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Identification of *Meloidogyne* spp. According to Morphological characteristics, PCR- SCAR Marker and 18S rDNA region in Iraq

Sarah. T. Hasan[1],Inad.D. Abood[2]
[1,2](Department of Plant Protection, College of Agriculture, University of Baghdad, Iraq)
Corresponding Author: Sarah. T. Hasan[1]

Abstract : *This study aimed at diagnosed Root- knot nematodes (RKNs), Meloidogyne spp., based on morphological characteristics of perineal pattern to adult female and molecular diagnoses. Preliminary results of morphological characteristics were showed two species of RKNs, M. javanica and M. incognita, with 73.33% and 20% percentage respectively. However only 6.67% of the root plants infected by both species. as well as molecular diagnoses were showed alignment with morphological characteristics when used several methods to extract genomic DNA (organic compounds, 5% chelex and freezing – boiling method) from single eggmass. 18S nuclear rDNA primer (Mel F/R) and Specific SCAR marker (Jav F/R) were used for detection species, results showed when used Mel F/R primer gave single bands approximately 902bp in all methods of DNA extract. While when used Specific SCAR marker (Jav F/R) results showed that M. javanica in samples that extract by 5% chelex and freezing – boiling methods when used Jav F/R with their that gave 670 bp. When sequencing PCR product for both species results were showed compatibility ratio between Iraq isolate (Meloidogyne javanica) with Meloidogyne javanica in GenBank (KF041325.1) was reached 100% while it reached 98% Iraq isolate (Meloidogyne incognita) with M.incognita that retrieved from GenBank (KU578066.1) in samples that extract by organic compounds after sent PCR product to Macrogen company to detection sequencing of these samples. The results of this present study the first molecular which confirmation and sequence data of presence of Meloidogyne javanica and Meloidogyne incognita in Iraq. Meloidogyne incognita sequences obtained were deposited in GenBank database with accession number (MG696872.1).*

Keywords: *Meloidogyne javanica, Meloidogyne incognita. Morphological characterize, perineal pattern, 18S nuclear rDNA.*

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I. Introduction

Root knot nematodes(RKNs) are causing major damage to many important economic groups [1]. description out of more than 90 species of Meloidogyne spp, [2]. In the cool areas found species, *Meloidogyne* chitwoodi, M. fallax and M. hapla, whilst *M. arenaria*, *M. incognita* and *M. javanica* are more common in warm climates region, tropical and subtropical regions of the world [3]. Accurate diagnosis of *Meloidogyne* species such as any other nematode species has been difficult because of several factors including; limited number of nematology taxonomists, inadequate funding to carry out research and also training of young scientists, wide host ranges, sexual dimorphisms, polyploidy and overlapping morphological characters [4]. It has developed different approaches to improve the accurate identification of the different types of nematodes [5]. The methods of identifying root knot nematodes are based on either morphological, biochemical and / or molecular approach, it is based on the traditional diagnosis of RKN primarily on morphological features, such as perineal patterns, data morphometric for females, males and the second stage events [6, 7]. However, the morphological data and morphological characterize require a great deal of work and are often inconclusive because it varies considerably within populations often differ and may not be enough to distinguish closely related Meloidogyne species [8,9]. Various molecular approaches have been designed to accurately determine the various Melodogen sex organs. This is primarily because DNA-based methods are fast and reliable compared to morphological or biochemical methods [10]. More molecular methods used in the diagnosis include mitochondrial DNA (mtDNA), restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLP), random amplified polymorphism DNA (RAPD), sequence characterized amplified region markers (SCAR-PCR), ribosomal DNA (rDNA), microsatellite DNA (satDNA), microarrays and real- time PCR (qPCR) [5,9,11,12 ,13, 14, 15, 16]. The nucleic acid of the nematodes is as in the rest of the eukaryotic of the Cistron, which is usually composed of hundreds of translated copies translated into small subunits (SSU) or called 18S and sub-units large subunit (LSU) or called 28S and also consists of internal interiors transcribed spacers (ITS) and external transcribed spacers (ETS), which are translated as 5.8S, and also consist of external modules that

