Electrophoresis of Proteins From Several Races of *Ditylenchus dipsaci*, Recovered from Dried Infested Courgette Tissue[1]

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SUMMARY

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Differences in protein composition of *Ditylenchus* spp. can be detected by electrophoresis. We used this technique to separate races of *D. dipsaci*. Six races of this nematode were used, bean, oat, garlic, lucerne, red clover, and teasel. All of them were recovered from dried tissue of infected courgettes. Different concentrations of proteins of each race were analysed by both polycrylamide gel electrophoresis and by isoelectric focusing. There was a lack of consistent differences among the races studied, except for the bean race, which showed the presence of one strong protein band that was different from the other five races.

Key words: *Ditylenchus dipsaci*, electrophoresis, isoelectric focusing, protein, race, SDS-PAGE, stem & bulb nematode.

RESUMO

Tenente, R.C.V. & A.A.F. EVANS, 1996. Eletroforese de proteinas de varias raças de *Ditylenchus dipsaci* extraídos de tecidos secos de abobrinha.
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Diferenças na composição de proteínas de nematóides do gênero *Ditylenchus* spp. podem ser detectadas através de eletroforese, sendo possível a separação de espécies. Neste trabalho foi estudada a possibilidade de usar esta técnica para diferenciar raças de *D. dipsaci*. Foram utilizadas seis raças: aveia, alho, alfafa, cardo, feijão e trevo vermelho. Os

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nematóides foram extraídos de tecidos secos de abobrinha infectada e as suspensões de nematóides com adição de SDS e mercaptoetanol correram em gel de poliacrilamida. Diferentes concentações de proteínas foram analisadas para cada.raça. Os resultados obtidos confirmam a falta de diferenças consistentes entre as populações estudadas de *D. dipsaci*, exceto para a raça do feijão, que mostrou claramente a presença de uma banda proteica mais forte, ausente nas outras cinco raças.

Palavras-chaves: *Ditylenchus dipsaci*, eletroforese, isoeletrocfocosina, nematóides dos caules e bulbos, proteínas, raças, SDS-PAGE.

INTRODUCTION

Differences in the composition of proteins, carbohydrates and lipids of nematodes can be used to separate species by electrophoresis. The majority of biochemical systematic studies on plant-parasitic nematodes have relied on electrophoresis to detect differences between species (Fox & Atkinson, 1986, for review). Isoelectric focusing (IEF) of soluble nematode proteins has been shown to be a practical and effective technique for the identification of closely related species and members of a species complex. Most of the work has been done on cyst-nematodes (Fleming & Marks, 1983; Fox & Atkinson, 1983; Marks & Fleming, 1985; Pozdol & Noel, 1984), but the IEF technique has also been effective in separating species of root-knot nematodes, *Meloidogyne* spp. (Lawson *et al.*, 1984; Venkatachari *et al.* 1991) and populations of *Pratylenchus brachyurus* (Payan & Dickson, 1990).

Several researchers obtained consistently distinct soluble protein profiles when comparing different races/species of nematodes by polyacrylamide gel electrophoresis (PAGE) (Eriksson & Granberg, 1969; Dickson *et al.*, 1970; Evans, 1971; Gysels, 1968; Hussey & Krusberg, 1971). Proteins of species and pathotypes of several *Globodera* and *Heterodera* have been compared using PAGE. Results found by (Jones *et al.*, 1970) supported an earlier suggestion that pathotypes should receive specific ranking, but failed to distinguish pathotypes within the subsequent defined species, *G. rostochiensis* and *G. pallida*. Stone & Williams (1974) verified that two pathotypes of *H. avenae*, which were distinguished by their ability to reproduce on barley (*Hordeum vulgare* L.), were electrophoretically identical and were considered subspecifically related. Franco (1979) observed that protein patterns from different *G. rostochiensis* populations

were identical, but differences were found in patterns from British and Peruvian populations of *G. pallida*. Also, species of *Aphelenchoides* were differed in protein banding patterns resolved by SDS-PAGE (Ibrahim *et al.*, 1994). Therefore, we were interested to compare protein patterns of six races of *D. dipsaci* by PAGE and IEF.

