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Detection of the *xyl3* gene in strains of *Fusarium oxysporum* f. sp. *vanillae*

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Abstract

The mechanisms of *Fusarium oxysporum* related to the degradation of structural components of the root, such as xylan, are very important since the colonization of this organ is a key piece in the establishment of the disease. The present study focused on detecting the gene coding for the xylanase *xyl3* enzyme in strains of *F. oxysporum* f. sp. *vanillae* and searching for homologues to this gene in sequences of other *formae speciales* and species of the *Fusarium* genus, in order to determine the phylogenetic relationships between xylanases within the *F. oxysporum* species complex, as well as to search for evidence of natural selection. The results indicated that, of the nine strains evaluated, only three had a copy of the *xyl3* gene. The phylogeny showed eight clades, where clade 3 was consistent with the classification of *xyl3*, while the other types of xylanases were grouped in clade 2. The natural selection test showed no evidence of positive selection within the phylogeny, suggesting that the neutral mutation is responsible for the diversity in the xylanase gene among the *F. oxysporum* species complex, leading to the proposal that the gene does not appear to have changed with colonization of new hosts.

Keywords:

mutations, positive selection, xylanase gene.

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Introduction

The fungus *Fusarium oxysporum* is a ubiquitous inhabitant of soils in almost all ecosystems. It is currently considered a complex of species, based on phylogenies carried out with different genes (O'Donell *et al*., 2009). *F. oxysporum* fulfills various ecological roles, although it is mainly considered a saprophyte (Abdul *et al*., 2016), it has an important role as a beneficial (Waweru *et al*., 2014) and pathogenic endophyte (Demers *et al*., 2015). The latter receive special attention since they cause very destructive diseases in various crops.

The pathogenic endophytes of *F. oxysporum* are named according to their specificity to the host, this is known as *formae speciales* (Edel-Hermann and Lecomte, 2019). Being a soil inhabitant, most of the *formae speciales* of *F. oxysporum* invade the plant through the root (Olivain *et al*., 2006; Turrá *et al*., 2015; Koyyapurath *et al*., 2016). Therefore, it is essential to understand the mechanisms that the fungus uses for the degradation of the main components of the root cell walls. These components include cellulose, hemicellulose and lignin (Pattathil *et al*., 2015).

Xylan is one of the structural components of hemicellulose and therefore, it is essential to determine the ability of the pathogen to degrade this polysaccharide (Pattathil *et al*., 2015). Due to the chemical complexity of xylan, a number of enzymes are needed to degrade it and break the resistance of the cell wall (De Vries and Visser, 2001; Kalluri *et al*., 2014).

Some pathogenic strains of *F. oxysporum* contain functional genes coding for different variants of the xylanase enzyme, for example, differential expression of the genes *xyl2* and *xyl3* has been observed during the colonization of tomato plants by *F. oxysporum* f. sp. *lycopersici*, with the *xyl3* gene being the one that showed activity in the roots (Ruiz-Roldán *et al*., 1999). It was also determined that the *xyl3* gene is expressed differentially between races and pathotypes of *F. oxysporum* f. sp. *ciceris* in chickpea, therefore, it can be used as a marker to differentiate between physiological races of this *forma specialis* (Jorge *et al*., 2005; Gurjar *et al*., 2009).

On the other hand, it was demonstrated that the presence and activity of two genes, *xyl3* and *xyl4*, are not directly related to the pathogenic capacity of *F. oxysporum* f. sp. *lycopersici* in tomato (Gómez-Gómez *et al*., 2002). *F. oxysporum* f. sp. *vanillae* is the cause of stem and root rot in vanilla (*Vanilla planifolia*), an orchid of high commercial value because it is the natural source of vanillin (Pinaria *et al*., 2010; Adame-García *et al*., 2015; González-Oviedo *et al*., 2022). For this pathogen, there is evidence of variation in the activity of lytic enzymes related to pathogenic differences found between different isolates of the fungus (Adame-García *et al*., 2011; Koyyappurath *et al*., 2015).

