Genetic Comparison of Pathogenic and Non-Pathogenic *Fusarium Oxysporum* Endophytes from *Vanilla* sp. in Mexico

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Abstract

Fusarium oxysporum is an ubiquitous inhabitant of soils with an important role as an endophyte in several plant species, being a neutral, benefic or pathogenic endophyte. Neutral and pathogenic strains have been reported for vanilla crops (*F. oxysporum* f. sp. *vanillae*), and a polyphyletic distribution of pathogenic strains has been demonstrated. However, there is no study of a molecular genetic comparison between both groups associated with vanilla. This study is aimed to evaluate if a genetic differentiation is possible between both groups. Different microsatellite regions were amplified and data were used for genetic and statistic comparisons between pathogenic and non-pathogenic endophytes. Further, the Sequence Type of TEF-gen was determined and a phylogeny was constructed. Also, *SIX* gene presence was evaluated using universal primers. Results show that, based on microsatellite data, a differentiation between the two groups is not possible, however, UPGMA and CA analysis suggest that the pathogenic group is a subgroup of the non-pathogenic group. Phylogeny confirms that the ability to infect vanilla is a polyphyletic trait, but comparison with UPGMA shows that some strains have a similar genotype by convergent evolution. A Bayesian approach suggests that strains can be divided into three genetic groups. For *SIX* genes, amplification was not possible, thus, we hypothesized three possible explanations. Genetic differentiation among pathogenic and non-pathogenic *F.* oxysporum endophytes from vanilla is not possible with microsatellite data, which corresponds with the Horizontal Gene Transference hypothesis. However, the *SIX* genes were no-detected in any pathogenic strain, three hypotheses are planted to explain this results.

Introduction

Fusarium is an ascomycetes genus present in all ecosystems on earth [1]. This genus is recognized by their ecological roles as a beneficial endophyte [2], plant pathogenic fungi [3–5], and animal pathogen, even for humans [6, 7]. The most studied *Fusarium* role is as a plant pathogen, for example, *F. graminearum* and *F. oxysporum*, which are considered the fourth and fifth places in the top ten of fungal pathogens in molecular plant pathology, respectively [8].

Fusarium oxysporum is widely studied around the world. This is a Species Complex constituted by pathogenic and non-pathogenic endophytes, and plant growth promoters [2, 9]. Pathogenic endophytes are named on the basis of its host; strains with the ability to infect some crops have only able to infect that host and no others, this is known as *formae speciales* [10].

The research in *F. oxysporum* has been focused on pathogenic endophytes, or *formae speciales*, to reveal levels of pathogenicity [11], mechanisms of virulence [12] and design control tools [13]. However, recent studies [14, 15] suggest that limiting the study of *F. oxysporum* to pathogenic strains could cause a loss of important genetic information that can help to understand the evolution and ecology of the pathogenicity [16].

The vanilla root rot disease is caused by *F. oxysporum* f. sp. *vanillae* [17] or *F. oxysporum* f. sp. *radicis-vanillae* [18]. This disease is considered the most important in vanilla production worldwide. The causal agent has been detected in producers countries such as Indonesia [19], Madagascar [18], Reunion Island and Comoros [20] and the center of origin and dispersion of the vanilla crop, Mexico [21, 22].

A phylogenetic study by Pinaria et al. [20] showed that pathogenic ability to vanilla is a polyphyletic trait among *F. oxysporum* Species Complex (FOSC). However, their results suggest that only Indonesian strains of *F. oxysporum* f. sp. *vanillae* are polyphyletic, but Mexican ones are monophyletic. Flores-de la Rosa et al. [23] show, with a wider sampling of the pathogen from Mexico, that Mexican strains are distributed in different branches of the phylogeny and have more pathogenesis points than Indonesian strains, suggesting a higher genetic diversity for Mexican pathogenic strains.

Two studies on *F. oxysporum* f. sp. *vanillae* have included non-pathogenic strains for phylogenetic analysis. Koyyappurath et al. [18] did a wide sampling of *F. oxysporum* endophytes, but phylogenetic diversity was very narrow-based on a TEF-1α

gene. Casillas-Isiordia et al. [22] showed that *F. oxysporum* pathogenic strains are closely related to non-pathogenic strains in wild genotypes of *Vanilla* sp.

As an implication of the polyphyletic nature of a *forma specialis*, currently it has been considered that horizontal gene transfer (HGT) is the mechanism responsible for acquired pathogenicity among different lineages of FOSC phylogeny, specifically horizontal movement of *Secreted In Xylem*, *SIX* genes [24, 25]. These genes apparently have a role as pathogenicity effectors causing Effector Triggered Susceptibility (ETS) according to the zigzag model [26]. A well-studied system of resistance and susceptibility based on *SIX* genes (*Avr*) presence/absence has been developed in the interaction between *F. oxysporum* f. sp. *lycopersici* and tomato [27].

However, there are no studies that look for some genetic differentiation between pathogenic and non-pathogenic strains in *F. oxysporum* f. sp. *vanillae* and to compare the genetic diversity of both groups. For this purpose, this study was aimed to genetically compare endophyte strains of *F. oxysporum*, pathogenic and non-pathogenic to vanilla, using microsatellite SSR developed specifically for FOSC, to determine if microsatellite variation sustains the polyphyletic hypothesis of ability to infect vanilla and determine the presence of some SIX genes.

Materials And Methods

Endophytes strains of Fusarium **sp.**

Two states from Mexico were sampled for vanilla plants with and without symptoms of stem and root rot disease. The state of Nayarit was sampled for the disease in wild genotypes of *V. pompona* [22] and pathogenicity of the same strains in *V. planifolia* was reported by Flores-de la Rosa et al. [23], strains from that study are labeled as “NAY” strains. The state of Veracruz, considered the center of diversity and dispersion of worldwide distributed vanilla genotypes, was sampled by Adame-García et al. [21] and some pathogenic endophytes with different levels of virulence were isolated, strains from that study are labeled as “JAGH” strains. The other strains were sampled from Francisco Sarabia and Papantla, Veracruz, the isolation and fast pathogenic proofs of these strains were carried out in *V. planifolia* following the protocol described before [21]. Koch´s postulates were applied twice.

DNA extraction, TEF gene amplification, and Mating Type identification

DNA extraction and TEF gene amplification of endophyte *Fusarium* strains were carried out according to a protocol established [28]. The amplification products were purified with the kit ExoSAP-IT PCR product Cleanup System and sequenced at the Pennsylvania State University Genomics Core Facility.