do not translate External non-transcribed spacers (NTS) [17]. The high similarities in the nucleotide sequence of the SSU and LSU regions and the large differences in the nucleotide sequence in the NTS and ITS regions are useful for the evolution and development of root knot nematodes [18]. Several regions as 18S and 28S ribosomal DNA (rDNA), have been used for phylogenetic analyses and characterization of Meloidogyne species. Ribosomal DNA has highly informative regions for diagnostic and phylogenetic studies of plant parasitic nematodes [19]. The 18S rDNA region was used for phylogenetic analysis of 12 Meloidogyne species, which revealed three well supported clades [20]. Power [21] indicted to the importance of genetic areas 5.8S, 18S, 26S, 28S in the molecular diagnosis of *Meloidogyne* species due to the high variability in nucleotide sequence in these regions compared to ITS and ETS regions. Are not found in the genetically proliferating species of *M. javanica*, *M. arenaria*, and *M. incognita*, which are differentiated by a single nucleotide or by a few nucleotides [22]. Also The 18S rDNA region was used in combination with a mtDNA region for phylogenetic analysis of these species, which revealed three major clades [23]. In Iraq this study the first paper for identification of RKNs, Therefore the objectives of study were to extract genomic DNA from RKNs by used several methods for that's. Hence, the objective of this study was identify and characterize RKN in Iraq by using morphological and molecular markers and DNA sequence data.

II. Material And Methods

Collection Samples

Samples of the roots of eggplant were collected from one of the fields of Babylon Governorate in Hashimia / Shomali Region region in Iraq. After observation the symptoms such as galls or knots on the root systems, the samples were brought to the laboratory and divided into two sections. The first section was placed in the refrigerator [24] until the diagnosis of nematodes was performed as soon as possible. The second section was used to infected seedling of tomato cultivar Super Marmande in a pot 1 kg volume in sunshade conditions to ensure continuity of nematode culture. *Meloidogyne* spp. were identified by using morphological characterize of Perineal pattern of the adult females according method that described by Taylor and Netscher [25] and Hartman and Sasser [26].

DNA extraction

Genomic DNA of RKN extract by using several methods of the extraction from single egg mass which collection from root plant.

DNA nematodes extract by using organic method: used Lysis buffer (10 mM Tris-Hcl (pH 8.0), 50 mM NaCl, 1M Dithiotheitol and 2.0 % SDS) that description by Al-Sammaraie [27]. one eggmass was place in Eppendorf containing 500 µl lysis buffer than added 30 µl of 10 mg / ml proteinase K, mix gently. following incubate tube for 1h at 56 ºC. after digestive was added 500 µl phenol/chloroform/ isoamylalcohol (25:24:1) then inverting gently to mix solution with sample. following by centrifuge for 5 minutes at 14,000 rpm/ min then transfer aqueous layer to a new tube then added 1 ml Absolute ethanol and inverting slowly several times. Sample put in freeze at -20 ºC for 30 min. Centrifuge sample at 14,000 rpm/min and decant ethanol. following by centrifuge at 13,000 rpm for 5 min and washing with 70% ethanol. Decant the ethanol after that dry the pellet for 15 min at room temperature to remove alcohol then dissolve DNA in 30 µl TE buffer. Sample saved in freeze at 4 ºC until used.

DNA extraction by Chelex resin: Eggmass was crushed in 240 µl TE with 200 µl of 5% chelex then added 20 µl of 10 mg / ml proteinase K, incubate sample at 56 ºC for 1h. After incubation vortex sample at high speed for 30 S. Finally centrifuge sample at 14,000 rpm /min and store DNA in 4 ºC until used. [28].

DNA extraction by freezing-boiling: The freezing-boiling method was followed to extract DNA from a single egg mass According to Demeter et.al.[29]with some modification of method .The modification included addition 300 µl distal water in Eppendorf tube that Containing single eggmass , then freezing tube in -37 ºC for overnight after that sample was incubation in thermos mixture at 99 ºC for 30 min with shaking 1000rpm/ min. Repeat the freezing - boiling five times to ensure access to DNA. Sample put in freeze at 4c until used.

PCR analyses and Sequencing: used the primer-pair MelF and MelR to amplify fragment of 18S nuclear rDNA gene [23] shown in table 1. The PCR amplification was done in a 25 µl reaction volume containing 5 µl of PCR Master Mix, INtRON Biotechnology company (MgCl2 (4Mm), dNTP (400µM) *Taq*-polymerase 2.5 Unit) and 1 µl from each primer and 3-5 µl of genomic DNA. PCR program was 7min initial denaturation at 94 ºC and following by 35 cycles of 1 min at 94 ºC, 1 min at 58 ºC for primer annealing and 1 min primer extension at 72 C and final extension step was one cycle at 72 C for 10 min. As well as used SCAR markers, this primer was recorded to be specific for DNA amplification of *M. javanica*, that was developed by Zijlstra et al [9] as shown in table 1. The PCR amplification was done in a 25 µl reaction volume containing 5 µl of PCR Master Mix, INtRON Biotechnology company and 1 µl from each primer and 3-5 µl of genomic DNA. The amplification temperature profile was: 5 min initial denaturing at 94 ºC and 10 min final extension at 72 ºC with the intervening 35 cycles of 30 Sec at 94 ºC, 30 Sec primer annealing at 64 ºC and 1min primer

