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Diversity and taxonomy of *Fusarium solani* isolated of wilted *Agave tequilana* var. azul plants

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ABSTRACT

Background/Objective. The objective of this work was to identify 24 strains of *F. solani* isolated from agave with wilt, with respect to the new phylogenetic species; determine their molecular similarity to *F. solani* f. spp.; determine their genetic diversity and their pathogenic capacity in agave, bean and corn.

Materials and Methods. Sequences of the ITS1-5.8S-ITS2 fragment of 24 agave isolates and those of *F. solani* f.spp., were compared with GenBank and FUSAROID-ID. Amplified 18S rRNA sequences were aligned with sequences reported of *F. solani* f. spp. *phaseoli* and *batatas*, defining the presence of introns. Genetic diversity was determined with the DNA RepPCR marker. Representative strains were tested against agave, bean and maize seedlings, evaluating their pathogenicity as root rot severity.

Results. Isolates morphologically identified as *F. solani*, GenBank placed them as *F. solani* or included in the FSSC, three strains were identified as *Xenoacremonium* sp. FUSAROID-ID defined that the sequences of *F. solani* were highly similar to those of *Neocosmospora martii*, *N. pseudoradicicola*, *N. solani* and *N. falciformis*. The ITS1-5.8S-ITS2 sequences and absence of introns in its SSU indicated that none is *F. solani* f. sp. *phaseoli*. Isolates obtained from agave were pathogenic to *A. tequilana* and a criollo corn cv, but not to *Fusarium*-resistant corn. No agave isolates were pathogenic to beans.

Conclusions. Four phylogenetic species of FSSC cause root rot in agave; *F. solani* isolates from agave did not affect *Fusarium*-resistant corn. It is safe to intercrop beans in agave.

Keywords: *Neocosmospora*, Rep-PCR, f. sp. *phaseoli*.

Introduction

Tequila is considered the quintessential Mexican alcoholic beverage. In 2022, 2.61 million tons of agave (*Agave tequilana* var. azul) stems "piñas", its essential raw material, were used, reaching a historical production volume of 651 million liters of tequila, of which 64% was exported (CRT, 2023). The crop requires six years to mature and, in that time, it is susceptible to agave wilt, a disease in which *Fusarium oxysporum* causes a vascular wilt or *Fusarium solani* induces a rot in the roots, lethal in maturity, and as such reduce production drastically (Aceves, 2003; Avila-Miranda *et al.*, 2010; Ramírez-Ramírez *et al.*, 2017). *F. solani* is a necrotrophic fungus, which forms long monophialides with non-catenulated apical microconidia and *Fusarium* macroconidia (Leslie and Summerell, 2006; Schroers *et al.*, 2016), although phylogenetic analyses indicated that members of that species made up the *Fusarium solani* species complex (FSSC), diverse and without a specific typification (Coleman *et al.*, 2009; Nalim *et al.*, 2011; O'Donnell, 2000; O'Donnell *et al.*, 2008; Short *et al.*, 2013; Zhang *et al.*, 2006). Therefore, in order to stabilize the taxonomy and nomenclature of the FSSC, phylogenetic species were recently recognized which were included in the genus *Neocosmopora* (Sandoval-Denis *et al.*, 2019, Crous *et al.,* 2021) or in the genus *Fusarium* (Geiser *et al.,* 2021). However, the plant pathogenicity of the FSSC, continues to be ambiguous, since, despite eleven *formae speciales* (f. spp.) called *phaseoli*, *pisi*, *cucurbitae*, *batatas*, *radicicola*, *robiniae*, *mori*, *piperis*, *eumartii*, *xanthoxyli* and *glycines* (Snyder and Hansen, 1941; McClure, 1951; Sakurai and Matuo, 1959; Sakurai and Matuo, 1961; Matuo and Sakurai, 1965; Roy *et al.*, 1989), having been described for over 50 years with a specific pathogenic capacity to a narrow group of species, in 2017, around 1,000 pathogenic relations with *F. solani* were reported with over 500 species of host plants (Farr and Rossman, 2017).