To determine what idiomorph of Mating Type gene is present in each strain a PCR-based identification of MAT-1 and MAT-2 was performed. For this, primers from diverse authors [29–31] were used. The MAT-1 idiomorph was detected with the pair of primers Falpha 1- F (5'-CGGTCAYGAGTATCTTCCTG-3’), and the Falpha 2-R (5’-GATGTAGATGGAGGGTTCAA-3’). Similarly, the MAT-2 idiomorph was detected using the forward and reverse primers FF1 (5’-GTATCTTCTGTCCACCACAG-3’) and Gfmat2c (5’-AGCGTCATTATTCGATCAAG-3’), respectively. The cycling conditions for MAT-1 amplification were as follows: initial denaturation at 95°C for 15 min; 35 cycles at 94°C for 1 min, 55°C for 30 s, and 72°C for 1 min; final extension at 72°C for 10 min. For amplification of MAT-2: initial denaturation at 95°C for 2 min, followed by 35 cycles of denaturation at 92°C for 30 s, 54°C for 40 s, 72°C for 2 min, and a final extension at 72°C for 7 min. The amplified products were observed by electrophoresis in 1% agarose gel, stained with EZ-vision and visualized under UV light. Sizes were estimated using a 100 bp ladder as reference.

Microsatellite Amplification And Sequencing

For analysis of genetic differentiation between pathogenic and non-pathogenic endophytes *F. oxysporum* strains, a microsatellite SSR approach was adopted. 10 microsatellite regions were amplified using primers developed by Demers *et al.* [32] (Table 1). PCR conditions were as follows: a total volume of 20 µL, consisting of 10X reaction buffer, 200 µM of each dNTP, 0.5 U Taq DNA polymerase, 0.4 µM of each primer and 50–100 ng of genomic DNA. Thermal conditions were an initial denaturalization at 94°C during 4 min, after this 35 cycles at 94°C for 1 min, annealing temperature (specified in Table 1) during 1 min, polymerization step was at 72°C for 1 min, and a final extension at 72°C during 7 min. PCR products were visualized in agarose gel at 2.5% in horizontal electrophoresis at 90V for 60 min. PCR products were dyed with EZ-VISION buffer (amresco ®) for UV visualization in a photo documentation system and purified with the kit ExoSAP-IT PCR product Cleanup System and sequenced at the Pennsylvania State University Genomics Core Facility.

Table 1
Primers sequences, temperature of annealing, size expected and motif repeated for each microsatellite locus developed for Demers et al. (2014).

Primer	Primer sequence (5´- 3´)	Annealing Temp.	Size expected (bp)	Motif repeated
FoAB11F	GGCCGCCCAGAAGAGGTAG	56°C	187–265	$(CACAGCA)_n$
FoAB11R	ATTGGAGCGGAAAAGAAACACG			
FoAD12F	TCGAGGAAGACACTGCACTAAAAA	56°C	185–203	$(AG)_n$
FoAD12R	CCCCAAGGTCAGACTCACTCAG			
FoAG11F	TGTAAGATGCTCTCTCT	56°C	125–195	$(CT)_n\ (GT)_m\ (GA)_k$
FoAG11R	AAAGGTAGTGATGCC			
FoDC5F	AGAAACAAGAACCCCATATCGC	60°C	102–132	$(TG)_n$
FoDC5R	ACTTAAACAGGAAAGGGACGGA			
FoDD7F	CGATTGACTACCGGGTGAACTTGT	56°C	329–335	$(CTT)_n$
FoDD7R	AGGGCGAGGGTGAGGGTGAGA			
FoDE7F	AACTCGCCATGCTTGACA	50°C	221–225	$(CGTT)_n$
FoDE7R	GAGGAGCGTGGCGACAT			
FoDF7F	GGCGTTGGGCGTTGCTAA	54°C	266–270	$(GT)_n$
FoDF7R	ATTTGTTGGGATTCCTTCAGAC			
FoED2F	ATGCCAGTCAAAGGGATAC	62°C	228–258	$(AGCTTGAGGAGAGGC)_n$
FoED2R	ACACAACAACACGCACAAG			
FoFA4F	TGGCACTGCTTCAAGGTTAGACT	56°C	364–368	$(AG)_n$
FoFA4R	ACCAATTTCATACAAGGGCACTC			
FoFE5F	GAACCCTTATCACTCCTA	52°C	446–450	$(GTT)_n$
FoFE5R	AGACTTATCCGCTACTTACC			

SIX genes amplification

To determine the presence or absence of *SIX* genes, primers developed by different authors and applied in many *formae speciales* were utilized (Table 2). Protocols established by the authors for PCR conditions and thermal programs, with some modifications in annealing temperature, were used (Table 2). PCR products were dyed with EZ-VISION buffer (amresco ®)

for UV visualization in a photo documentation system and purified with the kit ExoSAP-IT PCR product Cleanup System and sequenced at the Pennsylvania State University Genomics Core Facility.

Table 2
Primers sequences, temperature of annealing and reference for each *SIX* gene effector evaluated for amplification.

Primer	Primer sequence (5´- 3´)	Annealing Temp.	Reference
SIX1F	ATGGTACTCCTTGGCGCCCTC	60°C	Meldrum et al. 2012
SIX1R	TGACAATGCGACCACGCCTCG		
SIX2F	CAACGCCGTTTGAATAAGCA	55°C	van der Does et al. 2008
SIX2R	TCTATCCGCTTTCTTCTCTC		
SIX3F	CCAGCCAGAAGGCCAGTTT	55°C	van der Does et al. 2008
SIX3R	GGCAATTAACCACTCTGCC		
SIX4F	TCAGGCTTCACTTAGCATAC	55°C	Lievens et al. 2009
SIX4R	GCCGACCGAAAAACCCTAA		
SIX5F	ACACGCTCTACTACTCTTCA	55°C	Lievens et al. 2009
SIX5R	GAAAACCTCAACGCGGCAAA		
SIX6F	CTCTCCTGAACCATCAACTT	55°C	Lievens et al. 2009
SIX6R	CAAGACCAGGTGTAGGCATT		
SIX7F	CATCTTTTCGCCGACTTGGT	55°C	Lievens et al. 2009
SIX7R	CTTAGCACCCTTGAGTAACT		
SIX8F	TCGCCTGCATAACAGGTGCCG	60°C	Meldrum et al. 2012
SIX8R	TTGTGTAGAAACTGGACAGTCGATGC		
SIX9F2	CTTCTAGCAGTTGTAGCCAC	56°C	Laurence et al. 2015
SIX9R2	GTACGCCAGTTGACGCAAG		
SIX10F	AAAAAGCAGGCTCCATGAAGCTCTTGTGGTTG	56°C	Laurence et al. 2015
SIX10R	AGAAAGCTGGGTCCTACTTAGACCTGGTAATTGTT		
SIX11F	GATGTTCTCCAAAGCCATCC	56°C	Laurence et al. 2015
SIX11R	AGAATGCCACTCGGTGTGA		
SIX14F	TTGCCACCTATGCATACCG	56°C	Laurence et al. 2015
SIX14R	TCCACATTCCTAAGCGAACC		

Bioinformatics Analysis Of Molecular Data

Determination of Sequences Types of TEF-1α gene

To determine the number of Sequences Type obtained from the sampling, the TEF-1α sequences were compared using BLAST, with default settings, in the FUSARIUM-ID database [33] from the *Fusarium* Research Center of Penn State

University.

