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Morphological and molecular characterization of *Aphelenchus avenae* found in *Freesia* plants and soil in Mosul-Iraq.

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Abstract

The Mycetophagous nematode *Aphelenchus avenae* is diffusioned in soil and is found in associated with different plants but its ability to cause plant disease remains largely undetermined. *Aphelenchus avenae* were isolated from *Freesia* bulbs or corms infected with root rots caused by *Fusarium solani* , *Fusarium oxysporum* and *Rhizoctonia solani* culturing on Potato Dextrose Agar {PDA}. In addition to isolating them from soil surrounding the rhizosphere of Freesia bulus or corms. *Aphelenchus avenae* were isolated and It was determined by morphological , molecular ways by polymerase chain reaction {PCR} then sequence ITS region. sequence of *Aphelenchus avenae* isolates that have been first registered at the Gene Bank data base.

Keywords: *Aphelenchus avenae*, Mycetophagous nematodes, Morphology, Molecular.

1. Introduction

Aphelenchus avenae Bastian, 1865 Predominantly fungal, commonly found in soil and can found in plant crowns, leaf sheaths may be in bark of a few roots [1 , 2]. [3] Explain that relatively few register of pathogenicity are found in higher plants. The pathogenicity is negligible of this species, however it is likely a vector for other pathogens such as bacteria and fungi. It is unclear whether it can penetrate healthy roots or whether it only attacks plants that have been damaged or weakened by other causes [4]. It is recorded at first in Iraq about [5] when was isolated from wheat crowns infected with crown rot caused by *Fusarium* spp. in Summel-Distract-Duhok, Iraqi Kurdistan region. When incubated at 25°C, this nematode was able to grow and propagate the fungus on media grown on PDA with varying amounts of potato dextrose solution; diffusions of *A*. *avenae* in semi solid substrates was studied too [6].

[7 , 8 , 9, 10, 11 , 6] They all explained *A. avenae* feeds over fifty two genera of fungi mostly plant pathogens and is an important biological control agent.

A.avenae has been used in many studies as a biocontrol agent against soil-borne fungal pathogens, where *A*. *avenae* populations in soils infested by various pathogenic fungi such as *R*.

solani, *Fusarium oxysporum* size variable. The current study aimed to verify characterization of *A. avenae* morphologically and molecularly**.**

Figure1. Distribution of *A. avenae* around the world shown in red region.

Material and Methods:

Collection of sample:

The occurrence of *A. avenae* associated with Freesia bulbs or corms was examined from green house at the college of Agriculture and Forestry, University of Mosul . Specimens for morphological and molecular analysis from *Aphelenchus avenae* was collected from Freesia bulbs or corms by root rot and infested with *Fusarium solani* , *Fusarium oxysporum* and *Rhizoctonia solani* .After culturing Freesia bulbs or corms in potato dextrose agar (PDA) and incubating at 25 °C.

Nematodes were extracted from the medium according to [12] and then added back to the medium for scale-up. The nematodes were then pulled out and mounted on glass slides for identification. Over 25 slides were made for each female, male and egg.

Other nematodes were collected from rhizosphere of Freesia bulbs or corms extracted in the soil using the Baermann funnel method. Put a little amount of soil hundred grams on flimsy paper to a Baermann funnel until 24-48 hours. Nematodes were killed for morphological examination by heat and Fixed in TAF then treated with a slow glycerol lastly fixed on slides in anhydrous glycerol.

Morphological Identification:

The samples were examined using a light microscope with a magnification of 10x and 40x , Morphological identification is a method of classifying the base of a nematode based on its physical characteristics, such as stylet, tail, knobs and mouth, lip area, oesophagus, intestine, spicules and shape of egg.

Molecular Identification:

DNA Extraction:

A method for extracting DNA from nematodes has been describe it by [13] Proteinase K was used and RNase protocol for PCR test . the genomic DNA extraction and purification was used for using beads for Effective cell Lyses [14]. Refer to the Manufacturer's Recommendations, the extraction process was carried out. Using a nano droplet (ND-3000) {Fermentas scientific, Inc.},

the DNA concentration and purity were checked. At -20ºC The extracted {DNA} was retained Until it is used.

Polymerase chain reaction {PCR}.

The PCR interaction was magnification using the gene {Amp-PCR} system Nine thousand seven hundred {9700} thermal cycler {Applied Biosystem; USA}. ITS region was magnification using ITS1 {Forward} and ITS4 {Reverse} By {IDT ,Company; Canada} primer pair was supplied {Table 1} by [15] was described. Taq, PCR Pre Mix {Intron; Korea} The optimized was used in PCR recipe.

