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GENETIC VARIABILITY OF DIFFERENT ISOLATES OF *FUSARIUM* SPP. ISOLATED FROM IMPORTED BANANA FRUITS *MUSA* SPP.

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Abstract

This study was carried out in the Laboratory of Plant Phytology at the Department of Plant Protection at Faculty of Agriculture, University of Karbala, in order to isolate and diagnose twelve isolates of *Fusarium* spp. isolated from imported banana fruits that found in local markets. The fungal isolates were identified using polymerase chain reaction (PCR) and nucleotide sequencing of DNA multiples using ITS1 and ITS4. The results of the analysis of the sequence of the nitrogen bases of the double-stranded PCR products using the Basic Local Alignment Search Tool (BLAST) showed that all the isolates of the fungus are attributed to *Fusarium* Spp. The Nucleotide sequence analysis of isolation isolates in this study showed that there was a genetic variation among them, as well as between newly recorded isolates and other isolates belonging to the same fungus, which were established at the National Center for Biotechnology Information, NCBI). The results showed that nine isolate isolates were not previously registered with the National Center for Biotechnology Information (NCBI), so they were registered under entry numbers (MN093942, MN121160, MN121161, MN121533, MN132885, MN121544, MN123531, MN121577 and MN121630).

Key words: *Fusarium* spp., (PCR) Polymerase chain reaction, DNA, ITS1, ITS4 and banana.

Introduction

Fungi are organisms that are scattered in all ecosystems and in numbers up to five times the number of plant species. Many countries lack the exact definition of fungi. This reflects global public opinion that about 95% of fungi are still unknown and are expected to be detected (Hawksworth *et al.*, 1995; Yang *et al.*, 2007). Soil is the main repository of all microorganisms, including pathogenic fungi such as *Fusarium* spp., which is one of the most severe and dangerous fungi affecting crops and human health through its production of various fungal toxins, as it exists away from the human perspective, which increases the seriousness of the breadth of the range of the family, Resistance to extreme environmental conditions (Arif *et al.*, 2012). The need for accurate classification of fungi is one of the urgent needs of its importance in reaching rapid and efficient methods in managing the disease. It has been noted in previous studies that the dependence on morphological characteristics in the classification of fungi may give accurate results sometimes, but many researchers do not rely on these qualities because they require sufficient experience in the field of classification, especially in the closely convergent fungal groups, to the need for time and effort, as well as the fact that it is often inaccurate because of the environmental factors that affect Size, shapes and colors of spores and fungal colonies (Yang *et al.*, 2007; Zhang *et al.*, 2012 and Huang *et al.*, 2016). Molecular characterization techniques have contributed to their accuracy, sensitivity and ability to detect and study genetic differences and to eliminate the disadvantages of traditional methods in diagnosing many organisms (Wang *et al.*, 2008; Giantsis *et al.*, 2017). Polymerase chain reaction (PCR) is one of the molecular techniques used to select and amplify a specific area of the organism's genome, depending on differences in the DNA sequence of that region and hence the knowledge of genetic relationships in terms of similarities and the difference between fungal species that will support the phenotypic diagnosis of the studied fungus (Chandra *et al.*, 2008; Al-Sanae *et al.*, 2016). This technique has been used in the diagnosis of many microorganisms, including fungi such as *Fusarium* spp. and *R. Solani* (Arif *et al.*, 2012;

Schisler *et al.*, 2012; Alhussaini *et al.*, 2016 and Al-Fadhal *et al*., 2018). Due to the importance of accurate classification of fungus, the aim of this study was to isolate and diagnose twelve isolates of *Fusarium* spp. molecularly using polymerase chain reaction (PCR), to determine the sequence of nitrogen bases and to identify similarities and the genetic variation between these isolates and other isolates of *Fusarium* spp. is internationally recognized and is established in National Center for Biotechnology Information (NCBI).

Materials and Methods

• Source of fungus isolates *Fusarium* spp.: In order to isolate the fungi associated with imported banana fruits, samples of bananas were collected from local markets and brought to the fungus laboratory at the Faculty of Agriculture - University of Al-Kufa for the purpose of isolation. The samples were divided into sterile and nonsterile sections, all the samples were sterilized with an alcohol solution ethanol (70%) for 2 minutes, then washed with sterile distilled water to remove traces of sterile material and dried with filter paper to remove excess water. Pieces of bananas fruits and various parts of it Which show the following qualities (peels, crown area and pulp) were planted in Petri dishes on the Potato Dextrose Agar (PDA), supplemented with Chloramphenicol antibiotic at a concentration of 200 mg / L. Incubate the dishes at a temperature of 2 ± 25°C for four days. *Fusarium* Spp. isolates have been purified on the same food medium (PSA) following the hyphal tip (*Fusarium* spp.) and the morphological characters mentioned (Nelson *et al.*, 1983; Summerell *et al.*, 2003). The same fungal isolates were also identified using polymerase chain reaction (PCR) and nucleotide sequencing, according to the method described below.

