Characterization of *Fusarium* spp., a Phytopathogen of avocado (*Persea americana* Miller var. *drymifolia* (Schltdl. and Cham.)) in Michoacán, México

Caracterización de *Fusarium* spp., fitopatógeno de aguacate (*Persea americana* Miller var. *drymifolia* (Schltdl. y Cham.)) en Michoacán, México

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Abstract

Avocado has great socioeconomic importance in Mexico because of the benefits it generates for the production chain participants and the significant foreign exchange earnings engendered by the export of its fruit. However, this crop has phytosanitary problems, caused mainly by fungi, among which the genus *Fusarium* stands out. The objective of this study was to characterize *Fusarium* species that caused root rot in avocado trees in Michoacan, Mexico. In 19 isolates of *Fusarium* spp., polymerase chain reactions (PCR) with primers coding for *elongation factor* and *calmodulin* genes were performed. These sequences were compared in homology using BLAST analysis and aligned in MEGA 6.0. Cladograms were created based on maximum verisimilitude. The pathogenicity of the isolates was evaluated based on their virulence and severity in the avocado plants. Morphological and molecular analyses showed that 15 isolates belonged to *F. oxysporum* Schl and four to *F. solani* Mart. All isolates were pathogenic, with virulence ranging from 16 to 56 days. All isolates produced root rot and yellowing of leaves, with 63% producing wilting and 16% producing apical necrosis, the latter being the most severe.

Keywords

plant pathogen • root rot • crop disease • Ascomycetes • *Fusarium oxysporum* Schl • *Fusarium solani* Mart

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Resumen

El aguacate en México tiene gran importancia socioeconómica por los beneficios que genera para los participantes de la cadena productiva y la generación de divisas por la exportación de su fruta. Sin embargo, este cultivo presenta problemas fitosanitarios ocasionados por hongos entre los que destacan el género *Fusarium*. El objetivo de esta investigación fue caracterizar especies de *Fusarium* que producen pudrición de raíz en árboles de aguacate en Michoacán, México. En 19 aislados de *Fusarium* spp. la reacción en cadena de la polimerasa (PCR) se hizo con iniciadores que codifican para genes de *factor de elongación* y *calmodulina*. Estas secuencias se compararon en homología por medio de análisis BLAST y se alinearon en MEGA 6.0. Los cladogramas se hicieron con base a máxima verisimilitud. La patogenicidad de los aislados se evaluó de acuerdo con su virulencia y severidad en plantas de aguacate. Los análisis morfológicos y moleculares concluyeron que 15 aislados pertenecen a *Fusarium oxyspo*r*um* Schl. y cuatro a *Fusarium solani* Mart. Todos los aislados fueron patogénicos, presentaron con su virulencia que osciló entre 16 a 56 días. El total de los aislados produjo pudrición radical y amarillamiento de las hojas, marchitez en 63% y necrosis apical en 16%, este último síntoma fue el más severo.

Palabras clave

patógeno vegetal • pudrición de raíz • enfermedades de cultivos • Ascomycetes • *Fusarium oxysporum* Schl • *Fusarium solani* Mart

Introduction

In 2016, 583,978 ha globally were sown with avocado, yielding 5,689,985 t (13). During that same year, in Mexico, 180,546.43 ha with a production of 1,889.353.50 t was sown with avocado. The state of Michoacan accounted for 85% of this total (38). This strong performance in Michoacan was because the crop was established in an area that contained benign agroecological conditions (soil and climate) that favored its reproductive development (19).

However, this species has undergone genetic and physiological changes resulting from the massive establishment and intensive management of its production systems, thus limiting production and affecting its quality (39, 46). Plant diseases caused by *Fusarium* spp. in the tropics have increased in importance with the introduction of high-yield production systems and genetically uniform cultivars (36, 44). Symptoms caused by *Fusarium* spp. in avocado trees include leaf yellowing, interrupted growth of vegetative flushes, small leaves, and the premature abscission of leaves. If rotting is severe, the trees can die weeks after the first foliar symptoms appear or they barely survive for long periods. At the root level, the observed symptoms include the rotting of small roots with a dark brown-black hue that, over time, spreads to the thickest roots. The symptoms described differ from those caused by *Armillaria mellea* or *Rosellinia* sp. owing to the absence of rhizomorphs or abundant white mycelia, which are characteristics of these species and, for *Phytophthora cinnamomi*, due to the absence of secondary roots (23, 32, 33).

Fungal species have traditionally been identified by morphological techniques based on the comparison of characters. These techniques are often complicated and slow, given that different variables must be evaluated. This is in addition to the observation and recognition of the characteristics of the isolates (45).

Alternative diagnostic techniques, such as molecular tools, have developed in recent decades, Polymerase chain reaction (PCR)-based protocols and specific primers provide better-founded diagnoses, as well as taxonomic identifications at the species level (10). The limitations associated with biochemical, morphological, and cytological variability have been overcome by the development of DNA markers (2).

