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Research article

Fusarium species complexes associated with chickpea wilt and stem and root rot in wheat

Complejos de especies de *Fusarium* asociados con la marchitez del garbanzo y la pudrición del tallo y raíz en trigo

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ABSTRACT

In Mexico, chickpea (*Cicer arietinum*) and wheat (*Triticum aestivum*) are two important crops for national consumption and/or exportation. However, they are susceptible to various diseases caused by *Fusarium* fungi. The objective of this research was to identify *Fusarium* species associated with the wheat root and stem rot diseases, and chickpea wilt in the Mexican Bajio area, through DNA sequence analyses of three genomic regions *TEF*, ITS and *mtSSU*. The 114 isolates obtained were classified into eight taxonomic species and located in four *Fusarium* Species Complexes. In order of frequency, *F. oxysporum* (87.6%), *F. equiseti* (4.7%), *F. solani* (4.7%), *F. proliferatum* (1.5%) and *Fusarium* sp. (1.5%) were identified in chickpea; and *F. proliferatum* (44%), *F. nygamai* (22%), *Fusarium* sp. (16%), *F. thapsinum* (6%), *F. oxysporum* (6%), *F. andiyazi* (4%) and *F. equiseti* (2%) were recovered from wheat. The *TEF* gene had the highest number of phylogenetically informative sites (43.03%), followed by *mtSSU* (32.88%) and ITS (12.82%). Moreover, the phylogenetic reconstruction using *TEF* was the closest to what is currently recognized for *Fusarium* species. The resulted clustering from the concatenation of the ITS+*mtSSU* regions was the most conflicting, while the constructs with concatenated regions involving the *TEF* gene were the least conflicting. The results from this research indicate the presence of a significant number of *Fusarium* species that should be monitored for disease prevention and/or treatment programs in chickpea and wheat crops in Mexico.

Keywords: *Cicer arietinum*, *Fusarium* sp., molecular identification, *Triticum aestivum*

RESUMEN

En México, el garbanzo y el trigo son cultivos importantes para el consumo nacional y/o exportación. Sin embargo, son susceptibles a diversas enfermedades causadas por hongos. El objetivo de esta investigación fue identificar especies de *Fusarium* asociadas a las enfermedades de pudrición de raíz y tallo de trigo y marchitez del garbanzo en la zona del Bajío mexicano, mediante análisis de secuencias de DNA de las tres regiones genómicas *TEF*, ITS y *mtSSU*. Los 114 aislamientos obtenidos se clasificaron en ocho especies y se ubicaron en cuatro Complejos de Especies de *Fusarium*. En orden de frecuencia, se identificaron en garbanzo *F. oxysporum* (87.6%), *F. equiseti* (4.7%), *F. solani* (4.7%), *F. proliferatum* (1.5%) y *Fusarium* sp. (1.5%); y se recuperaron de trigo a *F. proliferatum* (44%), *F. nygamai*

(22%), *Fusarium* sp. (16%), *F. thapsinum* (6%), *F. oxysporum* (6%), *F. andiyazi* (4%) y *F. equiseti* (2%). El gen TEF tuvo el mayor número de sitios filogenéticamente informativos (43%), seguido de *mtSSU* (32.9%) e ITS (12.8%). Además, la reconstrucción filogenética usando TEF fue la más cercana a lo que actualmente se reconoce para las especies de *Fusarium*. El agrupamiento resultante de la concatenación de las regiones ITS+*mtSSU* fue el más conflictivo. Los resultados de la presente investigación indicaron la presencia de un número importante de especies de *Fusarium* que deben ser monitoreadas en programas de prevención y/o tratamiento de enfermedades en cultivos de garbanzo y trigo en México.

Palabras clave: *Cicer arietinum*, *Fusarium* sp., identificación molecular, *Triticum aestivum.*

INTRODUCTION

The *Fusarium* genus is one of the most important groups of phytopathogenic fungi affecting a wide variety of crops throughout the world. Furthermore, several *Fusarium* species synthesize many mycotoxins that affect the quality of food products (Summerell, 2019). Throughout *Fusarium* taxonomic history, three species concepts have been used: morphological, biological and phylogenetic, to differentiate the species within the genus (Leslie *et al*., 2001). These concepts were combined in current research to study *Fusarium* species (Summerell and Leslie, 2011). To date, there is no stable consensus on taxonomy within the genus. At present, around 400 phylogenetically distinct species (phylospecies) are estimated, and more than 80% of these were reported in the last 25 years. Nevertheless, about one third of the species are not extensively described (Summerell, 2019; O'Donnell *et al.*, 2022).

The use of DNA-based methods for the identification of pathogens has become routine during the last two decades. Some of the commonly sequence genomic regions for identification of *Fusarium* species include the translation elongation factor-1α (*tef-1α* or *TEF*), the RNA polymerase 1 and 2 (*RPB1* and *RPB2*), β-tubulin (*tub*) and histone (*his*) genes (O'Donnell *et al*., 2013; Summerell, 2019). Currently, it is recommended to use the *TEF* gene as routine barcoding for *Fusarium* species identification and encourage the *RPB1* and *RPB2* sequences to corroborate that classification (Geiser *et al.*, 2013; Laurence *et al.*, 2015; Summerell, 2019; O'Donnell *et al.*, 2022). As an attempt to simplify the taxonomy within the *Fusarium* genus and derived from the resolving power of DNA-based taxonomy, the terminology of *Fusarium* Species Complexes has been introduced, firstly for the *F. oxysporum* Species Complex (FOSC) (O'Donnell and Cigelnik, 1997). Nowadays, 23 species complexes have been reported (Laurence *et al.*, 2015). The term Species Complex is not formally defined, but generally implies a grouping of species that shared morphological characteristics and phylogenetic markers (O'Donnell *et al.*, 2013).