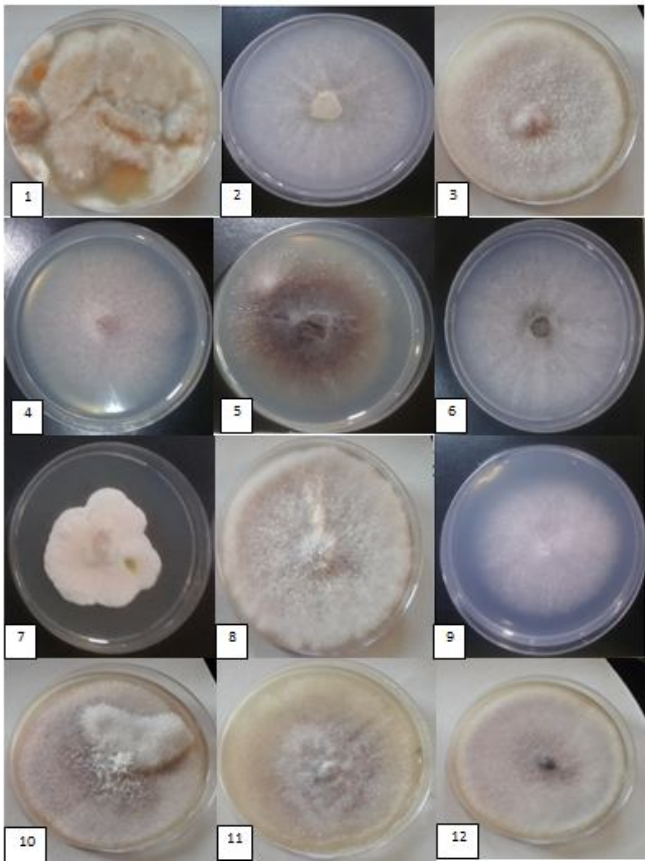

Fig. 1: The color and shapes of *Fusarium* spp. isolates on PDA.

• Molecular diagnosis of fungus isolates *Fusarium* spp. DNA extraction: The DNA was extracted for fungal isolates and was prescribed by Zymo Research and used by (Cat. No. D6005).

• Polymerase chain reaction (PCR): The polymerase reaction test was performed using the Maxime PCR PreMix (i-Taq), Cat. No. 25026) processed by the Korean company iNtRoN. The total polymerase chain reaction with a total volume of 20 microliters containing 1 µl of TCCGTA GGTGAACCTGCGG: ITS1 and TCCTC CGCTTA (TTGATATGC: TS4) and 1 µl of extracted DNA were carried out. All the above components are placed in the tubing fitted by the manufacturer and completed in water (Nuclease-free water) to 20 microliters. The DNA of *Fusarium* spp. isolates was doubled using the following steps and conditions of polymerase chain reaction (PCR): Initial denaturation of DNA for 5 minutes At 98°C followed by 35 cycles of final denaturation for 40 seconds at 94°C, primer annealing for 40 seconds at 55°C and initial elongation, (PCR-amplified product) for 1 min at 72°C and finalizing PCR by final elongation at 72°C (16°C). Add 10 microliters of DNA multiplied by polymerase chain reaction (PCR) to each well of the pre-prepared cacao gel layer. Five microliters of DNA (1Kb DNA ladder marker) were added to the hole on the left side of the added samples to determine the size of the DNA. Connect the power supply electrodes to the

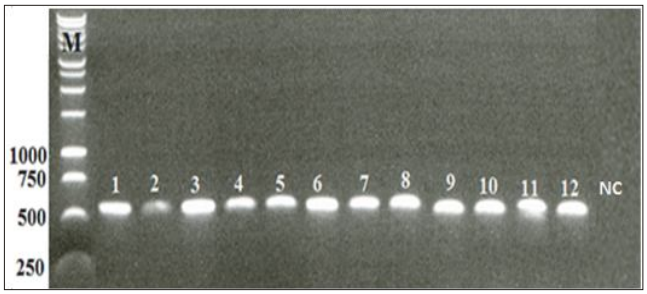

Fig. 2: DNA products amplified by polymerase chain reaction (PCR) from *Fusarium* spp. isolates (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12) isolated from imported Banana fruits into Iraq.