extension at 72 ºC. 5 µl of each PCR product were separated by 1.5% agarose in 1% TBE buffer at 70 V for 60-70 min, stained with 3 µl red safe stain, then gel was illuminated by ultraviolet light and photographed.

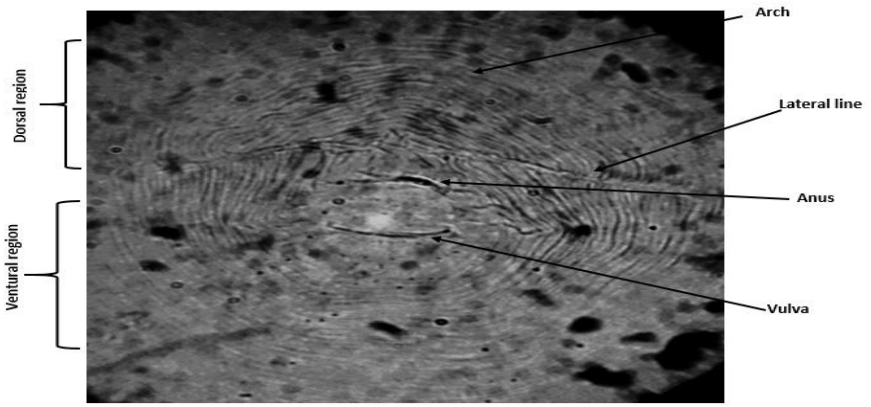
Table (1) Sequence of primers that used for amplifications of *Meloidogyne* species, specific SCAR marker and the partial sequences of 18S rDNA genus of *Meloidogyne* spp.

Primer name	Sequence of primer	Targeted region	Amplified species
Mel	F-TACGGACTGAGATAATGGT R- GGTTCAAGCCACTGCGA	18SrDNA	*Meloidogyne* spp.
Jav	F-GGTGCGCGATTGAACTGAGC R-AGGCCCTTCAGTGGAACTATAC	SCAR Marker	*M. javanica*

Sequence alignments and data analyses: The PCR products of SCAR marker and 18SrDNA partial sequences were sent to Macrogen company in Korea for determination of nucleotide sequences, and then the data of the nucleotides sequence of SCAR marker and 18SrDNA partial sequences were alignment analyzed by https://blast.ncbi.nlm.nih.gov/Blast.cgi in National center biological information (NCBI).

Results and Discussion

Morphological characterize of perineal pattern: The results of the survey showed that all samples of eggplant roots that's collected from the field were infected by root knot nematodes (RKNs). When determined type species by perineal pattern to adult female the results showed two types of species are identified. M. javanica by 73.33% and M. incognita by 20% and 6.67% of the container plants have been found on the two species together. Figure (1- A) that showed perineal pattern for *M. javanica*, which is characterized by existence of the lateral line in the middle of the pattern as this line distinguishes this type from other species. While figure (1-B) illustrates perineal pattern for *M.incognita* which is characterized by existence of high Squarish dorsal arch , which Located in upper dorsal region. These are results consistent with the results of previous studies [30, 31]



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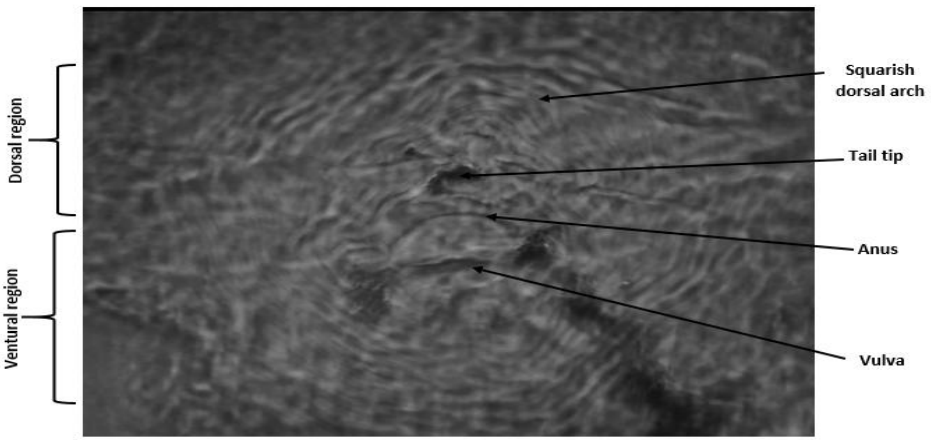

B

Figure 1. Morphological characterizes of perineal pattern to *Meloidogyne* spp from adult female (A) perineal patterns of *Meloidogyne javanica* (B) perineal patterns of *Meloidogyne incognita*.