In Mexico, the chickpea (*Cicer arietinum* L.) and the bread wheat (*Triticum aestivum* L.) are considered strategic crops with different final uses. Wheat production is mainly used for domestic consumption, and a large part of chickpea production is consigned to export.

Chickpea is an important dietary legume that provides a high-quality source of protein. The main producers are India, Australia and Pakistan, and other countries such as. Australia, Mexico and Russia are not large consumers of chickpeas, but are world exporters (Guerrero *et al.*, 2015; Singh and Vyas, 2021). The crop is susceptible to various types of abiotic and biotic stresses. Of the most widespread diseases, chickpea wilt, which occurs in 32 countries from six continents (Singh *et al.*, 2014), is caused by several *Fusarium* species that generate significant losses in production and quality. It has been reported that *F. oxysporum*, *F. redolens* and *F. solani* can infect chickpea plants at any time during their life´s cycle (Singh and Vyas, 2021; Zhou *et al.*, 2021). Moreover, *F. equiseti* has previously been identified infecting chickpeas in Ethiopia, India, Iran, and Canada (Younesi *et al.*, 2021; Zhou *et al.*, 2021); and *F. proliferatum* was found in diseased plant tissues in China, India, Spain, Pakistan, Iran, and other countries (Zhou *et al.*, 2021).

For its part, wheat is one of the most important crops for human consumption. Likewise, it is susceptible to several diseases caused by *Fusarium* species that are found in practically all areas of the world where this species is cultivated (Gilchrist-Saavedra *et al.*, 2005; Liu and Ogbonnaya, 2015). The root, stem and crown rot of wheat, which occur throughout the development cycle of the plant, are considered a serious threat to grain production in various parts around the world; and yield and grain quality losses can be considerable (Liu and Ogbonnaya, 2015). These rotting diseases are of a growing concern in the main wheat-producing areas in Mexico, although they remain poorly studied (Suaste-Franco *et al.*, 2020). The main *Fusarium* species causing root rot in wheat plants worldwide are *F. culmorum*, *F. graminearum*, *F. avenaceum*, *F. acuminatum* and *F. poa*e (Cook, 2010; Shikur-Gebremariam *et al.*, 2018). In Mexico, *F. graminearum*, *F. avenaceum*, *F. equiseti*, *F. proliferatum*, *F. poae*, *F. oxysporum* and *F. moniliforme* were associated with symptoms of root and stem rot, and head blight in crops of wheat (Gilchrist-Saavedra *et al.*, 2005; Leyva-Mir *et al.*, 2017).

From the above, the objective of this research was to identify, at the species level, *Fusarium* strains isolated from chickpea and wheat cultivated in central Mexico through DNA sequence analysis of three genomic regions and its phylogenetic inference.

MATERIALS AND METHODS

Fungal isolation and DNA extraction

A total of 68 *Fusarium* isolates were obtained and identified from wilted chickpea plants cultivated in the states of Guanajuato and Michoacán, Mexico (Luna-Paez *et al.,* 2004). Moreover, 55 isolates of *Fusarium* were obtained from wheat plants with symptoms of root or stem rot from wheat-cultivated areas in Guanajuato (Rangel-Castillo *et al.,* 2017) (Supplementary Table 1). The total DNA was extracted from 500 mg of fungal hyphae by a hexadecetyltrimethylammonium bromide (CTAB) procedure (Doyle and Doyle, 1987).

Amplification and sequencing of *TEF*, ITS and *mtSSU* regions

The *TEF* gene (≈ 800 bp) was amplified with the primers EF-1 (5' ATGGGTAAGGARGACAAGAC 3') and EF-2 (5' GGARGTACCAGRSATCTCATG 3'). The ITS region (≈ 1,100 pb) with the primers ITS-5HP (5' TCCGTAGGTGAACCTGCGC 3') and NL4 (5' GGTCCGTGTTTCAAGACGG 3') and *mtSSU* (≈ 700 pb) with MS1 (5' CAGCAGTCAAGAATATTAGTCA 3') y MS2 (5' GCGGATTATCGAATTAAATAAC 3'). The PCR reaction mix was prepared in a 25 µL final volume containing 200 µM dNTPs, 1U *Taq* DNA polymerase, Promega (USA), 1X buffer, 1.5 mM $MgCl_2$, 10 picomoles of primers, and 100 ng of DNA. The PCR thermocycling for the *TEF* gene consisted of an initial denaturation cycle at 94°C for 5 min, followed by 35 cycles [94°C for 1 min; 62°C for 30 s; 72°C for 1 min] and one final extension cycle of 72°C for 10 min. For the ITS region an initial denaturation cycle was applied at 94°C for 5 min, followed by 35 cycles [95°C for 1 min; 58°C for 1 min; 72°C for 2 min] and 72°C for 10 min of final extension. For the *mtSSU* gene one cycle of 95°C for 5 min was applied, followed by 35 cycles [95°C for 1 min; 52°C for 30 sec; 72°C for 1 min] and 72°C for 10 min of final extension. Amplifications were performed in a GeneAmp® PCR System 9700 thermocycler, Applied Biosystems (Massachusetts, USA). The amplified fragments were separated on 1% agarose and visualized by UV light after ethidium bromide staining.