Table 1: Determination of the proportions and genetic variation of *Fusarium* spp. isolates isolated in this study from imported banana fruits.

												Isolates of *Fusarium* spp.
											-	*F. equiseti* (1)
										-	91	*F. proliferatum*(2)
									-	94	94	*F. circinatum* (3)
								-	94	97	90	*F. oxysporum* f.sp. *cubense* (4)
							-	94	99	94	95	*F. verticillioides* (5)
						-	94	97	94	100	91	*F. proliferatum* (6)
					-	75	71	73	71	75	68	*F. oxysporum* f.sp. *ciceris* (7)
				-	90	81	77	76	76	81	72	*F. verticillioides* (8)
			-	80	75	99	95	98	94	99	90	*F. proliferatum* (9)
		-	91	76	71	91	96	90	95	91	92	*F. verticillioides* (10)
	-	96	95	77	71	94	100	93	99	94	95	*F. verticillioides* (11)
-	99	95	94	76	29	93	99	93	99	93	95	*F. verticillioides* (12)
F. verticillioides (12)	*F. verticillioides* (11)	*F. verticillioides* (10)	*F. proliferatum* (9)	*F. verticillioides* (8)	*F. oxysporum* f.sp. *ciceris* (7)	*F. proliferatum* (6)	*F. verticillioides* (5)	*F. oxysporum* f.sp. *cubense* (4)	*F. circinatum* (3)	*F. proliferatum*(2)	*F. equiseti* (1)	

power supply and operate at 150 mA for one hour. After the specimen completes, the cacao layer containing the DNA transcripts (UV transillumination) was examined and taken.

• Analysis of the sequence of DNA bases of pathogenic fungi: PCR amplifiers were fed by *Fusarium* spp. isolates by polymerase chain reaction (PCR) with ITS1 and ITS4 to Korean Macrogen for the sequencing of the nucleotide sequence and the front and rear directions of the double-stranded DNA All the sequences of the nitrogen bases were analyzed using the Basic Local Alignment Search Tool (BLAST) to compare them with the data available at the National Center for Biotechnology Information (NCBI), which belongs to the internationally recognized *Fusarium* spp.

Results and Discussion

• Isolation and diagnosis of isolates of *Fusarium* spp.: Twelve of imported banana fruit samples were isolated from *Fusarium* Spp. The samples across border crossings. These isolates were diagnosed based on the general characteristics mentioned (Nelson *et al.*, 1983; Summerell *et al.*, 2003), of which (Fig. 1).

The results of DNA extraction from these fungal isolates and exposure to polymerase chain reaction (PCR) showed the possibility of multiplying the DNA (DNA products) and different sizes ranged between 750-500 pairs of nitrogen base (bp) and using the front and rear buds (ITS1 and ITS4) (Fig. 2).

The results of the Nucleotide sequence analysis of the double nucleic acid isolates of isolated samples and

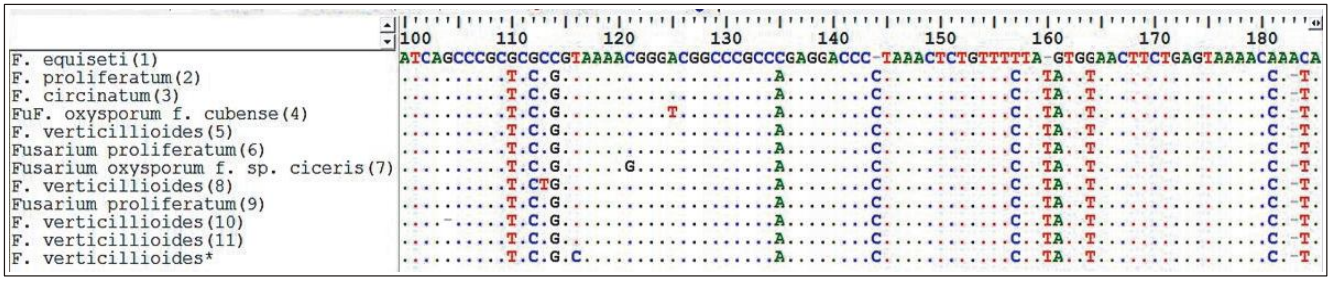

Fig. 3: Some similarities and differences in some areas of nucleotide sequencing alignments produced by PCR product amplified from *Fusarium* spp. isolates. Identical bases are represented by dots. The numbers on the right side of the name of the fungus represent the sequences of the nitrogen bases of the DNA products of the imported Banana fruits samples.

