Mesopotamia Journal of Agriculture

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EXISTENCE, MOLECULAR IDENTIFICATION AND GENETIC VARIATION OF NEW ISOLATES OF SEED GALL NEMATODE Anguina tritici PARASITIZING ON WHEAT AND BARLEY IN IRAQ

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Article information
Article history:
Received:12/9/2022
Accepted:20/1/2023
Available:31/3/2023

Keywords:
Anguina tritici, Infestation percentage, Disease incidence, Molecular Identification.

DOI:
10.33899/magrj.2023.135099.1197

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ABSTRACT

This study aimed to investigate the existence of wheat seed gall nematode Anguina tritici in the silos and some wheat fileds in Duhok province / Kurdistan Region - Iraq.,and diagnose four new isolates of this nematode on wheat and one isolate on barley collected from different loactions in Iraq.The results indiacted that wheat impurities samples were infested with seed galls by 66 % as the highest percentager in silo of Faidia during 2020, while the lowest (6%) recoded in the silo of Zakho during the same year.The highest disease incidence was recorded in the wheat fields of Akre by 34.6%,while the lowest (2%) in the wheat fields of Semel. Molecular identification results revealed that the bands of amplified DNA products of all nematode isolates were visualized by agarose gel electrophoresis in the same position ("~" up to 750 bp).Sequencing of the partial gene 5.8S rRNA gene confirmed that all nematode isolates belong to seed gall nematode A.tritici, also a description of their accession number by blast program showed the same percentage identitiy (100 %), and their comparision by a DNA Dot Plot emphasized that they are genetically similar. Results of phylogenetic tree analysis showed grouping of nematode isolates with each other and with the other two isolates of the same species from Iraq,whilst nucleotide variations increased with other nematode species of the same genus,and increased more with other nematode species of the same family (Anguinidae).

INTRODUCTION

Ear – cockle disease caused by seed gall nematode *Anguina tritici* (Steinbuch, 1799) Filipjev, 1936 (Anguinidae, Tylinchida) is one of the important and common diseases of wheat crop and spreads in the most areas grown by wheat in the world. This nematode is commonly found where the practice of sowing clean seeds is not followed (Mukhtar, *et al.*,2018). Wheat seed-gall nematode was the first recorded plant parasitic nematodes on wheat in 1743 England,and several species of *Anguina* are known that cause gall formation on seeds,leaves,and other aerial plant parts of grain crops and forage grasses (Agrios,2005).Ear - cockle disease leads to sustainable losses in the wheat crop in tropical and sub-tropical countries (Kort,1972).It can be found wherever wheat is grown,and this disease is still common in eastern Europe and in parts of Asia and Africa and in Australia (Agrios,2005).Since the first record

of *A.tritici* in Iraq in 1921 by Rao, this nematode remained an important pest on wheat (Ami and Taher,2013) and barley (Ami and Mustafa, 2017) in Iraq. *A.tritici* reduced wheat yield by 57 % in mexipak cv.(Fattah,1988) which seems to be very susceptible to wheat seed gall nematode,where percentage of infection reached 72.85% as recorded by Ami and Taher (2014). it was shown that *A. tritici* is one of the major problems in Duhok province, Kurdistan Region-Iraq,where the wheat was infected in most of the surveyed fields in Duhok province (Ami and Taher,2013). As well as most of the wheat (Both soft and durum c.vs) samples taken from silos of Duhok province were infested with seed galls caused by *A.tritici* (Ami and Taher , 2014).It is clear from the previous studies that races of *A.tritic* on wheat c.vs do not infect barley c.vs (Stephan and Antoon,1990;Al-Talib,1986 and Ami and Taher,2014). Molecular diagnostics was used to determine genetic identity of several species of *Angunia* including *A.tritici* (Powers, *et.al*., 2001 and Li, *et.al*.,2015).For *A.tritici* isolates, previous studies in Iraq stated that races isolates of this nematode species were genetically similar either within races on wheat (Ami, *et.al*.2019) or races isolates on wheat with each other and with high similarity (95.5%) with the race isolate on barley (Al-badrany, *et al.*, 2022).

Therefore,This study was conducted to verify the presence of this nematode in some areas of Dohuk province,whether within the wheat grains imported to the silos or in the wheat fields themselves,in addition to the genetically diagnosing of some new isolates found within infested wheat grains collected from province of Dohuk, Erbil and Kirkuk to compare them with each other and with the genetic structure of a new isolate found within barley grains from Ninawa province.

MATERIALS AND METHODS

1- Collection of seed gall samples during summer 2020 and 2022:

Fiv**e** samples of wheat grain impurities were collected during summer 2020 and 2022, each weighing approximately 10 kg were brought from silo of Zakho**,** Faydia and Shekhan to the laboratory of plant pathology at the department of plant protection in the College to calculate the Infestation Percentage for each sampleby taking 10 sub – samples,with a weight of 100 g from eaxch main sample after mixing it well, where number of seed galls and healthy grains were calculated, and it is worth noting that wheat seed galls were characterized by being smaller in size than a healthy grains and has a bluish black color mixed with brown color,then infestation percenatge was calculated according to the following equation: I.P (%) = (A ÷ B) x 100 where : I.P = Infection Percentage. A= Number of seed galls. B = Total number of seed galls and healthy grains.

2- Survey of wheat fields during March of the growing season 2021- 2022:

Field survey was conducted by choosing five wheat field area in each nine locations in Duhok province involved :Bersvi / Zakho, Derkar /Zakho, Heezawa / Zakho, Grig sindy / Zakho,Semel, Faydia, Shekhan and Akre.Number of infected

wheat plants with an ear – cockle disese was calculated depending on the typical symptoms of the disease, especially those related to the rolling or twisting of the leaves and using wood frame with an area of 1m² was used for this purpose by walking in the field in two axis vertical with each other and throwing it in two directions (left and right) alternatively then disease incidence was calculated depending on the following equation : Disease incidence (%) = (Number of infected plants ÷ Total number of plants in the wood frame) x 100.

3- Molecular identification of new isolations of seed gall nematode *Anguina tritici* on wheat and barley:

3-1: Nematode samples:

Five isolates of *A.tritici* were used for extraction of deoxyribonucleic acid **(**DNA) included : Four isolates for soft (bread) wheat seed gall which were collected from different locations included two isolates from Duhok province (from Faydia silo durinhg 2021 and 2022) and one isolate from each Haweeja / Kirkuk while the other from silo of Erbil province with one isolate of barley seed gall from Bashiqa / Ninawa province.