Although the taxonomy and identification of species of the genus *Fusarium* have been studied based on several genetic markers, it has not yet been possible to find a suitable marker for the identification of all its species (47). The high degree of variability within *F. oxysporum* has shown the complexity of the species (3).

A wide variety of molecular markers have been used to analyze the diversity of plant pathogenic *Fusarium* species at the genomic level: AFLPs in *F. graminearum* and *F. asiaticum* (34) and RAPD and ISSR in *F. graminearum* and *F. culmorum* (28, 40, 43). Attempts have been made to characterize *F. oxysporum* lines using different techniques, such as IGS and ITS regions; however, these have not provided favorable results (40, 41).

Ribosomal RNA genes have been used in phylogenetic studies of fungi. Some of the criticism against using rDNA markers include if they are used instead of biased base composition then this might influence the branching order of the tree and the unequal rates of evolution among lineages, nonindependence of sites and invariable sites, and among-site rate variation may contribute to artifactual topologies in the trees. To compensate for this, several protein-coding genes have been explored as phylogenetic markers (20).

Studies have shown that phylogenies obtained from the *elongation factor* gene are congruent with other molecular phylogenies for the recovery of monophyly from groups such as Metazoa, Fungi, Magnoliophyta, and Euglenozoa. This marker is important for providing a highly informative section between closely related species (17, 20). However, coding sequences of genes such as *calmodulin* (31) have been employed for molecular phylogenetic analysis of the *Gibberella fujikuroi* complex and other *Fusarium* species.

In the avocado belt of Michoacan, Mexico, there are no studies of species of the genus *Fusarium* that cause root rot in avocado trees. Therefore, there is no information on the phenotypic, genetic, and pathogenic diversity of *Fusarium* species. Thus, the objectives of the present study were to isolate and morphologically and molecularly identify *Fusarium* species, obtained from avocado roots, and determine their pathogenicity in avocado plants. The hypothesis was that in the Michoacan avocado belt, *Fusarium* spp. is associated with avocado trees that have root rot symptoms.

Materials and methods

Targeted samplings were conducted in 10 localities of nine municipalities (Ario de Rosales, Los Reyes, Nuevo Parangaricutiro, Periban, Tacambaro, Tancitaro, Tingambato, Uruapan (2), and Ziracuaretiro) located in the so-called avocado belt of the state of Michoacán,

México, with different elevations and agro-ecological zones (19). In each locality, three root samples were collected from five trees that showed wilting symptoms. Washing and disinfection of the root fragments were performed in the laboratory according to the protocol described by other researchers (1, 42). A total of 10 tissue sections per sample were seeded in a Petri dish with Bioxon® potato dextrose agar (PDA) culture medium. When the colony had grown, hyphal tips were transferred to Petri dishes containing Spezieller Nährstoffarmer Agar (SNA) with two pieces of 0.5 cm sterile filter paper to induce sporulation (24).

Monosporic cultures

Monosporic cultures were obtained for all isolates grown in SNA. From each isolate, two 5-mm disks of medium and mycelium were placed in 5-mL glass tubes containing 3 mL of sterile distilled water. The tubes were briefly shaken to separate the conidia from the mycelium and 25 µL was taken from the conidial suspension and dispersed in dishes containing 2% agar water. The dishes were incubated at 25°C in the darkness for 24 h. The germinated conidia were observed under a stereoscopic microscope Leica S9E and then individually transferred to dishes containing SNA and sterile paper, and incubated at 25°C. Once the cultures covered most of the medium in the dish, 3 mL of 25% sterile glycerol was added to remove the conidia from the mycelium. The conidial suspension (1 mL) was transferred to 2 mL cryogenic tubes and stored at -80°C. Aliquots of the stored conidial suspension of each isolate were used for subsequent characterization.

Cultural and morphological characterization

To stimulate the development of the cultures and conidia, fungal were incubated at 25°C in PDA medium and CLA (carnation leaves in 2% agar water) (24). After 15, 30, and 60 days of incubation, the following morphological characters were analyzed: type and thickness of the mycelium, and the colony color and agar pigmentation based on the Mathuen Handbook of Color (21) and the Color Picker tool for Mac OS X. The morphological characters of 30 macroconidia, microconidia, and chlamydospores were measured by calculating the ranges and mean values (24). In addition, information on the shape, size, and number of septa of macroconidia and microconidia, presence of mono and/or polyphialides, chlamydospores, and sterile coiled hyphae was recorded.

For the growth rate, mycelial disks 1 cm in diameter were taken and placed in the center of five Petri dishes containing PDA medium, after which they were incubated at 21°C. The growth was measured after 72 h. The daily growth rate (mm) was obtained from the resulting difference between the final diameter minus the initial diameter divided by the total number of days evaluated (24).

