PCR in its ability to detect small numbers of CRKN (i.e. small concentrations of template DNA). The LAMP assay was 100 times more sensitive than PCR in detecting the CRKN.

The DNA used in both PCR and LAMP assays is typically isolated from nematodes that are extracted from soil samples by sieving and decanting. We wanted to see if we could simplify the LAMP assays by removing the nematode isolation step and use DNA isolated directed from soil as the reaction template. We artificially inoculated soil with CRKN and used the Qiagen DNeasy PowerSoil Kit to isolate DNA directly from the soil. Using this soil-extracted DNA as template, we could detect CRKN in the soil using our LAMP assay (Figure 3). We did not detect CRKN in DNA isolated from uninoculated soil samples.

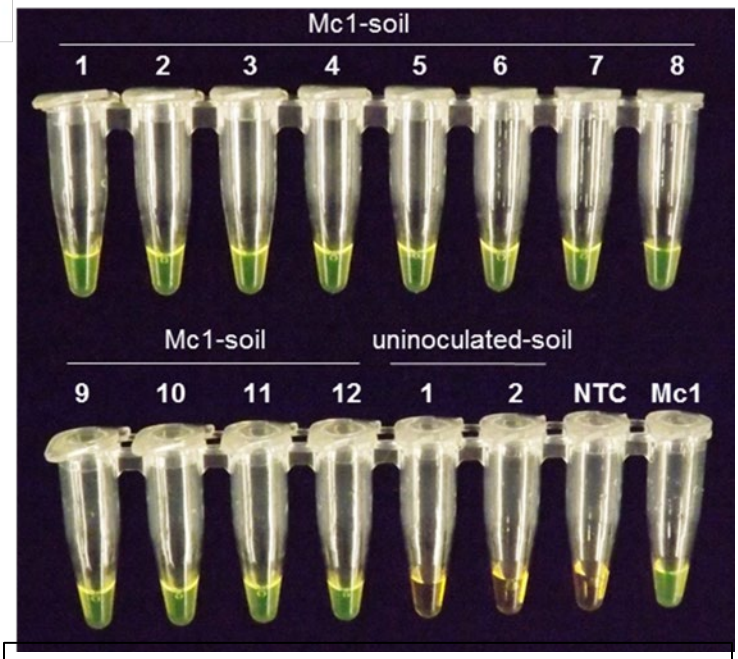

Figure 3. DNA was extracted directly from CRKN inoculated soil samples (Mc1-soil), or uninoculated-soil samples. SYBR Green I staining of LAMP products showed *M. chitwoodi* (CRKN) in all the 12 Mc1-soil samples. The LAMP reactions from two uninoculated-soil samples, No-template control (NTC) and Mc1 genomic DNA were used as controls.

Because of its capability of DNA amplification at isothermal conditions with high sensitivity and efficiency, the LAMP assay serves as a useful tool for CRKN identification. The LAMP assay presented here eliminates the need for nematode identification based on morphological features, which requires specialist expertise and training. Instead, diagnosticians and extension agents can perform this molecular assay on soil-extracted nematodes for a quick and easy test for the presence of CRKN. The LAMP assay is a plus-minus assay, which means that it will tell you if the nematode is present or not. At this stage, the assay is not quantitative. However, knowing that CRKN is present is useful because it can tell you if there is a CRKN problem. Considering that 1 juvenile nematode in 250 g of soil could result in significant population growth and serious crop losses if left uncontrolled (Elling, 2013; Ingham et al., 2000), determining the presence of CRKN is a significant step in the management process.

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Introduction for David Linnard Wheeler

Department of Plant Pathology at Washington State University

The new potato pathologist, Dr. David Linnard Wheeler, at Washington State University (WSU) officially started on 1/2/2020. David grew up in Lebanon, Pennsylvania as the youngest of seven children. From a young age, David was attracted to nature, science, art, and music. During college he studied Horticulture and Fine Arts in Philadelphia, Pennsylvania. During his undergrad, David became very interested in fungi, plants, and the interactions between these organisms. To quench his thirst to learn more, David pursued graduate school in the Pacific Northwest. Here, working with Dr. Dennis Johnson, David began to study early dying in potatoes and mint. Additionally, he pursued degrees in statistics and data science during this time.

After graduate school, David relocated to Bozeman, Montana, where, working with Montana State University, he studied disease epidemiology of row crops for about a year. While in Montana, David was fortunate enough to be offered the potato pathology position at WSU. He accepted the position and moved to Pullman, WA in late 2019. David looks forward to working with potato producers in the Pacific Northwest and teaching classes at WSU.

For fun, David enjoys spending time in the outdoors with his Newfoundland, Josh.