3-2: Extraction and purification of the nuclic acid DNA:

Second stage juveniles (J2) (≈50) of *A.tritici* were used for extraction of DNA and individually for each of the five nematode ispolates that included: 1D.W (wheat seed galls from Duhok province),2K.W (wheat seed galls from Kikuk province), 3E.W(wheat seed galls from Erbil province).,4N.B (Barley seed galls from Ninawa provinc).and 5 F.W(wheat seed galls from Faydiyi –Duhok province), by Bioscience Animal DNA preparation Kit (Jena Bioscience GmbH .07749 Jena Germany) method using worm Lysis buffer WLB), as described by Castagnone-Sereno *et al.*, (1995).

3 – 3: Amplification of the target DNA by Polymarase chain reaction (PCR):

Two universal primers were used for amplification of the internal transcribed spacer (ITS rDNA) region, which contains ITS1, 5.8S rRNA coding sequence, and ITS2 (Skantar *et al.,*2012) included:10 Picomoles (pmol) of forward primer-TW81(5'GTTTCCGTAGGTGAAC CTGC-3') and 10 pmol reverse primer-AB28 (5'ATATGCTTAAGTTCAGCGGGT-3') provided by Germany company (Jena Bioscience) for the five nematode isolates.The amplification was done as described by Michel and Sikora (2005).The amplified products and a phiX174 DNA / HaeIII marker were separated on 1.5 % agarose gel stained with Gel Red in 1 × TAE,where they were examined under UV light by UV Trans Illumination.

3- 4: DNA sequencing:

The five samples of PCR product 5.8S rRNA partial gene were sequenced by ABI Prism Terminator Sequencing Kit (Applied Biosystem) at Macrogen Molecular

Company of Korea. Chromatograms of 5.8S rRNA gene were edited and base calls checked using Finch TV program software.

3-5: Sequence alignment:

After submission sequences of the five isolates of *A. tritici* to GenBank in NCBI,their accession numbers were determined as follows : ON167528, ON167529, ON167530, ON167531 and ON426963 for each of nematode isolate 1D.W,2K.W, 3E.W.,4N.B. and 5 F.W.collected from silo of Faydia / Duhok province durinhg 2021, Haweeja / Kirkok province, silo of Erbil province, Bashiqa / Ninawa province (Barley seed gall isolate).and silo of Faydia / Duhok province durinhg 2022 respectively**.**The 5.8S rRNA gene sequence were applied to the Basic Local Alignment Search Tool (BLAST) which is a search tool that applies the sequence alignment (https://blast.ncbi.nlm.nih.gov/Blast.cgi#alnHdr_2218760357) and is available at the NCBI (National Center for Biotechnology Information) website for extraction percentage identity for the five nematode isolates with each other and with the other sequences for species selected by NCBI.In addition,DNA Dot Plot (A graphical method for comparing two biological sequences) was used as another method to compare sequences of the five isolates with each other and with the other sequences for species selected by NCBI.

3-6.: Construction of phylogenetic tree:

Phylogenetic tree was constructed using the NCBI BLAST program (https://www..ncbi.nlm.nih.gov/BLAST/) from which all nematode species that have the required percentage of genetic similarity with all isolates of *A.tritici* diagnosed in this study can be obtained, and it should be that those nematodes are in themselves either different isolates of the same species or species of the same genus or the same family (Anguinidae),then after selecting some of them phylogenetic tree was constructed .

RESULTS & DISCUSSION:

1-Infestation Percentage of wheat grains with seed galls caused by wheat seed **gall nematode *A. tritici* collected from silos in Duhok province :** The results of the existence of the seed galls within wheat grains impurities collected from different silos in Duhok province (Table,1) revealed that the highes infestation percentage was found in the samples collected from silo of Faidia (Fayda) with significant difference (as demonstrated by statistical analysis) with the other samples collected from silo of Zakho and Shekhan,while the lowest was recorded by sapmles collected from silo of Zakho,which did not differ significantly with samples collected from silo of Shekhan.At the same time th**e** highes infestation percentage was recorded during summer of 2020 with significant difference compared to the samples collected during 2022.And in general results indicated that the highes infestation percentage (66%) of wheat grains with seed galls was recorded by impurieties samples collected from silo of Fayda (Faidia) during summer 2020 with significant

Table (1): Infestation Percentage of wheat grain samples infected by wheat seed gall nematode *Anguina tritici* collected from different silos in Duhok province.

Location of Silo	Infestation Percentage (%) during summer 2020	Infestation Percentage (%) summer 2022	Effect of both growing seasons together
Zakho	6 d	18 b	12 b
Faidia (Fayda)	66 a	12 c	39 a
Shekhan	10 cd	20 b	15 b
Effect of each growing season	41 a	25 b	

- Impurities were collected from Silo laboratory in each location.
- Means with different letter for the effect of each factor (locations or growing season) or their interaction is significantly differed accoeding to Duncan's Multiple Range Test ($P \leq 0.05$).
- Each value for the interaction between locations and growing season is a mean of 10 sub – samples (replications).
- Each value for the effect of growing season is a mean of 30 values (3 locations x 10 replications).
- Each value for the effect of locations is a mean of 20 values (2 growing seasons x 10 replications).

difference with the samples collected from the other silos during both years (2020 and 2022),while the lowest infestation percentage (6%) was recorded by grain impurieties samples collected from silo of Zakho during the same year (2020) with significant difference with the other samples during both year except with those samples collected from silo of Shekan durin summer 2020.The variation in infestation percenatge between grain wheat impurities samples collected from the silos is due to the differences in wheat infection by ear – cockle disease in wheat fields from whichs crops were transferred to the silos after harvest.The increase of the infestation percentage in silo of Faydia as an a verage of both years is due to the great superiority of the infestation percentage in the wheat grain impuritie in silo of Faidia during summer 2020.

