DNA Extraction and PCR-Based Diagnosis of the Root-Knot Nematodes (*Meloidogyne* Species and Races) of Jordan

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ABSTRACT

Several methods of genomic DNA extraction from different developmental stages of Jordanian populations of root-knot nematodes species viz., *Meloidogyne javanica*, *M. incognita* (races 1 and 2) and *M. arenaria* (race 2), were evaluated. Two assays of DNA fingerprinting viz., Sequence Characterized Amplified Regions (SCAR) and Random Amplified Polymorphic DNA (RAPD) based Polymerase Chain Reaction (PCR) were used. Among the tested DNA extraction methods, a minipreparation method was the most efficient, cost and time effective for SCAR-PCR. Methods used for DNA extraction from single juveniles or females were more suitable for RAPD-PCR. Typical DNA products of 670, 420, or 1200 bp in size were specifically amplified by SCAR-PCR when DNA extracts of *M. javanica*, *M. arenaria* (race 2), or *M. incognita* (races 1 or 2), respectively, were used. Accordingly, *Meloidogyne* species in Jordan could be most reliably identified by using SCAR-PCR assay. Using RAPD-PCR primer PA-01 had produced RAPD DNA patterns with clear bands that clearly distinguished one species from the others and so allowed the identification of the three *Meloidogyne* species. Molecular biology techniques for the identification of *Meloidogyne* spp. could be particularly useful in cases of mixed populations of the three species and as a reliable quarantine tests.

Keywords: Diagnostic Assays, Molecular Fingerprints, RAPD-PCR, SCAR-PCR, Survey.

INTRODUCTION

Root-Knot Nematodes (RKNs) are the most economically important group of plant-parasitic nematodes worldwide, attacking nearly every crop grown (Sasser and Freckman, 1987). Over 80 species of *Meloidogyne* have been described, three of which are extremely polyphagous apomictic species viz., *M. incognita* (Kofoid and White) Chitwood, *M. javanica* (Treub) Chitwood, and *M. arenaria* (Neal) Chitwood that are distributed worldwide and account for the majority of crop losses due to RKNs (Xu *et al.*, 2001).

Precise identification of different *Meloidogyne* species is important for the design of efficient control plans (Cenis, 1993). Based on morphological characteristics and host preference, Abu-Gharbieh (1982a and 1982b) in Jordan identified two *Meloidogyne* species viz., *M. javanica* and *M. incognita* from soil and plant samples. Three species of RKNs were identified viz., *M. javanica*, *M. incognita* and *M. arenaria* based on nematode morphology (Barker *et al.*, 1985), North Carolina differential host test (Taylor and Sasser, 1978) and cytogenetics (Triantaphyllou, 1985). It is of great importance to confirm species identity based on

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DNA fingerprints, since it is the least affected by environmental conditions. DNA analysis has been widely used in nematode systematic (Powers *et al*., 2005) and for identification of nematodes (Williamson *et al*., 1997; Randig *et al*., 2002; Zijlstra *et al*., 2004). DNA-based methods that enable the detection, identification and determination of species composition of these nematodes in mixtures were developed (Cenis, 1993; Blok *et al*., 1997; Zijlstra *et al*., 2000; Xu *et al*., 2001). Random Amplified Polymorphic DNA (RAPD) fingerprinting is a rapid technique and was successfully used to distinguish the root-knot nematode species viz., *M. incognita*, *M. javanica, M. arenaria* and *M. hapla* (Cenis, 1993) and to study genetic polymorphism within *Meloidogyne* species (Castagnone-Sereno *et al*., 1994). Particularly, valuable features of RAPD analysis are that no previous knowledge of DNA sequence needed and can be performed easily on small amounts of DNA, such as DNA extracted from single nematode females (Cenis, 1993; Randig *et al*., 2001). Unique RAPD markers may be cloned to develop Sequence-Tagged Site (STS) markers or so called Sequence Characterized Amplified Region (SCAR) markers, and this approach has been utilized to develop PCR primers that allowed differentiation of *M. hapla* and *M. chitwoodi* (Williamson *et al*., 1997) and that allowed differentiation of *M. arenaria*, *M. javanica*, and *M. incognita* (Zijlstra *et al*., 2000; Qui *et al*., 2006). Therefore, the objectives of this study were to evaluate different DNA extraction methods from different developmental stages of the recently surveyed RKNs and to evaluate the extracted DNAs in RAPD- and SCAR-PCR based techniques for identifying *Meloidogyne* species and races in Jordan.

