ACTA UNIVERSITATIS SAPIENTIAE
AGRICULTURE AND ENVIRONMENT, **11** (2019) 5–15

DOI: 10.2478/ausae-2019-0001

First molecular identification of wheat seed gall nematode *Anguina tritici* races parasitized on wheat in Iraq

Sulaiman Naif AMI,[1] Ibrahim Esa TAHER,[1]
Fenik Sherzad HUSSEN,[2] Ayoub Ibrahim AHMED [3]

[1] Department of Plant Protection, College of Agricultural Engineering Sciences, University of Duhok, Kurdistan Region – Iraq, e-mail: sulaiman.ami@uod.ac
[*]Corresponding author: Ibrahim.esa@uod.ac

[2] Department of Biology, College of Science, University of Salahaddin, Kurdistan Region – Iraq, e-mail: fenik_aya@yahoo.com

[3] Department of Plant Protection, Technical Institute in Khabat, Erbil Polytechnic University, Kurdistan Region – Iraq, e-mail: ayoub.ibrahim@epu.edu.krd

Manuscript received December 07, 2018; revised January 23, 2019; Accepted January 30, 2019

Abstract. This study was conducted to identify races of wheat seed gall nematode *Anguina tritici* by using molecular identification for this nematode species parasitized on durum and bread wheat cultivars. Wheat seed galls of both cultivars were collected from two cities, Erbil and Duhok, from the Kurdistan Region and Iraq respectively. DNA was extracted from both nematode isolates (populations), and then PCR reactions were performed with Internal Transcribed Spacer (ITS) region using primers TW81/AB28 with 2 µl of template DNA of *A. tritici* for both nematode isolates on both wheat cultivars. The bands of both amplification products of PCR reactions were visualized in position about 800 bp on agarose gel, which indicates that both isolates of *A. tritici* belong to the same race. On the other hand, no nucleotide differences were observed between the two nematode isolations, as revealed by the sequence alignment of DNA of the internal transcribed spacer (ITS) region and phylogenetic tree, which emphasizes at the same time that both nematode isolates are of the same genetic structure or have the same identity and confirms their belonging to the same nematode race.

Keywords: molecular identification, *Anguina tritici*, races, wheat

1. Introduction

Ear-cockle disease is the oldest disease of wheat (*Triticum* spp.) [1], which was caused by the first recorded plant parasitic nematode in England by John Needham in 1743 – that is, wheat seed gall nematode *Anguina tritici* (Steinbuch, 1799; Filipjev, 1936) [2]. It is one of the major aerial diseases, and it causes sustainable losses in the wheat crop of tropical and sub-tropical countries [3]. It can be found in any place where wheat is grown, and this disease is still common in Eastern Europe and in parts of Africa and Asia [4]. Symptoms of nematode attack can be discerned at the seedling stage, but farmers generally fail to recognize the disease before harvesting and threshing of the plant [5]. Since its first record in Iraq by Rao in 1921 [6], *A. tritici* has remained an important nematode pest in Iraq, occurring in most wheat-growing areas, with a disease incidence from 22.9% up to 45% on the mexipac cv. of wheat [4]. *A. tritici* caused reduction in the wheat yield, reaching 57% in mexipak cv. [7], which increased to 75% on the same cultivar in Duhok Province in 1989 [8]. Ami et al. [9] reported that the percentage of infestation by galls reached its maximum value (50%) in bread wheat in Bashika, northern Iraq. Ear-cockle disease reduces the human consumption and market price of wheat [10], with significant reduction in the protein and gluten contents of the flour product of infested wheat with seed galls [11]. During the survey in Duhok Province in 2010–2011, it was shown that *A. tritici* was still one of the major problems in this region, reaching 50% in some wheat fields [12].

It is obvious from the previous studies that there are three races of *A. tritici* isolated from wheat plants that could not infect barley plants [11, 12, 13]. So, it was considered that there is another race on barely. Stephan et al. [14] stated that galls from durum wheat cultivars attacked only durum cultivars and from bread wheat infected only bread wheat to show the existence of durum wheat race and bread wheat race. In addition, another race of *A. tritici* was collected in 1998 and recorded for the first time by Stephan et al. [15] based on the differences of their obvious effects on wheat plants such as spike number and seed production. On the other hand, Taher [12] found that the *A. tritici* population from bread wheat has the ability to infect many durum cultivars. Therefore, this study aimed to clarify if there is any variation or similarity in the genetic structure of both populations of wheat seed gall nematode *A. tritici* parasitized on durum and bread wheat to verify – by using molecular identification technique – whether they belong to the same race or to two different races.

