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Molecular Identification of Isolates of *Ustilago Maydis* (Dc.) Corda the Causal Common Smut in Maize

J. M. Abed,^{1*} and S. H. Samer²

¹College of Agriculture , University of Anbar, Iraq

²College of Agriculture, University of Baghdad, Iraq

*Corresponding author's e-mail: ag.jasim.mahmood@uoanbar.edu.iq

Abstract. Aim of study was to isolate and identify *U. maydis* races that cause common smut disease using molecular methods. In addition to detect phylogenetic relation among isolates of local races and global races using Polymerase Chain Reaction (PCR) through phylogenetic tree using Internal Transcribed Spacer(ITS). DNA was extracted from 50 isolates of *U.maydis* and detected 34 bands from the isolates using gel electrophoresis including (8 Baghdad, 5 Babylon , 14 Waset and 7 Erbil) .Results revealed that detection of isolates was on ITS region and DNA diagnostic was detected at 650 base pair(bp) of all the isolates using ITS1 and ITS4 primers. Also the study included determination of sequencing the nitrogen bases for the isolates. Results indicated that Baghdad isolates of (Um 21,Um 22, Um23 , Um24, Um25, Um26, Um27, Um28) have relative relation with global race of FBI.. While Babylon isolates (Um 5, Um 6, Um7, Um8, Um 10) were had close relation with race 521. And isolates of Waset showed a variation in relationship of a number of global races, Isolate Um41 was closely related to 521, and isolates Um32, Um47, Um48 were closely to the race XA0609, and isolates Um30, Um33, Um36, Um37, Um38, Um43, Um44, Um46, Um49 they are relevant to the races CPO10.020a. However, isolates of Erbil (Um11, Um 12, Um 14, Um15, Um 16 , Um 17, Um19) were in relation with race FB1. Results of genetic origin demonstrated that of Baghdad ,Babylon ,Waset and Erbil have close relation with global FB1, 521 , CPO10.020a , XA0609 which has a1b1 genotype).

1. Introduction:

Maize, *Zea mays* L., is one of the important cereal crops, and it ranks third after wheat and rice crops in terms of cultivated area and global production, .Thus, it is an important food source for humans and animals [1]. Recently cultivation of this crop has expanded which has been accompanied by a large spread of pests. Among the most important diseases is the common smut caused by the prevalent *Ustilago maydis*, that can be observed wherever the maize corn crop is growing, it this disease may causes results in losses of up to 40% [2]. In a previous reports , the common disease caused a yield loss reaches 15% ([3]. The disease spreads quickly in all areas of maize fields and causes losses ranging between 4-40%, depending on the fungus races, the variety sensitivity, and the time and location of the infection.. This disease affects corn maize, sweet corn and Teosinte plant and is called Common smut, Biol smut or Blister smut [4]. As for [5], it has been found that this disease causes a great loss to corn when infected with Spaniel, as it cannot be used for food or oil production. The seasonal losses in the United States of America with this disease are estimated at 1.24 billion US dollars. The fungus has a number of strains that differ in their pathogenicity according to the different genes that control the compatibility between opposite



or compatible fungal hyphae forming diploid fungal strands [6]. The genotype-based diagnosis requires highly sensitive techniques and accurate linking between even the genetically close groups, on the other hand traditional approaches are inefficient and less. The ITS (Internal Transcribed Spacer) between conservative ribosomal genes especially the servative rRNA gene is more suitable for diagnosing species and strains. The ITS interfacial region is highly conservative due to the few evolutionary constraints and thus it is used with successfully in distinguishing species within the same genus of fungi [7]. The ITS sequences falls between the minor and major subunits of rDNA and the non-cloned NTS (Non transcribed subunits spacer) that separates ribosomal gene sets with significant inter-species variability as opposed to rRNA genes that are subject to evolutionary restrictions and thus are less conservative.. The remarkable development in plant pathology is mainly associated with molecular technique[8]. The technology is based on PCR, to amplify specific domains in the DNA as each organism has a unique fingerprint distinguishes it from other organisms. The emergence of molecular technologies through which led to a significant improvement in the sensitivity and specificity of microbiology diagnostics.. These methods have been successfully used in the molecular diagnosis of fungi [9] This study was suggested to Determining the fungus races present in central and northern Iraq using molecular methods.

