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Comparisons of Isozyme Phenotypes in Five *Meloidogyne* spp. with Isoelectric Focusing[1]

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Abstract: *Meloidogyne incognita* race 1, *M. javanica*, *M. arenaria* race 1, *M. hapla*, and an undescribed *Meloidogyne* sp. were analyzed by comparing isozyme phenotypes of esterase, malate dehydrogenase, phosphoglucomutase, isocitrate dehydrogenase, and α-glycerophosphate dehydrogenase. Isozyme phenotypes were obtained from single mature females by isoelectric focusing electrophoresis. Of these five isozymes, only esterase and phosphoglucomutase could be used to separate all five *Meloidogyne* spp.; however, the single esterase electromorphs were similar for *M. incognita* and *M. hapla*. Yet when both nematodes were run on the same gel, differences in their esterase phenotypes were detectable. Isozyme phenotypes from the other three isozymes revealed a great deal of similarity among *M. incognita*, *M. javanica*, *M. arenaria*, and the undescribed *Meloidogyne* sp.

Key words: biochemical systematics, electrophoresis, enzyme, esterase, α-glycerophosphate dehydrogenase, isocitrate dehydrogenase, isoelectric focusing, malate dehydrogenase, *Meloidogyne arenaria*, *M. hapla*, *M. incognita*, *M. javanica*, nematode, phosphoglucomutase.

Significant progress has been made using biochemical systematics to elucidate nematode taxonomy (1,5–7,10,12–15,19). Studies in this field with *Meloidogyne* spp. were conducted for the first time by comparing protein extracts from mass homogenates of females by polyacrylamide gel electrophoresis (PAGE) (4,5). The four major *Meloidogyne* spp.—*M. incognita*, *M. javanica*, *M. arenaria*, and *M. hapla*—each possessed characteristic enzyme phenotypes. In subsequent work enzyme phenotypes from single females of *Meloidogyne* spp. were analyzed using thin-slab polyacrylamide gel electrophoresis (6).

Recent studies confirmed that esterase and malate dehydrogenase phenotypes from single females resolved by a modified thin-slab PAGE system are diagnostic for the four most common *Meloidogyne* spp. (16). Also recently, an automated electrophoretic apparatus (Phast system, Pharmacia LKB, Piscataway, NJ), which processes thin polyacrylamide slab gels, has been used to identify *Meloidogyne* spp. (9). Genetic diversity also was observed in *Heterodera glycines* enzyme phenotypes from individual and mass homogenates of females by ultra-thin isoelectric focusing (IEF) (18). The IEF system was used for the first time in comparative studies of *Meloidogyne* spp. using egg proteins to differentiate the four major species (15). Our objective was to compare five enzyme systems of five *Meloidogyne* spp. by IEF using single female proteins.

Materials and Methods

The five *Meloidogyne* spp. were reared on 2-week-old tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) plants inoculated with approximately 500 freshly hatched juveniles. Local populations of *Meloidogyne incognita* race 1 (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood, *M. arenaria* race 1 (Neal) Chitwood, and an undescribed *Meloidogyne* species from Bradenton, Florida, were maintained at 27 C in a growth chamber. An isolate of *M. hapla* Chitwood originally from North Carolina was maintained in an air conditioned greenhouse. After 40–45 days, white females with protruding egg masses were dissected from roots and placed in deionized water in a BPI dish. The females then were transferred onto a 5-×-5-mm filter paper wick (Pharmacia LKB), which was soaked previously with 5 μl of a solution contain-

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ing 20% sucrose (w/v) and 2% (v/v) Triton X-100 (8) and arranged on a glass slide. The slide was placed on an aluminum foil-wrapped support over an ice block contained in a sandwich box (17). Each female was punctured and the contents were spread over the entire wick by folding the buffer-soaked wick over the homogenate and pressing with a needle.

Isoelectric focusing was performed with the LKB Ultrophor system (Pharmacia LKB) using precast thin layer gels each 25 × 11 × 0.1 cm. The gel pH range was 3.5–9.5, and the electrolytes used were 1 M H_3PO_4 (anode) and 1 M NaOH (cathode). The precast gels were cut into smaller sections for runs with 6–12 nematode preparations. Each gel was prefocused for 10 minutes and the nematode-saturated wicks were placed on the gel surface about 1 cm from the anode strip and spaced 0.5 cm apart. The run was continued for 1.5 hours at 5 C and a constant voltage of 1,500 V. At the end of every run, the pH gradient across the gel was measured immediately with a surface electrode at 0.5-cm spacings.