2-Disease incidence of surveyed wheat fields in Duhok province infected with ear - cockle disease caused by wheat seed gall nematode *A.tritici* during march 2022:

The results showed that some of the surveyed wheat fields were infected with ear – cockle disease, and the disease incidence varied among the fields,where the highest disease incidence (34.6 %) was recorded in wheat fields of Akre with a significant difference (as indicated by the statistical analysis) with the other wheat fields followed by disease incidence (24.4%) in the wheat fields of Bersvi /Zakho,whereas the lowest disease incidence (2%) was recorded in the wheat fields of Semel with a non significant difference compared to the disease incidence in the wheat fields of each of Grig sindy /Zakho (4.8%) and Faydia (5.2%) (Table,2).This discrepancy in the disease incidence in the surveyed wheat fields is attributed to the variation of factors or conditions suitable for the development of the disease in one region without

the another, such as planting of highly susceptible wheat cultivars to this disease in the fields with the highest disease incidence and the presence of the appropriate environmental conditions, such as availability of sufficient moisture that helps 2nd stage juveniles to climb on wheat seedlings, especially as some farmers resort to irrigating the fields due to the lack of rain during this year,or maybe to plant wheat seeds infested with seed galls, or planting seeds in the soil already infested with seed galls,where they play a role in increasing the disease incidence that have occurred in some fields, while the absence of infection or a decrease in a disease incidence in some wheat fields is due to planting of low susceptible wheat cultivars and using of dusty wheat seeds with fungicides, as well as using herbicides for the purpose of weed control in the wheat fields which play a significant role in reducing the disease.Also, some farmers resort to grazing wheat fields at a certain stage of wheat seedling age in order to increase the number of seedling tillers,that causes a significant decrease in the disease incidence due to animal feeding on the above part of the seedlings that contians nematode juveniles after climbing on them.

Table (2): Disease incidence percentage (D.I %) of surveyed wheat fields infected with ear - cockle disease caused by wheat seed gall nematode *A. tritici* in selected locations in Duhok province during growing season 2022

Location	Hezawa / Zakho	Grig sindy / Zakho	Derka / Zakho	Bersvi / Zakho	Semel	Shekhan	Akre	Fai-dia
D.I (%)	14.2 c	4.8 de	14.4 c	26.4 b	2 e	8.4 d	34.6 a	5.2 de

- Means with different letter (s) are significantly differ according to Duncan's Multiple Range Test ($P \leq 0.05$).
- Each value is an average of 5 replications (5 wheat fields).

3- Molecular identification of new isolates of seed gall nematode *Anguina tritici* on wheat and barley:

3-1 : Electrophorized of amplified partial 5.8S rRNA gene in 1.5 % Agarose gel: The bands of amplified DNA products of the Internal Transcribed Spacer (ITS) by P C R reaction with the use of two universal primers TW81-F and AB28 Actin-R for the five nematode isolates were visualized as showed by agarose gel electrophoresis in the same position that is **"~"** up to 750 bp (Figure,1), which indicates that both primers were efFective in amplifiaction of DNA extracted from 2nd juveniles of the five nematode isolates parasiizing on wheat or barley.These results came to some extent similar to what has been found by Ami and *et al.,* (2019) in diagnosing two isolates of wheat seed gall nematode with 800 bp,which is due to the use of the same primers in the amplificAtion process, so it came to confirm the increase of genetic similarity between them.

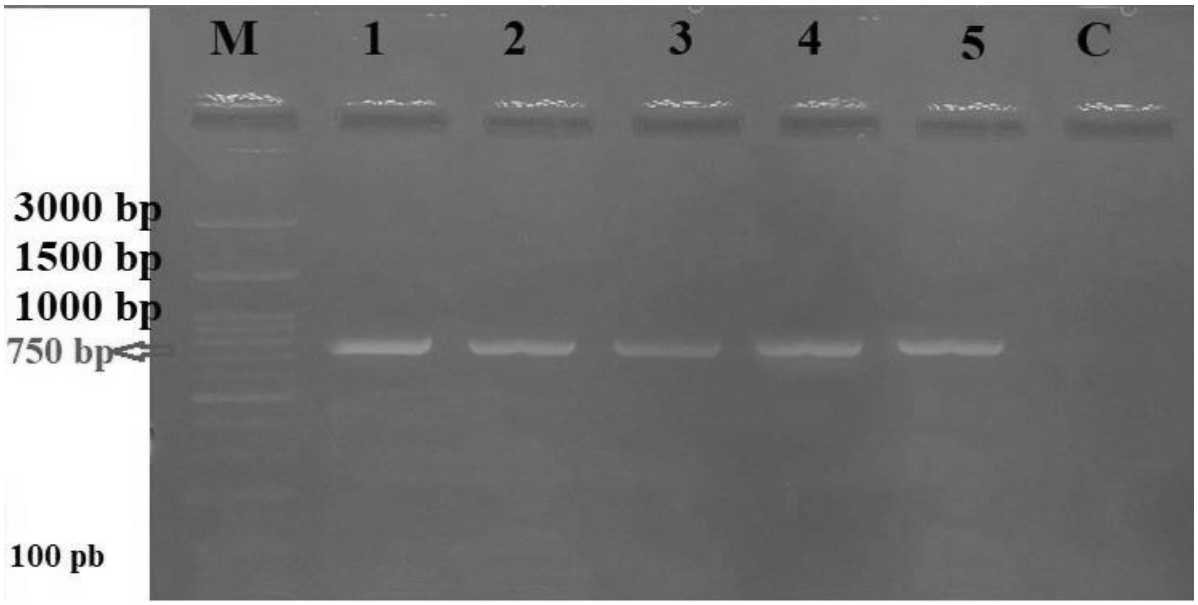

Figure (1): Agarose gel electrophoresis of the P C R products, M is a Marker ladder, Lanes 1,2,3,4 and 5 are positive product for pair of primers (TW81-F with AB28Actin-R) for all nematode isolates: 1D.W, 2K.W, 3E.W.,4N.B. and 5 F.W., where 4N.B.is nematode isolate on barley and the other on wheat and C is a negative control of sterile distilled water.