2. Materials and Methods

2.1 Sample collection

Samples of maize plants showing symptoms disease were collected from maize fields in the governorates of Baghdad, Wasit, Babylon and Erbil during autumn season of 2014. A hundred samples collected from (Baghdad 30 samples, Babylon 20 samples, Wasit 30 samples, Erbil 20 samples). The pathological symptoms appearing on the a ranis for this purpose, and the samples that were collected were placed in paper bags kept at room temperature for conducting subsequent tests.

2.2 DNA extraction of *Ustilago maydis* isolates

DNA was extracted in the laboratories of the Advanced Scientific Company ASCO (Baghdad - Iraq). The DNA was extracted from the Telio spores of the fungus using a standard DNA extraction kit produced by BIO BASIC INC and the Follow protocol was followed literally.

Quantification and qualification of the extracted DNA

The concentration and purity of the extracted DNA was determined prior to the amplification process using a Spectrophotometer, depending on the optical absorption, according to the following equation:

$$\text{DNA purity} = \frac{(260 \text{ nm}) \text{ optical absorption}}{((280 \text{ nm}) \text{ optical absorption})}$$

$$\text{DNA concentration ng} / \mu\text{l} = \text{optical absorption (260 nm)} \times 50 \mu\text{g} / \text{ml}$$

Polymerase Chain Reaction (PCR)

The amplification of ITS was carried using couple ITS-specific primers (ITS1 forward 5-TCC GTA GGT GAA CCT GCG G -3) and (ITS4 as reverse5- TCC TCC GCT TAT TGA TAT GC -3).

The PCR reaction mixture was prepared with a final volume of 50 μl for each sample, following the instructions of the supplied company (Promega - USA).

Agarose Gel Electrophoresis for DNA

The agarose gel prepared in concentration of 1.5% by dissolving 1.5 g of agarose in 1 liter of 1X TBE PRE to observe the products).

ITS sequencing

To determine the sequences of the target ITS in each the isolates, the amplified products were purified and sent to sequenced on ABI machine in Macrogen (company address).

2.3 Data Analyzes

The obtained sequences were BLASTn on NCBI The genetic relationship was estimated using ClustalW2 and MEGA 6 software [10].

3. Results and Discussion:

3.1 DNA isolation results

The DNA of the fungus *Ustilago maydis* was isolated from the teliospores. The DNA of 50 isolates from the pathogenic fungi was obtained. It was extracted and purified. Spectrophotometer, with a wavelength of 280/260 nm, and 5 ml of DNA isolated from each isolate were carried over to a gel agarose at a concentration of 0.8% distributed as follows: Baghdad eight isolates (Um21, Um22, Um23, Um24, Um25, Um26, Um27, Um28). Babylon five isolates solve the following symbols (Um4, Um5, Um6, Um7, Um10). The median of fourteen isolates (Um30, Um32, Um33, Um 36, 37 Um, Um38, Um41, 43 Um, 44 Um, Um45, 46 Um, 47 Um, 48 Um,. Um49) Erbil has seven isolates (Um11, Um12, Um14, Um15, Um16, Um17, Um19).

3.2 Molecular identification of U.maydis races

The results of using the primers ITS1 and ITS4 by adopting the reaction program after following the migration method using a 2% agarose gel and examining the gel under ultraviolet rays showed the presence of clear bands formed as a result of amplification in the isolates pathways, the bands showed clear similarity in the molecular size as the size reached approximately 650 base pairs (bp) Figures (1) and (2).

