Molecular Characterization of Nematodes Affected by Heavy Metal

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Abstract: Four *Meloidogynidae* (*M. incognita* EG., *M. incognita, M. Javanica* and *M. hapla*) isolates under heavy metals potentially of sewage irrigation were differed in disease severity. *Meloidogynidae* isolates were appeared low in disease severity on tomato plants compared with *M. incognita* EG. The lowest of infection due to effect of heavy metals of sewage irrigation. Four of *Meloidogynidae* isolates which differ in disease potential show genetic variability. Protein pattern, endo β-1-4 gluconase isozymes, and DNA fingerprint successfully revealed genetic diversity. DISC PAGE endo β-1-4 gluconase isozymes revealed 8 hands which differ in density, relative mobility and number among 4 *Meloidogynidae* isolates. SDS-PAGE of protein patterns varied among 4 isolates in number, density and molecular weight of protein species, 13, 16, 17 and 17 bands of *M. incognita*. EG; *M. incognita*, *M. gavanica* and *M. hapla* isolates, respectively. As well as reproducibility polymorphism revealed 10 common polypeptidies (monomorphic) with 50%; 7 specific polypeptidies (polymorphic) with 35% and 3 unique polypeptidies (protein marker) with 15%. RAPD-PCR for identification of 4 *Meloidogynitae* isolates were conducted on the screening primers and on the extraction of template DNA. Out of 9 sequence 3 mer primers were successful in identification of 4 isolates. PCR amplification yielded reproducible DNA fragments which differentiated the 4 isolates examined (number, density and molecular weight) also recognized between *M. incognita, M. gavacina, M. hapla* and specially compare with *M. incognita* E.G. The diversity among *Meloidogynitae* isolates due to the effects of heavy metals irrigation. DISC PAGE, SDS-PAGE and RAPD-PCR conformed the genetic variability among 4 *Meloidogynitae* isolates. [Journal of American Science 2010;6(8):122-130]. (ISSN: 1545-1003).

Key words: Meloidogynitae, DISC-PAGE, SDS-PAGE, protein finger-print, DNA fingerprint.

1. Introduction

Owing to a shortage of irrigation water, there is a great need to use untraditional types of irrigation e.g. Sewage water. Nowadays, sewage irrigation is increasingly varied as an exploit of this resource to overcome such problem and to get rid of sewage at the same time to alleviate sewage problems.

International experience has demonstrated beyond doubt that the reuse of sewage water or sewage sludge in agriculture offers major agronomic, environmental, as well as economic advantages (Ashraf et al., 1994).

However, in spite of sewage benefits, but it also contains pathogens, vector attraction, potentially toxic elements, increasing levels of nitrogen and phosphorus, salinity, inorganic, organic and biological contaminants, therefore requires avoiding potential environmental problems (MAFF, 1991).

Investigations using historic site soils provide available adjunct to the classic designs for assessing the environmental effects of sewage water. Potentially toxic elements (PTEs) are inorganic elements, which occur in sewage in higher concentrations than in soil. Some are essential trace elements (for instance Cu and Zn) needed for nutrition of plants, animals and man. Others such as Pb and Hg have no nutritional value. Their value is due to the long period of time required for repeated sewage application to accumulate PTEs in soil (Omran et al., 1996 and Neglaa, 2002).

Since there is little information concerning the genotoxic effect of sewage application in this site, therefore considerably more work must be performed based on the genetic studies to assess the influence at sewage irrigation in an area such as El-Gabal El-Asfar old farm. Therefore the objectives of this present study the genotoxic potentially of sewage irrigation on Nematoda lived in soil.

2. Material and Methods

Nematoda culture:

The natural population of nematoda used in this study were collected from old farm of El-Gabal El-Asfar (north-east of Cairo) irrigated with sewage water. Captured nematoda were brought as soon as possible to the Nematoda Lab. Plant Protection Inst. Agric. Research Center where were identified according to species. Nematoda belonging to Meloidogynidae (*M. hapla, M. incognita* and *M. javanica*) were kept while other species were discarded. They were cultured on tomato

(Lycopersicon esculentum cv. Castle rock; pot cultures were maintained in a greenhouse (approximately 25ºC; average relative humidity 80%; day length of 16 h.). Females were recovered using the method of Hussey (1971). Root systems were excised, cleaned of debris and cut into approximately 1.0-2.5 cm pieces and placed in a 1 liter Erlenmeyer flask with 250 ml of a 50% solution of liquid pectinase concentrate an agitated on a shaker overnight at room temperature. Released females were collected, washed with distilled water and transferred into 1.5 ml Eppendrof tubes and stored at -20ºC until required M. incognita, EG was obtained from the culture maintained on tomato plants cv. Castle rock in a greenhouse from Nematode Lab. Plant Protection Inst. Research Center.