The ITS region was magnification about 650bp according to the following program: pre-Denaturation at ninety-five degrees Celsius for three minutes.; (Denaturation at ninety-four degrees Celsius for 45sec; annealing at Fifty-two degrees Celsius for one minutes and reach at Seventy two degrees Celsius for one minutes} for 35 cycles; final extension at Seventy two degrees Celsius for seven minutes then holding at four degrees Celsius . Has been used one and a half percent agarose gel for amplicons were electrophoresed.

Table 1- The sequence of Primers that used in an experience

PRIMER	SEQUENCE	PRIMER SEQUENCE	TM {ºC}	GC%	SIZE OF PRODUCT {BP}
ITS	F	5′- TCCGTAGGTGAACCTGCGG -3′	60.3	50 %	550-600
	R	5′ TCCTCCGCTTATTGATATGC-3′	57.8	41 %	

The sequencing

According to [16] approximately twenty µl of molecular product with front primer { Seventeen pmol} per specimen was sent to sequencing {Macrogen, Korea}. Bellero-phon software accustomed to verify the chimera sequence. Sequences were Compatible with a current pertinent sequence Data-base at the National Center for Biotechnology Information {NCBl} using BLAST {Basic local alignment search tool} program [17]. Phylogenetic of neighbour- Joining Trees were Created by BLAST results as well as using the MEGA Version five Software [18 , 19].

RESULT AND DISCUSSION

Morphological identification

The results of the morphological examination under the light microscope indicated the following:

The morphology of male and female is cylinder shape (Figure 2.A), Head rounded to flattened not offset from body with 3 or 4 very faint head annules barely (Figure 2.B) , stylet present without basal knobs (Figure 2.C) , oesophageal glands overlap intestine dorsally (Figure 2.C) , female tail

short visibly rounded (Figure 2.E) , annules posterior to excretory pore , vulva posterior to located of hinder third of body (Figure 2.D) , spicules paired, slender, not fused, with minute rostrum, (Figure 2.F), eggs oval shape to elongated transparent, embryogenesis process can observe inside eggs(Figure 2. G&H) Ovary outstretched, variable in length (Figure 2.I), these result correspond to [1, 5 , 20 , 21] .

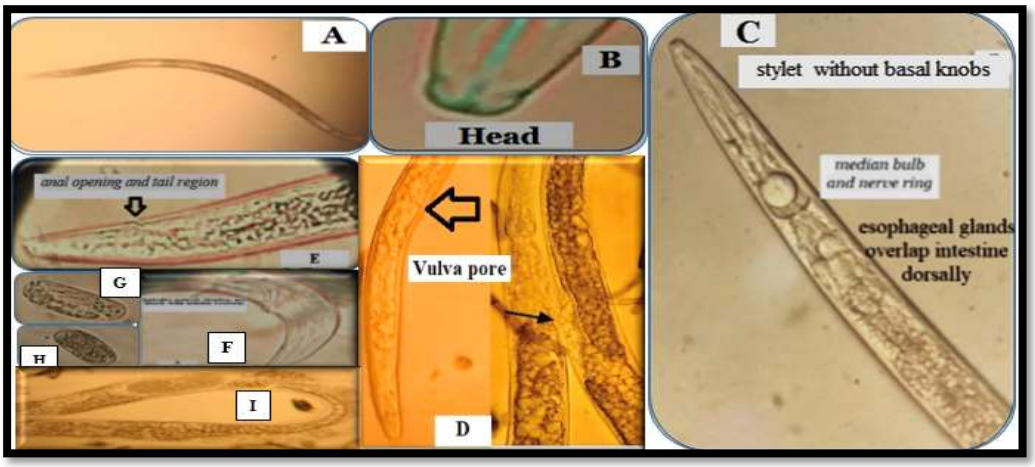

Figure2. *Aphelenchus avenae* (light microscopy) A: shape of nematode ; B: Head ; C: stylet , medium bulb , nerve ring and esophageal gland overlap intestine ; D: vulva and post vulval uterine Sac, E: anal opening and region of Tail F: shape of spicules , G&H : shape of egg , I: ovary.

Molecular Identification

A. aveneae other isolates have been identified by Molecular methods so they represent most widespread isolate nematodes from all tested samples. The 550bp Polymerase chain reaction {PCR} amplicon {Fig.3} was sequenced.

