THE APPLICATION OF MICROSCALE ISOELECTRIC FOCUSING ON STUDYING THE DIFFERENTIATION OF POPULATIONS OF *HETERODERA GLYCINES*

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تطبيق طريقة الفصل الكهربائي الدقيق لدراسة تمييز عشائر من نيماتودا الحوصلات لفول الصويا *HETERODERA GLYCINES*

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ملخص البحث:

تم بحث شقوق الإنزيمات لعشائر مختلفة من نيماتودا الحوصلات لفول الصويا باستعمال طريقة الفصل الكهربائي الدقيق. وجد تباين في الإنزيمات وهي الايستيريزات، بيروكسيديزات والبولي فينول أوكسيديز للمجاميع الحوصلية. الأشكال الإنزيمية كانت مميزة لكل عشيرة ويبدو أن إنزيم الايستريز غير التخصصي أداة تصنيفية مفيدة لدراسة التنوع الوراثي ضمن المجموعة الواحدة.

ABSTRACT

Enzyme patterns were investingated in different populations of **H. glycines** using a micro-slab isoelectric focusing method. Variability was found in esterases, peroxidases and poly-phenoloxidases. Characteristics of electrophoretograms are given for Race 3, Race 4, Shinsei and Saga populations. It appears that non-specific esterases are a useful tool for the study of genetic variability within individual populations.

INTRODUCTION

Earlier investigations on species of meloidogyne using micro-electrophoresis of single-females demonstrated genetic variability within populations of five species(2). This approach is obviously of great value for studying phenotypic variability of specific characters. Esterases' patterns were found to be the most valuable marker for genetic polymorphism in two races of Heterodera avenae (1,5). Investigations by using electrophoresis on individual females from the. selected population, showed that four strains of H. avenae differed in allelic frequencies(10). Comparative studies of nematode proteins by gel electrophoresis have provided information that has been helpful in nematode identification and in elucidating relationships among various nematode species(3,4,8,12,). Since proteins, which are the products of ribo nucleic acids, are almost a direct reflection of the structure and function of the genes, this type of study is most important in demonstrating polymorphism, and these results strongly indicate the need for more of this type of micro-studies, using specific enzymes to define the genetic variability of the cyst forming.

The thin layer isoelectric technique is obviously an ideal method for such studies, providing that it can be modified to detect the small quantities of proteins involved in single cyst analysis. Accordingly a number of investigations were undertaken to test the feasibility of achieving this.

MATERIALS AND METHODS

The population of Heterodera glycines were reared on soybean, Glycine max, var. Lee, in green house at 20-28 °C.

The freshly collected white cysts were washed and cleaned to free them of any debris. They then surface-steilized for 30 minutes, in 0.5% milton solution. The cysts, after sterilization, were washed in buffer solution and each individual cyst was crushed separaately in the same buffer in a capillary homoginizer (1.2MMID and 50 mm long) made especially for this purpose.

Small wells 3.2Mm- were removed from the gel by means of a piece of brass tubing. Approximately 5 microlitres of the sample,which contained 12-14mg of proteins, were transferred by an algamicrometer syringe to the wells.

A perspex bridge was constructed carrying a series of small-bore stainless steel tubes. The bridge was designed so that it chipped on to the edge of the electrophorsis plate and the sample tubes were arranged so that they entered the gel. Amodified method of Hunter and Burstone (6) to make it suitable for thin layer isoelectrically focused gels was used to develope the non-specific esterases. A histochemical method described by pearse (9) was utilized for demonstrating peroxidase activity. Poly- phenoloxidase activity was detected by the method of Hussey and Sasser(7).

RESULTS

Esterase activity:

The esterase patterns were very consistent over many separate experiments. It should be noted that while the esterase detection methods are very sensitive, selection of individual cysts for size is an important factor.

Esterase isoenzymes were detected in pH 4-6 following their separation of single extracts of populations propagated on "Lee". Nine to eleven bands were resolved in the narrow range from Shinsei, Saga, Race 3 and Race 4 and are shown in figure(1). Four to six of these bands had active sites of esterase whereas the other bands had weak activity. The common band of these populations were a,b,e,n and o with Ef 0.07, 0.20, 0.42, 0.96, and 0.98, and isoelectric points of 3.92, 4.30,4.76, 6.22, and 6.40(table 1). Three populations (Shinsei, Race 3 and Race 4) had four active sites c (Ef 0.28), d(Ef 0.38), f(Ef 0.48) and h(Ef 0.55) with isoelectric points of 4.48, 4.66, 4.90, and 5.06 respectively. An additional site k with weak activity at Ef 0.63 and PI value of 5.34 was only present in Shinsei population.