As a strategy to manage wilt in agave when it increases to anti-economic levels, crop rotation is carried out, with maize (*Zea mays*) being the main alternating group, although there are reports of severe epidemics, even after years of rotation. Likewise, the planting of beans (*Phaseolus vulgaris*) is recommended as a crop between rows in the first years to improve weed management and anticipate an economic benefit in the long cycle of the agave (Herrera-Pérez, 2017). Considering the lack of information of *F. solani* as a phytopathogen of *Agave tequilana*, the aims of this study were to define the taxonomy of 24 strains identified morphologically as *F. solani*, isolated from agave plants with wilt and symptoms of root and crown rot. Conserved fragment of the rRNA ITS-5.8S-ITS2 were sequenced and compared

into the FUSAROID-ID data base to locate them taxonomically, considering their similarity of sequences with the new phylogenetic species reported from the *Fusarium solani* species complex (FSSC). With these same sequences, an attempt was made to relate 24 agave strains with the 11 *F. solani* f. spp. to define, based on the presence of introns in the 18S rDNA (SSU) subunit, its molecular similarity to f. sp. *phaseoli*, which is pathogenic to beans. In addition, an attempt was also made to define the genetic diversity of the complete genome, using Rep-PCR fingerprints of repeated fragment amplification, and from the analysis of this diversity, determine the pathogenic capacity of representative strains on agave, maize and bean plants in order to confirm or define their host status.

Materials and Methods

Sampling symptomatic agave plants

Nine commercial fields in nine municipal areas in Jalisco, Mexico were sampled, in addition with another field with *A. americana* with similar symptoms in Comitán, Chiapas, Mexico. The sampling was aimed to obtain agave plants with symptoms of wilt and reddish necrosis in the base of the stem and crown (Figure 1 A and B), in order to obtain *Fusarium solani* isolations.

Fragments of healthy internal tissue, adjacent to necrotic tissue from the base of the stem or the crown, were obtained (Figure 1B). The fragments were disinfected in a mixture of chlorine, alcohol and sterilized water (1:1:8, v/v/v) for 1.5 min. and planted in Petri dishes with a potato-dextrose-agar (PDA) culture medium, incubated at 28 ºC for seven days. Fungi with long conidiophores with

Figure 1. Appearance of plants from which the *Fusarium solani* strains were isolated A) *Agave tequilana* var. azul plant, with symptoms of agave wilt. B) Typical reddish necrotic tissue in the crown and the base of the stem.

a single microconidium at the apex were selected, preferably with *Fusarium*-type macroconidia (Figure 2 A and B) (Booth, 1971; Leslie and Summerell, 2006).

To generate mycelia, the isolations were grown on sweet sterile cellophane, placed on PDA and incubated for ten days at 27 °C. The mycelium was cryofractured in liquid nitrogen and the DNA was extracted using the Zymo Research Plant/Seed DNA MiniPrep™ protocol, Cat. No. D6020. Its integrity and purity were confirmed by electrophoresis on a 0.8% agarose gel stained with SYBRSafe™ Invitrogen DNA gel stain, visualized using a Dark Reader™ blue light transilluminator.

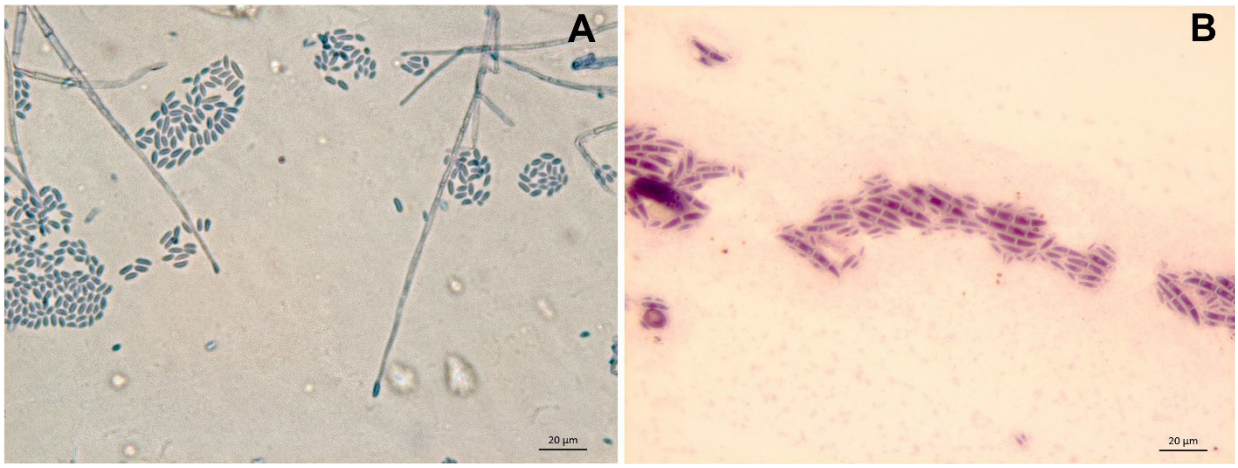

Figure 2. Microphotograph of the main morphological characteristics used to identify *Fusarium solani* isolations obtained from necrotic tissue in the crown or base of the stem of *Agave tequilana* var. azul plant. A). Long conidiophores with only one microconidium. B) *Fusarium* macroconidia.

