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Novel report on six *Fusarium* species associated with head blight and crown rot of wheat in Basra province, Iraq

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Abstract

Background: Wheat is the main cultivated crop and the highest cereal used up by humans in Iraq. Two *Fusarium* species causing *Fusarium* head blight (FHB) disease on wheat has been detected only in the middle of Iraq, and *Fusarium* crown rot (FCR) disease has been detected in several provinces, but it was limited to only three species. This study was conducted to deepen and enlarge our current understanding of the causal agents of these symptoms observed in the wheat fields and to isolate, purify, and identify (molecularly) the most common soil fungi occurring in the rhizosphere of seedlings showing, root, crown, stem, and head at symptom occurrence.

Results: Eighty-eight *Fusarium* isolates were isolated from 7 wheat cultivars planted in 14 fields in the south of Iraq, Basra province, by using polymerase chain reaction (PCR) technique. Based on molecular identification methods as well as the analysis of phylogenetic trees, the results of this study have verified that the main species belonging to the genus *Fusarium* causing FHB and FCR diseases on wheat in the studied areas are *F. pseudograminearum*, *F. graminearum*, *F. equiseti*, *F. culmorum*, *F. solani*, *F. avenaceum*, *F. chlamydosporum*, *F. cerealis*, and *F. nygamai*. The first 3 species have been identified previously in several Iraqi studies on wheat and barley. This is the first record of the last 6 *Fusarium* species from the wheat crop in Iraq as causative pathogens of FHB and FCR. Individual phylogenetic trees of 71 *Fusarium* isolates reconstructed based on the internal transcribed spacer (ITS) sequences showed that all isolates belonging to each of the 9 *Fusarium* species form monophyletic groups with identical isolates of the same species. Collective phylogenetic tree of the remaining 17 *Fusarium* isolates reconstructed based on translation elongation factor 1 alpha (TEF1-α) gene sequences showed that all isolates belonging to each of the 4 *Fusarium* species forms monophyletic groups with identical isolates of the same species.

Conclusion: Iraqi wheat cropping system is seriously threatened by FHB and FCR diseases. Other Iraqi provinces should be inspected to understand the distribution of both diseases.

Keywords: Cultivars, Identification, Morphology, Molecular, Phylogenetic tree

Introduction

Wheat (*Triticum aestivum* L. em. Thell) plays a noteworthy role in the world. More than 90 countries of the Third World are harboring a total population exceeding 4.5 billion wheat-consuming people as the basic carbohydrates (Braun et al. 2010). Bread wheat is the main cultivated crop and the highest cereal used up by humans in Iraq. In 2016, the harvested wheat area in Iraq was 920,096 ha, and the production quantity was 3,052,939 t (Food and Agriculture Organization of the United Nations (FAO) 2018).

Multiple *Fusarium* species are responsible for the two destructive wheat diseases: *Fusarium* head blight (FHB) and *Fusarium* crown rot (FCR). These two diseases are the most widespread cereal diseases that have presented in most regions all over the world. Also, they are the most detrimental diseases that infect wheat cropping systems and other cereal crops resulting in considerable yield losses every year (Smiley and Patterson 1996; Koch et al. 2006; Hogg et al. 2010).

The prevalent species accompanying these two diseases are *Fusarium graminearum* Schwabe, *Fusarium*

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culmorum (W.G. Smith) Saccardo, and *Fusarium pseudograminearum*, whereas *Fusarium equiseti* (Corda) Sacc., *Fusarium avenaceum* (Fries) Saccardo, *Fusarium poae* (Peck) Wollenw, *Fusarium crookwellense* Burgess, Nelson & Toussoun, *Fusarium sporotrichioides* Scherb., *Fusarium acuminatum* (Ellis and Everh.), and *Fusarium oxysporium* Schlecht. Emend. Snyder & Hansen have been identified as less commonly isolated species (Braithwaite et al. 1998; Bottalico and Perrone 2002; Monds et al. 2005).

Regarding FHB disease, also known as scab or ear blight, whiteheads, and pink mold, is mostly caused by *Fusarium graminearum* Schwabe (teleomorph: *Gibberella zeae* (Schwabe) Petch) (Wegulo et al. 2011). In addition to a complex of *Fusarium* species often associated with FHB disease, up to 16 *Fusarium* species have been isolated from infected wheat heads (Parry et al. 1995). The FCR disease is mainly caused by *Fusarium pseudograminearum* teleomorph: *Gibberella coronicola* (Aoki and O'Donnell 1999; Li et al. 2008). Along with Chakraborty et al. (2006), there are 11 species that could also cause FCR disease in some wheat cultivars.

Parry et al. (1995) stated that even though FHB and FCR mostly caused by the same *Fusarium* species, the relationship among them is still unclear. Identification of *Fusarium* species established upon the morphological characteristics detected on particular media is trustworthy methods (Burgess et al. 2001; Nelson et al. 1983), but it demands time and the necessary expertise; therefore, it is very difficult for non-specialist (Bluhm et al. 2002). Hence, the best diagnostic method for conclusive identification of *Fusarium* species is the polymerase chain reaction (PCR) techniques as molecular approaches (Niessen et al. 2004; Mulè et al. 2005; Demeke et al. 2005; Jurado et al. 2006).

To the best of our knowledge, *F.pseudograminearum* and *F. graminearum* causing FHB disease on wheat have been detected only in the middle of Iraq by Khudhair et al. (2015a). While FCR disease has been detected in several provinces in Iraq, it was limited to only three species, *F. pseudograminearum*, *F. graminearum*, and *F. equiseti* (Hameed et al. 2012; Khudhair et al. 2015b; Matny et al. 2017; Lahuf et al. 2018).

Bearing in mind all the above literatures on the wheat plant diseases and the field observation of the symptoms on wheat stem bases and reduction of the average wheat production associated with empty and head bleaching suspected to be pathogenic fungi belonging to the genus *Fusarium* such as FHB and FCR. This study was conducted to isolate, purify, and identify (molecularly) most fungi from the soil at the emerging seedling, root, crown, stem, head, and rhizosphere at symptom occurrence, and also to deepen and enlarge our current understanding of the causal agents of these symptoms observed in the wheat fields.

Materials and methods

Description of study fields and sampling

In 2017/2018, a total of 14 winter wheat fields in 7 districts of Basra province, i.e., Qurna/Mzeara'a (QM) 34° 38′ 66.5″ N, 73° 27′ 40″ E; Qurna Research Station (QRS) 34° 36′ 51.8″ N, 73° 54′ 41″ E; Thagar/Al-Izz1 (TI1) 34° 50′ 40.5″ N, 73° 03′ 68″ E; Thagar/Al-Izz2 (TI2) 34° 54′ 92.1″ N, 72° 84′ 09″ E; Thagar/Al-Izz3 (TI3) 34° 54′ 43.6″ N, 72° 87′ 72″ E; Thagar/Karakor (TK) 34° 51′ 91.1″ N, 73° 28′ 64″ E; Dair (D) 34° 09′ 05.2″ N, 74° 48′ 90″ E; Nashwa (N) 34° 15′ 46.5″ N, 74° 98′ 04″ E; Al-Modienh/Salih River (MSR) 34° 24′ 99.1″ N, 70° 76′ 14″ E, Al-Modienh/Salt Project (MSP) 34° 22′ 68.9″ N, 70° 89′ 05″ E; Talha/marshland 1 (ML1) 34° 15′ 00.7″ N, 71° 49′ 14″ E; Talha/marshland 2 (ML2) 34° 16′ 63.8″ N, 71° 55′ 44″ E; Al-Hammar Marsh (HM) 34° 07′ 68.9″ N, 71° 52′ 68″ E; and Huwair (H) 34° 36′ 08.9″ N, 71° 58′ 79″ E at the north of Basra/south of Iraq (Fig. 1) were chosen for the isolation and identification of the fungi associated with the bleaching and yellowing symptoms of wheat plant terminals (heads, roots, and stems), which were most commonly observed during the last 10 years. The area of the 14 selected fields range between 15 and 100 ha.