SDS-polyacrylamide gel electrophoresis :

Protein concentration was determined by Lowry's method (1951) using bovine serum albumin is a standard.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described by Laemmli (1970) under reducing conditions using 12% run gel and with 3.7% stacking gel in a dis continuous electrode buffer system (Ruppel and Cioli, 1977). Protein samples and molecular weight marker (Sigma) were denaturated and reducing completely before electrophoresis by mixing the samples with on equal volume of 2x sample buffer (Lucius et al., 1987) and heating the mixture at 95ºC in a water bath for 5 min. Treated samples centrifuged at 12.00 rpm for 10 min and chilled on ice before use. For subsequent fractions, the lanes well filled up to their full capacity (50 µl with 25 µg protein per ml.). The gel was stained with coomassi brillant blue R. 250.

Enzyme activity assay :

Endo-β-1,4 gluconase activity is determined (according to Yan-Hong et al., 2005) by the hydrolysis of 200 µl 1% CMC in 100 mM sodium acetate buffer containing 100 mM NaCl (pH 5.2) at 50ºC for 10 min. Dinitrosalicylic acid (0.5 ml) was added to stop the reaction by boiling in a water bath for 5 min and quick cooling to room temperature. The absorbance at 540 mm was measured (as standard assay). One unit of endo-β-1,4-glucanase activity is defined as the amount of enzyme that yields 1 µ mol glucose in 1 min at 20ºC.

Isozyme electrophoresis :

Isozyme variability as assayed by vertical polyacrylamide slab gel electrophoresis using 10% acrylamide, the nematoda were homogenized in 50 mM Tris HCL (pH 7.5) and 5% glycerol. Each sample was vertexed and centrifuged for 10 min at 10.000 rpm at 5ºC. The supernatant was transferred to new eppendrof tube and kept -20ºC. Advance of 10 µl supernatant of each sample was mixed 5 µl bromophenol blue, then a volume of 15 µl from this mixture was applied to each well. The gel was stained after electrophoresis with (1% CMC in 100 mM sodium acetate containing 100 mM NaCl (pH 5.2) and 0.5 ml Dinitrosalicylic acid and incubated at 37ºC in incubated at 37ºC in dark for complete staining (Janathan and Wendel, 1990).

Extraction of Nematoda DNA :

Approximately, 20 adult females were hand-picked from roots of each host plant and washed in a sterile plastic dish containing sterile water. They were ruptured with a pestle on a slide glass, and 150 µl of DNA-extraction buffer (200 mM Tris-HCl, pH 8.5; 250 mM NaCl, 25 mM EDTA and 0.5% SDS) was added. The homogenate was transferred to a 0.5 ml micro-centrifuge tube. After adding 75 µl of 3 mM potassium acetate, pH 5.2. The lysate was kept at -20ºC for 10 min. After centrifugation, the supernatant was transferred to a new micro-centrifuge tube. Nucleic acids were precipitated with on equal volume of isopropanol at room temperature for 30 min and pelleted by centrifugation at 15.000 rpm for 5 min. The pellet was washed with 70% ethanol, dried and dissolved in TE buffer (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA). The concentration of template DNA was adjusted to about 1 mg/ml (modified method of Cenis 1993).

PCR amplification:

Nine arbitrary primers was selected according to Orui (1999) (Table 1) were used for PCR amplification. The amplification was performed in 25 µl of reaction buffer (10 mM Tris HCl pH 8.3; 50 mM KCl, .5 mM MgCl2) with 100 µM of each dNTP, 0.1 µM primer, 10 U Taq DNA polymerase and 5 µl of crude DNA extract. The PCR thermal program in a DNA thermal cycles of 94ºC min, 36ºC for 1 min and 72ºC for 1 min. The amplified DNA as performed through 1.5% agarose gels. The gels were stained with ethidium bromide solution (0.5 µg/ml) and were photographed on a UV transilluminator.