Following a run the gels were rinsed in the stain reaction buffer for 2–3 minutes and stained for esterase (α- and β-naphthyl acetate), malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), α-glycerophosphate dehydrogenase (α-GPD) (2), and phosphoglucomutase (PGM) (20). The gels were maintained in the reaction mixture at 37 C for esterase and 25 C for the other enzymes and incubated in the dark until bands appeared. Each gel was photographed, fixed (5:5:1 v/v methanol, water, and glacial acetic acid) for 1 hour, dried, and stored between plastic sheets (Pharmacia LKB). Each enzyme system was evaluated initially from two or three females of each species and then confirmed at least four times using single mature females. The isoelectric point of each electromorph (i.e., a single resolved band) was determined by plotting the distance migrated by the electromorph on a regression line fitted to the pH gradient of the gel. The results presented are averages of a minimum of four observations.

Results

The relative intensity of staining reactions is related to the enzyme activity; thus only deeply stained and easily detectable bands (electromorphs) were considered. All isozymes migrated toward the cathode on the gels. Several isozyme phenotypes were detected for each enzyme system in all five *Meloidogyne* spp.

Five different phenotypes were detected for esterase; however, the single electromorphs resolved for *M. hapla* (pH 4.91) and *M. incognita* (pH 4.94) were similar (Figs. 1A, 2A). *Meloidogyne arenaria* also had a single electromorph at pH 4.71. Five electromorphs were detected in *M. javanica* at pH 4.69, 4.79, 4.86, 4.95, and 5.37. Two electromorphs were detected for *Meloidogyne* sp. at pH 4.91 and 5.03, the former being shared with *M. hapla.*

Five distinct phenotypes were detected for PGM (Figs. 1B, 2B). *Meloidogyne incognita, M. javanica, M. arenaria,* and *Meloidogyne* sp. shared three electromorphs at pH 4.76, 4.90, and 5.00. In addition, *M. incognita* had a band at pH 4.43, *Meloidogyne* sp. had a band at pH 4.55, *M. arenaria* had a band at pH 4.34, and *M. javanica* had two bands—one each at pH 4.34 and 4.50, with the former being shared with *M. arenaria.* Two electromorphs were detected in *M. hapla* at pH 4.56 and 5.08.

Three different phenotypes were detected in the MDH system, with *M. incognita, M. javanica,* and *Meloidogyne* sp. sharing a common phenotype composed of a single electromorph at pH 6.51 (Fig. 2C). The second phenotype was characteristic of *M. arenaria,* which possessed two electromorphs at pH 5.72 and 6.51. The latter was shared with *M. incognita, M. javanica* and *Meloidogyne* sp. The third phenotype, characteristic of *M. hapla,* had a single electromorph at pH 5.52.

The α-GPD system had two different phenotypes (Fig. 2D). A common phenotype was shared by *M. incognita, M. hapla,* and *Meloidogyne* sp. and consisted of two electromorphs at pH 5.54 and 5.74. The second phenotype was shared by *M. javani-*

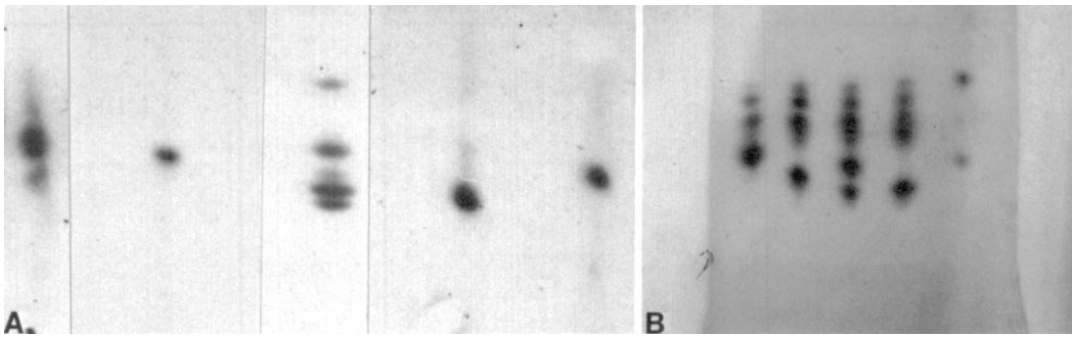

FIG. 1. Photograph of comparative isozyme patterns following isoelectric focusing of crude protein homogenates from single females of the undescribed *Meloidogyne* sp., *M. incognita, M. javanica, M. arenaria,* and *M. hapla* (from left to right). A) Esterase electromorphs. The photograph is a composite from two separate runs, with patterns from *M. javanica* and *Meloidogyne* sp. overlayed above the others. B) Phosphoglucomutase.

ca and *M. arenaria* and consisted of three electromorphs at pH 5.09, 5.25, and 5.54. One of the electromorphs belonging to this phenotype was shared by the first phenotype at pH 5.54.