PCR products sequencing and phylogenetic analyses

Selected products of *TEF*, ITS and *mtSSU* were sequenced in both strands with the same primers used in the PCR reactions. The sequences were edited and consensus sequences were created with BioEdit 7.1.3 (Hall, 1999). All DNA sequences were submitted to the GenBank database (Supplementary Table 1). The nucleotide sequences were aligned using the ClustalW tools implemented in MEGAX (Kumar *et al.,* 2018). For phylogenetic reconstructions, the three genomic regions were analyzed separately and concatenated. The most appropriate nucleotide substitution patterns were chosen using the program implemented in MEGAX (Kumar *et al.,* 2018). Phylogenetic trees were generated by applying the Maximum Likelihood method using MEGAX.

RESULTS

Species identification

The *TEF* gene amplification allowed us to obtain defined amplicons of about 800 bp. A total of 114 TEF sequences were obtained, revealing the identification of the species *F. oxysporum* (59 isolates: 56 from the chickpea root, two from the wheat root, and one from the wheat stem), *F. proliferatum* (23 isolates: 14 from the wheat stem and eight from the wheat root and one isolate from the chickpea root), *F. nygamai* (11 isolates: seven from the stem and four from the root of wheat), *F. thapsinum* (three isolates from the wheat stem), *F. andiyazi* (two isolates from the wheat stem and root), *F. equiseti* (three isolates from the chickpea root and one isolate from the wheat root). Furthermore, three isolates recovered from the chickpea root were identified as *F. solani*, while nine isolates (one isolate form the chickpea root, five from the wheat stem, and three from the wheat root) were not clearly identified at the species level (Table 1).

The most frequent species isolated from chickpea was *F. oxysporum* (87.6%), followed by *F. equiseti* (4.7%), *F. solani* (4.7%), *F. proliferatum* (1.5%) and *Fusarium* sp. (1.5%). Likewise, the most common *Fusarium* species isolated from wheat was *F. proliferatum* (44%), followed by *F. nygamai* (22%), *Fusarium* sp. (16%), *F. thapsinum* (6%), *F. oxysporum* (6%), *F. andiyazi* (4%), and *F. equiseti* (2%) (Table 1).

The ITS region amplifications allowed to verify the presence of defined amplicons of ≈ 1100 bp. A total of 103 isolates were sequenced, revealing the identification of the species *F. oxysporum* (54 isolates: 51 from the chickpea roots, two from the wheat roots and one from the wheat stem), *F. proliferatum* (22 isolates: all from the stem and root of wheat, except one isolate from the chickpea root), *F. nygamai* (nine isolates from the stem and root of wheat), *F. thapsinum* (two isolates from the wheat stem), *F. equiseti* (four isolates from the chickpea roots and one isolate from the wheat root). Furthermore, three isolates recovered from the chickpea root were identified as *F. solani*, while nine isolates (one isolate from the chickpea root, five from the wheat stem, and three from the wheat root) were not clearly identified at the species level (Table 1).

On the other hand, the *mtSSU* amplification allowed us to obtain defined amplicons of ≈ 700 bp. Sequence analysis allowed the recovery of nine sequences that revealed the presence of several *Fusarium* species, although some results were different from those obtained with the ITS and TEF

regions. The species *F. oxysporum* (47 isolates: 43 from the chickpea root, two from the wheat root and two from the wheat stem), *F. proliferatum* (14 isolates: all from the stem and the root of wheat) were identified. Three isolates from the chickpea roots were identified as *F. solani*. In addition, 26 isolates (16 from the wheat stem and 10 from the wheat root), and three isolates from the chickpea root were not clearly recognized at the species level using blast against the NCBI database (Table 1).

Phylogenetic analyses

The *TEF* gene presented the shortest alignment length with 495 bp, followed by *mtSSU* with a length of 660 bp and ITS with 967 bp. Moreover, the multiple alignment of the *TEF* sequences presented the highest number of variable sites (45.86%), followed by the *mtSSU* (33.33%) and the ITS region (13.24%). Similarly, the *TEF* gene had the most phylogenetically informative sites (43.03%), followed by the *mtSSU* (32.88%) and the ITS region (12.82%).

Phylogenetic analyses of the three genomic regions (ITS, *TEF* and *mtSSU*), separately and concatenated, revealed similar clustering of *Fusarium* isolates/species, with some discrepancies. In all cases, a total of four major clusters corresponding to four *Fusarium* Species Complexes were formed. The *Fusarium* Species Complex with the highest number of isolates (58 isolates) was *F. oxysposrum* (FOSC). While the *F. fujikuroi* Species Complex (FFSC) was represented by a larger number of species, namely *F. proliferatum* (23 isolates), *F. nygamai* (11 isolates), *F. thapsinum* (three isolates), *F. andiyazi* (two isolates), and some undefined species *F. terricola* or *F. sudanense* (nine isolates). The *F. incarnatum-equiseti* (FIESC) and *F. solani* (FSSC) Species Complexes were the least frequent, with four and three isolates, respectively (O'Donnell and Cigelnik, 1997).