Table(1):Distinguishable random primers selected from four Meloidogyne species producing polymorphism according to Orui (1999).

Primer	Sequence (5`-3`)	Distinguish able species
OPA-01	GAGGCCCTCC	A1, A2, J, 1, H
OPA-03	AGTCAGCCAC	A1, J, A2, , H
OPC-05	TGAGCGGACA	A1, A2, J, H
OPC-09	CTCTGGAGAC	A1, A2, J. H
OPC-19	CTGGGGACTT	A1, H, J.I
OPC-20	ACCCGGTCAC	A1, A2, J.I
OPF-01	ACGGATCCTG	A1, A2, J
OPG-03	GAGCCCTCCA	A1, A2, J. I

A1 : *M. aerenaria* (esterase type: A-1)J: *M. javanica*
A2: *M. aerenaria* (esterase type: A-2) I: M. incognita
H: *M. hapla* = on identical RAPD pattern among species

3. Results

The characteristics of water irrigation in Gabal El-Asfar are compared of particulates, nutrients, heavy metals hydrocarbons, and residuces of toxic compounds such as herbicides and pesticides. This is further supported by the trace metal data in the water and sediments (TAV, 1999). The domestic wastewater represents the main contribution of Gabal El-Asfar.

Table (1). Selected heavy metals in water and sediments in the Gabal El-Asfar drain.

Metals	Zn	Mn	Cu	Fe	Pb	Hg	Cd
Water (ppb)	0.076	0.35	0.54	0.45	0.32	0.37	0.40
Sediment (ppm)	164.21	481.70	0.75	2.45	95.3	0.44	0.15

Biological variation among 4 Moleidogynidae isolates were detected by protein content, protein fragments endo-β-,4 gluconase activity and isozymes and DNA finger print Protein content was determined in adult warm of each isolate related to BSA (Table, 2). It was revealed that, the protein content was varied among four isolates with 725, 540, 510 and 345 mg/g fresh tissue of M. incognita EG, M. incognita; M. javanica and M. hapla respectively.

Endo β-1,4-gluconase activity was assayed of each isolate, the specific activity of enzyme was 3.52, 4.21, 4.02 and 5.00 μ/mg protein of four M. incognita; M. javanica and M. hapla respectively (Table 2). The total enzyme activity was decreased in nematoda (3 isolates) under heavy metal potentially (2275, 2050.1, 1725.2 μ) with recovery 85.2, 72.3 and 65.4% of M. incognita, M. javanica and M. hapla respectively compared with M. incognita EG. (2552.5 μ) (Table 2).

Table (2). Endo β-,4 gluconase activity and characterization from Nematoda isolates under heavy metals potentially.

Nematoda isolates	Protein content (mg)	Total activity (U)	Specific activity (μ/mg)	Recovery %
***M. incognita* EG**	725	2552.5	3.52	100
M. incognita	540	2275.0	4.21	85.2
M. javanica	510	2050.1	4.02	72.3
M. hapla	345	1725.2	5.00	65.4

SDS-PAGE protein patterns was illustrated in (Fig. 1-a) and Table (3). The results showed the variation of Meloidogynidae isolates in protein pattern and density bands. The variability analysis among isolates appeared 20 protein fractions. Out of them 13, 16, 17 and 17 polypoeptides of M. incognita EG, M. incognita, M. javanica and M. hapla respectively. The molecular weight of polypeptides were determined related to protein markers (Table 3). Some polypeptides bands disappeared in M. incognita EG. 170, 125, 112, 105, 82, 63 and 47 KDa while appeared in other isolates under heavy metals potentially. The most prominent alteration (polymorphic bands) among Meloidogynitae isolates 7 bands (125, 120, 112, 105, 63, 47 an 20 KDa) with 35%. The prominent polypeptide bands in all isolates, 10bands (monomorphic or common fractions) were 165, 150, 130, 100, 95, 47, 33, 28, 15 and 10 KDa with percentage 50%. The unique polypeptides (genetic markers) with 75 KDa (M. incognita-EG); 85 KDa (M. incognita) and 170 KDa (M. hapla) with percentage 15% (Table 3).