DNA Extraction from Nematode: Many methods of genomic DNA extraction from single eggmass. The results of DNA extraction showed that the highest purity of DNA (260A/280A) was obtained when using organic extraction method table (2). The concentration of DNA was 293 µg / µl. This is results agree with Schneegurt et al. [32], which indicated Phenol / Chloroform efficiency to remove organic compounds (humus) and pigments found in soil nematodes. The freezing and boiling methods followed after organic method, which reached 1.40 and 33.2 µg / µl respectively. While the latter came in terms of purity and concentration of 5% Chelex method which gave 1.20 and 32.5 µg / µl, respectively. Jacobsen and Rasmussen [33] pointed to the efficiency of the chelex solution in extracting DNA from soil organisms by inhibiting the action of enzymes inhibiting the polymerase chain reaction and also removing the soil molecules. As well as chelex method was rapid and easy However, extraction in this way is characterized by obtaining monoclonal DNA, which requires an interaction Polymerization immediately after extraction to avoid contamination and destruction of DNA.

Table 2. concentration and purity of the nematodes DNA according to extraction methods.

Methods	DNA concentration (µg / µl)	DNA purity (A260/A280)
Organic Extraction	293.0	1.60
Freezing-Bioling method	33.2	1.40
Chelex 5%	32.5	1.20

The results of amplification of PCR reaction when using Mel F/R primer with all samples extracted by the three methods reported, this is a universal primer used to diagnose root-knot nematode within specific sequences in the 18S rDNA gene found in all species of the *Meloidogyne* within simple differences in the sequence of nucleotide of species, the results were showed all sample gave single band 902 bp (Fig.2-A). These results are agreement with the results of Tigano et al. [23] which referred to the efficiency of the Mel F/R primer, which was designed by researcher to diagnose *Meloidogyne* species, moreover enabled phylogenetic tree to be plotted according to the sequence of nucleotides in the 18S nuclear rDNA region. 18S rDNA region found in all species of *Meloidogyne* with a slight difference in the sequence of these nucleotides, which depends on the diagnosis. Another study indicated efficiency of this primer in identify eighteen isolate of root knot nematode species which were collected from coffee plantations across Nicaragua as well as comparison between these species by phylogenetic tree [34]. While PCR results when using the Jav F / R primer showed the presence of *M. javanica* in sample that extracted by the freezing-boiling and 5%Chelex methods, that gave single band 670 bp, whereas while did not give any bands when used with the DNA extracted in the organic compounds. This indicates the presence of *M. javanica* in the DNA extracted in the freezing-boiling and 5%Chelex (Fig. 2-B). These results are consistent with the results of Zijlstra et al. [9] Qui et al. [35] and Karajeh et al. [36] which indicate the efficiency of Jav F / R primers in detection of *M.javanica*.

