Characterisation of *Meloidogyne* species on Southern Herbs in Hainan island using perineal pattern and esterase phenotype and amplified mitochondrial DNA restriction fragment length polymorphism analysis

Mei-ying Fu, Mian-cai Chen, Tong-bin Xiao and Hui-fang Wang

[1]**Institute of Environment & Plant Protection, Hainan Academy of Agricultural Sciences, Hainan Key Laboratory for Control of Plant Diseases and Insect Pests , Haikou, 571100, China; e-mail: mcchen@263.net.**

Accepted for publication 12 October 2011

Summary. Various kinds of southern herbs have been planted in Hainan island because of the suitable climatic conditions. However, root-knot nematodes have become a serious problem to cultivation of southern herbs. Thirty *Meloidogyne* populations on southern herbs from Hainan island were characterised in terms of perineal pattern and esterase phenotype, and *Hinf*I and *Eco*RI was used to double digest the amplified products from the mitochondrial DNA (mtDNA) region between the *COII* and *lrRNA* genes. Perineal patterning and esterase phenotyping revealed that 17 of the populations were *M. incognita*, five were *M. javanica*, three were *M. arenaria*, four were *M. enterolobii* and one was *M. hispanica*. In order for rapid and reliable identification of *Meloidogyne* species, we used the primers C2F3 and 1108 to amplify the intergenic region between *COII* and *lrRNA* genes of mtDNA, and *Hinf*I and *Eco*RI were used to double digest the amplified products. The results showed that five *Meloidogyne* species can be distinguished. It is concluded that mtDNA-PCR-RFLP was a rapid and reliable approach for molecular identification of common *Meloidogyne* species.

Key words: Hainan**,** nematode identification, mtDNA-PCR-RFLP, root-knot nematodes, southern herbs.

Southern herbs are cultivated in Southeast Asia and Africa as medicinal material. Various kinds of southern herbs have been planted in Hainan island because of the suitable climatic conditions. According to several surveys, there were more than 30 kinds of southern herbs planted in Hainan island. For example, pepper, betel nut, morinda citrifolia, cinnamon, sandalwood, clove, *Amomum villosum* and others have been planted there on a large-scale (Yan, 2001). According to the statistics, the harvested area of betel nut were 20,733.3 ha, with the total output of 6.43 million metric tons and output value of 1.54 billion yuan in 2005. However, the damage caused by root-knot nematodes (*Meloidogyne* spp.) has a serious impact on yield and quality of southern herbs.

Root-knot nematodes are economically important plant pathogens, and more than 90 nominal species have been described. These root endoparasites are widely distributed from the tropical south to the temperate north, infecting many crop species and causing crop losses up to 70% in China (Xu *et al*., 1994). *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, *M. chitwoodi*, *M. fallax* and *M. hapla* accounted for more than 95% of the occurrences of this genus and are the most widespread species. In China, *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* cause serious economic losses, and the former three are distributed mainly in tropical regions, while the latter occur in cool temperate regions.

Effective management of root-knot nematodes often requires rapid and accurate species identification of target nematode populations. *Meloidogyne* species are traditionally differentiated from each other by morphological characters and isozyme phenotypes. Morphological identification demands considerable skill and could be unreliable due to significant intraspecific morphological variations in *Meloidogyne* spp. Isozyme

phenotyping has been shown to be a valuable means for precise identification of major *Meloidogyne* species (Esbenshade & Triantaphyllou, 1985a, b; Carneiro *et al*., 2000). In particular, esterase and malate dehydrogenase are frequently used. However, isozyme analysis is only performed with single females, not single second-stage juveniles (J2), males or eggs (Esbenshade & Triantaphyllou, 1990). Since the female stage is often unavailable in soil samples, the isozyme method requires the time and space to establish and maintain populations in culture from single egg masses in order to obtain females. Based on the polymerase chain reaction (PCR), developing methods to identify *Meloidogyne* species using individual single juvenile has been the objective of various studies. Several molecular methods which detect DNA polymorphisms between species have been used with DNA extracted from J2; for example, five major species of *Meloidogyne* were discriminated by amplification and restriction of the intergenic region between the *COII* and *lrRNA* genes in the mitochondrial genome (Powers & Harris, 1993). *Hinf*I was used to digest the amplification products from the mtDNA region between the *COII* and *lrRNA* genes, which enabled identification of five *Meloidogyne* species from China (Xu *et al*., 2004). Specific SCAR primers were designed to identify *M. incognita*, *M. javanica* and *M. arenaria*, which enabled identification of these species from single J2, and three PCR reactions were obtained from extracts of single J2 of these species (Meng *et al*., 2004).

