First molecular identification of *Fusarium oxysporum* causing Fusarium wilt of Armenian cucumber in Iraq

First molecular identification of *Fusarium oxysporum* causing Fusarium wilt of Armenian cucumber in Iraq

Huda Hazim Wafi AL-Taae[1] and Ali Kareem Al-Taae[1]

[1]Plant Protection Department., College of Agriculture and Forestry, Mosul University, Iraq

Email: aaltaae@yahoo.co.uk

Abstract. *Cucumis melo var. flexuosus* , which is commonly known as Armenian cucumber is one of the most economically important species of the family Cucurbitaceae. In June of 2018, wilt symptoms were observed on Armenian cucumber plants in Fadhilia ,Ninevah governorate ,North of Iraq. The fungus was isolated from diseased tissues and initially identified based on, its morphological characterizations using conidial and hyphal structures.. Pathogenicity of the fungus was also confirmed by Koch's postulates. Molecular identification of the fungal pathogen was done by amplifying the internal transcribed spacer (ITS) region of the conserved ribosomal DNA. All these ITS sequences showed homologous to those of *Fusarium oxysporum* isolates in the GenBank database with a similarity percentage of 99%.. To the best of our knowledge this is the first molecular record of *F. oxysporium* on the Armenian cucumber in Iraq .

1. Introduction

The Cucurbitaceae, also called cucurbits, plants of this family are grown around the tropics and in temperate areas. Those with edible fruits were among the earliest cultivated plants in both the old and new worlds. This family have about 965 species in around 95 genera and the most important them are Cucurbita, squash, pumpkin, zucchini, watermelon (*C. lanatus, C. colocynthis*) and Cucumis cucumber (*C. sativus*), and *Cucumis melo* var. flexuosus , which commonly known as Armenian cucumber [1].

The Armenian cucumber, *Cucumis melo var. flexuosus*, produce slender fruit which tastes like a cucumber and looks somewhat like a cucumber inside. It is actually a variety of muskmelon (*C. melo*), a species closely related to the cucumber (*C. sativus*). It is also known as the yard-long cucumber, snake cucumber, snake melon, and uri in Japan.. The skin is very thin, light green, and bump less. It has no bitterness and the fruit is almost always used without peeling. It is also sometimes called a "gutah".[2]. In general, cucumbers are susceptible to different diseases: mostly fungal and viral infections. the most common cucumber diseases include powdery mildew, bacterial wilt, cucumber mosaic, downy mildew and fusarium wilt. *Fusarium oxysporum f. sp. cucumerinum* (F.O.C) is the most common pathogen on cucumber plants causing Fusarium wilt on cucumber and reduced the yield [3; 4] .Also, soil borne pathogenic fungi. *Rhizoctonia solani, Pythium* spp., *Phytophthora* spp., *Macrophomina phaseolina* and *Sclerotium rolfsii* are the most common pathogens associated on cucumber plants causing damping-off and root rots to cucumber [5] . F.O.C was isolated from the infected roots of cucumbers and recorded in many areas [2; 6; 7]. I was found [8] that the F.O.C and *F. oxysporum* f.sp. *niveum* were to be externally and internally seed borne in cucumber and watermelon, respectively. In the last two decades, molecular tools have demonstrated a major impact on the

identification of plant pathogens. These molecular techniques can avoid many of the drawbacks associated with classical methods of pathogen identification and can also improve our pathogen detection in different environmental conditions. These techniques are more specific, sensitive and accurate than traditional methods, and do not demand specialized taxonomical expertise. Different molecular techniques were used for detection, identification, and genetic diversity analysis of some *F. oxysporum* isolates [4; 8; 9; 10]. However, in Iraq there are no molecular identification studies on wilt disease of Armenian cucumber plants in addition to the lack of studies on Armenian cucumber. Thus this aimed to identify the causal agent of Fusarium wilt morphologically and molecularly.