Table 2: Comparison among the similarity percentage of *Fusarium equiseti* isolates (1) isolated in this study from imported banana fruits with the other isolates of the same fungus previously registered at NCBI.

Fungus	Isolate name	Origin	The most similar sequences in GenBank database	
			Gen Bank Accession Number	Sequence similarity (%)
*F.equiseti**	Jubair1	Iraq	MN093942	100
F.equiseti	CBS307.94	Netherland	MH862468	98
F.equiseti	MRC2330	USA	MH582462	98
F.equiseti	MRC 1892	USA	MH582461	98
F.equiseti	MRC 1891	USA	MH582460	98
F.equiseti	05005	Switzerland	MG274305	98
F.equiseti	D20-2	China	KY365574	98
F.equiseti	D25-1	China	KY365564	98
F.equiseti	BRIP:64448	Australia	KU529157	98
F.equiseti	NRRL26419	USA	NR121457	98
F.equiseti	NRRL20697	USA	GQ505683	98
F.equiseti	HGUP17361.3	China	MK069606	98
F.equiseti	CBS185.34	Netherland	MH855481	98
F.equiseti	CBS107.07	Netherland	MH854570	98
F.equiseti	Z331	Poland	KP264661	98
F.equiseti	QH100	China	JF911784	98
F.equiseti	F106	Canada	JX534311	98
F.equiseti	F003	Canada	JX534247	98
F.equiseti	72L	Poland	KF889098	98
F.equiseti	CRW1	India	MK075026	97
F.equiseti	GKF38	Ghana	MK713438	97
F.equiseti	BN6	China	MH483995	97
* Insulation of *Fusarium equiseti* (1) isolates in this study.				

BLAST showed that all *Fusarium* spp. fungal isolates showed isolated genetic isolates in this study with distinct genetic differences in some areas of nucleotide sequencing alignments (Fig. 3 and Fig. 4) as well as among other isolates belonging to the same fungus registered at the National Biotechnology Information Center (NCBI).

The results of the Nucleotide sequence analysis of the double nucleic acid isolates from the isolated fungus isolates using BLAST showed that all fungal isolates of *Fusarium* spp. showed isolated isolates in this study. Genetic differences in the proportions and differences in nucleotide sequences sequencing analysis. The genotype of the genetic analysis showed clear differences in the similarity and variability of the isolates of *Fusarium* spp. where the isolating tree divided the isolates into two main groups. The first group included isolates, except for isolates 7 and 8, (11, 12, 3 and 5), which gave high ratios between 99 and 100%, which in turn gave different ratios of 94 to 95% with isolation No. 1 and showed the lowest ratios in this group with isolation No. 10, given rates ranged between 92-96% with all the isolates mentioned above. (Table 1). While the second major group, which included isolates 7 and 8, gave a similarity of 90% between the

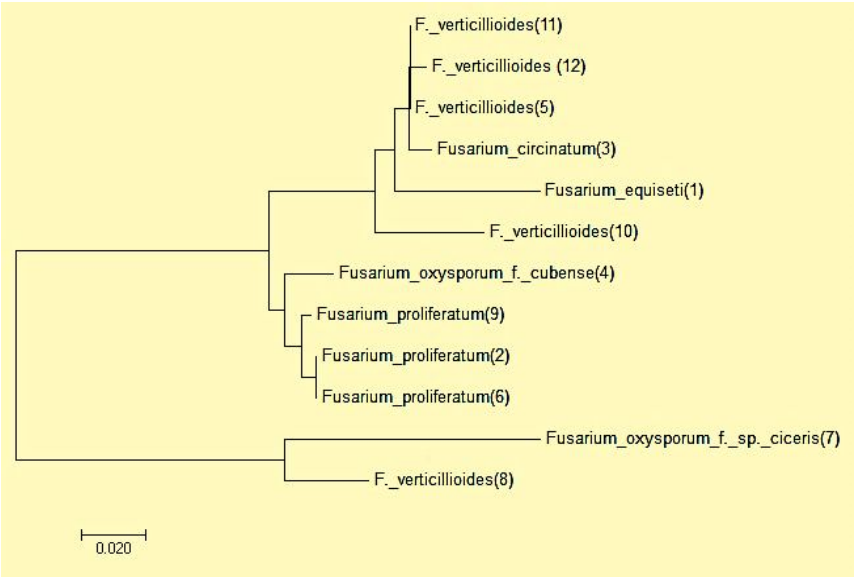

Fig. 4: A phylogenetic tree showing the genetic relationship among the isolates of *Fusarium* spp isolated in this study from the imported Banana fruits.