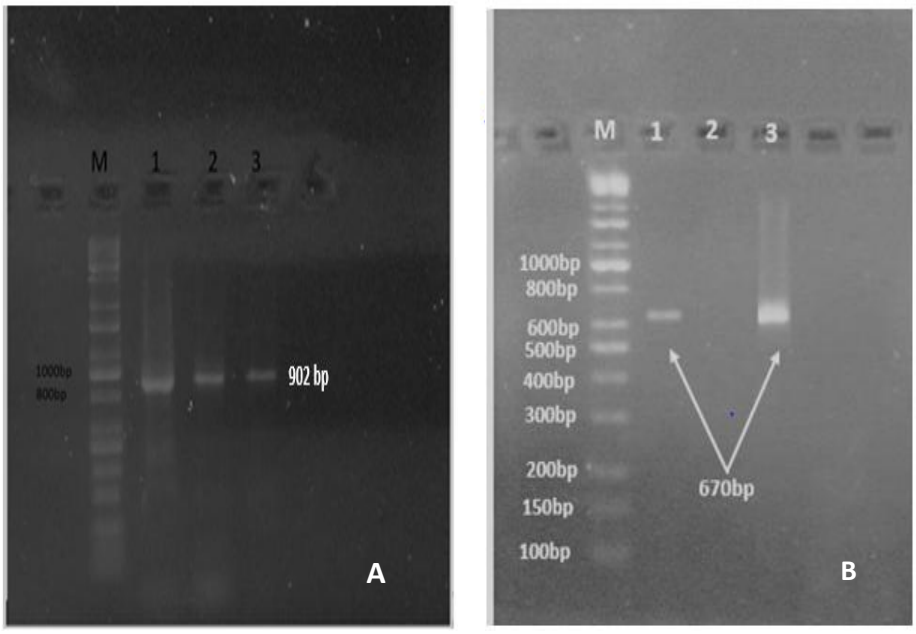

Fig.2. Gel picture showing the amplification patterns of 18S rDNA region by Mel F/R primer and SCAR markers. (A) amplification of Mel F/R in samples which DNA extracted by (1-3) Freezing – boiling, organic methods and 5% chelex 100 resin respectively. (B) amplification of Jav F/R primer in samples which extracted by (1-3) Freezing – boiling, organic methods and 5% chelex 100 resin respectively.

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SCAR marker and 18S rDNA Sequences: The sequence results were showed present *M. javanica* in samples which were extracted by Freezing-boiling and 5% chelex methods when used Jav F/R primer identified as *M.javanica*. when comparison between sequence of *M.javanica* in this present study with *M.javanica* sequence restore from GenBank (KF041325.1), the compatibility ratio of sequences between them was 100% without any type of substitution in their location, and Range of nucleotide from 130-345 (Fig.3). While result of sequence to sample that genomic DNA extraction by organic compounds when used Mel F primer that detection of 18S nuclear rDNA region compatibility ratio was 98% with *M.incognita* that retrieved from GenBank (KU578066.1) because found some variation in sequencing of Iraq isolate in many nucleotide locations of genomic DNA Fig. (4) that demonstrated found transition mutations (substituting a single ring structure for another single ring) as well as transversions (refers to the substitution of a (two ring) purine for a (one ring) pyrimidine or vice versa) . In table (3) we note occurred transition C>T, A>G, A>G, A>G and G>A in locations 839, 853,857 and 866 respectively. Moreover, transversions (T>G) occurred in location 846, 849 and 854 respectively. The results of molecular analysis indicate to agreement with morphological characterize of perineal pattern to these species. These result agree with the studies of Zijilstra et.al [9] Karajeh et.al [36] and Mirehki et.al [37] which indicate to efficiency of SCAR marker in diagnose *Meloidogyne* species. The SCAR marker technicality is easy, fast and unharmed because it does not include the use of radioactive isotopes. advantage of these markers for RKNs reveal in invaded soils demand further studies. Ever after SCAR marker is particular at species level, it will be specifically beneficial in situation of mixed infection. Furthermore, SCAR marker is characterized by three adjectives including species selectivity, critical and speed [38].

