Nematol. medit. (2001), 29: 63-66

Istituto di Nematologia Agraria, C.N.R., 70126 Bari, Italy

POLYMORPHISM OF ESTERASE ISOZYME ZYMOGRAMS OF *MELOIDOGYNE* POPULATIONS DETECTED BY PHASTSYSTEM

by

S. MOLINARI

Summary. Esterase isozyme patterns obtained with an automated electrophoretic apparatus have been used to detect phenotypic variability of forty-one *Meloidogyne* populations collected from countries throughout the world. Three previously undetected phenotypes have been found along with those commonly ascribed to the four most diffused species *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla*. Two of such undescribed phenotypes belonged to tropical populations. Nine different phenotypes could be distinguished thus indicating a high degree of polymorphism within the populations tested.

Isozyme electrophoretic patterns were first used as specific markers of *Meloidogyne* species by Dickson *et al.* (1971). Since then increasing numbers of *Meloidogyne* populations have been screened by this technique improving the rate and the reproducibility of the analysis by more and more sophisticated electrophoretic equipment (Esbenshade and Triantaphyllou, 1990). Automated systems, such as the PhastSystem, which became available at the end of the 1980s, are widely used at present for rapid species identification (Yong Fang *et al.*, 1998). Moreover, identification of populations has become easy, quick and reliable by using esterase zymograms compared with known standards and this is most useful for routine work. The minimal amount of proteins required by such methods permits the analysis of single females and identification of mixed populations which can give an estimation of the relative proportion of different *Meloidogyne* species in nematode-dynamics studies (Esbenshade and Triantaphyllou, 1990; Karssen *et al.*, 1995).

Previous surveys of high numbers of *Meloidogyne* populations from all over the world have demonstrated that zymograms are useful tools for testing genetic variability and evolutionary relationships within the genus (Esbenshade and Triantaphyllou, 1985, 1987). The PhastSystem has already been used to detect isozyme polymorphism of different nematode genera such as *Heterodera*, *Meloidogyne* and *Xiphinema*, and species identification of longidorids (Abrantes *et al.*, 1994; Molinari *et al.*, 1996, 1997; Lamberti *et al.*, 1999a, 1999b; Crozzoli *et al.*, 2000).

In this paper an additional survey of several *Meloidogyne* populations from all over the world based on esterase phenotypes was carried out by using the PhastSystem which has increased the availability of different esterase phenotypes mainly from tropical countries.

Materials and methods

Forty-one *Meloidogyne* populations collected from India, United States of America (California), Venezuela, Cuba, Portugal, Spain, Egypt, Tunisia, Switzerland and Italy were cultured

from a single egg mass on susceptible tomato cv. Roma VF in a glasshouse. Samples for electrophoresis were one or a few females hand-picked from infested roots. After rinsing in water, the nematodes were transferred to an Eppendorf-shaped, miniature homogenizer (Biomedix, UK) containing a minimal volume of a grinding buffer consisting of 20% sucrose, 0.1 M Trizma-Base, 0.08 M boric acid, pH 8.4, 2.5 mM EDTA, 5 µg bromophenol blue and the protease inhibitors PMSF (1 mM), pepstatin (1 µM) and leupeptin (1 µM). Then the females were homogenized on ice using a small plastic pestle connected to a rotor and centrifuged at 10,000 rpm for 3 min in a bench centrifuge. Supernatants (4 µl) were immediately used as samples in electrophoretic runs or stored at -80 °C.

Proteins were separated by native polyacrylamide gel electrophoresis using a PhastSystem equipment (Pharmacia Biotech, USA), which permits pre-programming of the chosen separation method. Reproducibility of the runs is ensured by microprocessor control and by the use of precast 0.45 mm thick gels whose separation zone is only 3.8 x 3.3 cm. A 2000 V power supply and an efficiently cooled thermostated separation bed allow field strengths as high as 500 V/cm to be achieved, thus giving high speed and high resolution separations. Two mini-gels can be placed in the separation bed so that up to 24 samples can be screened with each run, although the applicator combs used permitted a loading of 6 samples (approx. 4 µl/sample) per gel. The buffer for native PAGE (0.88 M L-alanine, and 0.25 M Tris, pH 8.8) is supplied in ready-to-use 3% agarose buffer strips. Precast gels consist of a 4.5% acrylamide stacking zone and a separation zone with an acrylamide gradient between 8 and 25% (2% crosslinking), buffered in 0.112 M acetate and 0.112 Tris, pH 6.4. A pre-run of 20 Vh (volthours) with the current set at 5 mA proceeded each run in order to initiate the moving boundary of the leading and trailing ions (acetate/L-alanine); the samples were automatically loaded and proteins separated according to their size and charge. The separation bed was cooled to 15 °C throughout the run which was stopped when the marker dye reached the bottom of the gel. The whole run usually lasted 145-150 Vh, approx. 30 min with the program used, giving a highly reproducible and quick separation.