3-2:Molecular Identification of seed gall nematode *A.tritici* isolates parasitizing on wheat and barley: Results of PCR product sequencing of the partial gene 5.8S rRNA gene confirmed that all nematode isolates detected in this study belong to seed gall nematode *A.tritici*,which was proven by sequence alignment of the ITS region of the five nematode isolates by BLAST program from Gen bank that there are no nucleotide differences between them, which means that they are genetically similar ,where the percent identity between them is 100% (Table,3).This results were confirmed by Dot plot extraction for each nematode isolate with the other (Figure.2),although the previous studies (Stephan and Antoon,1990; Al-Talib,1986 and Ami and Taher,2014) proved that wheat isolate does not infect barley and vice versa, that is,each isolate was considered as an independent race in itself. On the other hand and according to the sequence description illustrated by BLAST of the NCBI, sequence of the nematode isolates diagnosed in this study was used to compare with other stored isoltea of nematode species sequences.The results got from the BLAST indicated that the highest percent identity was 100% with sequences of several isolates of *A.tritici* such as Isolate AE. (KT900694.1) (Table, 3) on wheat from Iraq, while the lowest percent identity was 99.28 % with nematode isolate MNEK 3 (MT675020.1) of the same species on wheat from Iraq too as confirmed and illustrated by a dot plot extraction (Table,3 and Figure,3).In comparing with the other nematode species of the same genus the highest percent identity was 95.39 % with *A.agrostis* isolate IQY21 (OL405087.1) (Table, 3 and Figure,4).on barley from Iraq, whilst the lowest was 89.31 % with *A.paludicola* (AF396364.1) (Table, 3 and Figure,5). from Australia, and in comparing with some nematode species belong to the same Family (Anguinidae) percent identity was 87.17 and 86.87% as the highest

and the lowest percentage with each of *Ditylenchus dipsaci* isolate 710_G-(C58-ON)) (MG384729.1) and *D.weischeri* isolate 648_CT-(Road) **(**MG386858.1) respectively from Canada. (Table,3 and Figure,6 and 7 respectively).

Table (3): Percentage identitiy of the five isolations of seed gall nematode *A.tritici* diagnosed in this study compared to the other isolates of the same species or the same genus or the same family according to the blast of Genbank NCBI of partial 5.8S rRNA

Seq.	Nematode species,isolate and its host	Accession number	Percent identity %	Country
1	*A. tritici* isolate 1D.W. on wheat	ON167528.1	100	Iraq
2	*A. tritici* isolate 2 K.W. on wheat	ON167529.1	100	Iraq
3	*A. tritici* isolate 3 E. W. on wheat	ON167530.1	100	Iraq
4	*A.tritici* isolate 4 N.B. on barley	ON167531.1	100	Iraq
5	*A. tritici* isolate 5 F.W on wheat	ON426963.1	100	Iraq
6	*A.tritici* isolate AE. on wheat	KT900694.1	100	Iraq
7	*A. tritici* isolate MNEK 9 on wheat	MT675026.1	99.46	Iraq
8	*A. tritici* isolate MNEK 3 on wheat	**MT675020.1**	99.28	Iraq
9	*A. tritici* isolate MNEK 10 on barley	MT675027.1	100	Iraq
10	*A. Agrostis* isolate IQY21 on on barley	OL405087.1	95.39	Iraq
11	*A. agropyronifloris* on *Pascopyrum smithii*	MG321215.1	94.90	USA
12	*A. agrostis* isolate 74A on bentgrass	KM114437.1	**92.27**	USA
13	*A. graminis* isolated from leaf gall	AF396351.1	92.09	Russia
14	*Anguina agropyri* isolated from sem gall	AF396355.1	91.40	Estonia
15	*A. pacificae* isolate 74C on bluegrass	KM114440.1	91.29	USA
16	*A. funesta* isolate 74D on ryegras	KM114439.1	91.17	Auatralia
17	*A. phalaridis* isolated from seed gall	AF396352.1	91.50	Estonia
18	*A. paludicola* isolated from seed gall	AF396364.1	89.31	Auatralia
19	*A. obesa* Isolate Juvenile 1 isolated from seed gall	KX385108.1	90.67	Iran
20	*Ditylenchus dipsaci* isolate 710_G-(C58-ON) on garlic	MG384729.1	87.17	Canada
21	*D. weischeri* isolate 648_CT- (Road) on *Cirsiu arvense*	MG386858.1	86.87	Canada

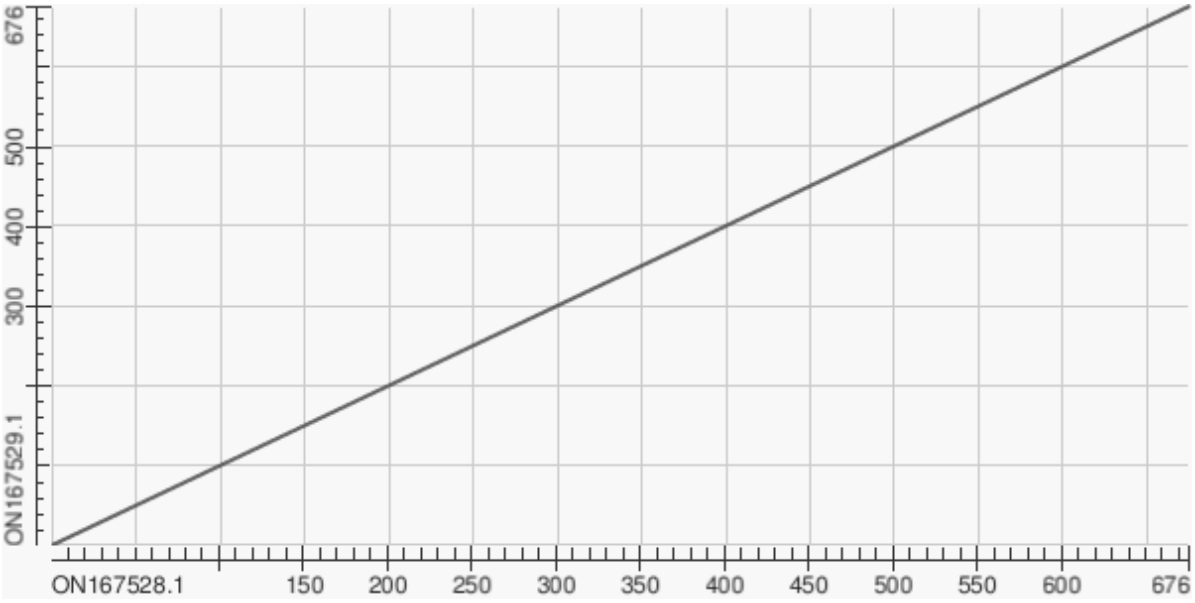

Figure (.2): A DNA Dot Plot of *A.tritici* isolate 1D.W. (ON167528.1) on wheat vs *A.tritici* isolate 2 K.W. (ON167529.1) on wheat (The other isolates of this nematode species diagnosed in this study followed the same path with each other).