Alignment And Phylogenetic Analysis Of Tef-1α

Multiple alignments of the 46 terminals were performed using CLUSTALW standard methods and settings (Bootstraps = 1000; Gap Open Penalties = 15; Gap Extend Penalties = 3). All strains identified as *Fusarium* species different from *F. oxysporum* were used as outgroups. Unweighted Parsimony analysis was performed using TNT 1.1 software [34] with a Winclada (1.94.1) interface. The search for the most parsimonious trees was executed with 1000 replicates each using a combination of algorithms (Ratchet + Drift + Sectorial Fusion + TBR-max). Inferences on clade robustness were derived with Bootstrap and Jackknife resampling (1000 repetitions with the same settings of principal search). A matrix of two-character state for presence (1) or absence (0) of pathogenicity toward *V. planifolia* in all the strains was constructed and optimization algorithms in Mesquite were used to reconstruct connectivity, and transitions in the hypothetical ancestral nodes. Optimizations were mapped on the strict consensus of optimal trees.

Statistical Analysis Of Microsatellite Data

To evaluate if genetic differentiation is possible to detect between pathogenic and non-pathogenic groups, an AMOVA analysis (999 permutations) was used with the numbers of repeated motifs in each strain. After this, genetic diversity for each locus was compared between the same groups using the Shannon Diversity Index and a chi-square proof was performed for the detection of significant differences, both analyses were conducted in GenAlEx 6.501. Clustering methods for differentiation among pathogen and non-pathogen endophytes were applied; a statistical and a genetic-distance based method were applied. UPGMA clustering method with Euclidean Similarity index was performed, using the number of motif repeats for each strain as input data, and a multivariate Correspondence Analysis was carried out, with two groups labeled (pathogenic and non-pathogenic); these analyses were performed in software PAST 3.14. To determine how many genetic groups can be observed in all the strains, a Bayesian approach was implemented; for this purpose, an admixture model with correlated allele frequency was used, and information of pathogenicity (positive and negative) was used as population prior information. A burn-in period of 25, 000 was applied in each run and 100,000 MCMC (Markov Chain Monte Carlo) in STRUCTURE software. The number of genetic groups (K) was evaluated (K = 1 to K = 10) and the best value was determined to apply the Evanno et al. [35] method based on changes of the log of the probability of data (Ln(Pr(D)) using the online software STRUCTURE HARVESTER v. 0.6.92 [36].

Results And Discussion

Identification of Fusarium **species and determination of Sequences Type of TEF-gene and Mating Type of** F. oxysporum

Different *Fusarium* species have been associated with vanilla plants, however, only *F. oxysporum* is reported as pathogenic at different levels [19, 21], suggesting a high genetic diversity of pathogenic endophyte strains [18, 37]. A total of 46 strains were used in this study. From these, nine were JAGH strains, 12 were NAY strains and all other 25 were isolated from Veracruz, 11 pathogenic and 14 non-pathogenic. BLAST of TEF-1α sequence in the FUSARIUM-ID database allowed the identification of six *Fusarium* species: *F. proliferatum* (JAGH1), *F. solani* (R21), *F. fujikuroi* (JF62), *F. lactis* (NAY8), *F. pseudocircinatum* (V4M, V12M) and *F. oxysporum* (all other strains). To our knowledge, this is the first report that associates *F. lactis* to *Vanilla* sp. 14 ST were identified in *F. oxysporum* associated to vanilla, with different levels of similarity to reference sequences, this is a relatively high number of ST when compared with other crops such as tomato [14] and cyclamen [15].

Both Mating Type idiomorph was observed, even in strains belonging to the same Sequence Type, demonstrating that the origin of these strains is not clonal. *MAT* gene has been associated with genetic diversity into a population of asexually

reproducing *Fusarium* [38] and with the differentiation of some physiological races of *F. oxysporum* [39]. Information about ST, *Fusarium* species identification, and Mating Type idiomorph are summarized in Table 3.

Table 3
Identification of *Fusarium* species relationed with *Vanilla* sp. and determination of Sequence Type and Mating Type of *Fusarium oxysporum*. ND = Non-Determinated.

Strain	Fusarium ID	Similarity (%)	Mating Type	Sequence Type
BC1	*F. oxysporum* NRRL22550	92.93	MAT-2	ST1
JAGH11	*F. oxysporum* NRRL22550	93.01	MAT-1	ST1
NAY12	*F. oxysporum* NRRL26449	97.88	MAT-1	ST2
V8M	*F. oxysporum* NRRL26449	99.26	MAT-2	ST2
JAGH5	*F. oxysporum* NRRL28367	88.07	MAT-1	ST3
JAGH5-1	*F. oxysporum* NRRL28406	97.71	MAT-1	ST4
NAY22	*F. oxysporum* NRRL28406	98.06	MAT-1	ST4
JF22	*F. oxysporum* NRRL31418	99.83	MAT-1	ST5
A3	*F. oxysporum* NRRL32885	99.84	MAT-1	ST6
28R1	*F. oxysporum* NRRL32885	99.38	MAT-1	ST6
A31	*F. oxysporum* NRRL32885	99.84	MAT-1	ST6
A32	*F. oxysporum* NRRL32885	99.69	MAT-1	ST6
NAY6	*F. oxysporum* NRRL32885	99.52	MAT-1	ST6
NAY7	*F. oxysporum* NRRL32885	99.37	MAT-2	ST6
V3	*F. oxysporum* NRRL36251	99.84	MAT-1	ST7
JAGH4	*F. oxysporum* NRRL36284	99.17	MAT-1	ST8
JAGH10	*F. oxysporum* NRRL36284	96.62	MAT-1	ST8
JF5	*F. oxysporum* NRRL36284	97.24	MAT-1	ST8
V6M	*F. oxysporum* NRRL36284	98.51	MAT-1	ST8
NAY13	*F. oxysporum* NRRL38290	97.26	MAT-2	ST9
NAY20	*F. oxysporum* NRRL38290	97.64	MAT-1	ST9
3R1	*F. oxysporum* NRRL38290	97.96	MAT-1	ST9
25R2	*F. oxysporum* NRRL38290	98.12	MAT-1	ST9
NAY1	*F. oxysporum* NRRL38290	98.06	MAT-1	ST9
NAY3	*F. oxysporum* NRRL38290	98.12	MAT-1	ST9
NAY9	*F. oxysporum* NRRL38290	98.07	MAT-1	ST9
NAY14	*F. oxysporum* NRRL38290	98.1	MAT-1	ST9
NAY21	*F. oxysporum* NRRL38290	98.27	MAT-2	ST9
R27	*F. oxysporum* NRRL38290	98.11	MAT-1	ST9
B3	*F. oxysporum* NRRL38514	99.68	MAT-1	ST10
JAGH2	*F. oxysporum* NRRL38514	99.84	MAT-1	ST10