2. Materials and Methods

2.1. Isolation and morphological identification

In June of 2018, wilt symptoms were seen on Armenian cucumber plants in Fadhilia of Ninevah governorate, Iraq. Samples were taken from symptomatic Armenian cucumber plants. Stem samples were sterilized by dipping in 1% NaOCl solution for 3–5 min and washed thoroughly with distilled water. The stem samples were cut with a sterilized blade and four pieces of diseased vascular tissues (5 mm) were placed on the surface of potato dextrose agar (PDA, Himedia, Mumbai) media. PDA was amended with streptomycin sulfate to minimize chances of any bacterial growth. Plates were incubated at 28 ± 2 °C and observed periodically. Fungal growth was purified for the purpose of definition and use in subsequent experiments. For morphological identification, single spore isolates were grown for 10–15 days on PDA medium [11] .Culture characteristics were determined from 10 to 15 day old PDA cultures. Microscopic features of conidia conidiophores and chlamydospores were also determined based on previous descriptions [8; 12].

2.2. Pathogenicity test

The pathogenicity of isolated fungus was evaluated on cucumber seedlings at the one-true-leaf stage. Their roots were dipped into a conidial suspension ($1x10^7$ conidia per ml) of the selected isolate for 30 min, after which seedlings were transplanted into sterilized soil in plastic pots (8.5 cm in diameter) and where incubated under greenhouse conditions. Final observation on disease development was made 30 days after inoculation.

2.3. Molecular identification

2.3.1. Genomic DNA extraction and PCR amplification

Pure cultures of *Fusarium* sp. were grown in potato dextrose broth (PDB) for 10 days at 25–28°C in the darkness. Mycelia were harvested by filtration through filter paper (Whatman No. 1). The harvested mycelia were used immediately for DNA extraction using Fungal/Bacterial/ Yeast DNA MiniPrep™ , Catalog No. D6005 according to the manufacturer `s procedures:

2.3.2. Amplification of ITS region of pathogen using PCR technique

The A ITS region of fungal pathogen was amplified using a forward primer (ITS1: 5′-TCCGTAGGTGAACCTGCGG-3′) and a reverse primer (ITS4:5′ TCCTCCGCTTATT GATATGC-3′) (Primers set supplied by IDT (Integrated DNA Technologies company, Canada.). The PCR amplification was performed in a total volume of 25µl containing 1.5µl DNA, 5 µl Taq PCR PreMix (Intron, Korea), 1µl of each primer (10 pmol) then distilled water was added into tube to a total volume of 25µl.The thermal cycling conditions were done as follows: Denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C for 45s, 52°C for 1 min and 72 °C for 1min with final incubation at 72 °C for 7 min using a thermal Cycler (Gene Amp, PCR system 9700; Applied Biosystem). The PCR products were separated by 1.5% agarose gel electrophoresis and visualized by exposure to ultraviolet light (302 nm) after red stain staining (Intron, Korea) [13].

2.3.3.Sequencing and bioinformatics analysis
The PCR products Sequencing was performed by national instrumentation center for environmental management (nicem) (http://nicem. Snu .ac. kr/main/? En _skin=index.html).Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http:// www. Ncbi .nlm .nih .gov) and BioEdit program.

3. Results and Discussions

3.1. Isolation and morphological identification
Armenian cucumber plants affected by wilt symptoms showed emergence of single and pure fungal colonies. The shape of the fungus colony was shown on the medium of the potato dextrose agar (PDA) with a white cotton appearance. The color of the colony changed with age to dark red. The diameter of the colony after eight days of incubation at a temperature of 28 ± 2 °C reached 8.5 cm in the microscopic examination, the spores of the fungus appeared in all three types. Microconida spores were characterized by a proliferating, oval or renal form consisting mostly of a single cell, which was carried on simple, non-truncated conidiophore and ranged between 13.2-4.3 x 4.4- 3.7 micrometers, and the large spores Macroconidia were crescent shaped, divided into three to five different cells (3-5) with a foot cell and apical cell they were born on short, branching and branching stands called sporodochium and macrocondia spores ranged between 35.5-8.7 x 17.2-8.3 micrometers. The chlamydiospores appeared in spherical and elliptical form and were found alone or in a short, peripheral or interstitial chain whose dimensions ranged between 18.7-18.2 x 20.3-18.9 micrometers. These characteristics correspond to *Fusarium oxysporum* and were in agreement to [14, 15] *, F.oxysporum* has been reported as a severe pathogen of many plants worldwide, as well as in Iraq [2; 16; 17; 18] . The registration of *F.oxysporum* on Armenian cucumber the first recorded in Iraq However, there has been no formal report of *F.oxysporum* causing wilt on Armenian cucumber in Iraq [19; 20] .

