VARIABILITY OF ENZYME PHENOTYPES IN A POPULATION OF *NACOBBUS ABERRANS* (NEMATODA: TYLENCHIDA) FROM CORDOBA, ARGENTINA

M.E. Doucet[1], E.E. Montamat[2] and A.L. Giayetto[3]

[1] *Laboratotio de Nematología, Centro de Zoologia Aplicada, Facultad de Ciencias Exactas, Fisicas y Naturales, Universidad Nacional de Córdoba, C.C. 122 (5000)*
[2] *Cátedra de Quimica Biológica, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba. C.C. 35, Suc. 16 (5016), Córdoba, Argentina*

Summary. Enzymatic variation was evaluated within a population of *Nacobbus aberrans* from the South of Córdoba, Argentina. Single females extracted from infected roots of quinoa (*Chenopodium album*) and tomato (*Lycopersicon esculentum*) were assayed for 15 enzymes by polyacrylamide gel electrophoresis. Of the 15 enzyme systems investigated, only esterases (EST), malate dehydrogenase (MDH), acid phosphatase (AcP), phosphoglucose isomerase (PGI), lactate dehydrogenase (LDH), ß hydroxibutyrate dehydrogenase (ß-BHD), malic enzyme (ME) and mannose phosphate isomerase (MPI) were detected. Seven distinct phenotypes were found in EST. No phenotypic differences in enzyme patterns were observed between females isolated from quinoa and tomato, indicating that the enzymatic variation was not related to the host plant.

Nacobbus aberrans is widely distributed in Argentina (Doucet, 1989). It is present in diverse geographical areas and is associated with cultivated and non-cultivated plants (Costilla, 1985; Doucet, 1992a). It has been demonstrated that there are significant differences in several morphometrics characters among populations of the species of different origin (Doucet and Di Rienzo, 1991).

Preliminary analyses of isosyme electrophoretic phenotypes revealed a certain level of variability of polymorphisms representing a higher number of genetic loci among and within populations (Doucet and Gardenal, 1992; Ibrahim *et al.*, 1997). Intraspecific variability is also implied by reports of several nematode populations differing in behaviour on a similar plant suggesting the existence of physiological races (Costilla, 1986; 1990). The group of the populations studied presented a marked heterogenicity with respect to host range and pathogenicity (Costilla, 1986; 1990; Baldwin and Cap, 1992). Therefore, it has been considered that *N. aberrans* would comprise different biological entities (Doucet and Di Rienzo, 1991; Doucet, 1992b). Host range and virulence, as well as other phenotypic characters, might simply represent alleles that vary in their frequencies within particular populations.

The aims of the present study were to assess the variation within a population of *N. aberrans* expressed by fifteen enzyme systems analyses; and to determine whether the host affects the enzyme profiles.

MATERIAL AND METHODS

Young females and egg masses of *N. aberrans* (Thorne) Thorne *et* Allen were removed from quinoa (*Chenopodium album* L.) infested roots collected in Rio Cuarto (Córdoba Province, Argentina). The population was reared, under glasshouse conditions, on tomato plants (*Lycopersicon esculentum* Mill.) inoculated with 100 infective juveniles (J2). After 60 days mature females were extracted from the roots. Only those females having small white egg masses were selected. Females collected from quinoa and tomato roots were transferred individually to microhematocrit tubes, previously heated and sealed at one end, containing 5 µl per tube of 0.5 M tris-HCl/40% glycerin and bromophenol blue 0.002%, pH 8.6. Nematodes were macerated on ice with a Hamilton microsyringe pestle. The homogenates were immediately frozen at -20 °C until subjected to electrophoresis in polyacrylamide gels (Ogita and Markert, 1979). For each enzyme analyzed, 30 single female extracts from quinoa and tomato were processed. After protein separation, the gels were stained for specific enzymes (Table I).

The intrapopulation variability was evaluated according to the number of bands and their electrophoretical mobility towards the anode considering the origin for each enzyme. The relative frequencies of the enzymatic patterns obtained from *N. aberrans* extracted from quinoa and tomato were also calculated.

RESULTS

A considerable intrapopulation variability was observed for each enzyme. Of the 15 enzyme systems analyzed only EST, MDH, AcP and PGI were highly reproducible; ten bands of EST activity were detected and seven distinct phenotypes were recognized.