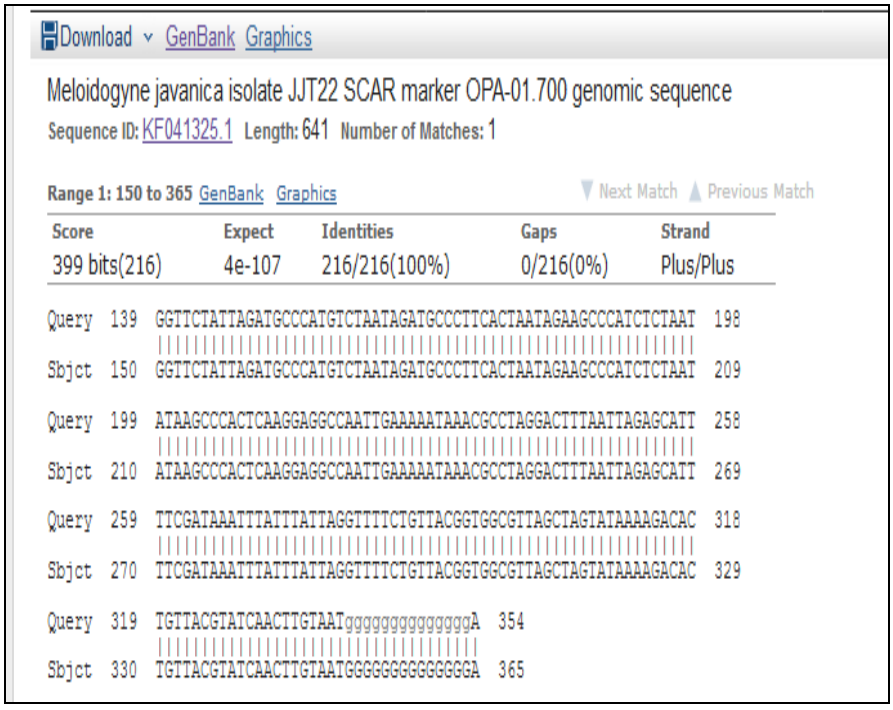

Figure 3. Alignment of isolate Iraqi sequences (*Meloidogyne javanica*) with sequence retrieved from GenBank when used SCAR marker F/R Jav.

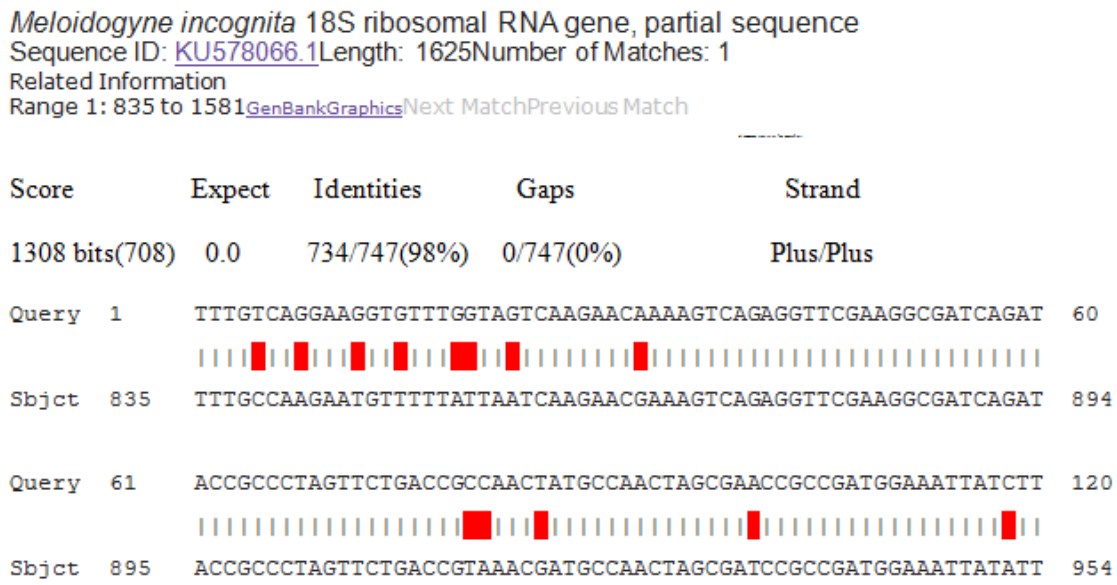

Fig 4. Alignment of isolate Iraqi sequences (*Meloidogyne incognita*) with sequence retrieved from GenBank when used 18S ribosomal RNA gene Mel F/R.

Table. 3 Variation of Partial Sequence of 18S ribosomal RNA gene for Iraq isolate *Meloidogyne incognita* and transition and transversion mutations compared with sequences of the 18S rRNA gene of the reference strains of *Meloidogyne incognita* in GenBank ID: KU578066.1.

Type of substitution	Location	Nucleotide	Range of nucleotide	Sequence ID	Score	Expect	Identities	SOURCE
Transition	**839**	**C>T**	835 to 1581	ID: KU578066.1	1308	0.0	98%	*Meloidogyne incognita*
Transition	**842**	**A>G**						
Transversion	**846**	**T>G**						
Transversion	**849**	**T>G**						
Transition	**853**	**A>G**						
Transversion	**854**	**T>G**						
Transition	**857**	**A>G**						
Transition	**866**	**G>A**						

III. Conclusion

This study presents the first report of identification of *Meloidogyne javanica* and *Meloidogyne incognita* in Iraq based on molecular diagnostic by using specific SCAR marker and 18S ribosomal DNA gene respectively. Moreover, it confirms the presence of transition and transversion mutations in genomic Iraq isolate, *Meloidogyne incognita*, compared with sequences isolates that was recoded in GenBank. Moreover this study shows that the Iraqi isolate (*M.incognita*) was registered in GenBank in accession number (MG696872.1) is the only reference to *M.incognita* which infected eggplant root in Iraq at NCBI when used 18s rDNA region to identify.