DISC-PAGE of endo β-,4-glucanase isozymes are shown in Fig. (1-b) and calculated in Table (4). The total number of isozymes 8 bands differ in number, relative mobility and density among four isolates such as 6,5, 4 and 4 bands for M. incognita EG, M. incognita, M. javanica and M. hapla respectively.

Table (3). Protein patterns of four Meloidogynidae isolates by SDS-PAGE.

MW (KDa)	Meloidogynidae				
	M. avenaria	M. incognita	M. javanica	M. hapla	Polymorphism
170	-	-	-	+	Unique
165	+++	+++	++++	+++	Monomorphic
150	++	++	++	+	Monomorphic
130	++	++	++	++	Monomorphic
125	-	++	++	++	Polymorphic
120	+	+	+	-	Polymorphic
112	-	++	++	++	Polymorphic
105	-	++	++	++	Polymorphic
100	++	+++	+++	++	Monomorphic
95	+++	+++	+++	+++	Monomorphic
82	-	++	-	-	Unique
75	+++	-	-	-	Unique
63	-	+++	++	++	Polymorphic
51	+++	-	++	++	Polymorphic
47	-	+	+	+	Monomorphic
33	+	++	++	+++	Monomorphic
28	++	+++	++	+	Monomorphic
20	+++	-	+++	+++	Polymorphic
15	+	++	++	++	Monomorphic
10	++	+++	+	+	Monomorphic
Total bands	13	16	17	17	20

Unique (genetic marker) Monomorphic (common protein pattern) Polymorphic (specific protein pattern)
Density band: ++++ Very strong. +++ Strong ++ Moerate, + Weak and - Absent band

Table (4). Eno-β-,4-gluconase isozymes analysis of Meloidogynidae isolates by DISC PAGE.

No.	Rf bands	Meloidogynidae isolates				Polymorphism
		M. incognita EG	M. incognita	M. javanica	M. hapla	
1	0.25	-	-	+	-	Unique
2	0.35	-	+	-	-	Unique
3	0.47	++	++	++	++	Monomorphic
4	0.51	+++	+++	+++	+++	Monomorphic
5	0.60	++	-	-	-	Unique
6	0.65	++++	++++	+++	++	Monomorphic
7	0.72	++	-	-	-	Unique
8	0.75	+++	++	-	++	Polymorphic
Total bans		6	5	4	4	8

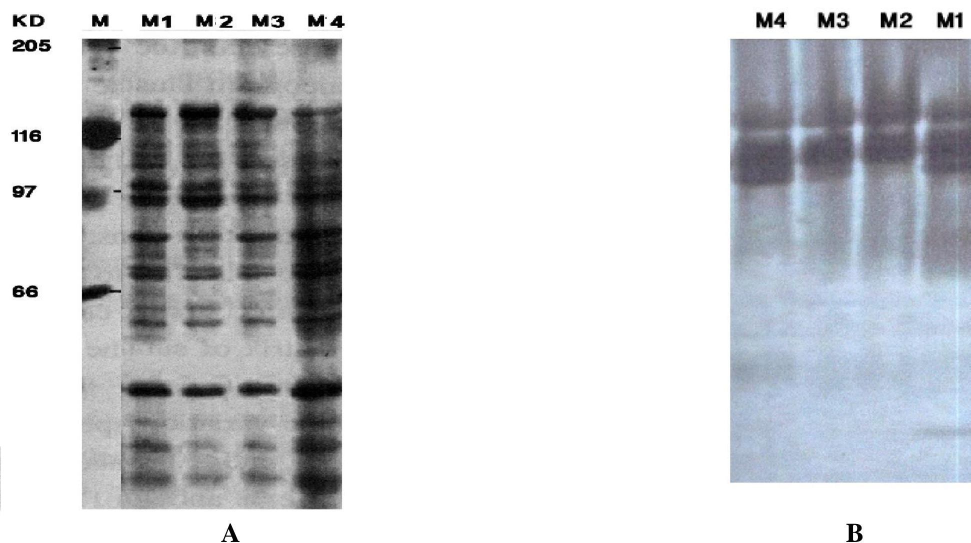

Fig. 1: Electrogram of protein and isozymes profiles of 4 Meloidogynitae.
1-A: SDS-PAGE (12%) of denaturated protein extracted from 4 isolates.
1-B: DISC-PAGE (10%) of native protein esterase isozymes.
M: Markers protein (KDa), M. incognita EG.(M1), M. incognita (M2), M. javanica (M3) and M. hapla isolates(M4).