The aim of this study was to research perineal patterns and esterase (Est) phenotypes and mtDNA-PCR-RFLP patterns of 30 *Meloidogyne* populations collected from Hainan of China. Our objectives were to determine the species identity of the populations and assess the utility of mtDNA polymorphism for reliable diagnosis of major root-knot nematodes occurring in Hainan.

MATERIAL AND METHODS

Nematode populations. Thirty *Meloidogyne* populations used in this study were listed in Table 1. All the populations were derived from single egg-masses from field populations and three single egg-masses were hand-picked from one field population. The original field populations were randomly collected from infected southern herbs in Hainan Island of China. Nematodes were routinely maintained on tomato at 20-28℃ in a glasshouse.

Perineal pattern slice. Perineal pattern temporary slides were made, using the method described by Taylor and Netscher (1974) with minor modifications. The infected roots were placed in 0.9% sodium chloride solution and the females were dissected out from the root under the dissecting microscope. The females were transferred to 45% lactic acid in a plastic Petri dish and the posterior end cut off with a very small scalpel. The perineal patterns were trimmed and transferred to water on a microscope slide, and a cover slip was applied. Perineal patterns are formed by expansion and alteration of the juvenile body and retain the lateral lines, the tail tip, and the phasmids.

Esterase analysis. Est were analysed according to the procedure of Esbenshade and Triantaphyllou (1985a) with minor modifications. The separating and stacking gels were homogeneous 7% and 4% polyacrylamide, respectively. Protein extracts from 20 females were loaded into the same lane. The bridge buffer was Tris/glycine pH 8.3. After electrophoresis, the gels were stained for Est for 20 min. Designation of Est phenotypes followed the scheme of Esbenshade and Triantaphyllou (1985b).

DNA preparation. Nematode egg-masses were hand-picked from infected tomato roots and J2 were hatched in water at 25°C Total genomic DNA was then extracted from *ca* 10 µl of packed J2 using the method of Cenis (1993). The quality and quantity of extracted DNA samples were checked by subjecting them to electrophoresis and Gold View staining together with uncut λDNA standards. For PCR amplification from single J2, individual J2 were hand-picked and ruptured with a steel needle in a 6 µl drop of lysis buffer (1×*ExTaq* PCR buffer 100 µg ml^{-1} proteinase K; TaKaRa Biotech, Dalian, China) on a glass slide. Nematode lysates were then transferred to PCR tubes and frozen for 30 min at -80°C. After freezing, samples were incubated for 1 h at 60°C and 15 min at 95°C before being processed for PCR amplification.

PCR-RFLP procedure. Primers C2F3 (5'-GGTCAATGTTCAGAAATTTGTGG-3') and 1108 (5'-TACCTTTGACCAATCACGCT-3') (Powers & Harris, 1993) were used to amplify the intergenic region between the *COII* and *lrRNA* genes in the mitochondrial genome of *Meloidogyne*. PCR was conducted in a 25 µl reaction volume containing10 ng of purified DNA or 5 µl of crude J2 lysate, 0.2 µM of each primer, 1× *Ex Taq* PCR buffer, 2 mM $Mgcl_2$, 0.2 mM of each dNTP and 1 U *Ex Taq* DNA Polymerase (Sangon, Shanghai, China). For amplification using purified DNA, reaction mixtures were subjected to a preheating at 94°C for 4 min, followed by 35 cycles of 1 min at 94°C, 1 min at 52°C and 2 min at 70°C, and a final incubation at 72°C for 5 min using a Mastercycler Personal thermal cycles. For amplification using crude J2 lysates, the number of cycles was raised to 40.