MATERIAL AND METHODS

Six races of *D. dipsaci*, bean, garlic, oat, teasel, lucerne and red clover were grown inside the fresh fruits of courgettes at 15°C, for 6 to 8 weeks (Hooper & Cowland, 1987). The infested fruits were then cut into thin slices and put in plastic trays for slow drying at room temperature.

Freshly extracted fourth-stage juveniles (J4) were obtained from these dried pieces of courgette tissue using the tray technique of Whitehead and Hemming (1965). Approximately 10,000 J4 of *D. dipsaci* from each race were washed by three centrifugations in distilled water. Subsequently, all preparations were stored at -80°C until needed for electrophoresis. This procedure was done separately for each race. After suspensions of nematodes were removed from the freezer they were held on ice at all times during this work. Nematodes were prepared for homogenisation by washing several times in protein extraction buffer containing proteinase inhibitors (1% SDS, 1% Betamercaptanol) before gentle grinding in a microhomogeniser (Biomedix Ltd, inner Middx, England) to produce a milky suspension. After centrifugation at 13,300g for one minute the supernatant was removed and the homogenisation repeated on the pellet with 10□l of extraction buffer. Both supernatants were mixed and clarified by centrifugation for 10 minutes at 4°C. The clear supernatant was removed, added to an SDS mercaptoethanol mixture containing Bromophenol-blue and loaded on to a freshly prepared gel.

Gels (12% or 8% polyacrylamide, 1% SDS, 1xTBE) were stained with Coomassie Brilliant blue - R250 stain solution (2%) prepared according to Allen *et al.* (1984). The diamine silver stain solution was prepared as described by Oakley *et al.* (1980). Both stain solutions were freshly prepared.

After electrophoresis, the gel was placed in a fixative solution of 20% Trichloroacetic acid (TCA) and shaken for 20 minutes at room temperature before transfer to a staining solution for 20 minutes at 50°C with shaking. Gels were destained in TCA at room temperature. Finally the gel was transferred to a plastic bag with 7% acetic acid solution, sealed and kept at 4°C.

To obtain a better separation of slowly migrating proteins, electrophoresis in 8% polyacrylamide gel was done as described above, except that staining was in the diamine silver stain.

Isoeletric focusing (IEF) was also used to determine the differences in proteins of *D. dipsaci* races. A commercially prepared gel (LKB Bromms Ampholine PAG plate pH 3.5-9.5) was used. Procedures for staining and destaining were the same as for SDS-PAGE.

RESULTS

SDS-PAGE (12% polyacrylamide).

The SDS-PAGE system resolved some slight variations in overall electrophoresis mobilities among the six *D. dipsaci* host races (Fig. 1A.), although the lower portion of the gel was not discernible photographically, because several bands were very faint. A single extra protein band was visible in the bean race (BR) which was absent from the others 5 races (near Marker 7). This band allowed the BR to be distinguished from the other 5 races (Fig. 1A).

SDS-PAGE (8% polyacrylamide).

A similar pattern was observed in the 8% polyacrylamide gel compared to the 12% gel, but better resolution was obtained for slowly migrating proteins (Fig. 1B). For instance beneath Marker 5, below the very intensely stained protein band, there are three bands that are seen more clearly than in Fig. 1A.

IEF (LKB prepared gel).

The results of isoelectric focusing of proteins from fourth-stage juveniles of *D. dipsaci* races, showed no differences in protein bands when diamine silver stain was used.

DISCUSSION

From the results of IEF of proteins of J4 *D. dipsaci* races, the diamine silver stain was not good enough for using in prepared LKB gel because it failed to show differences among the races. Meanwhile, the IEF is a effective technique for species identification and also for some populations of nematodes, that were

reported by Fleming & Marks (1983); Fox & Atkinson (1983); Lawson *et al.* (1984); Marks & Fleming (1985); Payan & Dickson (1990); Pozdol & Noel (1984); and Venkatachari *et al.* (1991), although they were worked with species of different genera, such as *Globodera*, *Meloidogyne*, and *Pratylenchus*.

