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Genetic diversity of the root-knot nematode *Meloidogyne enterolobii* and development of a SCAR marker for this guava-damaging species

M. Tigano[a], K. de Siqueira[a], P. Castagnone-Sereno[b], K. Mulet[b], P. Queiroz[a], M. dos Santos[a], C. Teixeira[a], M. Almeida[a], J. Silva[a] and R. Carneiro[a]*

[a]Embrapa Recursos Genéticos e Biotecnologia, CP 02372, 70770-917 Brasília, DF, Brazil; and [b]INRA-UMR1301, UNSA, CNRS-UMR6243, Interactions Biotiques et Santé Végétale, 400 route des Chappes, BP167, 06903 Sophia Antipolis, France

The objectives of this work were to evaluate the genetic variability of *Meloidogyne enterolobii* by molecular markers, and develop species-specific molecular markers for application in detection. Sixteen *M. enterolobii* isolates from different geographical regions (Brazil and other countries) and hosts were used in this study. The identification and purification of the populations were carried out based on isoenzyme phenotype. The DNA amplification of the intergenic region (IGS) of the rDNA and of the region between the cytochrome oxidase subunit II (*COII*) and 16S rRNA genes (mtDNA) produced specific fragments of the expected size for this nematode, i.e. 780 and 705 bp, respectively. Intraspecific variability among the isolates was evaluated with three different neutral molecular markers: AFLP, ISSR and RAPD. The results showed a low level of diversity among the isolates tested, indicating that *M. enterolobii* is a genetically homogeneous root-knot nematode species. The RAPD method allowed the identification of a species-specific RAPD fragment for *M. enterolobii*. This fragment was cloned and sequenced, and from the sequence obtained, a set of primers was designed and tested. The amplification of a 520-bp-long fragment occurred only for the 16 isolates of *M. enterolobii* and not for the 10 other *Meloidogyne* species tested. In addition, positive detection was achieved in a single individual female, egg-mass and second stage juvenile of this nematode. This SCAR species-specific marker for *M. enterolobii* represents a new molecular tool to be used in the detection of this nematode from field samples and as a routine diagnostic test for quarantine devices.

Keywords: AFLP, diagnostics, ISSR, molecular identification, *Psidium guajava*, RAPD

Introduction

The root-knot nematode (RKN) *Meloidogyne enterolobii* (Yang & Eisenback, 1983) was described from a population sampled in China and isolated from a tree species (*Enterolobium contortisiliquum*). This nematode was also reported from other regions in China, but mainly isolated from guava (*Psidium guajava*) (Xu *et al*., 2004). More recently, it was suggested that *M. enterolobii* was a senior synonym of *Meloidogyne mayaguensis* (Xu *et al*., 2004; Randig *et al*., 2009; G. Karssen, Plant Protection Service, PO Box 9102, 6700 HC Wageningen, the Netherlands), a species originally described in Puerto Rico, isolated from aubergine (*Solanum melongena*) roots. The presence of this parasite has increasingly been detected in different geographical regions from a wide range of hosts, including crops carrying genes of resistance to the main *Meloidogyne* spp. (Fargette *et al*., 1996; Blok *et al*., 2002; Carneiro *et al*., 2006; Brito *et al*., 2007).

*E-mail: recar@cenargen.embrapa.br

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Recently, *M. enterolobii* was detected in two commercial greenhouses in Switzerland on tomato (*Solanum lycopersicum* cv. Maxifort) rootstock resistant to *Meloidogyne* spp. (Kiewnick *et al*., 2008). Considering the risk of introduction and dissemination of this pest in the European region, *M. enterolobii* was recently added to the EPPO Alert List (EPPO, 2008).

In Brazil, *M. enterolobii* was originally detected in guava orchards in 2001 in Pernambuco and Bahia states (Carneiro *et al*., 2001). Since then, this nematode has been a matter of grave concern in the country because it has been spreading rapidly, making the cultivation of guava unviable in heavily infested areas (Carneiro *et al*., 2007; Siqueira *et al*., 2009).

Morphological identification demands considerable skill and could be unreliable because of significant intraspecific morphological variation in *Meloidogyne* spp. Because of its morphological resemblance to *M. incognita* when considering only the perineal patterns (Carneiro *et al*., 2001; Brito *et al*., 2004), *M. enterolobii* might have been misidentified in a number of surveys. Isozyme phenotyping has been shown to be a valuable tool for precise identification of major *Meloidogyne* species

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(Esbenshade & Triantaphyllou, 1990; Carneiro *et al.*, 1996, 2000). In all recent surveys in Brazil, *M. enterolobii* has been identified using esterase profile (Carneiro *et al.*, 2001; Siqueira *et al.*, 2009). However, the limitation of this technique is that second-stage juveniles (J2) cannot be reliably diagnosed, which hinders its use in routine examination of soil samples that often contain only J2. Regarding the use of molecular tools, the analyses of a specific mitochondrial DNA region (Blok *et al.*, 1997, 2002; Brito *et al.*, 2004; Xu *et al.*, 2004; Tigano *et al.*, 2005; Jeyaprakash *et al.*, 2006) and the intergenic region (IGS) from the ribosomal DNA (Adam *et al.*, 2007) were demonstrated as efficient in differentiating *M. enterolobii* from the most common *Meloidogyne* species, based on the size of PCR-amplified fragments. However, these markers have not yet been validated on a certain number of representative nematode populations and they cannot be used in multiplex diagnostics.