2. Materials and methods

A. Nematode samples

Two isolates (populations) of wheat galls were collected, involving galls of durum wheat from the Erbil silo in Erbil Province and galls of bread wheat from the Faydiyi silo in Duhok Province. These two different isolates were used as a source of *A. tritici* for nematode DNA extraction.

B. The extraction and purification of deoxyribonucleic acid (DNA)

DNA were extracted from several 2nd-stage juveniles (*Fig. 1*) for each nematode isolate from Erbil and Duhok provinces, using worm Lysis buffer (WLB), which consisted of 10 mM Tris pH 8.2, 50 mM KCl, 0.45% Tween 20, 2.5 mM MgCl2, 0.05% gelatine, and 60 µg/ml Proteinase K. Nematode juveniles were crushed on the clean slide with 10 µl WLB under binocular microscopes and then transferred to a new PCR tube on ice with an extra 10 µl of WLB. The samples were frozen at -80 °C for 10 minutes, and then samples were warmed up to room temperature, after which incubated in water bath at 60 °C for 1 h and followed by a 95 °C incubation for 10 min to completely lyse the cells, digest the proteins, and inactivate proteinase K. Subsequently, the tube was cooled on ice and centrifuged at 6,000 rpm for 30 sec. [16]. The supernatant material containing the DNA was gathered and stored at -20 °C or directly used for PCR.

C. The amplification of target nucleic acid DNA

Two primers were used for the amplification of the ITS-rRNA gene, which were TW81 (5'-GTTTCCGTAGGTGAACCTGC-3') and AB28 (5'-ATATGCTTAAGTTCAGCGGGT-3') for both nematode populations. The amplification was performed in 25 µl reactions containing 12.5 µl Red MyTaq™ (Mix Master Mix), 1µl of each primer, and the 2 µl of DNA template with 8.5 µl of a double distilled water to obtain a final volume of 25 µl. The conditions of the PCR reaction were 95 °C for 4 min, followed by 35 cycles of 95 °C for 40 sec, 56.5°C for 40 sec, 72°C for 1 min, and a final extension of 72 °C for 10 min [17]. The amplified products and a phiX174 DNA / HaeIII marker were separated on a 1% agarose gel stained with Gel Red in 1 × TAE and then examined under UV light.

D. DNA sequencing

This process was performed in the laboratories of My TACG Bioscience Genomics BioSci and Tech Company in Malaysia, where samples of amplified

DNA products for both nematode isolations were sent to determine the order of the four nitrogenous bases, including: Adenine (A), Guanine (G), Cytosine (C), and Thymine (T) in a strand of DNA. The BioEdit program was used for alignment and a sequence scanner to check the sequence quality before the alignment (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

After the submission sequences of *A. tritici* for both nematode isolates to GenBank in NCBI, the accession number was determined, which was for the Erbil isolate KT900694 while for the Duhok isolate KT900693.