Nucleic acid extraction

DNA extraction was performed using the protocol of Cenis (7). The pellet was resuspended in TE buffer and stored at -20°C until subsequent use. Quality was assessed by gel electrophoresis at 120 volts for 20 min and staining was performed with ethidium bromide, was after which it was observed in a MiniBis Pro® ultraviolet photo-documenter. The concentration and purity of the extracted DNA were measured in a Thermo Scientific NanoDrop spectrophotometer; 1 μL^{-1} of the sample was placed in a BioRad®

SmartSpec ultraviolet spectrophotometer, fitted with a Hellma-Analytics® TrayCell cell, and evaluated by readings at 260 and 280 nm.

Polymerase chain reaction and DNA sequencing

DNAs from fungal isolates were amplified by PCR based on sequences coding for elongation factor EF-1α (ATGGGTAAGGARGACAAGAC) and EF-2 (GGARGTACCAGTSATCATGGT) and calmodulin Cal-228F (GAGTTCAAGGAGGCCTTCTCCC) and Cal-737F (CATCTTTCTGGCCATCATGG). For amplification, a PCR reaction mixture composed of 2.5 µL 1M Tris pH 9.0, 2 µL dNTPs, 0.5 µL forward primer, 0.5 µL reverse primer at a final concentration of 200 nM, 11.3 µL dH_2O, 3 µL of 50mM $MgCl_2$, 0.2 µL Taq polymerase, and 2.5 µL of isolated DNA was used. PCR was performed in a Life Technologies® Veriti® thermal cycler under the following conditions: an initial denaturation cycle of 95°C for 4 min, followed by 35 denaturation cycles at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 2 min; final extension was performed at 72°C for 10 min (20, 29).

Amplification samples were electrophoretically analyzed in agarose gels at 2% Tris-borate-EDTA X (TBEX) with a molecular weight marker of 100 base pairs at 120 volts for 5 min and then at 80 volts for 60 min; the gels were then visualized in a MiniBis Pro® ultraviolet photo-documenter. The PCR product was purified and sequenced.

Phylogenetic analysis

The obtained sequences were compared in homology with sequences stored in GenBank using BLAST analysis, from where those that presented the best similarity index as well as those whose morphological characteristics and antecedents were relevant for the study were obtained. These sequences were aligned and edited using the MEGA 6.0 software program. Later, the best evolutionary model was sought for the construction of cladograms based on the analysis of maximum parsimony and maximum likelihood. The cladograms obtained were edited with the FigTree 1.4.2 application for Mac OSX.

Pathogenicity tests

Isolates were grown in CLA at 25°C for 15 days. Conidia were collected in sterile water and diluted to 6,072 conidia/mL^{-1}. The conidial suspension was used to inoculate 4-month-old avocado plants grown in sterile substrate and inoculations of the conidial suspensions were performed (42), for which 100 mL^{-1} of the solution of each isolate was added near the root of the plant, without causing damage to them, and five plants were inoculated per treatment including a control without inoculum. Subsequently, the severity and virulence of each isolate were evaluated.

A completely randomized experimental design was used. The virulence data were subjected to an analysis of variance and the means of the treatments were compared with Tukey's test ($p \leq 0.05$). Analyses were performed using SAS (35) version 9.1.

Results and discussion

Cultural and morphological analysis

A total of 19 isolates of *Fusarium* spp. were obtained from nine municipalities within the avocado belt of the state of Michoacan, Mexico. Monosporic cultures produced abundant, dense, aerial, and floccose mycelia in the PDA medium.

The colonies pigmented the culture medium, with 30% presenting gray coloration, 25% violet, 25% blue, and 20% yellow. Variation in colony coloration is a character of the genus *Fusarium* (5); however, pigments are not a representative taxonomic character of species and may vary within the same isolate, except for the yellow pigment that is characteristic of *F. thapsinum* Klittich (24, 47) (figure 1).

The growth rates ranged between 1.65 and 5.13 mm/day, which were similar to those obtained for *F. oxysporum* Schl (16), indicating that it can grow up to 1 mm per day at a temperature of 25°C; however, for the isolates of yellow coloration, its growth rate was 2.98–3.17 mm/day.