Gels were stained for esterase activity in the Development Unit of PhastSystem. This is a chamber for staining and de-staining maintained at a controlled temperature in which gels are gently stirred throughout the staining procedure. The staining solution consisting of α- and ß-naphtyl-acetate (0.2 mg/ml each) and Fast Blue RR (0.5 mg/ml) was filtered through 0.5 µm nitrocellulose filters and used immediately. The staining procedure was carried out at 37 °C for 30 min. Afterwards the staining gels were dried and scanned by means of a ScanJet II cx (Hewlett Packard). Image files were printed on photo quality paper.

Results and discussion

Nine different phenotype were detected in the forty-one *Meloidogyne* populations analyzed (Figs 1 and 2), taking into account only the major bands. Most of the populations were identified as *M. hapla* (H1), *M. incognita* (I1), *M. javanica* (J1-J2), *M. arenaria* (A1-A2). Three different phenotypes (sp1, sp2, sp3) could not be identified to species. Two of the phenotypes (sp1, sp2) belonged to populations from tropical countries (Venezuela, Cuba). A population from Venezuela and one from Cuba produced an identical zymogram (sp2); both populations were virulent against *Mi*-bearing tomato cultivars. Most of the populations (60%) showed the typical phenotype of *M. incognita*. The predominance of this species has been previously reported in an extensive survey carried out on 291 populations from all over the world (Esbenshade and Triantaphyllou, 1985). However, the three atypical profiles shown in this paper were

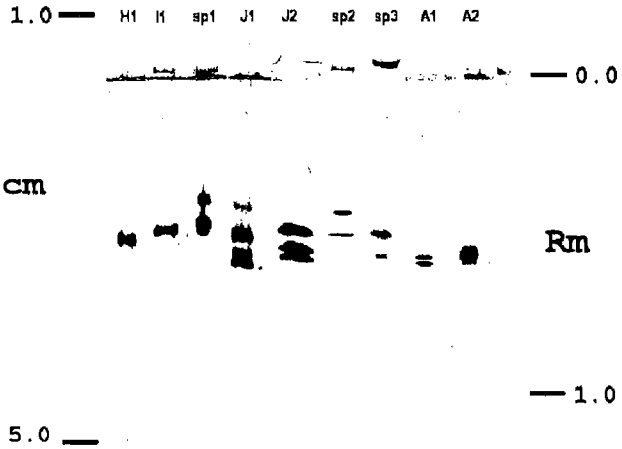

Fig. 1 - Esterase electrophoretical profiles of *Meloidogyne* populations. The figure is a print on ultra glossy picture paper of scanned images of minigels. Lanes are indicated as follows: *M. hapla* (H1), *M. incognita* (I1), *M. javanica* (J1-J2), *M. arenaria* (A1-A2), sp1, sp2 and sp3 are previously undetected phenotypes. Relative electrophoretic mobility (Rm) is 0.0 at the loading point and 1.0 at the front line visualized by bromophenol blue at the end of the run. The centimeter scale indicates the actual dimensions of the gel.

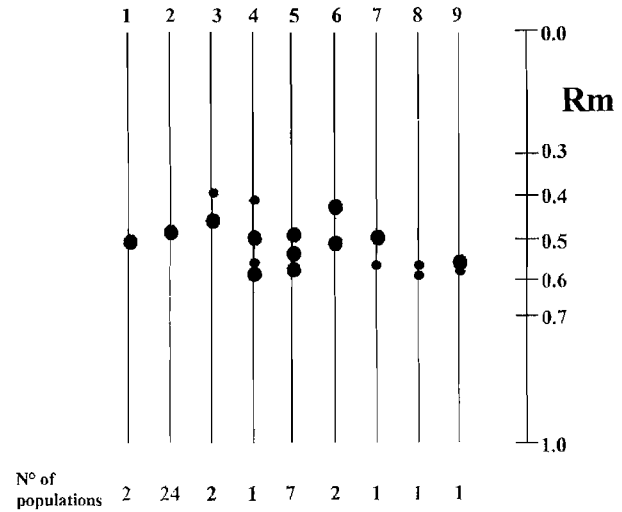

Fig. 2 - Esterase phenotypes observed in 41 *Meloidogyne* populations collected from all over the world. Lane numbers correspond to phenotype designations used in Fig. 1.

not reported in such or other previous work (Pais *et al.*, 1989; Cenis *et al.*, 1992; Carneiro *et al.*, 1996). A more accurate characterization of these undetermined populations by using morphometric parameters, different enzymes and DNA recombinant techniques are needed to define such populations as new species. This work will be attempted in the near future.

Finally, the data of this paper give evidence that the polymorphism of *Meloidogyne* spp. expressed as esterase phenotypes is progressively increasing as more populations from different geographic areas, especially the tropics, are screened.

Acknowledgements. The author thanks Prof. Franco Lamberti of the Nematology Institute of Bari, Italy, for providing *Meloidogyne* populations.

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Accepted for publication on 12 January 2001.