Phylogenetic reconstruction using TEF, which was the closest reconstruction to what is currently accepted for *Fusarium* species, revealed a close relationship between the *F. oxysporum* and *F. fujikuroi* Species Complexes. The *F. incarnatum-equiseti* Species Complex was the next most related to the two earlier complexes, while the *F. solani* Species Complex was the most distant (Fig. 1). Within the *F. fujikuroi* Species Complex (FFSC), two main clusters were formed; the first cluster was formed by the isolates of the *F. proliferatum* species, and the second cluster was formed by *F. nygamai*, *F. thapsinum*, *F. andiyazi*, and the group formed by the isolates for which the exact name to the species was not clearly defined (*Fusarium* sp.) (Fig. 1). Similarly, the phylogenetic reconstruction using *mtSSU* resembled the tree generated with the *TEF* gene, with the difference of low resolution among some isolates of the *F. fujikuroi* Complex Species. On the other hand, clustering based on the ITS region showed differences compared to the phylogenetic reconstruction based on the *TEF* gene, where the *F. incarnatum-equiseti* Species Complex was located within the *F. fujikuroi* Species Complex. Furthermore, the phylogenetic reconstruction using the concatenated regions TEF+*mtSSU* resembled the tree generated with the *TEF* gene. While the phylogenetic reconstructions based on *TEF*+ITS and *TEF*+ITS+*mtSSU* regions were similar to that of *TEF*, with the exception that the *F. incarnatum-equiseti* Species Complex was more closely related to the *F. oxysporum* Species Complex. On the contrary, the phylogenetic reconstruction based on the ITS+*mtSSU* regions was the most conflicting where isolates identified in the same species were separated into different clusters.

DISCUSSION

In the present study, TEF, ITS, and mtSSU sequence data allowed identification of 114 isolates representing eight species located in four Fusarium species complexes associated with chickpea wilt and wheat root and stem rot in the Bajío region of Mexico.

The most frequent species isolated from chickpea were *F, oxysporum* (87.6%), followed by *F. equiseti* (4.7%), *F. solani* (4.7%), *F. proliferatum* (1.5%) and *Fusarium* sp. (1.5%).

Table 1. *Fusarium* species identified in wheat and chickpea crops using three molecular markers (translation elongation factor-1α, internal transcribed spacer and small subunit ribosomal RNA gene).

DNA region	Translation elongation factor-1α (*TEF*)			Internal transcribed spacer (ITS)			Small subunit ribosomal RNA gene (*mtSSU*)		
Plant species	**Chickpea**	**Wheat**		**Chickpea**	**Wheat**		**Chickpea**	**Wheat**	
Fusarium species	**Roots**	**Roots**	**Stem**	**Roots**	**Roots**	**Stem**	**Roots**	**Roots**	**Stem**
F. oxysporum	56	2	1	51	2	1	43	2	2
F. proliferatum	1	8	14	1	7	14	-	5	9
F. nygamai	-	4	7	-	3	6	-	-	-
F. thapsinum	-	-	3	-	-	2	-	-	-
F. andiyazi	-	1	1	-	-	-	-	-	-
F. equiseti	3	1	-	4	1	-	-	-	-
F. solani	3	-	-	3	-	-	3	-	-
Fusarium sp.	1	3	5	1	3	5	3	10	16