The electrophoretic patterns obtained for lucerne, red clover, garlic, oat, teasel and bean were similar, and these races could not be distinguished. However, the bean race showed a single consistent extra protein band compared to the other five races, thus allowing consistent differentiation between the bean race and the others. But this work can not explain whether the polyacrylamide gel electrophoresis can be used to distinguish these races of *D. dipsaci*. Also, Jones *et al.* (1970) observed that this system had failed to distinguish pathotypes between the species of *G. rostochiensis* and *G. palida*, and similarities were reported by Stone & William (1974) of two pathotypes of *H. avenae*, that were identical when they were analysed by this system (PAGE). Nevertheless, Trudgill & Carpenter (1971), distinguished pathotypes of several cyst-forming nematodes using PAGE; also, Franco (1979) observed that the protein pattern were different from British and Peruvian populations of *G. palida*. Other workers, also found differences between nematode species by using the PAGE (Gysels, 1968; Dickson *et al.*, 1970; Evans, 1971; Hussey & Krusberg, 1971, and Ibrahim *et al.*, 1994).

It is possible that further work would find other races of *D. dipsaci* with distinct protein profiles as anlysed by PAGE and also, the present molecular techniques might give rise to a new method for distinguishing the population within *D. dipsaci*.

CONCLUSION

There has been a long search for finding consistent differences between races and although some have been found (e. g. giant race of bean) none has been found which covers all races. This is especially true for the use of gel electrophoresis of proteins from different races. These results confirm the lack of consistent differences in populations of this nematode (Figs. 1A and 1B), except for the bean race that shows a clear band which was absent from the other races.

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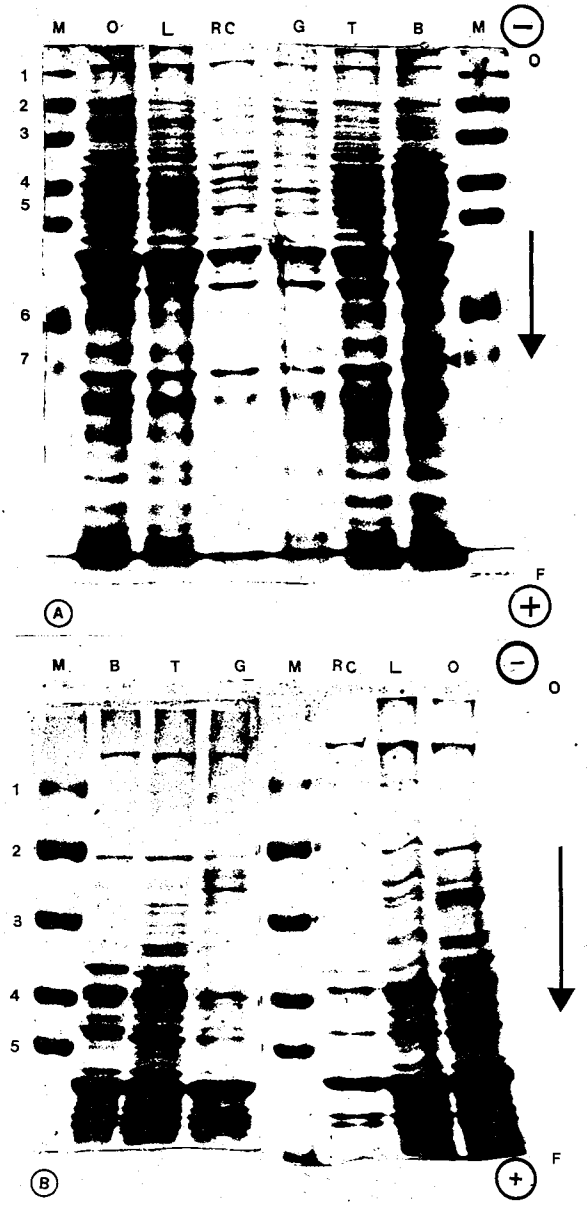

Figure 1. Protein patterns following polyacrylamide gel electrophoresis (A) 12% and (B) 8%, of extracts of six races of *Ditylenchus dipsaci* bean (B); teasel (T); garlic (G); red clover (R); lucerne (L) and oat (O). M= Molecular weight markers; standard mixture of seven proteins for electrophoresis (Sigma); 1 = 180 Kd; 2 = 115 Kd; 3 = 84 Kd; 4 = 58 Kd; 5 = 48 Kd; 6 = 36.5 Kd; 7 = 26.6 Kd.