3.3 PCR amplification

The results of the electrophoresis of 34 isolates Fig. (1) and (2) showed *U.maydis* isolates from different regions of the country that included four governorates: Baghdad (8) isolates, Babylon (5) isolates, Waset (14 isolates), then Erbil (7) isolates, as the results of the interaction process were shown for the DNA strings extracted using the prefixes ITS1, ITS4 targeting the ITS interface area The results of the migration of these isolates on a 2% agarose gel showed the presence of clear bands resulting from the process of replication in the pathways of isolates indicating that the initiator is bound to the complementary sequence of it in the template DNA. The resulting bands showed similarity in the partial size. The volumetric index and this result is similar to the expected size of 650 base pairs (bp) as in Fig. 1, 2. One of the most suitable regions for progeny diagnosis is the ITS inter- region within the rRNA conservative gene This is due to the possibility of phylogenetic analysis of closely related and closely related species, using the ITS1 and ITS4 primers from the 18s rRNA and 28s rRNA ribosomal region, in addition to the fact that the ITS region is highly conservative due to the few evolutionary constraints and therefore this region uses ITS to distinguish the same sex species. And the strains within the genus with high accuracy. These results are consistent with what he found [12 and 13] as this region was used by using the prefix pair ITS1, ITS4 to distinguish the species belonging to the genus *Ustilago*, and both 3 and 17 found that these prefixes were used to distinguish the species of the genus *Ustilago* sp. [6] The primers ITS1 and ITS4 were used to characterize the *U. maydis* races and the obtained results showed that the molecular size of the isolates used in the replication process was 650 base pairs.

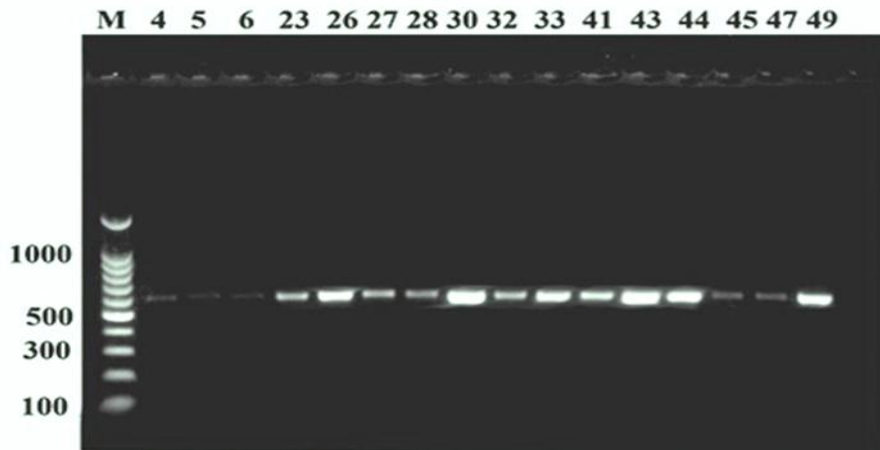


Figure (1) The results of the migration of a replication product of the ITS interconnection of the fungus *U. maydis* on a 2% agarose gel. M: the volumetric index represents 100-1500 base pairs, 4-49 represent the isolates

