Indian J. Nematol. 28 (2) : 105-109 (1998)

ESTERASE AND MALATE DEHYDROGENASE PATTERNS OF RACES OF *HETERODERA CAJANI* AND *H. ZEAE*

SHASHI SINGH, SATISH KUMAR, H.K. BAJAJ AND A.K. GANGULY*

Department of Nematology, CCS Haryana Agricultural University, Hisar-125 004

Abstract: The isozyme patterns of esterases and malate dehydrogenases of two races of *H. cajani* and three races of *H. zeae* were analysed by native polyacrylamide gel-electrophoresis. The reproducible banding patterns of races of both the nematodes differed in number, concentration and relative mobility.

Key words: Electrophoresis, Esterase, *Heterodera cajani, Heterodera zeae*, Malate dehydrogenase, Phenotypes, Race.

H. cajani Koshy, 1967 and *H. zeae* Koshy *et al.*, 1971 parasitize economically important crops in Northern India causing considerable losses. Walia & Bajaj (1986, 1988) reported the existence of two host races viz. pigeonpea race (PPR, race A) and clusterbean race (CBR, race B) in *H. cajani*, differentiable on the basis of their reproduction on clusterbean and sunhemp. Similarly Bajaj & Gupta (1994) reported two races of *H. zeae* differentiated on the basis of their abilities to reproduce on maize and vetiver. Since classical taxonomic studies comparatively ineffective in differentiating the host races, their identification based upon esterase (EST) and malate dehydrogenase (MDH) isozymes using polyacrylamide gel-electophoresis (PAGE) was attempted. Such techniques have recently been proved as effective methods of distinguishing genera, closely related species and races (Dickson *et al.*, 1971; Hussey & Krusberg, 1971; Dalmasso & Berge, 1978, 1983, Pozdol & Noel, 1984).

MATERIALS AND METHODS

Propagation of culture

H. cajani : Pure cultures of two populations of *H. cajani* collected around the roots of pigeonpea and clusterbean, Hisar were raised in isolation on their respective hosts in the screen house of Department of Nematology, CCS HAU, Hisar. They were subjected to host differential test as described by Walia and Bajaj (1986, 1988), to identify their host races which were consequently designated to represent race A (pigeonpea race) race B (clusterbean race) of this species.

H. zeae: Similarly, pure cultures of three populations of *H. zeae* viz., maize, Ambala; vetiver, Sonepat and vetiver, Hisar grown on their respective hosts were subjected to reproduce on vetiver, maize and both (Bajaj & Gupta, 1994) to identify their host races and were designated as race 1, 2 and 3, respectively.

20-30 white young females of each race of two species were homogenized separately in 20 µl of extraction medium (20% sucrose and 2% Triton x-100) for EST in microhaematocrite tubes (one end

* Division of Nematology, I.A.R.I., New Delhi.

scaled). After homogenization the macerates were frozen immediately after extraction in a freezer at -10°C in air tight labelled vials. Nematodes to be assayed for MDH activity were also extracted in a similar manner but in tris-buffer (Trudgill & Carpenter, 1971).

Shortly before electrophoresis, the frozen samples were thawed and centrifuged at 15000g for 15 minutes in a microhaemotocrite centrifuge maintained at 0°C. The clear aqueous phase was then used directly for electrophoresis. EST and MDH isozymes present in nematode samples were determined by native polyacrylamide gel-electrophoresis in a standard gel apparatus (Atto, Japan) using gel size 138 mm (W), 130 mm (L) x 1 mm thick.

The electrophoresis was carried out on 7% acrylamide gel pH 8.4 (for esterase) and pH 8.0 (for MDH) as per the procedure of Ganguly & Dasgupta (1989). The gels were visualised following the method of Janati *et al.* (1982).

After staining, the gels were rinsed with distilled water and fixed in 7% acetic acid distilled water solution and then photographed.

RESULTS AND DISCUSSION

The result of this investigation is presented in Fig. 1. Both the populations of *H. cajani* and three population of *H. zeae* were found to be polymorphic for esterase and MDH.

H. cajani

EST : Phenotype designated as Hc (EST3 were observed only in race A, with Rf at 0.675 and 0.766, but, race B exhibited a distinct phenotype designated as Hc (B) EST5 with Rf at 0.58, 0.633, 0.675, 0.675, and 0.766. Thus, three of the major bands (electrophoretic form) were detected common in race A and B but two distinct bands with Rf 0.58 and 0.633 were associated only with race B.