Spatial characterization of field infection

All the 14 selected fields were geo-referenced through GBS sites. To allow for the possibility of specific determination for these sites, reference point was matched to the adjacent geographical location names distinguishable in the spatial database.

Wheat cultivars

Seven soft wheat winter cultivars (Abu Ghraib 3 (AG3), Ebaa-99 (I99), Adana 99 (A99, Turkey), Bengal (Barcelona (B.)), Research 22 (local (Res. 22)), Rasheed (local (R.)), and AGRI-saaten (AGRI S., Germany)) were planted in the 14 studied fields. The last cultivar was planted for the first time in the south of Iraq. These seven cultivars were selected to represent those frequently grown in Basra province/Southern Iraq.

Sample collection

Soil samples

Soil samples were taken from the 14 selected fields at seedling emergence with a depth of 20–30 cm. Five to 10 soil samples (depending on the field area) were collected from random locations within each field using a digging tool, sited in particular air-tight plastic collection sacks, and placed in an icebox for transferring to the

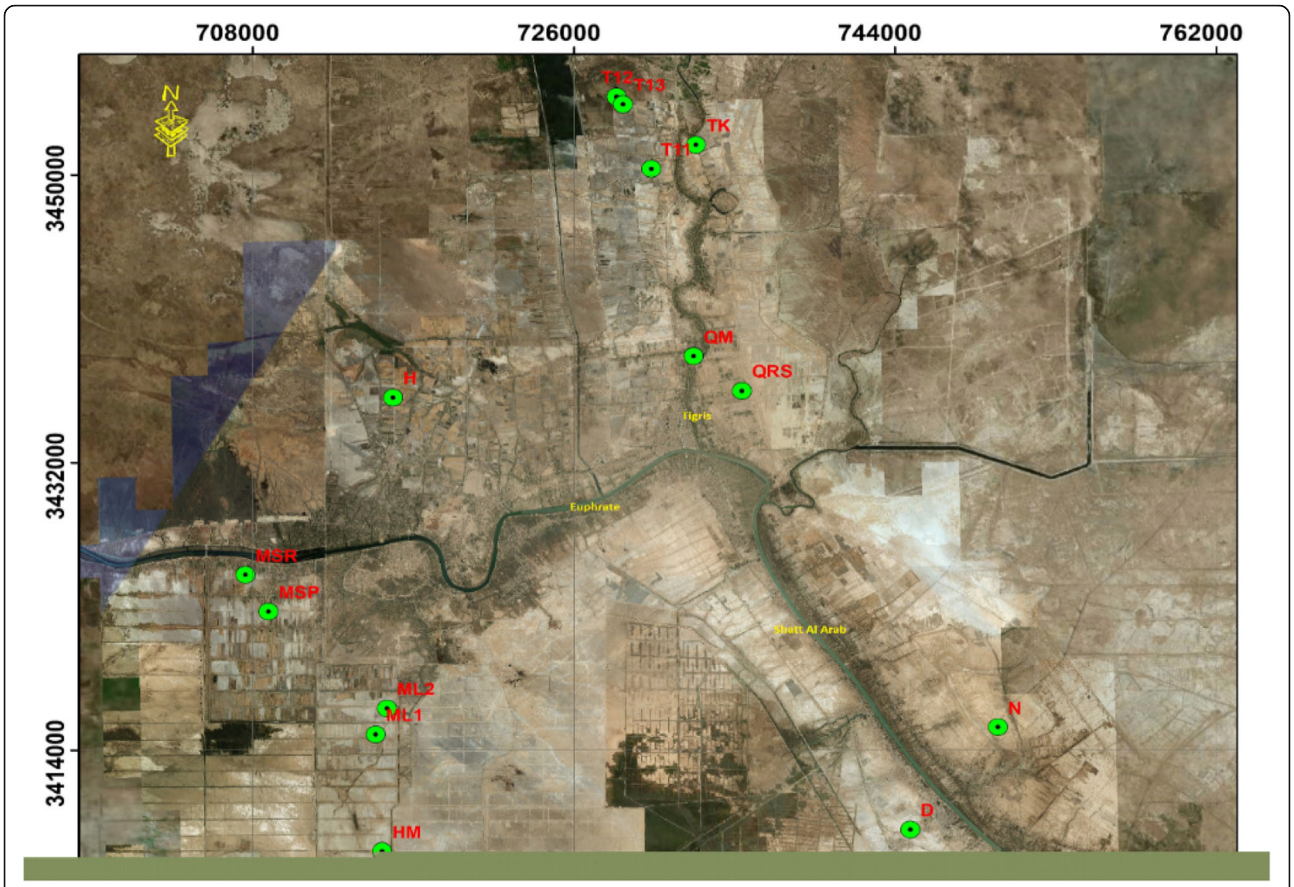

Fig. 1 Map of the south of Basra illustrating the 14 selected wheat fields where symptomatic wheat plants were collected in 2018/2019

laboratory. The soil samples were stored at 4 °C in the laboratory awaiting additional soil processing.

Plant samples

For every field, infected plants showing disease symptoms (blighted heads and stem tissues turned to tan or brown as well as senesce prematurely) were collected within 15–20 days before harvest (during the premature and ripening stage). In 2017/2018, samples were collected between April 5 and May 1. For every field, depending on field area, a complete 8–15 tillers/field were collected. At harvest time, the seeds from mature heads were individually collected, cautiously pulled off, combined, and air-dried. The seeds from each field were sited in polythene bags and stored in the dark at 10 °C before being tested in the experiments of fungal isolation.

Wheat plants with symptoms of partial or full white-heads and crown and stem browning were collected (Figs. 2 and 3). These symptomatic plants with their

Fig. 2 *Fusarium* crown rot (FCR) symptoms at ripening time in **1** Talha (HM) and **2** Thagar (TI2) wheat fields.

Fig. 3 *Fusarium* head blight (FHB) symptoms in **1** Qurna (QM) and **2** Talha (ML2) wheat field

roots and rhizosphere picked up from naturally diseased cultivated plants grows at the 14 selected fields in the south of Basra. All the samples were placed individually in air-tight plastic collection sacks in the fields, sited in a cool box for transferring to the laboratory, and stored at 4 °C for not more than 7 days before being tested.

Isolation of fungi

Each plant part (head, crown, stem base, and root) was thoroughly washed in running water for 10–15 min, soaked in 6% sodium hypochlorite for 2 min, rinsed in sterile distilled water several times, and then air-dried on a filter paper, while wheat grains were sterilized by moistening with 70% ethanol for 2 min, soaking in 6% sodium hypochlorite for 1 min, rinsing in sterile distilled water several times, and then air-dried on a filter paper. Then, they were placed in Petri dishes (9 cm) of full-strength potato dextrose agar (PDA), rose Bengal agar (RBA), malt extract agar (MEA), Czapek Dox Agar (CZA), malachite green agar (MGA), and Sabouraud dextrose agar (SDA) with three replicates for each, five disinfected tissue pieces per Petri dish with 2:1:2 plant tissue arrangement, and seven grains/Petri plate in 2:3:2 arrangement. The Petri dishes were incubated at 25 °C ± 2 for 7–14 days and checked daily for fungal growth. For better sporulation and chlamydospore production, all isolates were sub-cultured onto water agar (WA), quarter-strength potato dextrose agar (QPDA), half-strength potato dextrose agar (HPDA), full-strength potato dextrose agar (PDA), and synthetic nutrient agar (SNA with 1 cm^2 of sterilized filter paper placed onto the middle of it), with three replicates for each, incubated under similar conditions.

Isolation from soil

Several methods for soil dilution were employed in this study, i.e., malachite green agar (MGA), rose Bengal (RB), potato dextrose agar (PDA, Oxoid Limited) with chloroform, and dichloran rose Bengal agar (DRBC) base. Serial dilution of the collected soil samples ranging from 10^{-4} to 10^{-6} dilution using sterile distilled water and boiled sterile distilled water (100 °C) was carried out. For fungal isolation improvement, a sterilized pipette was used to transfer 1 ml of every single dilution to the surface of the abovementioned media. The Petri dishes were then incubated for 3–5 days at 25 °C ± 2 for fungal growth and purified onto PDA, as the best media displaying clear structures for all fungal isolates during this study.