The endo-β-,4-gluconase isozymes variability among isolates, showed some isozymes disappeared among 4 isolates. As well as one genetic marker (unique) of M. incognita and M. javanica while 2 genetic marker for M. incognita EG.; 3 common isozymes (monomorphic) in 4 isolates with 47% and 1 specific isozymes (polymorphic) with 1.25%.

DNA fingerprint : Total DNA preparation was found crucial for RAPD-PCR. The DNA yield was determined spectrophotometrically as 7 µg/20 g tissues. The DNA purity as indicated by 260/280 was 1.5. It is therefore essential to optimize the PCR conditions to obtain reproducible and interpretable results before-going on routine analysis.

The PCR reaction conditions, polymorphism among the four Meloidogynitae isolates were detected using different random primers by RAPD-PCR gave the best results of amplification expressed on average number of bands per primer. Of the three primers (Operon random primer) were screened in RAPD analysis is for their ability to produce sufficient amplification products. Three random primers namely OPA-03; OPC07 and OPE-01 were more stable and reproducible and gave sufficient polymorphism among 4 Meloidogynitae isolates. Therefore are focused our efforts on these primers are summarized in Table (5, 6) and Fig. (2).

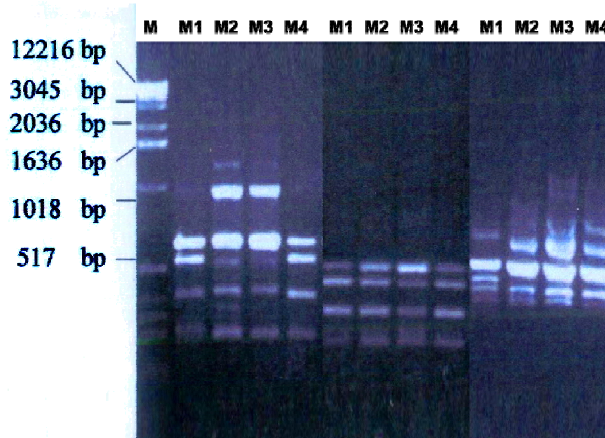

Fig. 2: Electrogram of agarose gel 1.5% showing DNA polymorphism based on RAPD analysis from the studies 4 Meloidogynitae against random primers (OPA-03, OPC-07 and OPF-1.
M:M. DNA molecular marker, M. incognita EG.(M1), M. incognita (M2), M. javanica (M3) and M. hapla isolates(M4).

Table 5. DNA fractopms of four Meloiodogynitae isolates using random primers by RAPD-PCR.

MW (bp)	ODA-03				OPC-07				OPF.01				Poly-morphism
	M1	M2	M3	M4	M1	M2	M3	M4	M1	M2	M3	M4	
1520	-	+	+	+	-	-	-	-	-	-	-	-	Polymorphic
1390	-	+++	+++	-	-	+	+	-	-	-	-	-	Polymorphic
1267	-	-	+	+	-	-	-	-	-	-	-	-	Polymorphic
1210	-	-	-	-	+	+	+	-	-	-	-	+	Polymorphic
1160	-	-	-	-	-	-	-	-	-	-	-	+	Unique
690	+++	+++	+++	+++	-	-	-	-	-	-	-	-	Polymorphic
815	-	-	-	-	-	-	-	-	-	-	-	+	Unique
650	-	+	+	+	+	+	+	+	-	-	-	+	Polymorphic
505	++	++	++	++	++	++	++	++	-	-	-	-	Polymorphic
500	+	-	-	-	-	-	+	+	-	-	-	-	Polymorphic
440	++	++	++	++	++	++	++	++	++	++	+	++	Monomorphic
460	++	++	++	++	++	++	++	++	++	++	++	++	Monomorphic
390	-	-	-	-	-	+	+	+	-	+	+	-	Polymorphic
375	-	-	-	-	-	+	+	+	-	-	+	-	Polymorphic
240	-	-	-	-	+	+	+	+	-	-	-	-	Polymorphic
290	-	-	-	-	+	+	+	+	+	+	+	+	Polymorphic
275	-	-	-	-	-	-	-	-	+	+	+	-	Polymorphic
250	-	-	-	-	-	-	-	-	+	+	+	+	Polymorphic
	5	7	8	7	7	10	11	9	5	6	7	8	18
	27				37				26				

