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New report of the pathogenic isolate of *Fusarium solani* isolated from Iraqi Potato tubers infected with *Fusarium* dry rot

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ABSTRACT

This study was carried out in the Plant Virology laboratory of the Plant Protection Department at the College of Agriculture, University of Kerbala, Iraq in order to isolate and diagnose the causal agent causing dry rot of potato tubers. The isolated fungus was identified using the polymerase chain reaction (PCR) technique and determining the nucleotide sequences of the amplified-PCR products using the primer pair ITS1 and ITS4. PCR amplification of DNA extracted from the isolated fungus showed the possibility of amplifying a 500bp PCR product. Analysis of the nucleotide sequence of the PCR-amplified product using the Basic Local Alignment Search Tool (BLAST) showed that the isolate identified in this study belong to the fungus *Fusarium solani*. A phylogenetic tree, constructed using the nucleotide sequences of *F.solani* isolated in this study and *F. isolates* previously registeredin the National Centre for Biotechnology Information (NCBI), showed three main clades. Among these *F. solani* isolates, the *F. solani* isolated in this study appeared in a separated clade with 99% similarity percentage with other *F. solani* isolates. Therefore, this isolate was recorded in GenBank under the accession number MN124374.

Key words : *Fusarium solani, Potato tubers*

Introduction

Fungi are diverse and widespread in all ecosystems and yet a high number of fungi species has not been identified (Yang *et al.,* 2007). *Fusarium* spp. is one of the important fungi that exist ubiquitously across the world in very large numbers. It causes diseases of a wide range of plants including potato, such asdry rot that is one of the serious diseases that impact potato tubers and caused by *Fusarium* (Gachango *et al.,* 2012). Accurate identification and diagnosis of pathogens are essential to protect plants against different diseases such as dry rot. Besides, several studies have indicated that fungal diagnosis using morphological characteristics could yield inaccurate results and sometimes requires considerable experience with fungal classification and taxonomy especially in classifying fungi that are morphologically alike such as *Fusarium* (O'Donnell *et al.,* 2008; Healy *et al.,* 2005). Therefore, to overcome these challenges, molecular methods have been used to identify many microorganisms including fungi that have economic importance (Schroeder *et al.,* 2013). Polymerase chain reaction

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(PCR) technology is one of the most important molecular techniques that effectively used in diagnose many fungi such as *Fusarium* spp. (AL-Abedy *et al.,* 2018). According to the nucleotide sequence, the similarities and differences between organisms can be revealed. One of the distinguishing features of the PCR method is the high precision in identifying genetic differences and to avoid the drawbacks of classical diagnostic techniques (Giantsis *et al.,* 2017). PCR approach is used to identify many microorganisms including fungi such as *Fusarium* and *R. solani* (Arif *et al.,* 2012; Alhussaini *et al.*, 2016; Al-Fadhal *et al.,* 2018). Due to the importance of precise diagnosis of pathogenic fungi, this work aims at isolation and molecular diagnosis of *Fusarium* isolated from infected potatoes.

Materials and Methods

Isolation of *Fusarium* Species

Potato tubers showing the symptoms of dry rot represented by the dry dark brown were collected from the local markets in Kerbala-Iraq (Fig. 1). Tubers were brought to the lab for isolating fungi associated with their infection. First, the tubers were carefully washed with water to remove dust and dirt. Then, they were chopped into small pieces and sterilized for 3 min with 1%sodium hypochlorite (NaOCl). Thereafter, sterilized potato pieces were thoroughly washed with distilled water to get rid of the sterile material and then dried with sterile filter paper. The potato pieces were put in Petri dishes containing potato dextrose agar (PDA) medium and 200 mL/L of the antibiotic chloramphenicol. The Petri dishes were incubated for 3 days at a temperature of 25±2°C. The fungal isolates were purified on the same PDA medium using the hyphal tip technique. The fungal isolates were initially diagnosed based on morphological characteristics using taxonomic keys.

