Development of a simple multiplex PCR protocol for identification of the tropical root-knot nematode species *Meloidogyne incognita, M. arenaria* and *M. javanica.*

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Abstract

More than 80 root-knot nematode species (*Meloidogyne* spp.) are known on a worldwide bases. The tropical species *Meloidogyne incognita, M. javanica* and *M. arenaria* are considered as the economically most important species with a extremely wide host range and high damage potential. As these species are morphologically very similar, fast and accurate PCR protocols are needed.

Next to identifying the quarantine species *M. chitwoodi, M. fallax*, and *M. enterolobii*, more than one PCR test was normally required for the tropical species. Therefore, a multiplex PCR protocol was developed for rapid identification these species. To identify *M. javanica* and *M. arenaria*, the available SCAR primers Mjav/Fjav and Far/Rar producing species specific products of 720bp and 420bp, respectively, were chosen. In order to develop a complementary primer for *M. incognita*, the 399bp product of the SCAR primer inc-K14-F/R was sequenced and the primers Mi2F4/Mi2R1 designed to produce a product of 300bp. This primer combination has produced reliable results in multiplex PCR assays with a number of different populations and no cross reaction was found with other *Meloidogyne* species. Furthermore, the amplified species specific products allow separation by high-resolution capillary electrophoresis and can be used in high-resolution-melting-curve analysis.

Introduction

Until recently, our lab routinely used the molecular diagnostic key by Adam et al. (2007) to identify *Meloidogyne* species. However, in some cases PCR amplification for *M. incognita* identification failed using the recommended MiF/MiR SCAR primers. As an alternative, SCAR primers inc-K14F/R proved more reliable for the correct identification of *M. incognita*). However, multiple PCR reactions (including a control for successful DNA extraction) are needed to identify a single tropical species.

Previous attempts to develop a multiplex PCR protocol failed because i) cross reaction of selected primers with other species and ii) unspecific amplicons appearing when new primers were designed from amplicon sequences of SCAR primers. Therefore, a re-evaluation of possible primer combinations were necessary for the development of a reliable and specific multiplex PCR assay.

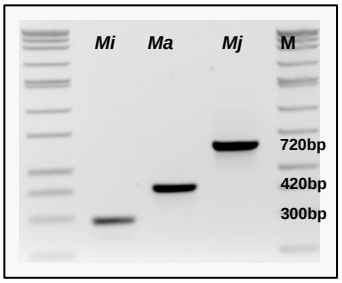

Figure 1. Example of multiplex PCR using primers Mi2F4/Mi2R1 (300bp), Far/Rar (420bp) and Fjv/Rjav (729bp) for identification of *M. incognita* (Mi), *M. arenaria* (a) and *M. javanica* (Mj). M = molecular marker

Material and methods

DNA was extracted from single second stage juveniles with a modified protocol by Kawasaki (1990). The amplicon for primer combination inc-K14F/R (399bp, Adam et al., 2007) was sequenced on an automated capillary sequencing robot (ABI3100). Primers Mi2F4/Mi2R1 were designed with an average annealing temperature of 55°C to 58°C using the program FastPCR (Vers. 6.0.188; Kalendar & Schulman, 2009)).

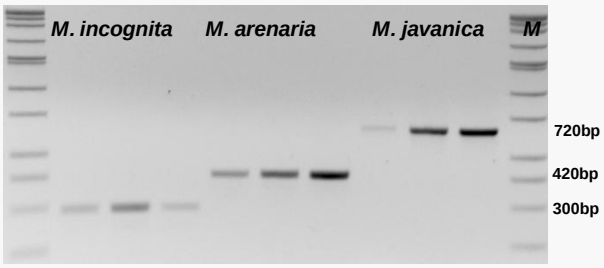

Figure 2. Example of multiplex PCR results for three different populations each of *M. incognita* (300bp; Germany, Taiwan, Switzerland), *M. arenaria* (420bp; Switzerland, Netherlands) and *M. javanica* (720bp; Netherlands, USA) on a 1.2% Agarose gel (70V, 1h.); M = molecular marker

Results

In contrast to previous attempts to develop a multiplex PCR protocol for the three tropical species, the tested combination of two SCAR primers and a new *M. incognita* primer proved to be reliable and specific for routine testing (Fig.1). In total 15 populations from 5 countries were evaluated. No cross reaction with DNA from other species (*M. hapla, M. fallax, M. chitwoodi, M. enterolobii, M. ethiopica*) was found. As a standard for *Meloidogyne* diagnostics, our lab uses single second stage juveniles as a source for DNA. Other sources (females, males) are also suitable for this assay. Mixtures of DNA from the three species were not yet evaluated. Varying DNA concentrations per reactions might lead to weaker bands especially after multiple cycles of freezing and thawing DNA (Fig. 2).

Conclusions

- The multiplex PCR assay provided reliable identification of *M. incognita, M. arenaria* and *M. javanica*
- The produced amplicons are easy to detect, independent from the gel-documentation system used
- The differences in amplicon length allow for additional uses of these primers such as high-resolution-melting-curve analysis

References

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