For identification, colony morphology and microscopic examinations of the obtained isolates of pure cultures were used to determine the structures of vegetation and reproduction. The identification was carried out morphologically to the genus level and molecularly to species.

Molecular identification

DNA extraction

Fungal genomic DNA prepared from a colony of each isolate was extracted using the Plant Genomic DNA Mini Kit (GP100) Geneaid protocol according to the manufacturer's instructions.

DNA electrophoresis

The process of DNA electrophoresis was performed according to Sambrook et al. (1989).

Polymerase chain reaction amplification

A total of 186 isolates of various fungal species from symptomatic wheat plants, their seeds, rhizosphere, and soil in the north of Basra were identified to genus or species based on the morphology, while 123 isolates out of the 186 isolates were molecularly identified to species by means of the PCR technique. The sequence of internal transcribed spacer (ITS) region was amplified using the universal primer pair ITS1 (F) (5′-TCC GTA GGT GAA CCT GCG G-3′) and ITS4 (R) (5′-TCC TCC GCT TAT TGA TAT GC-3′).

Amplification of genomic DNA was executed in a total volume of 25 μl consist of 5 μl Master Mix (Bioneer, Korea), 1.5 μl of each primer, 5 μl of the genomic DNA as a template, and 12 μl deionized sterile distilled water.

PCR reactions were accomplished in a Thermo-cycler (MyGenie96 Thermal Block, Bioneer, Korea) and the amplification program initiated with denaturation at 95 °C for activating the tag-polymerase for 5 min, followed by 35 cycles of 30-s denaturation at 95 °C, 30 s of annealing at 60 °C, and 45 s of extension at 72 °C with final extension at 72 °C for 5 min. In the last part, 1.5% agarose gel electrophoresis was dissolved in 1× Tris-borate-EDTA (TBE) buffer, stained with ethidium bromide, visualized under a UV transilluminator, and photographed by GeneSnap photo imaging system (SynGene).

DNA sequencing

The sequences of ITS1-5.8S rDNA and the purification for the amplified PCR products of 123 isolates were conducted in (MACROGEN Company, South Korea) "http://dna.macrogen.com." The obtained sequences were compared with those available in GeneBank and NCBI http://www.ncbi.nlm.nih.gov employing BLAST. Established upon the closest identification of BLAST analysis, 17 isolates from the *Fusarium* genus were matched in less than 97% of similarity with high expected values and inadequate maximum scores. As a result, the genomic DNA of these 17 *Fusarium* isolates were again amplified, purified, and sequenced using the translation elongation factor 1 α (TEF1-α) gene with primers EF-1 (5-′ATG GGT AAG GAG GAC AAG AC-3′) and EF-2 (5′-GGA AGT ACC AGT GAT CAT GTT-3′) as follows.

Amplification of genomic DNA was executed similar to that done for the ITS region. The amplification program initiated with denaturation at 95 °C to activate the tag-polymerase for 5 min, followed by 35 cycles of 30-s denaturation at 95 °C, 30 s of annealing at 51.5 °C, and 90 s of extension at 72 °C with final extension at 72 °C for 7 min. The purified PCR products of the 17 *Fusarium* isolates then sent to the same commercial company for sequencing in bi-directions using the above particular forward and reverse primers (EF-1 and EF-2).

Analysis of ITS region and TEF1-α gene for Fusarium species

The evolutionary history of all *Fusarium* spp. was concluded by using the maximum likelihood method and Kimura 2-parameter model (Kimura 1980). Initial trees for the heuristic search were achieved by applying the neighbor-joining method (nearest-neighbor interchange (NNI)). The evolutionary distances were computed using the maximum composite likelihood (MCL) approach, and the neighbor-joining tree reliabilities were estimated by the bootstrap method with 1100 replications. Evolutionary analyses were conducted in MEGA 7 (Kumar et al. 2018). *Aspergillus niveus* (MH865978.1) and *Alternaria ventricosa* (KM454880.1) sequences from GenBank were selected as the out-group.

Results

Molecular identification based on ITS1 and ITS4 primer pairs

Established upon the closest identification of BLAST analysis, the obtained nucleotide sequences of 123 isolates were compared to the GenBank databases and matched at diverse global similarity.

Molecular identification of *Fusarium* spp.

Our results of morphological investigation in association with DNA sequencing consistent with the first matching identification of BLAST sequence database in NCBI using distinct sequences proved the identity of 88 isolates out of the total 123 isolates that belong to the genus *Fusarium* in 9 species (*F. pseudograminearum*, *F. graminearum*, *F. culmorum*, *F. chlamydosprum*, *F. equiseti*, *F. solani*, *F. avenaceum*, *F. cerealis*, and *F. nygamai*). Seventy-one sequences of these isolates corresponded to the GenBank databases at 97–100% as global similarity (Additional file 1), whereas the remaining 17 *Fusarium* isolate sequences were matched to the published sequences that deposited in the GenBank sequence database in less than 97% of similarity with high expected values and inadequate maximum scores.

Therefore, the 71 *Fusarium* isolates deposited in the GenBank nucleotide databases under accession numbers MK271097–MK271110 for *Fusarium chlamydosporum* (14 isolates), MK271111–MK271115 for *Fusarium equiseti* (5 isolates), MK271231–MK271238 for *Fusarium solani* (8 isolates), MK271239–MK271246 for *Fusarium pseudograminearum* (8 isolates), MK271247–MK271265 for *Fusarium graminearum* (19 isolates), MK271266–MK271269 for *Fusarium avenaceum* (4 isolates), MK271270–MK271271 for *Fusarium cerealis* (2 isolates), MK281297–MK281306 for *Fusarium culmorum* (10 isolates), and MK271272 for *Fusarium nygamai* (one isolate) (Table 1).

Sequencing of TEF1-α gene

Established upon the closest identification of BLAST analysis, the obtained nucleotide sequences of the 17 sequences of *Fusarium* isolates were compared to the GenBank databases and matched at 99–100% (Table 2).

The results based on ITS sequences showed that only six isolates (ITS-22 (Fus-2), ITS-78 (Fus-11), ITS-85 (Fus-13), ITS-102 (Fus18), ITS-111 (Fus-20), and ITS-117 (Fus-22)) were similar in identification as indicated by the partial TEF1-α gene sequences, but with different percentage of identity. The first isolate (ITS-22) was identified as *Fusarium pseudograminearum*, and the latter isolates (ITS-78, ITS-85, ITS-102, ITS-111, and ITS-117) belong to *Fusarium culmorum*, whereas the remaining 11 isolates were completely different in identification.

Table 1 Identification of *Fusarium* taxa isolated from the selected wheat fields based on the sequencing of rDNA ITS1-5.8S-ITS4 region