Histological analyses performed in the root zone of vanilla infected with the pathogen have shown that, unlike other *formae speciales*, *F. oxysporum* f. sp. *vanillae* invades the root hair zone, penetrates through cortical cells, but does not colonize the vascular system, indicating that its root damage mechanisms are essential for disease establishment (Koyyappurath *et al*., 2016). However, there is no information on the enzymes that degrade important components of the root, such as xylan, and to date the mechanisms that this pathogen uses to establish the disease have not been clearly established.

The objective of the present work was to detect the presence of the *xyl3* gene in strains of *F. oxysporum* f. sp. v*anillae* that have shown different levels of pathogenicity, in order to determine phylogenetic relationships between xylanases within the *F. oxysporum* species complex, as well as to search for evidence of positive natural selection.

Materials and methods

Strains of *Fusarium oxysporum* f. sp. *vanillae*

Nine strains of *F. oxysporum* f. sp. *vanillae* were used, which were previously reported as pathogenic to vanilla (Adame-García *et al*., 2015) and which belong to the collection of vanilla pathogens under the protection of the Laboratory of Genetics and Plant-Microorganism

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Interactions of the Faculty of Agricultural Sciences of the Veracruzan University. Each fungal shelter consisted of PDA agar discs with mycelium immersed in sterile distilled water stored at 4 ° C. For their use, the strains were reactivated in PDA medium from the inoculation of 10 µl of the fungal suspension of the strain in shelter, incubated for seven days at 27 °C, with a period of 16 h of light and eight of darkness.

DNA extraction and amplification of the *xyl3* gene

DNA extraction was performed according to the protocol established by Adame-García *et al*. (2016). The conditions for the amplification of the *xyl3* gene were based on the protocol described by Gurjar *et al*. (2009), using the oligonucleotides *XYL3*-F (5'- GAC AAY AGC ATG AAG TGG GAT- 3') and *XYL3*-R (5'- ACA CCC CAD ACR GTR ATD CC-3'). The reaction mixture consisted of 1X PCR buffer, 2.5 mM of $MgCl_2$, 1 U of Taq DNA polymerase (Promega brand), 0.25 mM of dNTPs, 25 pmol of each oligonucleotide and 50 ng of genomic DNA, in a final volume of 25 µl.

The thermal cycle used for amplification was as follows: an initial denaturation phase at 94 °C for 5 min, 30 denaturation cycles at 94 °C for 1 min, annealing at 50 °C for 30 s and polymerization at 72 °C for 30 s and final extension at 72 °C for 10 min. PCR reactions were performed in a T100 thermal cycler (Bio-Rad®). PCR products were visualized in a 1.8% agarose gel in TAE buffer (80 V, 60 min), stained in 2% ethidium bromide (Promega) under UV light in a Gel Doc EZ Imager photodocumenter (Bio-Rad®), a 100 bp molecular weight marker (Promega®) was used to compare the size of the amplification product.

Subsequently, the amplification products were purified using the protocol of the Wizard® SV Gel and PCR Clean-Up System kit (Promega) and were sequenced using the Sanger sequencing method. The amplifications were repeated in triplicate.

Search for sequences homologous to the *xyl3* gene

The sequences were analyzed and edited in the Bioedit 7.2.5 software (Hall, 1999) to perform a Blast analysis (parameters offered by default) in the genbank database of the NCBI. For this, the analysis included genomes of different *formae speciales* of *F. oxysporum* as well as genomes of other species of the *Fusarium* genus obtained from different electronic databases of open access.

Sequence annealing and phylogenetic analysis

A group of 81 sequences of genes coding for the xylanase enzyme were used, which were annealed using the ClustalW algorithm (gap open= 15; gap extend= 3). The database consisted of 76 sequences of different *formae speciales* and five of other species of the *Fusarium* genus. Annealing was performed in the Bioedit 7.2.5 software (Hall, 1999). Unweighted parsimony analyses were performed with the TNT 1.1 software (Goloboff *et al*., 2008) using the Winclada interface (1.94.1). The search for the most parsimonious tree was executed with 1 000 replicates for each case, using a combination of algorithms (Ratchet + Drift + Sectorial Fusion + TBR-max). Inferences about clade robustness were derived with Bootstrap resampling (1 000 repetitions with the same search characteristics).