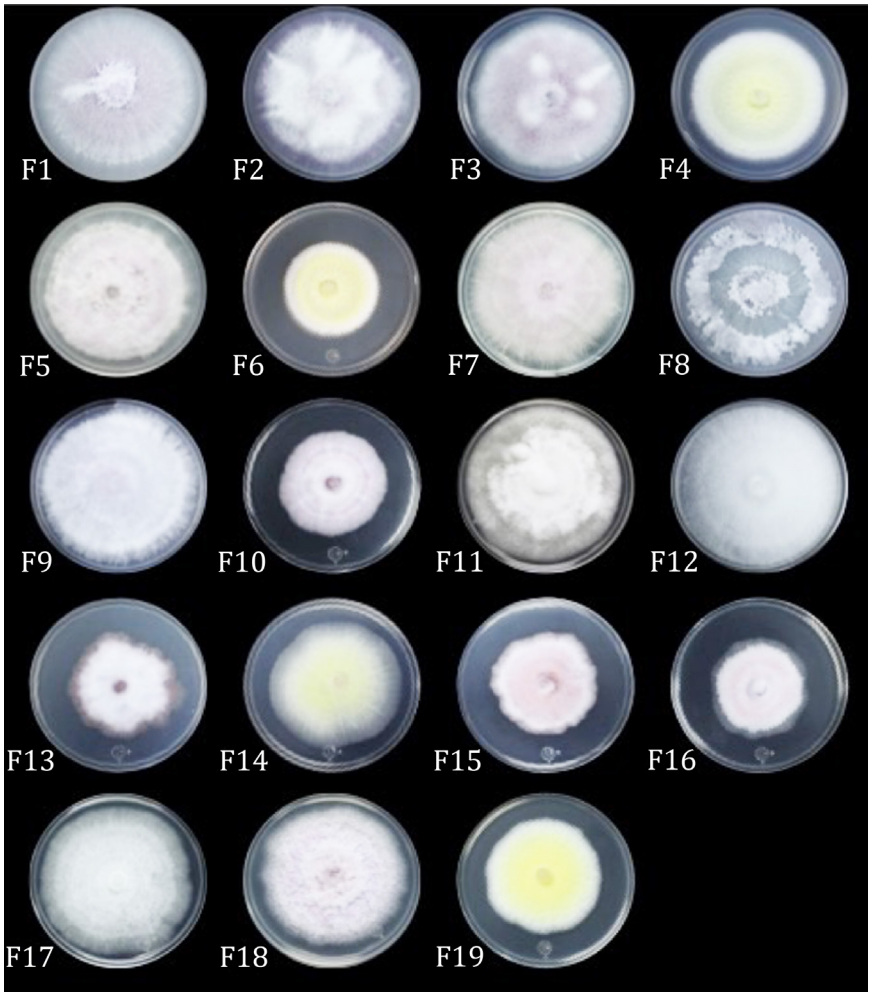

Figure 1. Comparison of 19 *Fusarium* isolates in PDA culture medium, obtained from 10 localities in the avocado belt of Michoacán, México.

Figura 1. Comparación de 19 aislados de *Fusarium* en medio de cultivo PDA, obtenidos de 10 localidades en la franja aguacatera de Michoacán, México.

In the CLA medium, the isolates produced abundant microconidia, which were oval, without septa, and arranged in fake heads. These characteristics match those reported previously for *F. oxysporum* and *F. solani* (27). The macroconidia, scarce in all isolates, were elongated, slightly curved, and septate. In the isolates F4, F6, F14, and F19, the macroconidia had five septa, with the apical cell rounded and the basal cell "foot"-shaped, being 8.98 to 9.42 µm wide and 51.12 to 56.32 µm long. For the remainder of the isolates, the macroconidia presented three septa, the apical cell was curved, and the basal cell "foot"-shaped, being 6.17 to 13.63 µm wide and 35.65 to 49.83 µm long. The macroconidia are considered the most important character for the identification of *Fusarium* species and in the CLA medium they are usually uniform (6, 24, 27, 35, 47). All isolates produced abundant chlamydospores of 12.5-17.6 µm long, globose shaped and smooth walled, terminals and intercalary in the hyphae, and were lonely and/or in small chains.

In the isolates F4, F6, F14, and F19, the chlamydospores were larger (19.3-20.4 µm diameter) than those of the other isolates, which matched with the size of the chlamydospores reported for *F. solani* (24, 27). Moreover, these four isolates presented rolled hyphae, both aerially and immersed in the culture medium. Rolled hyphae are also produced by *F. sterilihyphosum*, *F. mexicanum*, *F. circinatum,* and *F. pseudocircinatum* (24, 27, 35). Nevertheless, *F. sterilihyphosum* presents longer and thinner conidia, and absent chlamydospores (27). *F. circinatum* and *F. pseudocircinatum* present violet pigmentation in PDA medium and an absence of chlamydospores (24, 27, 30). In contrast, the isolates F4, F6, F14, and F19 produced abundant chlamydospores and yellow pigmentation in the culture medium (24) (figure 2, page 308).

Molecular identification

The DNA extracted from the 19 isolates had a concentration between 102 and 398 ng μL^{-1}, whereas the 260/280 nm absorbance ratio was 1.79-1.92, which coincides with the findings published previously (47), where a DNA sample with a value of -1.8 was accepted. The PCR based on the *elongation factor* and *calmodulin* genes provided fragments of a size greater than 600 base pairs; this size is among those reported for species of the genus *Fusarium* (2, 20, 29, 47). The selected GenBank sequences for phylogenetic analysis with *elongation factor* and *calmodulin* genes, by their similarity, are represented in table 1 (page 308) and table 2 (page 309).