Table 3: Comparison among the similarity percentage of *F. proliferatum* isolates (2) isolated in this study from imported banana fruits with the other isolates of the same fungus previously registered at NCBI.

Fungus	Isolate name	Origin	The most similar sequences in GenBank database	
			Gen Bank Accession Number	Sequence similarity (%)
*F. proliferatum**	Jubair2	Iraq	MN121160	100
F. proliferatum	PHVNO21	Philippines	MK252904	99
F. proliferatum	81L	Poland	MN018760	99
F. proliferatum	36L	Poland	MN018759	99
F. proliferatum	M5	India	MK751710	99
F. proliferatum	CCH	Malaysia	MK685139	99
F. proliferatum	OL4	Malaysia	MK685138	99
F. proliferatum	CM	China	MK644026	99
F. proliferatum	Z38	China	MH558052	99
F. proliferatum	181	China	MK590321	99
F. proliferatum	FMB-CP1-FR	Pakistan	MK583509	99
F. proliferatum	G small	USA	MK299989	99
F. proliferatum	F small	USA	MK299988	99
F. proliferatum	E small	USA	MK299987	99
F. proliferatum	D small	USA	MK299986	99
F. proliferatum	TN1345OM17	Tunisia	MH329789	99
F. proliferatum	JUF0040	Bangladesh	MK446840	99
* Isolation of *F. proliferatum* (2) isolates in this study.				

two isolates, with a clear difference from the other isolates. (Table 1).

It was also observed by comparing the sequence of the nitrogen bases of *Fusarium equiseti* (1) registered with (MN093942) with the data available at the National Center for Biotechnology Information (NCBI). The highest genetic predisposition (98%) was associated with most *F. equiseti* isolates from the Netherlands, the United States, Switzerland, Canada and China, while most of them were genetically separated from *F. equiseti* isolates isolated from India, Ghana and China (MK075026, MK713438 and MK713438, respectively) with a similarity of 97% (Table 2).

The results shown in table 3, show that the isolation of *Fusarium proliferatum* (2) registered (MN121160) gave 99% genetic heterogeneity with the isolates of *F. proliferatum* recorded at the National Center for Biotechnology Information (NCBI) and isolated from different countries Philippines and Poland India, Malaysia, China, Pakistan, the United States, Tunisia and Bangladesh.

Showed the isolation of fungi *F. oxysporum f. sp. cubense* (4) registered (MN121161) similarity genetically modified to 99% with the isolation of fungi *F. oxysporum f. sp. Cubense* Isolated in Pakistan (MF630984). As the proportions of similarity between isolated isolates in this study and other isolates recorded globally between 98-97% (Table 4).

It was also observed by comparing the sequence of

Table 4: Comparison among the similarity percentage *F. oxysporum* f. sp. *cubense* isolates (4) isolated in this study from imported banana fruits with the other isolates of the same fungus previously registered at NCBI.

Fungus	Isolate name	Origin	The most similar sequences in GenBank database	
			Gen Bank Accession Number	Sequence similarity (%)
F. oxysporum f. sp. *Cubense**	Jubair4	Iraq	MN121161	100
F. oxysporum f. sp. *cubense*	A51	Pakistan	MF630984	99
F. oxysporum f. sp. *cubense*	A60	Pakistan	MF540567	98
F. oxysporum f. sp. *cubense*	A52	Pakistan	MF630985	98
F. oxysporum f. sp. *cubense*	A14	Pakistan	MF540558	98
F. oxysporum f. sp. *cubense*	A43	Pakistan	MF630978	97
F. oxysporum f. sp. *cubense*	A39	Pakistan	MF630975	97
F. oxysporum f. sp. *cubense*	A32	Pakistan	MF630971	97
F. oxysporum f. sp. *cubense*	A13	Pakistan	MF540559	97
* Isolation of fungi *F. oxysporum f. sp. cubense* (4) isolated in this study.				

Table 5: Comparison among the similarity percentage of *Fusarium verticillioides* isolates (5) isolated in this study from imported banana fruits with the other isolates of the same fungus previously registered at NCBI.