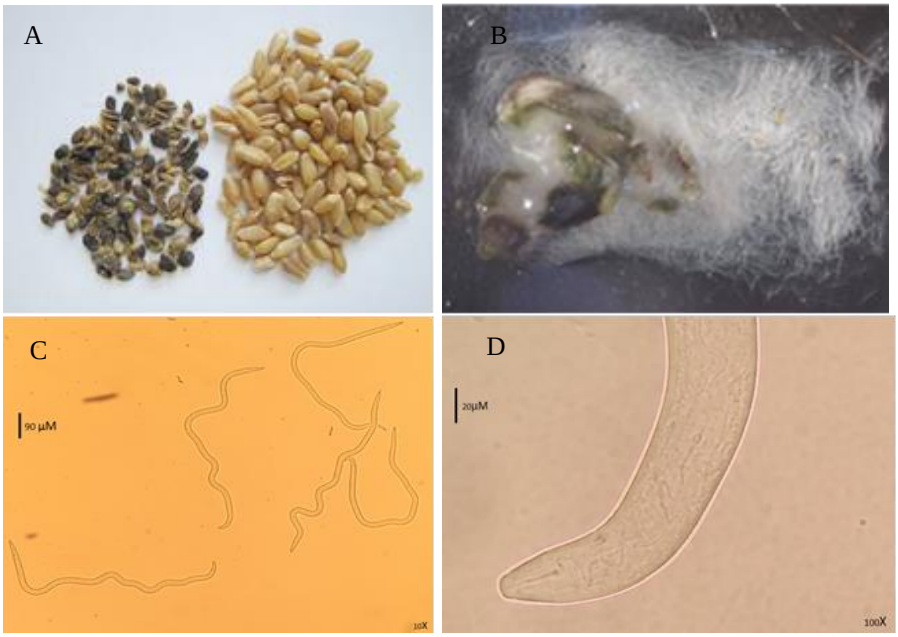


Figure 1. Healthy wheat seeds and galls (A), exiting of second-stage juveniles (J2) of *A. tritici* after opening the gall (B), free J2 of *A. tritici* (C), and anterior portion of J2 show stylet and esophagus (D)

E. Extraction of phylogenic tree

Phylogenic tree was extracted applying the NCBI BLAST program (https://www.ncbi.nlm.nih.gov/BLAST/) by which all nematode species that have the required percentage of genetic similarity with the studied nematode *A. tritici* can be obtained, and it is worth mentioning that those nematodes are in themselves species of the same genus or the same family, and by choosing some of them phylogenic tree was extracted.

3. Results and discussions

DNA sequencing of the Internal Transcribed Spacer ITS PCR-amplified product revealed a size of 800 bp among both isolates of wheat seed gall nematodes *A. tritici* from Erbil and Duhok provinces.

Fig. 2 illustrates the typical amplification products of PCR reactions with primers TW81/AB28, using 2 µl of template DNA of *A. tritici* for both isolates of Erbil and Duhok provinces. The bands of both amplification products of PCR reactions were visualized in position at about 800 bp on agarose gel, which indicates that both isolates of *A. tritici* from Erbil and Duhok provinces belong to the same race because they have the same size of bp. and genotype.

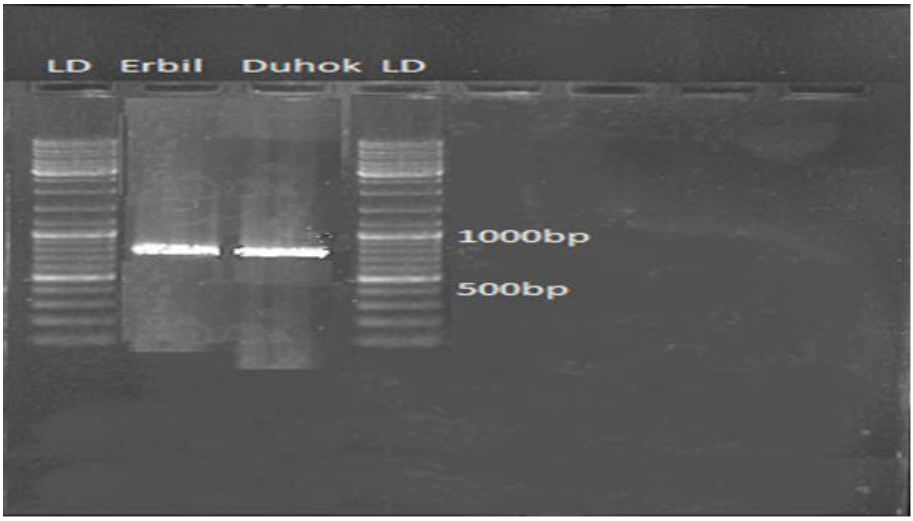


Figure 2. Agarose gel shows typical amplification products of PCR reactions with primers TW81/AB28, using 5 µl of template DNA of *Anguina tritici* of both isolates AE and AD from Erbil and Duhok provinces respectively.