Strain	Fusarium ID	Similarity (%)	Mating Type	Sequence Type
A33	*F. oxysporum* NRRL38597	100	MAT-1	ST11
BR1	*F. oxysporum* NRRL38597	99.68	MAT-1	ST11
JF3	*F. oxysporum* NRRL39464	95.88	MAT-1	ST12
JF12	*F. oxysporum* NRRL39464	100	MAT-1	ST12
R115	*F. oxysporum* NRRL39464	100	MAT-1	ST12
JAGH9	*F. oxysporum* NRRL40180	100	MAT-1	ST13
JAGH3	*F. oxysporum* NRRL40180	98.42	MAT-1	ST13
A8	*F. oxysporum* NRRL45881	99.84	MAT-2	ST14
V9M	*F. oxysporum* NRRL45881	100	MAT-1	ST14
JAGH1	*F. proliferatum* Br-1	99.08	ND	ND
R21	*F. solani* NRRL32755	99.24	ND	ND
JF62	*F. fujikuroi* complex NRRL31418	93.3	ND	ND
NAY8	*Fusarium lactis* NRRL31629	98.85	ND	ND
V4M	*F. pseudocircinatum* NRRL31631	97.07	ND	ND
V12	*F. pseudocircinatum* NRRL31631	97.39	ND	ND

Phylogenetic Analysis Of Tef-gene

Unweighted Parsimony analysis recovered 343 most parsimonious trees (L = 624; CI = 73; RI = 69). To summarize all the information a Strict Consensus Tree was performed and the resultant topology is presented (Fig. 1a). There are some clades composed only by pathogenic strains that are well bootstrap and jackknife supported. A clade composed of five strains, four pathogenic and one non-pathogenic (arrowed in Fig. 1a) suggests a lost pathogenicity event.

Polyphyletic nature of different *formae speciales* in FOSC has been suggested, some examples are *F. oxysporum* f. sp. *melonis* [40], *F. oxysporum* f. sp. *phaseoli* [41], *F. oxysporum* f. sp. *apii* [42] and many others. Pinaria et al. [20] showed that *F. oxysporum* f. sp. *vanillae* is polyphyletic in FOSC, based on TEF and mtSSU genes phylogeny. They observed that *F. oxysporum* f. sp. *vanillae* is present in the three clades proposed by O´Donell et al. [43] and in the two phylogenetic species recognized by Laurence et al. [25]. However, they suggest that only Indonesian strains of the pathogen have a polyphyletic origin while Mexican ones are monophyletic. Optimizations based on a parsimony approach allowed us to observe 11 points of pathogenicity origin inside the phylogeny, however, ancestral states are not well resolved by the optimization. A potential pathogenic ancestral state is present in the internode where the separation of *F. oxysporum* from other species is observed. Some clades are composed of strains from the two states, Veracruz and Nayarit, thus this means that some lineages are present in both places.

The number of clades in our TEF phylogeny was lower than the number of ST. However, a parsimony optimization allowed us to confirm that the pathogenic ability of some endophytes to vanilla, or *F. oxysporum* f. sp. *vanillae*, is a polyphyletic trait among FOSC in the Mexican strains of the pathogen [23] and are not monophyletic as proposed by Pinaria et al [20]. Interestingly, some pathogenic strains as A3 surged in a clade where all members and the theoretical ancestor are non-pathogenic. Conversely, BC1 strain is the only non-pathogenic strain in a pathogenic clade (Fig. 1a). This phenomenon can

be explained on the basis of Horizontal Gene Transfer, a mechanism that has been demonstrated to play a very important role in the evolution of plant pathogenic fungi [44].

Genetic Differentiation And Diversity

Results from the AMOVA with the two populations (pathogen and non-pathogen) shows that all variation is observed and explained within groups and not among them. A genetic differentiation based on our microsatellite data among pathogen and non-pathogen endophytes to vanilla is not possible. For comparison of diversity among the groups a Shannon index was calculated for every locus and compared with a chi-square, showing a significant difference among the pathogenic and non-pathogenic groups in locus FoFA4 ($p = 0.037$). The results are summarized in Table 4.

Table 4
Shannon´s Diversity Index comparing between pathogenic and non-pathogenic groups. Significant differences are represented by asterisk ($p < 0.05$).

	FoAB11	FoAD12	FoAG11	FoAG11	FoAG11	FoDC5	FoDD7	FoDE7	FoDF7	FoFA4
Pathogenic	0.726	1.594	2.539	2.500	2.437	1.844	2.563	0.314	1.693	1.704
Non-Pathogenic	0.994	1.871	2.600	2.831	2.636	2.033	2.242	0.567	1.989	2.186*

Clustering UPGMA method showed that some pathogenic strains are very close genetically, however, there is not a unique cluster grouping all pathogenic endophytes (Fig. 1B). A comparison among TEF-1α phylogenetic tree and microsatellite data UPGMA revealed that some pathogenic genotypes are similar by convergent evolution. A Correspondence Analysis showed that pathogenic endophytes group are a subgroup inside the non-pathogenic one, supporting conclusions observed in UPGMA dendrogram (Fig. 1B). Different diversity indexes were estimated showing low variation among pathogenic and non-pathogenic groups (Table 5). For determination of the most likely number of genetic groups (k) in our strains collection, a Bayesian approach was applied. Our results showed that the most probable number of groups is $k = 3$ based in a total variation on logK ($\Delta k = 4.6218$).

For the detection of genetic differentiation between pathogenic and non-pathogenic endophytes, molecular data from microsatellite were analyzed. The variation of these markers was explained better by a stepwise model than with a multiple-step model because very low variations were observed in sister strains of *F. oxysporum* [45]. Dendrogram UPGMA shows that there is not an exclusive cluster that grouped all pathogenic strains. This result is consistent with the polyphyletic distribution of this forma special in the FOSC, because if a group is monophyletic a very similar pattern of repetition of motifs is predicted, as in *F. oxysporum* f. sp. *ciceris* [32]. However, a very high diversity grade was observed in contrast to other studies [46] where a very slow diversity of *F. oxysporum* f. sp. *vanillae* isolated from India was found.

A comparison between the phylogenetic tree and dendrogram shows that some strains with a genotype very similar are the result of convergent evolution. This has been observed in other systems with other pathogenic fungi to plants, insects and humans and is explained by a co-evolutionary process [47]. The coevolution process of *F. oxysporum* f. sp. *vanillae* with vanilla in Mexico is particularly interesting given the ancestral distribution of this plant. All genotypes of vanilla cultivated around the world have their single-origin center in Papantla, Mexico [48], but genotypes in Papantla have multiple origins from southeast Mexico and Mesoamerica [49].

