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Enzyme phenotypes of Brazilian populations of *Meloidogyne* spp.

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Summary – Enzyme phenotype, especially esterases and malate dehydrogenase, were used to characterize 90 *Meloidogyne* spp. populations from different regions of Brazil, using a simplified technique for routine analyses. Soluble proteins from macerates of individual females were separated by horizontal electrophoresis on 1 mm-thick-polyacrylamide gels (7 %). Of the populations studied, 24 were *M. incognita,* 39 *M. javanica,* six *M. arenaria,* two *M. exigua,* eight *M. hapla,* two *M. graminicola,* and nine *Meloidogyne* spp. Among the undescribed species, *Meloidogyne* sp.1 is similar to *M. javanica* on perineal pattern and can be distinguished by the lack of one electrophoretic esterase band and its ability to reproduce on pepper. *Meloidogyne* sp. 2, the most virulent parasite of coffee roots in Paraná State, is an undescribed species. Unfortunately, enzymatic profiles provide insufficient information to separate the host races in *M. incognita* and *M. arenaria.*

Résumé – ***Phénotypes enzymatiques de populations brésiliennes de* Meloidogyne *spp.*** – Des phénotypes enzymatiques (estérases et malate deshydrogénases) ont été utilisés pour la caractérisation de 90 populations de *Meloidogyne* originaires de différentes régions du Brésil. Nous avons employé une technique simplifiée pour les analyses de routine. Les protéines solubles sont séparées à partir d'une seule femelle par électrophorèse horizontale, en gel de 1 mm d'épaisseur à 7 % de polyacrylamide. Les populations se répartissent comme suit : 24 *M. incognita,* 39 *M. javanica,* six *M. arenaria,* deux *M. exigua,* huit *M. hapla,* deux *M. graminicola* et neuf *Meloidogyne* spp. Parmi les espèces atypiques, *Meloidogyne* sp. 1 ressemble à *M. javanica* par la configuration périnéale et peut être différenciée en électrophorèse par l'absence d'une bande estérasique et sa capacité à se développer sur le poivron. *Meloidogyne* sp. 2, le plus virulent parasite du caféier dans l'état du Paraná, correspond à une espèce non encore décrite. Les profils enzymatiques ne donnent malheureusement aucune information sur les races de *M. incognita* et *M. arenaria.*

Key-words : biochemical systematics, Brazil, electrophoresis, enzyme phenotype, identification, *Meloidogyne,* nematodes.

Root-knot nematodes, *Meloidogyne* spp., are among the most destructive plant-parasitic nematodes in Brazil. The unambiguous identification of these nematodes is essential before management practices can be optimized. Currently in Brazil, identification of the most common and agronomically important species is made by microscopic examination of perineal patterns of females and by differential host tests. Identification by these procedures is a difficult task, even for well-qualified taxonomists. Biochemical studies have demonstrated that major species of *Meloidogyne* can be differentiated by species-specific enzyme phenotypes using the isoesterases, Est, and the malate dehydrogenases, Mdh (Dalmasso & Bergé, 1978; Esbenshade & Triantaphyllou, 1985 *a,* 1990).

The present paper reports the Est and Mdh phenotypes, differential host tests and perineal patterns of 90 populations of *Meloidogyne* spp. collected from different regions of Brazil. It also presents a modification of a simple technique for routine isozyme analysis of *Meloidogyne* populations.

Material and methods

Ninety *Meloidogyne* spp. populations originating from the Brazilian States of Mato Grosso, Minas Gerais, Paraná, and Rio Grande do Sul were examinated (Table 1). Species identification was based first on esterase and malate dehydrogenase phenotypes (Esbenshade & Triantaphyllou, 1990). Other complementary studies were made : perineal morphology (Eisenback *et al.,* 1980) and differential host tests (Hartman & Sasser, 1985). Nematodes were collected from their natural host, and they were propagated on tomato (*Lycopersicon esculentum* Mill.) cv. Santa Cruz in a greenhouse at 25 to 30 °C. The differential host test was made only with the populations that reproduced on tomato. Electrophoresis was performed in 7 % polyacrylamide gel slabs (11 × 18 cm, 1 mm thick) in a Cl-18 Permatron apparatus using a modification of the technique proposed by Smithies (1955). Forty young females were collected from each natural field host or from tomato roots and individually macerated (fourteen females to *M. exigua*) in 2-3 µl of extraction buffer (Dalmasso & Bergé, 1978). The suspension was absorbed by Whatman 3 MM filter paper (1.5 × 4.0 mm), and each piece of paper was loaded with a fine forceps, into wells, in the separating gel, and a drop of bromophenol blue 100 was added. The buffer system proposed by Scandalios (1969) was used. Electrophoresis was carried out for approximately 2 h, at 5 °C, 80 volts, until the bromo-