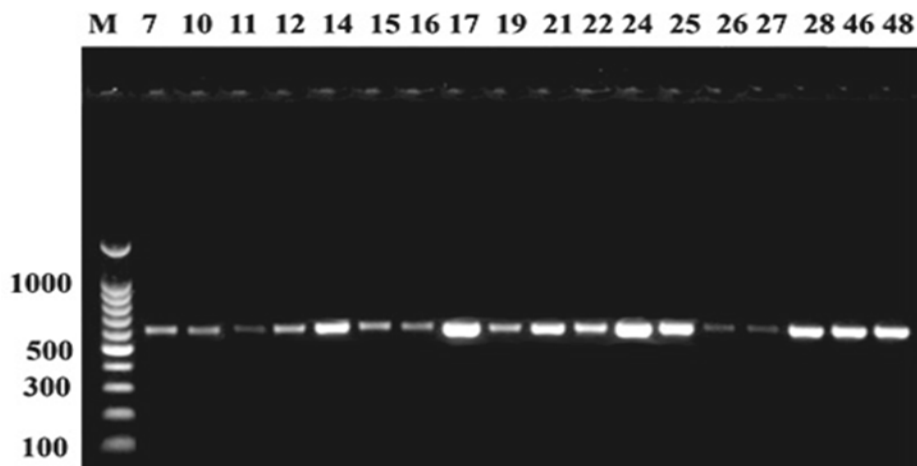


Fig. (2) Results of the migration of a replication product of the ITS intra-zone of the fungus *U. maydis* on a 2% agarose gel. M: the volumetric index represents 100-1500 base pairs, 7-48 represent isolates

3.4 Sequences

The 34 isolates were sent to the Korean company Macrogen to determine the sequence of nitrogenous bases for them, and these isolates bear the following symbols: Baghdad eight isolates, Babylon five isolates, Wasit fourteen isolates, Erbil, seven isolates.

Phylogenetic analysis

Genetic kinship analysis and phylogenetic tree diagramming was carried out by relying on (neighbor-joining) of various isolates of the local *U. maydis* and compared with different strains of the sequence of nitrogenous bases analyzed depending on the ITS interface region, after identifying these strains taken from the National Center for Biotechnology Information. National Center Biotechnology Information (NCBI) and GenBank. The results of the comparison between local isolates and global strains that have been doubled according to the ITS region, whose nitrogenous sequences have been identified, are sequencing to determine the similarities, differences, and degree of relationship between them and the global strains, using the MEGA6 program and the ClustalW2 program connected to the Internet (www). Ebi.ac.uk, and the results of the analysis indicated that clusters formed or formed for

genetically related strains. The results in Fig. 3 showed that Baghdad governorate isolates (Um21, Um22, Um23, Um24, Um25, Um26, Um27, Um28) were close. The link for the FB1 strain corresponds to 90-93%, while the Babylonian isolates (Um4, Um5, Um6, Um7, Um10) were closer to the global strain 521 with a match ratio of 93% (Figure 4). Whereas the isolates of Wasit Governorate showed a clear variation in the degree of their relationship to a number of international strains with a match (86-90%) Figure (8), the isolate Um41 was closely related to the strain 521, while the isolates carrying the symbol Um32, Um47 and Um48 were closer to the XA0609 strain. The isolates Um30, Um33, Um36, Um37, Um38, Um43, Um44, Um46, Um49 were related to the strain CPO10.020a. The results of the analysis (Fig. 5) indicated that Erbil isolates (Um11, Um12, Um14, Um15, Um16, Um17, Um19) were related to the FB1 strain with a match ratio of 93%. It is clear from the mentioned results that the isolates that were tested and analyzed belong to the FB1 strains 521, CPO10.020a, XA0609 and these strains carry the a1b1 genotype (3 and 11). Figure (6) showed the degree of kinship between the local slips under study under study, which represents the results of the analysis of these isolates representing the four studied governorates. The high degree of genetic similarity of the *U.maydis* communities isolated from different regions of Iraq may be due to the emerging development in those strains. Through time, which may be one of the most important reasons for the natural selection of ancestors. Some of the studied isolates showed some type of heterogeneity. Different environments produce new strains, and this leads to evolution and genetic diversity as it appeared in isolates of the pathogen.