Isolate	NCBI Blast identification	Accession no.	% ID	Field	Source	Primer	E.V
TS-3	*F. chlamydosporum*	MK271097	100	N	Head	ITS1	0.0
ITS-6	*F. nygamai*	MK271272	100	N	Root	ITS1	0.0
ITS-7	*F. equiseti*	MK271114	100	N	Root	ITS1	0.0
ITS-8	*F. chlamydosporum*	MK271104	100	N	Root	ITS1	0.0
ITS-10	*F. equiseti*	MK271115	100	QM	Root	ITS1	0.0
ITS-13	*F. chlamydosporum*	MK271105	100	HM	Root	ITS1	0.0
ITS-14	*F. equiseti*	MK271111	100	H	Head	ITS1	0.0
ITS-15	*F. chlamydosporum*	MK271100	100	HM	Stem	ITS1	0.0
ITS-19	*F. graminearum*	MK271263	100	TI3	Rhizo	ITS1	0.0
ITS-20	*F. pseudograminearum*	MK271243	100	MSR	Root	ITS1	0.0
ITS-21	*F. culmorum*	MK281297	100	D	Head	ITS1	0.0
ITS-23	F. pseudograminearum	MK271241	100	MSR	Stem	ITS1	0.0
ITS-24	*F. pseudograminearum*	MK271246	100	TK	Seed	ITS1	0.0
ITS-29	*F. solani*	MK271231	100	TI1	Head	ITS1	0.0
ITS-30	*F. solani*	MK271232	100	D	Head	ITS1	0.0
ITS-33	*F. graminearum*	MK271264	100	N	Rhizo	ITS1	0.0
ITS-34	*F. avenaceum*	MK271268	100	MSP	Root	ITS1	0.0
ITS-35	*F. avenaceum*	MK271267	100	N	Stem	ITS1	0.0
ITS-36	*F. graminearum*	MK271248	100	H	Stem	ITS1	0.0
ITS-37	*F. graminearum*	MK271249	100	QRS	Stem	ITS1	0.0
ITS-38	*F. graminearum*	MK271250	100	QRS	Stem	ITS1	0.0
ITS-39	*F. graminearum*	MK271259	100	N	Root	ITS1	0.0
ITS-40	*F. culmorum*	MK281298	100	HM	Stem	ITS1	0.0
ITS-41	*F. chlamydosporum*	MK271106	100	HM	Root	ITS1	0.0
ITS-42	*F. chlamydosporum*	MK271107	100	LM1	Root	ITS1	0.0
ITS-43	*F. chlamydosporum*	MK271109	100	HM	Rhizo	ITS1	0.0
ITS-52	*F. solani*	MK271235	100	TI1	Stem	ITS1	0.0
ITS-54	*F graminearum*	MK271260	100	LM2	Root	ITS1	0.0
ITS-56	*F. avenaceum*	MK271269	100	TI3	Rhizo	ITS1	0.0
ITS-57	*F. chlamydosporum*	MK271101	100	MSP	Stem	ITS1	0.0
ITS-59	*F. graminearum*	MK271265	100	LM2	Rhizo	ITS1	0.0
ITS-63	*F. culmorum*	MK281299	100	D	Head	ITS1	0.0
ITS-64	*F. graminearum*	MK271261	100	TI3	Root	ITS1	0.0
ITS-67	*F. graminearum*	MK271247	100	QRS	Head	ITS1	0.0
ITS-68	*F. pseudograminearum*	MK271239	100	TI1	Head	ITS1	0.0
ITS-69	*F. culmorum*	MK281300	100	TI3	Rhizo	ITS1	0.0
ITS-71	*F. graminearum*	MK271251	100	TI3	Stem	ITS1	0.0
ITS-72	*F. chlamydosporum*	MK271102	100	TI2	Stem	ITS1	0.0
ITS-73	*F. solani*	MK271236	100	TI2	Root	ITS1	0.0
ITS-74	*F. culmorum*	MK281301	100	TI2	Root	ITS1	0.0
ITS-75	*F. culmorum*	MK281302	100	MSP	Stem	ITS1	0.0
ITS-77	*F. culmorum*	MK281303	100	MSP	Root	ITS1	0.0
ITS-79	*F. culmorum*	MK281304	100	TI3	Stem	ITS1	0.0

Table 1 Identification of *Fusarium* taxa isolated from the selected wheat fields based on the sequencing of rDNA ITS1-5.8S-ITS4 region *(Continued)*

Isolate	NCBI Blast identification	Accession no.	% ID	Field	Source	Primer	E.V
ITS-80	*F. pseudograminearum*	MK271244	100	TI3	Root	ITS1	0.0
ITS-86	*F. equiseti*	MK271113	100	HM	Stem	ITS1	0.0
ITS-88	*F. chlamydosporum*	MK271098	100	LM1	Head	ITS1	0.0
ITS-92	*F. solani*	MK271238	100	QM	Rhizo	ITS1	0.0
ITS-94	*F. chlamydosporum*	MK271108	100	N	Root	ITS1	0.0
ITS-97	*F. chlamydosporum*	MK271103	100	QM	Stem	ITS1	0.0
ITS-99	*F. culmorum*	MK281305	100	ML2	Root	ITS1	0.0
ITS-103	*F. graminearum*	MK271252	100	QRS	Stem	ITS1	0.0
ITS-105	*F. graminearum*	MK271253	100	TI3	Stem	ITS1	0.0
ITS-106	*F. pseudograminearum*	MK271240	100	LM1	Head	ITS1	0.0
ITS-107	*F. pseudograminearum*	MK271245	100	LM2	Root	ITS1	0.0
ITS-108	*F. pseudograminearum*	MK271242	100	MSP	Stem	ITS1	0.0
ITS-109	*F. graminearum*	MK271254	100	LM2	Stem	ITS1	0.0
ITS-110	*F. graminearum*	MK271262	100	N	Root	ITS1	0.0
ITS-112	*F. cerealis*	MK271270	100	QRS	Root	ITS1	0.0
ITS-114	*F. graminearum*	MK271255	100	QRS	Stem	ITS1	0.0
ITS-115	*F. cerealis*	MK271271	100	QRS	Root	ITS1	0.0
ITS-118	*F. culmorum*	MK281306	100	QRS	Root	ITS1	0.0
ITS-119	*F. equiseti*	MK271112	100	LM1	Head	ITS1	0.0
ITS-122	*F. graminearum*	MK271256	100	TI2	Stem	ITS1	0.0
ITS-123	*F. graminearum*	MK271257	100	TI2	Stem	ITS1	0.0
ITS-125	*F. graminearum*	MK271258	100	H	Stem	ITS1	0.0
ITS-126	*F. avenaceum*	MK271266	100	D	Head	ITS1	0.0
ITS-129	*F. solani*	MK271237	100	TI1	Root	ITS1	0.0
ITS-130	*F. solani*	MK271233	100	TI1	Head	ITS1	0.0
ITS-131	*F. chlamydosporum*	MK271110	100	TK	Soil	ITS1	0.0
ITS-142	*F. cf. solani*	MK271234	100	TI1	Head	ITS1	0.0
ITS-146	*F. chlamydosporum*	MK271099	100	TK	Head	ITS1	0.0

The expectation value (describes the number of different alignments you may expect to see fortuitously when searching a database of a certain size)
%ID percentage of identity, *E.V E* value

Phylogenetic analyses of ITS region

Individual evolutionary analysis of the obtained nine *Fusarium* species (*F. pseudograminearum*, *F. graminearum*, *F. culmorum*, *F. chlamydosprum*, *F. equiseti*, *F. solani*, *F. avenaceum*, *F. cerealis*, and *F. nygamai*) contained within nucleotide sequences from a number of valid and verified DNA sequences together with the sequences of current study executed using MEGA X software program (Kumar et al. 2018).

Analysis of ITS region for *Fusarium* species

Polymerase chain reaction (PCR) analysis was established by using universal primers (ITS1 and ITS4) to confirm the quality of DNA amplification from 71 Iraqi *Fusarium* isolates. A DNA fragment of the ITS region for these 71 obtained *Fusarium* isolates varied ranging between 456 and 650 bp. According to White et al. (1990), the length of the obtained DNA fragment had the ideal conditions for amplification. Analysis of BLASTn establish on this region attributes 97–100% global similarities of query coverage identity with the ITS sequences of 9 species belonging to *Fusarium* genus (Table 1). In order to reconstruct the ITS trees, multiple alignment of ≈ 550 bp segment of ITS gene for a number of various *Fusarium* taxa. ITS sequences of *Aspergillus niveus* (MH865978) and *Alternaria ventricosa* (KM454880) were used as the out-group species.