Three sites g(Ef 0.51), I(Ef 0.58) and j(Ef 0.62) with PIS of 4.98, 5.14 and 5.26 were also present only in Saga population and Race 3 and m (Ef 0.78) in Shinsei and Saga populations at isoelectric points of 5.44 and 5.66 respectively.

Peroxidase activity:

Single cyst extracts were separated in the ampholine pH range 3-10 and tested for peroxidase activity. Six populations had a unique and characteristic pattern(Figure 2).

An iso-electrophoretogram showing the number and the Ef values of the isoenzymes found in the six populations is illustrated in Table (2). These isoenzymes had Ef values of 0.08, 0.28, 0.59, 0.82 and 0.89. The common bands were d (Ef 0.82) and e (Ef 0.89) with PIS of 8.07 and 8.42. Three populations (Race 3, Race 4, and Tenn.) had one active site a (Ef 0.08) with PI of 3.88 which was not observed in Shinsei, Saga and Akita populations. An additional band was detected, b (Ef 0.28) in Shinsei population and c (Ef 0.59) in Saga and Akita populations at isoelectric points of 4.90 and 6.50 respectively.

Poly-phenoloxidase activity (ppo):

The patterns of ppo activity from all populations of **H.glycines** were also commpared (Figure 3). Three intense bands were located for all populations at Ef 0.62, 0.69 and 0.74, while other faint bands at Ef 0.20, 0.26, 0,33 and 0.40 were also visible (Table 3). All the bands were present in the pH range 4.60 to 7.14.

Discussion:

While electrofocusing in thin layer permits the comparisons of many small samples simultaneously, there are occasions when this is inadequate. Often when nemaatodes which are not freely available are to be included in comparsons, there are logistic problem associated with obtaining an adequate quantity of matreial. Additionally, nematodes may mature very slowly on resistant hosts making it difficult to obtain adequate quantities. A somewhat different problem arises when it is desired to study allele frequencies in which individualy must be examined In all of these cases a micro-method is invaluable and it was found that the thin layer technique readily lends itself to such adaptations.

The data obtained in this study for the esterase system showed that this system was the most satisfactory one for investigating by this method and it is hoped that it will be exploited in future studies to further clarify isoenzymes of esterases, in racial complexes. However, single nematode extracts were first used by Dalmasso and Berge (2), who reported that the esterases