Using DNA molecular markers

The following were used as DNA molecular markers: The fragment ITS1-5.8S-ITS2, obtained with the primers ITS1 (5'-TCCTCCGCTTATYGATATGC-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), and the small subunit of rDNA (SSU) amplified with the primers NS1 (5'-GTAGTCATATGCTTGTCTC-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') (White *et al.*, 1990). The PCR reaction was performed in a 50 µL cocktail containing 30 µL of deionized water, 10 µL of 5X MyFi™ buffer reaction (1 mM of dNTPs and 3 mM of $MgCl_2$), 2 µL (1 µL for each primer at 20 µM), 2 µL of MyFi™ Bioline™ polymerase and 6 µL of the DNA template. Amplification was carried out in a Gradient Palm thermocycler (Corbett Life Sciences™) with the following amplification program: An initial denaturation at 95 ºC for 2 min; 30 cycles with denaturation at 94 ºC for 1 min, annealing at 61 ºC for 2 min and an extension at 72 ºC for 2 min and a final extension at 72 ºC

for 6 min. The amplifications were visualized in a 1% agarose gel, stained with SYBERsafe™ and photo-documented with a Canon Powershot™ A620 camera. The size of the amplifications was determined by comparing them with the Bioline™ HyperLadder 100bp plus DNA marker. The amplified fragments were sequenced in the National Laboratory of Genetics of Biodiversity of the CINVESTAV (Irapuato, Guanajuato, Mexico).

To define the molecular taxonomic location of the strains, the ITS1-5.8S-ITS2 fragment of each strain was compared with the nucleotide collection (nr/nt) of the GenBank, and reports were obtained of highly similar sequences with the Mega BLAST tool. In addition, the sequences of this same tool were obtained, which were added to the GenBank by O'Donnell *et al.* (2008), Lombard *et al.* (2015), Rupe *et al.* (2001), Suga *et al.* (2000b) and Zaccardelli *et al.* (2008) of the 11 f. spp. of *F. solani* reported to date: *batatas, cucurbitae*, *eumartii*, *glycines, mori*, *phaseoli, piperis, pisi*, *radicicola*, *robiniae* and *xanthoxyli*. All these sequences were compared in the FUSARIOID-ID database (https://www.fusarium.org/page/Pairwise%20ID) to associate them to phylogenetic species defined from the FSSC (Crous *et al.*, 2021). Using the program Mega 11, a phylogenetic tree was obtained grouped with the UPMGA method, defining its evolutionary distance with the Maximum Composite Likelihood method (Tamura *et al.*, 2021).

To analyze the SN5-SN8 fragment included in the SSU sequence of the isolated agave strains, they were aligned using the program Mega 11 (Tamura *et al.*, 2021), thus aligning the sequences of the fragment SN5-SN8 of three f. sp. *phaseoli* strains with Gen Bank accession numbers AF150481, AF150482, AF150483 and one of the f. sp. *batatas* with accession number AF150485 (Suga *et al.*, 2000a). This strategy was carried out to verify, in the alignments, the presence of introns in this fragment of the SSU in f. sp. *phaseoli*, and to determine their absence in f. sp. *batatas*, considering that this is a trait that remains in f. spp of *F. solani*, which helps in its molecular identification (Suga *et al.*, 2000a). In this way, the presence of introns and the insert length in base pairs could be determined in the *F. solani* strains obtained from *A. tequilana*.

To determine the diversity of the complete genome of the strains, genetic fingerprinting were generated from fragments repeated with the Rep-PCR DNA marker, using primers 1R (5- IIIIC GICGICATCIGGC-3') and REP 2I- (5' ICGICTTATCIGGCCTAC-3') (Versalovic *et al.*, 1991) and the protocol by Rademaker and Brujin (1998) to amplify, with a few modifications. The PCR cocktail (25 µL) contained 2.5 µL of buffer10X (500 mM KCl and 100 mM Tris-HCl [pH 8.3], 100 µL gelatin, 1% Triton, 1.5 mg/mL BSA), 2.5 µL of 2 mM dNTPs, 1.25 µL of 1.5 mM $MgCl_2$, 2 µL of 0.25 µM of each primer, 0.5 µL of 5U of Taq DNA polymerase Amplificasa™ (BioTecMol) and 3 µL of the DNA. The amplification program consisted in a denaturation at 95 °C for 6 min, followed by 30 cycles with