The MDH and PGI systems revealed three phenotypic groups with different band patterns for each enzyme. A null allele in MDH2 and the typical design of a locus with two alleles in PGI suggest a heterozygoty. Two phenotypes were observed for AcP each characterized by two electromorphs (Figs 1 and 2).

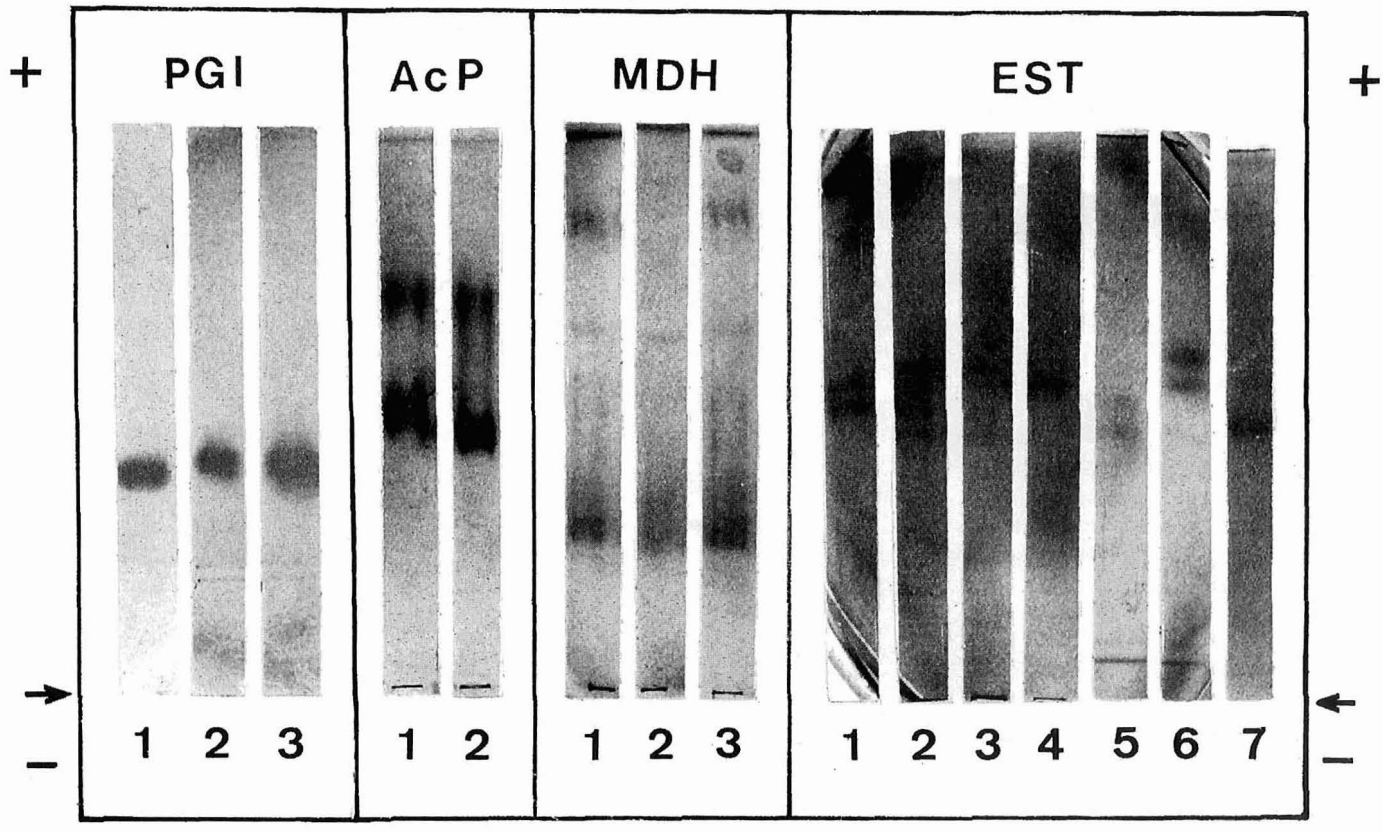

Fig. 1. Electrophoretic phenotypes of phosphoglucose isomerase (PGI), acid phosphatase (AcP), malate dehydrogenase (MDH), and esterases (EST) detected in a population of *Nacobbus aberrans*. Arrows indicate the origin.

