CHARACTERISATION OF ROOT-KNOT NEMATODES (*Meloidogyne spp.*,) FROM SELECTED LEGUMES IN MBEERE DISTRICT USING ISOENZYME PHENOTYPES

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Abstract

Cowpeas are widely grown in Eastern Africa and Southeast Asia primarily as a leafy vegetable. The protein content of the leafy cowpea parts consumed annually in Africa and Asia is equivalent of 5 million tonnes (t) of dry cowpea seeds and that this represents as much as 30% of the total food legume production in the lowland tropic (Steele *et al.,* 1985).

Pigeon pea (*Cajanus cajan* (*L*) Millsp.) is an important grain legume crop of rain-fed agriculture in the semi-arid tropics. Main pigeon pea producing regions are the Indian sub-continent, Central America and Southern and Eastern Africa. Pigeon pea is produced as a vegetable or export grain crop in southern and eastern Africa. In Kenya, pigeon pea is the third most widely grown pulse crop, and is one of the fastest growing cash crops with an annual growth rate of 3% in the last decade. Green pigeon pea is exported from Kenya to Europe (Snapp *et al*., 2003).

Materials and Methods

Study sites and sampling

Sampling was done in three selected sites in Mbeere district (Gachoka, Mwea, and Siakago zones). Each of the sites was divided into 2 localities from which 10 cowpeas and pigeon peas infected by RKNs were randomly selected and uprooted. They were packaged in paper bags and transported to Kenyatta University where infected plants were used to raise pure cultures.

Raising pure cultures

To raise pure cultures for the isoenzyme analysis, single females were handpicked from the infected plants, and inoculated on tomato (*Lycoperscion esculentum)* seedlings variety moneymaker. The seedlings were maintained under greenhouse conditions at 20-28^{0}C for 35 days. Plants were then harvested and females used for isoezyme characterization.

Isoezyme analysis

Sample preparation

Ten young females per plant isolate were isolated under a dissecting microscope. After the isolation, the females were rinsed in reagent-grade water and transferred to an ice-cold 1.5 ml eppendorf tube containing 60 µl extraction buffer (20% sucrose, 2 % triton X-100 and 0.01% Bromophenol blue) (Esbenshade and Triantaphyllou, 1985) and squashed to release body contents. Samples were centrifuged in a micro centrifuge at 12 000 g 4 ^{0}C for 1 min.

PAGE electrophoresis and enzyme staining

Electrophoresis was done on thin slab native polyacrylamide gradient gel (8-25%) at 4 ^{0}C. The gel electrophoresis was carried out as described by Karssen *et al*ybITzJαIhzSGT3α3GJIIezGTHJAJαSGTHGG%αIrzS4TJ5αJue66T4%JIKzAT4H65GH44IszS

species produced a distinct band or bands. M. *javanica* produced three bands. M. javanica predominated Siakago zone.

Plate 1: Gel photo showing malate dehydrogenase (Mdh) and esterase (Est) phenotypes of the representative specimens of M. javanica (Siakago samples) lane 4: MJS5(Mdh-Est phenotype) (N1-J3); 6: MJS1 (N1-J3); 7: MJS10 (N1-J3); 8: MJS9 (N1-J3); 9: MJS4 (N1-J3); 10: MJS2 (N1-J3); 11: MJS3 (N1-J3); 12: MJS7 (N1-J3)

All the lanes have *M. javanica*

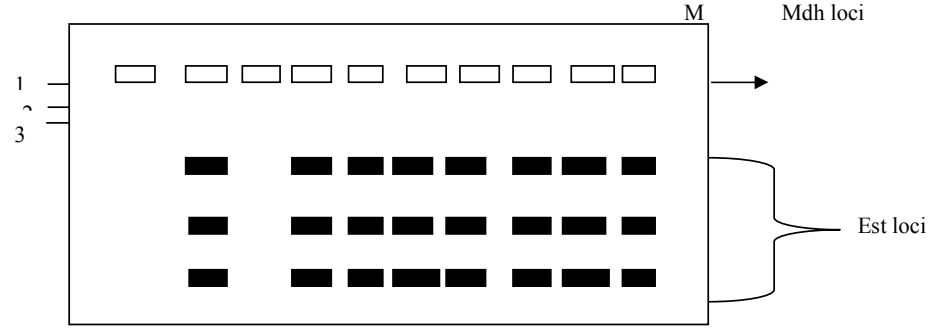

Fig.1: A schematic representation of the gel image shown in plate 1. Esterase loci are in black while Mdh locus is in white.