Table1. *Meloidogyne* populations used in this study

Species	Popula-tions	Origin	Primary host	Est	mtDNA-PCR-RFLP	
					PCR product (bp)	*Hinf*I and *Eco*RI digestion (bp)
M. incognita	MIWN1	Wanning, Hainan	*Morinda citrifolia*	I2	1700	1200, 400, 100
	MIWN2	Wanning, Hainan	*Morinda citrifolia*	I2	1700	1200, 400, 100
	MIDZ1	Danzhou, Hainan	*Morinda citrifolia*	I2	1700	1200, 400, 100
	MIHK1	Haikou, Hainan	*Morinda citrifolia*	I2	1700	1200, 400, 100
	MIHK2	Haikou, Hainan	*Morinda citrifolia*	I2	1700	1200, 400, 100
	MIDA	Dingan, Hainan	*Morinda citrifolia*	I2	1700	1200, 400, 100
	MIWN3	Wanning, Hainan	*Morinda citrifolia*	I2	1700	1200, 400, 100
	MITC	Tunchang, Hainan	Fructus alpiniae oxyphyllae	I2	1700	1200, 400, 100
	MIWN4	Wanning, Hainan	Fructus alpiniae oxyphyllae	I2	1700	1200, 400, 100
	MILS	Lingshui, Hainan	Fructus alpiniae oxyphyllae	I2	1700	1200, 400, 100
	MIWN5	Wanning, Hainan	Fructus alpiniae oxyphyllae	I2	1700	1200, 400, 100
	MIHK3	Haikou, Hainan	*Catharanthus roseus*	I2	1700	1200, 400, 100
	MIWC1	Wenchang, Hainan	*Catharanthus roseus*	I2	1700	1200, 400, 100
	MIWC2	Wenchang, Hainan	*Catharanthus roseus*	I2	1700	1200, 400, 100
	MIWN6	Wanning, Hainan	Alpinia officinarum	I2	1700	1200, 400, 100
	MIHK4	Haikou, Hainan	Betel nut	I2	1700	1200, 400, 100
	MIDZ2	Danzhou, Hainan	Clove	I2	1700	1200, 400, 100
M. javanica	MJWC	Wenchang, Hainan	*Catharanthus roseus*	J3	1700	1200, 500, 400, 100
	MJTC	Tunchang, Hainan	*Aquilaria*	J3	1700	1200, 500, 400, 100
	MJHK	Haikou, Hainan	*Catharanthus roseus*	J3	1700	1200, 500, 400, 100
	MJDA1	Dingan, Hainan	*Morinda citrifolia*	J3	1700	1200, 500, 400, 100
	MJDA2	Dingan, Hainan	*Morinda citrifolia*	J3	1700	1200, 500, 400, 100
M. arenaria	MAWN1	Wanning, Hainan	*Morinda citrifolia*	A2	1100	1100
	MADZ	Danzhou, Hainan	*Morinda citrifolia*	A2	1100	1100
	MAWN2	Wanning, Hainan	*Morinda citrifolia*	A2	1100	1100
M. hispanica	MHDZ	Danzhou, Hainan	*Morinda citrifolia*	S2-M1	1700	1200, 500
M. enterolobii	MEWC	Wenchang, Hainan	*Aquilaria*	VS1-S1	700	700
	MEHK1	Haikou, Hainan	*Aquilaria*	VS1-S1	700	700
	MEHK2	Haikou, Hainan	*Morinda citrifolia*	VS1-S1	700	700
	MEWN	Wanning, Hainan	Clove	VS1-S1	700	700