Diversity indexes showed that there is a higher diversity in the non-pathogenic endophytes than in the pathogenic ones. The Shannon index has been used successfully to evaluate diversity among phytopathogenic fungi using molecular markers [50]. The higher diversity in non-pathogenic strains suggests that pathogenic fungi are a small sub-group that has acquired abilities to infect but belong to the non-pathogenic group.

The CA analysis (Fig. 2) supports the inclusion of pathogenic strains in the non-pathogenic group. Inami *et al.* [51] determined that *F. oxysporum* f. sp. *lycopersici* share a common ancestor with non-pathogenic strains obtained from wild tomatoes in Peru, thus, the pathogenic strains are a subgroup of a bigger group of non-pathogenic strains. Evanno et al. [35] method estimated that the most likely number of genetic groups of our collection of endophytes is k = 3, suggesting that pathogenic could be composed by two groups.

In conclusion, genetic differentiation among pathogenic (*F. oxysporum* f. sp. *vanillae*) and non-pathogenic endophytes to vanilla are not obtained when microsatellite data are used for determination. However, these endophytes have a great genetic diversity observed in the number of ST of TEF gene, diversity indexes and clustering method of microsatellite variation. Phylogeny and microsatellite variation support the polyphyletic ability to infect vanilla trough the FOSC [20, 23, 32].

Amplification of SIX genes effectors

All pathogenic and non-pathogenic strains were evaluated for the detection of all currently know *SIX* genes effectors. Proofs for each pair of primers were repeated three times with DNA extractions from different Petri dishes but no amplification was obtained. The positive control gene TEF-1α was always amplified, showing the viability of DNA samples and PCR reactive. For the *SIX1* gene, a strain of *F. oxysporum* f. sp. *cubense* was used as positive control and a unique product with expected size was obtained, showing the viability of thermal conditions and primer design.

Ma et al. [24] demonstrated that the *F. oxysporum* genome can be divided into two different parts, the Core Genome consisting of the chromosomes conserved in the Species Complex and inherited vertically; and Specific Lineage Regions that are supernumerary chromosomes with the ability to confer pathogenic features to non-pathogenic strains (Horizontal Gene Transference). In these chromosomes the *SIX* genes are carried, which have been observed to play an essential role in susceptibility/resistance reaction in the *F. oxysporum* f. sp. *lycopersici* – tomato system [27, 52]. The role of *SIX* genes in the plant-pathogen interaction is of the pathogenicity effector, disrupting the immunity triggered by MAMP´s in the plant and triggering newly the susceptibility, according to with zigzag model [26].

These *SIX* genes are found in other *formae speciales*, but their role in the interaction with their hosts are not clarified [53, 54]. In recent studies, evidence for the horizontal transference of these genes was obtained showing that *SIX* genes are a monophyletic group depending on their host [25, 55]. Contrary to waited, amplification for *SIX* genes was not obtained from all endophytes to vanilla strains, pathogenic and non-pathogenic. Positive controls (TEF gene and an *F. oxysporum* f. sp. *cubense* strain) were amplified with a unique product at the expected size. Primers used in this study have been used in many others studies with different *formae speciales* [25, 56–59], even in natural ecosystems were they allowed detection of some *SIX* genes at very low levels directly from the uncultivated soil [60]. *SIX* genes sequences are highly conserved and few variations at nucleotides are responsible for host specificity [61, 62].

We offer three possible explanations for the lack of *SIX* genes amplifications: 1) regions to annealing for primers in our strains are very variable and recognition is impossible having no amplification as result, 2) the responsible genes for infection and specificity are different to what were evaluated in this study. Recently, it has been determined that *F. oxysporum* f. sp. *radicis-cucumerinum*, a root rotting pathogen, do not have *SIX1* in the LS genome, but *SIX6* and other less common *SIX* genes are present [63], moreover, many others putative *SIX* genes with potential effector activity are found on the basis of the location of a miniature Impala transposon (*mimp*) associated to *SIX* genes, with evidence also of horizontal transference [64]; and 3) interaction between plant and pathogen is located at the beginning of the zigzag model and no effectors are needed for establishment of the disease. The presence of a MAMP has been associated with slow and moderate virulent strains of *F. oxysporum* f. sp. *vanillae*, while absence is associated with highly virulent ones [21, Luna-Rodríguez *et al., submitted for publication*]. Moreover, Koyyappurath *et al.* [18] observed that the damage on vanilla caused by a pathogenic endophyte is limited to the first layers of the root, not to the vascular system, proposing to change the name of the forma special to *F. oxysporum* f. sp. *radicis-vanillae*. In fact, resistance to the disease is observed because a

high level of lignification on the root hypodermis is present [65], while the activity of *SIX* genes has been observed when a pathogenic strain colonizes the vascular system only [66].

In conclusion, a genetic comparison among pathogenic and non-pathogenic endophytes using microsatellite data does not allow discrimination of both groups, however, phylogeny and genetic distances suggest that some pathogenic genotypes are similar because of convergent evolution. There is evidence for a great genetic diversity of endophyte *F. oxysporum* in the vanilla crop, based on microsatellite, TEF-1α sequences types and MT idiomorphs. Amplification of the currently known SIX genes was not possible, further studies are necessary for this field.

Declarations

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References

1. Summerell BA, Laurence MH, Liew ECY, Leslie JF (2010) Biogeography and phylogeography of Fusarium: a review. Fungal Divers 44:3–13. https://doi.org/10.1007/s13225-010-0060-2
2. Machungo C, Losenge T, Kahangi E et al (2009) Effect of endophytic Fusarium oxysporum on growth of tissue-cultured banana plants. J Hort Sci 2:160–167
3. Freeman S, Sharon M, Maymon M et al (2013) Fusarium euwallaceae sp. nov.–a symbiotic fungus of Euwallacea sp., an invasive ambrosia beetle in Israel and California. Mycologia 105:1595–1606. https://doi.org/10.3852/13-066
4. Martínez E, Martínez P, Guillén D et al (2015) Diversidad de Fusarium en las raíces de caña de azúcar (Saccharum officinarum) en el estado de Morelos, México. Rev Mex Micol 42:33–43
5. Gordon TR (2017) Fusarium oxysporum and the Fusarium Wilt Syndrome. Annu Rev Phytopathol 55:23–39. https://doi.org/10.1146/annurev-phyto-080615-095919
6. Guarro J, Madrid MP, Roncero MIG et al (2004) Fusarium oxysporum as a multihost model for the genetic dissection of fungal virulence in plants and mammals Fusarium oxysporum as a multihost model for the genetic dissection of fungal virulence in plants and mammals. Infect Immun 72:1760–1766. https://doi.org/10.1128/IAI.72.3.1760
7. Jain PK, Gupta VK, Misra AK et al (2011) Current Status of Fusarium Infection in Human and Animal. Asian J Anim Vet Adv 6:201–227. https://doi.org/10.3923/ajava.2011.201.227