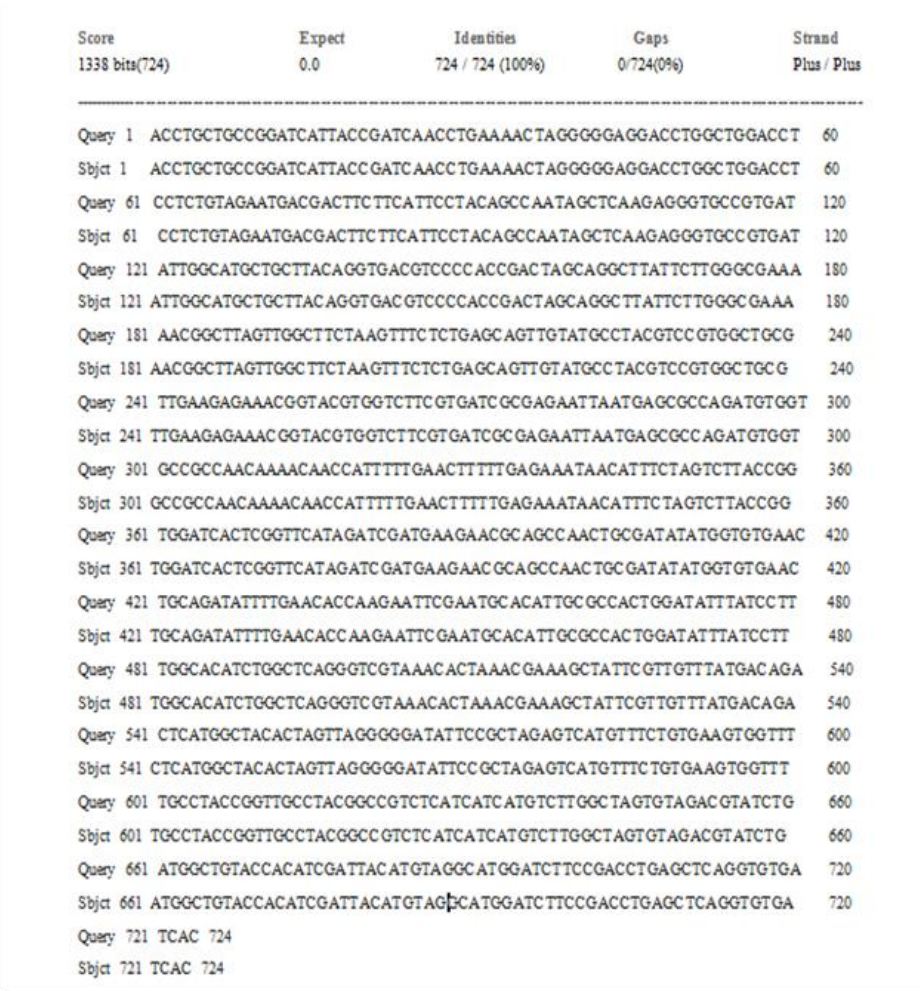
On the other hand, no nucleotide differences were observed between either of the isolations by comparing the DNA sequence alignment of the internal transcribed spacer (ITS) region for both nematode isolates, as clarified in *Fig. 3* and *Fig. 4*. Their genetic similarity analysis, as illustrated by the phylogenetic tree (*Fig. 5*), revealed that both nematode isolates are of the same genetic structure or have the same identity, which also confirms their belonging to the same nematode race.