M1 : *M. incognita* EG M2 = *M. incognita*; M3: *M. javanica*, M4= *M. hapla*
Unique = Genetic marker Polymorphic (PAF) = Specific amplified fragment
Monomorphic (MAF) = Common amplified fragment (TAF) Total amplified fragment

Table 6. Polymorphism and genetic marker among four Meloidogynitae isolates using random primers by RAP-PCR.

Primer	Polymorphism				Genetic marker				
	TAF	MAF	PAF	Unique	MW. (bp)	M1	M2	M3	M4
OPA-03	9	4	4	1	500	+	-	-	-
OPC-07	11	6	5	-	-	-	-	-	-
OPF-01	11	4	2	5	1210 1160 815 650 375	- - - - -	- - - - -	- - - - +	+ + + + -
Total bands	31	14	11	6	6	1	-	1	4
Percentage	-	45.16	35.48	19.35	19.35	3.22	-	3.23	12.90

The RAPD-PCR analysis of DNA extracted from 4 Meloidogynitae isolates revealed with different molecular weight ranged from 1520 to 250 bp of primers OPA-03, OPC-07 and OPF.01 respectively. The DNA amplified fragments of 4 isolates were varied in number, density and molecular weight. The variability analysis among 4 isolates showed some DNA amplified fragments absent or/and present in some isolates (Table 5, 6). The polymorphism analysis among isolates revealed 18 amplified bands, 2 monomorphic amplified bands (common in all isolates) with 11%; polymorphic amplified bands

(specific bands) with 78% and 6 unique bands (genetic markers) with 11%. The genetic markers were 500 bp (M. incognita EG, 375 bp (*M.javanica*), (1210, 1160, 815 and 650 bp (*M. javanica*).

4. Discussion

Four Meloidogynitae (M. incognita, M. javanica and M. hapla) isolated under heavy metals potentially of sewage irrigation were reduced in disease severity on tomato plants compared with M. incognita EG which increased in disease severity ones. An assessment of the potential environmental impacts caused by recycling sewage water to agricultural land has been attempted on the El-Gabal El-Asfar concerning the genetic alteration caused by this stress to living organism. The farm has been irrigated with sewage for more than 80 years and there are environmental and health concerns about soil, water and crop contamination at the site and also about potentially toxic heavy metals (Wally et al., 1987).

In the present study, three adult worms isolates of laboratory regard collected from ad form of El-Gabal El-Asfar at Egypt were investigated genetic diversity. As well as identified isolate were obtained from Plant protection Research Institute and originally selected on the basis of different pathogenicity.

Several techniques of molecular biology for studies of genetic diversity in Meloidogynitae have been used (Florence et al., 2004; Jamjoom, 2006 and Haggag and El-Sherbiny, 2006).

Zone electrophoresis in a supporting medium such as polyacrylamide is a sensitive method for detecting minor difference in identical molecules. The most detectable differences revealed by this method are amino acid substitution resulting in a charged difference in a protein molecule, either because the substituted amino acid itself carries the different charge or because its substitution results in a configurationally change in the molecule and consequently the overall net charge changes due to covering or uncovering ionizable groups (Hubby and Throckmarton, 1965).

Electrophoretic protein banding pattern of an organism can be used to elucidate reliable biochemical genetic markers of this organism. It can also provide information about structural genes and their regulatory systems which control the biosynthetic pathways of that protein banding pattern (Abdel Salam et al., 1992).

The observed changes in protein banding patterns in the present study could be reasonably interpreted to be the result of gene mutation. This conclusion is in accordance with Abdel Salam et al. (1992). However, other investigators Gamal El-Din et al. (1988) traced such changes back to the induction of chromosomal abnormalities such as bridges breakes, laggards and micronuclei which can lead to loss of some of the genetic material. Therefore, some electrophoretic bands disappeared due to the deletion of their corresponding bands.