8. Dean R, Van Kan JAL, Pretorius ZA et al (2012) The Top 10 fungal pathogens in molecular plant pathology. Mol Plant Pathol 13:414–430. https://doi.org/10.1111/j.1364-3703.2011.00783.x
9. Ting ASY, Meon S, Kadir J et al (2008) Endophytic microorganisms as potential growth promoters of banana. Biocontrol 53:541–553. https://doi.org/10.1007/s10526-007-9093-1
10. Edel-Hermann V, Lecomte C (2019) Current status of Fusarium oxysporum formae speciales and races. Phytopathology 109:512–530. https://doi.org/https://doi.org/10.1094/PHYTO-08-18-0320-RVW
11. Sasaki K, Nakahara K, Shigyo M et al (2015) Detection and quantification of onion isolates of Fusarium oxysporum f. sp. cepae in onion plant. J. Gen. plant Pathol. v. 81
12. Stewart JE, Abdo Z, Dumroese RK et al (2012) Virulence of Fusarium oxysporum and F. commune to Douglas-fir (Pseudotsuga menziesii) seedlings. For Pathol 42:220–228. https://doi.org/10.1111/j.1439-0329.2011.00746.x
13. Carvalho DDC, Lobo Júnior M, Martins I et al (2014) Biological control of Fusarium oxysporum f. sp. phaseoli by Trichoderma harzianum and its use for common bean seed treatment. Trop Plant Pathol 39:384–391. https://doi.org/10.1590/S1982-56762014000500005
14. Demers JE, Gugino BK, Jiménez-Gasco M del M (2015) Highly diverse endophytic and soil Fusarium oxysporum populations associated with field-grown tomato plants. Appl Environ Microbiol 81:81–90. https://doi.org/10.1128/AEM.02590-14
15. Lecomte C, Edel-Hermann V, Cannesan M-A et al (2016) Fusarium oxysporum f. sp. cyclaminis: underestimated genetic diversity. Eur J Plant Pathol 145
16. Magdama F, Monserrate-Maggi L, Serrano L et al (2019) Comparative analysis uncovers the limitations of current molecular detection methods for Fusarium oxysporum f. sp. cubense race 4 strains. PLoS One 14:e0222727. https://doi.org/10.1371/journal.pone.0222727
17. Ploetz RC (2006) Fusarium induced diseases of tropical, perennial crops. Phytopathology 96:648–652. https://doi.org/10.1094/PHYTO-96-0648
18. Koyyappurath S, Atuahiva T, Le Guen R et al (2016) Fusarium oxysporum f. sp. radicis-vanillae is the causal agent of root and stem rot of vanilla. Plant Pathol 65:612–625. https://doi.org/10.1111/ppa.12445
19. Pinaria AG, Liew ECY, Burgess LW (2010) Fusarium species associated with vanilla stem rot in Indonesia. Australas Plant Pathol 39:176–183. https://doi.org/10.1071/AP09079
20. Pinaria AG, Laurence MH, Burgess LW, Liew ECY (2015) Phylogeny and origin of Fusarium oxysporum f. sp. vanillae in Indonesia. Plant Pathol 64:1358–1365. https://doi.org/10.1111/ppa.12365
21. Adame-García J, Rodriguez-Guerra R, Iglesias-Andreu LG et al (2015) Molecular identification and pathogenic variation of fusarium species isolated from Vanilla planifolia in Papantla Mexico. Bot Sci 93:669–678. https://doi.org/10.17129/botsci.142
22. Casillas-Isiordia R, Flores de la Rosa FR, Can-chulim Á et al (2017) Fusarium sp. asssociated with Vanilla sp. rot in Nayarit, Mexico. ARPN J Agric Biol Sci 12:43–50
23. Flores-de la Rosa FR, De Luna E, Adame-García J et al (2018) Phylogenetic position and nucleotide diversity of Fusarium oxysporum f. sp. vanillae worldwide based on translation elongation factor 1α sequences. Plant Pathol 67:1278–1285. https://doi.org/10.1111/ppa.12847
24. Ma L-J, van der Does HC, Borkovich KA et al (2010) Comparative genomics reveals mobile pathogenicity chromosomes in Fusarium. Nature 464:367–373. https://doi.org/10.1038/nature08850
25. Laurence MH, Summerell BA, Liew ECY (2015) Fusarium oxysporum f. sp. canariensis: Evidence for horizontal gene transfer of putative pathogenicity genes. Plant Pathol 64:1068–1075. https://doi.org/10.1111/ppa.12350
26. Jones JDG, Dangl JL (2006) The plant immune system. Nature 444:323
27. Essarioui A, Mokrini F, Afechtal M (2016) Molecular interactions between tomato and its wilt pathogen Fusarium oxysporum f. sp. Lycopersici. 66–74