Fungus	Isolate name	Origin	The most similar sequences in GenBank database	
			Gen Bank Accession Number	Sequence similarity (%)
*F. verticillioides**	Jubair5	Iraq	MN121533	100
F. verticillioides	HS17	China	KY426418	99
F. verticillioides	AYMA3	Egypt	MK643346	99
F. verticillioides	SWTPF34	India	MG840709	99
F. verticillioides	SWTPF16	India	MG840691	99
F. verticillioides	APBSWTPF69	India	MG569624	99
F. verticillioides	KNCLF2	India	MH591464	99
F. verticillioides	ATS105	India	MF411134	99
F. verticillioides	5005	Switzerland	MG274298	99
F. verticillioides	CM1	Canada	MG515226	99
F. verticillioides	A106	India	KY436182	99
F. verticillioides	M03S C1	USA	KX681583	99
F. verticillioides	H02S C2	USA	KX681582	99
F. verticillioides	DET-51	Brazil	KX385056	99
F. verticillioides	DET-3	Brazil	KX385055	99
F. verticillioides	TVD Fungal-culture 137	Canada	KF494135	94
F. verticillioides	TVD Fungal-culture 136	Canada	KF494134	94

* *Fusarium verticillioides* (5) isolates isolated in this study.

the nitrogen bases of the *Fusarium verticillioides* (5) recorded in data available at the National Center for Biotechnology Information (NCBI) under the entry number (MN121533). The highest percentage of similarity (99%) was with most Isolates isolated from China, Egypt, India, Switzerland, Canada and the United States. The

Table 6: Comparison among the similarity percentage of *Fusarium proliferatum* isolates (6) isolated in this study from imported banana fruits with the other isolates of the same fungus previously registered at NCBI.

Fungus	Isolate name	Origin	The most similar sequences in GenBank database	
			Gen Bank Accession Number	Sequence similarity (%)
*F. proliferatum**	Jubair6	Iraq	MN132885	100
F. proliferatum	Strain 10	India	KJ847729	98
F. proliferatum	KTSR 9	Srilank	KF013253	98
F. proliferatum	A Small	USA	MK299983	98
F. proliferatum	B Small	USA	MK299984	98
F. proliferatum	D Small	USA	MK299986	98
F. proliferatum	G Small	USA	MK299989	98
F. proliferatum	KUYZ001B-2-7	China	MH517439	98
F. proliferatum	KUYZ015B-5	China	MH520726	98
F. proliferatum	KUYZ023B-3	China	MH547107	98
F. proliferatum	SWM2	Iraq	LC384878	98
F. proliferatum	XPSZX	China	MH548896	98
F. proliferatum	FMB0158	Pakistan	MK372915	98
F. proliferatum	ZXY5	China	MK158221	98
F. proliferatum	ZXY6	China	MK158224	98
F. proliferatum	E Small	USA	MK299987	98
F. proliferatum	FMB-CP1-FR	Pakistan	MK583509	98
F. proliferatum	Z38	China	MH558052	98
F. proliferatum	CM	China	MK644026	98
F. proliferatum	M5	India	MK761710	98
F. proliferatum	PC91	Iran	MK765917	98

* Isolation of *F. proliferatum* (6) isolates in this study.

Table 7: Comparison among the similarity percentage of *F. oxysporum* f. sp. *ciceris* isolates (7) isolated in this study from imported banana fruits with the other isolates of the same fungus previously registered at NCBI.

Fungus	Isolate name	Origin	The most similar sequences in GenBank database	
			Gen Bank Accession Number	Sequence similarity (%)
F. oxysporum f. sp. *Cubense**	Jubair4	Iraq	MN121161	100
F. oxysporum f. sp. *ciceris* *	Jubair7	Iraq	MN121544	100
F. oxysporum f. sp. *Ciceris*	Foc50	India	JF727559	99
F. oxysporum f. sp. *Ciceris*	Foc49	India	KP295936	96
F. oxysporum f. sp. *Ciceris*	Foc48	India	KP295935	96
* Isolation of fungi *F. oxysporum f. sp. ciceris* (7) isolated in this study.				

lowest genetic similarity (94%) was found with isolation isolates isolated from Canada (TVD Fungal-culture 137 and TVD Fungal-culture 136) (Table 5).

