Phylogeny, distribution, and pathogenicity of fusarioid fungi associated with chickpea wilt in Sinaloa and Sonora, Mexico

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Research Article

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Abstract

Wilt disease complex, is the most important disease of chickpeas (*Cicer arietinum* L.) in the production areas from Mexico. Disease symptoms include root rot, yellowing, wilting, poor growth, discoloration of vascular tissues, and death of plants. This study aimed to identify the fusarioid species associated with chickpea wilt in northwest Mexico by the combination of phylogenetic analyses and morphological characterization, as well as to determine their pathogenicity and virulence on chickpea seedlings. A total of 80 isolates of fusarioid fungi were obtained from symptomatic plants of 16 chickpea fields distributed in Sinaloa and Sonora, Mexico. Subsequently, a subset of 41 isolates representing the range of geographic origin was selected for further morphological characterization, phylogeny, and pathogenicity tests. Phylogenetic analyses of partial fragments of the translation elongation factor 1-alpha (*tef1-α*) and RNA polymerase second largest subunit (*rpb2*) genes were used to determine the identity of 26 *Fusarium* isolates and 15 *Neocosmospora* isolates to species level. Pathogenicity tests were performed on chickpea seedlings (cv. Blanco Sinaloa) under greenhouse conditions. Phylogenetic analyses of 41 fungal isolates of this study allowed the identification of *Fusarium languescens* (51.2%), *Neocosmospora falciformis* (36.6%), *F. nirenbergiae* (7.3%), and *F. verticillioides* (4.9%). All fungal isolates were found to be pathogenic on chickpea seedlings and a significant difference in virulence was observed. To our knowledge, *F. languescens* and *F. nirenbergiae*, belonging to the *Fusarium oxysporum* species complex, as well as *F. verticillioides* belonging to the *Fusarium fujikuroi* species complex are recorded for the first time as causal agents of chickpea wilt in Mexico and worldwide.

Main text

Chickpea (*Cicer arietinum* L.) belongs to the Fabaceae family and is the third most important legume worldwide after beans (*Phaseolus vulgaris*) and peas (*Pisum sativum*). Likewise, chickpea is a vital source of edible protein in many developing countries (Faruk and Khatun, 2020). Mexico ranks as the twelfth largest producer in the world with a production of 171,968 tons, the main producing states being: Sinaloa, Michoacán, Sonora, Guanajuato and Baja California Sur (SIAP 2022).

In general, the disease known as wilt, caused by soil borne fungi, has been mainly associated with fusarioid genera such as *Fusarium* and *Neocosmospora*, which are the main limiting factors in chickpea production worldwide (Bekele et al. 2021). Symptoms of the disease can develop at any stage of plant growth and affect plants grouped in patches or appear spread across a field (Jiménez-Díaz et al. 2015; Jendoubi et al. 2017; Zhou et al. 2021). The early wilt and seedling collapse can be observed in susceptible genotypes within 25 days after sowing in the field (Jiménez-Díaz et al. 2015) but symptoms are more visible in the early stages of flowering, 6 to 8 weeks after sowing and can also appear up to podding stage. The typical symptoms are foliar yellowing to drying of the lower leaves followed by complete wilting of the plant (Jiménez-Díaz et al. 2015; Hale et al. 2020). The roots show a brown discoloration of the internal tissues that can be seen when they are split vertically or cross-sectioned (Jiménez-Díaz et al. 2015).

This disease reduces chickpea production due to decreased yield and grain weight. Losses can reach up to 100% of the total harvest in highly infested fields and under favorable conditions (Jendoubi et al. 2017). Chickpea wilt is present around the world and has been recorded causing severe losses in countries such as India, Burma, Bangladesh, Chile, Ethiopia, Iran, Mexico, Nepal, Pakistan, among others (Dhawale and Dhale 2021).

To date, *F. oxysporum* sensu lato and *F. solani* sensu lato have been commonly reported as the main pathogens causing chickpea wilt worldwide; however, other *Fusarium* species associated with chickpea wilt are *F. proliferatum*, *F. nygamai*, *F. phyllophilum*, *F. dlamini* (Duarte et al., 2016), *F. redolens* (Jiménez-Fernández et al. 2011; Bouhadida et al. 2017; Younesi et al. 2021; Moparthi et al. 2021; Zaim and Bekkar 2022; Armstrong-Cho et al. 2023), *F. equiseti* (Younesi et al. 2021; Zhou et al. 2021), *F. hostae, F. acuminatum* (Younesi et al. 2021), *F. poae* (Moparthi et al. 2021), *F. culmorum* (Moparthi et al. 2021; Zhou et al. 2021), *F. sporotrichioides* (Zhou et al. 2021), and *F. avenaceum* (Armstrong-Cho et al. 2023).

Fusarium oxysporum sensu lato and *Neocosmospora falciformis* have been reported as the species of fusaroid fungi affecting chickpea plants in Mexico (Fierros-Leyva et al. 2019; Velarde-Félix et al. 2022); however, detailed studies on the phylogenetic identity and virulence of the cryptic species belonging to the genera *Fusarium* and *Neocosmospora* have not been carried out. This study aimed to identify the fusarioid species associated with chickpea wilt in northwest Mexico by the combination of phylogenetic analyses and morphological characterization, as well as to determine their pathogenicity and virulence on chickpea seedlings.

Materials and methods

Sample collection

During the 2019 growing season, surveys were carried out in 16 commercial chickpea fields distributed in the main production area in northwest Mexico (Sinaloa and Sonora states). A total of 80 chickpea plants (cv. Blanco Sinaloa 92) showing root rot, yellowing, wilting, poor growth, discoloration of vascular tissues, and death of plants were collected.

Isolation, purification, and conservation of fungi

Fusarioid fungi were obtained using the procedures described by Crous et al. (2009). For isolation, pieces of roots (5 mm long) were taken from the margin between necrotic and healthy tissues, surface disinfested by dipping in 2% sodium hypochlorite solution (NaOCl) for 1 min, rinsed two times with sterile distilled water, and dried on sterilized paper. The pieces were placed in Petri plates with potato dextrose agar (PDA) (Difco, USA). The plates were incubated at 25 ºC for 4 days in darkness and then mycelial plugs (5 mm in diameter) from the edge of active growth of *Fusarium*-like colonies were transferred to Petri dishes with fresh PDA and incubated at 25°C for 10 days. Pure cultures were obtained by transferring single germinated conidia to fresh PDA under a dissecting microscope. The fungal isolates

used in the present study were deposited in the Culture Collection of Phytopathogenic Fungi at the Research Center for Food and Development (Culiacán, Sinaloa, Mexico). Mycelial plugs of the fungal isolates were maintained in sterile distilled water at 4°C and in 10% glycerol at -80°C.

