Fundam. appl. Nematol., 1993, **16** (2), 187-191

USE OF ESTERASE PATTERNS OF FEMALES AND GALLED ROOTS FOR THE IDENTIFICATION OF SPECIES OF *MELOIDOGYNE*

Said K. Ibrahim and Roland N. Perry

Entomology and Nematology Department, AFRC IACR, Rothamsted Experimental Station, Harpenden, Hertfordshire, AL5 2JQ, UK.

Accepted for publication 7 August 1992.

Key-words : electrophoresis, esterase, *Meloidogyne*, root-knot nematode, taxonomy.

The impact of root-knot nematodes (*Meloidogyne* spp.) on crops and the relationship between nematode and host differ between species. Thus, accurate identification is important but the use of morphological techniques is very difficult, even for well qualified taxonomists (Eisenback, 1985; Hirschmann, 1985; Hussey, 1990). Isozyme electrophoresis using nonspecific esterase patterns can distinguish between different species (Hussey *et al.*, 1972; Dalmasso & Bergé, 1978, 1983; Janati *et al.*, 1982; Fargette, 1987*a*; Nobbs *et al.*, 1992) and has been reported to be a reliable diagnostic character for species identification (Esbenshade & Triantaphyllou, 1990). Detection of esterase activity using electrophoresis techniques involves obtaining females either by using chemical " macerating " enzymes (Hussey, 1971) or root dissection (Jones, 1980); both approaches require considerable time to obtain sufficient material. Dissection of the giant cell complex is difficult at the early stage of infection and more difficult later when lateral roots emerge and adult female nematodes rupture easily (Jones, 1980).

Ishibashi (1970) and Bergé and Dalmasso (1975) reported that esterase patterns of *Meloidogyne* females dissected from galled roots varied when nematodes were cultured on different hosts. By contrast, Dickson *et al.* (1971), Hussey *et al.* (1972), Fargette (1987*b*) and Pais and Abrantes (1989), working with different populations of the same species, failed to show host-induced differences in esterase patterns.

The objective of this study was to determine if galled roots can be used as the basis of a rapid technique to detect esterase activity of female *Meloidogyne,* thus avoiding the need to separate females from the roots. Using this method, the consistency of patterns from *M. javanica, M. incognita* and *M. arenaria* on three different hosts was also examined.

Materials and methods

Nematode cultures and enzyme extraction

Populations of *M. javanica* Race 1 (North Carolina State University Acc. No. 83), *M. incognita* (from Portugal), *M. arenaria* Race 1 (NCSU Acc. No. 351), *M. hapla* (from Zimbabwe) and *M. enterolobii* (from Dr. M. Fargette, ORSTOM, France) were cultured on tomato, *Lycopersicon esculentum* (cv. Pixie), and *M. graminicola* (from Dr. R. A. Plowright, International Institute of Parasitology, St. Albans, UK) was cultured on upland rice (cv. IR-36); pot cultures were maintained in a glasshouse (25-27 °C; average relative humidity 80 %; day length of 16 h). For experiments using different hosts, *M. javanica, M. incognita* and *M. arenaria* were cultured on tomato, tobacco (*Nicotiana tabacum* cv. Whiteburley) and aubergine (*Solanum melongena* cv. Blackbell). Control extractions were done on uninfected roots from each host plant.

For tests on females, root galls were rinsed with glass distilled water (GDW) and females were dissected from root tissue and placed in an ice bath. Twenty females of each species were transferred into a centrifuge tube; a 40 µl solution of extraction buffer, containing 20 % glycerol, 2 % Triton X-100 and 0.01 g bromphenol blue as marker dye, was added to the tube. The nematodes were homogenized in the extraction tube with a small plastic pestle and centrifuged at 10 000 rpm for 10 min at 4 °C. The clarified supernatant was introduced immediately into the electrophoresis cell. The nematodes remained in an ice bath throughout this procedure.

For tests on infected roots, areas of galled roots containing females were cut, washed with GDW, and a weighed amount (300 mg wet weight) was placed in a microcentrifuge tube and either used immediately for analysis or stored at – 20 °C. Surface water was carefully removed with paper tissue. Carborundum powder was added and roots were homogenised in 40 µl of extraction buffer using a glass rod. The homogenate was centrifuged at 14 000 rpm for 10 min at 4°C. The clear supernatant was used for esterase determinations as above. Uninfected roots were processed in exactly the same manner.