MATERIALS AND METHODS

Preparation of Pure Nematode Cultures

Eighty-three populations of the root-knot nematodes *Meloidogyne* spp. previously collected from Jordan (Abu-Gharbieh *et al*., 2005; Karajeh *et al*., 2006) were used. Single egg-mass populations of RKNs were developed by inoculating individual egg-masses and culturing them on susceptible tomato seedlings cv. GS 12. They were further propagated into additional pots and maintained under growth room conditions adjusted at 25 + 3^{o} C air temperature and 16: 8 hour light-dark intervals. Three *Meloidogyne* spp. were previously identified ; viz., *M. javanica*, *M. incognita* (races 1 and 2), *M. arenaria* (race 2), based on studies of nematode morphology, host preference (based on the North Carolina differential host test), cytogenetics and DNA fingerprinting. (Karajeh, 2004; Karajeh *et al*., 2006).

Nematode Genomic DNA Extraction

Due to difficulties in collecting relatively large amounts of RKNs, several methods of genomic DNA extraction from different developmental RKN stages including eggs, single or many 2nd stage juveniles and females were used and compared.

DNA Extraction from Eggs, Juveniles and Females

A minipreparation procedure was followed after modification of previously reported methods by Cenis (1993) and Swain *et al.* (1995). Several hundreds of juveniles, 10-15 egg-masses, or 10 females were collected from each population culture (originating from single egg-mass), placed in a 1.5-ml microtube and disinfested with 500 µl of 0.5 % sodium hypochlorite for 5 minutes. After a brief centrifugation in a microcentrifuge at 8000 rpm (Eppendorf, Model 3150, Germany) and washing of the pellet with sterile water, 400 µl of extraction buffer (250 mM Tris-HCl, pH 8.0; 250 mM sodium chloride; 50 mM Ethylene Diaminetetraacetic Acid (EDTA) and 0.5 % Sodium Dodicyl Sulfate, SDS), and also 10 µl of β-mercaptoethanol (1% then modified to 2.5%) was added. Nematodes were crushed with a conical grinder that fits the tube exactly and acted by hand for 5 minutes. The lysate was placed in the freezer (at -20oC) for 20 minutes after the addition of 0.5 volume of 3M sodium acetate at pH 5.2. The tubes were then centrifuged at 13000

rpm for 2 minutes and the supernatant was transferred to another sterile tube. Two volumes of cold absolute ethanol (at -20oC) were added and left in the freezer at -20oC for 1 hr and the precipitated DNA was pelletted by centrifugation at 14000 rpm for 3 minutes. The pellet was washed with 70% ethanol, air dried for 1 hour in an electrical oven at 25o C and re-suspended in 50 µl of 1X PCR buffer (10 mM Tris-HCl, pH 8.3; 50 mM KCl, 2.5 mM MgCl2, 0.1% Triton X-100, 0.01% gelatin). Some DNA samples were re-suspended in nuclease-free water or in TE buffer (10 mM Tris-HCl (pH 7.4), 1mM EDTA (pH 8.0).

Proteinase K enzyme according to Williamson *et al*. (1997) and De Ley *et al*. (2002) methods was also used. Several hundreds of juveniles or few of mature females were picked, transferred to a 1.5ml microtube containing 50 µl nematode lysis buffer (1X PCR buffer with 100 µg /ml proteinase K), and crushed using an aerosol tip before freezing at –20^{o} C for 1 hour. Incubation was then performed at 60^{o}C for 1 hour, followed by inactivation of proteinase K by incubation at 94^{o}C for 10 minutes. Centrifugation was done at 13000 rpm for 2 minutes and supernatants (DNA extracts) were stored in the freezer (at -20 ^{o} C).