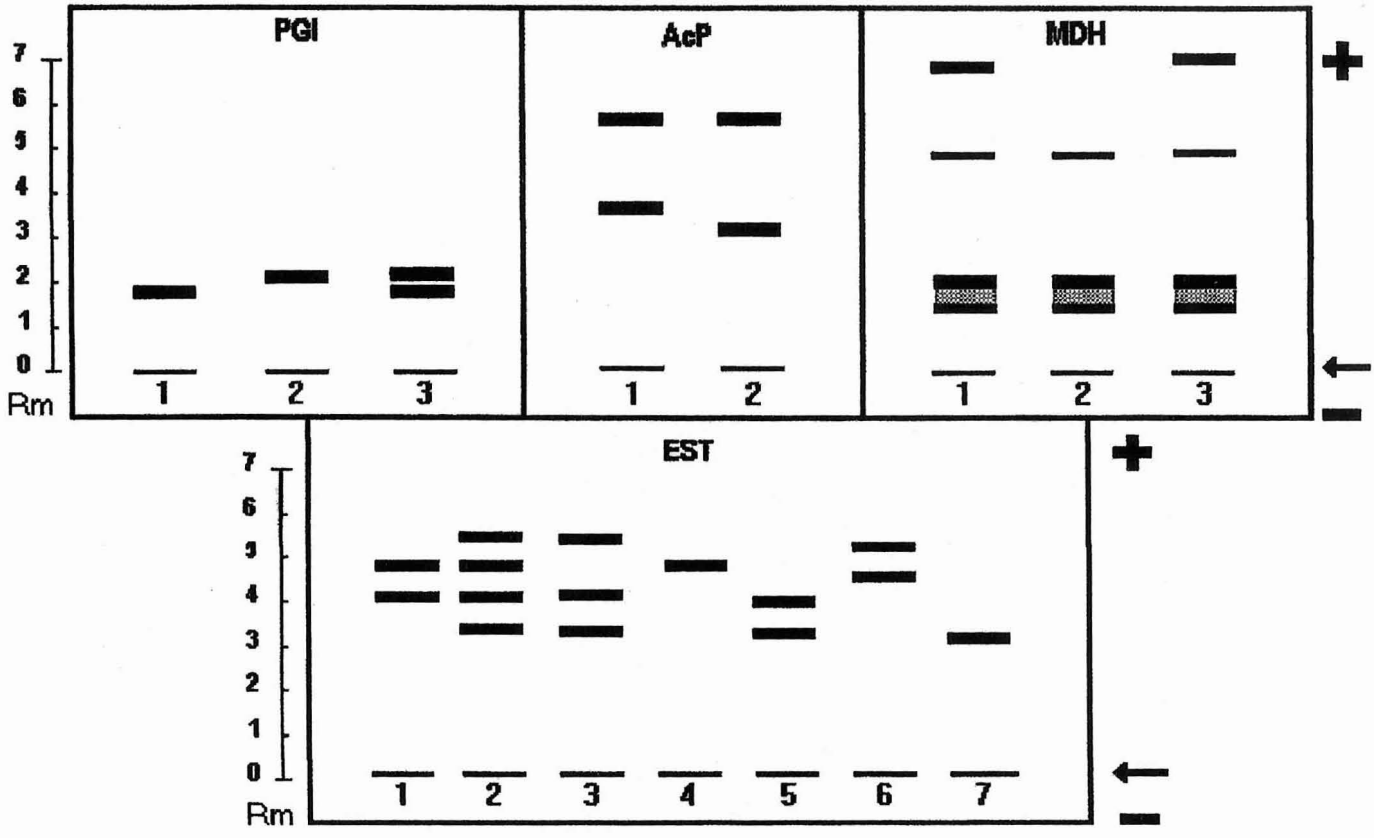

Fig. 2. Schematic representation of electrophoretic patterns of a population of *N. aberrans*. PGI - AcP - MDH - EST. Arrows indicate the origin.

Table I. Enzyme examinated, stain and references used in the investigation of *Nacobbus aberrans*.