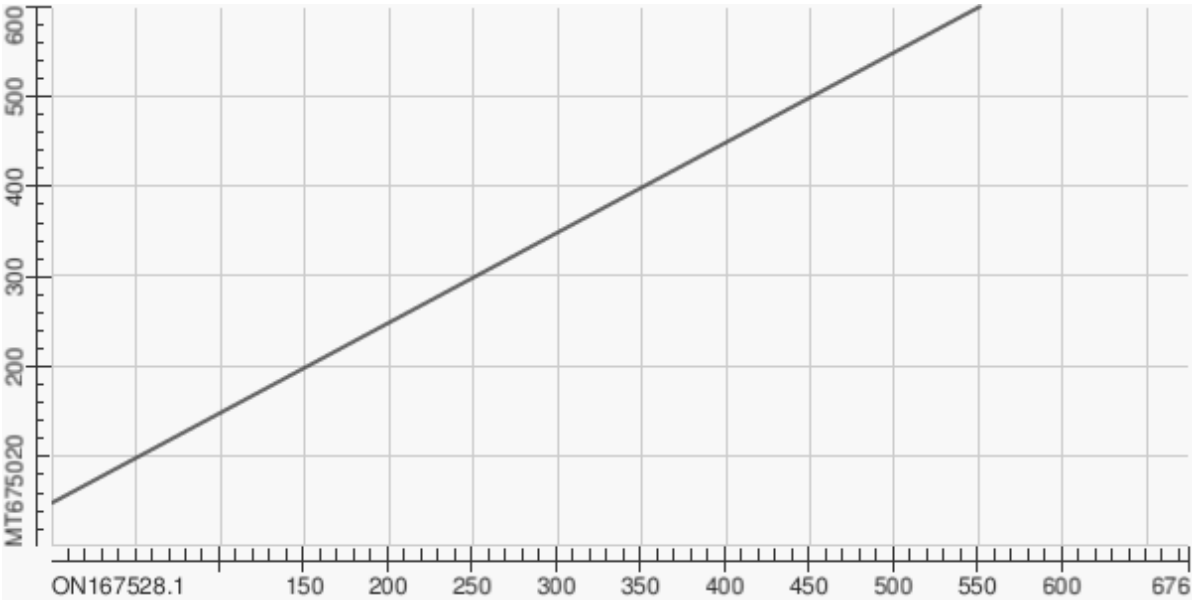

Figure (3): A DNA Dot Plot of *A.tritici* isolate 1D .W. (ON167528.1) on wheat vs *A. tritici* isolate MNEK 3 (MT675020.1) on wheat from Kirkuk - Iraq (The other isolates of nematode species diagnosed in this study followed the same path).

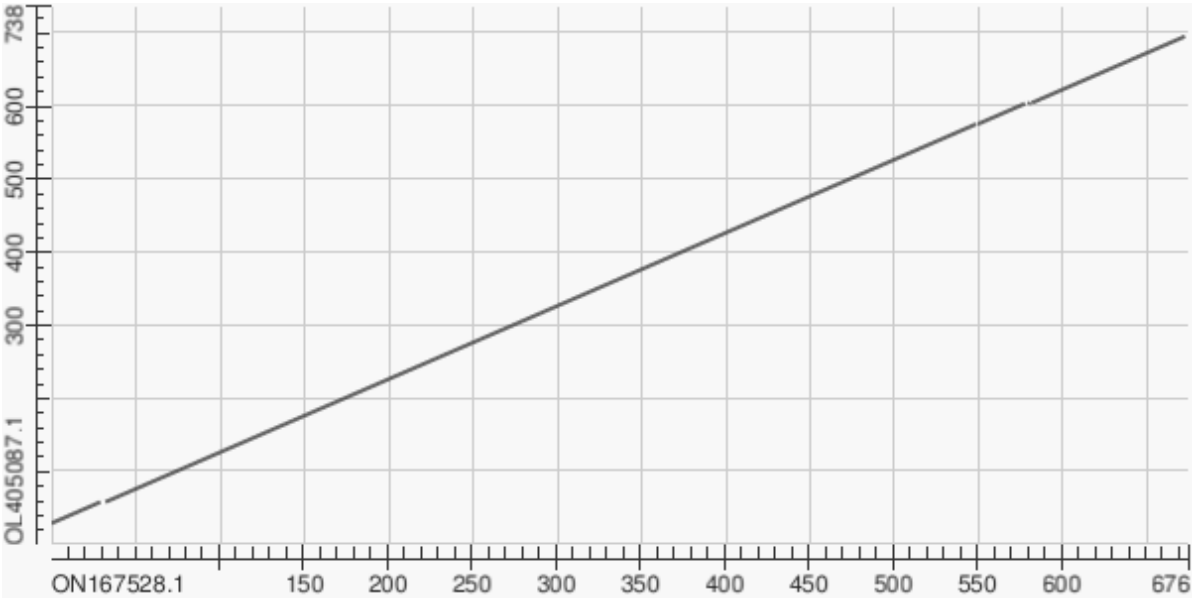

Figure (4): A DNA Dot Plot of *A.tritici* isolate 1D .W.on wheat ON167528.1 vs *A. agrostis* isolate IQY21 (OL405087.1) on barley from Iraq (The other isoltes of nematode species diagnosed in this study followed the same path)