Positive selection detection by codon

The modified Nei-Gojobori model was applied to determine the parameters dN and dS (Nei and Gojobori, 1986). The calculations were performed using a maximum likelihood method based on the phylogenetic tree previously obtained. The general time-reversible (GTR) model was applied as a nucleotide substitution model and a standard genetic code was selected, this analysis was performed with the MEGA 7 software (Kumar *et al*., 2016).

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Results and discussion

Amplification of the *xyl3* gene in *F. oxysporum* f. sp. *vanillae*

Only three of the nine strains of *F. oxysporum* f. sp. *vanillae* (JAGH5, JAGH10, JAGH12) were positive for amplification of the *xyl3* gene. A single 0.7 kb product with no nonspecific bands was observed. The sequencing process allowed obtaining three sequences with high definition in the electropherogram. The Blast analysis linked all sequences to the xylanase *xyl3* gene of *F. oxysporum* f. sp. *lycopersici* (accession number AF052582.1) with 99% similarity.

These results allowed a genotyped division of *F. oxysporum* f. sp. *vanillae* into two groups, one in which the *xyl3* gene is present and one in which it is absent. Since so far there were no reports of any type of xylanase in *F. oxysporum* f. sp. *vanillae*, the present study reports for the first time the detection of the *xyl3* gene in this *forma specialis*. In addition, since the amplification reactions of the gene did not generate products in all the strains previously studied by Adame-García *et al*. (2015), it is stated that there are different genotypes within this *forma specialis* and that among these differences is the xylanase 3 enzyme (XYL3).

The *xyl3* gene has been used to distinguish races of *F. oxysporum* f. sp. *ciceris* (Gurjar *et al*., 2009) and its differential activity has supported the differentiation of pathotypes (Jorge *et al*., 2005). It is noteworthy that the strains of *F. oxysporum* f. sp. *vanillae* that generated amplification products of the *xyl3* gene belong to the group of moderate virulence for vanilla (Adame-García *et al*., 2015), while it has been demonstrated that several structural motifs of xylan have changed during the evolution of plant groups (Peña *et al*., 2016).

Such information will be valuable in determining how much the diversity of xylanase enzymes of *F. oxysporum* f. sp. *vanillae* is related to strains that present a higher degree of pathogenicity, considering the structural composition of xylans of the cell wall of the roots of *V. planifolia* and in comparison with *Vanilla pompona*, which has the characteristic of being one of the species of the genus most resistant to pathogens (Soto-Arenas and Solano Gómez, 2007).

Search for the xylanase *xyl3* gene in genomes of *Fusarium* spp. and *formae speciales* of *F. oxysporum*

The Blast analysis in each genome found in the NCBI database allowed identifying some copies of the xylanase *xyl3* gene in different species and *formae speciales* of *F. oxysporum*. Table 1 shows the percentages of similarity achieved with the sequences of this study.

Table 1. Results of the Blast analysis of the *xyl3* gene of *F. oxysporum* f. sp. *vanillae* performed against *formae speciales* genomes of *F. oxysporum* and *Fusarium* spp.