A modified proteinase K method was adapted for extracting genomic DNA from eggs (Williamson *et al*., 1997; De Ley *et al*., 2002). Fifty egg-masses were collected from single egg-mass population, placed in a 1.5 ml microtube and disinfested with 500 µl of 0.5% NaOCl for 5 minutes. After a brief centrifugation in a microcentrifuge at 8000 rpm and washing the pellet with sterile water, 100 µl of 1X PCR buffer containing 100 µg/ ml proteinase K was added to the tube. The solution was vortexed for a moment then incubated in an electrical oven at 37^{o}C for 24 hours. The solution was then vortexed thoroughly, incubated at 55^{o}C for 10 minutes and at 95^{o}C for 3 minutes to inactivate proteinase K. The lysate was incubated in the freezer (at -20^{o} C) for 20 minutes after the addition of 0.5 volume of 3 M sodium acetate at pH 5.2. The tubes were then centrifuged at 13000 rpm for 3 minutes and the supernatant was transferred to another sterile tube. Two volumes of cold absolute ethanol (at -20^{o}C) were added and left in the freezer at -20^{o}C for 1 hour. The precipitated DNA was pelletted by centrifugation at 14000 rpm for 3 minutes, washed with 70% ethanol, air dried for 2 hours at room temperature and re-suspended in 50 µl of 1X PCR buffer.

DNA Extraction from Single Female or Juvenile

Two methods of DNA extraction from single females and one method from single juveniles were used and compared:

Sodium Dodicyl Sulfate (SDS) method: One female was excised from galled plant root, washed in a drop of tap water on a sterile Petri-dish, surface sterilized in a drop of 0.5% NaOCl, washed in a drop of distilled water, transferred to 25 µl of the minipreparation extraction buffer and crushed. DNA extraction performed as previously described. The DNA extracts were re-suspended in 25 µl of 1X PCR buffer.

Sodium hydroxide (NaOH) method: One female was excised from galled plant root, washed in a drop of tap water on a sterile Petri-dish, surface sterilized in a drop of 0.5 % NaOCl, washed in drop of distilled water. The female was sucked to the opening of 100 µl tip by micropipette, transferred to PCR tube, overlaid with 20 µl of 0.25 M NaOH, incubated at 25^{o} C in an electrical oven for 24 hours, heated at 99^{o} C for 3 minutes, allowed to cool at room temperature, spanned down, and neutralized with 4 µl 1M HCl, 10 µl 0.5 M Tris-HCl (pH 8.0), and 5 µl 2 % Triton X-100. The solution was again heated at 99^{o} C for 3 minutes, spined down, and allowed to cool at room temperature (Floyd *et al*., 2002).

One 2nd stage juvenile was picked, placed in one-drop (20 µl) of nuclease-free water on a sterile Petridish,

and directly crushed with a sterile aerosol tip. The lysate was stored in the freezer (at –20°C) and further directly used for DNA amplification.

Nematode DNA Amplification and Visualization

a- SCAR Based PCR Assay

SCAR based PCR primers developed by Zijlstra *et al.* (2000) were used and synthesized by Alpha DNA (Montreal, Canada) shown in (Table 2). The primer pairs were reported to be specific for amplifying the DNA of *M. arenaria*, *M. incognita*, and *M. javanica*, respectively. For DNA amplification, the thermocycler (Eppendorf Personal Cycler, Germany) was programmed as follows: initial DNA denaturation step at 95° C for 2 minutes followed by 45 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 1 minute and DNA synthesis at 72°C for 2 minutes and a final extension step at 72°C for 10 minutes. The thermocycler lid was preheated at 104° C to prevent water condensation during the reaction. Reaction products of SCAR-PCR were loaded, electrophoretically separated, and UV-visualized (Sambrook *et al*., 1989).

b- RAPD Based PCR Assay

Amplification reactions were carried out using RAPD based PCR primer PA-01 (Alpha DNA, Montreal, Canada) that its sequence (Table 2) was found useful by Cenis (1993) to differentiate *Meloidogyne* species. Reaction conditions, time intervals, and the number of cycles were optimized. Amplification reactions for RAPD-PCR were performed in 25 µl reaction volumes containing 1X PCR buffer, 200 µM each of dATP, dCTP, dGTP and dTTP, 1 unit of *Taq* DNA polymerase, the 10-mer primer (PA-01) at a final concentration of 0.3 µM and 1.5 µl of total extracted DNA. The polymerase chain reaction was run under the following conditions: initial DNA denaturation step at 95°C for 2 minutes followed by 50 cycles of denaturation at 94°C for 30 seconds, annealing at 36°C for 1 minute, and extension at 72°C for 2 minutes with ramp time 0.3°C per second for heating from 36 to 72°C and a final extension step at 72°C for 5 minutes.