Table 1 *Meloidogyne* spp. populations used in the study

Species	Code	Geographical origin[a]	Original host plant
M. enterolobii	Me1	Godet, Guadeloupe, FWI	Tomato
	Me2	Jobos, Isabella, Puerto Rico	Aubergine
	Me3	Martinique, FWI	Guava
	Me4	Qezaltenaco, Guatemala	Coffee
	Me5	Guanacaste, Costa Rica	Coffee
	Me6	Maloua, Congo	Potato
	Me7	Petrolina, PE, Brazil	Guava
	Me8	Limoeiro do Norte, CE, Brazil	Guava
	Me9	São João da Barra, RJ, Brazil	Guava
	Me10	Pedro Canário, ES, Brazil	Guava
	Me11	Reginópolis, SP, Brazil	Pepper
	Me12	Tupã, SP, Brazil	Guava
	Me13	Campos Novos Paulista, SP, Brazil	Pepper
	Me14	Pirajú, SP, Brazil	Pepper
	Me15	Santa Mariana, PR, Brazil	Guava
	Me16	Novo Horizonte do Sul, MS, Brazil	Guava
M. arenaria	Mar1	Florida, USA	Peanut
	Mar2	Martinique, FWI	Banana
M. ethiopica	Meth1	RS, Brazil	Kiwi
	Meth2	Casa Blanca, Chile	Grapevine
	Meth3	Curicó, Chile	Grapevine
M. exigua	Mex	MG, Brazil	Coffee
M. floridensis	Mflor	Florida, USA	Peach
M. hapla	Mhap	SP, Brazil	Strawberry
M. hispanica	Mhisp	PE, Brazil	Sugar cane
M. incognita	Minc1	SP, Brazil	Coffee
	Minc2	PR, Brazil	Cotton/Coffee
	Minc3	BA, Brazil	Pumpkin
M. inornata	Minor	SP, Brazil	Yakon
M. javanica	Mjav	PE, Brazil	Tomato
M. paranaensis	Mpar	PR, Brazil	Coffee
Meloidogyne sp.	Sp1	DF, Brazil	Cucumber
Meloidogyne sp.	Sp2	RS, Brazil	Lavender

[a]FWI: French West Indies; Brazilian states: BA, Bahia; CE: Ceará; DF: Distrito Federal; ES: Espírito Santo; MG: Minas Gerais; MS: Mato Grosso do Sul; PE: Pernambuco; PR: Paraná; RJ: Rio de Janeiro; RS: Rio Grande do Sul; SP: São Paulo.

Recently, a new species-specific satellite DNA family was identified for *M. enterolobii*, and shown to be a good molecular marker for use in the diagnostics of this nematode (Randig *et al.*, 2009).

Regarding the genetic diversity in *M. enterolobii*, RFLP and RAPD analyses showed a low variability among the few isolates used in these studies (Fargette *et al.*, 1996; Blok *et al.*, 1997). Other neutral molecular markers, such as AFLP and ISSR, have been used to analyse the genetic diversity of *Meloidogyne* species (Semblat *et al.*, 1998; Fargette *et al.*, 2005; Carneiro *et al.*, 2008).

In this study, the analyses of molecular markers [amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISRR) and random amplified polymorphic DNA (RAPD)] were applied to examine the genetic variation of *M. enterolobii* isolates obtained from different geographical regions. Furthermore, a species-specific DNA fragment obtained from the RAPD analyses allowed the development of a SCAR marker as a new tool for the diagnosis of this economically important pest.

Materials and methods

Identification of nematode populations and genomic DNA extraction

Sixteen populations of *M. enterolobii* originating from Brazil and other countries and 17 *Meloidogyne* spp. were identified and purified using isozyme analyses (Carneiro & Almeida, 2001) (Table 1). They were multiplied under greenhouse conditions on tomato cv. Santa Clara plants. The eggs were extracted from infested tomato roots according to McClure *et al.* (1973) and stored at −80°C until use. For each nematode population genomic DNA was purified from aliquots of 200–300 μL eggs by a phenol-chloroform method (Sambrook *et al.*, 1989).

IGS and mitochondrial DNA analysis

The primers C2F3 (5′-GGTCAATGTTCAGAAATTTGT GG-3′) and 1108 (5′-TACCTTTGACCAATCACGCT-3′) designed by Powers & Harris (1993) were used to amplify the mitochondrial DNA region between the cytochome oxidase subunit II (*COII*) and 16S rRNA genes (mtDNA). The IGS of the ribosomal DNA (rDNA) was amplified using the primers 194 (5′-TTAACTTGCCAGATCGG ACG-3′) and 195 (5′-TCTAATGAGCCGTACGC-3′), proposed by Blok *et al.* (1997) to differentiate *M. enterolobii* from other major tropical root-knot nematodes. Amplifications were performed using 25-μL volumes containing 12 ng DNA, 200 μM dNTPs (Pharmacia Biotech), 0·4 μM primers, 2 U *Taq* DNA polymerase (Phoneutria Biotecnologia & Serviços) and 1 × *Taq* DNA polymerase reaction buffer. The temperature profile for all reactions was 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 50°C

for 30 s and 72°C for 2 min, with a final extension at 72°C for 7 min.