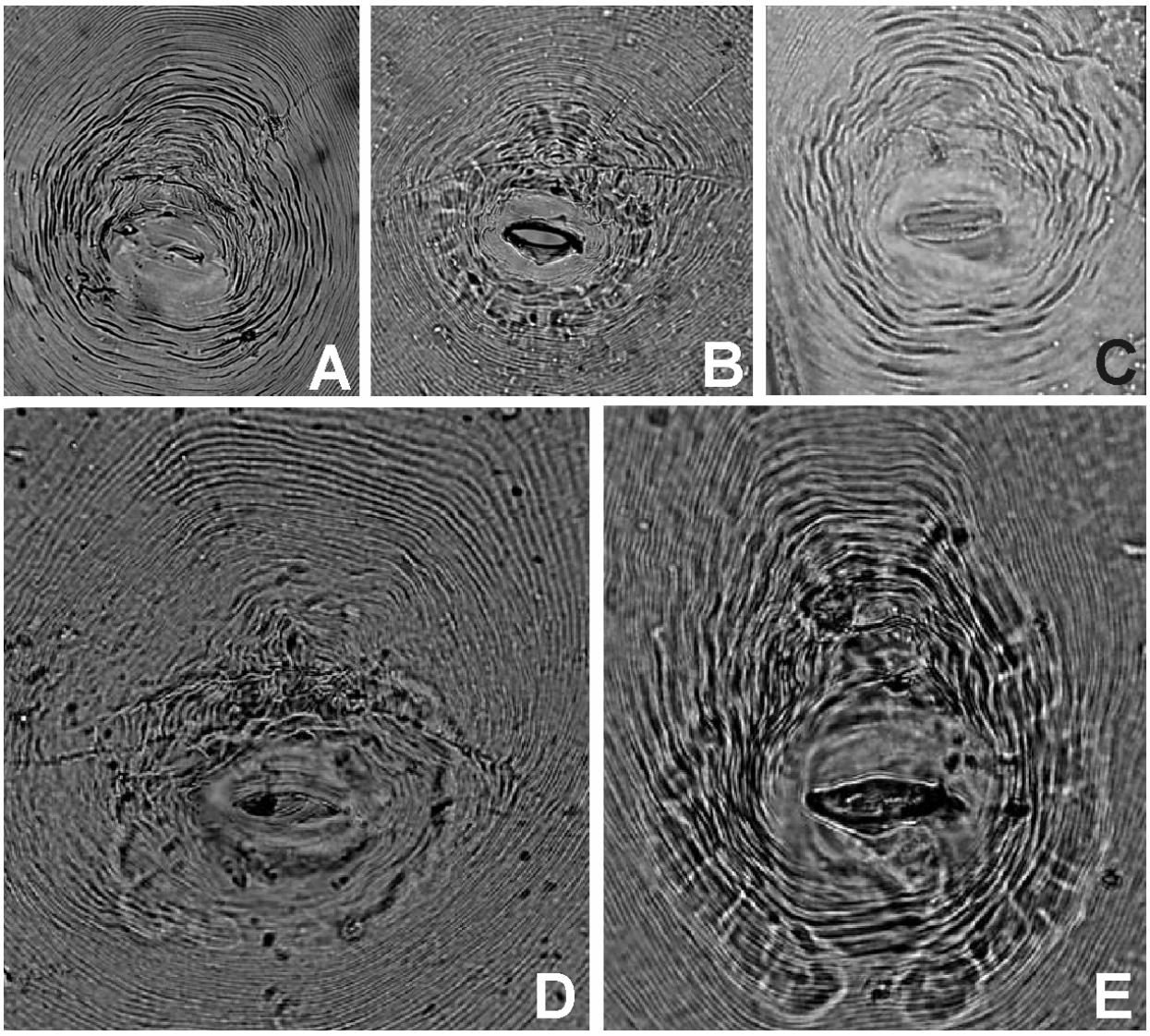

Fig. 1. Perineal patterns of five *Meloidogyne* species. A: MIWN1 (*M. incognita*); B: MJWC (*M. javanica*); C: MADZ (*M. arenaria*); D: MHDZ (*M. hispanica*); E: MEWC (*M. enterolobii*).

Photograph of perineal pattern A (*M. incognita*): Elongated with more or less flattened dorsal arch. Striae smooth to wavy and with no lateral lines. Perineal pattern B (*M. javanica*): Perineal pattern with the definite incisures on the lateral lines separating the striae of the dorsal and ventral sectors. They lead to the tail terminus. Dorsal arch rounded to flattened. Perineal pattern C (*M. arenaria*): Striae wavy obvious with same forking at lateral lines and short irregular striae near lateral lines. Perineal pattern D (*M. hispanica*): Perineal pattern with folds along both lateral lines. Striae rounded to flattened. Striae of the dorsal arch into a square. Perineal pattern E (*M. enterolobii*): Perineal pattern with no lateral lines. Striae smooth to wavy and have high dorsal arch.

Following amplification, 5 µl of PCR products were separated on a 1.0% agarose gel, stained with Gold View, and visualised under UV illumination. Using *Hinf*I and *Eco*RI to double digestion of amplified products was conducted in a 20 µl volume containing 5 µl of PCR product, 2 µl of 10×restriction buffer H and 1 µl of each enzyme (*Hinf*I and *Eco*RI) (TaKaRa Biotech, Dalian, China) and 11 µl sterile water. Double digestion was allowed to proceed for 6 h at 37°C. Restricted products were assayed on a 1.5% agarose gel.

RESULTS

Perineal pattern. Large variations were found in root-knot nematode perineal patterns. Even perineal pattern of different populations of the same root-knot nematode was very different. In this study, we have listed typical perineal patterns of five *Meloidogyne* species (Fig. 1).