Table 1. *Origin, species identification, differential host plant reactions and enzyme phenotypes of* Meloidogyne *populations from Brazil.*

States *	Origin	Species	Differential host plant reactions **		Enzyme phenotype ***	
	Crops (number of populations)		+	–	Est	Mdh
PR RS	Coffee (1), soybean (1) Artichoke (1), tomato (1), lettuce (1), beet (1), pepper (2), peach tree (1), carrot (2)	*M. incognita* race 1	pepper, watermelon, tomato	cotton, tobacco, peanut	I 1 I 2	N 1
PR	Coffee (5), cotton (1), bean (1), soybean (1)	*M. incognita* race 2	pepper, watermelon tomato	cotton, peanut	I 1	N 1
PR	Cotton (1), mulberry tree (1), coffee (1), bean (1)	*M. incognita* race 3	cotton, pepper, watermelon, tomato	tobacco, peanut	I 1	N 1
PR	Cotton (1)	*M. incognita* race 4	cotton, tobacco, pepper, tomato watermelon	peanut	I 2	N 1
RS, PR, PE, MT	soybean (13), artichoke (3), peach trees (4), cucumber (1), beet (3), broccoli (1), green bean (2), squash (2), carrot (1), tomato (6), bean (2), lettuce (1)	*M. javanica*	tobacco, watermelon, tomato	cotton, pepper, peanut	J 3	N 1
PR	corn (1), mulberry (1), rice (1), cassava (1), tomato (1), sugar cane (1), *Mimosa scabrella* (1)	*Meloidogyne* sp. 1	tobacco, pepper, watermelon, tomato	cotton, peanut	J 2	N 1
RS	tomato (2), soybean (2), kiwi (1), lettuce (1)	*M. arenaria* race 2	tobacco, pepper, watermelon, tomato	cotton, peanut	A 2 A 3	N 1
MG, RS	coffee (1), kiwi (1), tomato (5), lettuce (1)	*M. hapla*	tobacco, pepper, peanut, tomato	cotton, watermelon	H 1	H 2
MG	coffee (1)	*M. exigua*	pepper, tomato, watermelon	cotton, tobacco, peanut	VF 1	N 1
MT	rubber tree (1)	*M. exigua*	–	tomato	VF 1	–
PR	coffee (1)	*Meloidogyne* sp. 2	tobacco, tomato, watermelon	cotton, pepper, peanut	F 1	N 1
RS	rice (2)	*M. graminicola*	–	–	VS 1	–

* MG - Minas Gerais, MT - Mato Grosso, PE - Pernambuco, PR - Paraná, RS - Rio Grande do Sul.

** Cotton cv. Deltapine, Tobacco cv.NC95, Pepper cv. California Wonder, Watermelon cv. Charleston Gray, Peanut cv. Florunner, Tomato cv. Rutgers; (+) suceptible host, (–) resistant host.

*** Phenotype designations : Est - esterase, Mdh - Malate dehydrogenase.

phenol blue had migrated 8 cm into the gel. On each gel slab a paper with protein extracts of three females of *M. javanica* was included as a reference.

Gels were stained for esterase activity for 30 min (Scandalios, 1969) and for malate dehydrogenase for 45 min (Harris & Hopkinson, 1976). Gels were washed, stored, and placed in 10 % acetic acid and 40 % methyl alcohol solution for 30 min. Gels were pressed between two cellophane sheets to dry. Enzyme phenotypes were designated with letter(s) and a number indicating the number of bands as described by Esbenshade and Triantaphyllou (1985 *b*, 1990).

Results

Eleven bands for esterase (Est) activity were detected among the 90 populations of *Meloidogyne*. A distinct Est-phenotype was associated with most of the populations of the major species and it was possible to detect atypical populations (Figs 1, 2). Two bands for malate dehydrogenase (Mdh) activity, phenotypes N1 and H2, were detected in the examined populations (Figs 1, 3). All the species, except *M. hapla* and *M. graminicola*, had the phenotype N1 (Rm 0.15).