Amplified fragment length polymorphism (AFLP) analysis

For each sample, approximately 1 μg genomic DNA was digested with *Eco*RI and ligated to *Eco*RI adaptors in a single 20-μL overnight reaction at 37°C (Suazo & Hall, 1999). The digestion-ligation reactions were diluted with TE buffer to a final volume of 200 μL and stored at −20°C. A series of 30 primers (19-mer) (Integrated DNA Technologies) were used, consisting of the *Eco*RI adapter sequence GACTGCGTACCAATTCAGT plus three 3′ selective nucleotides (AGT, ACT, AGC, ATT, GGC, CAG, TGG, CCT, ACC, TCG, ATA, AGG, AAT, AAC, GCC, CGA, GGG, CTC, CAT, TTA, TTG, TAC, GAC, GTG, CCG, TCT, GAG, TGC, CGT and CAC). Amplification conditions and the electrophoresis of the PCR products were performed using the protocols described by Suazo & Hall (1999).

Inter-simple sequence repeat (ISSR) analysis

The amplification reactions were performed in a 13-μL volume containing 9 ng genomic DNA, using the PCR conditions described by Carneiro *et al.* (2008). The following ISSR primers (Integrated DNA Technologies) were used: (GA)8C, $(CA)_8G$, $(CCA)_5$, $(CA)_8RT$, $(GT)_8$ YA, $(ATG)_6$, $(GACA)_4$, $(GTC)_6$, $(GTG)_6$, $(GAG)_4GC$, $(CTC)_4GC$, $(GAGA)_4GG$, $(GA)_8T$, $(AC)_8T$, $(AC)_8YC$, $VHV(TGT)_5$, $BDB(ACA)_5$ and $HVH(TG)_7$.

Random amplified polymorphic DNA (RAPD) analysis

The RAPD-PCR reactions were performed in a volume of 13 μL containing 9 ng genomic DNA, using the PCR conditions described by Carneiro *et al.* (2008). The following 35 random 10-mer oligonucleotide primers (Operon Technologies) were used in the analysis: A4, A5, A13, A14, A15, A17, A18, AB11, B1, B5, B6, B20, C7, C9, C16, C18, D5, D8, D13, E14, E18, E19, G2, G3, G4, G5, G6, G13, K7, K16, K19, K20, M20, N7 and R7.

Phylogenetic analyses

The genetic diversity and relationships of the 16 *M. enterolobii* populations listed in Table 1 were assessed by AFLP, ISSR and RAPD analyses as described above, using *M. exigua* and *M. incognita* (populations Mex and Minc1, respectively) as outgroup taxa. Amplified bands were scored as present or absent from the digitized photographs of the gels. Experiments were repeated at least once and only DNA fragments consistently present between repeated gels were recorded and considered as binary characters. For each of the three molecular markers, DNA fingerprints of the populations were converted into a binary matrix, and phylogenetic reconstruction was performed using the neighbour-joining (NJ) algorithm (Saitou & Nei, 1987) implemented in PAUP* version 4b10 (Swofford, 2002). The data were assumed to be unordered with no *a priori* weighting. For all analyses, 1000 bootstrap replicates were performed to test the node support of the generated trees. Moreover, as the AFLP, ISSR and RAPD markers amplified here could reasonably be considered as independent characters, the three datasets were combined to run a global NJ analysis, according to the total-evidence approach (Huelsenbeck *et al.*, 1996), using the same computer settings as in the three individual analyses.

Development of sequence-characterized amplified regions (SCAR) primers

RAPD analysis, using the same conditions as previously described, were used to compare *M. enterolobii* to other *Meloidogyne* species. RAPD bands present only in *M. enterolobii* were selected, excised from the agarose gel using a QIAquick gel extraction kit (QIAGEN®), and cloned into the pGEM®T Easy vector (Promega, Inc.), according to the manufacturers' instructions. For each fragment, sequencing of the inserts was done on two independent clones by Macrogen. From each sequence obtained, a pair of putative specific primers was designed and synthesised by Integrated DNA Technologies. Amplification using the SCAR primers were performed as described for the RAPD analysis, using the following conditions: 5 min at 94°C; 28 cycles of 30 s at 94°C, 30 s at 62°C and 1 min at 72°C; plus a final extension step of 8 min at 72°C. DNA from individual nematodes (female and J2 juvenile) was obtained using a simplification of the procedure of Castagnone *et al.* (2005), and a tenth of the resulting DNA extract was used as template for PCR amplification.

Results

Identification and genetic variability of populations

The 16 *M. enterolobii* isolates were identified using the esterase phenotype (VS1-S1) with two major bands (Rm 0·7, 0·9). Occasionally, these bands resolved in two minor bands (Rm: 0·75, 0·95). The malate dehydrogenase phenotype N1a (Rm 1·4) was also unique and detected in all populations of *M. enterolobii*. In this study, the identification of all the isolates was confirmed by the analyses of molecular markers. Genomic DNA from these isolates was successfully amplified for the IGS of the rDNA and the mtDNA region. The size of DNA band amplified from all *M. enterolobii* populations was 780 and 705 bp for IGS and mtDNA, respectively (data not shown).