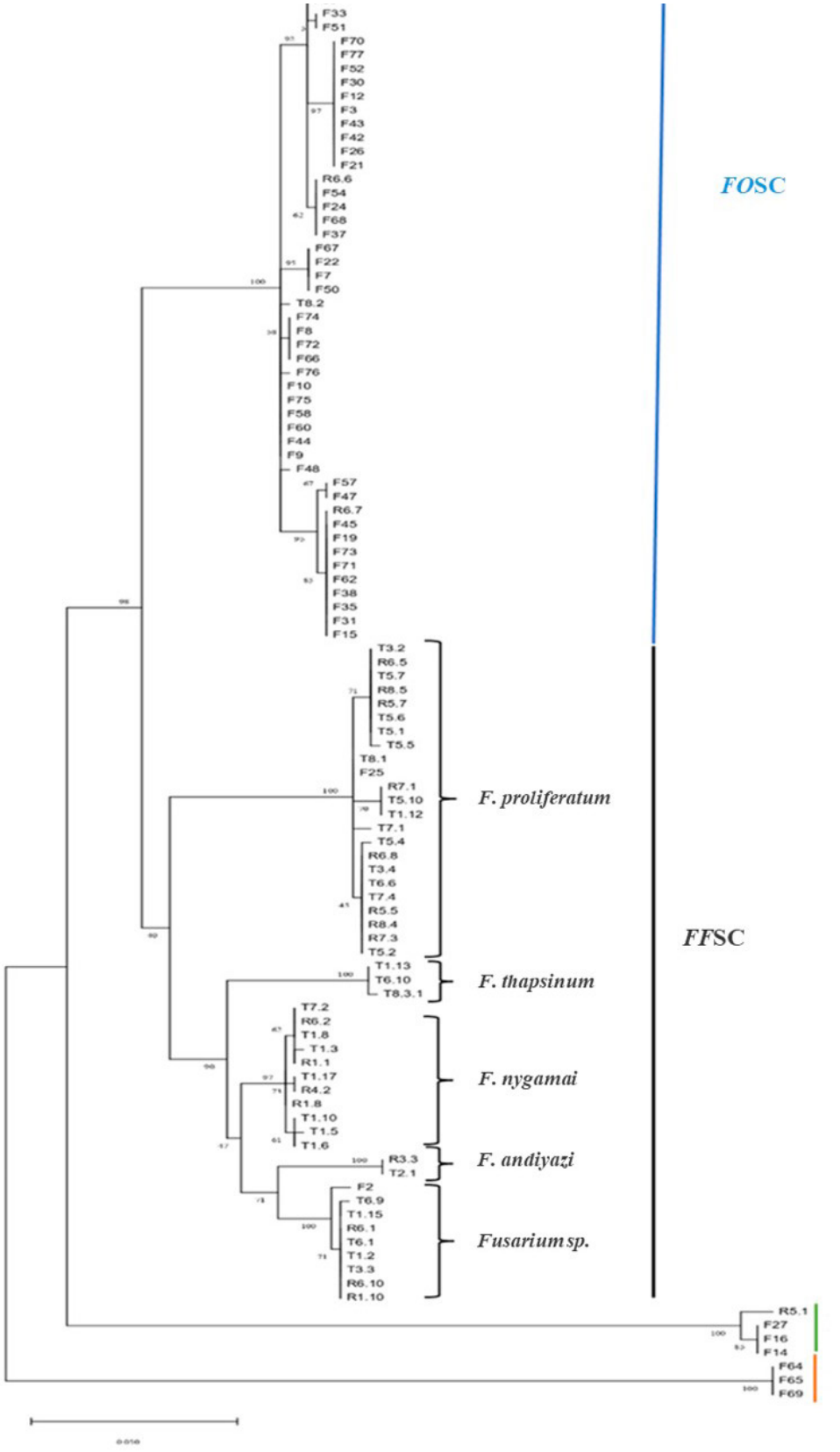

Figure. 1 Phylogenetic tree based on *TEF* gene sequences for isolates from the *F. oxysposrum* (FOSC), *F. fujikuroi* (FFSC), *F. solani* (FSSC) and *F. incarnatum-equiseti* (FIESC) Species Complexes obtained from wheat and chickpea plants in Mexico. Evolutionary history was inferred using the maximum likelihood method and the Kimura 2-parameter model.

Pathogenic strains of *F. oxysporum* cause vascular wilt and crust rot in a wide variety of agricultural crops, which promoted its inclusion among the ten main pathogens according to its scientific and economic importance in plant pathology (Dean *et al.*, 2012). This fungus exhibits two pathotypes in the field, one causes symptoms of yellowing and the other wilting in the affected plants, and both limit chickpea production in various parts of the world (Jiménez-Díaz *et al.*, 2015; Singh and Vyas, 2021). The wilt disease caused by *F. oxysporum* is considered the most dangerous, and the impact could be so severe that grain production may be lost entirely (Singh and Vyas, 2021). Likewise, *F. solani* has been reported in the chickpea crop (Cabral *et al.*, 2016); while *F. equiseti* has been registered as causal agent of root and seedlings rots of chickpea in Australia and India (Nene *et al.*, 1996). In addition, *F. proliferatum* was recently found to cause chickpea wilt in Cuba (Duarte-Leal *et al.*, 2020). For the unidentified species of the *F. fujikuroi* Specie Complex (*F. terricola* or *F. sudanense*), two species recently reported as new (Moussa *et al.*, 2017), there is still no information on its pathogenicity in chickpea cultivation. In Mexico, only the *F. oxysporum* and *F. solani* were previously reported to cause wilt of chickpea (Guerrero *et al.*, 2015; Fierros Leyva *et al.*, 2019).

The most frequent *Fusarium* species isolated from wheat were *F. proliferatum* (44%), *F. nygamai* (22%), *Fusarium* sp. (16%), *F. thapsinum* (6%), *F. oxysporum* (6%), *F. andiyazi* (4%), and *F. equiseti* (2%) (O'Donnell and Cigelnik, 1997). Several *Fusarium* species cause crown and root rot diseases such as. *F. graminearum*, *F. culmorum*, and *F. pseudograminearum* being the most important epidemiologically (Cook, 2010). However, other species, such as *F. proliferatum* (Nirenberg and O'Donnell, 1998; Leyva-Mir *et al.*, 2017), *F. equiseti* (Shikur-Gebremariam *et al.*, 2018), *F. oxysporum* (Smiley and Patterson, 1996) have been associated with the wheat root disease. *F. oxysporum* was reported as nonpathogenic by Shikur-Gebremariam *et al.* (2018). However, Demirci and Dane (2003) reported *F. oxysporum* as weakly pathogenic on winter wheat, while some isolates have been able to kill wheat seedlings under greenhouse conditions (Smiley and Patterson, 1996). On the other hand, *F. equiseti*, considered the most widespread non-pathogenic *Fusarium* species (Leslie and Summerell, 2006), was considered by other authors a pathogenic fungus of wheat, causing the root and/or crown diseases (Fernandez *et al.*, 2014; Shikur-Gebremariam *et al.*, 2018). For its part, *F. nygamai* was reported as one of the species causing root and crown rot in wheat, although it was the species that has caused the least severe damage (Abedi-Tizaki and Zafari, 2015). However, *F. nygamai* is commonly known to cause the root rot in sorghum, and it is associated with other plant species: cotton, rice, maize, millet, tobacco and beets (Leslie and Summerell, 2006; Cao *et al.*, 2018; Chala *et al.*, 2019). Parallelly, *F. andiyazi* was found to trigger crown rot in durum wheat by Zidan *et al.* (2020), although it mainly affects other species viz. rice, corn, sugarcane and sorghum (Leyva-Madrigal *et al.*, 2015; Yao *et al.*, 2020). By contrast, *F. thapsinum*, which is the most important causal agent of corn and stem rot in sorghum (Nirenberg and O'Donnell, 1998; Leyva-Madrigal *et al.*, 2015; Chala *et al.*, 2019), no association of *F. thapsinum* with stem and root rot in bread wheat has been reported to date. For the unidentified species of the *F. fujikuroi* Species Complex (*F. terricola* or *F. sudanense*), only *F. sudanense* was studied to determine its effect on seedling blight and seed rot of wheat (Larran *et al.*, 2020). Subject to conducting experiments on the pathogenic potential of these species in wheat, a possible explanation for their presence in the collected samples would be the rotation practice of wheat with other crops, possibly hosts of these species, in the Bajio areas of Mexico.