RESULTS

Several methods of genomic DNA extraction from eggs, juveniles or females were used and compared in their suitability for RAPD and SCAR based PCR assays. A comparison among the DNA extraction methods with respect to nematode developmental stage used in the extraction and the need for nematode crushing, cost, time, expected presence of DNA inhibitors, and relative amount of extracted DNA was shown in Table (1). In the minipreparation method of DNA extraction, the amount of extracted genomic DNA was increased by increasing the concentration of β-mercaptoethanol from 1 to 2.5 % (data not shown). Crushing of 2nd stage juveniles, eggs, and females was greatly enhanced by using a conical grinder that fits the tube exactly rather than by using an aerosol barrier tip. Yet, in both cases, some juveniles and eggs were left uncrushed as judged by microscopic examination but their proportion was greatly reduced by using conical grinder. DNA extracted by this method (from eggs in Figure 1) was then visualized under UV light as a compact high molecular weight band. The bands of RNA (Figure 1) were identified by their quick breakdown after short storage in the freezer compared to the more stable DNA bands. In the DNA rehydration step, 1X PCR buffer was more efficient compared with either TE buffer or distilled water in DNA protection during rehydration. Therefore, the amount of rehydrated DNA was higher and resulted in improving DNA amplification by PCR. In proteinase K method of DNA extraction, increasing the concentration of proteinase K from 50 to 100 µg /ml in the lysis solution yielded three folds higher amount of extracted genomic DNA (data not shown). Reducing the temperature of lysate

incubation from 65 to 60 °C have significantly increased the activity of proteinase K and so led to an increase in the amount of extracted genomic DNA.

Long incubation (at 37° C for 24 hours) was needed for efficient DNA extraction from eggs using the modified proteinase K procedure. The long incubation could allow proteinase K molecules to pass through the natural opening in the egg shell and cause lysis of egg content and liberate DNA molecules. Furthermore vortixing at 14000 rpm for 30 seconds followed by incubation at 55° C for 10 minutes had increased the amount of extracted DNA. Proteinase K inactivation at 95° C for 3 minutes and DNA precipitation and washing followed by protein precipitation had reduced greatly the expected inhibitory effect of the presence of proteinase K molecules in DNA extracts.

The time needed for DNA extraction from single juvenile was shorter for direct crushing of single juveniles in a drop of nuclease-free water (around 12 minutes) than the time needed for SDS method (around 3 hours) or for the NaOH method (around 24 hours). The SDS method was superior by offering DNA extracts suitable for PCR compared with the other two methods.

Sequence characterized amplified region-PCR reaction conditions were optimized. The optimum annealing temperature was found to be 50º C and fixed for all primer pairs used in SCAR-PCR amplification (Table 2). Using the Fjav/ Rjav primer pair, a typical DNA product of 670 bp in size was amplified when extracted DNA from *M. javanica* was used as a template DNA (Figure 2A). No amplification could be observed when DNA extracts from *M. arenaria* or *M. incognita* (race 1 or 2) were used as a template DNA. Similarly, using the Far/Rar primer pair, a typical DNA product of 420 bp in size was amplified when extracted DNA from *M. arenaria* (race 2) was used as a template DNA (Figure 2A). No amplification could be observed when DNA extracts from *M. javanica* or *M. incognita* (race 1 or 2) were used as a template DNA. Similarly, using the Finc/Rinc primer pair, a typical DNA product of 1200 bp in size was amplified when extracted DNA from *M. incognita* (race 1 or 2) was used as a template DNA. No amplification could be observed when DNA extracts from *M. javanica* and *M. arenaria* (race 2) were used as a template DNA (Figure 2A).

Typical DNA products of 670, 420, or 1200 bp in size were amplified when extracted DNA of *M. javanica*, *M. arenaria* (race 2), or *M. incognita* (race 1 or 2), respectively, were used as template DNA (Figure 2A).

The RAPD-PCR reaction conditions were optimized and suited for the thermal cycler. The optimum annealing temperature was found to be 36º C for PA-01 primer. Amplification of DNA by RAPD-PCR was successfully obtained using DNA extracts from minipreparation and modified proteinase K methods and to a less extent from single females or juveniles.