3.2. Pathogenicity test
Symptoms of the pathogenicity examination appeared on cucumber seedlings as yellowing and wilt in the leaves followed by paleness and dryness of the leaves and stems after one month of the emergence of symptoms of infection., Often the disease symptoms began from one side of the seedlings, and when the stems and roots of the infected seedlings cut brown color tissues were observed particularly in wooden vasiculars. The final colony and conidia characteristics were analyzed to confirm that the isolated fungus was the same with what were used for the pathogenicity test.

3.3. Sequencing and bioinformatics analysis
To confirm the morphological identification, the internal transcribed spacer (ITS) region of the Fusarium isolate was amplified with universal primers ITS1 and ITS4.The isolate was partially diagnosed after conformity with the copies at the gene bank at National Center Biotechnology Information (NCBI) genes gave 99% (diagnostic accuracy) match with isolation FJ545397.1.The Query cover of the sequence was 99% Error Value 0.0 .The results obtained showed that 2 variations: first C>A second T>G, Transversion have shown 99% compatibility as showed in table 1.

Table1. Represent Type of Polymorphism of 16srRNA Gene from *Fusarium oxysporum* Isolate

Type of substitution	Location	Nucleotide	Sequence ID	Score	Expect	Identities
Transvertion	76	C>A	ID: FJ545397.1	816	0.0	99%
Transvertion	171	T>G				

The phylogenetic analysis results showed the close genetic relationship among *F. oxysporum* isolated in this study (indicated with black prism) and those worldwide deposited in genbank database Figure (1) particularly with *F.oxysporum* strains accession Nos . FJ545397.1, MK163439.1, MG669223.1, KJ767070.1 , KY100124.1, MG020684.1, KM260335.1 and KY114955.1 from Ghana . China , India , Malaysia , amexico , Pakistan and Tunisia respectively. The nucleotide sequence of ITS region of the Iraqi isolate has been assigned in the GenBank with Accession No. MN128597.1. Thus, *F.oxysporum* was identified as the causative agent of Fusarium wilt of Armenian cucumber in Iraq (Fig. 1). The registration of *F.oxysporum* on Armenian cucumber has not been formal reported [19, 20] .Thus, to the best of our knowledge, this is the first molecular report of Fusarium wilt of Armenian cucumber in Iraq .