denaturation at 94 °C for 6 min, annealing at 40 °C for 1 min, an extension at 65 °C for 8 min and a final extension at 65 °C for 16 min. The genetic fingerprinting were visualized in agarose gels at 2.1%, with electrophoresis at 4 °C, 40V and 40 mA for 20 h. Diversity was determined based on standardized matrices of presence-absence of amplified fragments of different base pair numbers in relation to the HyperLadder100bp plus molecular weight marker from Bioline™. A phylogenetic tree was generated using the Manhattan average dissimilarity coefficient, with the Neighbor-Joining method, using the NTSYSpc program, version 2.21 (Rohlf, 2009).

Pathogenicity tests

In order to evaluate the pathogenicity of representative strains of the genetic diversity of *F. solani*, in three independent bioassays, *Agave tequilana* plants; maize seedlings of the Magno (Aspros ™), Ocelote and Faisán hybrids (Asgrow™) reported as resistant to root rot by *Fusarium* and a traditional variety of "Morado" maize; as well as "Flor de Mayo" bean seedlings were inoculated with the FsH, FsC1, FsC7 and FsCA strains. Additionally, plants of the three species that were not inoculated were developed and evaluated as non-inoculated control (NI). Confrontations were evaluated by a completely randomized design with four repetitions. The plants were established in a greenhouse, in pots with a peat and sandy soil substrate, sterilized in an oven at 100 °C for 24 h. The inoculum of each strain was grown in PDA for 10 days, pre-germinating a conidial suspension by shaking it in a minimal synthetic medium (Okon *et al.*, 1973) at 150 rpm and 28 °C for 12 h. The substrate was inoculated with 4 x 10^5 conidia/mL, placing 1 mL of the suspension in three equidistant points around the plant, without injuring the roots. The severity of the root rot was quantified as a percentage of the internally necrotic lenght of ten root fragments, each 10 cm long per plant. The treatments were evaluated 90 days after inoculation (DDI) in agave plants and 42 DDI in maize and bean plants. The statistical analysis was carried out with Proc ANOVA and Duncan's multiple range test at a significance level of $P \leq 0.05$ with the program SAS, version 9.0 (The SAS Institute Inc., Cary, NC).

Results and Discussion

Twenty-four isolates, morphologically identified as *F. solani* (Table 1) were obtained. They were all isolated from agave plants with wilt. The cultures of strains FsP1, FsDr and FsCA had a clearly smaller diameter, in PDA medium after 5 days (Figure 3 A), in comparison with the diameter reached by the rest of the strains (Figure 3 B).

Table 1. Designation of *F. solani* and *Xenoacremonium* spp strains and ubication of the commercial *Agave tequilana* var azul fields where strains were obtained by municipality and production zone in Jalisco, Mexico.

F. solani strain	Municipality, State	Agave production zone
FsF, FsG, FsH, FsP1, FsP2, FsP4	Acatic, Jal.	Altos Sur
FsP	Tototlán, Jal.	Ciénega
FsC	Tepatitlán, Jal.	Altos Sur
FsQ	Atotonilco, Jal.	Ciénaga
FsO	Tecolotlán, Jal.	Sierra de Amula
FsA, FsK	Tala, Jal.	Valles
FsC7	Comitán, Chis.	
FsDr	San Gabriel, Jal.	Sur
FsCR, FsM, FsI	Teuchitlán, Jal.	Valles
FsA3, FsCA, FsC1, FsD2, FsM1, FsSub, F3	Zapotlanejo, Jal.	Centro

Figure 3. Appearance of fungal cultures after five days of growing in PDA at 28 °C. A) FsDr strain, identified molecularly as *Xenoacremonium* sp.and B) *Fusarium solani* FsP strain. C) Microphotograph of mycelium and conidiophore of the FsDr strain without macroconidia.