Using RAPD–PCR, PA-01 primer produced DNA patterns with clear bands that clearly distinguished any species from the others and so allowed the identification of the three *Meloidogyne* species. After optimizing amplification with PA-01, certain bands were consistent and diagnostic. The size of these bands was 600 and 700 bp for *M. javanica*, 600 bp for *M. arenaria* (race 2), 600 and 850 bp for *M. incognita* without consistent variation in the number and size of the amplified DNA fragments was observed between the two races (Figure 2 B).

DISCUSSION

The results presented in this study show that it is possible to use RAPD-PCR to identify the three *Meloidogyne* species in Jordan. Amplification with PA-01 primer produced diagnostic DNA patterns at the species level. The number of 10-mers of random sequences is virtually unlimited. This is a clear advantage over isozyme studies, in which the number of

available enzymatic systems is limited (Cenis, 1993). One notable feature of RAPD-PCR in relation to RFLP studies, that require microgram amounts of DNA, is its ability to work with crude preparations of DNA in picogram amounts. This makes it possible to analyze using RAPD-PCR with as little material as a single juvenile (Cenis, 1993) or single females (Randig *et al*., 2001). The use of single juveniles is useful in studies of genetic variation, diagnosis of mixed populations and in the identification of *Meloidogyne* species from infested soil prior to the growing season.

The minipreparation method of DNA extraction was relatively efficient since it is highly reproducible for the isolation of nematode DNA from second stage juveniles, adults and even from few egg masses when compared to other protocols of nematode DNA extraction. It eliminates the need of utilizing liquid nitrogen lyophilized material, phenolization procedure, proteinase K treatments and also incubation steps. As a consequence, it allows for processing of many nematode samples per day and is also relatively inexpensive.

Extraction of genomic DNA from single female was done using the SDS based method described herein. This extraction method has several advantages over the method of DNA extraction from single female reported by Randig *et al*. (2001). This method comprises no use of phenol or chloroform, no use of proteinase K, and no long incubation (overnight) therefore safer, time saving, and relatively inexpensive. The major advantage of the extraction of single female DNA and for RAPD-PCR is that it avoids the preliminary space- and time-consuming step of nematode production on host plants (eight weeks on average) needed for RAPD analysis of *Meloidogyne* species. This extraction method could be convenient and reliable tool for epidemiological and ecological studies of RKNs. These results are in general agreement with earlier findings of Cenis (1993) and Swain *et al*. (1995). However, differences in the source and purity of the DNA may result in different fingerprints. This makes RAPD-PCR not a very suitable method for routine identification purposes. Moreover, the use of short, non-specific primers will allow amplification of any contaminant DNA (including microbial contaminants), possibly present in extracts, which will confuse the nematode pattern.

The three primer pairs of SCAR-PCR (Fjav /Rjav, Finc /Rinc, and Far /Rar) were able to direct amplification of single fragments with a specific size from the target DNA of *M. javanica*, *M. incognita*, and *M. arenaria*, respectively. Size and specificity of the amplified DNA products agree with the studies of Zijlstra *et al*. (2000). The SCAR-PCR technique is simple, rapid (3 hours depending on the thermal cycler), and safe because it does not involve the use of radioactive isotopes. Utility of SCAR-PCR for RKN detection in infested soils requires further studies. Since SCAR-PCR is specific at species level, it will be particularly useful in cases of mixed populations. Reliable identification of the quarantine root-knot nematodes viz., *M. chitwoodi* and *M. fallax* became possible and practical by using SCAR-PCR based techniques (Zijlstra, 2008). Moreover, SCAR-PCR based technique is characterized by the three Ss: species selectivity, sensitivity and speed (Arnheim and Erlich, 1992) that makes it possible to use for plant resistance management, reliable routine quarantine and research purposes.

Table 1: A summarized comparison among the used genomic DNA extraction procedures.

Procedure	Nematode stage	Need for manual crushing	Relative DNA yield	Estimated time needed (hrs)	Expected presence of DNA inhibitors	Relative Cost
Minipreparation [1]	All	Yes	High	5	Low	Low
Proteinase K [2]	Juveniles and females	Yes	Moderate	1.5	High	High
Modified Proteinase K	Eggs	No	Low	25	Low	High
Direct crushing	Single juveniles	Yes	Low	0.2	High	Very low
SDS based method	Single females	Yes	Low	3	Low	Low
NaOH based method [3]	Single females	No	Low	24	High	Low

(1) According to *Cenis*, 1993

(2) According to *Williamson et al*., 1997 and *De Ley et al*., 2002.