Electrophoresis and esterase visualisation

The esterase isoenzymes present in the nematode and galled root samples were determined by native polyacry-

lamide gel electrophoresis in either a mini-slab gel apparatus (Atto, Japan) using gel size 90 mm W, 80 mm L × 1 mm thick or a standard gel apparatus (Atto, Japan) using gel size 138 mmW, 130 mmL × 1 mm thick. Gels were replicated at least three times. A 3 % acrylamide stacking gel and 7 % acrylamide separation gel were used. Samples of 20-25 µl were injected into the electrophoresis cell. The buffer system was essentially that of Laemmli (1970), except that Triton X-100 was substituted for dodecyl sulfate (SDS). A constant current of 15 mA was applied until the marker dye reached the stacking gel when the current was increased to 20 mA. When the marker dye reached the bottom of the separating gel, the power was turned off and the gel removed.

Esterase activity was determined by incubating the gels at 37 °C for 15-30 min in a freshly prepared solution of 100 mg Fast Blue RR Salt, 50 µg α, β-napthyl acetate and 50 µg α-napthyl butyrate dissolved in 5 ml acetone made up to 100 ml with 0.2 M tris-chloride buffer, pH 6.6. The reaction was stopped by adding 10 % acetic acid. Relative electrophoretic mobility (Rm) for each enzyme band was calculated as the ratio of its movement to that of the marker dye.

Results

Six different patterns of nonspecific α and β esterase (Dalmasso & Bergé, 1978), detected either from females or galled root extracts on polyacrylamide slab gel electrophoresis, were consistent and highly reproducible for each *Meloidogyne* species tested (Fig. 1A). *M. javanica* had three strong bands located at Rm 0.35, 0.42 and 0.47 and two very weak bands at Rm 0.39 and 0.49, while *M. incognita* had one strong band and one weak band at Rm 0.38 and 0.40, respectively; the band at Rm 0.62 was not consistently visible. *M. arenaria* exhibited two strong bands at Rm 0.42 and 0.45 and two faint bands at Rm 0.35 and 0.40. *M. hapla* had a strong and a weak band at Rm 0.39 and 0.40, respectively, and *M. enterolobii* gave four bands at Rm 0.23, 0.29, 0.32 and 0.38. Only one diffused band at Rm 0.20 was detected from female extracts of *M. graminicola*. Roots infected with *M. graminicola* showed five extra bands, two strong bands at Rm 0.52 and 0.75 and three weak bands at Rm 0.56, 0.58 and 0.72. All esterase bands, even the weak ones, detected from the female extracts were clearly observed in infected root extracts (Fig. 1A). There were two extra bands (at Rm 0.23 and 0.65) detected from infected tomato roots which were not detected in the control (Fig. 1A).

The Rm values for isoenzyme patterns of female extracts of *M. javanica, M. incognita* and *M. arenaria* dissected from three different hosts (tomato, tobacco and aubergine) were identical for each species (Fig. 1B) and consistent irrespective of whether dissected females or homogenized galled root pieces were used (Fig. 1C). Uninfected plant roots exhibited very weak bands; for example, tomato had one weak band at Rm 0.71 and a diffuse band at Rm 0.12, while tobacco and aubergine each gave two weak bands but at different positions : Rm 0.19 and 0.67; Rm 0.50 and 0.73, respectively.

Discussion

This work shows that galled root pieces can be used as the basis of a rapid method to characterise and identify the main species of *Meloidogyne* using polyacrylamide gel electrophoresis to detect nonspecific esterase phenotypes. The use of galled roots saves considerable time as females do not have to be dissected from the roots; the method also avoids protein contamination and enzyme degradation which may be associated with other protocols (Dickson *et al.*, 1971; Jones, 1980). Esterase phenotypes of *M. javanica, M. incognita* and *M. arenaria* remained constant irrespective of whether they were cultured on tomato, tobacco or aubergine or whether isolated females or galled roots were used.

Comparison of the results of this study with previous reports shows some differences in certain Rm values and in the total number of bands that were resolved. Such variations could be the result of different techniques and/or laboratory conditions (Esbenshade & Triantaphyllou, 1985). In addition, many of the published reports did not include plates of the original gels so comparison of band resolution is not possible; procedures utilized in this study produced very consistent and sharp bands.