In the present research, the *TEF* gene had the highest number of variable sites (45.86%), followed by *mtSSU* (33.33%) and ITS region (13.24%). Likewise, *TEF* gene presented the highest number of phylogenetically informative sites (43.03%), followed by *mtSSU* (32.88%) and ITS (12.82%). Possibly, the greater variability in the intron-rich regions of protein-coding genes allows these genes to exhibit more variability (Geiser *et al.*, 2021) and therefore, might be better candidates for *Fusarium* DNA barcoding (Summerell, 2019). The use of the ITS region as a barcode, recommended for fungi, has several advantages, but this region is not variable enough to discriminate closely related species for most *Fusarium* Species Complexes since it does not tend to evolve at a similar rate to that of speciation (Al-Hatmi *et al.*, 2016). In contrast, protein-coding genes tend to contain higher information content and tend to evolve at a faster rate than ITS regions (Al-Hatmi *et al.*, 2016; Summerell, 2019).

The obtained clustering of the *Fusarium* Species Complexes and the distribution of the species within the *F. fujikuroi* Specie Complex (FFSC), principally with the *TEF* gene, were similar to that obtained by Geiser *et al.* (2021) based on exonic nucleotide sequences of 19 orthologous protein-coding genes, and also to that reported by Al-Hatmi *et al.* (2016) using *TEF*, topoisomerase I (TOP1) and phosphoglycerate kinase (PGK); and to that obtained by O'Donnell *et al.* (2013) based on RPB1 and RPB2 genes. For the *Fusarium* genus, *TEF* gene is highly recommended for species identification and phylogenetic reconstruction of the different *Fusarium* Species Complexes (O'Donnell *et al.*, 2013; Al-Hatmi *et al.*, 2016; Summerell, 2019) since no non-orthologous copies have been detected and universal primers to amplify the same *TEF* regions across the entire phylogenetic breadth of the genus have been designed (Geiser *et al.*, 2013; O'Donnell *et al.*, 2013).

Generally, phylogenetic reconstructions based on concatenated regions resulted on lower resolution than the construct obtained with the *TEF* gene. Concatenated datasets that included *TEF* were the least conflicting, reinforcing the utility of *TEF* for Fusarium phylogenetics. The resulted clustering from the concatenation of the ITS+*mtSSU* regions

was the most conflicting for our data since isolates from the same species were separated into different clusters. This could be due to the different rates of evolution of these two genomic regions, resulting in different levels of genetic variation (Mitchell and Zuccaro, 2006). The tree´s construction from concatenated data is a common practice for phylogenetic analyses. However, depending on the analyzed genomic regions, species, statistical methods, among other factors, trees based on concatenated sequences may or may not agree with individual trees (Thiergart *et al.*, 2014; Al-Hatmi *et al.*, 2016; Geiser *et al.*, 2021). Potential sources of phylogenetic conflict between individual gene trees and concatenated alignment trees, may include uncertain orthology or hidden paralogy, horizontal gene transfer, introgression between species, or unknown factors such as misspecification of a single-fits-all model genes (Jayaswal *et al.*, 2014).

CONCLUSIONS

Eight *Fusarium* species associated with stem and root rot of wheat and chickpea wilt grown in Mexico were identified. The species *F. oxysporum* was the most frequent in chickpea, but *F. equiseti*, *F. solani* and *F. proliferatum* were also identified. From wheat, *F. proliferatum* was the most frequent, and *F. nygamai*, *F. thapsinum*, *F. oxysporum*, *F. andiyazi* and *F. equiseti* were also recovered. The diversity of the identified species was reflected in the presence of four *Fusarium* Species Complexes. The *F. oxysporum* Species Complex was the most frequent, while the *F. fujikuroi* Species Complex was represented by a greater number of species (*F. proliferatum*, *F. nygamai*, *F. thapsinum*, *F. andiyazi*, and *Fusarium* sp.). The *F. incarnatum-equiseti* and *F. solani* Species Complexes were the least frequent. On the other hand, concatenation of genomic regions for the reconstruction of the phylogenetic tree of the species did not improve the tree obtained with the *TEF* gene. *TEF* remains recommended for species-level identification within the genus *Fusarium*.

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AUTHOR PARTICIPATION

VM.E. Project and design planning, laboratory protocol development, writing original draft-review, funding acquisition.