Calmodulin

Phylogenetic analysis of the *calmodulin* gene coding region was performed with 640 characters in a GTR substitution model. The cladogram for maximum plausibility showed a monophyletic group that grouped the isolates *of F. solani* Mart, two monophyletic groups of *F. oxysporum* Schl, and a sister polyphyletic group of these. Results coincided with those from other research (31, 48); therefore, *calmodulin*-coding sequences are effective in the phylogenetic analysis of highly related *Fusarium* species (figure 3, page 309).

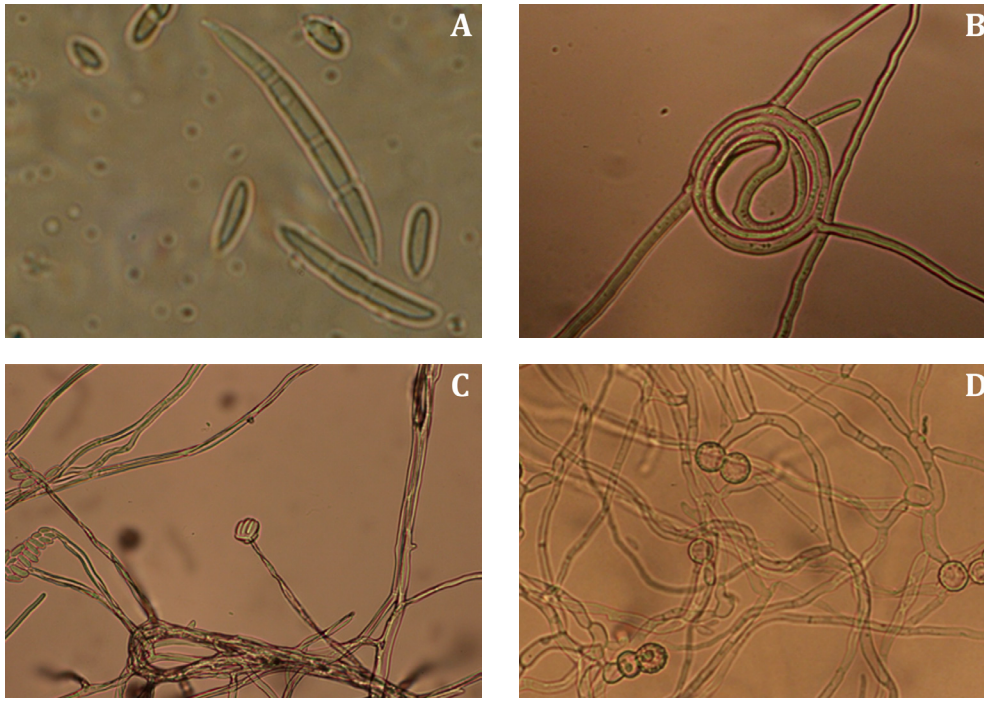

Figure 2. Reproductive structures of *Fusarium* spp. A) Macroconidia, B) Rolled hyphae, C) Monophialides, D) Chlamydospores.

Figura 2. Estructuras reproductivas de *Fusarium* spp. A) Macroconidios, B) Hifas enrolladas, C) Monofialidess, D) Clamidosporas.

Table 1. Coding sequences for the *elongation factor* gene obtained from GenBank.

Tabla 1. Secuencias codificantes para el gen *factor de elongación* obtenidas del GenBank.

Accession	Species	Origin	Percentage of homology
KJ648634	*Fusarium oxysporum*	MALAYSIA	99.71
KM092365	*Fusarium oxysporum*	NEW ZEALAND	99.85
DQ452430	*Fusarium oxysporum*	SOUTH KOREA	99.41
KF225015	*Fusarium oxysporum*	FRANCE	99.70
JF740853	*Fusarium oxysporum*	USA	99.70
JF740725	*Fusarium oxysporum*	USA	99.70
AF160271	*Fusarium pseudocircinatum*	USA	
KM823607	*Fusarium mexicanum*	MEXICO	
AF160295	*Fusarium circinatum*	USA	
DQ452858	*Fusarium sterilihyphosum*	BRAZIL	
JF740850	*Fusarium solani*	USA	99.85
DQ247542	*Fusarium solani*	USA	99.25
JF740784	*Fusarium solani*	USA	99.40
KF022238	*Fusarium gramindzrum*		

Table 2. Coding sequences for *calmodulin* gene obtained from GenBank.
Tabla 2. Secuencias codificantes para el gen *calmodulina* obtenidas del GenBank.

