DIAGNOSTIC IDENTIFICATION OF *MELOIDOGYNE* SPECIES TO EXPEDITE PATHOGEN DETECTION IN ROW CROPS

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Abstract

Root-knot nematodes, or *Meloidogyne* spp., are one of the most significant plant parasitic nematodes found on cotton in the United States. This research focuses on the implementation of species identification of *Meloidogyne* based upon molecular techniques that are much quicker than current standards in the state of Alabama. The objectives of this project are to 1) implement a molecular identification protocol for species identification for the state of Alabama, 2) collect root-knot nematode samples from Auburn research stations and grower fields for species identification and species location mapping, and 3) implement microplot trials to track species interaction throughout a growing season. The methods included for this involve "smashing" individual nematodes for DNA, PCR (Polymerase Chain Reaction) for the amplification of the DNA, and gel electrophoresis to check PCR band sizes to identify specific species. These methods for extraction of nematodes from the soil and roots will be performed via gravity sieving and sucrose centrifugation methods. As this project has recently been initiated, the results are brief. A molecular technique has been implemented, and currently the only species to be identified is *Meloidogyne incognita*. As this project is very new, no recommendations can be made, but it seems that as expected, *M. incognita* is very prevalent in most cotton throughout Alabama.

Introduction

Root-knot nematodes (*Meloidogyne spp.*) cause significant losses of yield in almost all crop varieties grown in the southeastern United States. As one of the most commonly found plant parasitic nematodes throughout the world, it is estimated to account for approximately 14% of crop yield loss per year. This accounts for an estimated 100 billion dollars per year (American Phytopathological Society, 2015). In the state of Alabama alone, around 2.1% of total cotton yield was lost during the 2014 season, which comes out to around 5,000 bales total (Lawrence et al. 2014). Once a field has become infested with these nematodes, a quick management strategy must be implemented in order for the grower to maximize yield. This yield maximization must be financially practical as well. An effective management option that is financially viable for containment of root-knot nematode populations is through crop rotation. By knowing the specific species of *Meloidogyne* present in the field, a grower can easily implement a crop rotation that the species will not be able to grow on. For example, if a grower has a cotton field with an infestation identified as *Meloidogyne incognita*, then the grower can rotate to something such as peanut next season. Since peanut is not a viable host for *M. incognita*, this should help contain and potentially eliminate the infestation. Thus, it is important to have quick and efficient ways to identify root-knot nematode species in the field in order to determine how to manage the infestation.

A common way to identify *Meloidogyne* species is through a host differential test. This test involves inoculating one population of root-knot nematodes on multiple types of crops to determine which plants the nematodes infect. Based upon what plants end up with galling on the roots from nematode pressure, process of elimination allows for species identification (Barker et al. 1985). However, this test can take anywhere from 30 to 45 days in order to obtain the results. Thus, a more time efficient method is desired. Currently, molecular identification allows for identification of species at a rate of 3 to 4 days (Adam et al. 2006), however, this technique is only accurate 50% of the time. Thus, the DNA analysis is a much more efficient method than host differential tests, although we must improve the accuracy.

In this study, a molecular identification method using PCR (polymerase chain reaction) and gel electrophoresis with all known primer sets were attempted to identify various *Meloidogyne* species throughout the state of Alabama. The objective of this study is to implement an assay that can quickly and accurately identify root-knot nematodes for Auburn University and the rest of the state as well.

Materials and Methods

Preparation of second stage juveniles (J2s) for PCR amplification

Root-knot eggs were extracted from roots and soil of cotton plants from fields in Alabama, as well as from plants previously inoculated with root-knot nematodes planted in the greenhouse. Root-knot eggs were extracted by shaking the roots in a 10% NaOCl solution for 4 minutes, followed by the collection of eggs on a 25 µm sieve. Extracted eggs were then transferred to a modified Baermann funnel and incubated in warm water at 31° C for 3 to 5 days. This allowed for the eggs to hatch into juveniles. Individual juveniles were then picked out and placed into 20 µl of water, where they were smashed by a pipette tip and used as DNA template for PCR.

PCR amplification and gel electrophoresis of individual nematode samples

Once the sample had been smashed and prepared as the DNA template, it was added to a PCR mix containing 9 µl of DNA template per sample, 20µM of both forward and reverse primers, and 2x JumpStart REDTaq ReadyMix as described by Powers and Harris (1993). Various primer sets were screened to determine which sets are the most efficient and provided the highest percentage of successful amplifications. Both species-specific primers as well as primers that differentiate species were screened. The final decision on what primer sets to use for the diagnostic assay will be determined by how well they perform on a consistent basis. Based on current research, primer sets JB3 and JB5 were used as a confirmation run that showed if *Meloidogyne* DNA was present (Kiewnick, S. et. al. 2014) by amplifying the COI region of the genetic sequence. The second primer sets used were species-specific primers as described by Adam, Blok, and Phillips (2006). The collected samples will be run on PCR via a Labnet MultiGene PCR machine. At the end of the PCR run, gel electrophoresis was run on a 1% agarose gel to separate the amplified bands, allowing for proper identification of *Meloidogyne* species to be determined.