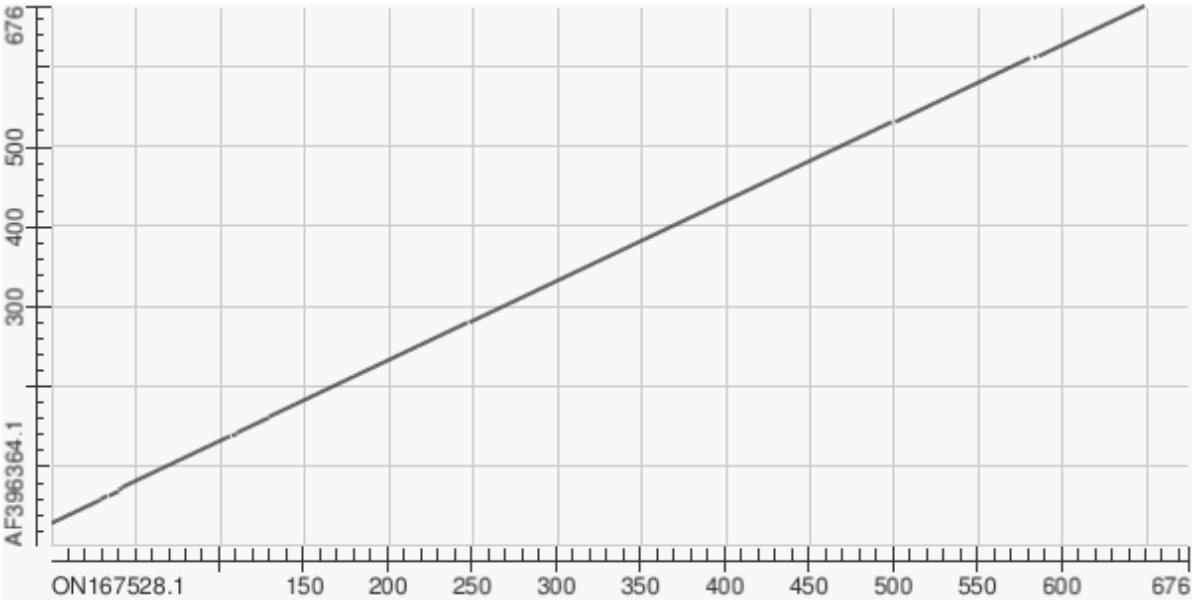

Figure (5): A DNA Dot Plot of *A.tritici* isolate 1D .W. (ON167528.1) on wheat vs grass seed gall nematode *A. paludicola* (AF396364.1) from Australia (The other isolates of nematode species diagnosed in this study followed the same path).

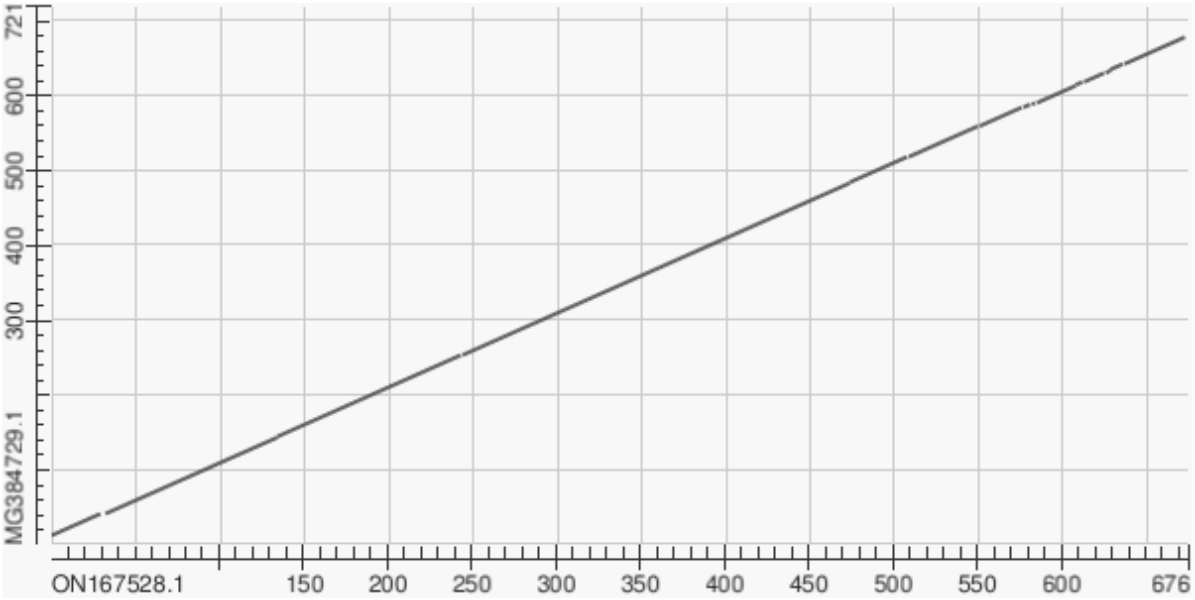

Figure (6): A DNA Dot Plot of *A.tritici* isolate 1D .W. (ON167528.1) on wheat vs *D. dipsaci* isolate 710_G – (C58-ON) (C58-ON) (MG384729.1) on garlic from Canaad (The other isolates of nematode species diagnosed in this study followed the same path).

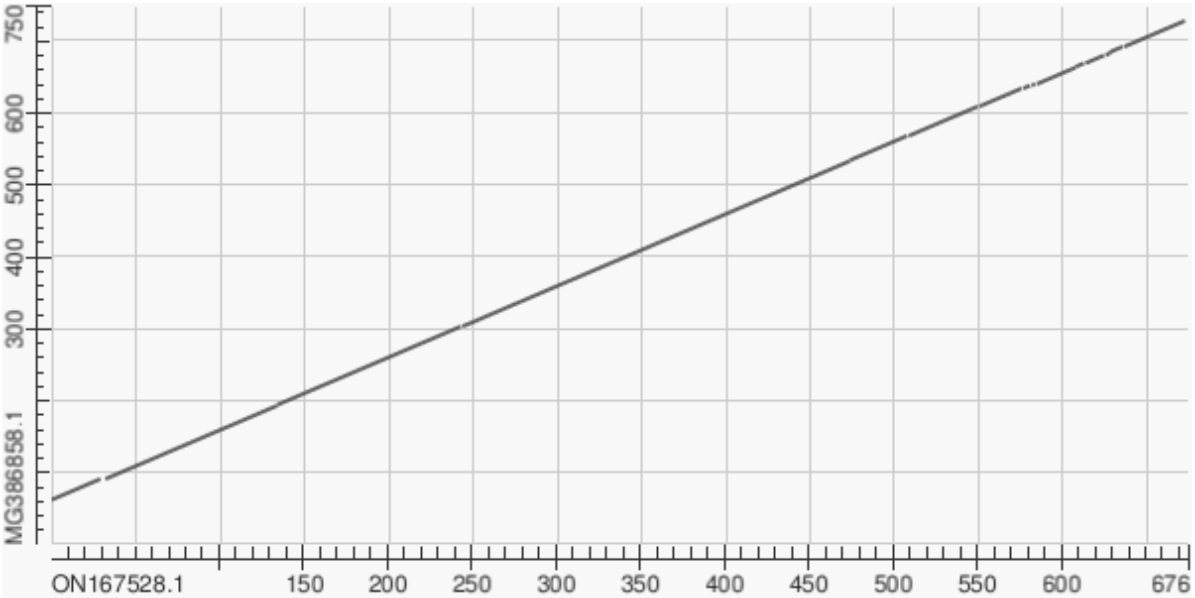

Figure (7): A DNA Dot Plot of *A.tritici* isolate 1D .W.on wheat ON167528.1 vs *D. weischeri* isolate 648_CT- (Road) **(**MG386858.1**)** on Cirsiu arvense from Canada (The other isolates diagnosed in this study followed the same path)

