THE USE OF HETEROLOGOUS CLONED DNA PROBES TO DISTINGUISH BETWEEN RACES OF MELOIDOGYNE INCognITA

BY

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DNA from one population each of the four races of Meloidogyne incognita was restriction endonuclease digested and Southern blotted onto nitrocellulose filters. The filters were probed with a 7kb fragment consisting of the rDNA repeat of Caenorhabditis elegans (pCe7) and with a 3.1kb fragment, which contains part of the 18s ribosomal RNA gene from Schistosoma mansoni (pSM389). The results show that not only does rDNA from these species hybridise strongly with all four races of M. incognita, but the restriction fragment patterns produced allow the differentiation of two of the M. incognita races.

Keywords: Meloidogyne incognita, rDNA, polymorphism

The genus Meloidogyne comprises a widely distributed group of plant-parasitic nematodes usually known as root-knot nematodes. Although there are approximately 60 described species of root-knot nematodes, most attention has been focused on Meloidogyne incognita, M. javanica and M. arenaria which account for the majority of crop losses caused by the root-knot nematodes. An extensive survey of about 1300 Meloidogyne populations from over 70 countries representing the primary food production regions of the world found that at least one of these species occurred in 95% of the samples (Carter & Sasser, 1982).

Accurate and reliable identification of these nematodes is fundamental for the design of control strategies. The diagnosis of root-knot nematode species was originally based on morphological characteristics, host preferences (Eisenback et al., 1981) and biochemical criteria (Esbenshade & Triantaphyllou, 1985) but the development of nucleic acid technology has provided a new approach to plant nematode species identification. Characteristic restriction digest patterns of repetitive DNA have allowed the major Meloidogyne species to be distinguished (Currant et al., 1986). Recently, use of polymerase chain reaction (PCR) has resulted in a more rapid means of differentiating these species (Harris et al., 1990; Cenis 1993). Identification of a specific oligonucleotide

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probe has been useful for diagnosis of *M. incognita* (Chacón et al., 1991). However, the success of these techniques has been limited, so far, to differentiating between species rather than races. Race specific diagnosis can only be achieved using the time consuming host differential test (Sasser & Carter, 1985).

One approach to the problem of distinguishing between closely related parasite populations has been the use of cloned DNA probes to identify restriction fragment length polymorphisms (RFLPs). In principle, DNA that is free from coding restraint evolves rapidly and so introns, pseudo-genes and ribosomal gene spacers are good sources of sequence variation between species. Some of the most useful probes have been derived from repetitive DNA or multi-copy genes since these sequences are rapidly detected by Southern analysis. Ribosomal DNA probes, which have the combined advantages of high copy number and sequence variation, have been used successfully to discriminate human parasites such as *Echinococcus* and *Brugia* species (McManus & Simpson, 1985; Cameron et al., 1988; Rishi & McManus, 1989).

Here we describe the use of such probes that cross-hybridise with all nematode DNAs but readily distinguish two *M. incognita* races by RFLPs. The first probe is a 3.1kb fragment of the 18s rRNA gene of *Schistosoma mansoni* (Simpson et al., 1984) and the second is a 7kb fragment corresponding to a single rDNA repeat from the free-living soil nematode *Caenorhabditis elegans* (Files & Hirsh, 1981).

**MATERIALS AND METHODS**

*Nematode populations*

Populations of *Meloidogyne* were maintained at BBSRC Institute of Arable Crops Research, Rothamsted and had the following origins: *M. incognita* R1 (originally from North Carolina State University: NCSU) (NCSU#78), *M. incognita* R2 (NCSU#1135), *M. incognita* R3 (NCSU#285) and *M. incognita* R4 (NCSU#401). Eggs and second stage juveniles were collected and prepared for DNA extraction as detailed previously (Chacón et al., 1991).

*DNA extraction and endonuclease digestions*

DNA was prepared from 0.1-1 ml of packed nematodes essentially as described by Simpson et al. (1982), with the exception that the purification step by CsCl centrifugation was replaced by phenol extraction and ethanol precipitation (Sambrook et al., 1989).

Three μg of DNA from each nematode was digested overnight at 37°C with *Eco* RI and *Hind* III (Boehringer Mannheim) in the buffers suggested by the manufacturer. The reactions were stopped by heating for 10 min at 65°C. Double digestions with *Eco* RI and *Hind* III were performed simultaneously in the buffer recommended by the manufacturers.