Species	Strain	Genbank accession	Similarity (%)
F. oxysporum f. sp. *lycopersici*	4287	NC-030997	99
F. oxysporum f. sp. *lycopersici*	MN25	JH650838	99
F. oxysporum f. sp. *pisi*	HDV247	JH651390	99
F. oxysporum f. sp. *radicis-lycopersici*	26 381	JH650976	99
F. oxysporum f. sp. *vasinfectum*	25 433	JH657940	98
F. oxysporum f. sp. *cubense* tropical race 4	54 006	JH658292	92

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Species	Strain	Genbank accession	Similarity (%)
F. oxysporum f. sp. *conglutinans* race 2	54 008	KK033209	98
F. oxysporum f. sp. *raphani*	54 005	JH658394	99
F. oxysporum f. sp. *melonis*	26 406	JH659333	99
F. oxysporum	Fo47	JH717908	99
F. oxysporum	FOSC 3-a	JH717848	98
F. oxysporum f. sp. *cubense* race 1	race 1	KB730516	99
F. oxysporum f. sp. *cubense* race 4	race 4	KB726570	96
F. oxysporum f. sp. *medicaginis*		KV442496	99
F. oxysporum	Fo5176	AFQF01000985	98
F. oxysporum	UASWSAC1	JNNQ01001126	99
F. oxysporum f. sp. *cubense*	C1HIR-9889	MBFV01000633	91
F. oxysporum f. sp. *conglutinans*	1	LPZQ01011374	98
F. oxysporum	JCM 11502	BCHB01000008	96
F. oxysporum f. sp. *cucumerinum*	Foc013	MABJ01000473	91
F. oxysporum f. sp. *niveum*	Fon005	MAKY01000369	99
F. oxysporum f. sp. *cucumerinum*	Foc001	MAKZ01000123	98
F. oxysporum f. sp. *cucumerinum*	Foc018	MABM01000088	98
F. oxysporum f. sp. *cucumerinum*	Foc021	MABL01000269	98
F. oxysporum f. sp. *cucumerinum*	Foc015	MABK01000132	99
F. oxysporum f. sp. *cucumerinum*	Foc030	MABN01001749	98
F. oxysporum f. sp. *radicis-cucumerinum*	Forc031	MABS01000137	99
F. oxysporum f. sp. *cucumerinum*	Foc035	MABO01000683	99
F. oxysporum f. sp. *radicis-cucumerinum*	Forc016	MABQ01000104	99
F. oxysporum f. sp. *niveum*	Fon019	MAMH01001256	99
F. oxysporum f. sp. *radicis-cucumerinum*	Forc024	MABR01000083	99
F. oxysporum f. sp. *niveum*	Fon002	MALA01000310	99
F. oxysporum f. sp. *niveum*	Fon013	MALC01000494	99
F. oxysporum f. sp. *niveum*	Fon010	MALB01000085	99

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Species	Strain	Genbank accession	Similarity (%)
F. oxysporum f. sp. *niveum*	Fon015	MALD01000061	99
F. oxysporum f. sp. *niveum*	Fon020	MALE01000131	99
F. oxysporum f. sp. *niveum*	Fon037	MALF01000365	99
F. oxysporum f. sp. *niveum*	Fon021	MALG01000164	99
F. oxysporum f. sp. *lycopersici*	Fol004	MALH01000304	99
F. oxysporum f. sp. *lycopersici*	Fol007	MALI01000160	99
F. oxysporum f. sp. *lycopersici*	Fol026	MALK01000267	99
F. oxysporum f. sp. *lycopersici*	Fol014	MALJ01000177	99
F. oxysporum f. sp. *lycopersici*	Fol018	MALL01000293	99
F. oxysporum f. sp. *lycopersici*	Fol016	MALM01000304	99
F. oxysporum f. sp. *lycopersici*	Fol038	MALO01000385	99
F. oxysporum f. sp. *lycopersici*	Fol029	MALN01000525	99
F. oxysporum f. sp. *lycopersici*	Fol069	MALP01000090	99
F. oxysporum f. sp. *lycopersici*	Fol072	MALQ01000181	99
F. oxysporum f. sp. *lycopersici*	Fol073	MALR01000973	99
F. oxysporum f. sp. *lycopersici*	Fol074	MALS01000346	99
F. oxysporum	FoMN14	MALU01000082	99
F. oxysporum f. sp. *lycopersici*	Fol075	MALT01000042	99
F. oxysporum f. sp. *lycopersici*	4 287	MALW01000519	99
F. oxysporum f. sp. *melonis*	Fom005	MALY01000333	99
F. oxysporum f. sp. *melonis*	Fom004	MALX01000210	99
F. oxysporum f. sp. *melonis*	Fom006	MALZ01000374	99
F. oxysporum f. sp. *melonis*	Fom009	MAMA01000314	99
F. oxysporum f. sp. *melonis*	Fom011	MAMC01000335	99
F. oxysporum f. sp. *melonis*	Fom010	MAMB01000159	99
F. oxysporum f. sp. *melonis*	Fom013	MAME01000015	99