Score	Expect	Identities	Gaps	Strand
1338 bits(724)	0.0	724 / 724 (100%)	0/724(0%)	Plus / Plus

```
Query  1    ACCTGCTGCCGGATCATTACCGATCAACCTGAAAACTAGGGGGAGGACCTGGCTGGACCT  60
Sbjct  1    ACCTGCTGCCGGATCATTACCGATCAACCTGAAAACTAGGGGGAGGACCTGGCTGGACCT  60
Query  61   CCTCTGTAGAATGACGACTTCTTCATTCCTACAGCCAATAGCTCAAGAGGGTGCCGTGAT  120
Sbjct  61   CCTCTGTAGAATGACGACTTCTTCATTCCTACAGCCAATAGCTCAAGAGGGTGCCGTGAT  120
Query  121  ATTGGCATGCTGCTTACAGGTGACGTCCCCACCGACTAGCAGGCTTATTCTTGGGCGAAA  180
Sbjct  121  ATTGGCATGCTGCTTACAGGTGACGTCCCCACCGACTAGCAGGCTTATTCTTGGGCGAAA  180

Query  181  AACGGCTTAGTTGGCTTCTAAGTTTCTCTGAGCAGTTGTATGCCTACGTCCGTGGCTGCG  240
Sbjct  181  AACGGCTTAGTTGGCTTCTAAGTTTCTCTGAGCAGTTGTATGCCTACGTCCGTGGCTGCG  240
Query  241  TTGAAGAGAAACGGTACGTGGTCTTCGTGATCGCGAGAATTAATGAGCGCCAGATGTGGT  300
Sbjct  241  TTGAAGAGAAACGGTACGTGGTCTTCGTGATCGCGAGAATTAATGAGCGCCAGATGTGGT  300
Query  301  GCCGCCAACAAAACAACCATTTTTGAACTTTTTGAGAAATAACATTTCTAGTCTTACCGG  360
Sbjct  301  GCCGCCAACAAAACAACCATTTTTGAACTTTTTGAGAAATAACATTTCTAGTCTTACCGG  360
Query  361  TGGATCACTCGGTTCATAGATCGATGAAGAACGCAGCCAACTGCGATATATGGTGTGAAC  420
Sbjct  361  TGGATCACTCGGTTCATAGATCGATGAAGAACGCAGCCAACTGCGATATATGGTGTGAAC  420
Query  421  TGCAGATATTTTGAACACCAAGAATTCGAATGCACATTGCGCCACTGGATATTTATCCTT  480
Sbjct  421  TGCAGATATTTTGAACACCAAGAATTCGAATGCACATTGCGCCACTGGATATTTATCCTT  480
Query  481  TGGCACATCTGGCTCAGGGTCGTAAACACTAAACGAAAGCTATTCGTTGTTTATGACAGA  540
Sbjct  481  TGGCACATCTGGCTCAGGGTCGTAAACACTAAACGAAAGCTATTCGTTGTTTATGACAGA  540
Query  541  CTCATGGCTACACTAGTTAGGGGGATATTCCGCTAGAGTCATGTTTCTGTGAAGTGGTTT  600
Sbjct  541  CTCATGGCTACACTAGTTAGGGGGATATTCCGCTAGAGTCATGTTTCTGTGAAGTGGTTT  600
Query  601  TGCCTACCGGTTGCCTACGGCCGTCTCATCATCATGTCTTGGCTAGTGTAGACGTATCTG  660
Sbjct  601  TGCCTACCGGTTGCCTACGGCCGTCTCATCATCATGTCTTGGCTAGTGTAGACGTATCTG  660
Query  661  ATGGCTGTACCACATCGATTACATGTAGGCATGGATCTTCCGACCTGAGCTCAGGTGTGA  720
Sbjct  661  ATGGCTGTACCACATCGATTACATGTAGGCATGGATCTTCCGACCTGAGCTCAGGTGTGA  720
Query  721  TCAC  724
Sbjct  721  TCAC  724
```

Figure 3. Anguina tritici isolate AE Internal Transcribed Spacer1, Partial Sequence; 5.8S Ribosomal RNA gene, Complete Sequence; and Internal Transcribed Spacer 2, partial sequence. Sequence ID: KT900694.1; Length: 724; Number of Matches: 1