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References

[1]. J. N., Sasser, and, D. W. Freckman. A world perspective on nematology: The role of the society. (1987): 7-14 in: Vistas on Nematology: A Commemoration of the Twenty-fifth Anniversary of the Society of Nematologists. J. A. Veech and D. W. Dickson, eds. Society of Nematologists, Hyattsville, MD..

[2]. D. J. Hunt and Z. A. Handoo. "Taxonomy, identification and principal species." Root-knot nematodes 1 (2009): 55-88.

[3]. R. N. Perry, M. Moens and J. L. Starr .Root knot nematodes. (Maurice Moens, Roland N Perry, & J. L. Starr, Eds) Current (pp. 1-101). CAB International.2009.

[4]. C.M.G. Oliveira, A.R. Monteiro, and V. C. Blok. "Morphological and molecular diagnostics for plant-parasitic nematodes: working together to get the identification done." Tropical Plant Pathology 36 (2), 2011: 65-73.

[5]. V.C. Blok, and T. O. Powers. "Biochemical and molecular identification" Root-knot nematodes (2009): 98-118.

[6]. S.B. Jepson. Identification of root-knot nematodes (*Meloidogyne* species). CAB International, Wallingford, United Kingdom.1987.

[7]. R.M.D.G. Carneiro and E.T. Cofcewicz - Plant-parasitic nematodes of coffee. Taxonomy of coffee-parasitic root-knot nematodes, *Meloidogyne* spp. In: Plant-parasitic Nematodes of Coffee (Souza R.M., ed.). Springer Science + Busines Media B. V., Dordrecht, Netherlands. (2008) 87-122.

[8]. H. Hirschmann. "The genus *Meloidogyne* and morphological characters differentiating its species." (1985).

[9]. C. Zijlstra, D.T.H.M . Donkers-Venne and M. Fargette . "Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays." Nematology 2(8), 2000: 847-853.

[10]. T.O. Powers, P.G. Mullin, T.S. Harris and L.A. Sutton. and R. S. Higgins. "Incorporating molecular identification of *Meloidogyne* spp. into a large-scale regional nematode survey." Journal of Nematology, 37(2) ,2005: 226.

[11]. T. Powers. "Nematode molecular diagnostics: from bands to barcodes." Annu. Rev. Phytopathol, .(42), 2004: 367-383.

[12]. M. Fargette, V. Lollier, M. Phillips, V. Blok and R. Frutos,"AFLP analysis of the genetic diversity of *Meloidogyne chitwoodi* and *M. fallax*, major agricultural pests." Comptes Rendus Biologies , 328.(5) , 2005: 455-462.

[13]. M.A.M. Adam, M.S. Phillips and V.C. Blok."Molecular diagnostic key for identification of single juveniles of seven common and economically important species of root- knot nematode (*Meloidogyne* spp.)." Plant Pathology 56 (1) :2007, 190-197.

[14]. N . Meštrović, P. Castagnone-Sereno, and M. Plohl. "Interplay of selective pressure and stochastic events directs evolution of the MEL172 satellite DNA library in root-knot nematodes." Molecular biology and evolution 23(12), 2006: 2316-2325.

[15]. M.H.M. Holterman, M. Oggenfuss, J.E. Frey and S. Kiewnick. Evaluation of High- resolution Melting Curve Analysis as a New Tool for Root-knot Nematode Diagnostics. Journal of Phytopathology, 160(2), 2012: 59-66.

[16]. V.C. Blok, J. Wishart, M. Fargette, K. Berthier, and M. S. Phillips. "Mitochondrial DNA differences distinguishing *Meloidogyne mayaguensis* from the major species of tropical root-knot nematodes." Nematology 4, (7), 2002: 773-781.

[17]. S.D. Berry, M. Fargette, V.W. Spaull, S. Morand, and P. Cadet,. Detection and quantification of root-knot nematode (*Meloidogyne javanica*), lesion nematode (*Pratylenchus zeae*) and dagger nematode (*Xiphinema elongatum*) parasites of sugarcane using real-time PCR. Molecular and Cellular Probes, 22(3), 2008: 168-176.

[18]. D.M. Hillis, and M. T. Dixon. "Ribosomal DNA: molecular evolution and phylogenetic inference." The Quarterly review of biology, 66 (4) ,1991: 411-453.

[19]. R.D.M. Page and E.C. Holmes. Molecular evolution: Aphylogenetic approach. Blackwell Science Ltd., Oxford, UK, 352 pp. (1998).