Novel esterase bands were found in extracts from infected plant roots. These bands were associated with the plant response to nematode invasion and could not be detected either in healthy root tissue or in extracts from dissected females. Although there is no information about esterase activity in root tissues, increase in the enzymatic activity of infected roots has been reported by Veech and Endo (1969) and Ibrahim (1991). Additional studies are required to define the role of these bands in the plant response to nematode invasion.

Acknowledgements

The authors are grateful to The Leverhulme Trust for financial support and to Dr. Keith Davies for providing nematode material and for much helpful discussion.

References

Bergé, J. B. & Dalmasso, A. (1975). Caractéristiques biochimiques de quelques populations de *Meloidogyne hapla* et *Meloidogyne* spp. *Cah. ORSTOM Sér. Biol.*, 10 : 263-271.

Dalmasso, A. & Bergé, J. B. (1978). Molecular polymorphism and phylogenetic relationship in some *Meloidogyne* spp. Application to the taxonomy of *Meloidogyne*. *J. Nematol.*, 10 : 323-332.

Dalmasso, A. & Bergé, J. B. (1983). Enzyme polymorphism and the concept of parthenogenetic species, exemplified by *Meloidogyne*. *In :* Stone, A. R., Platt, H. M. & Khalil, L. F. (Eds). *Concepts in nematode systematics*. New York & London, Academic Press : 187-196.

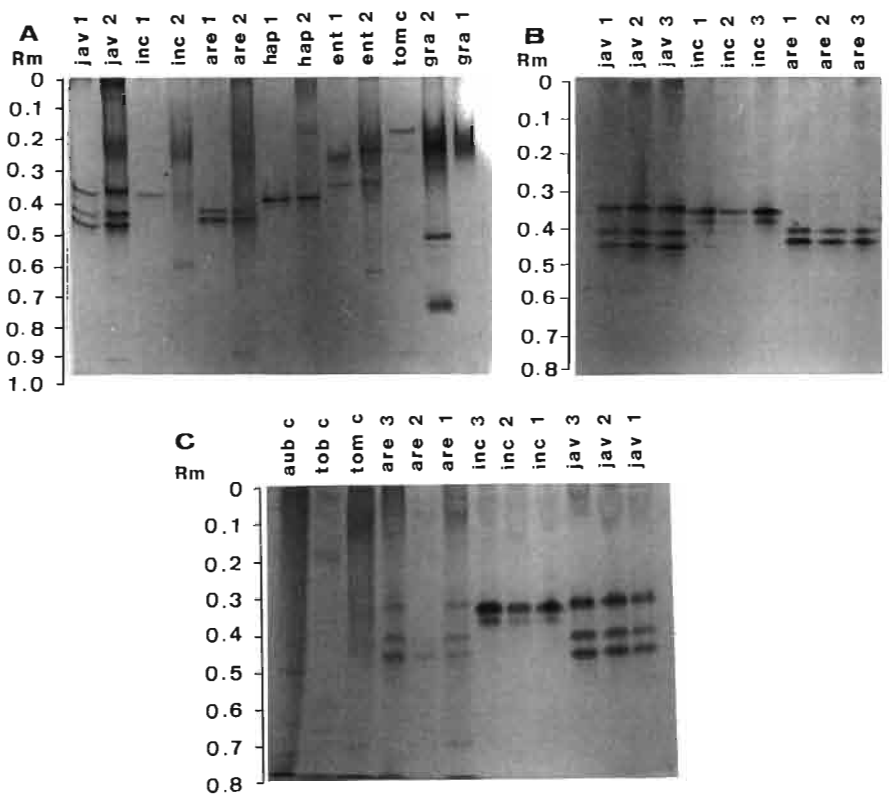

Fig. 1. A : Esterase phenotypes of *Meloidogyne* spp. from (1) females and (2) galled root pieces. All species cultured on tomato plants, except for *M. graminicola* cultured on rice; B : Esterase phenotypes of females of three *Meloidogyne* species cultured on (1) tomato (2) tobacco and (3) aubergine; C : Esterase phenotypes of galled root pieces of three *Meloidogyne* pieces cultured on (1) tomato (2) tobacco and (3) aubergine; (c) uninfected root pieces.