The fragment ITS1-5.8S-ITS2 of the isolations obtained from *Agave tequilana* plants was sequenced and these sequences were integrated into the GenBank database with accession codes FsCR MK027255; FsP2 MK027256; FsC1 MK027257; FsM MK027258; FsSub MK027259; FsA3 MK027260; FsD2 MK027261; FsM1 MK027262; FsP4 MK027263; FsF3 MK027264; FsA KU878136; FsC KU878137; FsF KU878138; FsG KU878139; FsH KU878140; FsK KU878141; FsO KU878142;:FsP KU878143; FsQ KU878144; FsC7 KU878145; XP1 MK027287; XCA MK027288; XDr MK027289. When comparing these sequences with the collection of nucleotides (nr/nt), the taxonomy of 21 isolations such as *F. solani* and members of the FSSC were molecularly ratified, for coinciding with 47 to 242 reports of highly similar sequences. FsCA, FsDr and FsP1 strains, with the same search criteria, were not highly similar to *F. solani* or

the FSSC, but they were similar to the genus *Xenoacremonium*, with 15 reports and 17 of high similarity with *Xenoacremonium falcatum* and *X. recifei*, which, along with the previously described morphological characteristics and the description by Lombard *et al.* (2015), was taxonomically not considered to be *F. solani*. When the fragment ITS1-5.8S-ITS2 of the different isolates of *F. solani* were compared with the FUSARIOID-ID data base, they were taxonomically located in the genus *Neocosmospora* according to the new phylogenetic species (Sandoval-Denis *et al.*, 2019; Geiser *et al.*, 2021). Therefore, the 21 *F. solani* strains were subdivided into *Neocosmospora falciformis* (syn of *Fusarium falciforme*) FsM1, FsP, FsD2, FsI, FsA3 and FsC1; *N. solani* (syn of *F. solani*) FsP4, FsF3; *N. martii* (syn of *F. martii*) FsQ, FsP2, FsC7, FsC, FsCr and *N. pseudoradicicola* (syn of *F. pseudoradicicola*) FsK, FsH, FsG, FsF, FsA, FsSub, FsO and FsM. This taxonomic subclassification helped to give a greater definition of its diversity, unlike considering them only *F. solani*. The evolutionary distance dendrogram generated with the ITS1-5.8S-ITS2 fragment sequences of the 21 strains (Figure 4), showed a considerable distance from most of the f. spp., with the exception of f. sp. *eumartii*, which was located next to the *N. martii* strains, and the f. sp. *radicicola*, which was located among the *N. falciformis* strains. All the strains isolated from *Agave tequilana* plants were evolutionarily distant from the f.sp. *phaseoli*. According to reports by O'Donnell *et al.* (2000); Suga *et al.* (2000b) and Zaccardelli *et al.* (2008), strains from different *F. solani* f. spp. tend to group closely when their ITS sequences are analyzed phylogenetically and are considered biologically and phylogenetically different species (Coleman, 2016). However, the strains taken from agave could not be considered a special form, as they belong to four distinct phylogenetic species without a pathogenic specificity, since they all share tequila agave as a host, behaving as necrotrophic with a wide range of hosts (Mengiste, 2012).

The sequences of the SSU of rDNA of the 21 strains of *F. solani* were obtained and included in the GenBank data base, with the following accession numbers: FsC1 MK027267; FsA MK027268; FsA3 MK027269; FsH MK027270; FsO MK027271; FsG MK027272; FsF MK027273: FsF3 MK027274; FsD2 MK027275; FsM MK027276; FsM1 MK027277; FsC MK027278; FsC7 MK027279; FsCR MK027280; FsK MK027281; FsQ MK027282; FsP MK027283; FsP2 MK027284; FsP4 MK027285; FsSub MK027286.

These sequences were cut according to the beginning and end of the fragment NS5-NS8 (Suga *et al.*, 2000a), and when aligning these fragments with the sequences of the f. spp. *phaseoli* and *batatas*, f. sp. *phaseoli* in sequence AF150481 was verified to have two inserts and measures 1,457 pb; sequence AF150482 has one insert and a length of 1,025 pb and sequence AF150483 has one insert and a length of 1048 pb, while sequence AF150484 of the f. sp. *batatas* has no inserts and has a length of 616 pb. With these sequences as a reference, it was determined that