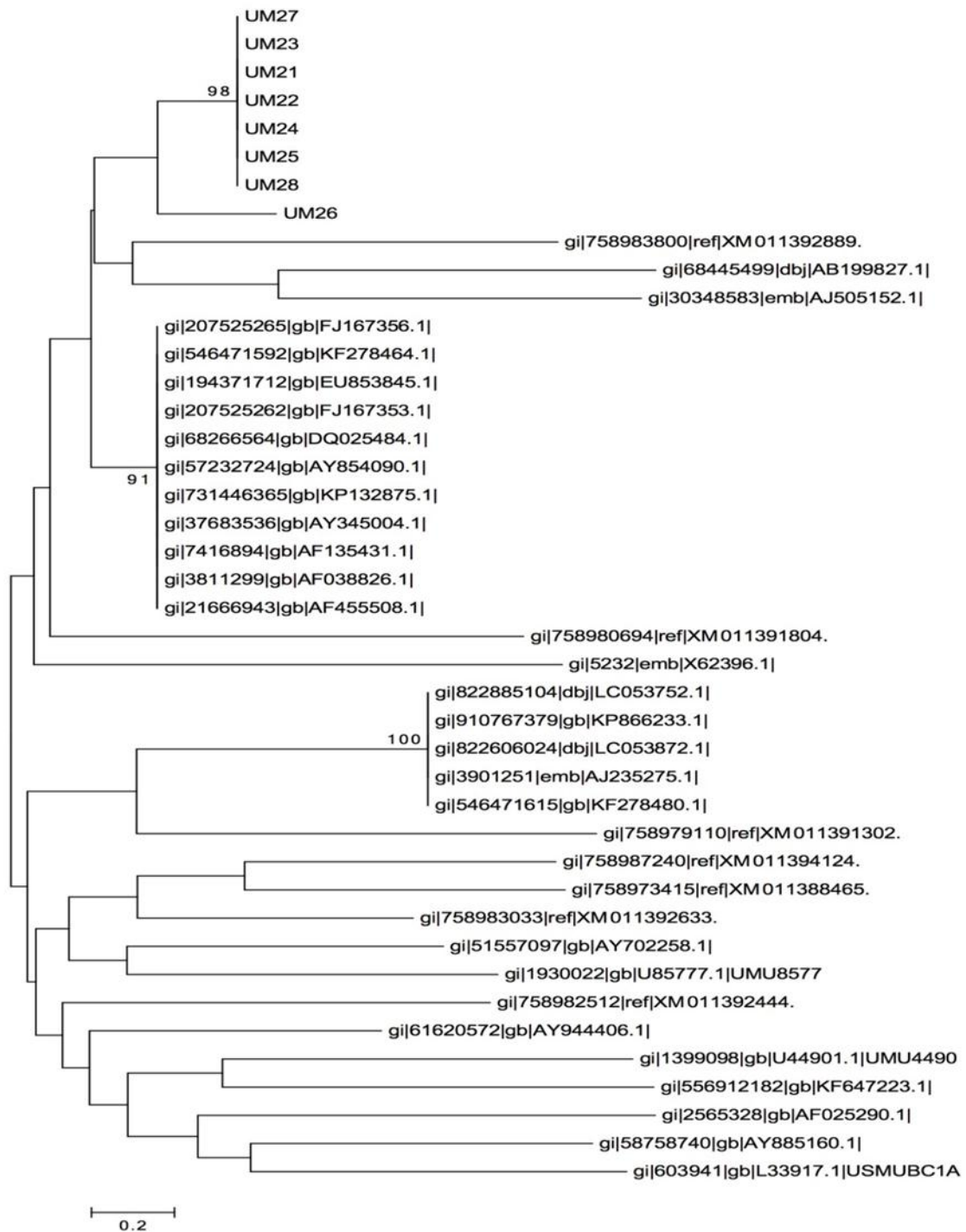


Figure (3) represents the kinship (link) between *U. maydis* isolates in Baghdad governorate and the standard fungus strains available in the genebank. (Um21 - Um28) represents the isolation of Baghdad