MDH : Six bands of MDH activity were detected among two races of *H. cajani* studied. These bands were designated numerically 1 to 6, according to increasing electrophoretic mobility towards the anode (Fig. 1). MDH phenotypes of two races were designated as Hc (A)MDH3 and Hc (B)MDH4 for race A and race B, respectively. The relative migration rate of MDH phenotypes were 0.25, 0.269 and 0.294 for race A and 0.117, 0.294, 0.318 and 0.353 for race B. One band at 0.294 was common in both races of *H. cajani*.

H. zeae

EST : Distinct phenotypes designated as Hz(1) EST3 and Hz(3) EST3 were detected in the EST system for race 1 and race 3, respectively. They differed from the race 2 phenotype designated as Hz(2) EST2. Races 1 and 3 had three electromorphs located at Rf 0.384, 0.430 and 0.477. However, race 2 shared two bands with race 1 and 3 (Rf 0.43 and 0.488). The first band of race 2 with Rf. 0.43 were matched with the second band of race 1 and race 3.

MDH : The phenotypes resolved for race 1, 2 and race 3 were Hz (1)MDH3, Hz (2)MDH3 and Hz (3)MDH5, respectively. The common relative migration rates of the MDH bands were 0.210 and 0.40 for all the three races of *H. zeae* and at 0.179 in race 1 and race 3. Additional bands with Rf at 0.388 and 0.73 were observed only in race 3 and a band at Rf 0.701 only in the race 2. From the data presented in this report clearly shows that EST and MDH isozymes pattern were useful for discriminating races of *H. cajani* and *H. zeae*.

Fig. 1. Est
race

Fig. 2 : Est
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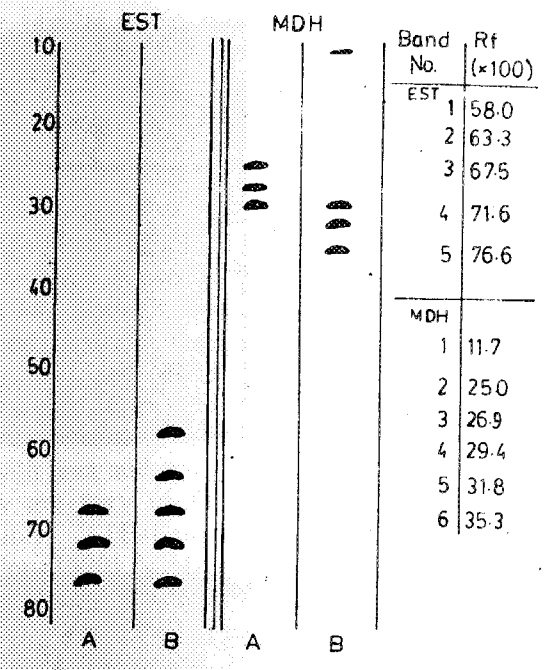

Fig. 1. Esterase and malate dehydrogenase phenotypes in pigeonpea race (Race A) and clusterbean race (Race B) of *H. cajani.*

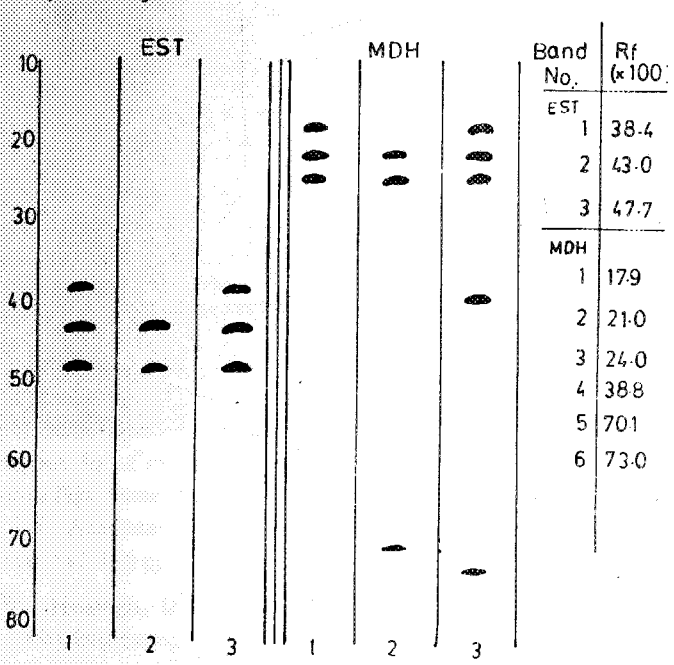

Fig. 2 : Esterase and malate dehydrogenase phenotypes in Maize, Ambala (race 1), Vetiver, Sonepat (race 2) and Vetiver Hisar (race 3) of *H. zeae.*