DNA extraction, PCR amplification, and sequencing

For molecular identification of 41 isolates of fusarioid fungi, aerial mycelium (~50 mg) from 8-day-old culture was directly scraped from the medium using a sterile spatula and placed in 1.5-mL microtubes. Total genomic DNA was extracted according to the CTAB method (Doyle and Doyle 1990). The extracted DNA was re-suspended in 30 µL of nuclease-free water and stored at − 20 ºC until further use.

Partial fragments of the translation elongation factor 1-alpha (*tef1-α*) and RNA polymerase second largest subunit (*rpb2*) genes were amplified by PCR using the primers pairs EF1-728F/EF1-986R (Carbone and Kohn 1999) and RBP2-5F2/RPB2-7cR (Liu et al. 1999), respectively. The PCR conditions were as follows: an initial denaturation step at 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 30 s; annealing for 30 s at 54 and 57°C; extension at 72°C for 30 and 90 s for 728F/EF1-986R and RBP2-5F2/RPB2-7cR, respectively; and a final extension step at 72°C for 5 min. The PCR assays were conducted in a Bio-Rad C1000 thermocycler (Bio-Rad, USA). The PCR products were separated by electrophoresis in 1% agarose gel stained with ethidium bromide and viewed under ultraviolet light. The amplicons were purified and sequenced by Macrogen (Macrogen Inc., Seoul, Korea).

Phylogenetic analyses

The phylogeny was reconstructed by concatenated analyses from *tef1-α* and *rpb2* sequences datasets. DNA sequences were edited in BioEdit version 7.0.5.3. (Hall 1999) and compared in the NCBI nucleotide database. Alignments were produced with MUSCLE (Edgar 2004) implemented in MEGA 11 (Tamura et al. 2021), using reference sequences from type organisms of *Fusarium* spp. and *Neocosmospora* spp. The independent alignments of each locus were combined (*tef1-α* + *rpb2*) for phylogenetic inference. The best-fitting partitioning scheme for the combined alignment was selected using unlinked branch lengths, the greedy algorithm, and the Akaike Information Criterion (AIC) in PartitionFinder v 1.1.1 (Lanfear et al. 2012). Finally, the Maximum Likelihood phylogenetic analysis was carried out in RAXML v 7.2.8 (Stamatakis 2006), using the GTRGAMMAI model for each partition identified by PartitionFinder by 1000 bootstrap replications and all positions containing gaps were considered. The phylograms were edited by FigTree v.1.4.2 (Rambaut 2014). All sequences generated in this study were deposited in GenBank (Table 1).

Table 1
Origin of *Fusarium* and *Neocosmospora* isolates obtained from chickpea plants with wilt symptoms in Sinaloa and Sonora, Mexico.

Isolate code	Site	Origin (Municipality, State)	Collection date	GenBank accession number	
				tef1-α	*rpb2*
CCLF78	14	Guasave, Sinaloa	February 2019	OQ930594	OQ930557
CCLF79	5	Guasave, Sinaloa	February 2019	OQ930578	OQ930543
CCLF80	10	Salvador Alvarado, Sinaloa	February 2019	OQ930615	OQ930576
CCLF81	9	Salvador Alvarado, Sinaloa	February 2019	OQ930600	OQ930563
CCLF82	12	Guasave, Sinaloa	February 2019	OQ930590	OQ930553
CCLF85	16	Hermosillo, Sonora	June 2019	OQ930584	OQ930548
CCLF90	16	Hermosillo, Sonora	June 2019	OQ930585	OQ930549
CCLF91	7	Salvador Alvarado, Sinaloa	January 2019	OQ930580	OQ930545
CCLF95	13	Guasave, Sinaloa	February 2019	OQ930593	OQ930556
CCLF96	1	Culiacán, Sinaloa	January 2019	OQ930588	OQ930552
CCLF97	12	Guasave, Sinaloa	February 2019	OQ930592	OQ930555
CCLF99	15	Guasave, Sinaloa	February 2019	OQ930596	OQ930559
CCLF101	3	Angostura, Sinaloa	January 2019	OQ880416	OQ880414
CCLF102	16	Hermosillo, Sonora	June 2019	OQ930603	OQ930566
CCLF103	16	Hermosillo, Sonora	June 2019	OQ930604	-
CCLF105	16	Hermosillo, Sonora	June 2019	OQ930602	OQ930565
CCLF107	2	Mocorito, Sinaloa	January 2019	OQ930597	OQ930560
CCLF110	6	Salvador Alvarado, Sinaloa	January 2019	OQ930613	OQ930574
CCLF111	9	Salvador Alvarado, Sinaloa	February 2019	OQ930601	OQ930564
CCLF113	1	Culiacán, Sinaloa	January 2019	OQ880415	OQ880413
CCLF114	16	Hermosillo, Sonora	June 2019	OQ930605	OQ930567
CCLF115	16	Hermosillo, Sonora	June 2019	OQ930610	OQ930571
CCLF117	16	Hermosillo, Sonora	June 2019	OQ930583	OQ930547
CCLF118	5	Guasave, Sinaloa	January 2019	OQ930598	OQ930561
CCLF120	5	Guasave, Sinaloa	January 2019	OQ930579	OQ930544

Isolate code	Site	Origin (Municipality, State)	Collection date	GenBank accession number	
				tef1-α	*rpb2*
CCLF122	4	Salvador Alvarado, Sinaloa	January 2019	OQ930612	OQ930573
CCLF123	12	Guasave, Sinaloa	February 2019	OQ930591	OQ930554
CCLF125	16	Hermosillo, Sonora	June 2019	OQ930586	OQ930550
CCLF127	13	Guasave, Sinaloa	February 2019	OQ930611	OQ930572
CCLF128	15	Guasave, Sinaloa	February 2019	OQ930595	OQ930558
CCLF129	8	Salvador Alvarado, Sinaloa	January 2019	OQ930599	OQ930562
CCLF130	16	Hermosillo, Sonora	January 2019	OQ930608	OQ930569
CCLF133	16	Hermosillo, Sonora	June 2019	OQ930606	OQ930568
CCLF134	10	Salvador Alvarado, Sinaloa	February 2019	OQ930614	OQ930575
CCLF136	12	Guasave, Sinaloa	February 2019	OQ930589	-
CCLF137	11	Salvador Alvarado, Sinaloa	February 2019	OQ930616	OQ930577
CCLF138	16	Hermosillo, Sonora	June 2019	OQ930587	OQ930551
CCLF142	16	Hermosillo, Sonora	June 2019	OQ930582	-
CCLF144	16	Hermosillo, Sonora	June 2019	OQ930607	-
CCLF145	16	Hermosillo, Sonora	June 2019	OQ930609	OQ930570
CCLF146	16	Hermosillo, Sonora	June 2019	OQ930581	OQ930546