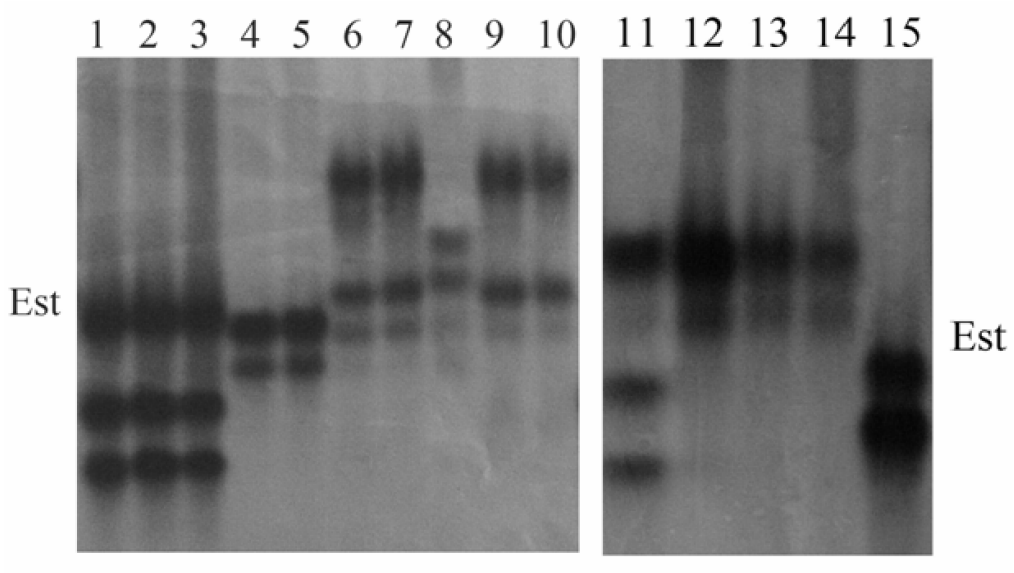

Fig. 2. Est phenotypes of some populations of *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, *M. enterolobii* and *M. hispanica* from Hainan island. 1: MJWC (Est phenotype J3); 2: MJTC (J3); 3: MJHK (J3); 4: MIWN1 (I2); 5: MIWN2 (I2); 6: MEWC (VS1-S1); 7: MEHK1 (VS1-S1); 8: MHDZ (S2-M1); 9: MEHK2 (VS1-S1); 10: MEWN (VS1-S1); 11: MJDA1 (J3); 12: MIDZ1 (I2); 13: MIHK1 (I2); 14: MIDA (I2); 15: MAWN1 (A2).

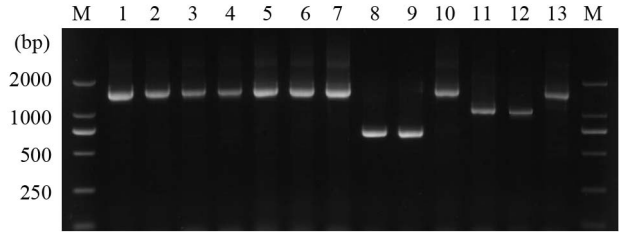

Fig. 3. PCR products amplified from representative populations of *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, *M. enterolobii* and *M. hispanica* from Hainan Province using primers C2F3 and 1108. 1: MJWC; 2: MJTC; 3: MJHK; 4: MJDA1; 5: MIWN1; 6: MIWN2; 7: MIDZ1; 8: MEWC; 9: MEHK1; 10: MHDZ; 11: MAWN1; 12: MADZ; 13: MIDA; M: DL2000 DNA marker (TaKaRa Biotech, Dalian China).

MtDNA-PCR-RFLP analysis. Using mtDNA primers C2F3 and 1108, an amplification product was obtained from either purified DNA or crude J2 lysate from each of the populations listed in Table 1. Fragments of three different sizes were amplified (Table 1, Fig. 2). Specifically, the populations of *M. incognita*, *M. javanica* and *M. hispanica* had a PCR product of 1700bp; the *M. arenaria* populations produced a smaller product of 1100bp and the *M. enterolobii* populations produced a product of 700bp (Fig. 3). When the amplification products were double-digested at the same time with the restriction enzyme *Hinf*I and *EcoR*I, distinctive enzyme phenotype-correlated RFLP profiles were generated that allowed the differentiation of the five species studied (Fig. 4). Different restriction sites on the 1700bp product separated *M. javanica*, *M. incognita* and *M. hispanica* from each other. Four fragments of 1200, 500, 400 and 100bp were generated in all the *M. javanica* populations; three fragments of 1200, 400 and 100bp were produced in all the *M. incognita* populations; and two fragments of 1200 and 500bp occurred in the *M. hispanica*. The 700bp fragment characteristic of *M. enterolobii* and 1100bp fragment characteristic of *M. arenaria* were not restricted by *Hinf*I and *EcoR*I.