The phylogenetic trees of the 71 *Fusarium* isolates reconstructed based on ITS sequences, using neighbor-joining analysis, showed that all isolates belonging to each of the 9 *Fusarium* species form monophyletic

Table 2 Identification of 17 *Fusarium* taxa isolated from the selected wheat fields based on the sequencing of TEF1-α gene region in bidirectional and ITS1-5.8 rDNA-ITS4

Isolate	NCBI Blast identification based on TEF primers	Accession no.	%ID	NCBI Blast identification based on ITS primers	Accession no.	%ID
Fus-2	*F. pseudograminearum*	MG670360.1	99	*F. pseudograminearum*	MH865930.1	95
Fus-5	*F. pseudograminearum*	MG570082.1	100	*F. culmorum*	MH828227.1	94
Fus-6	*F. culmorum*	MG670244.1	100	*F. pseudograminearum*	MH715262.1	88
Fus-7	*F. culmorum*	MG670244.1	100	*Fusarium* sp.	KY931512.1	95
Fus-8	*F. culmorum*	GU370472.1	99	*Fusarium* sp.	JQ756987.1	80
Fus-9	*F. culmorum*	MG670244.1	99	*F. pseudograminearum*	MH333076.1	80
Fus-10	*F. pseudograminearum*	MG570082.1	100	*F. asiaticum*	KY272788.1	94
Fus-11	*F. culmorum*	MG670244.1	99	*F. culmorum*	KJ466109.1	95
Fus-13	*F. culmorum*	MG670244.1	100	*F. culmorum*	MH864973.1	87
Fus-15	*F. equiseti*	KP881271.1	100	*Fusarium* sp.	FJ840527.1	80
Fus-16	*F. pseudograminearum*	MG570082.1	100	*F. culmorum*	KF889093.1	89
Fus-17	*F. pseudograminearum*	MG570082.1	100	*F. graminearum*	KY426415.1	87
Fus-18	*F. culmorum*	JF740860.1	100	*F. culmorum*	MH864973.1	93
Fus-20	*F. culmorum*	MG670244.1	100	*F. culmorum*	MH864973.1	86
Fus-22	*F. culmorum*	MG670244.1	100	*F. culmorum*	KP726896.1	92
Fus-23	*F. equiseti*	KT224322.1	99	*Fusarium* sp.	KU693456.1	96
Fus-25	*F. solani*	MH300508.1	99	*Fusarium* sp.	KF293367.1	94

%ID percentage of identity

groups with identical isolates of the same species. The phylogenetic trees displayed that the 14 isolates of *F. chlamydosporum*, 5 isolates of *F. equiseti*, 19 isolates of *F. graminearum*, 4 isolates of *F. avenaceum*, 10 isolates of *F. culmorum*, 8 isolates of *F. solani*, 8 isolates of *F. pseudograminearum*, 2 isolates of F. cerealis, and 1 isolate of *F. nygamai* clustered with a number of reference isolates of the *F. chlamydosporum*, *F. equiseti*, *F. graminearum*, *F. avenaceum*, *F. culmorum*, *F. solani*, *F. pseudograminearum*, *F. cerealis*, and *F. nygamai*, respectively (Figs. 4, 5, 6, 7, 8, 9, 10, 11, and 12).

Phylogenetic analyses of TEF1-α gene

Collective evolutionary analysis of the obtained four *Fusarium* species (*F. pseudograminearum*, *F. culmorum*, *F. equiseti*, and *F. solani*) contained within nucleotide sequences from a number of valid and verified DNA sequences together with the sequences of current study executed using MEGA X software program (Kumar et al. 2018).

Analysis of TEF1-α gene for *Fusarium* species

The length of TEF1-α gene for the 17 obtained *Fusarium* isolates ranges between 670 and 740 bp. The analysis of BLASTn established on this region attributes 99–100% global similarities of query coverage identity with bidirectional of the EF-1F and EF-2R sequences of four *Fusarium* species (*F. culmorum*, *F. equiseti*, *F. pseudograminearum*, and *F. solani*) (Table 2). In order to reconstruct the TEF1-α gene trees, multiple alignment of ≈ 750 bp segment of TEF1-α gene for a number of various *Fusarium* taxa. TEF1-α gene sequence of *Alternaria tenuissima* (LC136861.1) was used as the outgroup species.

The collective phylogenetic tree of the 17 *Fusarium* isolates reconstructed based on TEF1-α gene sequences, using neighbor-joining analysis, showed that all isolates belonging to each of the 4 *Fusarium* species form monophyletic groups with identical isolates of the same species (Fig. 13). The phylogenetic tree displayed that the 2 isolates of *F. equiseti*, 9 isolates of *F. culmorum*, 1 isolate of *F. solani*, and 5 isolates of *F. pseudograminearum* clustered with a number of reference isolates of the *F. equiseti*, *F. culmorum*, *F. solani*, and *F. pseudograminearum*, respectively.

Discussion

Based on molecular identification methods as well as the analysis of phylogenetic trees, the results of this study have verified that the main species belonging to the genus *Fusarium* isolated and identified from wheat cropping system causing FHB and FCR diseases in the south of Iraq, Basra province, Iraq, are *F. graminearum*, *F. culmorum*, *F. pseudograminearum*, *F. solani*, *F. equiseti*, *F. avenaceum*, *F. chlamydosporum*, *F. cerealis*, and *F. nygamai*.

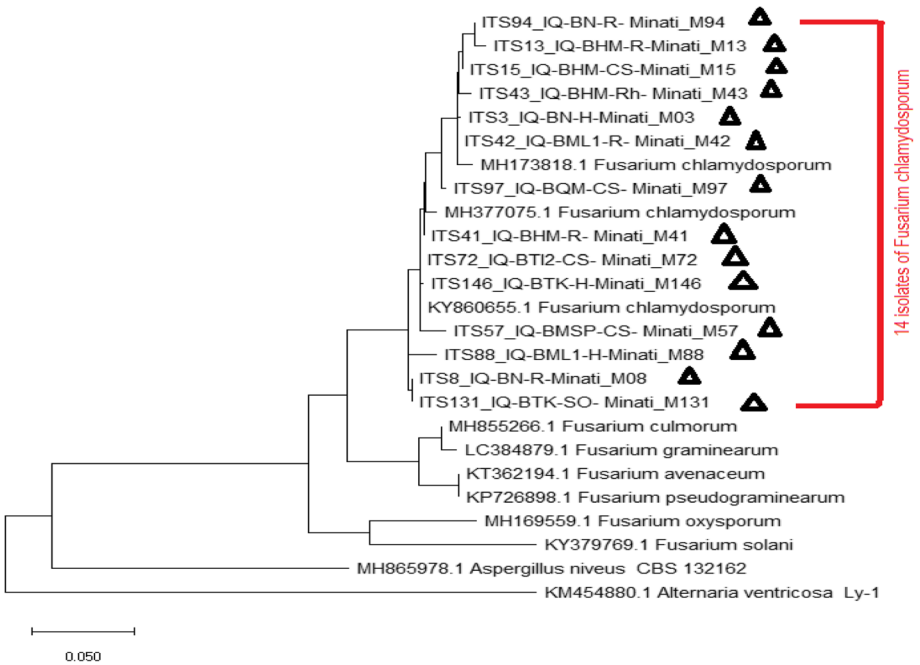

Fig. 4 Phylogenetic tree generated with the ITS-rDNA sequences of the *Fusarium chlamydosporum* (14), as inferred from the maximum likelihood (ML) analyses (determined by black triangles) and related species retrieved from GenBank. ITS sequences of *Aspergillus niveus* (MH865978) and *Alternaria ventricosa* (KM454880) were used as the out-group species

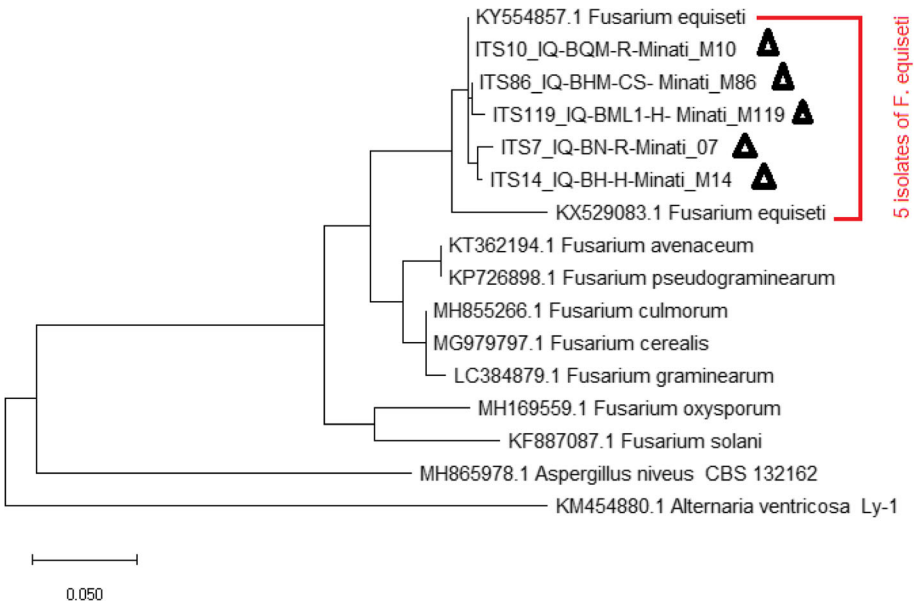

Fig. 5 Phylogenetic tree generated with the ITS-rDNA sequences of the *Fusarium equiseti* (5), as inferred from the maximum likelihood (ML) analyses (determined by black triangles) and related species retrieved from GenBank. ITS sequences of *Aspergillus niveus* (MH865978) and *Alternaria ventricosa* (KM454880) were used as the out-group species