(3) According to *Floyd et al*., 2002.

Table 2: Nucleotide sequences of Meloidogyne species-specific primers and expected sizes of their typical amplified DNA bands.

Primer Name	Primer Sequence (5¯ to 3¯)	DNA Typical band (bp)	Specificity
Far [2]	TCGGCGATAGAGGTAAATGAC	420	*M. arenaria*
Rar	TCGGCGATAGACACTACAACT		
Finc	CTCTGCCCAATGAGCTGTCC	1200	*M. incognita*
Rinc	CTCTGCCCTCACATTAAG		
Fjav	GGTGCGCGATTGAACTGAGC	670	*M. javanica*
Rjav	CAGGCCCTTCAGTGGAACTATAC		
PA-01 [3]	CAGGCCCTTC	Unique patterns	Differential

[1] SCAR-PCR primers were developed by Zijlstra *et al*., 2000.

[2] RAPD-PCR primer.

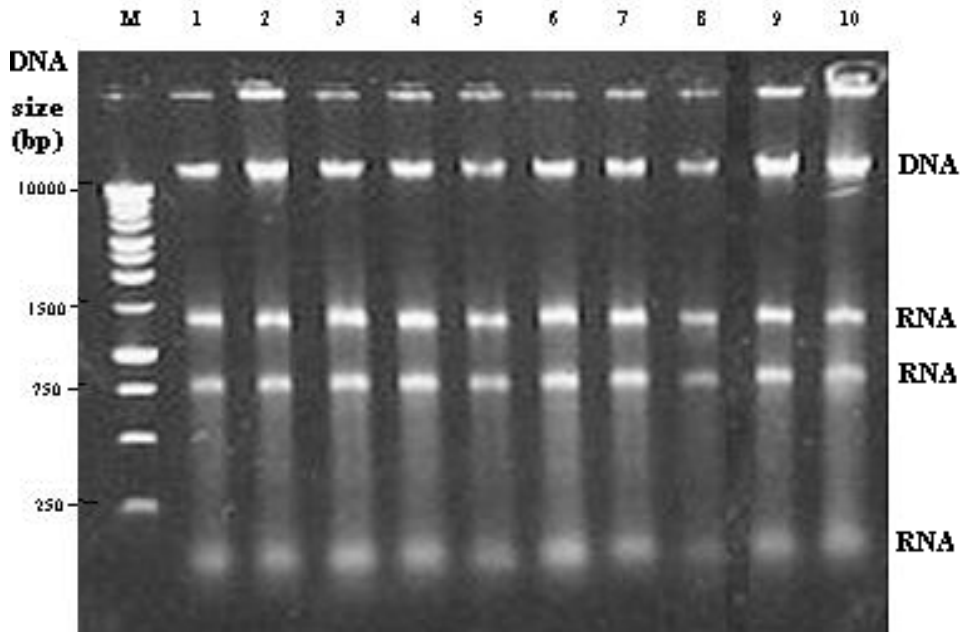

Figure 1: Total nucleic acids (DNA and RNA) extracted from different *Meloidogyne* populations (*Lanes* 1-10: P35-P40, P82, P28, P30 and P71) extracted from eggs using the minipreparation method. *Lane* M; 1kb-DNA ladder.