28. Adame-García J, Flores de la Rosa FR, Ricaño-rodríguez J, Luna Rodríguez M (2016) Adequacy of a protocol for amplification of EF-1α gene of Fusarium oxysporum f. sp. vanillae. ARPN J Agric Biol Sci 11:236–241
29. Arie T, Kaneko I, Yoshida T et al (2000) Mating-type genes from asexual phytopathogenic ascomycetes Fusarium oxysporum and Alternaria alternata. Mol Plant Microbe Interact 13:1330–1339. https://doi.org/10.1094/MPMI.2000.13.12.1330
30. Fourie G, Steenkamp ET, Gordon TR, Viljoen A (2009) Evolutionary Relationships among the Fusarium oxysporum f. sp. cubense Vegetative Compatibility Groups. Appl Environ Microbiol 75:4770–4781. https://doi.org/10.1128/AEM.00370-09
31. Steenkamp ET, Wingfield BD, Coutinho TA et al (2000) PCR-based identification of MAT-1 and MAT-2 in the Gibberella fujikuroi species complex. Appl Environ Microbiol 66:4378–4382. https://doi.org/10.1128/AEM.66.10.4378-4382.2000
32. Demers JE, Garzón CD, Jiménez-Gasco M del M (2014) Striking genetic similarity between races of Fusarium oxysporum f. sp. ciceris confirms a monophyletic origin and clonal evolution of the chickpea vascular wilt pathogen. Eur J Plant Pathol 139:303–318. https://doi.org/10.1007/s10658-014-0387-8
33. Geiser DM, Jiménez-Gasco MDM, Kang S et al (2004) FUSARIUM-ID v. 1.0: A DNA sequence database for identifying *Fusarium*. Eur J Plant Pathol 110:473–479. https://doi.org/10.1023/B:EJPP.0000032386.75915.a0
34. Goloboff PA, Farris JS, Nixon KC (2008) TNT, a free program for phylogenetic analysis. Cladistics 24:774–786. https://doi.org/10.1111/j.1096-0031.2008.00217.x
35. Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. Mol Ecol 14:2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x
36. Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361. https://doi.org/10.1007/s12686-011-9548-7
37. Adame-García J, Trigos Á, Iglesias-Andreu LG et al (2011) Isozymic and pathogenic variations of Fusarium spp. associated with vanilla stem and root rotting. Trop Subtrop Agroecosystems 299–306
38. Kerényi Z, Moretti A, Waalwijk C et al (2004) Mating Type Sequences in Asexually Reproducing Fusarium Species. Appl Environ Microbiol 70:4419–4423. https://doi.org/10.1128/AEM.70.8.4419
39. Kashyap PL, Rai S, Kumar S, Srivastava AK (2016) Genetic diversity, mating types and phylogenetic analysis of Indian races of Fusarium oxysporum f. sp. ciceris from chickpea. Arch Phytopathol Plant Prot 49:533–553. https://doi.org/10.1080/03235408.2016.1243024
40. Mirtalebi M, Banihashemi Z, Linde CC (2013) Phylogenetic relationships of Fusarium oxysporum f. sp. melonis in Iran. Eur. J. plant Pathol. v. 136
41. Cantuarias-Avilés T, Mourão Filho F, de AA, Stuchi ES, et al (2012) Rootstocks for high fruit yield and quality of “Tahiti” lime under rain-fed conditions. Sci Hortic (Amsterdam) 142:105–111. https://doi.org/10.1016/j.scienta.2012.05.008
42. Epstein L, Kaur S, Chang PL et al (2016) Races of the Celery Pathogen Fusarium oxysporum f. sp. apii Are Polyphyletic. Phytopathology 107:463–473. https://doi.org/10.1094/PHYTO-04-16-0174-R
43. O’Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc Natl Acad Sci U S A 95:2044–2049. https://doi.org/10.1073/pnas.95.5.2044
44. Soanes D, Richards TA (2014) Horizontal Gene Transfer in Eukaryotic Plant Pathogens. Annu Rev Phytopathol 52:583–614. https://doi.org/10.1146/annurev-phyto-102313-050127
45. Demers JE, Jiménez-Gasco M del M (2016) Evolution of Nine Microsatellite Loci in the Fungus Fusarium oxysporum. J Mol Evol 82:27–37. https://doi.org/10.1007/s00239-015-9725-5
46. Vijayan AK, Sithara L, Sreelakshmi KP et al (2012) Molecular diversity of Fusarium oxysporum causing rot diseases of vanilla in south India. Arch Phytopathol Plant Prot 45:1319–1326. https://doi.org/10.1080/03235408.2012.673265

47. Shang Y, Xiao G, Zheng P et al (2016) Divergent and Convergent Evolution of Fungal Pathogenicity. Genome Biol Evol 8:1374–1387. https://doi.org/10.1093/gbe/evw082
48. Bory S, Grisoni M, Duval MF, Besse P (2008) Biodiversity and preservation of vanilla: Present state of knowledge. Genet Resour Crop Evol 55:551–571. https://doi.org/10.1007/s10722-007-9260-3
49. Lubinsky P, Bory S, Hernández Hernández J et al (2008) Origins and dispersal of cultivated vanilla (Vanilla planifolia Jacks. [Orchidaceae]). Econ Bot 62:127–138. https://doi.org/10.1007/s12231-008-9014-y
50. Klaubauf S, Inselsbacher E, Zechmeister-Boltenstern S et al (2010) Molecular diversity of fungal communities in agricultural soils from Lower Austria. Fungal Divers 44:65–75. https://doi.org/10.1007/s13225-010-0053-1
51. Inami K, Kashiwa T, Kawabe M et al (2014) The Tomato Wilt Fungus Fusarium oxysporum f. sp. lycopersici shares Common Ancestors with Nonpathogenic F. oxysporum isolated from Wild Tomatoes in the Peruvian Andes. Microbes Environ 29:200–210. https://doi.org/10.1264/jsme2.ME13184
52. Takken F, Rep M (2010) The arms race between tomato and Fusarium oxysporum. Mol Plant Pathol 11:309–314. https://doi.org/10.1111/j.1364-3703.2009.00605.x
53. Guo L, Han L, Yang L et al (2014) Genome and Transcriptome Analysis of the Fungal Pathogen Fusarium oxysporum f. sp. cubense Causing Banana Vascular Wilt Disease. PLoS One 9:e95543
54. Taylor A, Vágány V, Jackson AC et al (2016) Identification of pathogenicity-related genes in Fusarium oxysporum f. sp. cepae. Mol Plant Pathol 17:1032–1047. https://doi.org/10.1111/mpp.12346
55. Czislowski E, Fraser-Smith S, Zander M et al (2017) Investigating the diversity of effector genes in the banana pathogen, *Fusarium oxysporum* f. sp. *cubense*, reveals evidence of horizontal gene transfer. Mol Plant Pathol. https://doi.org/10.1111/mpp.12594
56. van der Does HC, Duyvesteijn RGE, Goltstein PM et al (2008) Expression of effector gene SIX1 of Fusarium oxysporum requires living plant cells. Fungal Genet Biol 45:1257–1264. https://doi.org/10.1016/j.fgb.2008.06.002
57. Lievens B, Houterman PM, Rep M (2009) Effector gene screening allows unambiguous identification of Fusarium oxysporum f. sp. lycopersici races and discrimination from other formae speciales. FEMS Microbiol Lett 300:201–215. https://doi.org/10.1111/j.1574-6968.2009.01783.x
58. Chakrabarti A, Rep M, Wang B et al (2011) Variation in potential effector genes distinguishing Australian and non-Australian isolates of the cotton wilt pathogen Fusarium oxysporum f.sp. vasinfectum. Plant Pathol 60:232–243. https://doi.org/10.1111/j.1365-3059.2010.02363.x
59. Meldrum RA, Fraser-Smith S, Tran-Nguyen LTT et al (2012) Presence of putative pathogenicity genes in isolates of Fusarium oxysporum f. sp. cubense from Australia. Australas Plant Pathol 41:551–557. https://doi.org/10.1007/s13313-012-0122-x
60. Rocha LO, Laurence MH, Ludowici VA et al (2016) Putative effector genes detected in Fusarium oxysporum from natural ecosystems of Australia. Plant Pathol 65:914–929. https://doi.org/10.1111/ppa.12472
61. Thatcher LF, Gardiner DM, Kazan K, Manners JM (2012) A Highly Conserved Effector in *Fusarium oxysporum* Is Required for Full Virulence on *Arabidopsis*. Mol Plant-Microbe Interact 25:180–190. https://doi.org/10.1094/MPMI-08-11-0212
62. van Dam P, de Sain M, ter Horst A et al (2017) Comparative genomics-based markers: discrimination of host-specificity in *Fusarium oxysporum*. Appl Environ Microbiol AEM.01868-17. https://doi.org/10.1128/AEM.01868-17
63. van Dam P, Fokkens L, Ayukawa Y et al (2017) A mobile pathogenicity chromosome in Fusarium oxysporum for infection of multiple cucurbit species. Sci Rep 7:9042. https://doi.org/10.1038/s41598-017-07995-y
64. van Dam P, Rep M (2017) The Distribution of Miniature Impala Elements and SIX Genes in the Fusarium Genus is Suggestive of Horizontal Gene Transfer. J Mol Evol 85:14–25. https://doi.org/10.1007/s00239-017-9801-0
65. Koyyappurath S, Conéjéro G, Dijoux JB et al (2015) Differential Responses of Vanilla Accessions to Root Rot and Colonization by Fusarium oxysporum f. sp. radicis-vanillae. Front Plant Sci 6:1–16.