Score	Expect	Identities	Gaps	Strand
1338 bits(724)	0.0	724 / 724 (100%)	0/724(0%)	Plus / Plus

```
Query  1    ACCTGCTGCCGGATCATTACCGATCAACCTGAAAACTAGGGGGAGGACCTGGCTGGACCT   60
Sbjct  1    ACCTGCTGCCGGATCATTACCGATCAACCTGAAAACTAGGGGGAGGACCTGGCTGGACCT   60
Query  61   CCTCTGTAGAATGACGACTTCTTCATTCCTACAGCCAATAGCTCAAGAGGGTGCCGTGAT   120
Sbjct  61   CCTCTGTAGAATGACGACTTCTTCATTCCTACAGCCAATAGCTCAAGAGGGTGCCGTGAT   120
Query  121  ATTGGCATGCTGCTTACAGGTGACGTCCCCACCGACTAGCAGGCTTATTCTTGGGCGAAA   180
Sbjct  121  ATTGGCATGCTGCTTACAGGTGACGTCCCCACCGACTAGCAGGCTTATTCTTGGGCGAAA   180
Query  181  AACGGCTTAGTTGGCTTCTAAGTTTCTCTGAGCAGTTGTATGCCTACGTCCGTGGCTGCG   240
Sbjct  181  AACGGCTTAGTTGGCTTCTAAGTTTCTCTGAGCAGTTGTATGCCTACGTCCGTGGCTGCG   240
Query  241  TTGAAGAGAAACGGTACGTGGTCTTCGTGATCGCGAGAATTAATGAGCGCCAGATGTGGT   300
Sbjct  241  TTGAAGAGAAACGGTACGTGGTCTTCGTGATCGCGAGAATTAATGAGCGCCAGATGTGGT   300
Query  301  GCCGCCAACAAAACAACCATTTTTGAACTTTTTGAGAAATAACATTTCTAGTCTTACCGG   360
Sbjct  301  GCCGCCAACAAAACAACCATTTTTGAACTTTTTGAGAAATAACATTTCTAGTCTTACCGG   360
Query  361  TGGATCACTCGGTTCATAGATCGATGAAGAACGCAGCCAACTGCGATATATGGTGTGAAC   420
Sbjct  361  TGGATCACTCGGTTCATAGATCGATGAAGAACGCAGCCAACTGCGATATATGGTGTGAAC   420
Query  421  TGCAGATATTTTGAACACCAAGAATTCGAATGCACATTGCGCCACTGGATATTTATCCTT   480
Sbjct  421  TGCAGATATTTTGAACACCAAGAATTCGAATGCACATTGCGCCACTGGATATTTATCCTT   480
Query  481  TGGCACATCTGGCTCAGGGTCGTAAACACTAAACGAAAGCTATTCGTTGTTTATGACAGA   540
Sbjct  481  TGGCACATCTGGCTCAGGGTCGTAAACACTAAACGAAAGCTATTCGTTGTTTATGACAGA   540
Query  541  CTCATGGCTACACTAGTTAGGGGGATATTCCGCTAGAGTCATGTTTCTGTGAAGTGGTTT   600
Sbjct  541  CTCATGGCTACACTAGTTAGGGGGATATTCCGCTAGAGTCATGTTTCTGTGAAGTGGTTT   600
Query  601  TGCCTACCGGTTGCCTACGGCCGTCTCATCATCATGTCTTGGCTAGTGTAGACGTATCTG   660
Sbjct  601  TGCCTACCGGTTGCCTACGGCCGTCTCATCATCATGTCTTGGCTAGTGTAGACGTATCTG   660
Query  661  ATGGCTGTACCACATCGATTACATGTAGGCATGGATCTTCCGACCTGAGCTCAGGTGTGA   720
Sbjct  661  ATGGCTGTACCACATCGATTACATGTAGCATGGATCTTCCGACCTGAGCTCAGGTGTGA   720
Query  721  TCAC  724
Sbjct  721  TCAC  724
```

Figure 4. *Anguina tritici* isolate AD Internal Transcribed Spacer1, Partial Sequence; 5.8S Ribosomal RNA gene, Complete Sequence; and Internal Transcribed Spacer 2, Partial Sequence. Sequence ID: KT900693.1; Length: 724; Number of Matches: 1

Phylogenetic tree (*Fig. 5*) was extracted to determine the amount of genetic change per time unit between both nematode isolates and some other isolates and species of *Anguina* as well as some nematode species belonging to the family Anguinidae such as stem and bulb nematodes *Ditylenchus* since they belong to the same ancestors.