Est phenotype I1 (Rm 0.38) was detected in 14 *M. incognita* populations and the phenotype I_2 (Rm 0.39) with a minor band (Rm 0,53) was detected in ten populations (Table 1; Figs 1, 2). The *M. incognita* races 1, 2 and 3, which originated from Paraná (PR) State, had the phenotype I1 and races 1 from Rio Grande do Sul (RS) State and 4 from PR State, the phenotype I2 (Table 1). All populations exhibited typical *M. incognita* perineal patterns. The reactions to the differential host plants are given in Table 1.

Est phenotype J3 (Figs 1, 2) was detected in 39 *M. javanica* populations (Table 1). These populations are frequently observed in southern Brazil on soybean, peach tree, mulberry, potato, tomatoes, bean, etc. About 20 % of *M. javanica* populations were in mixture with *M. arenaria*. The atypical phenotype J2 (*Meloidogyne* sp. 1), a population from the northwest Paraná State, where it was found on bean, corn, mulberry, rice, cassava, melon, sugar cane, and *Mimosa scabrella* Benth., exhibited a typical perineal pattern of *M. javanica* (Fig. 4). In differential host test this population reproduced on tobacco, pepper, watermelon, and tomato.

Phenotypes A2 and A3 (Figs 1, 3) were detected from six populations of *M. arenaria* from tomato, soybean, kiwi or lettuce. The two phenotypes had the same differential host plants (race 2) : tobacco, pepper, watermelon, and tomato were good hosts, cotton and peanut were not hosts (Table 1). The perineal patterns were typical of *M. arenaria*.

Est phenotype H1 (Rm 0.45) was detected in seven populations of *M. hapla* (Figs 1, 2), isolated from kiwi, lettuce and tomato in RS State, and one population from

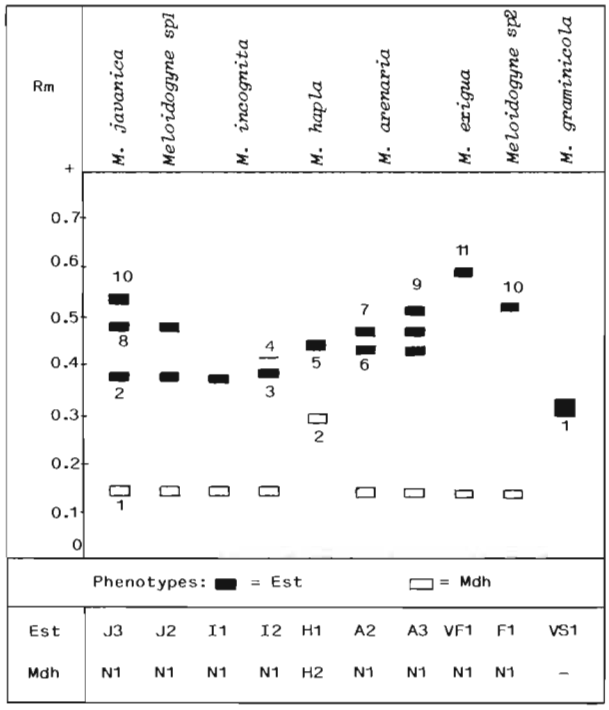

Fig. 1. *Phenotypes of esterase (Est) and malate dehydrogenase (Mdh) observed in 90 Brazilian populations of* Meloidogyne *spp.*

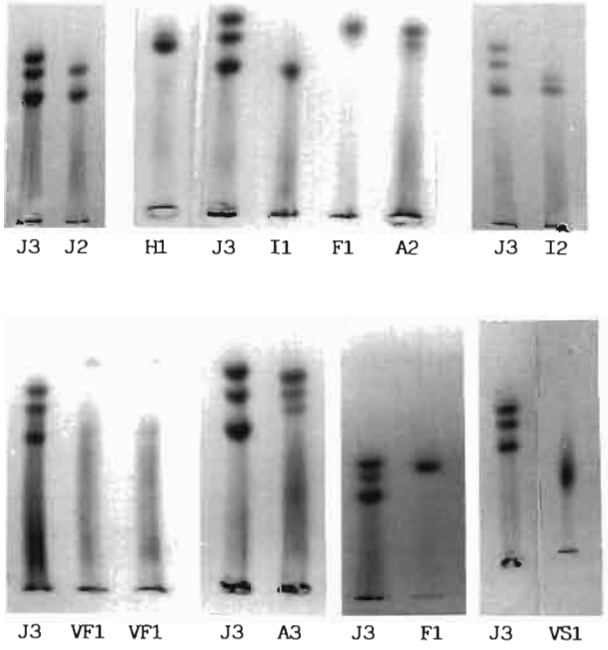

Fig. 2. *Eleven esterase phenotypes found in 90 Brazilian populations of* Meloidogyne *(For explanation of phenotype designations see Fig. 1).*