From Gachoka samples two species were identified: *M. javanica* and *M. arenaria*
M. javanica had a specific phenotype (N1-J3) while *M. arenaria* had the phenotype (N1-A2)

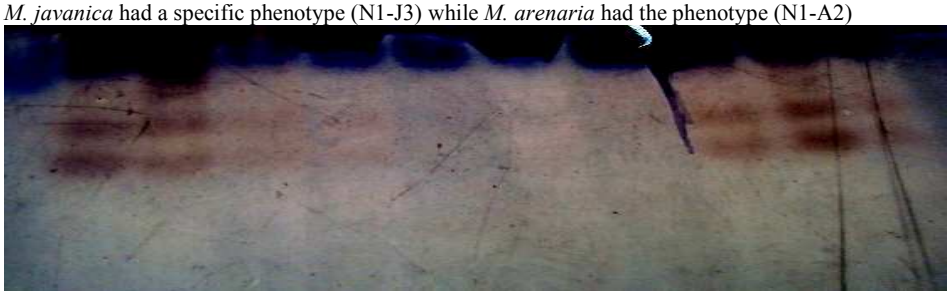

Plate 2: Malate dehydrogenase (Mdh) and Esterase (Est) phenotypes of representative specimens of M.arenaria and M. javanica (Gachoka samples).Lane 2:MJG5(Mdh-Est phenotype)(N1-A2); 3: MJG3 (N1-J3); 4: MJG10 (N1-J3); 5: MJG9 (N1-A2);7: MJG2 (N1-J3); 9: MJG1 (N1-A2);10: MJG6 (N1-J3); 11: MJG7 (N1-A2)

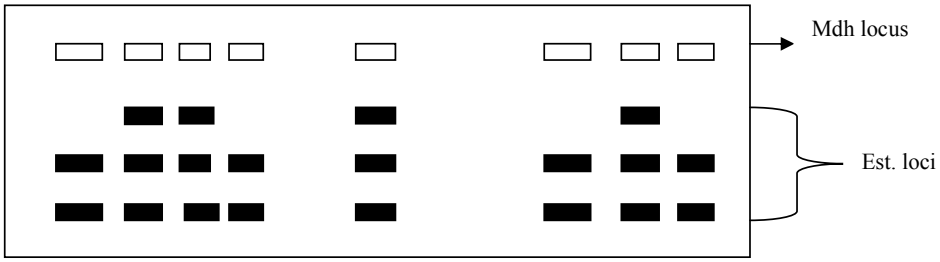

Fig. 2: A schematic representation of the gel image shown in plate 2. Est. loci in black. Mdh locus in white.

In Mwea samples two species were identified. *M. arenaria* was the most predominant in this region. *M. javanica* occurred in a few specimens.

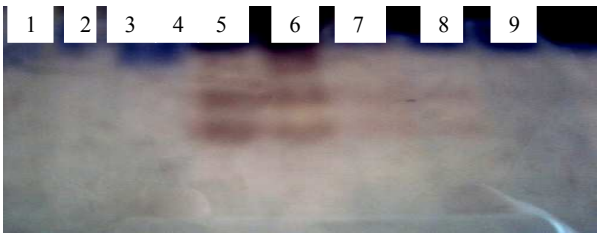

Plate 3: Gel photo showing malate dehydrogenase (Mdh) and esterase(Est) phenotypes of the representatives of M. arenaria and M. javanica of Mwea samples lane5:MJM8 (N1-A2); 6: MJM2 (N1-J3); 7: MJM 3(N1-A2); 8: MJM9 (N1-A2)

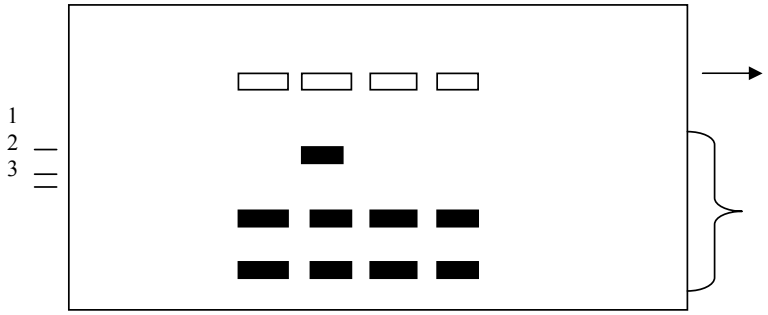

Fig. 3: A schematic representation of the gel image shown in plate 3. Est. loci in black