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Species	Strain	Genbank accession	Similarity (%)
F. oxysporum f. sp. *melonis*	Fom012	MAMD01000050	99
F. oxysporum f. sp. *lycopersici*	Fol002	MAMG01000077	99
F. oxysporum f. sp. *melonis*	Fom016	MAMF01000406	99
F. oxysporum f. sp. *cucumerinum*	Foc011	MABT01000535	91
F. oxysporum f. sp. *ciceris*	38-1	MEHF01000035	99
F. oxysporum f. sp. *melongenae*	14 004	MPIL01001435	93
F. verticilliodes	7 600	XM-018901170	88
F. fujikuroi	58 289	HF679026	88
F. culmorum		LT598661	80
F. graminearum		NC-026476	80
F. pseudograminearum	CS309	NC-031953	80

Phylogeny of the *xyl3* gene

Parsimony analysis generated a single most parsimonious tree (Figure 1). In this topology, eight clades with appropriate bootstrap support were retrieved. Clade 3 contains the sequence corresponding to *F. oxysporum* f. sp. *lycopersici xyl3* (AF052582) and the three sequences of the xylanase gene of *F. oxysporum* f. sp. *vanillae*, this corroborates that these strains contain a homologous gene of *xyl3*. It is noteworthy to note that this clade contains most of the strains of *F. oxysporum* f. sp. *lycopersici* and only one strain of *F. oxysporum* f. sp. *medicaginis*.