Morphology

For morphological characterization, two isolates of each species were selected as representatives based on preliminary phylogenetic analyses. *Fusarium* and *Neocosmospora* isolates were incubated at 25°C with a 12-h photoperiod for 15 days on PDA (Difco, USA) and synthetic nutrient agar (SNA) media (Leslie and Summerell 2006) to examine the shape and size of macro, microconidia, and chlamydospores using an Axio Imager M2 microscope (Zeiss, Germany). Images were documented using an Axiocam 305 (Zeiss, Germany) and processed using ZEN 2.3 SP1 imaging software (Zeiss, Germany). For each isolate, three replicates were used and radial growth was measured after 10 days of incubation at 25°C in the dark. The colony diameter of isolate was measured perpendicularly in two directions. The experiment was repeated once.

Pathogenicity and virulence tests

The pathogenicity of 26 *Fusarium* and 15 *Neocosmospora* isolates was verified by inoculating chickpea seedlings of a susceptible genotype (cv. Blanco Sinaloa-92) under greenhouse conditions. For inoculum preparation, each fungal isolate was cultured on SNA medium at 25°C for 12 days. The mycelial growth and conidia were scraped with a slide, placed in sterile distilled water, and liquefied for 10 s using a waring blender. The inoculum suspension was adjusted to a concentration of 1×10^5 conidia mL^{-1} and Tween 20® was added.

Chickpea seeds were sown in 128-cavity polystyrene trays containing an autoclaved mixture of peat moss and sand (2:1). Seedlings were regularly watered to keep the growth substrate in a wet condition. Fifteen-day-old chickpea seedlings were carefully removed from their cavities. The roots were washed with sterile distilled water and inoculated by immersion of roots in the spore suspension for 30 min. Once the time had elapsed, the seedlings were placed again in trays with sterile substrate and kept in a greenhouse at a temperature of 25 to 35°C. Each isolate was inoculated on seven plants and the experiment was repeated twice. The roots of 10 control seedlings were immersed in sterile distilled water.

The observation of symptom progress was performed daily and the evaluation of disease severity was carried out 30 days after inoculation using a 5-category visual scale, where 0 = no visible symptoms, 1 = less than 25% foliage diseased, 2 = 25 to 50% foliage diseased, 3 = 50 to 75% foliage diseased, 4 = more than 75% foliage diseased. The scale values were transformed to percent values and virulence assay data were analyzed. Normality and homogeneity of variances were first checked according to Kolmogorov-Smirnov and Levene tests, respectively. Variances of the two experiments were not statistically different for each test; therefore, the raw data for the two repeats of each experiment were combined for subsequent analysis. Then, data were subjected to analyses of variance (ANOVA) and means were compared by Fisher's least significant difference (LSD) test, at 5% probability using PROC GLM in SAS (version 9.3; SAS Institute, Cary, NC). Uninoculated controls were excluded from statistical analysis.

Results

Fungal isolation

Chickpea wilt is a prevalent disease in northwest Mexico and symptoms were observed in all chickpea fields that were sampled in this study. A total of 80 fungal isolates were obtained from symptomatic chickpea roots collected in 16 commercial fields distributed in the states of Sinaloa ($n = 15$) and Sonora ($n = 1$) in Mexico. Subsequently, 41 isolates were selected as representatives and were included in the phylogenetic analyses and pathogenicity tests (Table 1).

Phylogenetic analyses

The phylogenetic analyses inferred under the Maximum Likelihood criterion provided sufficient information to distinguish two *Fusarium* species (*F. languescens* and *F. nirenbergiae*) belonging to the

Fusarium oxysporum species complex (Fig. 1), one *Fusarium* species (*F. verticillioides*) belonging to the *Fusarium fujikuroi* species complex (Fig. 2), and to *Neocosmospora falciformis* (Fig. 3) associated with chickpea wilt in northwest Mexico.

Morphology

Fusarium languescens isolates exhibited floccose colonies with abundant aerial mycelium and pigmentation varied from white to pale rosy on PDA (Fig. 4). On SNA, aerial mycelium was sparse with abundant sporulation. Macroconidia were falcate, hyaline, dorsiventrally curved with almost parallel sides tapering slightly towards both ends, 1 to 3–septate, of 21.5–35.8 × 3.1–4.2 µm. Microconidia were hyaline, falcate to ellipsoidal, aseptate or 1–septate, of 5.1–8.1 × 2.5–3.3 µm, and forming small false heads on the tips of the phialides. Chlamydospores were globose to subglobose, and formed terminally.

Fusarium nirenbergiae isolates showed cottony colonies with abundant light purple aerial mycelia and its pigmentation varied from white to violet color on the underside of PDA (Fig. 4). On SNA, macroconidia were hyaline, of 19.6–45.4 × 3.5–4.9 µm, with 2 to 5–septate, most commonly had three septa, with curved apical cells and foot-shaped basal cells. Microconidia were oval, aseptate or 1–septate, of 6.2–11.7 × 2.3–4.2 µm, and arranged in small false heads on short monophialides. Chlamydospores were aseptate, hyaline to light brown, globose and were produced terminally or intercalary.

Fusarium verticillioides isolates exhibited cottony colonies and its pigmentation was initially white but with violet pigments with age on PDA (Fig. 4). Macroconidia were slightly falcate to almost straight, hyaline, 3 to 5–septate and measuring 30.8–52.2 × 3.4–4.3 µm. Microconidia were oval to club shaped, hyaline, aseptate, of 3.9–16.3 × 2.6–4.5 µm, and developed in long chains.

Neocosmospora falciformis isolates exhibited white-greyish to pale-cream colonies on PDA (Fig. 4). Macroconidia were slender, falcate, hyaline, 3 to 5–septate, of 35.4–47.9 × 3.5 to 5.0 µm. Microconidia were ellipsoidal to reniform, sometimes with a truncate base, and 0 to 1–septate, of 9.0 to 12.3 × 2.8 to 5.2 µm. Chlamydospores were globose to subglobose, formed singly or in short chains, intercalary and thin- to thick-walled.