Enzyme	Enzyme Commission Number	Reference
Hexokinase (HK)	E.C. 2.7.1.1	Esbenshade and Triantaphyllou, 1987
Catalase (CAT)	E.C. 1.11.1.6	Esbenshade and Triantaphyllou, 1987
Glucose 6 phosphate dehydrogenase (G-6-PIDH)	E.C. 1.1.1.49	Montamat *et al.*, 1987
Phosphoglucomutase (PGM)	E.C. 2.7.5.1.	Montamat *et al.*, 1987
Isocitrate dehidrogenase (ICD)	E.C. 1.1.1.42	Esbenshade and Triantaphyllou, 1987
Alcohol dehydrogenase NADP+ dependent (ADH NADP+)	E.C. 1.1.1.2	Montamat *et al.*, 1987
Aspartate aminotransferase (AAT)	E.C. 2.6.1.1	Montamat *et al.*, 1987
Malate dehidrogenase (MDH)	E.C. 1.1.1.37	Esbenshade and Triantaphyllou, 1987
Malic enzyme (ME)	E.C. 1.1.1.40	Montamat *et al.*, 1987
Lactate dehydrogenase (LDH)	E.C. 1.1.1.27	Esbenshade and Triantaphyllou, 1987
ß Hydroxybutyrate dehydrogenase (ßHBD)	E.C. 1.1.1.30	Esbenshade and Triantaphyllou, 1987
Acid phosphatase (AcP)	E.C. 3.1.3.2	Esbenshade and Triantaphyllou, 1987
Phosphoglucose isomerase (PGI)	E.C. 5.3.1.9	Esbenshade and Triantaphyllou, 1987
Esterases (EST)	E.C. 3.1.1.1	Esbenshade and Triantaphyllou, 1987
Mannose-phosphate isomerase (MPI)	E.C. 5.3.1.8	Esbenshade and Triantaphyllou, 1987

LDH and ßHBD were monomorphic presenting only one band with high mobility (data not shown).

The analysis of ME and MPI indicated a certain variability. However these enzymes were not considered due to the low activity observed. HK, CAT, G-6-PDH, PGM, IDH, ADH NADP+ and AAT did not show activity. Female nematode extracted from quinoa exhibited enzymatic patterns identical to those obtained from females extracted from tomato. The relative frequencies of the phenotypes were also similar ($\chi^2 < 0.05$) (Table II).

DISCUSSION

The taxonomic status of *N. aberrans is* controversial because of its phenotypic variation. Intrapopulation variability in relation to enzymes in *N. aberrans* was suggested (Doucet and Gardenal, 1992; Ibrahim *et al.*, 1997) and is confirmed by the present results. EST appears as the most variable enzyme. Enzymes such as CAT, HK and AAT did not show activity as in previous studies where 20 females of the same population were analyzed (Doucet and Gardenal, 1992). MDH proved

Table II. Frequency of enzyme phenotypes from *N. aberrans* cultured on two host plants.

Enzyme	Phenotype	Relative frequency	
		Chenopodium album	*Lycopersicon esculentum*
PGI	1	6.50	11.50
	2	87.00	84.60
	3	6.50	3.80
AcP	1	73.33	54.55
	2	26.67	45.45
MDH	1	21.43	40.00
	2	64.28	53.33
	3	14.28	6.66
EST	1	20.00	21.43
	2	16.00	21.43
	3	4.00	7.14
	4	28.00	14.28
	5	16.00	14.28
	6	12.00	17.86
	7	4.00	3.57

to be monomorphic although it was related to different populations, whereas three distinct phenotypes have been revealed in the present work. On the contrary, it was reported that ME and EST showed polymorphism; however, this polymorphism could only be observed in EST. The intrapopulation variability detected in our investigation by EST analysis is not consistent with results obtained in recent studies (Ibrahim et *al.*, 1997). This is probably due to the use of different techniques. The most extensive enzymatic variation was detected in EST. This can be useful for comparative studies of populations of *N. aberrans*. However, further biochemical studies should be conducted in order to find a better marker for diagnostic purposes.

Comparison of the frequencies of the enzyme phenotypes obtained from single females isolated from quinoa or tomato did not reveal any influence of the plant host on the genetic expression. A similar situation has been demonstrated for other nematode species in relation to several hosts (Esbenshade and Trantaphyllou, 1987; Fargette, 1987; Ibrahim and Rave, 1995).

Analysies of the enzymes used and morphological and morphometrical variation already observed in this particular population (Doucet and Di Rienzo, 1991), confirm that *N. aberrans* must be considered as a particularly variable species.