The results showed that the isolated isolates *Fusarium proliferatum* (6) registered (MN132885 (gave the genetic similarity of the recorded isolation with isolates belonging to the same fungi recorded in the National Center for Biotechnology Information (NCBI) were 98% among isolates isolated from India (KJ847729 and MK761710). Sri Lanka (KF013253), United States (MK299983, MK299984, MK299986, MK299987, MK299989), China (MH517439, MH520726, MH547107, MH548896, MK158221, MK158224, MH558052 and MK644026). (Table 6).

The results Showed the isolation of fungi *F. oxysporum f. sp. ciceris* (7) registered (MN121544) similarity genetically modified to 99% with the isolation of fungi *F. oxysporum f. sp. ciceris* previously registered in India (JF727559), while the lowest proportion was 96% with previously registered fungal isolates in India (KP295936 and KP295936) (Table 7).

Also observed by comparing the sequence of the nitrogen bases of the *F.vericillioides* (8) registered (MN123531) with the data available at the National Center for Biotechnology Information (NCBI), the highest genetic similarity was observed with isolation of isolated *F. verticillioides* from India With a similarity of 97%, while it differed from other isolates of the same fungus *F. verticillioides* and by a similarity of 96% (Table 8).

The results shown in table 9, show that the isolates of *F. verticillioides* (10) registered (MN121577) were the most closest genetically to isolates *F. verticillioides* Chinese (MF063030, MN121060, MF063031), Egyptian (MK450468, MF373436) and the Indian (MH591464 and

Table 8: Comparison among the similarity percentage of *F.vericillioides* isolates (8) isolated in this study from imported banana fruits with the other isolates of the same fungus previously registered at NCBI.

Fungus	Isolate name	Origin	The most similar sequences in GenBank database	
			Gen Bank Accession Number	Sequence similarity (%)
*F.verticillioides**	Jubair8	Iraq	MN123531	100
F.verticillioides	FM5	India	HQ995664	97
F.verticillioides	TED39	India	JQ894497	96
F.verticillioides	PMC22	China	MH981594	96
F.verticillioides	PMP23B	China	MH933695	96
F.verticillioides	YP15A	China	MH933688	96
F.verticillioides	NIHHS402	Korea	KY555003	96
F.verticillioides	CBS127178	USA	MH864460	96
F.verticillioides	BH22	China	MH712166	96
F.verticillioides	BIONCLF13	India	MH681583	96
F.verticillioides	238	Iran	MF351534	96
F.verticillioides	Lyc31	China	KX013201	96
F.verticillioides	AS19	Iran	KX761893	96
F.verticillioides	ZJtyt	China	KX119132	96
F.verticillioides	ST-R-28	China	KU258729	96
F.verticillioides	OSMSS	India	KR017037	96
F.verticillioides	MP02	India	KT598349	96
F.verticillioides	OCTJ	China	KR183784	96
* Isolated fungi *F.verticillioides* (8) isolated in this study.				

Table 9: Comparison among the similarity percentage of *F. verticillioides* (10) isolated in this study from imported banana fruits with the other isolates of the same fungus previously registered at NCBI.

Fungus	Isolate name	Origin	The most similar sequences in GenBank database	
			Gen Bank Accession Number	Sequence similarity (%)
*F. verticillioides**	Jubair10	Iraq	MN121577	100
F. verticillioides	S8	China	MF063030	95
F. verticillioides	QN0629	China	MN121060	95
F. verticillioides	Gm2	Egypt	MK450468	95
F. verticillioides	KNCLF2	India	MH591464	95
F. verticillioides	Z-Kh-F4	Egypt	MF373436	95
F. verticillioides	ATS105	India	MF411134	95
F. verticillioides	C3	China	MF063031	95
F. verticillioides	BRM051205	Brazil	MK987187	94
F. verticillioides	BRM051204	Brazil	MK987186	94
F. verticillioides	NJAU 1012	China	MK881639	94
F. verticillioides	Fv1	Iran	MK790136	94
F. verticillioides	171779	Mexico	MK174969	94
F. verticillioides	CF2	Taiwan	MK649933	94
F. verticillioides	CF1	Taiwan	MK649932	94
F. verticillioides	AYMA3	Egypt	MK643346	94
F. verticillioides	YC-IK8	China	MK517965	94
* The isolation of *F. verticillioides* (10) isolates in this study.				

Table 10: Comparison among the similarity percentage of *F. verticillioides* (12) isolated in this study from imported banana fruits with the other isolates of the same fungus previously registered at NCBI.