[20]. V.C. Blok. "Achievements in and future prospects for molecular diagnostics of plant-parasitic nematodes." Canadian Journal of Plant Pathology, 27(2), 2005: 176-185.

[21]. I.T. De Ley, P. De Ley, A. Vierstraete, G. Karssen, G., Moens, M. and J. Vanfleteren,. Phylogenetic analyses of *Meloidogyne* small subunit rDNA. Journal of nematology, 34(4),2002: p.319.

[22]. A.F. Bird and H. R. Wallace. "The influence of temperature on Meloidogyne hapla and M. javanica." Nematologica, 11(4), 1965: 581-589.

[23]. T. Powers. Nematode molecular diagnostics: from bands to barcodes. Annual Review of Phytopathology, **42**, 2004:367-383.

[24]. V. C. Blok."Achievements in and future prospects for molecular diagnostics of plant-parasitic nematodes." Canadian Journal of Plant Pathology, 27 (2), 2005: 176-185.

[25]. A.F. Bird and H.R. Wallace . The influence of temperature on *Meloidogyne hapla* and *M.javanica* , Nematologica 11, 1965:581-589.

[26]. D.P. Taylor and C. Netscher . "An improved technique for preparing perineal patterns of Meloidogyne spp." Nematologica 20 (2) 1974: 268-269.

[27]. K.M. Hartman and J.N .Sasser . "Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology." (1985).

[28]. H.K.I. Al-Sammarraie. Comparison between Two Different DNA Extraction Techniques Taken from Buccal Swabs Suitable for Genetic Analyzer. 19 (3), 2016: 108-113.

[29]. S.P. Stock. Molecular approaches and the taxonomy of Insect-parasitic and pathogenic Nematodes. In: Stock SP ET AL. (Eds.) Insect pathogens: Molecular approaches and techniques, Oxfordshire: CABI international, Oxfordshire, UK, 2009: 71-94.

[30]. J . Demeler, S. Ramünke, S .Wolken, D. Ianiello, L. Rinaldi, Ianiello, D., J.B. Gahutu, G. Cringoli, V. G. Samson-Himmelstjerna,. and J. Krücken. Discrimination of gastrointestinal nematode eggs from crude fecal egg preparations by inhibitor-resistant conventional and real-time PCR. PloS one, 8(4), 2013: p. e61285.

[31]. H. A. Al-Saaedy. "Root-knot nematodes on eggplant in Iraq." Nematologia Mediterranea, 1986 :14.2

[32]. R. F. Al-Sabie and S.N. Ami.. Identification of races of root – knot nematodes *Meloidogyne* spp. In northern Iraq. Arab Journal of plant protection, (8), 1990:83-87.

[33]. M.A. Schneegurt, S.Y. Dore and C.F. Kulpa. Direct extraction of DNA from soils for studies in microbial ecology. Curr.Issues Mol. Biol. (5), 2003: 1–8.

[34]. C.S. Jacobsen and O. F. Rasmussen. "Development and application of a new method to extract bacterial DNA from soil based on separation of bacteria from soil with cation-exchange resin." Applied and Environmental Microbiology 58(8) ,1992: 2458-2462.

[35]. J.J. Qiu, B.B. Westerdahl, C. Anderson and V.M. Williamson."Sensitive PCR detection of Meloidogyne arenaria, M. incognita, and M. javanica extracted from soil." Journal of nematology 38.4 (2006): 434.

[36]. M. R. Karajeh., W. I. Abu-Gharbieh, and S. A. Masoud. "DNA extraction and PCR-based diagnosis of the root-knot nematodes (Meloidogyne species and races) of Jordan." Jordan Journal of Agricultural Sciences 6.3 (2010).

[37]. K. Mirehki., M. Abdollahi, M. Mohaghegh Dovlat Abadi and N. Ghazalbash, ‘‘Application of Morphological Characters and PCR-SCAR Markers for Identification of Dominant Species of Root-knot Nematode (*Meloidogyne* spp.) in Glasshouses of Cold Region of Kohgiluyeh and Boyer-Ahmad Province, Iran “Agricultural biotechnology, 13(1), 2014.

[38]. N. Arnheim and H. Erlich. Polymerase chain reaction strategy. Annual Review of Biochemistry, 61, 1992: 131-156.

Sarah. T. Hasan " Identification of *Meloidogyne* spp. According to Morphological characteristics, PCR- SCAR Marker and 18S rDNA region in Iraq. “IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 11.1 (2018): 50-56.