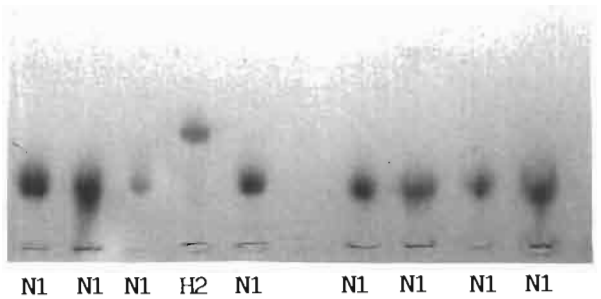

Fig. 3. *Two malate dehydrogenase phenotypes found in 90 Brazilian populations of* Meloidogyne *(For explanation of phenotype designations see Fig. 1).*

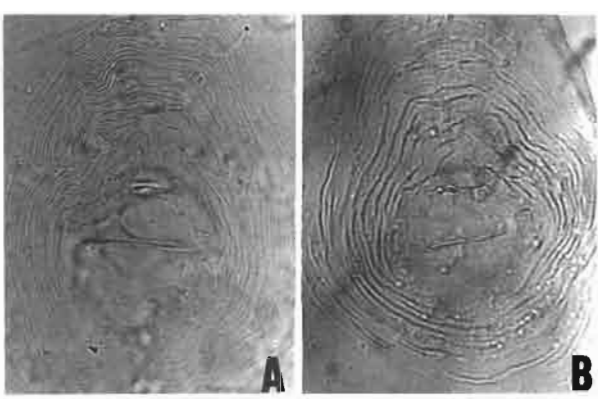

Fig. 4. *Light micrograph of* Meloidogyne *perineal patterns. A :* Meloidogyne *sp. 1; B :* Meloidogyne *sp. 2.*

coffee from Minas Gerais (MG) State. Only *M. hapla* had a species specific Mdh phenotype designated H2 (Figs 1, 3), with one major band (Rm 0.3). These populations exhibited typical *M. hapla* perineal patterns. The reactions to differential host plants are given in Table 1.

Phenotype VF1 (Figs 1, 2) with a fast band (Rm 0.6) was observed in two of *M. exigua* populations from coffee and rubber trees (Table 1). These two populations showed the typical perineal pattern of this species, but on the basis of differential plants these two populations are different : the population from rubber tree did not reproduce on tomato, and the population from coffee reproduced on tomato, tobacco and watermelon (Table 1). The population from rubber trees in Michelin Plantation in Mato Grosso (MT) State was mixed with *M. incognita.*

Another phenotype F1 (*Meloidogyne* sp. 2) with a large band (Rm 0.55) was observed in one population isolated from coffee (Figs 1, 2; Table 1). This population in differential host tests reproduced on tobacco, watermelon, and tomato, but did not reproduce on cotton, pepper, and peanut. Its perineal patterns is typical of *M. incognita.*

Phenotype VS1 (Rm 0.33), with a very slow and large band of high enzymatic activity (Figs 1, 2), was detected in two *M. graminicola* population from rice in RS State (Table 1). Its Mdh phenotype could not be characterized because the band was not clearly stained in this assay. The perineal pattern is typical of *M. graminicola* and the population did not reproduce on tomato (Table 1).

Discussion

This study confirmed that esterase (Est) phenotypes are useful biochemical characters for identifying major *Meloidogyne* species. They are also useful in detecting new taxonomic forms within the genus (Esbenshade & Triantaphyllou, 1990).

Although the observed enzyme patterns were stable and repeatable, the mobility of a given band in different gels varied within narrow limits. Also, because of variations in technique and laboratory conditions, the relative mobility of the enzyme bands in this study differed slightly from other reports (Bergé & Dalmasso, 1975; Dalmasso & Bergé, 1978, 1983; Janati *et al.,* 1982; Esbenshade & Triantaphyllou, 1985 *a, b;* Fargette, 1987; Pais & Abrantes, 1989).