The genetic variability of these 16 *M. enterolobii* isolates was assessed using AFLP, ISSR and RAPD molecular markers. The patterns obtained for each type of marker, after electrophoresis of the amplified products are illustrated in Fig. 1. Overall, a total of 1242 reproducible

Table 2 Characteristics of the molecular markers generated to evaluate the genetic diversity of *Meloidogyne enterolobii*

	AFLP	ISSR	RAPD
No. primers used	30	20	35
Amplification range (bp)	200–3000	300–2500	200–4500
No. reproducible amplified fragments	470	321	451
No. polymorphic fragments (%)	20 (4·3)	209 (65·1)	142 (31·5)

amplified fragments were generated, among which 371 (29·87%) were polymorphic (Table 2). Under the experimental conditions, the three types of markers revealed different levels of resolution (i.e. genetic diversity), with pairwise genetic distance among the 16 *M. enterolobii* isolates analysed ranging from 0 to 0·02553, from 0·0969 to 0·31776, and from 0·02217 to 0·14412 in the AFLP, ISSR and RAPD analyses, respectively.

All the reproducible amplified bands from the AFLP, ISSR and RAPD analyses were recorded to build binary matrices, from which phylogenetic analyses were performed. The NJ trees obtained for each of the three types of marker were similar, and showed that all the *M. enterolobii* isolates belonged to one single cluster (with 100% bootstrap support), without any supported subspecific clustering of the isolates related to either their original host or original geographic region (data not shown). After combining data together into one single analysis, the same tree topology was observed (Fig. 2), leading to the conclusion that the resulting tree provided an accurate estimate of the relationships of the isolates studied.

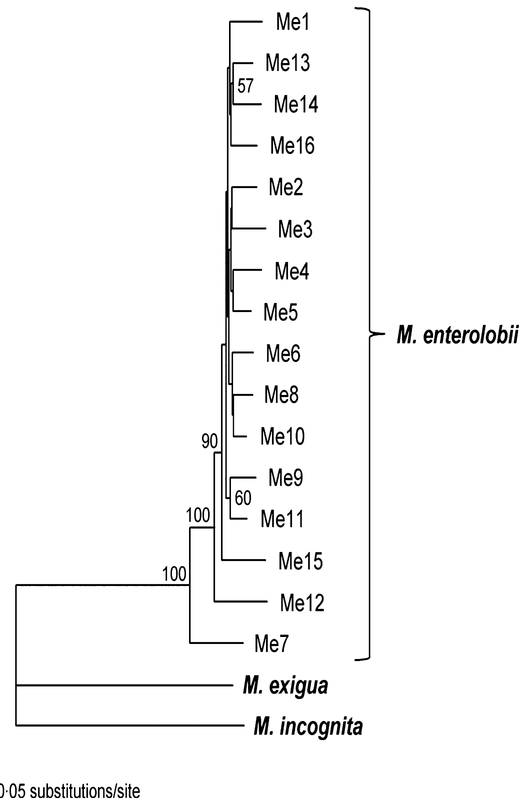

Figure 2 Neighbour-joining tree showing the relationships of *Meloidogyne enterolobii* isolates, using a concatenation of AFLP, ISSR and RAPD datasets. Bootstrap values (>50%) based on 1000 replicates are given.

Development of a SCAR marker specific for *M. enterolobii*

From the analysis of the RAPD fingerprints of the *Meloidogyne* spp. isolates tested, some of the random primers allowed the amplification of fragments specific for *M. enterolobii*. Among them, primer K7 produced an amplified band of approximately 600 bp in the pattern of the *M. enterolobii* isolates, whilst no band of the same size was observed for the isolates from the other species tested (Fig. 3). After cloning and sequencing of this DNA fragment, a pair of putative SCAR primers, defined as MK7-F (5′-GATCAGAGGCGGGCGCATTGCGA-3′) and MK7-R (5′-CGAACTCGCTCGAACTCGAC-3′),

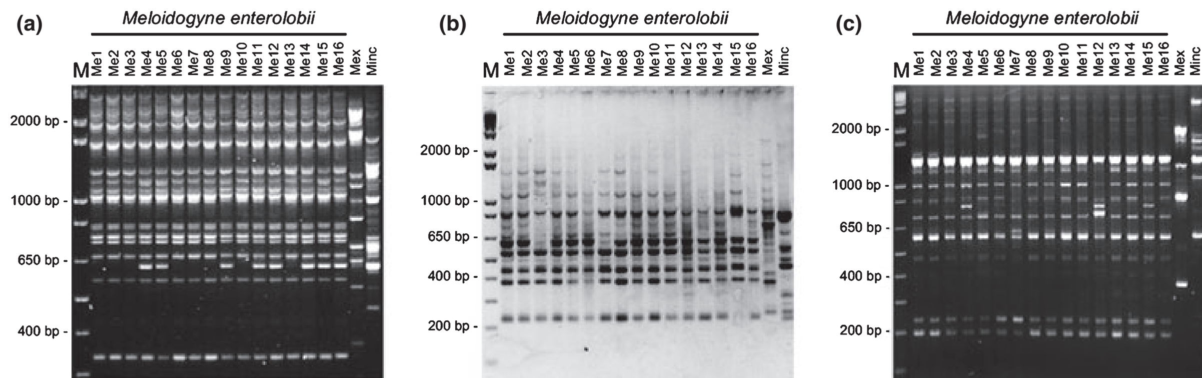

Figure 1 Examples of the genetic variability among *Meloidogyne enterolobii* isolates detected by the analyses of: (a) AFLP13 (5′-GACTGCG TACCAATTCAGTAATT- 3′), (b) ISSR 01 (5′-GAGAGAGAGAGAGAG-3′) and (c) RAPD OPR7 (5′-ACTGGCCTGA-3′). M = 1-kb Plus DNA Ladder. For population codes, see Table 1.