The IDH system had two distinct phenotypes (Fig. 2E). *Meloidogyne incognita, M. javanica, M. arenaria,* and *Meloidogyne* sp. shared a common phenotype, each with six electromorphs at pH values of 5.66, 5.85, 6.06, 6.54, 6.89, and 7.06. Six electromorphs were detected for *M. hapla* of which three electromorphs—one each at pH 5.66, 5.85, and 6.06—were homologous with those of the remaining four species; three additional bands were detected at pH 5.19, 5.31, and 5.49.

DISCUSSION

Specific isozyme phenotypes were resolved with consistency from single females of *Meloidogyne* spp. by IEF electrophoresis. This system allows for the rapid and simultaneous testing of single females from several *Meloidogyne* spp. of the same age. Isoelectric points of isozymes with only slight differences in pH can be readily resolved and detected. This attribute enables more efficient studies on biochemical systematics of nematodes, and, because single individuals can be analyzed, it affords another mechanism for studying polymorphism within *Meloidogyne* spp. Our results from IEF electrophoresis confirm previous reports (3,5–9) that certain isozyme phenotypes are useful in identifying species of economic importance within *Meloidogyne* spp.

In our study only PGM phenotypes were distinctly characteristic for all five species of *Meloidogyne* and, hence, were diagnostic for the species. Although the PGM phenotypes of *M. arenaria* and *M. javanica* were similar, there was one additional electromorph detected in *M. javanica.*

Esterase phenotypes have proven useful in differentiating the four major *Meloidogyne* spp. (7,9,11). Our studies confirm this fact, but the close similarity of the single electromorphs from *M. incognita* and *M. hapla* warrants further study; especially with gels with a narrow pH range, such as 4–6. A narrower pH range would help with the resolution of electromorphs with similar isoelectric points.

On comparison of our data with earlier studies (6,9,16) in which isozymes were differentiated by electrophoretic techniques on the basis of molecular weight and electrical charge, there is a close similarity in the total number of electromorphs resolved for esterase. Both the PAGE (9) and IEF system resolved one electromorph each for single females of *M. incognita* and *M. hapla*; however, the PAGE system revealed three electromorphs in *M. javanica* and 1–3 in *M. arenaria,* whereas the IEF system resolved five electromorphs in *M. javanica* and one in *M. arenaria.* Clearly, analysis of esterase electromorphs from protein ex-