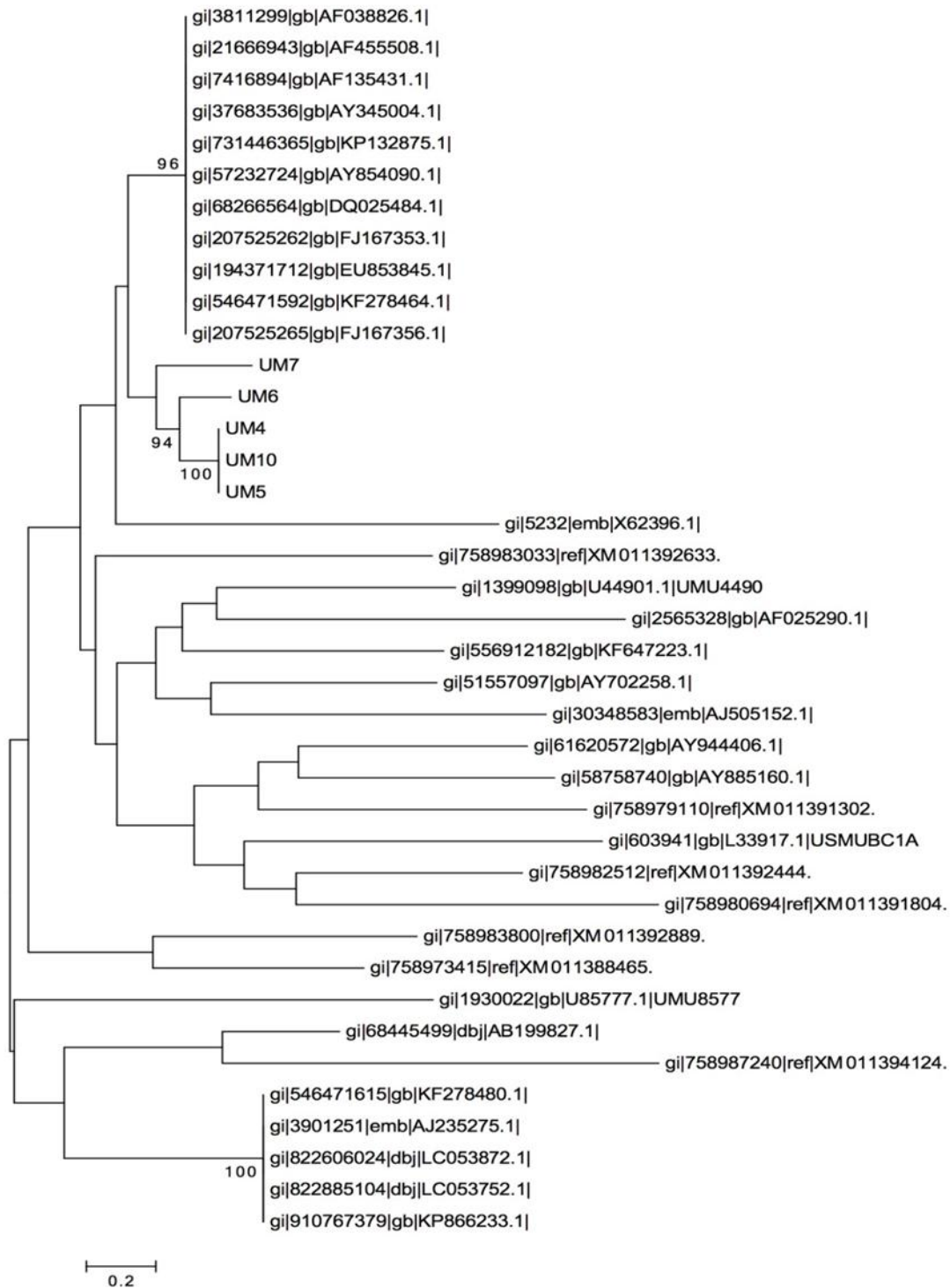


Figure (4) represents the kinship (link) between *U.maydis* isolates in Babylon and the standard fungus strains available in the genebank. Um4 –Um10 represents the isolates of Babylon