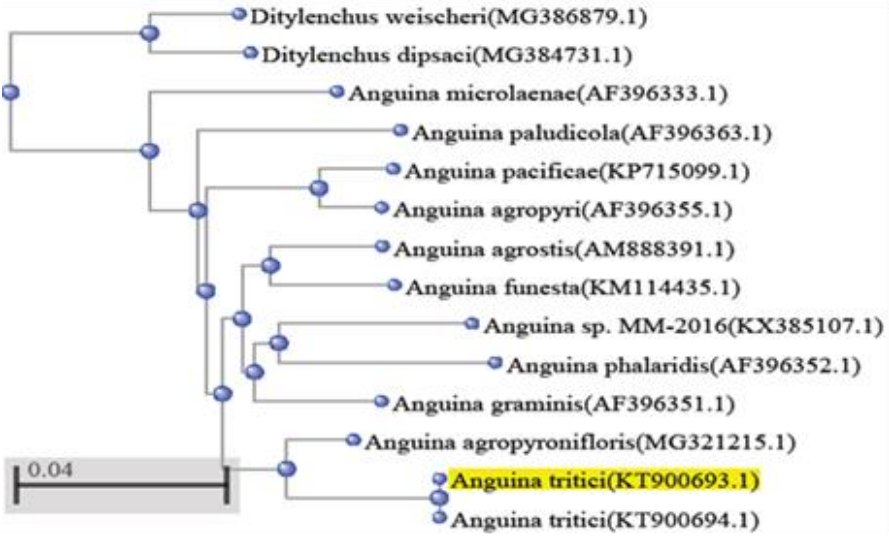


Figure 5. Best Maximum Likelihood Phylogenetic tree based on rDNA ITS sequences within isolates and some species of *Anguina* and two species of *Ditylenchus*. Branch lengths are proportional to the number of inferred changes.

The horizontal lines or branches in this tree represent an evolutionary pedigree that changes over time: the longest the branch in the horizontal dimension, the largest the amount of alteration. The scale bar at the base of the figure displays a scale for this. In this case, the line segment with the number 0.04 shows the length of branch that represents an amount of genetic alteration by 0.04. The units of branch length are nucleotide substitutions per site, which is the number of changes or substitutions divided by the sequence length. The vertical lines are used to lay out the tree visual, and therefore they simply show the connection of horizontal lines with each other and for how long they are unpertinent.

It seems from the phylogenetic tree that the closest species to both nematode isolates is *A. agropyroniflori* with a genetic similarity of 95%, while nucleotide differences increased with other nematode species where more nucleotide differences were observed with *D. dipsaci*, in which the genetic similarity between both isolates and this species reached 86% (*Table 1*).

Table 1. Genetic similarity percentage of isolates AE and AD of wheat seed gall nematode *A. tritici*, which have the accession numbers of KT900694 and KT900693, respectively, with some other nematode species composition [%] of the selected essential oils [13]

Nematode species	Accession number or sequence ID	Genetic similarity percentage with both isolates of *A. tritici*
A. agropyronifloris	MG321215	95
A. agrostis	AM888391	92
A. pacificae	KP715099	91
A. funesta	KM114435	91
A. obesa	KX385107	91
A. graminis	AF396351	92
A. agropyri	AF396355	91
A. phalaridis	AF396352	90
A. paludicola	AF396363	90
A. microlaenae	AF396333	90
Ditylenchus weischeri	MG386879	87
D. dipsaci	MG384731	86

This results clarified that all research related to wheat seed gall nematode *A. tritici* on wheat in Iraq which considered that both durum wheat isolate and bread wheat isolate of that nematode to be different races are not really accurate. Both isolates are the same, but the infection of durum or bread wheat by this nematode is due to the susceptibility of wheat cultivars, environmental circumstances, planting date, the application of both pesticides and herbicides by farmers, and the agricultural processes that one way or another affect the pathogenicity of *A. tritici* [11, 12].

Taher [12] rejected the idea that nematodes isolated from bread wheat invaded only bread wheat cultivars and those from durum wheat attacked only durum wheat cultivars. Thus, several races had been recorded by Stephan et al. [14] in addition to another new race of *A. tritici*, collected in 1998 and recorded for the first time by Stephan et al. [15] based on differences in their pathogenicity on wheat plants such as spike number and seed production.

These three races mentioned by previous studies do not resemble the J_2 population – used in the experiment carried out by Taher [12] – regarding its behaviour of infection, and this might indicate new races of *A. tritici* which have ability to infect different cultivars of durum and bread wheat but incapable of infecting barley cultivars. This result was obtained by Mustafa [11], who reported that *A. tritici* isolated from wheat plants could not parasite on barley plants. Al-Talib et al. [13] recorded a new race of barely plant named barley race. It is clear that there are two races of wheat and barley, but this molecular study emphasized for the first time that both nematode isolates from durum and bread wheat have the same genetic characteristics depending on the amplification products of PCR reactions. Therefore, we can say that *A. tritici* consists of two races, including wheat and barley races, while the pathogenicity of wheat race varies by different cultivars of wheat as well as the infection behaviour and severity may change in different regions and under different environmental circumstances. Also, we recommend the implementation of further molecular studies on different populations for the further clarification of these nematode races in Iraq. According to this result, *A. tritici* on durum and bread wheat belong to the same race or one race, which means that there are two races of *A. tritici* in Iraq: one on wheat and the other one on barley.