Molecular Diagnostics of *F.solani*

The diagnosis of the pathogenic fungal isolates was carried out in the Virology Laboratory of the College of Agriculture at the University of Kerbala using PCR technique and the nucleotide sequence was determined following the procedure discussed in the following sections. DNA was extracted from the fungal isolate according to the procedure presented by Zymo Research Corp. using its Quick-DNA Fungal/Bacterial kit (Cat. No. D6005).

Fig. 1. Potato tubers showing symptoms of dry rot disease.

PCR amplification and DAN sequencing of rDNA-ITS region

The partial ITS region of each DNA extracted from each *F. solani* isolate was PCR-amplified using the universal primer pair: ITS1 (TCCGTTGGTGAACCAGCGG) and ITS4 (TCCTCCGC TTATGATATGC) (White *et al.,* 1990). PCR amplification was done using *Taq* DNA polymerase (Roche, Cat. No. 11 146 173 001) in a final volume of 20 mL PCR reaction mixture containing 2 mL 10X PCR buffer, 1 mL each primer (10 pmol), 2 ìldNTPs (2 mM), 3 mL template DNA (30 ng/µL), and 1 unit *Taq* polymerase. Each sample volume was then completed to 20 mL by adding nuclease-free water.

PCR amplification was performed using the following conditions: initial denaturation at 94 °C for 1 min followed by 35 cycles each consisting of final denaturation at 94 °C for 30 sec, annealing temperature at 55 °C for 30 sec, initial extension for 1 min, and final extension at 72°C for 5 min (Zhang *et al.*, 2012). PCR-amplified products were electrophoretically separated on a 1% agarose gel for 140 min at 80 V, 400 mA and visualized with ethidium bromide staining under UV illumination and images were captured using VilberLourmat, Taiwan gel documentation system.

Nucleotide Sequence Analysis

For DNA sequencing, the PCR-amplified products were gel-purified using the *Favor Prep PCR Purification Kit* (Cat. No. FAGCK 001, Favorgen, Tawan) and sent along with the primer pair (ITS1 and ITS4) to the Macrogen DNA sequencing service in Korea. PCR products were directly sequenced in both di-

rections using the respective forward and reverse primers. The obtained nucleotide sequences were aligned and compared with the sequences belonged to the *F. solani* isolates in the NCBI database using the Basic Local Alignment Search Tool (BLAST) (Zhang *et al.*, 2012). Using the MEGA6 software, multiple alignments of the nucleotide sequences and construction of phylogenetic trees were performed using the Neighbor-joining method (Tamura *et al.*, 2013).

Results and Discussion

Isolation and Diagnosis of *Fusarium* spp. from Infected Potato Tubers

F. solani isolated from the infected potato tubers produced sparse to abundant, pink to red mycelium (Fig.2). Macroconidia are sickle-shaped with a slightly blunted apical end blunt and have 3 to 4 septa on average. Microconidia are abundant, oval to kidney-shaped, and formed in false heads on very long monophialides (Ke *et al.*, 2016).

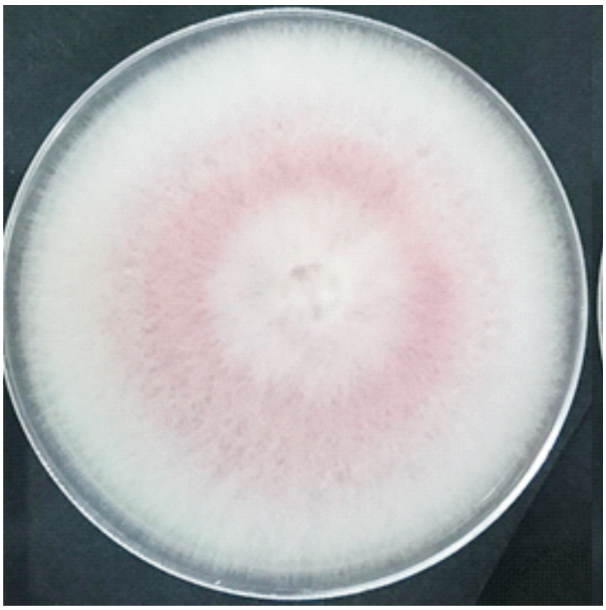

Fig. 2. *Fusarium solani* isolate growing on the PDA medium.