In this study, two esterase phenotypes were detected. The esterases of the b system were strongly stained and hydrolyze both β and α-naphthylacetate. Only the major bands of b-esterases were considered in this study since they were consistent and visible with the β-naphthylacetate.

Table 1: summary of isoenzyme phenotypes (Malate dehydrogenase and Esterase) and the number of specimen associated with each phenotype.

Species	Isoenzyme phenotypes	Number of specimens
M. javanica	N1-J3	58
M. arenaria	N1-A2	32

Discussion

The species identity of ninety root-knot nematodes affecting cow peas and pigeon peas in Mbeere was revealed using isoenzyme analysis. The species *M. javanica* was identified from 64.4% of the samples while *M. arenaria* had 35.5%. Isoenzyme phenotypes have been shown to be reliable criteria for the identification of the major *Meloidogyne* species (Esbenshade and Triantaphyllou, 1985a). Among the isoenzymes used, esterase phenotypes are highly polymorphic and can be used for the differentiation of various species (Triantaphyllou, 1985; Fargette, 1987; Carneiro *et al.,* 2000).

The Mdh phenotype produced a single band for all the samples. It is therefore not possible to differentiate the different species using this phenotype. The samples were then subjected to the Est. phenotype which showed polymorphism as each species produced a distinct band or bands. A letter was designated suggesting the particular nematode species followed by a number indicating the number of major bands of enzymatic activity. Thus, *M. javanica* had the phenotype J3 while *M. arenaria* had A2. Species – specific phenotype J3 was detected in 58 of the samples from Gachoka, Mwea and Siakago. Phenotype A2 was found in the remaining 38 samples.

Differences in activities of the isoenzyme were indicated by the color intensity of the bands of the different alleles from the same locus. This may be due to the catalytic action of the enzymes that are influenced by epigenic modulation, other possibilities like low esterase activity of the band and the physiological state of the females.Malate dehydrogenase phenotype was designated for convenience, by the letter N, standing for "non-specific phenotype," and a number indicating the number of bands of activity. The most prevalent phenotype of Mdh had one major band hence named N1. This was identified in *M. javanica* and *M. arenaria.*

This study confirmed previous reports that the esterase phenotypes is the most useful biochemical character for identification of major *Meloidogyne* species. (Dalmasso *et al.,* 1983; Dickson *et al.,* 1971; Hussey *et al*., 1972). Enzyme patterns are stable and repeatable but the mobility of a given band in different gels can vary within a narrow range. This could be due to slight variations in laboratory conditions including extraction procedures, handling of the samples following extraction, sample storage and electrophoretic conditions of separation (Rollinson 1980). Such factors or conditions may vary among laboratories and from one electrophoretic run to the other in the same laboratory.

Conclusion

Isoenzyme phenotypes are useful tools for identification of *Meloidogyne* species since they are fast and more accurate than relying on morphological characters. However, isoenzyme profiles can only be performed with use of females and not the second- stage juveniles (J2). The females are not found in the soil unlike the J2 therefore, the isoenzyme profiles require time and space to establish and maintain populations in culture from a single egg to adult. Identification of root-knot nematodes is important for making crop management decisions, deployment of cultivars because of differences in reproduction on different cultivars resulting from host status and resistance specificities, and for monitoring population movement particularly in quarantine species.

Recommedations

This study allowed identification of root-knot nematodes affecting cow peas and pigeon peas in Mbeere district. The following recommendations can be made:

1) More identification can be done on other crops in Mbeere in order to establish the species diversity of *Meloidogyne* species.
2) Resistant genes in root-knot nematode resistant plants should be investigated and these plants adapted in crop rotation with susceptible plants.
3) Use of molecular markers to validate identification using isoenzyme profiles.

Acknowlegdement

The first author is grateful to the funding of this project through Prof. Waceke Wanjohi, Department of Agricultural Sciences and Technology (K.U).

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