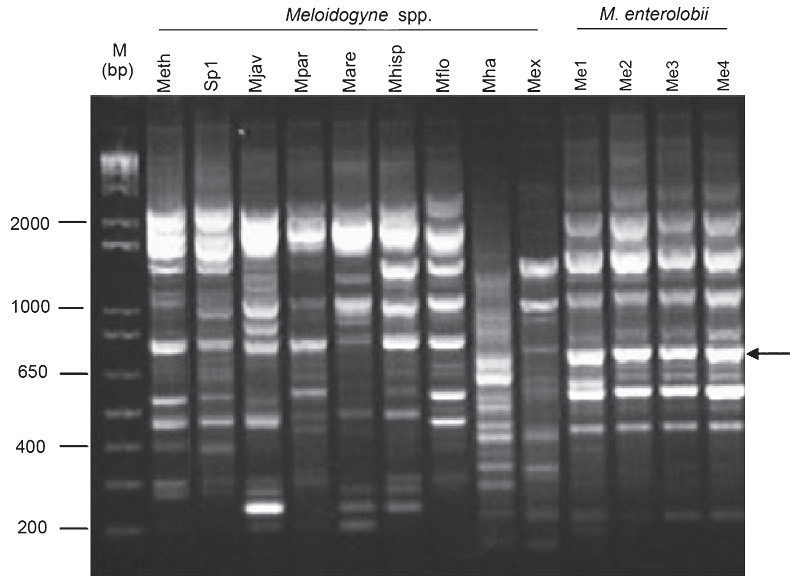

Figure 3 RAPD patterns for 13 *Meloidogyne* spp. isolates generated with the primer K7. Arrowhead indicates the specific band for *Meloidogyne enterolobii* isolates. M = 1-kb Plus DNA Ladder. For population codes, see Table 1.

was designed. Using these primers, a fragment of 520 bp was amplified for *M. enterolobii*. Primer reliability was assessed using the 16 distinct isolates of *M. enterolobii*, and the 12 other *Meloidogyne* species listed in Table 1. The expected 520-bp DNA fragment was amplified for all the *M. enterolobii* isolates, whilst no amplification was observed in the other nematode species tested (Fig. 4). With the primer set defined above, the same specific amplified fragment was obtained with DNA from a single individual female (Fig. 5), egg-mass and J2 (Fig. 6) of *M. enterolobii*, demonstrating the sensitivity of the detection.

Discussion

The specific identification of the populations used in this study was made based on their typical isozyme phenotypes. All populations of *M. enterolobii* presented the same phenotypes for esterase (VS1-S1) and malate dehydrogenase (N1a). These phenotypes were reported first for *M. enterolobii* from China (Esbenshade & Triantaphyllou, 1985) and later for *M. mayaguensis* from West Africa (Fargette, 1987; Fargette *et al.*, 1996), Martinique (Carneiro *et al.*, 2000), Brazil (Carneiro *et al.*, 2001; Siqueira *et al.*, 2009), USA (Brito *et al.*, 2004) and Venezuela (Molinari *et al.*, 2005). This identification was confirmed using two molecular markers which were previously reported to easily separate *M. enterolobii* from other common RKN species by the size of the PCR products. The IGS product amplified here for all the populations agrees with the size reported for this nematode (Blok *et al.*, 1997; Adam *et al.*, 2007). This marker was recently used in a proposed molecular diagnostic key to separate *M. enterolobii* from other common and economically important species of RKN (Adam *et al.*, 2007). During the first step of this RKN molecular diagnostic key, the size of IGS amplification immediately allows the clear separation of *M. enterolobii* from the other

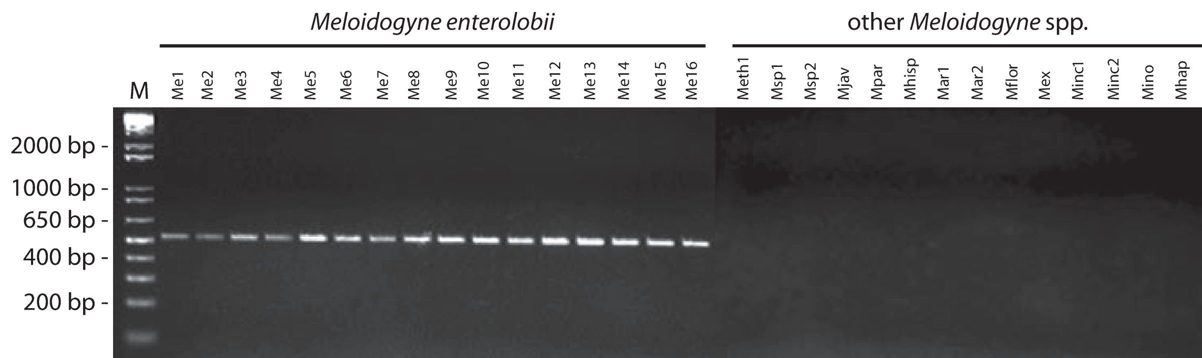

Figure 4 Specific amplification using the SCAR primers MK7F/R in *Meloidogyne enterolobii* populations (genomic DNA extracted from eggs). M = 1-kb Plus DNA Ladder. For population codes, see Table 1.