Distribution of fungal species

Fusarium languescens was the most frequently identified species (51.2%), followed by *Neocosmospora falciformis* (36.6%), *F. nirenbergiae* (7.3%), and *F. verticillioides* (4.9%) from the 41 isolates of fusarioid fungi obtained from chickpea symptomatic samples. The distribution of fusarioid species varied among the isolates obtained from chickpea fields in the two states in northwest Mexico (Fig. 5). *Fusarium languescens* was found in chickpea fields from both states (Sinaloa and Sonora). *Fusarium nirenbergiae* and *F. verticillioides* were only recorded in Sinaloa. Whereas, *N. falciformis* was identified in the states of Sinaloa and Sonora.

Pathogenicity tests

All isolates of *Fusarium* spp. and *Neocosmospora falciformis* were pathogenic in chickpea plants. Inoculated plants developed symptoms of root rot and yellowing, whereas control plants remained symptomless. Fungal colonies were re-isolated from all symptomatic plants and were found to be morphologically identical to the original isolates inoculated on chickpea seedlings, thus fulfilling Koch's postulates. In addition, there were significant differences ($P \leq 0.05$) in disease severity produced by the different fungal isolates tested, and variability in the virulence of the isolates was observed (Fig. 6). Among the fusarioid species tested in this study, *F. languescens* and *N. falciformis* were most virulent species, followed by *F. nirenbergiae* and *F. verticillioides* (Fig. 6).

Discussion

The phylogenetic analyses using *tef1-α* and *rpb2* sequences dataset provided sufficient information to identify four fusarioid species, including *Fusarium languescens, F. nirenbergiae*, *F. verticillioides*, and *Neocosmospora falciformis.* In this sense, some studies have identified *Fusarium* and *Neocosmospora* species based on multilocus phylogenetic analysis using sequence dataset of the *Cal, tef1-α, IGS, rpb2* and *tub2* markers; however, it has been shown that the *tef1-α* and *rpb2* sequences are those that provide the best phylogenetic resolution to discriminate cryptic species within both genera of fusarioid fungi (Crespo et al. 2019; Lombard et al. 2019; Zhou et al. 2021; Crous et al. 2021; Mirghasempour et al. 2022).

In this study, *F. languescens* was the most frequently isolated and distributed species in symptomatic samples from the three states of Mexico. Likewise, all the cultural and morphological characters of the two representative isolates coincided with those reported by Lombard et al. (2019). This fungal species was first associated with tomato wilt (*Solanum lycopersicum*) in Morocco, Israel and the Netherlands, as well as in maize in South Africa (Lombard et al. 2019). Therefore, to our knowledge, this is the first worldwide report of *F. languescens* as a chickpea pathogen.

Fusarium nirenbergiae was described by Lombard et al. (2019) and isolated from carnation (*Dianthus caryophyllus*) in the Netherlands. The morphological characters observed in our study clearly agree with those reported by Lombard et al. (2019) and Aiello et al. (2021). Likewise, the three isolates of *F. nirenbergiae* used in our study were pathogenic in chickpea plants and caused wilting. Similarly, wilt symptom caused by *F. nirenbergiae* has also been reported in passion fruit (*Passiflora edulis*) in Italy, in common bean (*Phaseolus vulgaris*) in Brazil, as well as yam (*Dioscorea polystachya*), a maple-like tree (*Acer negundo*) and rot corm in saffron (*Crocus sativus*) in China (Zhao et al. 2019; Dongzhen et al. 2020; Aiello et al. 2021; De Carvalho et al. 2022; Mirghasempour et al. 2022; Mirghasempour et al. 2022). Until now, little information is available on the pathogenicity and host range affected by *F. nirenbergiae* isolates. To the best of our knowledge, this is the first report of *F. nirenbergiae* causing wilt symptoms in chickpea in Mexico and worldwide.

In the present study, two isolates of *F. verticillioides*, belonging to the FFSC were identified. This complex is one of the best studied and contains more than 60 phylogenetically distinct species recognized and distributed in different ecologies (Yilmaz et al. 2021). The characteristics of the *F. verticillioides* colonies coincided with those described by Guo et al. (2021); while, the features of microconidia and macroconidia were similar to those reported by Yilmaz et al. (2021). Several studies have recorded *F. verticillioides* causing wilt symptoms in yams, root rot in tobacco and wilt in guava (Dongzhen et al. 2020; Gai et al. 2021; Gangaraj et al. 2021); however, this is the first report of *F. verticillioides* as the causal agent of chickpea wilt in Mexico and worldwide.

Based on the phylogenetic analysis, 15 isolates included in this study were identified as *N. falciformi*s. Morphological features of the two representative isolates were consistent with the description of *N. falciformis* (Sandoval-Denis et al. 2019). *Neocosmospora* (previously named *F. solani* species complex) is a genus that can be morphologically recognized by both sexual and asexual morphs, exhibit generally consistent ecological behavior, lack trichothecene mycotoxins, and form a strongly supported monophyletic group with more than 75 phylogenetic species (Crous et al. 2021). In Mexico, *N. falciformis* was already known as a pathogen of chickpea (Velarde-Felix et al. 2022), as well as causing root rot and wilt symptoms in several crops such as lima bean (*Phaseolus lunatus*) (Sousa et al. 2017), onion (Tirado-Ramirez et al. 2018), tomato (Vega-Gutierrez et al. 2019a), papaya (Vega-Gutierrez et al. 2019b), maize (Douriet-Angulo et al. 2019), tomatillo (*Physalis ixocarpa*) (Ayala-Armenta et al. 2020), and common bean (Diaz-Najera et al. 2021).

Overall, this study provides the first overview about phylogeny, biodiversity, and pathogenicity of fusarioid species associated with chickpea wilt in Sinaloa and Sonora, Mexico. *Fusarium languescens* and *F. nirenbergiae*, belonging to the FOSC, as well as *F. verticillioides* belonging to the FFSC are recorded for the first time as causal agents of chickpea wilt in Mexico and worldwide. Further studies on fungicide seed treatment, biocontrol agents, as well as on the response of chickpea cultivars to pathogenic fusarioid fungi are needed to establish effective strategies for the integrated management of wilt disease in chickpea fields in Mexico.