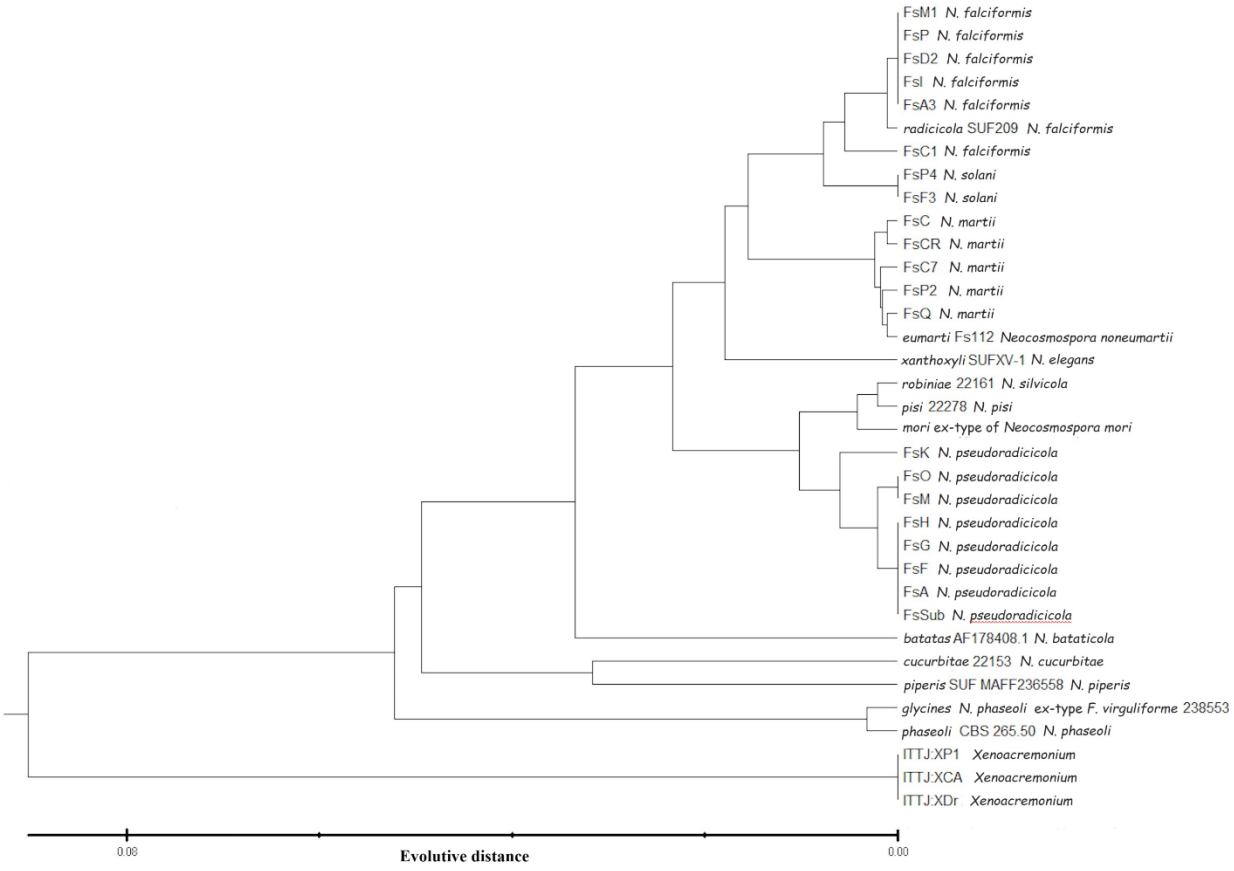

Figure 4. Phylogenetic tree of evolutive history constructed with UPGMA method from strains identified as *Fusarium solani* in the GenBank and classified as phylogenetic species of *Neocosmospora* using FUSARIOID-ID nucleotide base, analyzing ITS1-5.8S-ITS2 sequences comparing with those of eleven *F. solani* f. spp.

none of the *Neocosmopora* strains isolated from agave have inserts in the fragment NS5-NS8 and all have a length of 616 pb., therefore none are similar to the f. sp. *phaseoli*, which would support the suggestion of intercropping beans between the rows of agave during the rainy season, or rotating with this crop when the incidence of agave wilt has increased, reducing the risk of increasing the inoculum of *Neocosmodpora* spp. by infecting and colonizing the beans.

An analysis of the Rep-PCR fingerprints obtained with the complete genome of the strains taken from agave, showed no clear association between the phylogenetic species *N. falciformis*, *N. martii*, *N. solani* and *N. pseudoradicicola* (Figure 5) as seen with the diversity shown in the dendrogram with only the sequence ITS1-5.8S-ITS2. Nevertheless, if five groups are considered at a dissimilarity coefficient of 0.30, most of the strains from three phylogenetic species remain in the same