ACKNOWLEDGEMENTS

The authors thank the Academia Nacional de Agronomía y Veterinaria, the Consejo de Investigaciones Científicas y Tecnológicas de la Provincia de Córdoba (CONICOR) (Res. 1618/98) and the Secretaría de Ciencia y Técnica (Res. 257/98) (Universidad Nacional de Córdoba), Argentina for their financial support.

LITERATURE CITED

Baldwin J.G. and Cap G.B., 1992 - Systematics of *Nacobbus*, the false root-knot nematode. Pp. 101 -112. In: *Nematology from molecule to ecosystem.* F.J. Gommers and P.W.T. Maas. Eds. Invergowfie, Scotland: European Society of Nematologists.

Costilla M.A., 1985 - El falso nematode del nudo *Nacobbus aberrans* (Thorne, 1935) Thorne & Allen, 1944 y su relación con el cultivo de papa en el noroeste argentino. *Revista industrial y agrícola de Tucumán, Argentina, 62*: 79-97.

Costilla M.A., 1986 - Comportamiento de diferentes poblaciones del falso nematodo del nudo *Nacobbus aberrans* (Thorne, 1935) Thorne y Allen, 1944 en cultivos de papa. VI Jornadas Fitosanitarias Argentinas, Actas, pp. 255-261; 8-11 de Abril, Neuquén, Argentina.

Costilla M.A., 1990 - Comportamiento e importancia de tres poblaciones de *Nacobbus aberrans* (Thorne, 1935) Thorne y Allen, 1944 en tomate y pimiento en tres localidades del noroeste argentino. *Nematropica, 20*: 2-3 (Abstr.)

Doucet M.E., 1989 - The genus *Nacobbus* Thorne & Allen, 1944 in Argentina. 1. Study of a population of *N. aberrans* (Thorne, 1935) Thorne & Allen, 1944 on *Chenopodium album* L. from Rio Cuarto, Province of Córdoba. *Revue de Nématoldgie, 12*: 17-26.

Doucet M.E., 1992a - Asociaciones entre nematodos fitófagos y malezas en la República Argentina. *Agriscientia, 9*:103-112.

Doucet M.E., 1992b - El complejo *Nacobbus* Thorne & Allen, 1944 (Nemata: Tylenchida) en Argentina. VIII Jornadas Fitosanitarias Argentinas, 8-11 de Setiembre 1992, Paraná Entre Ríos, Argentina. (Abstr.)

Doucet M.E. and Di Rienzo J.A., 1991 - EI género *Nacobbus* (Thorne, 1935) Thorne y Allen, 1944 en Argentina. 3. Caracterización morfológica y morfométrica de poblaciones de *Nacobbus aberrans* (Thorne, 1935) Thorne y Allen, 1944. *Nematropica, 21*: 19-35.

Doucet M.E. and Gardenal C.N., 1992 - The genus *Nacobbus* in Argentina. 4. Preliminary comparison of populations of *Nacobbus aberrans* (Thorne, 1935) Thorne y Allen, 1944 by means of isozyme phenotypes. *Nematropica, 22*: 243-246.

Esbenshade P.R. and Triantaphyllou A.C., 1987 - Enzymatic relationships and evolution in the genus *Meldidogyne* (Nematoda: Tylenchida). *Journal of Nematology, 19*: 8-18.

Fargette M., 1987 - Use of esterase phenotype in the taxonomy of the genus *Meloidogyne*. 1. Stability of the esterase phenotype. *Revue de Nematologie, 10*: 39-43.

Ibrahim S.K., Baldwin J.G., Roberts P.A. and Hyman B.C., 1997 - Genetic variation in *Nacobbus aberrans*: an aproach toward taxonomic resolution. *Journal of Nematology, 29*: 241-249.

Ibrahim S.K. and Rowe J.A., 1995 - Use of isoelectric focusing and polyacrylamide gel electrophoresis of non-specific esterase phenotypes for identification of the cyst nematodes *Heterodera* species. *Fundamental and Applied Nematology, 18*:189-196.

Montamat E.E., Arauzo S., Cazzulo J.J. and Subías E. 1987 - Characterization by electrophoretic zymograms of 19 *Trypanosoma cruzi* clones derived from two chronic chagasic patients. *Comparative Biochemistry and Physiology, 87*: 417-422.

Ogita S.O. and Markert C., 1979 - A miniaturized system for electrophoresis on polyacrylarnide gels. *Analytical Biochemistry, 99*: 233-241.

Accepted for publication on 15 October 2001