Declarations

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Author contribution Conceptualization: CICB, RSGE, and JMTP; Design and methodology: CICB, RSGE, JLF, VVH, and JMTP; Experiment execution and analysis: CICB, GAMR, KYLM, and JMTP; The first original draft preparation: CICB; Writing, rewriting and editing: CICB and JMTP; supervision: JMTP; all authors read and approved the final manuscript.

Data availability The data that support the findings are available from the corresponding author upon reasonable request.

Conflict of interest The authors have no conflicts of interest to declare.

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Figures

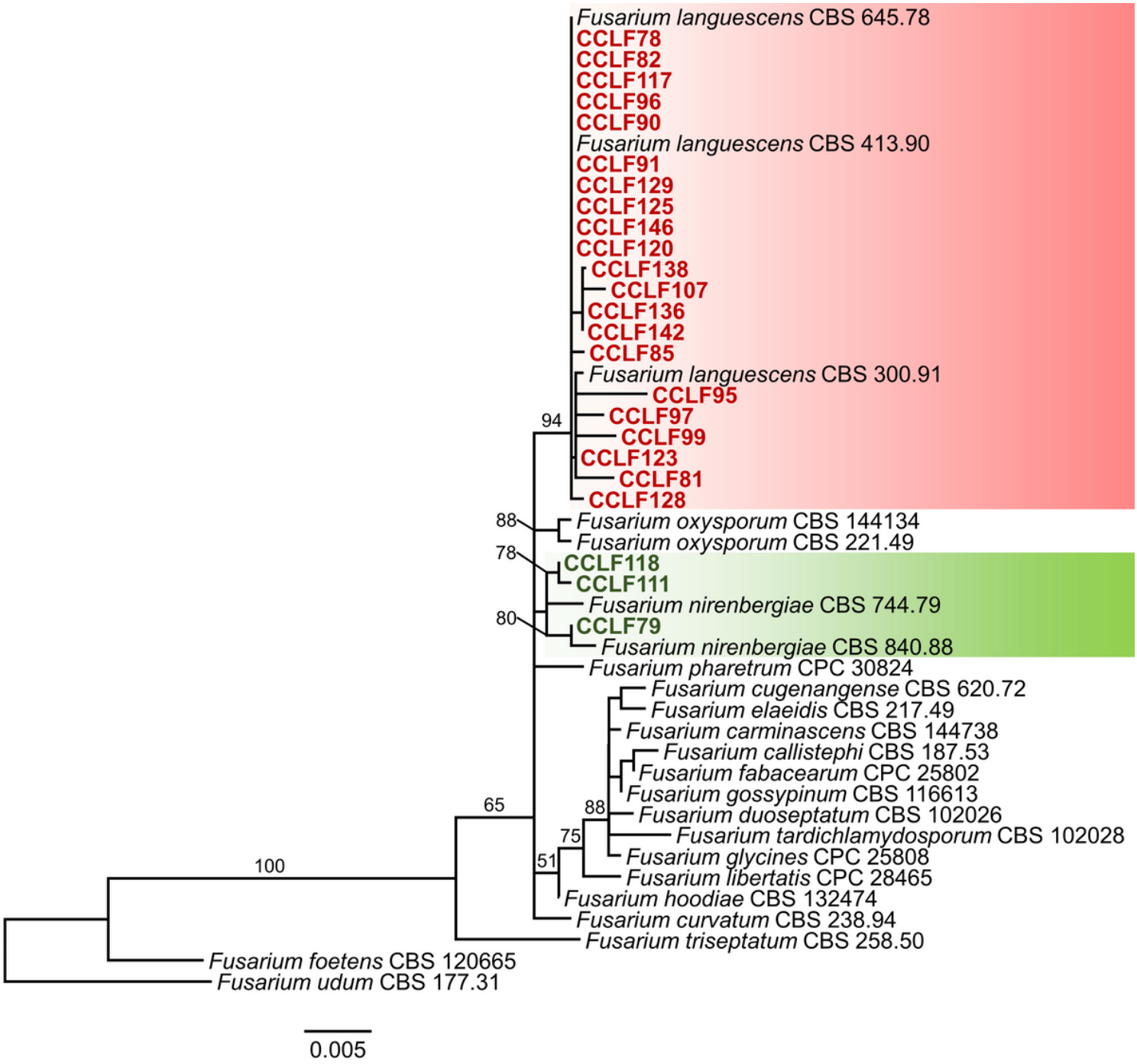

Figure 1

Maximum likelihood phylogenetic tree inferred from a combined alignment of *tef1-α* and *rpb2* sequence dataset for isolates of the *Fusarium oxysporum* species complex. The tree was rooted to *Fusarium foetens* CBS 120665 and *F. udum* CBS 17731. Values above the branches represent bootstrap support values (>50%). Isolates from this study are indicated in red and green.