Fungus	Isolate name	Origin	The most similar sequences in GenBank database	
			Gen Bank Accession Number	Sequence similarity (%)
*F. verticillioides**	Jubair12	Iraq	MN121630	100
F. verticillioides	AYMA3	Egypt	MK643346	99
F. verticillioides	SWTPF34	India	MG840709	99
F. verticillioides	SWTPF16	India	MG840691	99
F. verticillioides	APBSWTPF69	India	MG569624	99
F. verticillioides	5005	Switzerland	MG274305	99
F. verticillioides	CM1	Canada	MG515226	99
F. verticillioides	A106	India	KY436182	99
F. verticillioides	M03S C1	USA	KX681583	99
F. verticillioides	H02S C2	USA	KX681582	99
F. verticillioides	DET-51	Brazil	KX385056	99
F. verticillioides	DET-3	Brazil	KX385055	99
F. verticillioides	PF	India	KP307911	99
F. verticillioides	IHEM 9835	Italy	KP132245	99
F. verticillioides	QN0629	China	MN121060	99
F. verticillioides	QZ1906	China	MN049928	99
F. verticillioides	KNCLF2	India	MH591464	99
F. verticillioides	ATS105	India	MF411134	99
F. verticillioides	HS17	China	KY426418	99
F. verticillioides	A105	India	KX607113	99
F. verticillioides	CBPPR0048	Brazil	KT211540	99
F. verticillioides	TVD Fungal-culture 137	Canada	KF494135	99
F. verticillioides	TVD Fungal-culture 136	Canada	KF494134	99
* The isolation of *F. verticillioides* (12) is isolated in this study.				

MF411134) by 95%. The previously identified isolates F. verticillioides showed a 94% sequestration of NBI isolates with isolates *F. verticillioides* isolated in this study.

The results shown in table 10, show that the isolates of *F. verticillioides* (12) recorded (MN121630) showed a genetically identical phenotype of 99% with each of the *F. verticillioides* isolates previously identified in Egypt (MK643346) and India (MG840709, MG840691, MG569624 and The United States (KX681583 and KX681582), Brazil (KX385056, KX385055 and KT211540), Italy (KP132245), China (MN121060, MN049928 and KY426418).

The results of this study conclude that all *Fusarium* spp. isolates were genetically different among them. The results showed that nine of these isolates were previously unregistered at the National Center for Biotechnology Information (NCBI), so they were registered under entry numbers MN093942, MN121160, MN121161, MN121533, MN132885, MN121544, MN123531, MN121577 and MN121630. It was also observed that the recorded isolates showed a high genetic correlation with previously registered isolates at the National Center for Biotechnology Information (NCBI), indicating that the entry of some agricultural products as banana fruits through the border crossing points is a source of entry and spread of many dangerous pathogens Including Fusarium spp., which is included in this study With different genetically modified isolates, which may be highly pathogenic and produce dangerous fungal toxins that are harmful to human health. In this study, polymerase chain reaction (PCR) was used to diagnose isolates of *Fusarium* spp., which is known for its high accuracy in the diagnosis of many organisms, including fungus *Fusarium* spp. and *Aspergillus* spp. and others (Huang *et al*., 2016) to get rid of diagnostic problems based on morphological characters. Despite the usefulness of phenotypic diagnosis in the detection of fungi under study in smaller groups before the introduction of other methods of diagnosis, there are many problems that accompany the diagnosis of phenotypic fungi, we need the diagnosis to the experience of high, especially in the fungal species close similar among them, such as some types of fungus Fusarium spp. As well as the need for considerable time and effort (Yang *et al*., 2007, Hsuan *et al*., 2011 and Zhang *et al*., 2012). There are also other factors that affect these phenotypes, such as the type and nature of the medium growth, humidity and lighting, which can affect the color and forms and sizes of spores and fungal colonies developing. Some researchers found that there is an error in the phenotypic classification of many fungi that were identified in previous studies, including Fusarium spp. Such as *Fusarium verticillioides* and *Fuarium subglutinans* when're, it was again diagnosed using polymerase chain reaction (PCR) (Giantsis *et al*., 2017). The Molecular Diagnostics method, based on differences in the trans-spaced DNA sequence, has been highly effective in diagnosing many fungi such as *Fusarium* spp. and *Cladosporium* spp. and *Fusarium verticillioides* (Chandra *et al*., 2008; Hsuan *et al*., 2011; Arif *et al*., 2012 and Alhussaini *et al*., 2016). Accurate diagnosis of pathogenic fungi is a necessary need to access an effective method or methods in disease management, quarantine purposes to protect agricultural crops and other natural resources. (Stanis *et al*., 2016).

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