Molecular Diagnosis of *F.solani* Using PCR

For confirmation of the morphological identification of *F. solani*, PCR amplification of DNA extracted from this isolates showed the possibility of amplifying a 500bp PCR using the ITS1-ITS4 primers (Fig. 3).

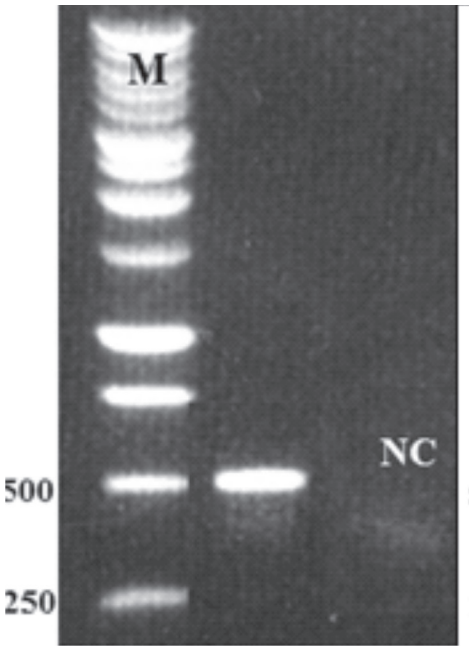

Fig. 3. DNA product amplified by polymerase chain reaction (PCR) from *F. solani* isolated from potato tubers showing dry rot. M= 1Kbp DNA ladder marker. NC: Negative control (no template DNA added).

The results of nucleotide sequence analysis revealed clear differences in the nucleotide sequence in some regions of the extracted DNA. However, this isolate showed sequence similarity of 99% with the nearest sequence similarity of *F. solani* isolate previously deposited at NCBI. Figure 4 presents aomparison of nucleotide sequence alignment of the studied fungal isolates with other isolates of the same fungal species already identified in the NCBI database.

It can be concluded from this study that the *F. solani* isolate investigated in this work is genetically different than other isolates of the same species from database resources of NCBI. Therefore, it has been added to the NCBI database as a new *F. solani* pathogen with the accession number MN124374.

As it was mentioned earlier, diagnosing fungus species according to their morphological characteristics only can result in inaccurate results. Therefore, a molecular method using PCR technique has been used in this work for more accurate classification of the most pathogenic *F. solani* isolate. PCR technology has been widely used as an alternative to morphological methods for its high accuracy in identification of various microorganisms including