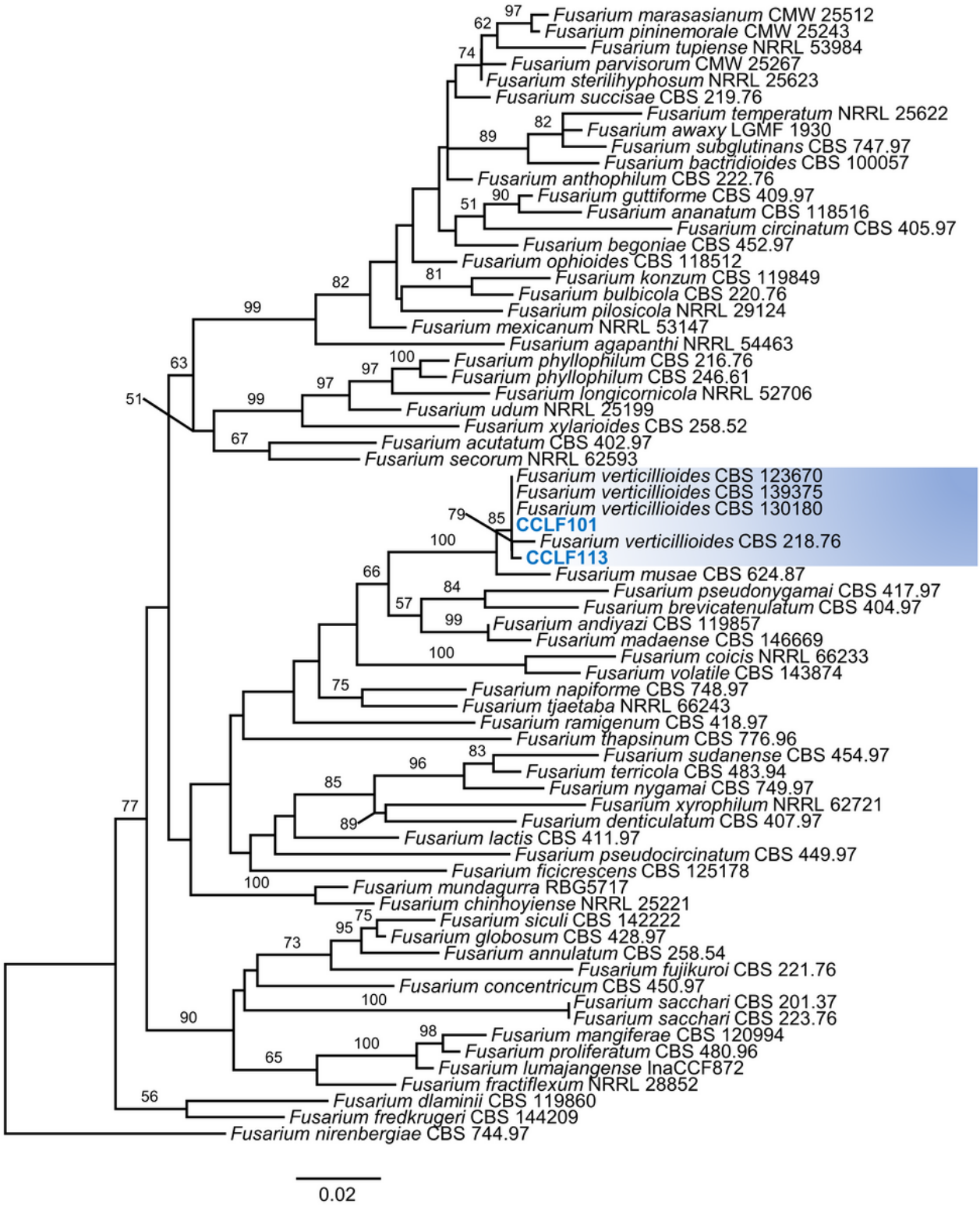

Figure 2

Maximum likelihood phylogenetic tree inferred from a combined alignment of *tef1-α* and *rpb2* sequence dataset for isolates of the *Fusarium fujikuroi* species complex. The tree was rooted to *Fusarium nirenbergie* CBS 744.97. Values above the branches represent bootstrap support values (>50%). Isolates from this study are indicated in blue.

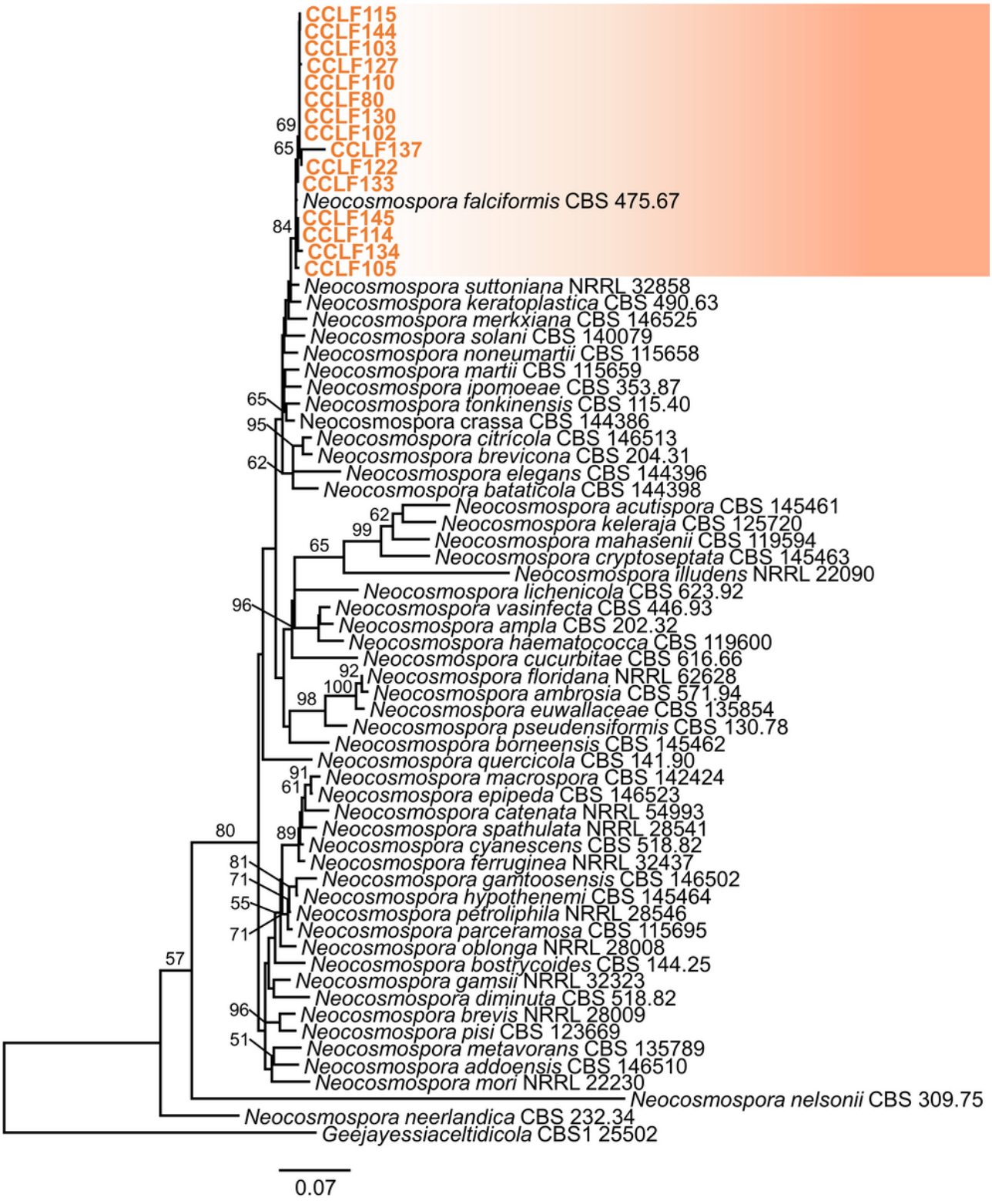

Figure 3

Maximum likelihood phylogenetic tree inferred from a combined alignment of *tef1-α* and *rpb2* sequence dataset for isolates of *Neocosmospora* spp. The tree was rooted to *Geejayessia celtidicola* CBS 125503. Values above the branches represent bootstrap support values (>50%). Isolates from this study are indicated in orange.