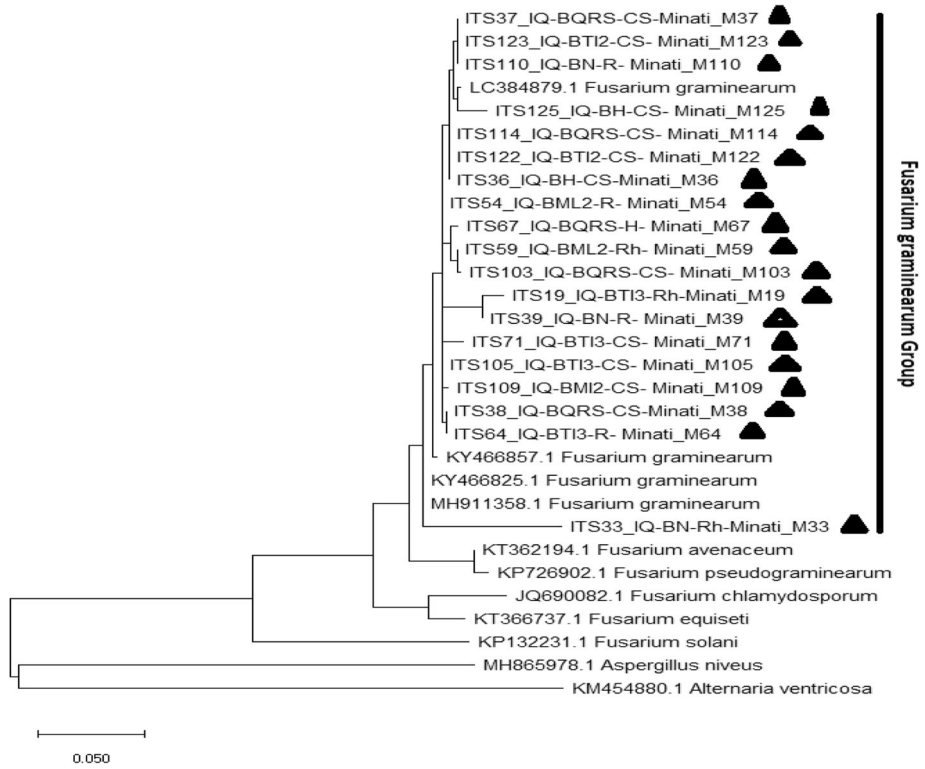

Fig. 6 Phylogenetic tree generated with the ITS-rDNA sequences of the *Fusarium graminearum* (19), as inferred from the maximum likelihood (ML) analyses (determined by black triangles) and related species retrieved from GenBank. ITS sequences of *Aspergillus niveus* (MH865978) and *Alternaria ventricosa* (KM454880) were used as the out-group species

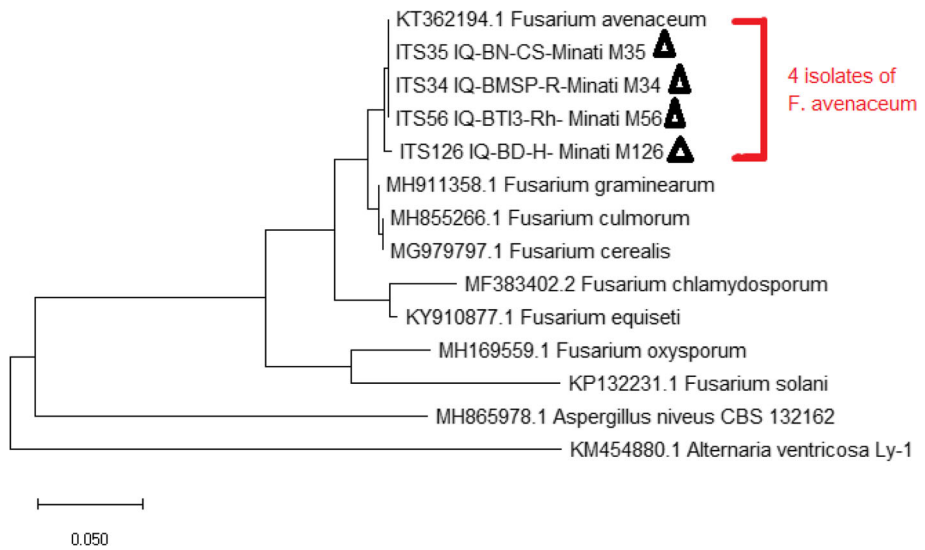

Fig. 7 Phylogenetic tree generated with the ITS-rDNA sequences of the *Fusarium avenaceum* (4), as inferred from the maximum likelihood (ML) analyses (determined by black triangles) and related species retrieved from GenBank. ITS sequences of *Aspergillus niveus* (MH865978) and *Alternaria ventricosa* (KM454880) were used as the out-group species

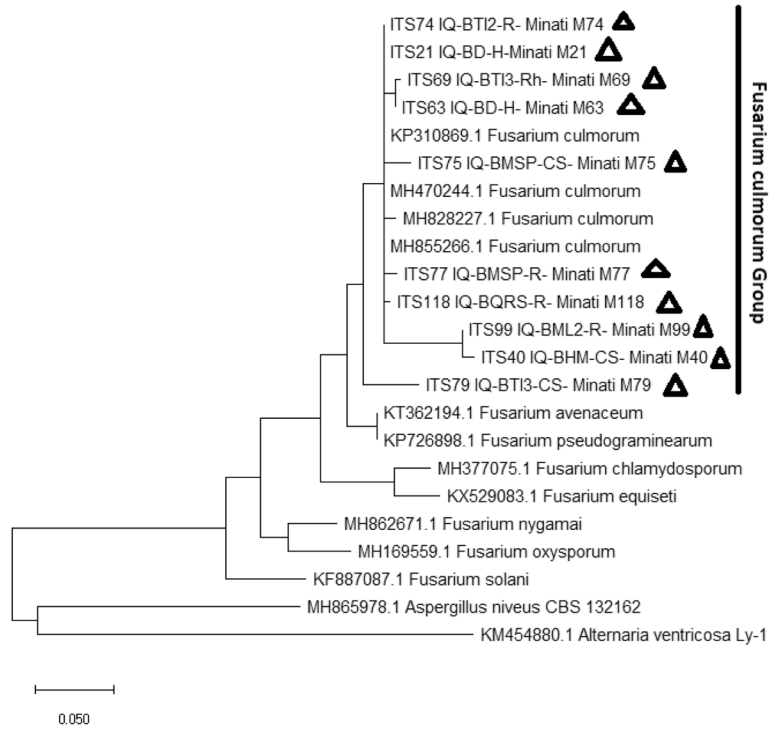

Fig. 8 Phylogenetic tree generated with the ITS-rDNA sequences of the *Fusarium culmorum* (10), as inferred from the maximum likelihood (ML) analyses (determined by black triangles) and related species retrieved from GenBank. ITS sequences of *Aspergillus niveus* (MH865978) and *Alternaria ventricosa* (KM454880) were used as the out-group species