4. Conclusions

Molecular identification of wheat seed gall nematode *Anguina tritici* parasitized on durum and bread wheat cultivars which were collected from two cities, Erbil and Duhok, from the Kurdistan Region and Iraq, respectively, emphasized that both nematode isolates are of the same genetic structure or have the same identity and confirmed their belonging to the same nematode race.

References

[1] Bhatti, S., Dahiya, R. S., Dhawan, S. C. (1978), New record of tundu and ear-cockle incidence in barley. *Nematol*., 331–332.

[2] Perry, R. N., Moens, M. (2006), *Plant nematology*. British Library, London, UK: CAB International.

[3] Kort, J. (1972), Nematode diseases of cereals of temperate climates. In: J. M. Webster (ed.), *Economic Nematology*, 97–126.

[4] Al-Beldawi, A. S., Stephan, Z. A., Alwan, H. (1974), General survey of wheat gall disease in Iraq. *Iraq Agric. Bull.* 29, 48–69.

[5] Khan, M. R., Athar, M. (1996), Response of wheat cultivars to different inoculum levels of *Anguina tritici*. *Nematol. Medit.* 24, 269–272.
[6] Rao, R. S. R. (1921), A preliminary list of insect pest of Iraq. Dept. of Agric. Iraq, *Bull.* 7.
[7] Fattah, F. A. (1988), Effect of methods on incidence of ear-cockle and tundu on wheat under field conditions. *Plant and soil* 109, 195–198.
[8] Stephan, Z. A., Antoon, B. G. (1990), Biotypes of earcockle nematode *Anguina tritici* in Iraq. *Current Nematology* 1, 85–8.
[9] Ami, S. N., Mohammad, H. Y., Younis, S. A. (2004), Study on ear-cockle disease in some regions of Nineveh province – North of Iraq. *Iraqi J. for Agric. Sci*. 5, 95–100.
[10] Paruthi, I. J., Bhatti, D. S. (1988), Effect on consumption and marketing of wheat contaminated with seed gall of *Anguina tritici in Haryana. Agric. Uni. J. of Res*. 18, 173–176.
[11] Mustafa, S. A. (2009), Study on wheat and barley ear-cockle disease caused by nematode *Anguina tritici* in Erbil province. M.Sc. thesis, College of Agriculture, University of Salahaddin-Erbil.
[12] Taher, I. E. (2012), Pathogenicity, biology and control of wheat seed gall nematode *Anguina tritici*. M.Sc. thesis, College of Agriculture, University of Duhok, Duhok, Kurdistan Region, Iraq.
[13] Al-Talib, N. Y., Al-Taae, A. K. M., Nimer, S. M., Stephan, Z. A., Al-Beldawi, A. S. (1986), New record of *Anguina tritici* on barley from *Iraq. Int. Nemat. Net. Newsl*. 3, 25–27.
[14] Stephan, Z. A., Hammadi, A. I., Antoon, B. G. (1991), Races of ear-cockle nematode *Anguina tritici* in Iraq. Dept., State Board of Agric. Res. and Educ., Abu-Ghraib, Baghdad, Iraq. *Basrah J. of Agric. Sci.* 4, 1–2.
[15] Stephan, Z. A., Hassan, M. S., Hammadi, A. I., Antoon, B. G. (2000), New race of wheat gall nematode *Anguina tritici* and susceptibility of some cultivars to this race. *Iraqi J. of Agric*. 5, 1–5.
[16] Waeyenberge, L., Ryss, A., Moens, M., Pinochet, J., Vrain, T. C. (2000), Molecular characterization of 18 *Pratylenchus* species using rDNA restriction fragment length polymorphism. *Nematol*. 2, 135–142.
[17] Michel, L., Sikora, R. A. (2005), Plant parasitic nematodes in subtropical and tropical agriculture. 2nd Edition. CABI Bioscience, Egham, UK.