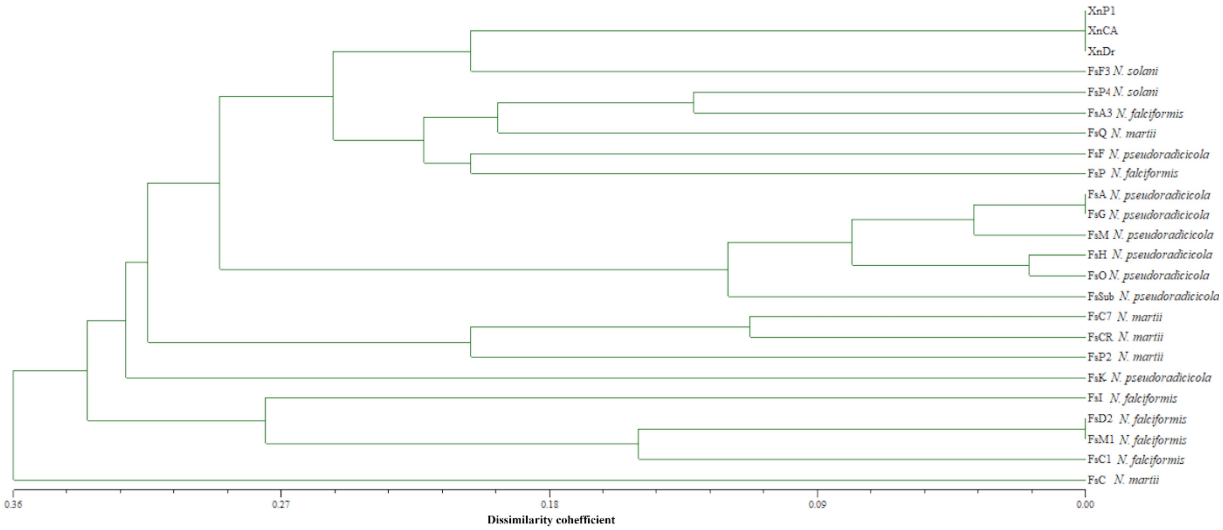

Figure 5. Diversity analysis of fingerprints obtained with the DNA marker Rep-PCR from strains of *Fusarium solani* identified as four phylogenetic species of *Neocosmospora* and *Xenoacremonium* spp. isolated from *Agave tequilana* var azul with agave wilt.

genetic diversity group. However, a diversity group was also formed, consisting of strains from all the four phylogenetic species, including the *Xenoacremonium* strains. This may explain that, although in ITSs and 1α elongation factor conserved genes, these phylogenetic species are very similar, their complete genome has a higher degree of diversity, but there is affinity between some strains.

When inoculating four representative strains from diversity groups and evaluating their pathogenicity as the severity of necrotic tissue, it was determined that the FsH, FsC1 and FsC7 strains inoculated in agave led to a severity of 16.35, 15.10 and 14.65 % respectively, without statistical differences between them. However, in the plants inoculated with the FsCA strain, no greater severity of root tissue necrosis was observed compared to the non-inoculated control (Table 2). This corroborates the pathogenicity of the FsH, FsC1 and FsC7 strains in agave, as previously reported by Ramírez-Ramírez *et al.* (2017) with the FsG strain.

When inoculating the strains FsC7, FsC1 and FsH and FsCA in four maize varieties, strains FsC7 and FsH induced more severe necrosis in "Morado" maize plants, with averages of 9.9 and 8.1% respectively. In a second level of significance, the C1 strain caused root necrosis by 4.8 and 4.7% in Magno and "Morado" maize, respectively. However, the strains FsH, FsC7 and FsC1, when inoculated in the hybrids Ocelote® and Faisán®, which are sold as resistant to root rot induced by *Fusarium* in maize, displayed no greater root rot severity than the non-inoculated control (Table 3). Considering that, despite this bioassay having been brief, being conducted in a greenhouse and only including four genetic materials in maize, it

Table 2. Severity (%) of root rot in agave plants inoculated with *Neocosmospora pseudoradicicola*, *N. falciformis*, *N. martii* and *Xenoacremonium* sp. obtained from rotten crown tissue of *Agave tequilana* var azul plants with agave wilt.

F. solani strains	Severity (%)	DMRT[z]
FsH *N. pseudoradicicola*	16.35	A
FsC1 *N. falciformis*	15.10	A
FsC7 *N. martii*	14.65	A
FsCA *Xenoacremonium* sp.	8.45	B
NI	8.33	B

[z]Means followed with the same letter are not significatively different. Duncan´s Multiple Rage Test (DMRT) (* ≤ 0.05).

Table 3. Severity (%) of root rot in four commercial and one native cultivar of corn (*Zea mays*) inoculated with the strains FsH (*Neocosmospora pseudoradicicola*), FsC7 (*N. falciformis*), FsC1 (*N. martii*) y FsCA (*Xenoacremonium* sp.), obtained from rotten crown of agave plants (*Agave tequilana* Weber var. azul) with agave wilt.