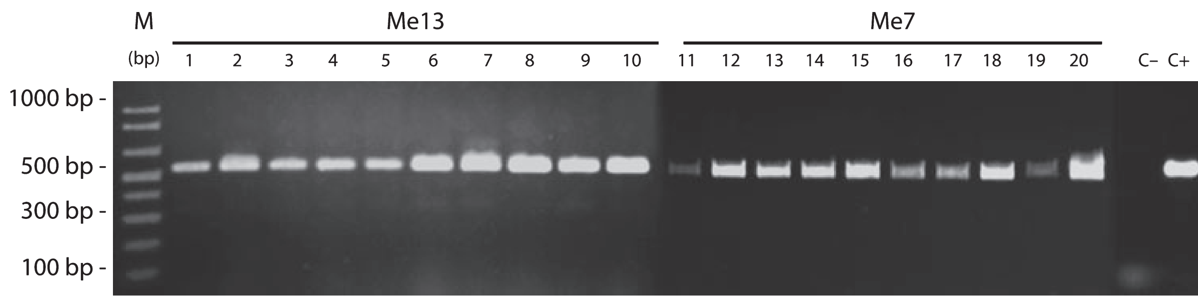

Figure 5 PCR amplification from single individual females of two populations of *Meloidogyne enterolobii*, using the SCAR primers MK7F/R. C+ and C− represent positive (genomic DNA) and negative (water) controls, respectively. M = 1-kb Plus DNA Ladder. For population codes, see Table 1.

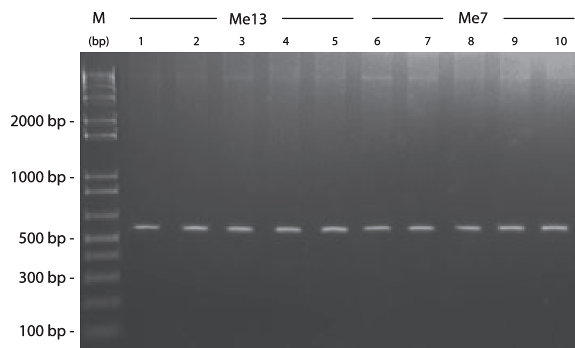

Figure 6 PCR amplification from single individual second stage juveniles of two populations of *Meloidogyne enterolobii*, using the SCAR primers MK7F/R. M = 1-kb Plus DNA Ladder. For population codes, see Table 1.

Meloidogyne species. For the mtDNA analyses, the same fragment was also amplified for all *M. enterolobii* populations, confirming the size of the PCR product already reported for this nematode (Blok *et al.*, 2002; Brito *et al.*, 2004; Tigano *et al.*, 2005; Jeyaprakash *et al.*, 2006). The present data validated the use of these markers as a molecular identification tool, considering that the previous results were obtained for a small number of populations, whilst the present work used a large collection of populations from Brazil and other tropical regions, and from various host plants.

SCAR markers have been developed to discriminate the three major RKN species occurring in neotropical and temperate areas or in crop protected systems, i.e. *M. incognita*, *M. javanica* and *M. arenaria* (Zijlstra, 2000; Zijlstra *et al.*, 2000; Fourie *et al.*, 2001). Specific SCAR markers were also defined to identify the three main RKN species parasitizing coffee crops in Brazil, i.e. *M. exigua*, *M. incognita* and *M. paranaensis* (Randig *et al.*, 2002), and their use of multiplex procedures enabled the specific identification of *Meloidogyne* spp. in a field survey of coffee plantations in Brazil (Carneiro *et al.*, 2005). The SCAR marker MK7F/R developed in this study proved to be species-specific for *M. enterolobii* when compared with several other common *Meloidogyne* species, and was validated on 16 *M. enterolobii* populations from different geographical regions and hosts. Moreover, it allowed implementation of a PCR method that is very easily interpreted and used for *M. enterolobii* routine diagnostics, because this molecular marker produces a positive or negative signal, with no amplification at all with other species. Another marker of this kind, based on satellite DNA sequences, was also recently developed for *M. enterolobii* (Randig *et al.*, 2009). The method proved to be sensitive enough to detect a single nematode, whatever its developmental stage. Moreover, since a tenth of the extract from a single nematode was enough for detection, the rest of the sample could eventually be used for additional PCR reactions if necessary to confirm results. Globally, the results enlarge the range of RKN species that can be unambiguously identified using the SCAR approach, and should allow the implementation of a simple and very sensitive method for the detection of *M. enterolobii* from field samples, as well as a routine diagnostic test for quarantine purposes. Since *M. enterolobii* has been spreading rapidly in guava crops in Brazil as a result of the planting of infected seedlings (Carneiro *et al.*, 2007; Siqueira *et al.*, 2009), an easy way to detect this species on guava seedlings (females and egg-masses), in organic manure or soil (J2) would be of vital use in restricting the sale of contaminated seedlings and preventing the spread of this RKN into different areas in the country (Carneiro *et al.*, 2007). Although *M. enterolobii* has not yet been recognised as a quarantine organism by the Brazilian Ministry of Agriculture (MAPA), it was recently included in the EPPO Alert List in Europe (EPPO, 2008).