Disappearance of some bands could also be explained on the basis of a mutational event at the regulatory genes which are suppressed at transcription level. Meanwhile, the appearance of new bands could be explained on the basis of a mutational event at the regulatory system of unexpressed gene(s) that activate them (Abd El-Salam et al., 1992).

Gel electrophoresis is a widely used tool in studies of genetic variability. The electrophoretic differences reflect the allelic variations of S. mansoni enzymes which might due to mutational events occurring in the shistosoma under stress and affecting the loci controlling the synthesis of isozymes.

Several factors may be considered as primary determinants of the number of bands observed on a gel, including. (a) The number of coding genes, (2) Their allelic states (homozygous or heterozygous) and (3) The quaternary of the protein products. The simplest case involves a single region of salinity with variant electromorphs (allozymes) observed in different individuals. Because allozymes are usually codominanly inherited, the presence and number of bands are depending on the number of polypeptide subunits contained in the active enzyme (Kahler and Allard, 1970).

An electrophoretic analysis was made on the homogenate of progeny of four M. Meloidogynitae for isozymes in order to determine isozymes. Gluconase isozyme enzymes that characterized by their common activity on many dinitrosalicylic acid substrates (Janathan and Weadel et al., 1990). The group of gluconase isozymes is one of the largest and most complicated system that has been intensively studied in many organisms of animal kingdom in vertebrates, gluconase isozymes are mostly monomeric. Consequently, each band of esterase activity reflects a structure of one polypeptide chain. Hence, each band on the gel represents the end product of one locus (allele). As revealed from Est zymograms, all bands were anndelly migrated and their distributions were varied, some bands were present in a certain Meloidogynitae were lacking in another. A total number of 3 were present in all isolates (monomorphic) and could considered as common bans for all 4 isolates. Bands No. 1, No. 2 and No. 5 observed only in M. incognita, M. javanica ES, M. incognita and M. javanica respectively which could be a result of gene expressed under stress. The result indicates that there were allelic variants in this

locus in all Meloidogynitae. Suggesting a polymorphic type of inheritance for this enzyme.

In this study, the genetic variability among the four studied Meloidogynitae using three random primers based on RAPD-PCR analysis. Initial screening of nine random primers resulted in only three primers that can produce. Informative, polymorphic products resolvable by agarose electrophoresis. The primers sequencesd (EC% range 50-80%) and manufacturer codes are given in material and methods. As postulated by Simpson, et al. (1993). The primer that has a high G+C content generates more amplified products.

In the present study RAPD-PCR markers were successfully used to discriminate variations among the five S. mansoni. RAPD-PCR markers were successfully used to discriminate variations among the 4 isolates, RAPD markers tend to reside in regions with many repeated sequences and therefore in non-coding regions which are more susceptible to mutations. Consequently, they usually reveal more polymorphisms compared with isozymes or RFLPS, which are mostly representative of a conserval genome regions (Welsh and Clellend,1990).

The introduction of RAPD-PCR technique has amplified the possibilities of polymorphisms analysis, as it allowed the use of small arbitrary nucleotide segments without the need of a previous knowledge of genes and/or genomic sequences (Welsh and Mc Clellend, 1990). Also, RAPD assay clearly has certain proactively advantages for detecting DNA variation. It is technically less demanding, cheaper and quicker than other molecular techniques (Stothard and Rolliknson, 1996).

Rollinson et al. (1998) reported that, snails resistant to infection occur naturally and there is a genetic basis for this resistance, in B. glabrata resistance to S. mansoni is known to be polygenic trait. So, we have initiated a preliminary search for snail genomic regions linked to, or involved in resistance by using a RAPD-PCR based approach in conjunction with progeny individual methods.

The present study, demonstrated the utility of RAPD-PCR method for the differentiation of Meloidogynitae isolates. The isolates studied were selected on the basis of different location and pathogenic by well characterized resistance, susceptibility phenotypes upon exposure to Meloidogynitae. Reproducible and inheritable stable polymorphic markes for Meloidogynitae were identified with one out of the ten arbitrary primers tested. Several of the primers produced monomorphic bands among the stocks, or the polymorphism identified were not reproducible. In previous studies, genetic diversity among Meloidogynitae snails was evaluated either by allozyme or restriction fragment length.

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