S. S. Support in writing and bioinformatics.

RC.M,E (Master of Science), GC.A,J, JC. K,M, VR.A and SF.R. Bachelor´s students who participated in the project to obtain their degree thesis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary table 1. Isolate names and GenBank accession numbers of three genomic regions

Isolate ID	ITS	mtSSU	TEF	Species name	Source	*Host*
F10	EU091001.1	EU418431.1	EU091043.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F12	ON411466	ON417370	KC113014.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F13	EU091002.1	ON417371	KC113015.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F14	EU091036.1	ON417372	EU091073.1	*Fusarium equiseti*	Root	*Cicer arietinum*
F15	EU091003.1	ON417373	EU091044.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F16	EU091035.1	ON417374	KC113037.1	*Fusarium equiseti*	Root	*Cicer arietinum*
F19	ON411467	ON417375	KC113030.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F2	EU091040.1	ND	EU091074.1	*Fusarium* sp.	Root	*Cicer arietinum*
F21	EU091004.1	ON417376	EU091045.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F22	EU091005.1	ND	EU091046.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F23	EU091006.1	ON417377	EU091047.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F24	ON411468	ON417378	KC113016.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F25	EU091039.1	ND	EU091072.1	*Fusarium proliferatum*	Root	*Cicer arietinum*
F26	EU124522.1	ON417379	EU091048.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F27	ND	ND	ON419841	*Fusarium equiseti*	Root	*Cicer arietinum*
F28	ND	ND	ON419842	*Fusarium oxysporum*	Root	*Cicer arietinum*
F29	ON411469	ON417380	KC113018.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F3	ND	ON417366	KC113012.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F30	EU091007.1	ON417381	KC113019.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F31	EU091008.1	ND	EU091049.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F32	ND	ND	ON419843	*Fusarium oxysporum*	Root	*Cicer arietinum*
F33	ND	ND	ON419844	*Fusarium oxysporum*	Root	*Cicer arietinum*
F34	ND	ND	ON419845	*Fusarium oxysporum*	Root	*Cicer arietinum*
F35	EU091009.1	ON417382	EU091050.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F36*	EU091010.1	ON417383	ND	*Fusarium oxysporum*	Root	*Cicer arietinum*
F37	EU091011.1	ON417384	EU091051.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F38	EU091012.1	ON417385	EU091052.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F40*	ON411470	ND	ND	*Fusarium oxysporum*	Root	*Cicer arietinum*
F41	EU091013.1	ON417386	EU091053.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F42	EU091014.1	ND	ON419846	*Fusarium oxysporum*	Root	*Cicer arietinum*
F43	EU091015.1	ND	EU091055.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F44	ON411471	EU418432.1	EU091056.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F45	EU091016.1	ND	ON419847	*Fusarium oxysporum*	Root	*Cicer arietinum*
F47	EU091017.1	ON417387	EU091057.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F48	EU091018.1	ON417388	EU091058.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F5*	EU091019.1	ND	ND	*Fusarium oxysporum*	Root	*Cicer arietinum*
F50	ND	ND	ON419848	*Fusarium oxysporum*	Root	*Cicer arietinum*
F51	ND	ND	ON419849	*Fusarium oxysporum*	Root	*Cicer arietinum*
F52	ON411472	ON417389	KC113022.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F53	EU091020.1	ON417390	EU091059.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F54	ON411473	ON417391	KC113023.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F55	EU091021.1	ON417392	EU091060.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F56*	EU091022.1	ND	ND	*Fusarium oxysporum*	Root	*Cicer arietinum*
F57	EU091023.1	ON417393	EU091061.1	*Fusarium oxysporum*	Root	*Cicer arietinum*

(*Continued*)