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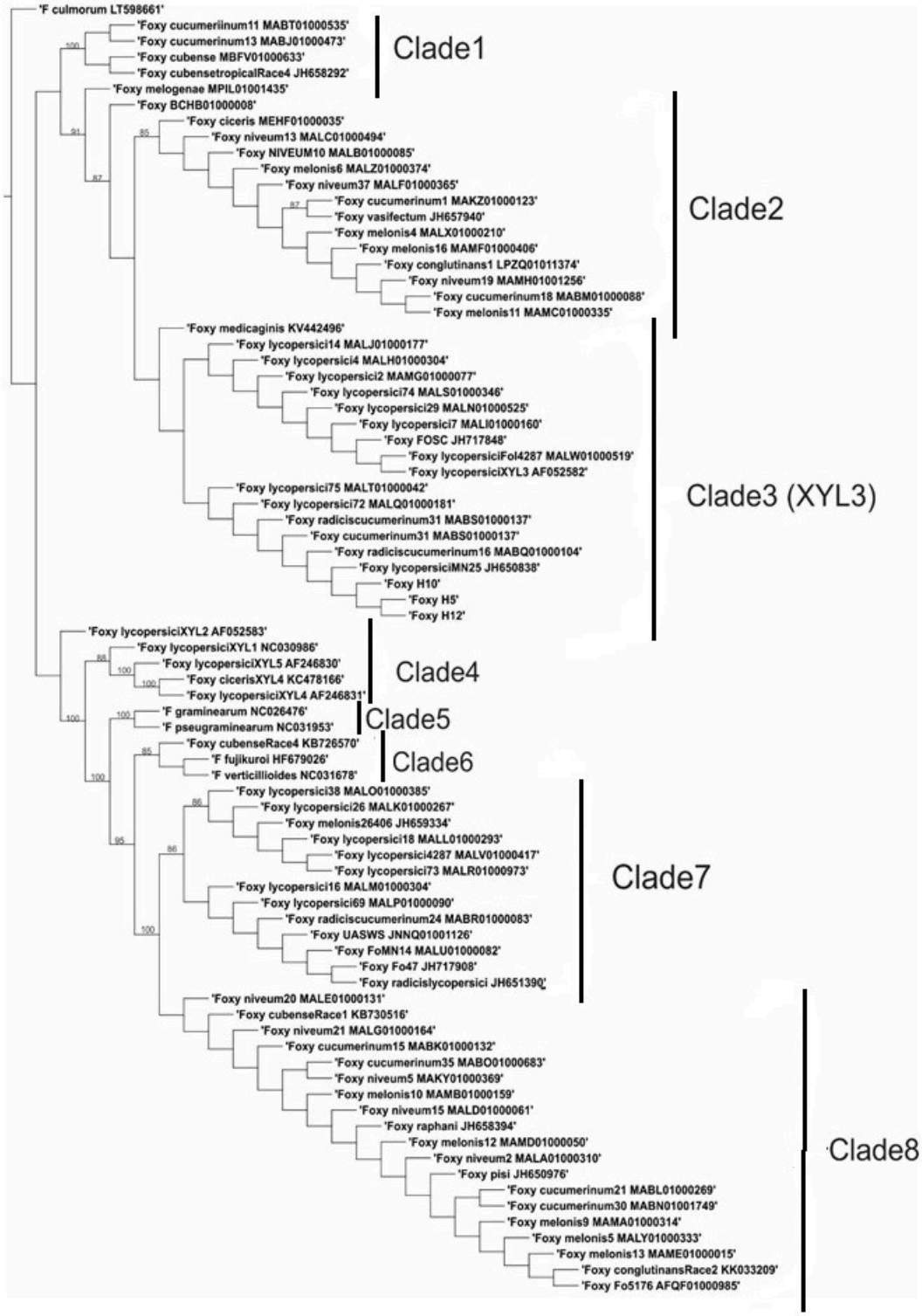
Figure 1. Most parsimonious tree (L= 1825; Ci=73; Ri= 96). Obtained from the database of coding sequences of xylanases of *F. oxysporum* and *Fusarium* spp.



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Other isoforms of the xylanase gene of *F. oxysporum* f. sp. *lycopersici* (*xyl1*, *xyl4*, *xyl5*) and *xyl4* of *F. oxysporum* f. sp. *ciceris* are grouped in clade 4 and are sisters of a lineage with only the *xyl2* isoform of *F. oxysporum* f. sp. *lycopersici*. Next to this clade, there are two small clades, the first composed of xylanase of *F. graminearum* (NC026476) and *F. pseudograminearum* (NC031953) and the second composed of xylanase of *F. verticillioides* (NC031678), *F. fujikuroi* (NC031678) and a strain of *F. oxysporum* f. sp. *cubense* RT4 (KB726570).

These two clades are very distinctive because these sequences were used as outer groups along with *F. culmorum* (LT598661). Clades 1, 2, and 8 have no sequences of *F. oxysporum* f. sp. *lycopersici*; those clades are composed of pathogenic strains of cucurbits, bananas and other plants. Some individual lineages were not adequately resolved. Eight well-supported clades were obtained for the phylogeny of the xylanase gene. A previous classification was proposed for the genes of the xylanase enzyme, they were classified as *xyl1* (Ruiz *et al*., 1999), *xyl2* and *xyl3* (Ruiz *et al*., 1999), *xyl4* and *xyl5* (Gómez-Gómez *et al*., 2002). Nevertheless, in the phylogeny shown in the present study, all *xyl* genes, except for *xyl3*, are located in the same clade (Clade 2; Figure 1).

Detection of positive selection at each codon

The model by Nei-Gojobori (1986) was used to determine whether some codons of the sequence of the xylanase *xyl3* gene are affected by positive selection. For this purpose, 108 codons were analyzed for synonymous and nonsynonymous mutations. The most common amino acid found was glycine, with four different codons, GGC (eight times), GGG (five times), GGA (two times), GGT (one time). This shows that synonymous mutations are frequently present in xylanase *xyl3* sequences. No significant results were observed regarding positive selection for other amino acids.