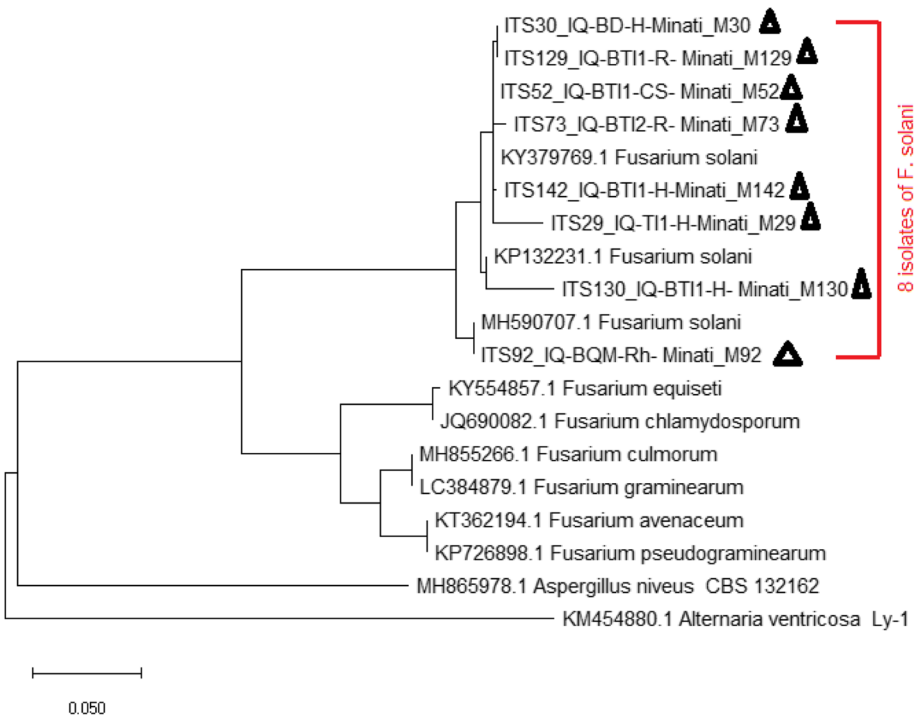

Fig. 9 Phylogenetic tree generated with the ITS-rDNA sequences of the *Fusarium solani* (8), as inferred from the maximum likelihood (ML) analyses (determined by black triangles) and related species retrieved from GenBank. ITS sequences of *Aspergillus niveus* (MH865978) and *Alternaria ventricosa* (KM454880) were used as the out-group species

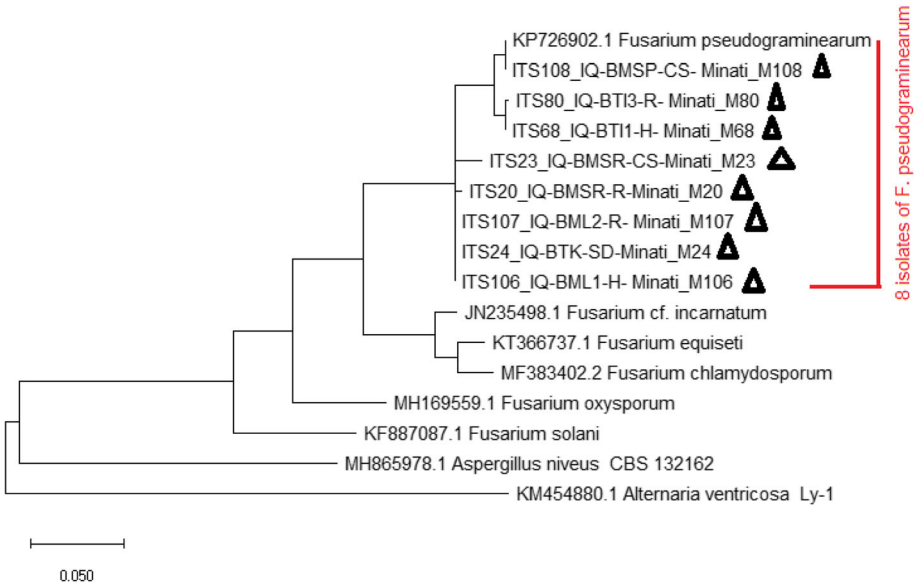

Fig. 10 Phylogenetic tree generated with the ITS-rDNA sequences of the *Fusarium pseudograminearum* (8), as inferred from the maximum likelihood (ML) analyses (determined by black triangles) and related species retrieved from GenBank. ITS sequences of *Aspergillus niveus* (MH865978) and *Alternaria ventricosa* (KM454880) were used as the out-group species

In previous Iraqi studies, three species of the genus *Fusarium* (*F. pseudograminearum*, *F. graminearum*, and *F. equiseti*) have been isolated and identified from wheat and barley as causative pathogens of FCR disease by Hameed et al. (2012), Matny et al. (2012), Khudhair et al. (2014), Khudhair et al. (2015a), Matny et al. (2017), and Lahuf et al. (2018). However, to the best of our knowledge, this is the first isolation and identification of the six new *Fusarium* species (*F. culmorum*, *F. chlamydosporum*, *F. solani*, *F. avenaceum*, *F. cerealis*, and *F. nygamai*) from the wheat crop in Iraq as causative pathogens of FHB and FCR.

In relation to FCR, as mentioned earlier, it is mainly caused by *F. pseuograminearum* (Aoki and O'Donnell 1999; Li et al. 2008). Akinsanmi et al. (2004) cited by (Chakraborty et al. 2006) pointed out that there are 10 species including *F. avenaceum*, *F. graminearum*, *F. proliferatum*, *F. subglutinans*, *F. tricinctum*, *F. acuminatum*, *F. babinda*, *F. crookwellense*, *F. pseudograminearum*, and *F. torulosum* that could also cause FCR disease in wheat cultivars.

Consequently, it can be clearly stated that our result is in accordance with the earlier published Iraqi studies on the occurrence of *F.pseudograminearum*, *F. graminearum*,

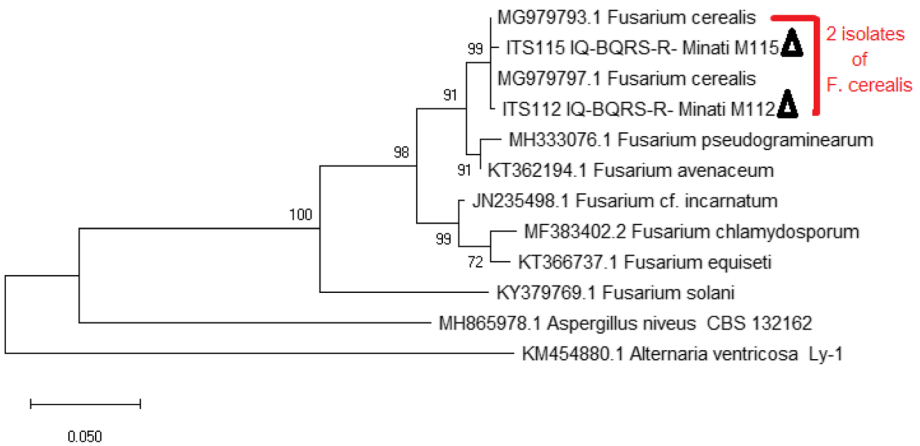

Fig. 11 Phylogenetic tree generated with the ITS-rDNA sequences of the *Fusarium cerealis* (2), as inferred from the maximum likelihood (ML) analyses (determined by black triangles) and related species retrieved from GenBank. ITS sequences of *Aspergillus niveus* (MH865978) and *Alternaria ventricosa* (KM454880) were used as the out-group species