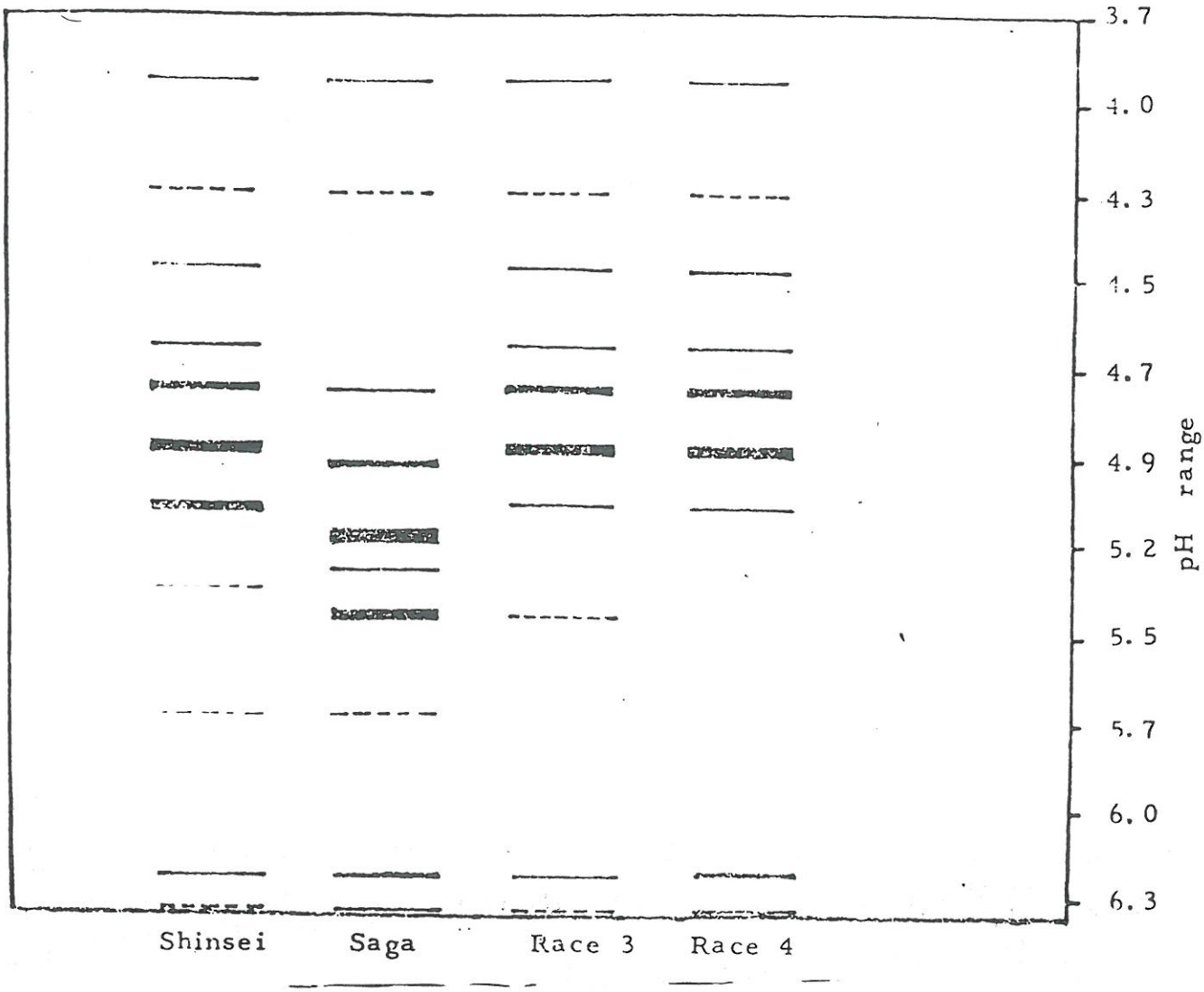

Fig. 1. Comparison of the esterase activity of extracts from single systs of four populations of H. gly cines cultured on Lee, separated in the pH range 4_6.

Table 1. Analysis of isoelectrophoretogram to show the relative E s and Pls of esterase isoenzymes detected from single extracts of four populations of H. glycines separated in the ampholine range pH 4-6. cultured on Lee.

Esterase isoenzyme	E_f	PI values	Shinsei	Saga	Race 3	Race 4
a	0.07	3.92	+	+	+	+
b	0.20	4.30	-	-	-	-
c	0.28	4.48	+		+	+
d	0.38	4.66	+		+	+
e	0.42	4.76	++	+	++	++
f	0.48	4.90	+++		+++	+++
g	0.51	4.98		++		
h	0.55	5.06	+++		++	++
i	0.58	5.14		+++		
j	0.62	5.26		+		
k	0.63	5.34	-			
l	0.66	5.44		+++	-	
m	0.78	5.66	-	-		
n	0.96	6.22	+	++	+	++
o	0.98	6.40	-	+	-	+

Reletive Intensities are indicated by the number of (+) signs.
Bands at the limit of the detectiion are indecated by (-).