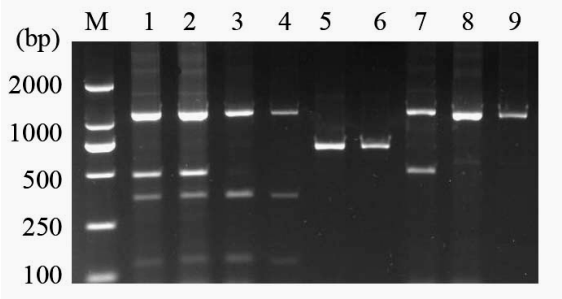

Fig. 4. *Hinf*I and *EcoR*I-restriction patterns of PCR products amplified from representative populations of *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, *M. enterolobii* and *M. hispanica* from Hainan island using primers C2F3 and 1108. 1: MJWC; 2: MJTC; 3: MIWN1; 4: MIWN2; 5: MEWC; 6: MEHK1; 7: MHDZ; 8: MAWN1; 9: MADZ; M: DL2000 DNA marker (TaKaRa Biotech, Dalian China).

DISCUSSION

The species identity of 30 *Meloidogyne* populations collected on southern herbs from Hainan island in China was revealed by perineal patterning and esterase phenotyping and confirmed by mtDNA-PCR-RFLP analysis in the present study. *Meloidogyne incognita*, *M. javanica*, *M. arenaria*, *M. enterolobii* and *M. hispanica* were identified by 56.7, 16.7, 10,13.3 and 3.3% of the total samples, respectively. *Meloidogyne incognita* was the predominant species on southern herbs from Hainan island in China. *Meloidogyne enterolobii* was rare in other regions of China, although it has been reported on distributing in Hainan island and Guangdong province (Xu, 2004; Zhuo, 2008). *Meloidogyne hispanica* was first reported on papaya from Hainan island in 2007 (Wang, 2007), and in this study it was found on *Morinda citrifolia* from Hainan island in China.

Since isozyme phenotypes have been shown to be reliable criteria for identification of major *Meloidogyne* species (Esbenshade & Triantaphyllou, 1985b), we used the traditional Est phenotype and perineal pattern as the means of identification in this study. Five root-knot nematodes were identified from the populations on seven southern herbs with the different Est phenotypes (*i.e*., I2, J3, A2, S2-M1, VS1-S1) and perineal patterns. One populaition has been identified as *M. hispanica*, its Est phenotype was S2-M1; moreover, it was only found from Hainan island in China. *Meloidogyne enterolobii* was originally found infecting pacara earpod trees in Hainan island of China (Yang & Eisenback, 1983). It has so far been recorded only from Hainan island (Zhao, 2000; Xu, 2004) and Guangdong Province (Zhuo, 2008). *Meloidogyne enterolobii* was regarded as a considerable threat to agriculture because it is able to reproduce on many crops, *e.g*., tomato, potato, *Cajanus cajan*, *Psidium guajava*, *Capsicum annuum, Vigna sinensis* and others. In this paper, four populations have been identified as *M. enterolobii* on *Morinda citrifolia*, *aquilaria* and *clove*, its Est phenotype is VS1-S1. However, previous studies have shown that *M. mayaguensis* had female perinean pattern, host range and cytogenetic features similar to those of *M. enterolobii* (Yang & Eisenback, 1983; Esbenshade & Triantaphyllou, 1985; Rammah & Hirschmann, 1988). In addition, populations of *M. mayaguensis* showed an Est phenotype (VS1-S1) identical to that of *M. enterolobii* (Esbenshade & Triantaphyllou, 1985; Fargette & Braaksma, 1990; Xu *et al*., 2004). *Meloidogyne mayaguensis* was differentiated from *M. enterolobii* only by some minor morphological differences and by a different malate dehydrogenase phenotype (N3c in *M. mayaguensis vs* N1a in *M. enterolobii*). Interestingly, the sequence comparison revealed that the 662bp (data not shown) sequence between primers C2F3 and 1108 for *M. enterolobii* in the present study was identical to that reported for *M. mayaguensis*. The mtDNA sequence evidence presented here suggests that *M. mayaguensis* could be conspecific with *M. enterolobii*, as it was soon suggested by Hunt & Handoo (2009). The taxonomic relationship of the two species needs to be studied in more detaily in the future.