Accession	Species	Origin	Percentage of homology
FR828826	*Fusarium oxysporum*	ITALY	99.02
KF466332	*Fusarium oxysporum*	USA	99.56
AJ560774	*Fusarium oxysporum*	ITALY	99.41
AF158365	*Fusarium oxysporum*	USA	99.70
GU737372	*Fusarium pseudocircinatum*	MEXICO	
KF597833	*Fusarium circinatum*	BRAZIL	
GU737387	*Fusarium sterilihyphosum*	USA	
JN614904	*Fusarium solani*	ARGENTINA	99.34
HQ412317	*Fusarium solani*	CHINA	95.89
HQ412319	*Fusarium solani*	CHINA	95.74
HQ412318	*Fusarium solani*	CHINA	95.69
KP753394	*Fusarium mangiferae*	USA	

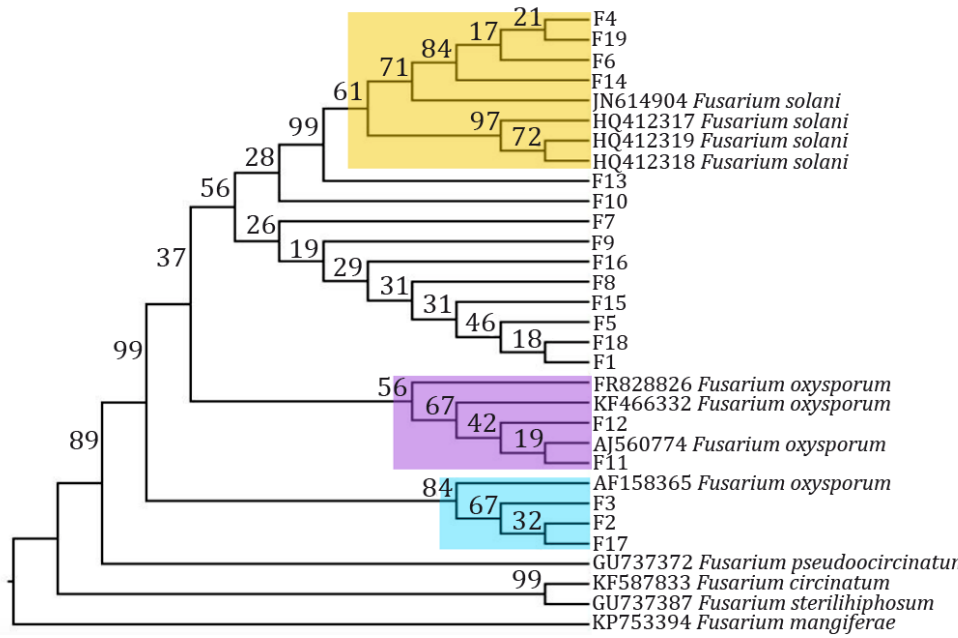

Figure 3. Cladogram product of the analysis of *Fusarium* sequences coding for *calmodulin* gene based on maximum likelihood with 500 repetitions in bootstrap.
Figura 3. Cladograma producto del análisis de secuencias de *Fusarium* codificantes del gen *calmodulina* con base en máxima verosimilitud con 500 repeticiones en bootstrap.

Elongation factor

Phylogenetic analysis of the *elongation factor* gene coding region was based on 632 characters and a GTR+G+I substitution model. The cladogram for maximum likelihood showed a different topology, with seven monophyletic groups, five of them for *F. oxysporum*, one for *F. solani,* and a group with those species that shared the character of a coiled hyphae. This resolution coincides with other researchers (18, 26, 47, 48) who stated that phylogenetic analyses performed based on protein-coding markers, and especially the region for the *elongation factor*, had a higher resolution than other primers such as *calmodulin* and ITS (figure 4).

Pathogenicity tests

The 19 isolates inoculated in avocado plants were pathogenic and they varied in time, virulence, and severity of the disease. For virulence, the analysis of variance performed on the obtained data detected highly significant differences ($p \leq 0.05$). The time elapsed for the first symptoms to appear varied between species. For *F. solani,* it fluctuated between 51 and 56 days, whereas for *F. oxysporum* it ranged from 16 to 46 days (figure 5, page 311).

The disease severity determined in avocado seedlings inoculated with the 19 isolates of the two species of the genus *Fusarium* showed variability, which was reflected by reduced plant growth.