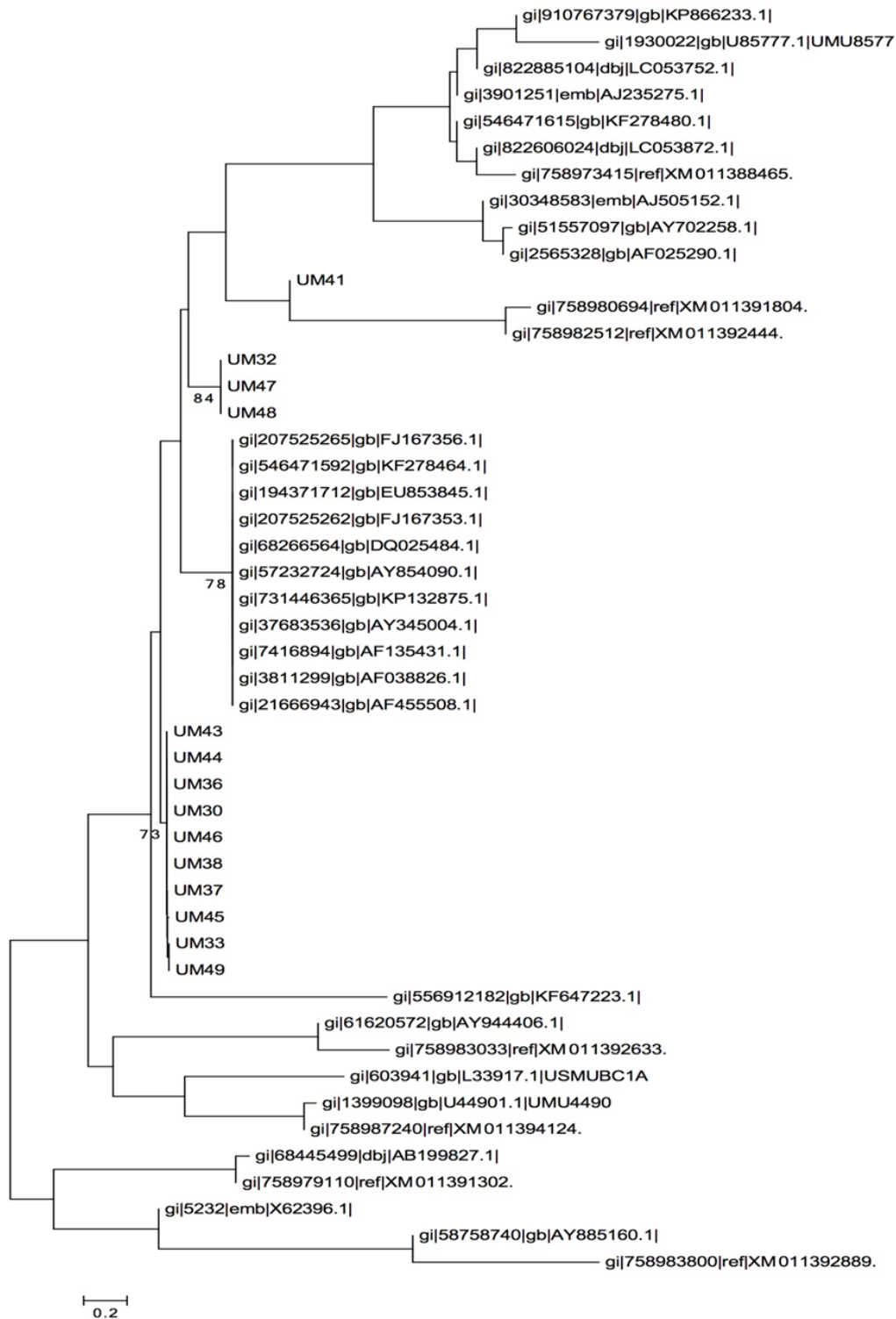


Figure (5) represents the kinship (link) between *U.maydis* isolates in Wasit and the standard fungus strains available in the genebank. (Um30-Um49) were Wasit isolates