https://doi.org/10.3389/fpls.2015.01125

66. Niño-Sanchez J, Tello V, Casado-del Castillo V et al (2015) Gene expression patterns and dynamics of the colonization of common bean (Phaseolus vulgaris L.) by highly virulent and weakly virulent strains of Fusarium oxysporum. Front Microbiol 6:1–14. https://doi.org/10.3389/fmicb.2015.00234

Figures

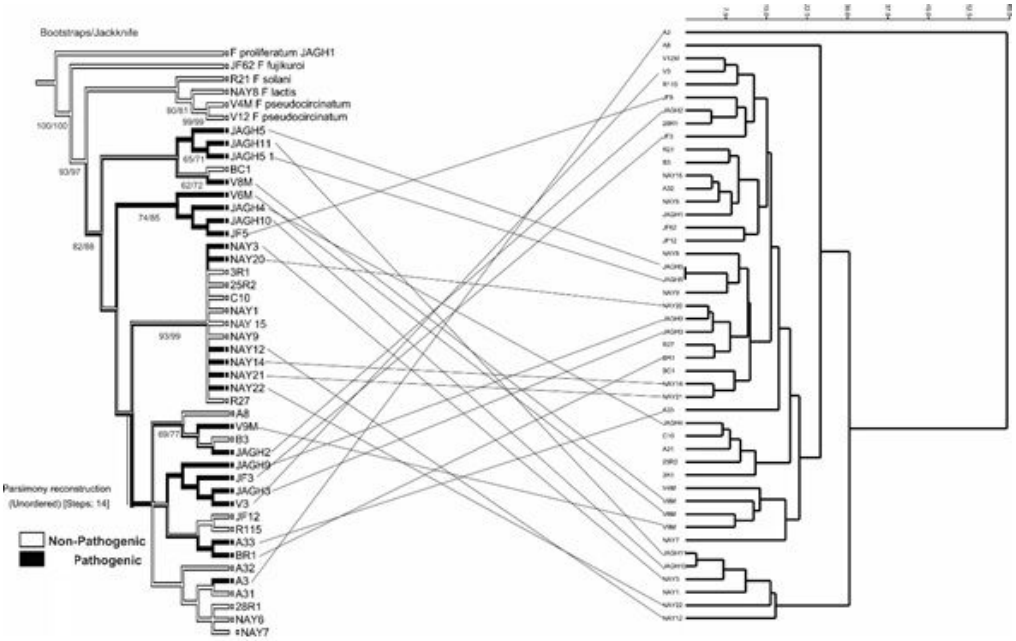

Figure 1

Comparison between phylogenetic relationships (A) and genetic similarity (B) of F. oxysporum endophytes associated with V. planifolia. The ancestral state reconstruction is observed; dark terminals are marked as pathogenic endophytes (F. oxysporum f. sp. vanillae) while white is non-pathogenic. Lines link the same strain in the phylogenetic tree and UPGMA dendrogram. This shows that some strains are genetically very close, however, these strains belong to different lineages. This result supports the polyphyletic nature of F. oxysporum f. sp. vanillae.

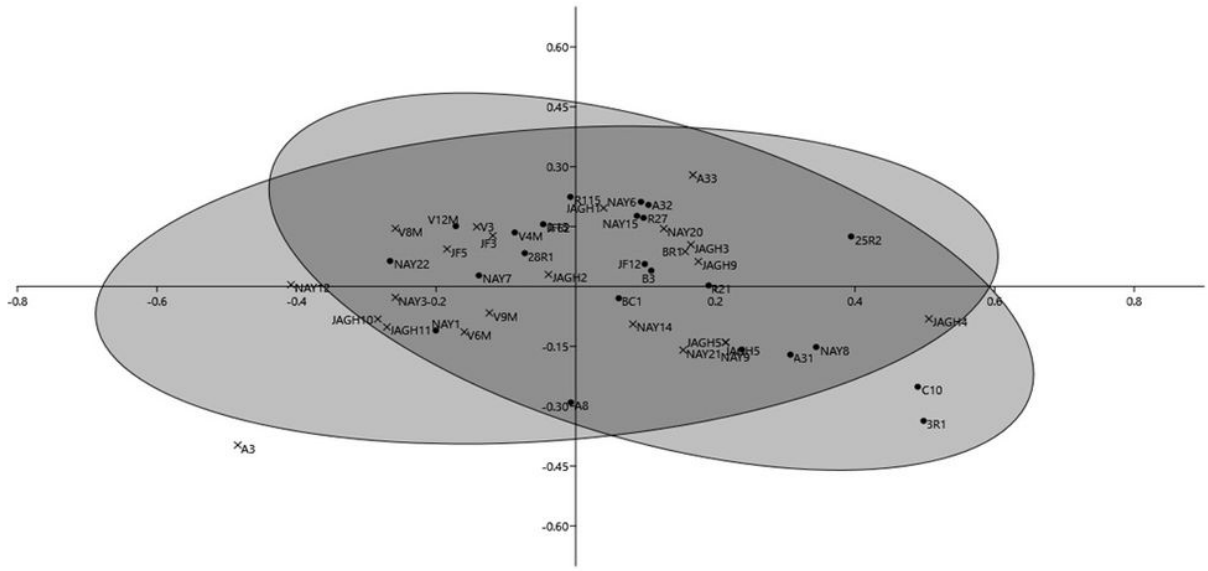

Figure 2

Correspondence Analysis of microsatellite data. The axis explained 68.119% of the total variance. Discrimination between pathogenic and non-pathogenic groups is not possible. The great majority of strains are grouped in the center of the graph, suggesting that microsatellite data is not an efficient determinant of pathogenicity.