With the increase in multiple cropping, damage caused by root-knot nematodes is getting worse. Effective control and management often requires rapid and accurate species identification of target nematode populations. Based on the extensive application of PCR technology, the mtDNA-PCR-RFLP assay has been used to diagnose the major *Meloidogyne* species in recent studies. Powers and Harris (1993) designed primers C2F3 and 1108 to amplify the intergenic region between the mitochondrial *COII* and *lrRNA* genes to discriminate five major *Meloidogyne* species. *Hinf*I restriction patterns of the C2F3 and 1108 amplification products from *M. incognita* and *M. javanica* have been reported in several studies with different results (Powers & Harris, 1993; Williamson *et al*., 1994; Orui, 1998; Blok *et al*., 2002; Sun *et al*., 2005). While Powers and Harris (1993) found three restriction fragments of 1000, 400, 300bp for *M. incognita*, and two fragments of 1000 and 700bp for *M. javanica*, the other investigators reported two products of 1300 and 400bp for *M. incognita*, and no restriction of the 1700bp product for *M. javanica*. We have not found anyone using two restriction enzymes to digest root-knot nematodes in previous studies. In this study, we used the *Hinf*I and *Eco*RI to double digest at the same time for 30 *Meloidogyne* species, and the results showed that all populations of *M. incognita* can be digested into three restriction fragments of about 1200, 400 and 100bp, almost all the populations of *M. javanica* can be digested into four restriction fragments of about 1200, 500, 400 and 100 bp, the population of *M. hispanica* can be digested into two restriction fragments of about 1200 and 500 bp, and the PCR product of specific region on mtDNA of *M. arenaria* and *M. enterolobii* cannot be digested. All the populations of *M. javanica* have two restriction sites on 1700 bp fragments, and it has four restriction fragments because two restriction enzymes were used to digest randomly. Different restriction sites on the 1700bp

product separated *M. javanica*, *M. incognita* and *M. hispanica* from each other. It concluded that mtDNA-PCR-RFLP is a rapid and reliable approach for molecular identification of common *Meloidogyne* species.

ACKNOWLEDGEMENT

This work was funded by the Special Fund for Agro-scientific Research in the Public Interest of China (201103018).

REFERENCES

BLOK, V.C., WISHART, J., FARGETTE, M., BERTHIER, K. & PHILLIPS, M. S. 2002. Mitochondrial DNA differences distinguishing *Meloidogyne mayaguensis* from the major species of tropical root-knot nematodes. *Nematology* 4: 773-781.

CARNEIRO, R.M.D.G., ALMEIDA, M.R.A. & QUENEHERVE, P. 2000. Enzyme phenotypes of *Meloidogyne* spp. populations. *Nematology* 2: 645-654.

CENIS, J.L. 1993. Identification of four major *Meloidogyne* spp. by random amplified polymorphic DNA (RAPD-PCR). *Phytopathology* 83: 76-80

ESBENSHADE, P.R. & TRIANTAPHYLLOU, A.C. 1985A. Electrophoretic methods for the study of root-knot nematode enzymes. In: Barker KR, Carter CC and Sasser JN (eds), *An Advanced Treatise on* Meloidogyne*, Vol 2, Methodology*. North Carolina State University Graphics, Raleigh, USA, pp. 115-123.

ESBENSHADE, P.R. & TRIANTAPHYLLOU, A.C. 1985B. Use of enzyme phenotypes for identification of *Meloidogyne* species. *Journal of Nematology* 17: 6-20.

ESBENSHADE, P.R. & TRIANTAPHYLLOU, A.C. 1990. Isozyme phenotypes for the identification of *Meloidogyne* species*. Journal of Nematology* 22: 10-5.

FARGETTE, M. & BRAAKSMA, R. 1990. Use of the esterase phenotype in the taxonomy of the genus *Meloidogyne*. 3. A study of some 'B' race lines and their taxonomic position. *Revue de Nematologie* 13: 375-386.

HUNT, D.J. & HANDOO, Z.A. 2009. Taxonomy, identification and principal species. In: *Root-knot nematodes*. R. Perry, M. Moens, J. L. Starr Eds. CABI Publishing, Wallingford, 496 p.