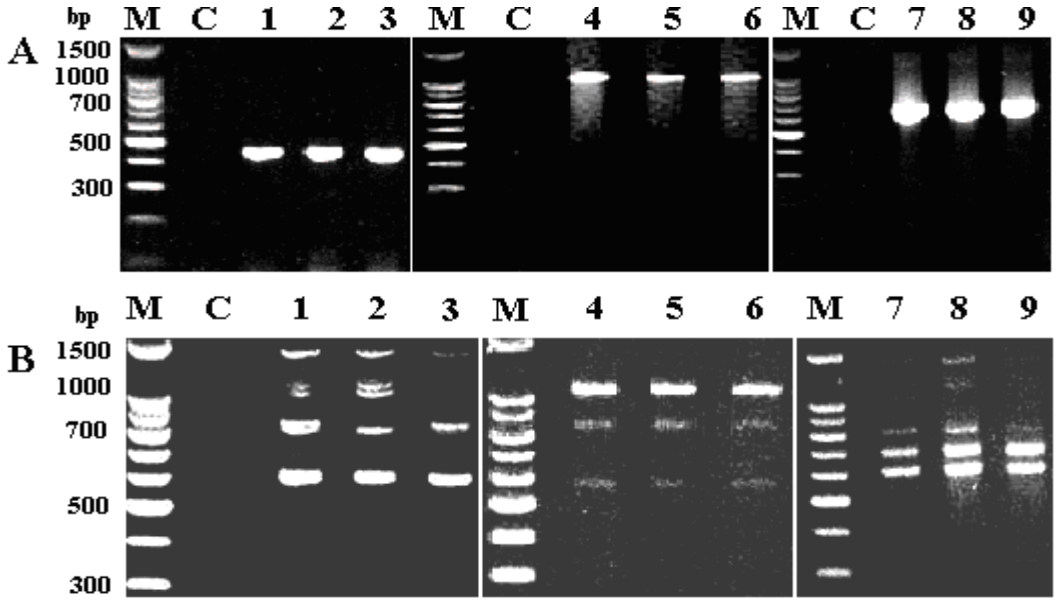

Figure 2: Ethidium bromide stained agarose gel electrophoresis of PCR amplified from extracted DNAs of the three *Meloidogyne* species. A: Amplified DNA amplification products (670, 420 and 1200 bp) using species-specific SCAR-PCR primer pairs (Fjav/Rjav, Far/Rar, and Finc/Rinc, respectively); B: Amplified DNA products of RAPD–PCR using PA – 01 primer using PA-01 primer, *lanes* 1-3: *M. arenaria* populations; *lane* 4: *M. incognita* (race 1); *lanes* 5 and 6: *M. incognita* (race 2) populations; *lanes* 7-9: *M. javanica* populations; *lanes* C: Water control; *lanes* M: 100 bp-DNA ladder.

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عزل الـ DNA والتشخيص المعتمد على الـ PCR لأنواع وسلالات نيماتودا تعقد الجذور (*Meloidogyne* Species And Races) في الأردن

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ملخص

تم في هذه الدراسة تقييم عدة طرق لعزل الـ DNA من الأطوار المختلفة للمجتمعات الأردنية من نيماتودا تعقد الجذور (*Meloidogyne javanica* و *M. incognita* (السلالتين 1 و 2) و *M. arenaria* (السلالة 2). حيث استخدم فحصي البصمة الوراثية لـ DNA لتشخيص أنواع النيماتودا معتمداً على تفاعل البلمرة المتسلسل PCR، وهما الفحص المعتمد على المناطق المضخمة ذات التسلسل المعروف (SCAR-PCR)، والفحص المعتمد على الـ DNA المضخم عشوائياً متعدد الأشكال (RAPD-PCR). من بين الطرق المستخدمة في عزل الـ DNA، كانت طريقة minipreparation هي الأكثر فعالية وأقل كلفة ووقت، خاصة في فحص SCAR-PCR. وكانت الطرق المستخدمة لعزل الـ DNA من اليافعات أو الإناث المفردة أكثر ملاءمة لفحص الـ RAPD-PCR. تم تضخيم نواتج نوعية من الـ DNA بحجم 670 أو 420 أو 1200 زوج قاعدة على التوالي عندما استخدم الـ DNA المعزول من *M. javanica* أو *M. arenaria* (السلالة 2) أو *M. incognita* (السلالتين 1 و 2) على التوالي. لذا يمكن تعريف هذه الأنواع من نيماتودا تعقد الجذور بالاعتماد على فحص SCAR-PCR. كذلك تبين أن البادئة PA-01 أنتجت أنماط RAPD patterns بحزم واضحة قد ميزت بوضوح كل نوع عن الآخر عند الاعتماد على فحص RAPD ، مما أسهم في تعريف الأنواع الثلاثة من نيماتودا تعقد الجذور. تعدّ التقنيات الحيوية مفيدة خاصة في حالة وجود مجتمعات خليطة من الأنواع الثلاثة وكفحوص تشخيصية يعتمد عليها في الحجر الزراعي.

الكلمات الدالة: الفحوص التشخيصية، البصمات الوراثية، RAPD-PCR، SCAR-PCR، الحصر.

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