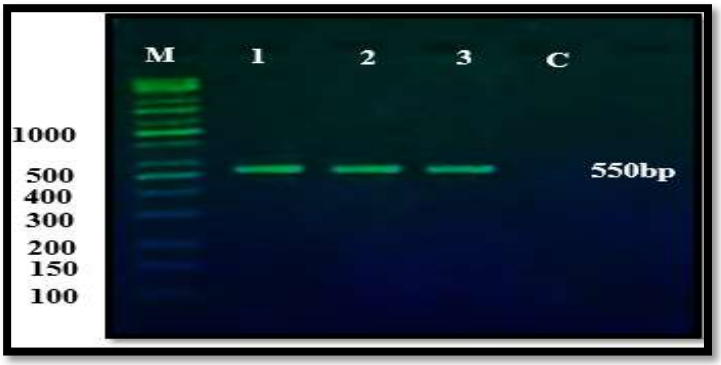

Figure3. The agarose-gel electrophoresis of the amplified Polymerase chain reaction output {550 bp} Lane M, Marker of DNA {10pb}, Lane one-three: ITS of region amplified bands; C which mean Control.

Good sequencing fineness was Aligned to get a closest relative with very similar and an N.J. Tree was created {Table 2}.

Table2. Sequencing Results of the New Strain**.**

	Accession	*Country*	*Source*	*Compatibility*
1.	**ID:** MN190280.1	**Serbia**	***Aphelenchus avenae***	**99%**
2.	**ID:** AF119048.1	**Japan:Iwate**	***Aphelenchus avenae***	**95%**
3.	**ID:** AB368919.1	**Japan**	***Aphelenchus avenae***	**95%**
4.	**ID:** ON112362.1	**China**	***Aphelenchus avenae***	**91%**
5.	**ID:** KJ155784.1	**India**	***Aphelenchus avenae***	**91%**
6.	**ID:** OK560107.1	**Russia**	***Aphelenchus avenae***	**96%**

The sequence with a similarity less from 99%, was placed to the GenBank database as well as given the serial code {ON140587.1}

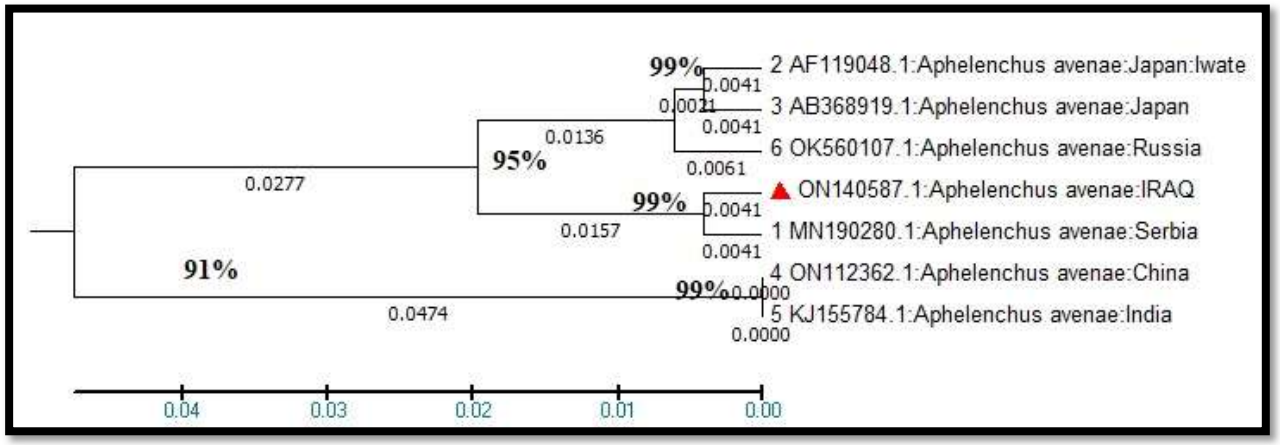

Figure4. The phylogenetic tree of *A. aveneae*

A. aveneae were identified with {99%} similarity to the closer neighbor (figure 4 & table 2) , the isolates showed in a dismiss clade (Red triangle) in the an NJ. tree. This mention that it is a new strains.

CONCLUSION

The isolation results showed the presence of *Aphelenchus aveneae* together with *F. solani* , *F. oxysporum* and *R. solani* culturing on Potato Dextrose Agar (PDA). Sequencing revealed that *Aphelenchus aveneae* was the first record on *Freesia* plants in the studied region and thus, was registered at the Gene-Bank database.

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