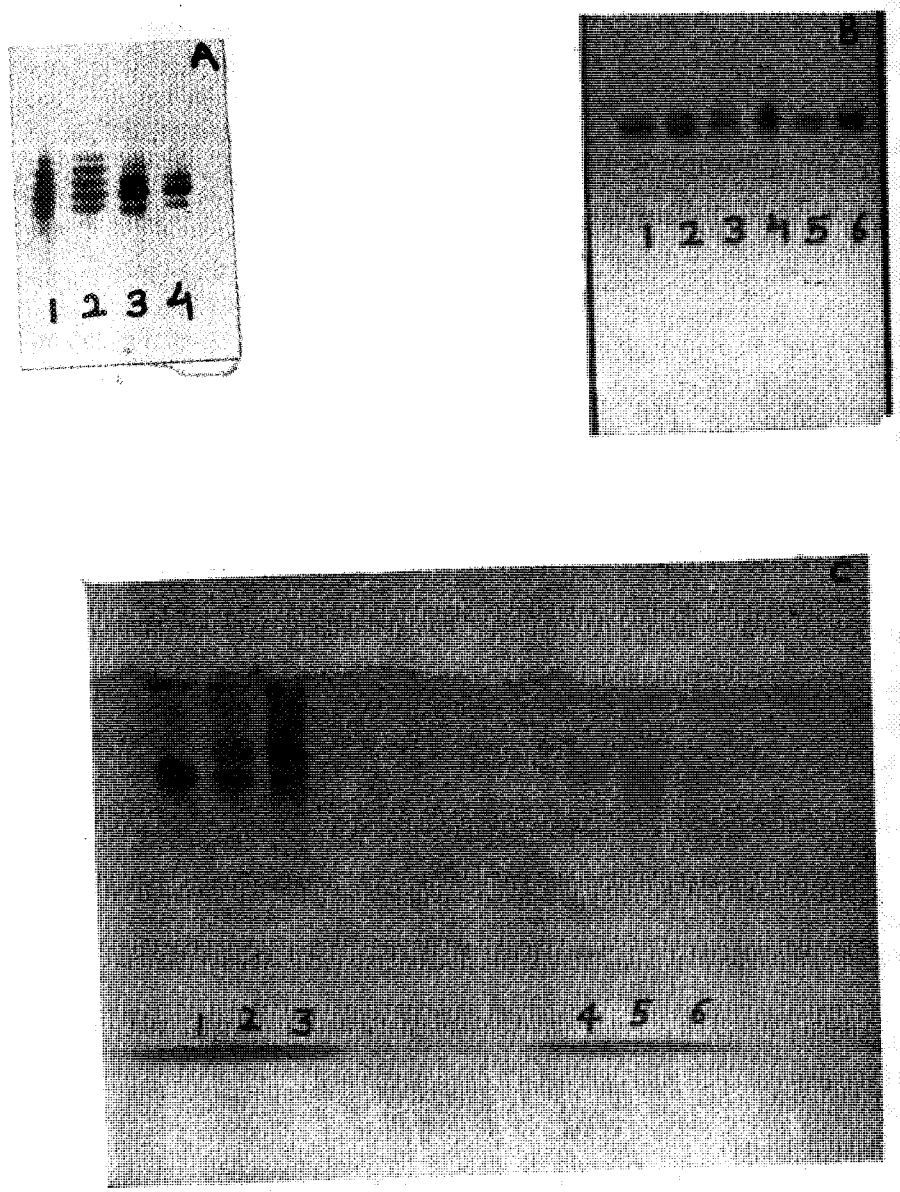

Fig. 3.

(a) Esterase phenotypes of *H. cajani* : 1-2 race-B, 3-4, race -A.

(b) Esterase phenotypes of *H. zeae* : 1-2 race-1, 3-4 race-3, 5-6 race-2.

(c) Malate dehydrogenase phenotypes -*H. cajani* : 1 race A, 2 and 3 race B; *H. zeae;* 4 race-3, 5 race-1 and 6 race-2.

Similar results were obtained by Huettel *et al.*, (1982) for citrus and banana races of *Radopholus similis* which were later on designated as *R. citrophilus* and *R. similis*, respectively. Four races of *H.glycines* were separated successfully into two groups by using SDS-PAGE system and this system also exhibited numerous banding differences between *H. glycines* and *H. schachtii*, distinguishing each from *H. trifolii* and *H. lespedezae* (Pozdol & Noel, 1984). EST and MDH phenotypes of different races of *M. incognita, M. hapla* and *M. arenaria* are, however, not correlated with host and cytogenetic races though they are very useful in species identification (Esbenshade & Triantaphyllou, 1990). Nobbs *et al.* (1992) reported that non-specific EST banding patterns can be used in the separation of *H. elachista, H. oryzicola, H. oryzae* and *H. sacchari* and help to resolve the problems of morphological similarities.

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Accepted for publication, March, 1998

B; *H. zeae;* 4 race-3, 5 race-