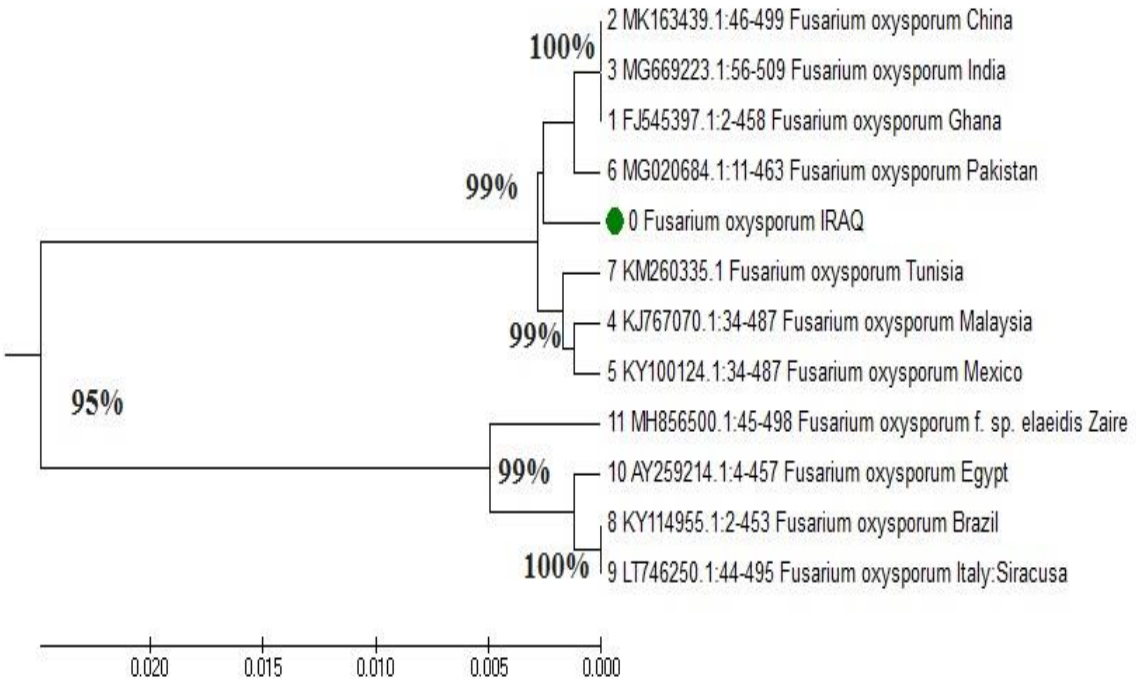

Figure 1. Phylogenetic tree constructed by the neighbor-joining method showing the phylogenetic relationships of *Fusarium oxysporum* compared with the reference sequences from gene bank

4. Conclusion

In this study, first molecular report of *F. oxysporium* causing fusarium wilt of Armenian cucumber plants in Iraq was achived.

References

[1] Christenhusz M J M and Byng J W 2016 The number of known plants species in the world and its annual increase. *Phytotaxa*. **261 (3)** 201–217.
[2] Al-Taae A K et al 2013 Priliminary study on Fressia Rot and Wilt . *Mesopotamia J. Agric.* **41 (1)** 303-214.
[3] Michail S H et al 1989 Cross protection of watermelon and cucumber plants against wilt by prior inoculation with an irrespective forms specialis of *Fusarium oxysporum*. *Acta Phytopathol. et Entomol.Hungarica* **24 3-4** 301-309.
[4] Martinez R M et al 2003 First report of *Fusarium oxysporum* in spain. *Plant pathology*. **52 (3)** 410.
[5] Bayraktar H F et al 2008 Use of RAPD and ISSR markers in detection of genetic variation and population structure among *Fusarium oxysporum f.sp. ciceris* isolates on chickpea in Turkey. *J Phytopathol* **156**: 146–154.
[6] Huang Z X 1990 Occurrence of cucumber wilt disease and identification of pathogen in Shanghai suburbs. *Acta Agricul. Shanghai* **6 (2)** 57-62.
[7] Huang Z S et al 1994 Identification of pathogenic races and integrated control of Fusarium wilt of cucumber in China. *Acta Agriculture Boreali Sinica* **9 (4)** 81-86.
[8] Barnett H L and Hunter B B 2006 *Illusrated Genera of Imperfect Fungi*. (Minneapolis, USA: Burgess publishing company).
[9] Honnareddy N and Dubey S C 2006 Pathogenic and molecular characterization of Indian isolates of *Fusarium oxysporum* f.sp. *ciceris* causing chickpea wilt. *Curr Sci* **91** 661-666.
[10] Leslie J F and Summerell B A 2006 *The Fusarium Laboratory Manual* (Hoboken, New Jersey,USA: Black Well Publishing)
[11] Nelson P E et al 1983 *Fusarium species: an illustrated manual for identification* (Pennsylvania, USA: The Pennsylvania State University Press).
[12] Lievens B M et al 2008 Recent developments in the molecular discrimination of formae speciales of *Fusarium oxysporum*. *Pest Manag Sci* **64** 781–788.
[13] Sambrook J E et al 1989 *Molecular Cloning: A Laboratory Manual* (New York, USA: Cold Spring Harbor Laboratory).
[14] Summerell, BA et al 2003 A utilitarian approach to Fusarium identification .*Plant Dis*. **87(2)**117-128.
[15] Bedlan G 1986 The most important fungal diseases of cucumbers. *Pflanzenschutz*. **9** 8-11.
[16] Ogura H 1992 Dispersal of *Fusarium oxysporum f.sp. cucumerinum* in soil. *Res. Rep. Agricul. Sci.* **40** 1-8.
[17] Singh B P et al 2006 Molecular characterization of *Fusarium oxysporum f.sp. ciceri* causing wilt of chickpea. *Afr J Biotechnol* **5**: 497-502.
[18] Vincelli P and Tisserat N 2008 Nucleic acid-based pathogen detection in applied plant pathology *Plant Dis. J*. **92** 660-669.
[19] Mathur R S 1968 *The fungi and plant diseases of Iraq* (Baghdad,Iraq: Ministry of Agriculture).
[20] Mustafa F H 1974 *A list of the common plant diseases in Iraq (Bull. 74)* (Baghdad, Iraq: Ministry of Agriculture).