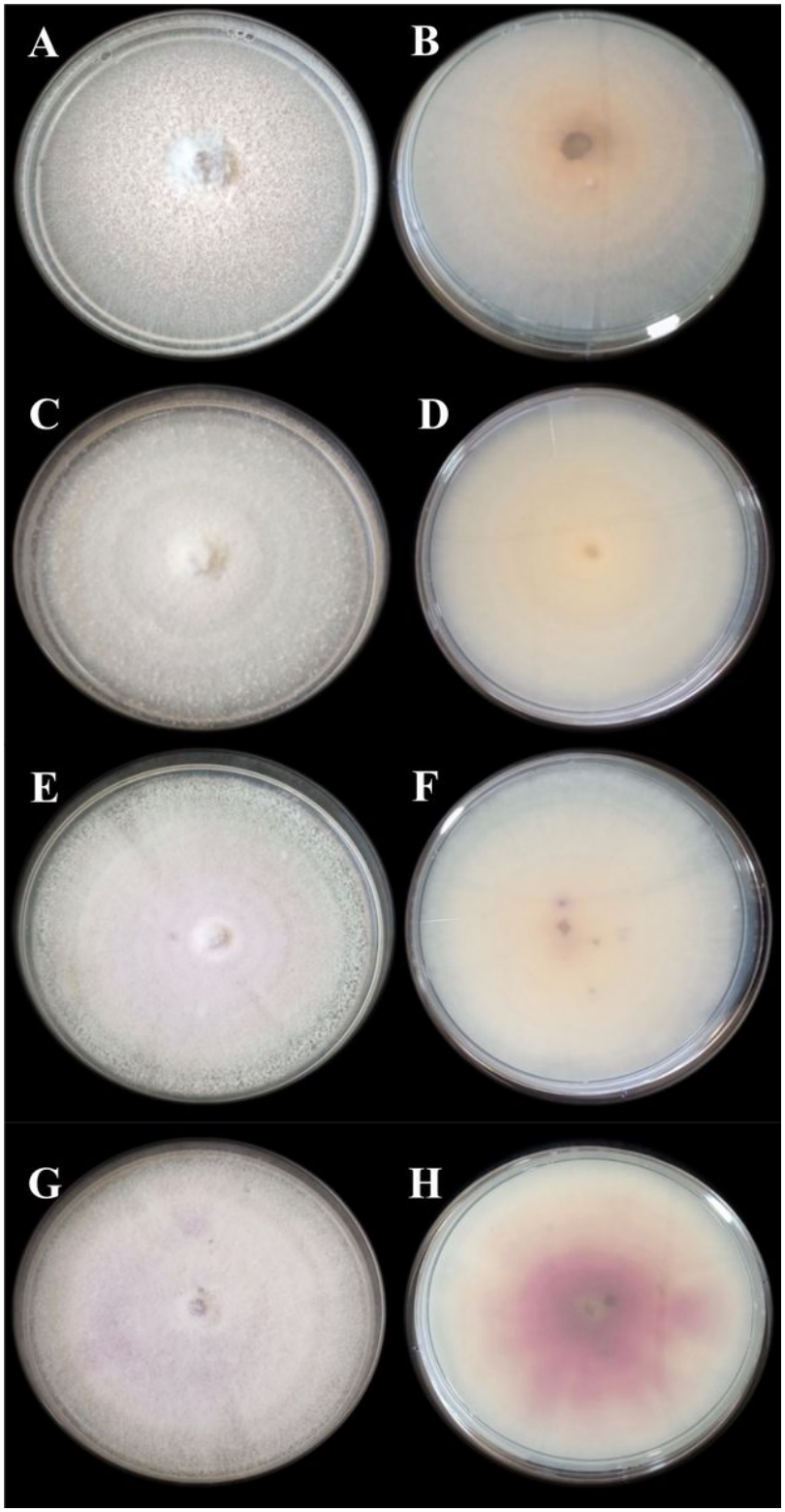

Figure 4

Colony morphology of four fusarioid species after 9 days incubation at 25 °C under continuous dark (Left = upper view of colony; Right = reverse view of colony). (**A–B)** *Fusarium verticillioides* **(C–D)** *Neocosmospora falciformis.* **(E–F)** *F. languescens.* **(G–H)** *F. nirenbergiae*