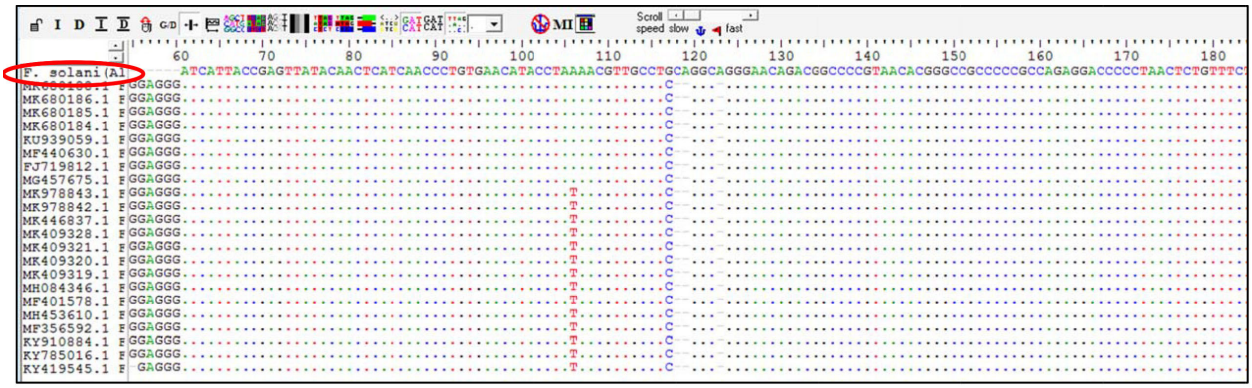

Fig. 4. Similarities and differences in sequence alignment in some regions of the DNA of the studied *F. solani* isolate and the most similar isolates of the same species deposited at NCBI Neighbor-Joining treein Figure 4 depicts that *F. solani* isolate appeared in a separate clade from the clade of other isolates of the same species.

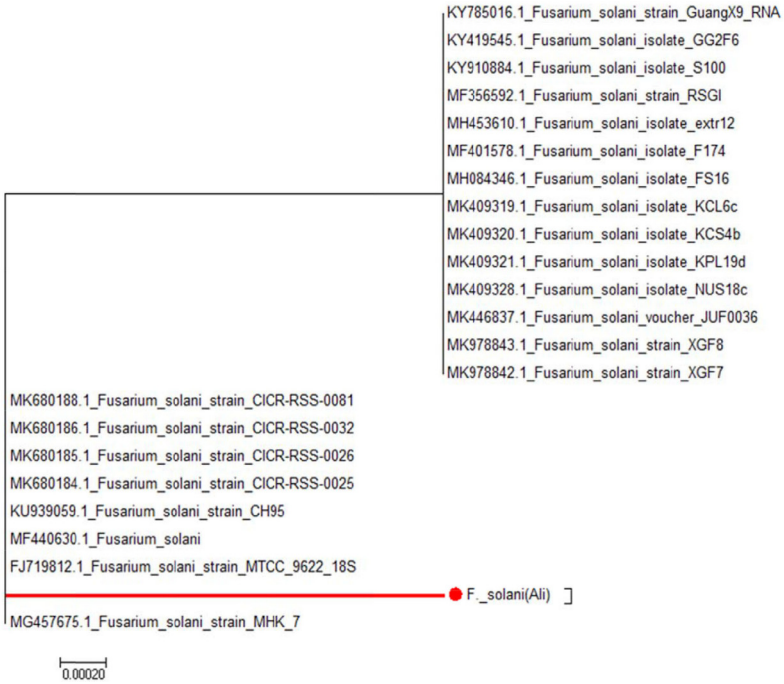

Fig. 5. Neighbor-joining tree showing the genetic relationship between the studied *Fusarium solani* isolate and the most similar isolates of the same species from the NCBI database.

Fusarium spp. and *Aspergillus* spp. etc. (Huang, 2006; AL-Abedy *et al.,* 2018). However, morphological methods still play a significant role in fungal taxonomy especially in classifying fungi in smaller groups. But, they require high experience in taxonomy as some fungal species are morphologically similar but genetically different as in *Fusarium* (O'Donnell *et al.,* 2008). Besides, morphological methods can be time and effort consuming as the size, shapes, and colors of fungal spores and colonies can be affected by environmental factors which make morphological identification of these fungal species more challenging (Zhang *et al.,* 2012).

In previous study, it was found an error in morphological taxonomy of certain *Fusarium*spp. such as *Fusarium subglutinans* and *Fusarium verticillioides* when the diagnosis was re-implemented using the PCR technique (Giantsis *et al.,* 2017). The DNA sequence in the internal transcribed spacer (ITS) region showed high effectiveness in diagnosing different fungal species including *Cladosporium* and *Fusarium* (Arif *et al.,* 2012; Alhussaini *et al.,* 2016; Al-

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