3-3:Phylogenetic tree inferences: Phylogenetic tree (Figure.8 and 9) was constructed for determination amount of genetic changes per time unit between nematode isolates that have been diagnosed in this study and other isolates and species of *Anguina* in addition to certain species of the same family (Anguinidae) and ancestors as in stem and bulb nematodes *Ditylenchus*.The horizontal branches in each phylogenetic tree express the evolution of lineage that alterates over time, so the

longest branch in the horizontal dimension represents the higher amount of alteration.The scale bar in the bottom of each figure shows a scale for this.Therfore, the line segment with the number 0.01 (Figure,8) or 0.03 (Figure,9) shows the length of branches which expresses the quantity of genetic changes by 0.01 and 0.03 in each figure respectively.The branch length unit is nucleotide substitutions per site,which is the number of substitutions or changes divided by the length of the sequence.The vertical lines exhibit the connection of the horizontal lines with each other and for which duration they are non pertinent. The results of the phylogenetic analysis showed grouping or clustering of the five investigated nematode isolates with each other and with the other two isolates of the same species from Iraq on expected lines in one cluster to be the most closest nematode species to them that included isolate AE and MNEK1 with an accession KT900694.1and MT675027.1 parasitized on wheat and barley respectively followed by isolate MNEK9 (MT675026.1) then MNEK3 (MT675020.1) of the same species parasitized on wheat from Iraq too respectively,whilst nucleotide differences increased with the other nematode species of the same genus where more differences were noticed with *A.paludicola* (AF396364.1) from Australia (diagnosed in Belgium) (Figure,8),then genetic change increased more with other nematode species of the same family (Anguinidae) such as *Ditylenchus weischeri* isolate 648_CT- (Road) (MG386858.1) from Canada(Figure ,9).Thus, results of this study emphasized that both seed gall nematodes either on wheat or barley do not differ genetically and have the same genetic characteristics,even though the previous studies in Iraq (Stephan and Antoon,1990; Al-Talib,1986 and Ami and Taher,2014) confirm their differences in pathoginicity and behavior of infection which indicate that each isolate (on wheat and barley) belongs to a special race.The differences in their pathogenicity may be

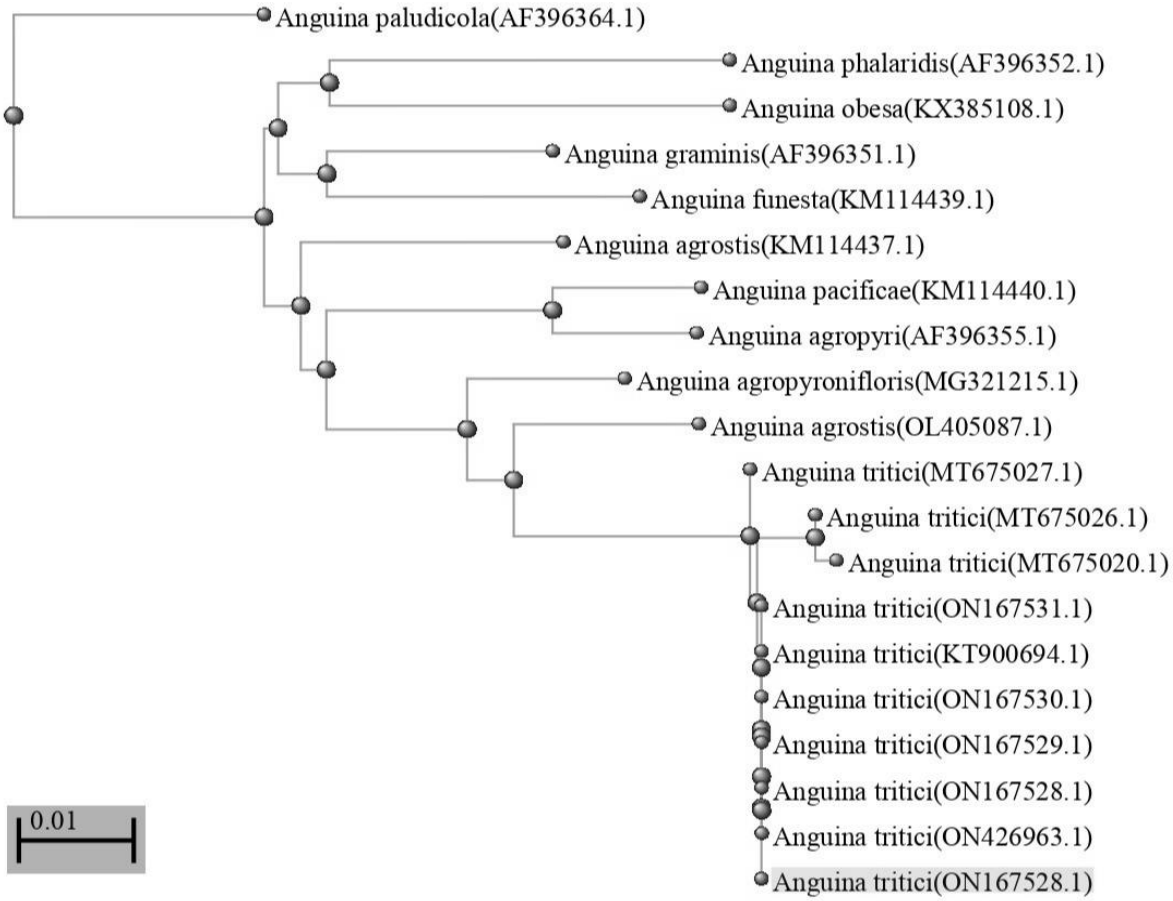

Figure (8): Phylogenetic tree produced by Neighbor joining method using Blast Pairwise alignments showing phylogenetic positioning of the five isolates of *A. tritici* diagnosed in this stud with some of the similar species and another species of the the same genus. Branch lengths are proportional to the number of inferred changes.

due to the adaptation of the individuals of each race to its preferred host, either through their attraction to the root secretions (allelochemical) then climbing on the plant to feed later on the components of the plant cells, where they prefer them over the components of the other host.