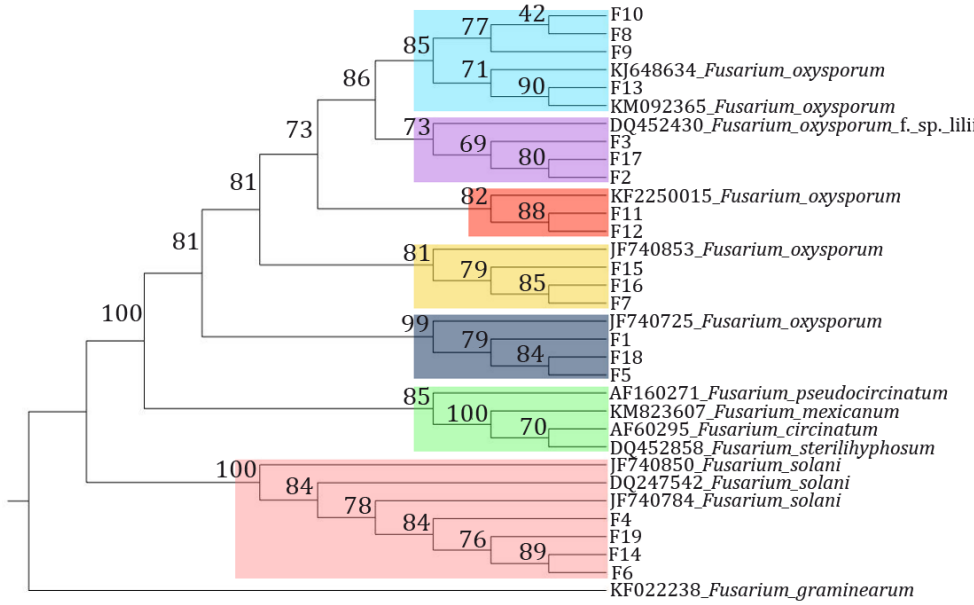

Figure 4. Cladogram representative of the analysis of sequences of *Fusarium* spp. coding for *elongation factor* gene realized based on maximum likelihood with 500 repetitions in bootstrap.

Figura 4. Cladograma representativo del análisis de secuencias de *Fusarium* spp. codificantes para el gen *factor de elongación* realizado con base en Máxima verosimilitud con 500 repeticiones en bootstrap.

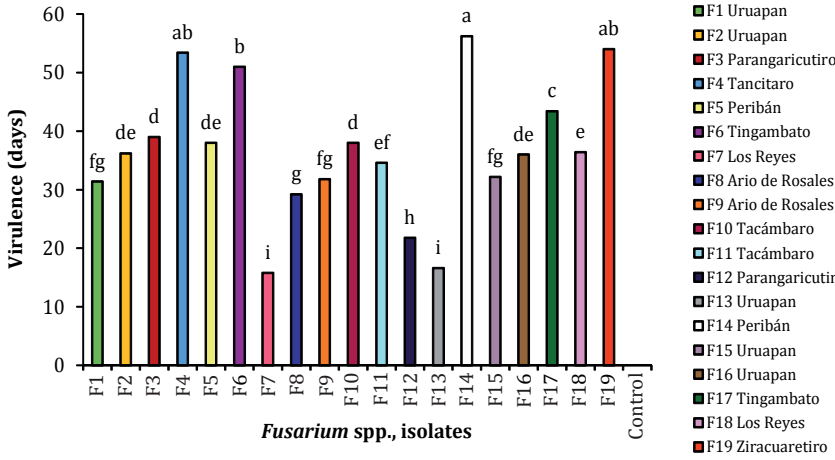

Figure 5. Virulence of the 19 *Fusarium* spp. isolates in avocado plants. Means with a different letter on a bar are statistically different (Tukey' s test p≤0.05).

Figura 5. Virulencia de 19 aislados de *Fusarium* spp. en plantas de aguacate. Medias con una letra diferente sobre cada barra son estadísticamente diferentes (Prueba de Tukey p≤0,05).

At the root level, the 19 isolates showed a reduction in volume with respect to the control, as well as a reddish-brown coloration in their tissue indicating necrosis (figure 6, page 312). These symptoms coincided with those reported in vanilla (22) and asparagus (11, 12), where *F. oxysporum* caused root rot in the former case, and root and crown rot in mature plants, seedlings, and young transplants in the latter case (8, 25). Similar symptoms were caused by *F. paranaense* belonging to the *F. solani* species complex in the root rot of soybeans (*Glycine max* (L.) Merrill). It has been reported that *F. solani* is secondary in citrus root rot because it does not cause plant death, despite colonizing a large part of the root, nevertheless it reduces the vigor of the plant (8), as noted in the present study.

In the aerial part of the plant, *Fusarium* isolates produced variability in the symptoms. Thus, in four isolates, yellowing was observed, in 12 apical wilting, and in three apical death compared with the control plant that remained healthy. These symptoms have previously been reported in other studies (4, 15, 22), which observed that *F. oxysporum* caused general decay, chlorosis, and necrosis in the stems. It has also been reported that *F. solani* in lisianthus (*Eustoma grandiflorum*) produced wilting, defoliation, and subsequent death of the plant (42). It has been reported that in a group of *Fusarium* species isolated from maize, *F. subglutinans* and *F. verticillioides* were the most pathogenic and produced similar symptoms to those observed initially in the present study (14).

The symptoms observed were yellowing, wilting, and apical necrosis. The most virulent isolate was F7, which was obtained in the municipality of Los Reyes, and corresponds to *F. oxysporum* Schl. This coincides with the results reported where *F. oxysporum* f. sp. *radicis-vanillie* caused yellowing and defoliation at 15 days after inoculation (22). *F. solani* has been shown to cause root rot at 35 days (8), which differed from that reported in other studies, where *F. solani* produced root and crown rot 2 weeks after inoculation (15).