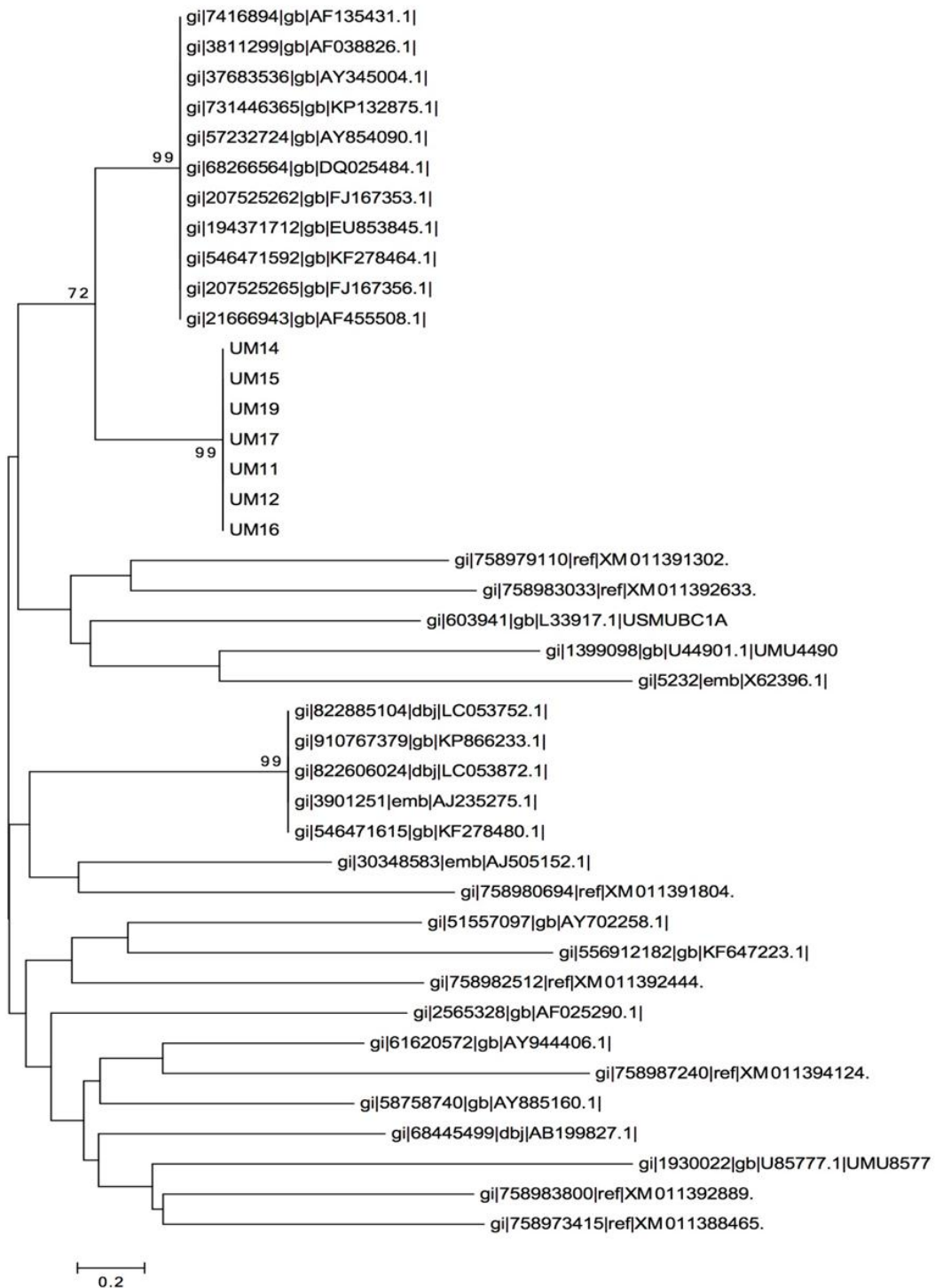


Figure (6) represents the kinship (link) between *U.maydis* isolates in Erbil and the standard fungus strains available in the genebank. As (Um11 - Um19) the isolates of Erbil

4. Conclusion

U.maydis isolates from the governorates of (Baghdad, Babylon, Wasit, Erbil) were genetically close to the global strains FB1, 521, CPO10.020a, XA0609 carrying the genotype a1b1, results of electrophoresis using the ITS The molecular size was approximately 650 base pairs for all *U.maydis* isolates.

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