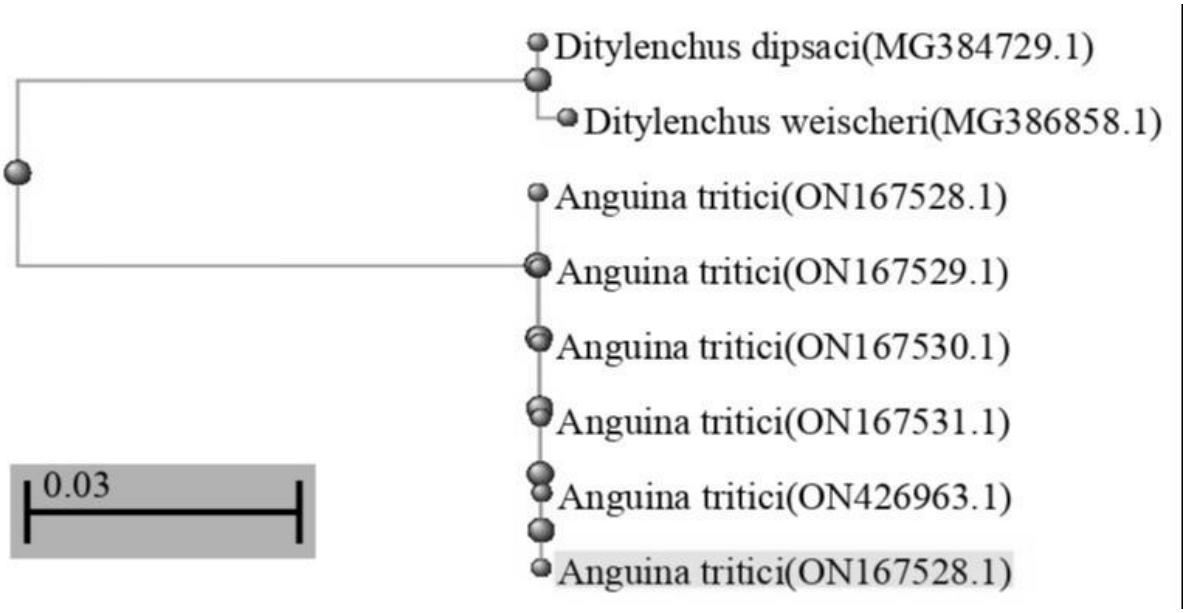

Figure (9): Phylogenetic tree produced by Neighbor joining method using Blast Pairwise alignments showing phylogenetic positioning of the five isolates of *A. tritici* diagnosed in this study with two selected species of the same family (Anguinidae). Branch lengths express number of inferred changes

CONCLUSIONS

Existence of seed gall nematode *Anguina tritici* was recorded within the impurities of wheat samples in the silos of Duhok province, in addition of its existence in the most wheat fields in the same province. Molecular identification and through sequenc analysis detected that all nematode isolates either on wheat or barley belong to seed gall nematode *A.tritici.* Evidences of sequence alignment by blast program, a DNA Dot Plot and phylogenetic tree analysis revealed that all nematode isolates diagnosed in this study are genetically similar.

ACKNOWLEDGMENT

The authors would like to thank and appreciate the University of Duhok,College of Agricultural Engineering Sciences, Department of Plant protection for their support throughout the research period.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

التواجد والتشخيص الجزيئي والتباين الوراثي لعزلات جديدة من نيماتودا تثالل الحبوب Anguina tritici المتطفلة على الحنطة والشعير في العراق

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الخلاصة

هدفت هذه الدراسة إلى التحري عن نيماتودا تثلل حبوب الحنطة Anguina tritici المسببة لمرض تثالل الحبوب في سايلوات وبعض حقول الحنطة في محافظة دهوك/ اقليم كوردستان– العراق وتشخيص أربع عزلات جديدة من هذه النيماتودا على الحنطة وعزلة واحدة منهاعلى الشعير جمعت من مناطق مختلفة من العراق. أظهرت النتائج تلوث عينات شوائب حبوب الحنطة بثاليل الحبوب بنسبة 66٪ كاعلى نسبة تلوث في عام 2020 في سايلو مدينة فايدة، فيما سجلت اقلها(6٪) في سايلو زاخو خلال السنة ذاتها .اما عن نسبة الاصابة في حقول الحنطة فقد سجلت اعلاها(34.6٪) في حقول مدينة عقرة واقلها(2٪) في حقول مدينة سميل.أظهر الترحيل الكهربائي لنواتج تضخيم الحامض النووي DNA باستخدام تفاعل البلمرة المتسلسل(PCR) لعزلات هذه النيماتودا على الحنطة والشعير حزما متشابها(≈ 750 bp زوج قاعدة)،كما اثبتت نتائج تحليل التتابع النيكليوتيدات أن جميع عزلات هذه النيماتودا تنتمي إلى النوع Anguina tritici وتبين من مقارنة تسلسل النيكليوتيدات لمنطقة التباعد الداخلي(ITS) للحامض النووي لعزلات هذه النيماتودا باستخدام برنامج BLAST من بنك الجينات الدولي وتأكيدها بالمخطط التنقيطي (DNA Dot Plot) انها متشابهة وراثيا بنسبة 100%.أظهرت نتائج تحليل شجرة النشوء والتطور او الشجرة الوراثية ان عزلات النيماتودا المشخصة في هذه الدراسة تشكلت في مجموعة متماثلة مع بعضها البعض ومع عزلتين من نفس النوع من العراق، بينما ازدادت الفروقات النوكليوتيدية مع أنواع النيماتودا الأخرى من نفس الجنس وازداد التباين الوراثي بوتيرة اكثر مع ألانواع الأخرى من نفس العائلة (Anguinidae).

الكلمات المفتاحية: Anguina tritici، نسبة التلويث، نسبة الاصابة، التشخيص الجزيئي.

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