MENG, Q.P., LONG, H. & XU, J.H. 2004. PCR assays for rapid and sensitive identification of three major root-knot nematodes, *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. *Journal of Plant Pathology* 34 (3): 204-210.

ORUI, Y. 1998. Identification of Japanese species of the genus *Meloidogyne* (Nematoda: Meloidogynidae) by PCR-RFLP analysis. *Applied Entomology and Zoology* 33: 43-51.

POWERS, T.O., HARRIS, T.S. 1993. A polymerase chain reaction method for identification of five major *Meloidogyne* species. *Journal of Nematology* 25: 1-6.

RAMMAH, A. & HIRSCHMANN, H. 1988. *Meloidogyne mayaguensis* n. sp. (Meloidogynidae), a root-knot nematode from Puerto Rico. *Journal of Nematology* 20: 58-69.

SUN, L.H., LIAO, J.L., LI, X.D. 2005. Analysis of root-knot nematode species and populations based on mitochondrial DNA. *Journal of Plant Pathology* 35: 134-140.

Taylor, D.P. & Netscher, C. 1974. An improved techique for preparing perineal patterns of *Meloidogyne* spp. *Nematologica* 20: 268-269.

WANG, X., LI, H.M., HU, Y.J. 2007. New record of root-knot nematode species in China - *M. hispanica*. *Journal of Plant Pathology* 37: 321-324.

WILLIAMSON, V.M., CASWELL-CHEN, E.P., WU F.F. & HANSON, D. 1994. PCR for nematode identification. In: Lamberti F, De Giorgi C and Bird DM (eds), *Advanced Molecular Plant Nematology*, Plenum Press, New York, pp.119-127.

Xu, J., Wei, D., Zhan, Y, Fu, P. & Cheng, H. 1994. Species and occurrence of nematade parasites of vegetables growing in plastic houses in Jiangsu Province. *Journal of Nanjing Agricultural University* 17: 51-55

Xu, J.H., Liu, P.L., Meng, Q.P. 2004. Characterization of *Meloidogyne* species from China using isozyme phenotypes and amplified mitochondrial DNA restriction fragment length polyerphism. *European Journal of Plant Pathology* 110: 309-315.

YAN, B.W. 2001. Talk on Hainan's southern drug. *Biology Teaching* 26: 39-41

YANG, B. & EISENBACK, J.D. 1983. *Meloidogyne enterolobii* n.sp. (Meloidogynidae), a root-knot nematode parasitising pacara earpod tree in China. *Journal of Nematology* 15: 381-391.

ZHUO, K., HU, M.X., LIAO, J.L. 2008. Identification of *Meloidogyne enterolobii* in Guangdong Province and Hainan Province*. Journal of Huazhong Agricultural University* 27: 193-197.

ZHAO, H. 2000. Identification and morphological comparison of the four common species of *Meloidogyne* from China. *Journal of Plant Pathology* 30: 288

Mei-ying Fu, Mian-cai Chen, Tong-bin Xiao, Hui-fang Wang. Определение видов *Meloidogyne* от «южных трав» острова Хайнань по данным морфологии перивульварной пластинки и RFLP mtDNA.
Резюме. Различные виды целебных т.н. «южных трав» культивируются на о-ве Хайнань. В последнее время значительной проблемой их выращивания стали галлообразующие нематоды. Тридцать популяций *Meloidogyne* с «южных трав» Хайнаня были исследованы методом изучения перивульварной платинки, спектров эстераз, а также при помощи рестрикции ферментами *Hinf*I и *Eco*RI ПЦР-продукта – участка митохондриальной ДНК (mtDNA) между генами *COII* и *lrRNA*. Изучение перивульварных пластинок и фенотипы эстераз показали, что 17 популяций представляют собой *M. incognita*, пять - *M. javanica*, три - *M. arenaria*, четыре - *M. enterolobii* и одна - *M. hispanica*. Для быстрого определения видов *Meloidogyne* использовали праймеры C2F3 и 1108 для амплификации участка mtDNA между генами *COII* и *lrRNA*, который затем подвергали двойной рестрикции в помощью *Hinf*I и *Eco*RI. Результаты этого анализа выявляют присутствие 5 видов *Meloidogyne*. Метод mtDNA-PCR-RFLP – быстрый и надежный подход к определению видов *Meloidogyne*.