Corn variety	Inoculated strain	Severity (%)	DMRT[z]
Morado	FsC7	9.9	A
Morado	FsH	8.1	AB
Magno	FsC1	4.8	BC
Morado	FsC1	4.7	BC
Morado	FsCA	4.2	CD
Magno	FsC7	3.3	CD
Magno	FsH	2.9	CD
Faisán	FsH	2.8	CD
Faisán	FsCA	2.8	CD
Ocelote	FsC7	2.5	CD
Magno	FsCA	1.7	CD
Faisán	FsC7	1.7	CD
Ocelote	FsC1	1.5	CD
Ocelote	FsH	1.4	CD
Faisán	FsC1	1.1	CD
Ocelote	FsCA	1.0	CD
Ocelote	NI	0.7	CD
Morado	NI	0.7	CD
Faisán	NI	0.5	D
Magno	NI	0.5	D

[z] Means followed with the same letter are not significatively different. Duncan's Multiple Rage Test (DMRT) (* ≤ 0.05).

shows that among the evaluated maize materials, there is diversity in the susceptibility to *Neocosmospora* spp. strains isolated from *A. tequilana*. This is important if we consider that the rotation with non-host crops is one of the cheapest strategies to handle soil borne plant pathogens in the field, when attempting to reduce its level of inoculum in the soil. Yet if this rotation is not carried out adequately, the inoculum of the pathogen is not reduced and losses in the crop that is trying to be protected remain or are even increased, such as in the maize-soybean rotation performed as a strategy for the management of *Fusarium virguliforme* (*F. solani* f. sp. *glycines*), causing sudden death in soybean (SDS), which, when performed for several cycles, did not reduce the severity of the disease in soybean, since the maize materials used were evaluated as susceptible to infection by *F. virguliforme*, although this infection did not reduce its production (Xing and Westphal, 2009).

In the "Flor de Mayo" bean plants, the inoculation of strains FsC1, FsC7 y FsH, did not induce more root necrosis than that observed in the non-inoculated control treatment, nor that quantified with the inoculation of the FsCa strain of *Xenoacremonium* sp of 0.28 y 0.17% de severity respectively (Table 4).

Table 4. Severity (%) of root rot in bean (*Phaseolus vulgare* cv. Flor de Mayo) plants inoculated with strains of *Neocosmospora pseudoradicicola*, *N. falciformis*, *N. martii* y *Xenoacremonium* sp., isolated from rotten crown tissue of *Agave tequilana* var. azul with agave wilt.

Inoculated strain	Severity (%)	DMRT[z]
FsC1 *N. falciformis*	0.43	A
NI	0.28	AB
FsC7 *N. martii*	0.21	AB
FsCA *Xenoacremonium* sp.	0.17	AB
FsH *N. pseudoradicicola*	0.03	B

[z]Means followed with the same letter are not significatively different. Duncan's Multiple Rage Test (DMRT) (* ≤ 0.05).

Conclusions

Strains morphologically identified as *F. solani* were primarily isolated from tequila agave plants with symptoms of wilting and necrosis at the base of the stem and crown.

When comparing the ITS1-5.8S-ITS2 sequences of strains obtained from *Agave tequilana* in the GenBank data base, were molecularly identified as *Fusarium solani* and were included in the *F. solani* species complex (FSSC).

A comparison of these same sequences with the FUSAROID-ID database led to determining a high similarity with those from the *Neocosmospora martii*, *N. pseudoradicicola*, *N. solani* and *N. falciformis* phylogenetic species, which are reported as synonymous with *Fusarium martii*, *F. pseudoradicicola*, *F. solani* and *F. falciformis*, displaying their genetic variability.

Sequences of the fragment ITS1-5.8S-ITS2 of the isolations obtained from *Agave tequilana* were molecularly different from the *F. solani* f. spp, with the exception of the f. sp. *eumartii* which is very similar to *N. martii* and the f. sp. *radicicola*, which is very similar to *N. falciformis*. This information, along with the absence of introns in the sequence of the 18S (SSU) fraction, supports that none of the strains obtained from *A. tequilana* belong to the f. sp. *phasioli*.

Strains representative of *Neocosmospora* isolated from *A. tequilana* were phytopathogenic to agave, "Morado" maize and to maize varieties without resistance to *Fusarium*. On the other hand, maize varieties resistant to *Fusarium* were not susceptible.

In pathogenicity tests, none of the strains was pathogenic to the inoculated bean plants, which seems to confirm the molecular findings in this regard.

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