This study also reports an assessment of the genetic diversity of *M. enterolobii*. The neutral molecular markers used here, i.e. AFLP, ISSR and RAPD, allowed the evaluation of polymorphism among *M. enterolobii* populations from Brazil and other countries. Although AFLP analyses have already been used to evaluate the genetic diversity of some *Meloidogyne* spp. populations, this study reports the first use among nematodes of a simplified AFLP protocol. This protocol has already been applied to the study of genetic variability in honey bees

and fungi (Suazo & Hall, 1999; Boucias *et al.*, 2000; Tigano *et al.*, 2006). However, the level of polymorphism detected here with AFLP was lower than that with the ISSR and RAPD markers, which was quite unexpected compared to previous studies (Semblat *et al.*, 1998; Fargette *et al.*, 2005). This is probably because of the lower resolution of the agarose gels used in the simplified protocol compared to that of the polyacrylamide gels in the original methodology. Globally, the overall variability revealed by the three markers indicated a high level of homogeneity among the isolates tested, confirming that *M. enterolobii* is a genetically homogenous RKN species, although it displays a very wide host range and worldwide distribution. Such a relative lack of genetic diversity had already been reported for a limited number of populations based on RFLP (Fargette *et al.*, 1996) and RAPD studies (Blok *et al.*, 1997). Obviously, this feature could be linked in part to the obligatory mitotic parthenogenetic mode of reproduction of this species (Yang & Eisenback, 1983), where the egg develops directly into an embryo without any fertilization/recombination event. Concerning the particular case of the *M. enterolobii* isolates sampled in Brazil (10 of the 16 analysed in the present study), a complementary hypothesis may be raised to further explain the low polymorphism detected among them. Indeed, since the nematode was only recently detected in the country (Carneiro *et al.*, 2001), it may be assumed that there has not been enough time since the first introduction to allow some intraspecific diversification of the populations. Regarding crop management, the low polymorphism detected here in *M. enterolobii* populations implies that similar methods of control may be implemented against this pathogen, such as plant resistance, crop-rotation or biological control, independent of the region of application.

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References

Adam MAM, Phillips MS, Blok VC, 2007. Molecular diagnostic key for identification of single juveniles of seven common and economically important species of root-knot nematode (*Meloidogyne* spp.). *Plant Pathology* **56**, 190–7.

Blok VC, Phillips MS, McNicol JW, Fargette M, 1997. Comparison of sequences from the ribosomal DNA intergenic region of *Meloidogyne mayaguensis* and other major tropical root-knot nematodes. *Journal of Nematology* **29**, 16–22.

Blok VC, Wishart J, Fargette M, Berthier K, Phillips MS, 2002. Mitochondrial DNA differences distinguishing *Meloidogyne mayaguensis* from the major species of tropical root-knot nematodes. *Nematology* **4**, 773–81.

Boucias D, Stokes C, Suazo A, Funderburk J, 2000. AFLP analysis of the entomopathogen *Nomuraea rileyi*. *Mycologia* **92**, 638–48.

Brito JA, Powers TO, Mullin PG, Inserra RN, Dickson DW, 2004. Morphological and molecular characterization of *Meloidogyne mayaguensis* isolates from Florida. *Journal of Nematology* **36**, 232–40.

Brito JA, Stanley JD, Mendes ML, Cetintas R, Dickson DW, 2007. Host status of selected cultivars plants to *Meloidogyne mayaguensis* from Florida. *Journal of Nematology* **36**, 232–40.

Carneiro RMDG, Almeida MRAA, 2001. Técnica de eletroforese usada no estudo de enzimas dos nematóides de galhas para identificação de espécies. *Nematologia Brasileira* **25**, 555–60.

Carneiro RMDG, Almeida MRA, Carneiro RG, 1996. Enzyme phenotypes of Brazilian isolates of *Meloidogyne* spp. *Fundamental and Applied Nematology* **19**, 555–60.

Carneiro RMDG, Almeida MRA, Quénéhervé P, 2000. Enzyme phenotype of *Meloidogyne* spp. populations. *Nematology* **2**, 645–54.

Carneiro RMDG, Moreira WA, Almeida MRA, Gomes ACMM, 2001. Primeiro registro de *Meloidogyne mayaguensis* em goiabeira no Brasil. *Nematologia Brasileira* **25**, 223–8.

Carneiro RMDG, Randig O, Almeida MRA, Gonçalves W, 2005. Identificação e caracterização de espécies de *Meloidogyne* em cafeeiro nos Estados de São Paulo e Minas Gerais através dos fenótipos de esterase e SCAR-Multiplex-PCR. *Nematologia Brasileira* **29**, 233–41.

Carneiro RMDG, Almeida MRA, Braga RS, Almeida CA, Gioria R, 2006. Primeiro registro de *Meloidogyne mayaguensis* parasitando plantas de tomate e pimentão resistentes à meloidoginose no estado de São Paulo. *Nematologia Brasileira* **30**, 81–6.

Carneiro RMDG, Cirotto PA, Quintanilha AP, Silva DB, Carneiro RG, 2007. Resistance to *Meloidogyne mayaguensis* in *Psidium* spp. accessions and their grafting compatibility with *P. guajava* cv. Paluma. *Fitopatologia Brasileira* **32**, 281–4.

Carneiro RMDG, dos Santos MFA, Almeida MRA, Mota FC, Gomes ACMM, Tigano MS, 2008. Diversity of *Meloidogyne arenaria* using morphological, cytological and molecular approaches. *Nematology* **10**, 819–34.

Castagnone C, Abad P, Castagnone-Sereno P, 2005. Satellite DNA-based species-specific identification of single individuals of the pinewood nematode *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae). *European Journal of Plant Pathology* **112**, 191–3.

EPPO, 2008. An emerging root-knot nematode, *Meloidogyne enterolobii*: addition to the EPPO Alert List. EPPO Reporting Service, 2008/105.