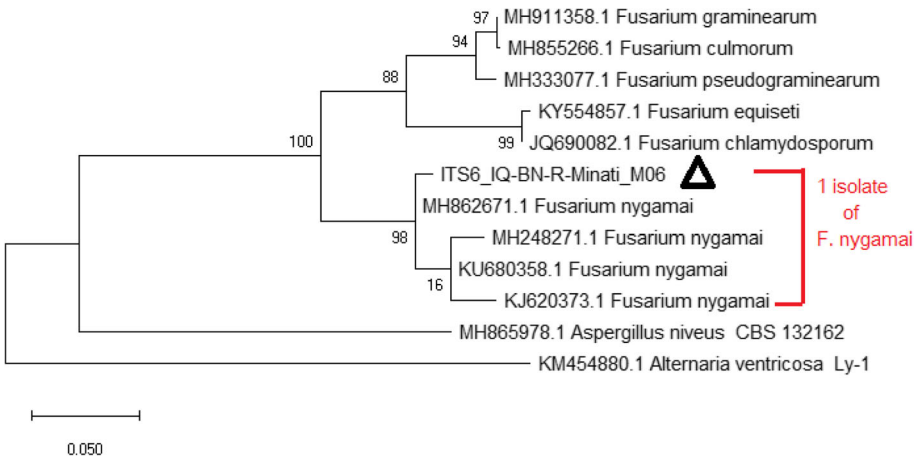

Fig. 12 Phylogenetic tree generated with the ITS-rDNA sequence of the *Fusarium nygamai* (1), as inferred from the maximum likelihood (ML) analyses (determined by black triangles) and related species retrieved from GenBank. ITS sequences of *Aspergillus niveus* (MH865978) and *Alternaria ventricosa* (KM454880) were used as the out-group species

and *F. equiseti* in wheat cropping system as causal pathogens of FCR disease. Moreover, we identified and recorded six *Fusarium* spp. (*F. solani*, *F. chlamydosporum*, *F. avenaceum*, *F. culmorum*, *F. cerealis*, and *F. nygamai*) as new causative pathogens of FCR disease in wheat cropping system in Iraq.

In terms of FHB, to the best of our knowledge, FHB disease on wheat has not yet been detected in all of wheat-harvested areas of the south of Iraq, Basra province. Importantly, in this study, we isolated and identified seven *Fusarium* spp. (*F. solani*, *F. culmorum*, *F. pseudograminearum*, *F. equiseti*, *F. graminearum*, *F. avenaceum*, and *F. chlamydosporum*) from wheat head to be recorded for the first time in Iraq as causal pathogens of FHB disease.

Even though *Fusarium graminearum* is known to be the main cause of FHB on wheat worldwide (Wegulo et al. 2011), there are another 16 Fusarium species (as a complex of these species) often isolated from the wheat crop in Australia and other countries, which could cause FHB disease on wheat as well (Parry et al. 1995). The distribution of these FHB causal pathogens globally

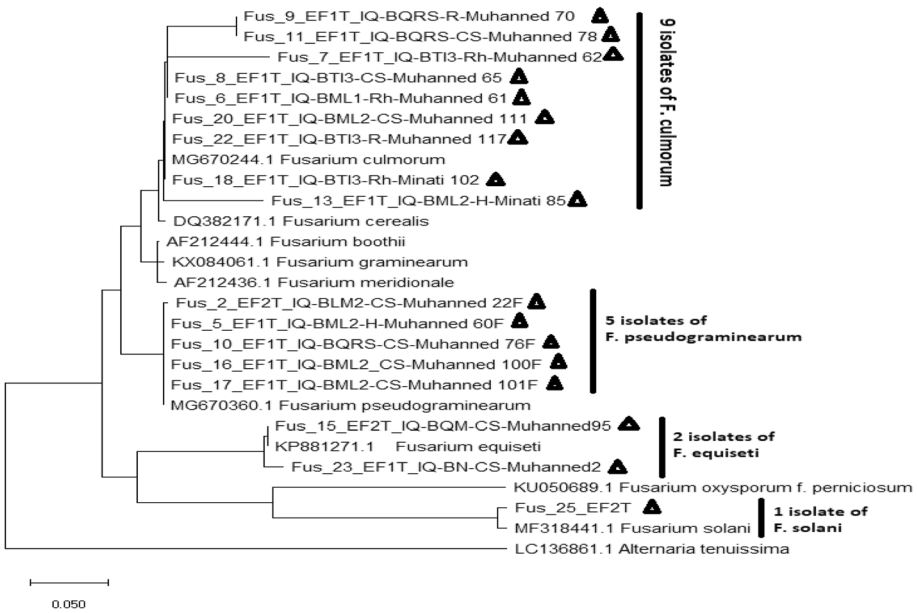

Fig. 13 Phylogenetic tree generated with the TEF1-α sequences of the 17 *Fusarium* isolates, as inferred from the maximum likelihood (ML) analyses (determined by black triangles) and related species retrieved from GenBank. TEF1-α gene sequence of *Alternaria tenuissima* (LC136861.1) was used as the out-group species

differs in occurrence among the harvested wheat areas and is affected by the weather and environmental conditions. More frequently, some or all these pathogens can occur concurrently in wheat heads (Xu and Nicholson 2009). Worldwide, *F. graminearum* is the most occurring species associated with FHB disease (Leslie and Summerell 2006, Leslie & Summerell, 2008), as it has been recognized in Europe, South and North America, and Asia. Other causal agents producing FHB have also been documented in Australia, Europe, and Asia (Xu and Nicholson 2009; Gale et al. 2002; Gale 2003; Gale et al. 2007; Alvarez et al. 2010).

The noticeable occurrence of FHB and FCR diseases in wheat- and barley-harvested areas in Iraq during the last few years associated with the obvious alteration in weather conditions represented by fluctuated temperature and high humidity companied with rainfall density and distribution. Additionally, the periodical increase of these two diseases in the last a few years might be attributed to the applying of impracticable agricultural practices, such as sowing uncertified seeds, conservation tillage, retaining wheat debris in the fields, and continuous winter wheat cropping without effective crop rotation (Khudhair et al. 2014). As stated by Lamprecht et al. (2006), opportunity of FCR disease can increase when using continuous wheat cropping in the same fields.

Conclusion

According to the obtained results, the Iraqi wheat cropping system is seriously threatened by FHB and FCR diseases. Seven *Fusarium* spp. were isolated from wheat head to be recorded for the first time in Iraq as causal pathogens of FHB disease, while nine *Fusarium* spp. were identified as causative pathogens of FCR disease. Further studies on these FHB and FCR causal pathogens in the Iraqi wheat cropping system should be conducted evaluating the potential yield losses and cultivar tolerance and determining mycotoxin produced. Likewise, it is necessary to survey the remaining Iraqi provinces especially the north and middle of Iraq to understand the distribution of both diseases and their responses to the different geographies and weather conditions.

Additional file

Additional file 1: Identification of general fungal taxa (123) isolated from symptomatic head, stem, root, seed and rhizosphere as well as from soil of the 14 selected fields based on morphological analysis and sequencing of rDNA ITS1-5.8S-ITS4 region during this study. (DOCX 40 kb)

Abbreviations

A99: Adana 99; AG3: Abu Ghraib 3; AGRI S.: AGRI-saaten; B.: Barcelona; CLA: Carnation leaf agar; CZA: Czapek Dox agar; D: Dair district; DRBC: Dichloran rose Bengal agar; FCR: Fusarium crown rot; FHB: Fusarium head blight; H: Huwair district; HM: Al-Hammar marshland; HPDA: Half potato dextrose agar; ITS: Internal transcribed spacer; MEA: Malt extract agar; MGA: Malachite green agar; ML: Maximum likelihood; ML1: Talha district/marshland 1; ML2: Talha district/marshland 2; MSP: Modienh district/salt project region; MSR: Modienh district/Salih River region; N: Nashwa district; PCR: Polymerase chain reaction; PDA: Potato dextrose agar; QM: Qurna district/Mazearaa region; QPDA: Quarter potato dextrose agar; QRS: Qurna Research Station; R.: Rasheed; RBA: Rose Bengal agar; Res.22: Research 22; SDA: Sabouraud dextrose agar; SNA: Synthetic nutrient agar; TEF1-α: Translation elongation factor 1 alpha; TI1: Thagar district/field 1; TI2: Thagar district/field 2; TI3: Thagar district/field 3; TK: Thagar district/Karakor region; WA: Water agar

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Authors' contributions

All work was done by MH under the supervision and advice of MK. MH was a major contributor in writing the manuscript. Both authors read and approved the final manuscript.

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Availability of data and materials

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Ethics approval and consent to participate

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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