Isolate ID	ITS	mtSSU	TEF	Species name	Source	*Host*
F58	EU091024.1	ND	EU091062.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F59	EU091025.1	ON417394	KC113040.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F6	ND	ND	KC113013.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F60	EU091034.1	ON417395	EU091063.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F61	ND	ND	ON419850	*Fusarium oxysporum*	Root	*Cicer arietinum*
F62	EU091026.1	ON417396	EU091064.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F63	ON411474	ON417397	KC113024.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F64	ON411475	ON417398	KC113039.1	*Fusarium solani*	Root	*Cicer arietinum*
F65	EU091037.1	ON417399	EU091070.1	*Fusarium solani*	Root	*Cicer arietinum*
F66	EU091027.1	ON417400	KC113027.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F67	EU091028.1	ON417401	EU091065.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F68	ON411476	ON417402	EU091066.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F69	EU091038.1	ON417403	EU091071.1	*Fusarium solani*	Root	*Cicer arietinum*
F7	EU091029.1	ON417367	KC113031.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F70	ON411477	ON417404	KC113026.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F71	EU091030.1	ON417405	EU091067.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F72	EU124523.1	ON417406	EU091068.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F73	EU091031.1	ON417407	KC113029.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F74	EU091032.1	ON417408	EU091069.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F75	ON411478	ON417409	KC113028.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F76	ON411479	ON417410	KC113036.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F77	ON411480	ON417411	KC113025.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F8	EU091033.1	ON417368	EU091041.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
F9	EU124521.1	ON417369	EU091042.1	*Fusarium oxysporum*	Root	*Cicer arietinum*
R1.1	ON411481	KX218400.1	ON419869	*Fusarium nygamai*	Root	*Triticum aestivum*
R1.10	ON411482	ON417413	KU508362.1	*Fusarium* sp.	Root	*Triticum aestivum*
R1.5*	ND	ON417412	ND	*Fusarium oxysporum*	Root	*Triticum aestivum*
R1.8	ND	KX218397.1	ON419851	*Fusarium nygamai*	Root	*Triticum aestivum*
R3.3	ON411483	KX218396.1	ON419870	*Fusarium andiyazi*	Root	*Triticum aestivum*
R4.2	ON411484	ON417414	KU508370.1	*Fusarium nygamai*	Root	*Triticum aestivum*
R5.1	ON411485	KX218405.1	ON419871	*Fusarium equiseti*	Root	*Triticum aestivum*
R5.5	ON411486	ND	KU508357.1	*Fusarium proliferatum*	Root	*Triticum aestivum*
R5.7	ND	ON417415	KU508353.1	*Fusarium proliferatum*	Root	*Triticum aestivum*
R6.1	ND	KX218410.1	ON419852	*Fusarium* sp.	Root	*Triticum aestivum*
R6.10	ON411490	ND	KU508363.1	*Fusarium* sp.	Root	*Triticum aestivum*
R6.2	ON411487	ON417416	ON419868	*Fusarium nygamai*	Root	*Triticum aestivum*
R6.5	ON411488	ND	KU508349.1	*Fusarium proliferatum*	Root	*Triticum aestivum*
R6.6	ND	KX218407.1	ON419853	*Fusarium oxysporum*	Root	*Triticum aestivum*
R6.7	ON411489	ON417417	KU508359.1	*Fusarium oxysporum*	Root	*Triticum aestivum*
R6.8	ND	ON417418	ON419854	*Fusarium proliferatum*	Root	*Triticum aestivum*
R7.1	ON411491	KX218413.1	ON419867	*Fusarium proliferatum*	Root	*Triticum aestivum*
R7.3	ON411492	KX218412.1	KU508355.1	*Fusarium proliferatum*	Root	*Triticum aestivum*
R8.4	ON411493	ND	ON419855	*Fusarium proliferatum*	Root	*Triticum aestivum*
R8.5	ON411494	ND	KU508352.1	*Fusarium proliferatum*	Root	*Triticum aestivum*
T1.10	ON411498	ON417422	KU508369.1	*Fusarium nygamai*	Stem	*Triticum aestivum*

(*Continued*)

Isolate ID	ITS	mtSSU	TEF	Species name	Source	*Host*
T1.12	ON411499	ND	KU508347.1	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T1.13	ON411500	ON417423	KU508368.1	*Fusarium thapsinum*	Stem	*Triticum aestivum*
T1.15	ON411501	KX218399.1	KU508361.1	*Fusarium* sp.	Stem	*Triticum aestivum*
T1.17	ON411502	ON417424	KU508371.1	*Fusarium nygamai*	Stem	*Triticum aestivum*
T1.2	ND	KX218402.1	KU508365.1	*Fusarium* sp.	Stem	*Triticum aestivum*
T1.3	ON411495	ON417419	ON419866	*Fusarium nygamai*	Stem	*Triticum aestivum*
T1.5	ON411496	ON417420	ON419865	*Fusarium nygamai*	Stem	*Triticum aestivum*
T1.6	ND	ON417421	ON419856	*Fusarium nygamai*	Stem	*Triticum aestivum*
T1.8	ON411497	KX218401.1	KU508373.1	*Fusarium nygamai*	Stem	*Triticum aestivum*
T2.1	ON411503	KX218395.1	KU508360.1	*Fusarium andiyazi*	Stem	*Triticum aestivum*
T3.2	ON411504	ND	KU508350.1	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T3.3	ON411505	ON417425	KU508364.1	*Fusarium* sp.	Stem	*Triticum aestivum*
T3.4	ON411506	KX218403.1	ON419872	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T4.1*	ON411507	ON417426	ND	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T5.1	ON411508	ON417427	ON419864	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T5.10	ON411515	ND	ON419857	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T5.2	ON411509	ON417428	KU508354.1	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T5.3*	ON411510	KX218415.1	ND	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T5.4	ON411511	ON417429	ON419863	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T5.5	ON411512	ON417430	ON419862	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T5.6	ON411513	KX218416.1	ON419873	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T5.7	ON411514	ND	KU508351.1	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T6.1	ON411516	ON417431	KU508366.1	*Fusarium* sp.	Stem	*Triticum aestivum*
T6.10	ND	KX218409.1	KU508367.1	*Fusarium thapsinum*	Stem	*Triticum aestivum*
T6.6	ND	KX218417.1	ON419858	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T6.9	ON411517	ON417432	ON419861	*Fusarium* sp.	Stem	*Triticum aestivum*
T7.1	ON411518	ON417433	ON419860	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T7.2	ON411519	ON417434	KU508372.1	*Fusarium nygamai*	Stem	*Triticum aestivum*
T7.4	ON411520	KX218408.1	ON419874	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T8.1	ON411521	ON417435	KU508358.1	*Fusarium proliferatum*	Stem	*Triticum aestivum*
T8.2	ON411522	KX218406.1	ON419875	*Fusarium oxysporum*	Stem	*Triticum aestivum*
T8.31	ON411523	ON417436	ON419859	*Fusarium thapsinum*	Stem	*Triticum aestivum*
E5.1*	ON411524	ON417437	ND	*Fusarium* sp.	Spike	*Triticum aestivum*
E5.2*	ON411525	ON417438	ND	*Fusarium* sp.	Spike	*Triticum aestivum*

ND Not available

* Isolates not used in the construction of the tree with the TEF gene