Brazilian populations of *M. incognita* had two esterase phenotype I1 or I2 showing some variability among the populations (Figs 1, 2). The same phenotypes were observed by Pais and Abrantes (1989). This variation could not be associated with the host race 1-4. Unfortunately electrophoresis profiles do not provide enough information for separating the host races of *M. incognita.*

Most of the *M. javanica* populations showed a single esterase phenotype (J3) not found in any other *Meloidogyne* species (Esbenshade & Triantaphyllou, 1990). The atypical form J2 (Figs 1, 2) named *Meloidogyne* sp. 1, which was reared through eight to ten generations on tomato, showed a different and stable phenotype (J2). It produced two bands of esterase activity in positions 2 and 8. The J2 population has also one malate dehydrogenase band in position 1 (N1) and the same perineal pattern as *M. javanica.* It can also be distinguished from *M. javanica* (J3) by a differential host test, in that it reproduces on pepper. This population has the same differential host response as *M. javanica* (pepper), as reported by Rammah and Hirschmann (1990), but it can be differentiated from this race of *M. javanica* (J3) by esterase phenotype J2. Additional studies are needed to clarify other morphological traits and to estimate the

degree of genetic similarity and phylogenetic relationships between *M. javanica* and *Meloidogyne* sp. 1.

The phenotypes A3 and A2 (Figs 1, 2) were species specific for *M. arenaria* and the lack of resolution of band 9 in A2 was apparently related to the use of an individual female, the low esterase activity of this band, or physiological conditions of the females. These populations of *M. arenaria* from Rio Gande do Sul State (A2, A3) had different differential host plants from the races 1 and 2 described by Hartman and Sasser (1985), but were similar to those from West Africa (Fargette, 1987) because they developed on pepper, but not on peanut (Table 1).

The characterization of *M. hapla* was based on esterase phenotype H1 and malate dehydrogenase H2 (Figs 1-3). The populations from two geographical regions (RS and MG States) had the same differential host plants. The *M. hapla* population from coffee (MG State) was identified previously as *M. exigua* probably because of the root symptoms. This population causes typical galls with small diameters in coffee, similar to those caused by *M. exigua* but different from those caused by other species occuring on coffee in Brazil (Campos *et al.*, 1990).

The characterization of *M. exigua* was based on the study of a few populations, using a large number of macerated females, because the esterase activity for this species was very low. The esterase phenotype VF1 (Figs 1, 2) found in *M. exigua* populations was first characterized by Esbenshade and Triantaphyllou (1990). *Meloidogyne exigua* from coffee (MG) was different from the rubber tree population (MT) : only the first population was able to reproduce on susceptible tomato plants. Santos and Triantaphyllou (1992) observed variability in populations of *Meloidogyne* spp. from coffee. This variability has resulted in frequent misidentification of these species in Brazil.

The phenotype F_1 (Figs 1, 2), designated as *Meloidogyne* sp. 2 was first characterized by Janati *et al.* (1982) and later by Esbenshade and Triantaphyllow (1985 *b*) in six populations of an undescribed species obtained from coffee from Brazil, Peru, and Surinam. A similar Est phenotype (F1) was observed in two populations of *M. incognita acrita* from Germany (Bergé & Dalmasso, 1975) and from West Africa (Fargette, 1987), in *M. querciana* (Esbenshade & Triantaphyllou, 1985 *b*) and recently in a new species from coffee, *M. konaensis* (Eisenback *et al.*, 1994). The Mdh phenotype (N1) is different than that of *M. querciana* (N3a). Although the perineal patterns of *Meloidogyne* sp. 1 were similar to those of *M. incognita* (Fig. 4), results of the host tests were similar to those reported for *M. javanica* or *M. arenaria* race 2. *Meloidogyne* sp. a has been found to attack coffee in Brazil, and accounts for approximately 52 % of all root- knot nematode infestations of coffee plantations in northwest Paraná State. This population has been named *M. incognita* biotype IAPAR. Its effects were reported to be more severe than *M. incognita* (Carneiro, 1993). The Est phenotype (F1) is the most useful character to differentiate this new species from *M. incognita* in coffee plantations surveys in Brazil. Examination of additional taxonomic characters must be undertaken to clarify its identification.

Meloidogyne graminicola, Est phenotype VS1 (Figs 1, 2), was distributed throughout rice plantations in RS State. Surveys have shown a substantial increase in distribution of this species. The damage caused by *M. graminicola* is frequently confused with that caused by the rice water weevil, *Orizophagus oryzae* Costa Lima, on irrigated rice (Sperandio & Amaral, 1994).

The method described in this paper is simple, inexpensive and allows routine enzyme studies of *Meloidogyne* species. Results confirm that use of esterase and malate dehydrogenase phenotypes are a rapid and efficient method to : *i*) characterize *Meloidogyne* species and detect atypical forms; *ii*) carry out extensive field surveys to determine the frequency and relative distribution of *Meloidogyne* spp. and *iii*) detect mixed species populations for purification prior to conducting studies on plant resistance or host specificity.

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