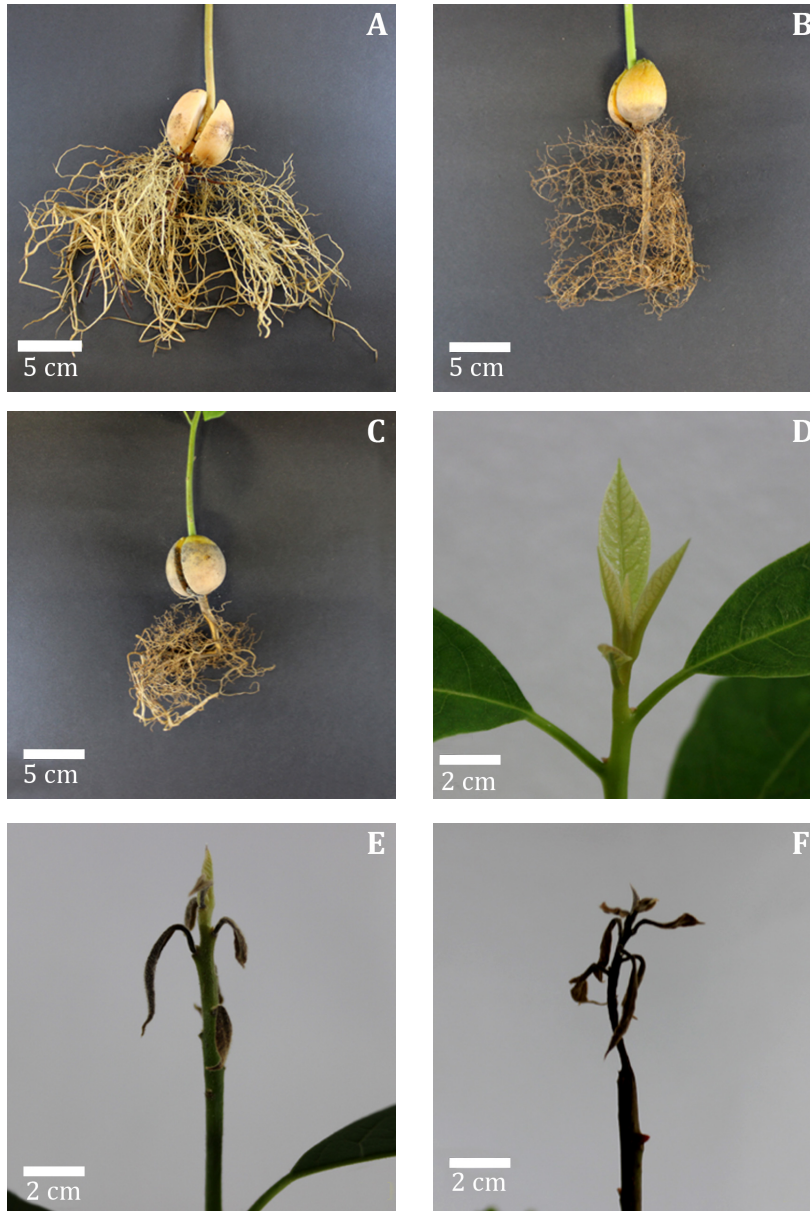

Figure 6. Symptoms observed in avocado root *Persea americana* Mill. *drymifolia* variety caused by the isolates of *F. oxysporum* and *F. solani*. (A) Healthy roots, (B) Reddish-brown coloration roots, (C) Apical necrosis, (D) Healthy plant, (E) Wilting of tips, (F) Death of seedling.

Figura 6. Síntomas observados en raíz de aguacate *Persea americana* Mill. variedad *drymifolia* ocasionados por las cepas de *F. oxysporum* y *F. solani*. (A) Raíces sanas, (B) Raíces de coloración marrón-rojiza, (C) Necrosis apical, (D), Planta sana, (E) Marchitamiento de puntas, (F) Muerte de plántula.

Conclusions

Cultural characterization and phylogenetic analysis derived from molecular characterization confirmed that 15 isolates belonged to *F. oxysporum* (F1, F2, F3, F5, F7, F8, F9, F10, F11, F12, F13, F15, F16, F17, and F18) and four to *F. solani* (F4, F6, F14, and F19).

The EF-1α obtained the best resolution for the identification of the *Fusarium* species.

F. oxysporum and *F. solani* produced root rot in avocado trees in the avocado belt of the state of Michoacan, Mexico.

F. oxysporum and *F. solani* were pathogenic in avocado plants (*P. americana* Miller *drymifolia* variety).

The species of *F. oxysporum* were more virulent than the ones from *F. solani* by producing root rot quickly, and thus yellowing, wilting, and apical necrosis in the aerial parts and finally plant death.

The F7 isolate, collected in the municipality of Los Reyes, was the most virulent and caused the death of avocado plants in 16 days. The isolates of *F. solani* produced less damage, with F4 and F14 only causing apical wilting.

The pathogenic variability exhibited by the *Fusarium* spp. isolates in avocado seedlings could help to establish strategies that enable the control and management of this phytopathogen more efficient for avocado cultivation in Michoacán, México, from the nursery stage to field plantations.

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