Esbenshade PR, Triantaphyllou AC, 1985. Use of enzyme phenotypes for identification of *Meloidogyne* species. *Journal of Nematology* **17**, 6–20.

Esbenshade PR, Triantaphyllou AC, 1990. Isozyme phenotypes for the identification of *Meloidogyne* species. *Journal of Nematology* **22**, 10–5.

Fargette M, 1987. Use of the esterase phenotype in the taxonomy of the genus *Meloidogyne*. 2. Esterase phenotype observed in

West African populations and their characterization. *Revue de Nématologie* **10**, 45–56.

Fargette M, Phillips MS, Blok V, Waugh R, Trudgill DL, 1996. An RFLP study of relationships between species, isolates and resistance-breaking lines of tropical species of *Meloidogyne*. *Fundamental and Applied Nematology* **19**, 193–200.

Fargette M, Lollier V, Phillips M, Blok V, Frutos R, 2005. AFLP analysis of the genetic diversity of *Meloidogyne chitwoodi* and *M. fallax*, major agricultural pests. *Comptes Rendus Biologies* **328**, 455–62.

Fourie H, Zijlstra C, McDonald AH, 2001. Identification of root-knot nematode species occurring in South Africa using the SCAR-PCR technique. *Nematology* **3**, 675–80.

Huelsenbeck JP, Bull JJ, Cunningham CW, 1996. Combining data in phylogenetic analysis. *Trends in Ecology and Evolution* **11**, 152–8.

Jeyaprakash A, Tigano MS, Brito J, Carneiro RMDG, Dickson DW, 2006. Differentiation of *Meloidogyne floridensis* from *M. arenaria* using high-fidelity PCR amplified mitochondrial AT-rich sequences. *Nematropica* **36**, 1–12.

Kiewnick S, Karssen G, Brito JA, Oggenfuss M, Frey JE, 2008. First report of root-knot nematode *Meloidogyne enterolobii* on tomato and cucumber. *Plant Disease* **92**, 1370.

McClure M, Kruk TH, Migashi I, 1973. A method for obtaining quantities of clear *Meloidogyne* eggs. *Journal of Nematology* **5**, 230.

Molinari S, Lamberti F, Crozzoli R, Sharma SB, Sanchez-Portales L, 2005. Isozyme patterns of exotic *Meloidogyne* spp. populations. *Nematologia Mediterranea* **33**, 61–5.

Powers TO, Harris TS, 1993. A polymerase chain reaction method for identification of five major Meloidogyne species. *Journal of Nematology* **25**, 1–6.

Randig O, Bongiovanni M, Carneiro RMDG, Castagnone-Sereno P, 2002. Genetic diversity of root-knot nematodes from Brazil and development of SCAR markers specific for the coffee-damaging species. *Genome* **45**, 862–70.

Randig O, Deau F, Santos MFA, Tigano MS, Carneiro RMDG, Catagnone-Sereno P, 2009. A novel species-specific satellite DNA family in the invasive root-knot nematode *Meloidogyne mayaguensis* and its potential use for diagnostics. *European Journal of Plant Pathology* **125**, 485–95.

Saitou N, Nei M, 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4**, 406–25.

Sambrook J, Fritsch EF, Maniatis T, 1989. *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY, USA: Cold Spring Harbor Laboratory Press.

Semblat JP, Wajnberg E, Dalmasso A, Abad P, Castagnone-Sereno P, 1998. High-resolution DNA fingerprinting of parthenogenetic root-knot nematodes using AFLP analysis. *Molecular Ecology* **7**, 119–25.

Siqueira KMS, Freitas VM, Almeida MRA *et al.*, 2009. Detecção de *Meloidogyne mayaguensis* em goiabeira e mamoeiro no estado de Goiás, usando marcadores moleculares. *Tropical Plant Pathology* **34**, 256–60.

Suazo A, Hall HG, 1999. Modification of the AFLP protocol applied to honey bee (*Apis mellifera* L.) DNA. *BioTechniques* **26**, 704–9.

Swofford DL, 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods)*, version 4. Sunderland, MA, USA: Sinauer Associates.

Tigano MS, Carneiro RMDG, Jeyaprakash A, Dickson DW, Adams BJ, 2005. Phylogeny of *Meloidogyne* spp. based on 18S rDNA and the intergenic region of mitochondrial DNA sequences. *Nematology* **7**, 851–62.

Tigano MS, Adams B, Maimala S, Boucias D, 2006. Genetic diversity of *Hirsutella thompsonii* isolates from Thailand based on AFLP analysis and partial *β-tubulin* gene sequences. *Genetics and Molecular Biology* **29**, 715–21.

Xu J, Liu P, Meng Q, Long H, 2004. Characterization of *Meloidogyne* species from China using isozyme, phenotypes and amplified mitochondrial DNA restriction fragment length polymorphism. *European Journal of Plant Pathology* **110**, 309–15.

Yang B, Eisenback JD, 1983. *Meloidogyne enterolobii* n. sp. (Meloidogynidae), a root-knot nematode parasitizing pacara earpod tree in China. *Journal of Nematology* **15**, 381–91.

Zijlstra C, 2000. Identification of *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* based on SCAR–PCR: a powerful way of enabling reliable identification of populations or individuals that share common traits. *European Journal of Plant Pathology* **106**, 283–90.

Zijlstra C, Donkers-Venne DTHM, Fargette M, 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified regions (SCAR) based PCR assays. *Nematology* **2**, 847–53.