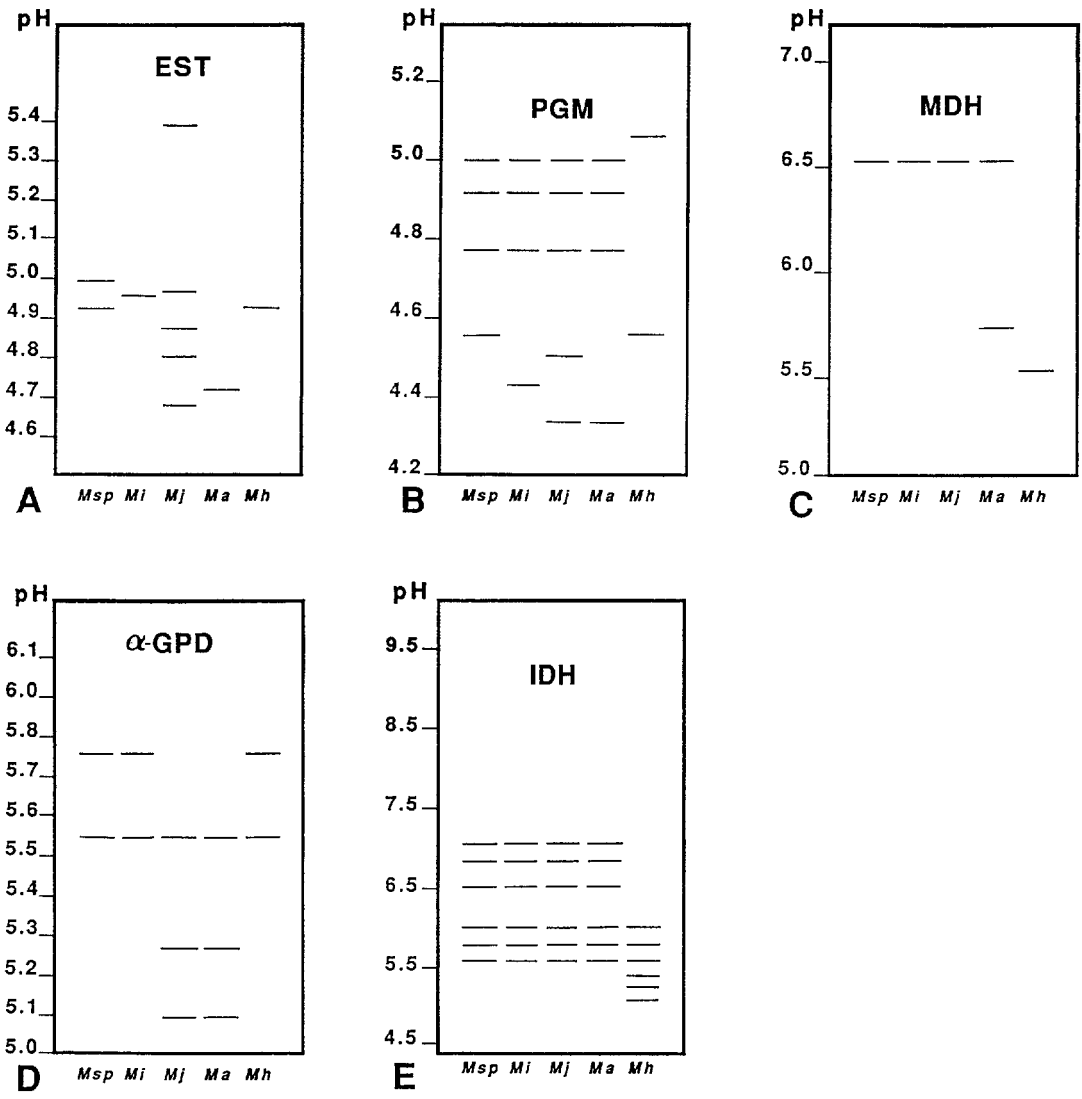

FIG. 2. Diagrammatic sketch of comparative isozyme phenotypes following isoelectric focusing of crude protein homogenates from single females of five *Meloidogyne* spp. M. sp. = undescribed *Meloidogyne* sp., Mi = *M. incognita*, Mj = *M. javanica*, Ma = *M. arenaria*, Mh = *M. hapla*. A) Esterase. B) Phosphoglucomutase. C) Malate dehydrogenase. D) α-Glycerophosphate dehydrogenase. E) Isocitrate dehydrogenase.

tracted from mass homogenates of *M. javanica* females by PAGE reveal more than three electromorphs (5), thus the resolution of five electromorphs for esterase from this species by IEF is not disconcerting.

Studies with different thin-slab methods of PAGE showed that the MDH phenotypes are useful in differentiating only *M. hapla* from the other major *Meloidogyne* spp. (6,16); however, IEF differentiated *M. hapla* and *M. arenaria* from the remaining species. *Meloidogyne arenaria* was distinct from the remaining four species with respect to MDH in that it had two electromorphs, whereas the remaining species had only one. Our findings agree with an earlier report (9) that the MDH system easily separates *M. incognita* and *M. hapla* despite a close similarity in esterase electromorphs between the two species.

The α-GPD system resolved a common phenotype for *M. hapla, M. incognita,* and *Meloidogyne* sp. Of the five enzymes detected for *M. hapla,* this was the only one

having similarities in phenotype patterns with two of the other *Meloidogyne* spp. The IDH system was useful for distinguishing *M. hapla* from the remaining four species because all others shared a common phenotype. The homology of IDH and α-GPD phenotypes for *M. arenaria* and *M. javanica* supports their placement in one main group based on discriminant alleles (3).

Future investigations on single-paired crosses with inbred lines of facultatively parthenogenetic *Meloidogyne* spp. using the agar-plate mating technique (8) may be amenable for genetic analyses of polymorphic enzymes. Since enzyme proteins are a direct manifestation of genes, their utility in research on polymorphism helps establish speciation on a genetic basis. Also, future work needs to encompass numerous populations within a species to determine intraspecific variability.

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