DICKSON, D. W., HUISINGH, D. & SASSER, J. N. (1971). Dehydrogenases, acid and alkaline phosphatases, and esterases for chemotaxonomy of selected *Meloidogyne, Ditylenchus, Heterodera* and *Aphelenchus* spp. *J. Nematol.,* 3 : 1-16.

EISENBACK, J. D. (1985). Diagnostic characters useful in the identification of the four most common species of root-knot nematodes (*Meloidogyne* spp.). *In :* Sasser, J. N. & Carter, C. C. (Eds). *An advanced treatise on* Meloidogyne. *Volume 1. Biology and control,* Raleigh : North Carolina State University Graphics : 95-112.

ESBENSHADE, P. R. & TRIANTAPHYLLOU, A. C. (1985). Use of enzyme phenotypes for identification of *Meloidogyne* species. *J. Nematol.,* 17 : 6-20.

ESBENSHADE, P. R. & TRIANTAPHYLLOU, A. C. (1990). Isozyme phenotypes for the identification of *Meloidogyne* species. *J. Nematol.,* 22 : 10-15.

FARGETTE, M. (1987*a*). Use of the esterase phenotype in the taxonomy of the genus *Meloidogyne*. 1. Stability of the esterase phenotype. *Revue Nématol.,* 10 : 39-43.

FARGETTE, M. (1987*b*). Use of the esterase phenotype in the taxonomy of the genus *Meloidogyne*. 2. Esterase phenotypes observed in West African populations and their characterization. *Revue Nématol.,* 10 : 45-55.

HIRSCHMANN, H. (1985). The genus *Meloidogyne* and morphological characters differentiating its species. *In :* Sasser, J. N. & Carter, C. C. (Eds). *An advanced treatise on* Meloidogyne. *Volume 1. Biology and control.* Raleigh, North Carolina State University Graphics : 79-93.

HUSSEY, R. S. (1971). A technique for obtaining quantities of living *Meloidogyne* females. *J. Nematol.,* 3 : 99-100.

HUSSEY, R. S. (1990). Biochemical and molecular methods of identifying *Meloidogyne* species. *J. Nematol.,* 22 : 8-9.

HUSSEY, R. S., SASSER, J. N. & HUISINGH, D. (1972). Disc-electrophoretic studies of soluble proteins and enzymes of *Meloidogyne incognita* and *M. arenaria. J. Nematol.,* 4 : 183-189.

IBRAHIM, S. K. (1991). Peroxidase isoenzymes from *Meloidogyne* spp. cultured on different hosts. *Revue Nématol.,* 14 : 335-344.

ISHIBASHI, N. (1970). Variations of the electrophoretic protein patterns of Heteroderidae (Nematoda : Tylenchida) depending on the developmental stage of the nematode and on the growing conditions of the host plants. *Appl. Ent. Zool.,* 5 : 23-32.

JANATI, A., BERGÉ, J. B., TRIANTAPHYLLOU, A. C. & DALMASSO, A. (1982). Nouvelles données sur l'utilisation des isoestérases pour l'identification des *Meloidogyne. Revue Nématol.,* 5 : 147-154.

JONES, M. G. K. (1980). Micro-gel electrophoretic examination of soluble proteins in giant transfer celles and associated root-knot nematodes *(Meloidogyne javanica)* in balsam roots. *Physiol. Pl. Pathol.,* 16 : 359-367.

LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature,* 227 : 680-685.

NOBBS, J. M., IBRAHIM, S. K. & ROWE, J. (1992). A comparison of the four cyst nematode species, *Heterodera elachista, H. oryzicola, H. oryzae* and *H. sacchari* (Nematoda : Heteroderidae), known to attack rice *(Oryzae sativa)* using morphological and biochemical techniques. *Fund. appl. Nematol.,* 15 : 551-562.

PAIS, C. S. & ABRANTES, I. M. de O. (1989). Esterase and malate dehydrogenase phenotypes in Portugese populations of *Meloidogyne* species. *J. Nematol.,* 21 : 342-346.

VEECH, J. A. & ENDO, B. Y. (1969). The histochemical localization of several enzymes of soybeans infected with the root-knot nematode *Meloidogyne incognita acrita. J. Nematol.,* 1 : 265-276.