Results and Discussion

There is currently a 100% obtainable success rate for DNA amplification via PCR with the JB3 and JB5 primer sets using the previously described methods (Figure 1). All isolates successfully show amplified product. The last two lanes with no product in Figure 1 are negative controls, where water was used instead of DNA. Thus, no product is expected there. *M. incognita* primer sets have successfully allowed for amplification of approximately 50% at best when using *M. incognita* DNA, as can be seen in Figure 2. It is hoped that going forward, a higher success rate can be achieved, as well as the identification of other species besides *M. incognita.*

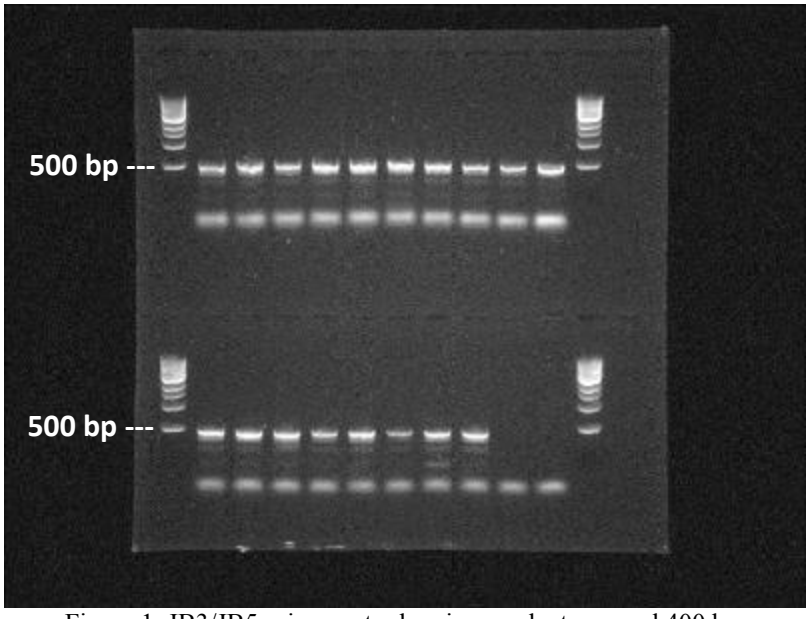

Figure 1. JB3/JB5 primer sets showing products around 400 bp.

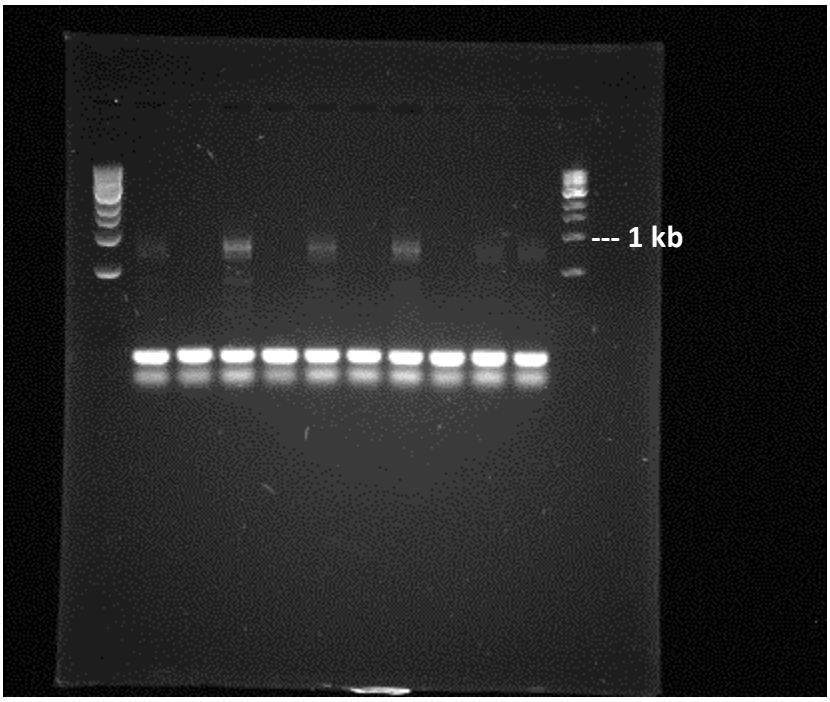

Figure 2. *M. incognita* primer set showing 900 bp fragment on some samples.

Summary

A successful assay is currently being used at Auburn University for the amplification of root-knot nematode DNA for species identification. This assay proves to be a quicker and more efficient method than previous techniques used by this research group. While it has successfully been implemented, there is much work still to be done. The success rate of species primers needs to be above 50%. A success rate as close to 100% as possible must be obtained. The next steps in this project include increasing the success rate as well as finding and identifying as many species of *Meloidogyne* in the state of Alabama as possible.

References

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