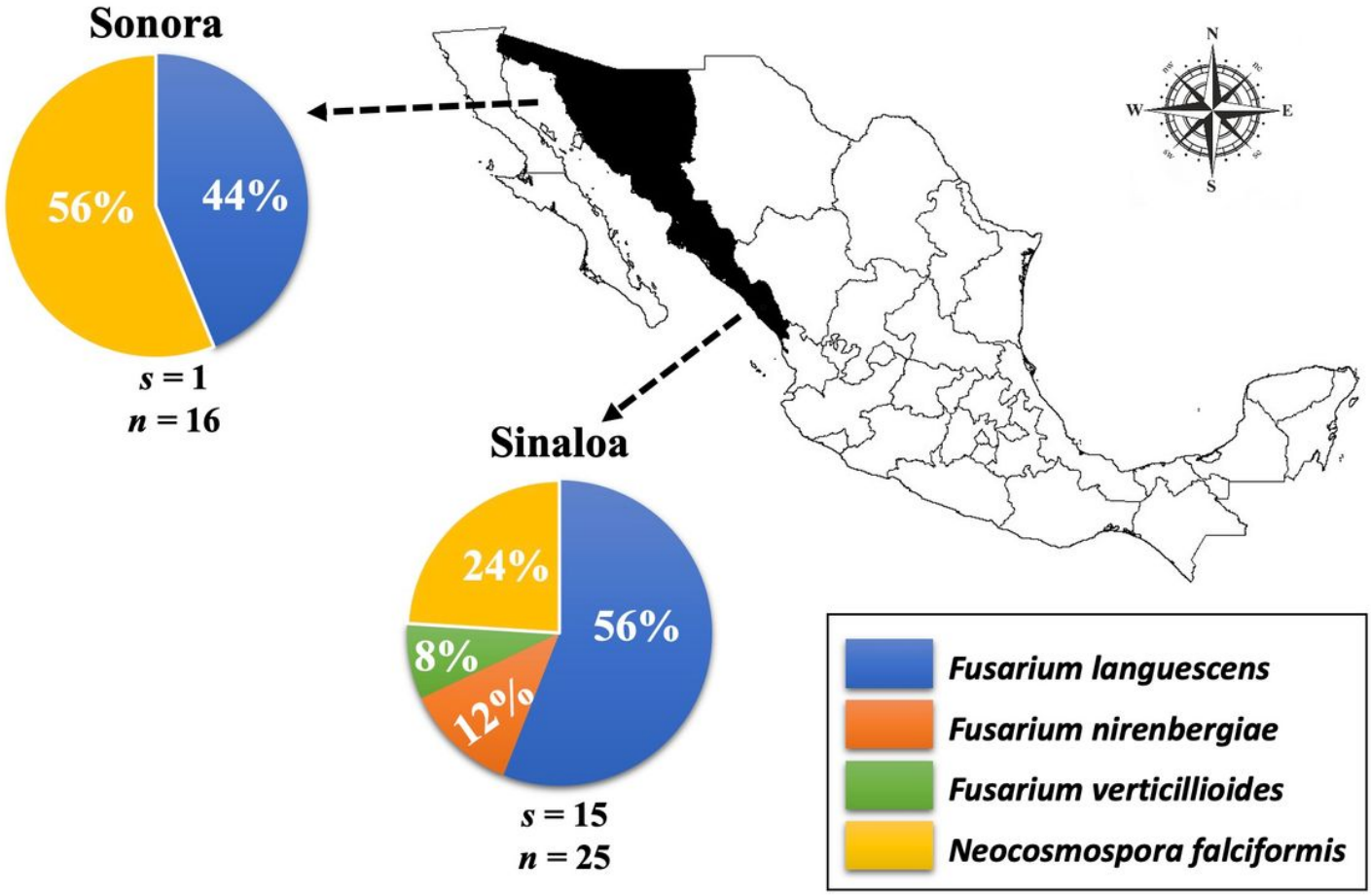

Figure 5

Collection sites of fusarioid fungi associated with chickpea wilt in Mexico. Circles represent association frequency of each species with chickpea plants showing symptoms of wilting in each population sampled, "*s*" is the number of commercial fields sampled in each population and "*n*" is the number of isolates analyzed in each population.

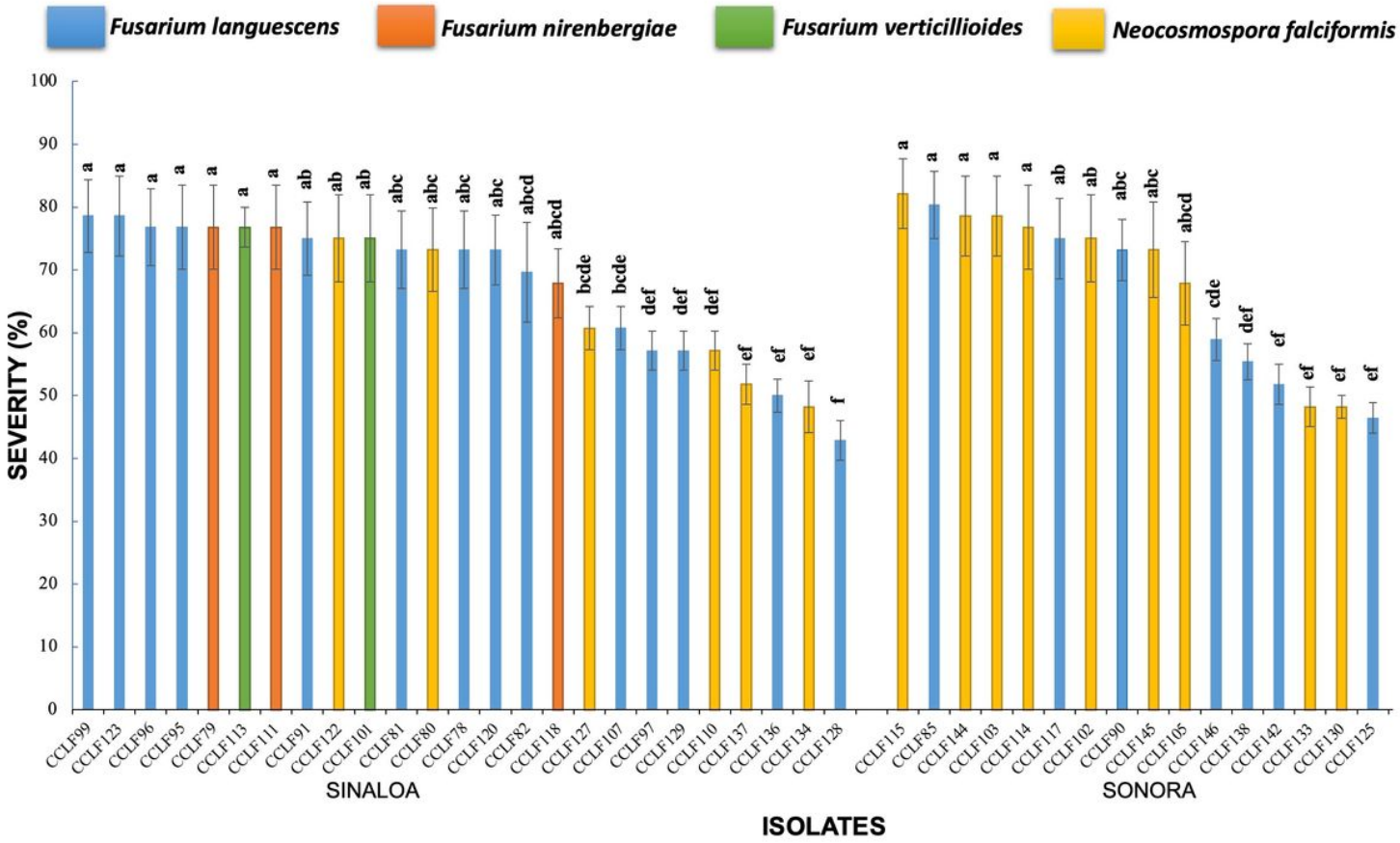

Figure 6

Disease severity caused by fusarioid fungi associated with chickpea wilt in Sinaloa and Sonora, Mexico, 30 days after inoculation onto roots of chickpea cv. Blanco Sinaloa. Error bars represent standard error. Columns with the same letter do not differ significantly, according to Fisher's LSD test ($P \leq 0.05$).