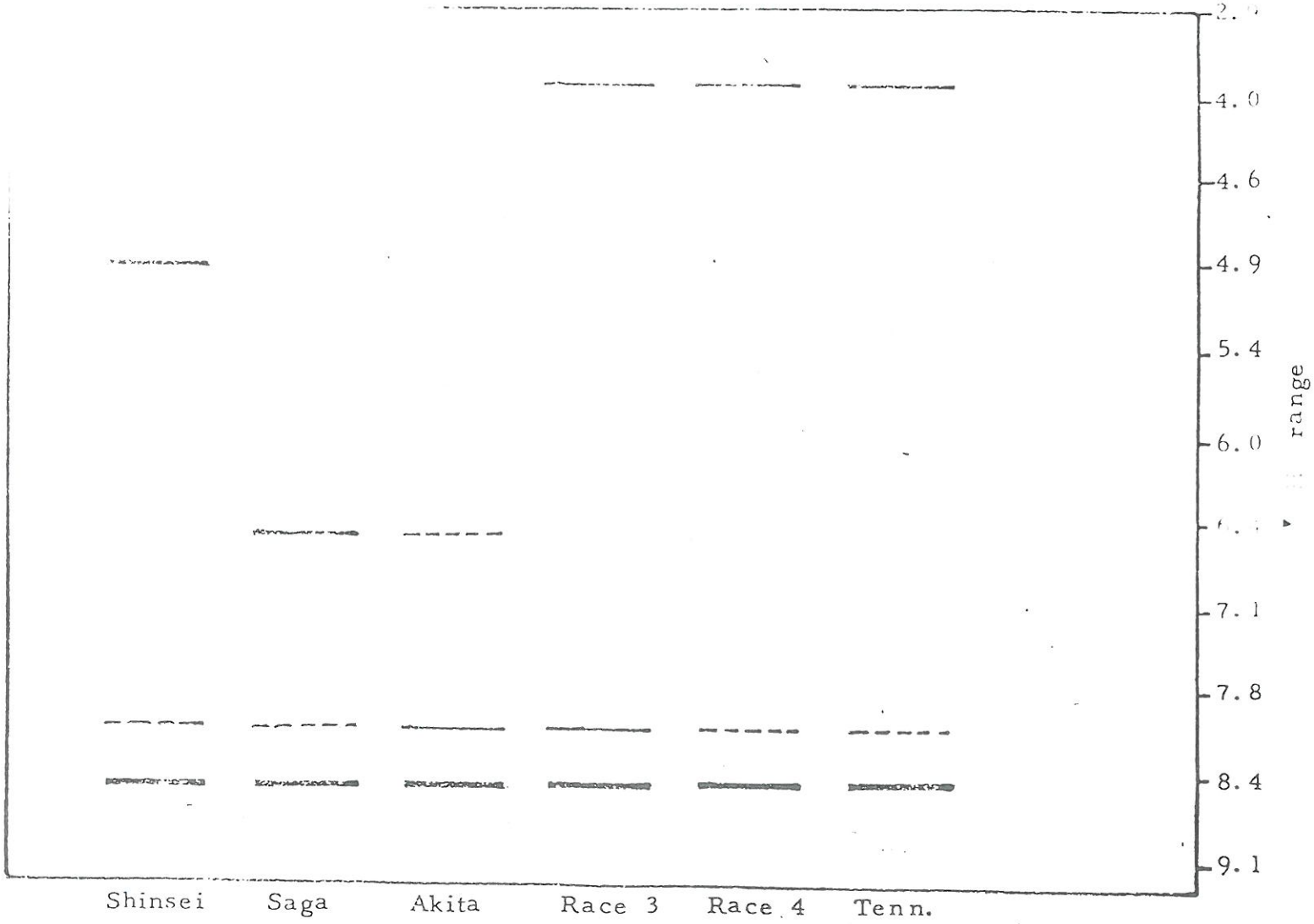

Fig. 2. Comparison of the peraxidase activity of extracts from single cyats of six populatiions of H. glycines, cultured on Lee, separated in the pH range 3- 10.

Table 2. Analysis of isoelectr ophoretogram to show the relative E_fs and PIs of peroxidase isoenzymesm detected from single extracts of six populations of H.glycines, separated in the ampholine range 3-10, cultured on Lee.

Peroxidase isoenzyme	E_f	PI values	Shinsei	Saga	Akita	Race 3	Race 4	Tenn.
a	0.08	3.88				- +	++	++
b	0.28	4.90	++					
c	0.59	6.50		++	-			
d	0.82	8.07	-	-	+	+	-	-
e	0.89	8.42	+++	+++	+++	+++	+++	+++

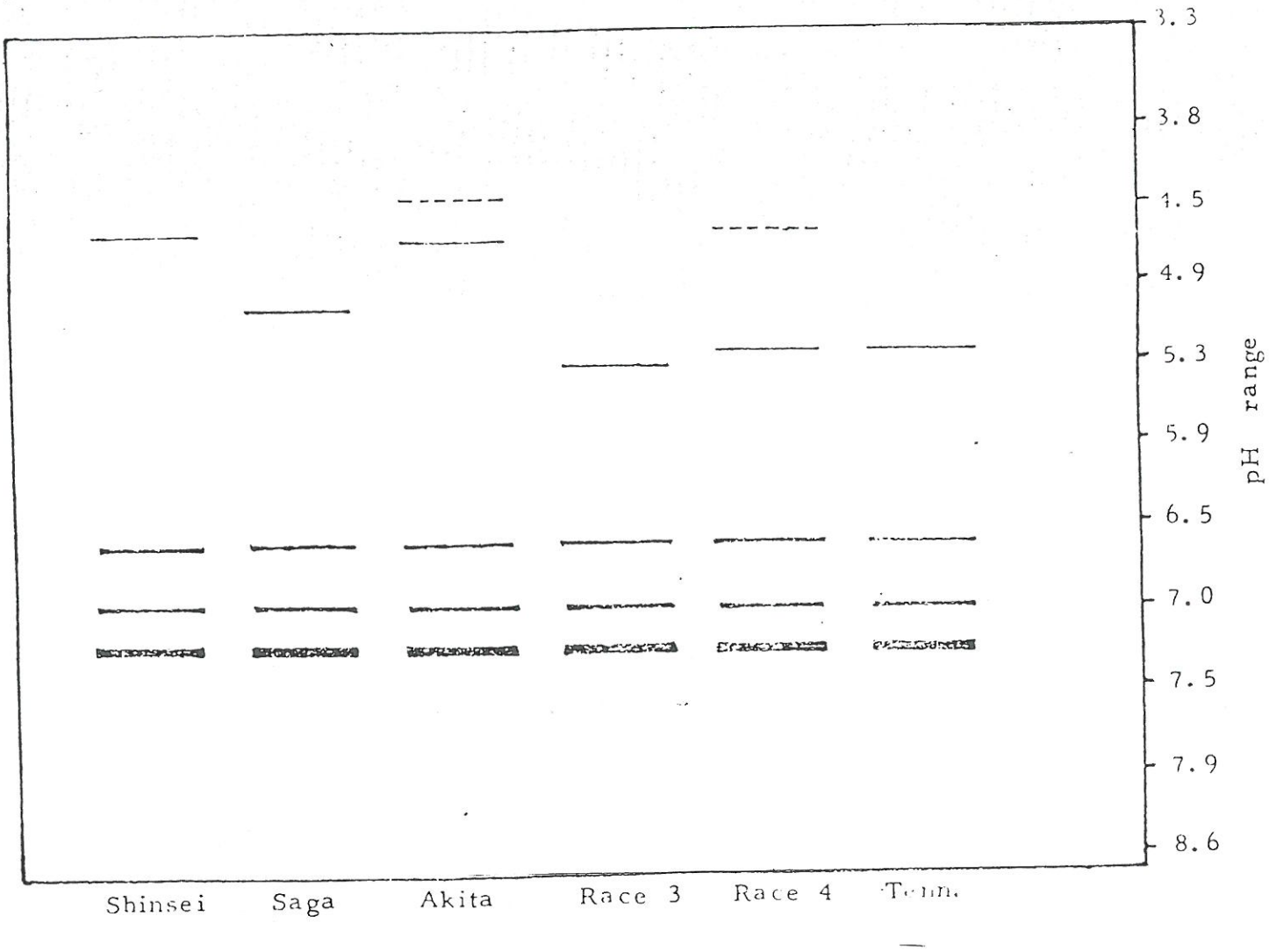

Fig. 3. Comparison of the polyphenolixidase activity of extracts from single cysts of six populations of H.glycines, cultutred on Lee, separated in the pH range 4-9.

Table 3. Analysis of isoelectrophoretogram to show the relative E_fs and PIs of polyphenoloxidase isoenzymes detected from single extracts of six populations of H. glycines, separated in the ampholine range 4-9, cultured on Lee.

Polyphenoloxidase isoenzyme	E_f	PI values	Shinsei	Saga	Akita	Race 3	Race 4	Tenn.
a	0.20	4.60			-	-		
b	0.26	4.67	+		+		-	
c	0.33	5.06		+				
d	0.40	5.28				+	+	+
e	0.62	6.64	++	++	++	++	++	++
f	0.69	7.00	++	++	++	++	++	++
g	0.74	7.22	+++	+++	+++	+++	+++	+++

showed differences in patterns from five species of Meloidogyne and some other populations using micro-electrophoresis performed in small tubes. Recently, Berge et al (1) and Gillois et al (5) found that two loci "B" and "b" were concerned in the synthesis of esterase in races of H.avenae. In this work it was found the six populations of H. glycines could be differentiated on the basis of esterases, peroxidases and poly-phenoloxidases using the single cyst technique. These results are in good agreement with those obtained by larger scale methods.

The data obtained in this study may be of practical value for taxonomic purposes. Molecular identification is especially useful for classifying mixed populations. However, it is hoped that this technique may play an important role in future work particularly with respect to determination of allele frequencies.

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