To evaluate the differences between xylanase genes according to clade division, natural selection tests based on codons were performed between the different lineages indicated by the gene tree. Synonymous mutations were found to be more abundant and common than nonsynonymous mutations, which in turn is evidence of neutral mutations (Nei and Gojobori, 1986). This approach has been used in other genes to detect positive selection; that is, evidence that natural selection gives rise to the diversity of some gene (Zhang *et al*., 2005; Hughes and Friedman, 2008; Metzger and Thomas, 2010). According to an exhaustive search of the scientific literature, this is the first study with an approach of positive selection tests on codons used for the analysis of genes in relation to pathogenicity in *F. oxysporum*.

Differences in the amplification of the xylanase *xyl3* gene in strains of *F. oxysporum* f. sp. *vanillae* can be explained on the basis of the polyphyletic distribution of this *forma specialis* among the *F. oxysporum* Species Complex (Pinaria *et al*., 2015; Flores-de la Rosa *et al*., 2018). Some pathogenicity effectors move horizontally between different lineages of *F. oxysporum*, giving pathogenic capacity to these lineages. Some of these new pathogenic lineages contain the *xyl3* gene in their genomes, while others do not, so there are pathogenic strains with and without activity of the gene (Laurence *et al*., 2015).

Conclusions

This research showed that the presence of the *xyl3* gene is not a characteristic of all strains of *F. oxysporum* f. sp. *vanillae*, even the presence of the gene could be associated with moderate virulence. Phylogeny suggests different types of *xyl* genes; however, no evidence of positive selection was observed in the coding sequences for this gene in *F. oxysporum*.

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Bibliography

1 Abdul-Karim, N. F.; Mohd, M.; Izham-Mohd, N. M and Zakaria, L. 2016. Saprophytic and potentially pathogenic *Fusarium* species from peat soil in Perak and Pahang. Trop Life Sci. Res. 27(1):1-20. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4807956/.

2 Adame-García, J.; Trigos, Á.; Iglesias-Andreu, L. G.; Flores-Estévez, N. and Luna-Rodríguez, M. 2011. Isozymic and pathogenic variations of *Fusarium* spp. associated with vanilla stem and root rotting. Trop subtrop agroecosystems. 13(3):299-306. https://www.revista.ccba.uady.mx/ojs/index.php/TSA/article/view/1330/663.

3 Adame-García, J.; Rodriguez-Guerra, R.; Iglesias-Andreu, L. G.; Ramos-Prado, J. M. and Luna-Rodríguez, M. 2015. Molecular identification and pathogenic variation of *Fusarium* species isolated from *Vanilla planifolia* in Papantla Mexico. Bot. Sci. 93(3):669-678. https://doi.org/10.17129/botsci.142.

4 Adame-García, J.; Flores-Rosa, F. R.; Ricaño-Rodríguez, J. and Luna-Rodríguez, M. 2016. Adequacy of a protocol for amplification of EF-1# gene of *Fusarium oxysporum* f. sp. *vanillae*. ARPN J. Agric. Biol. Sci. 11(6):236-241. http://www.arpnjournals.org/jabs/ research-papers/rp-2016/jabs-0616-804.pdf.

5 Demers, J. E.; Gugino, B. K. and Jiménez-Gasco, M. M. 2015. Highly diverse endophytic and soil *Fusarium oxysporum* populations associated with field-grown tomato plants. Appl. Environ. Microbiol. 81(1):81-90. https://doi.org/10.1128/AEM.02590-14.

6 De-Vries, R. P. and Visser J. 2001. *Aspergillus* enzymes involved in degradation of plant cell wall polysaccharides. Microbiol Mol. Biol Rev. 65(4):497-522. https://doi.org/10.1128/MMBR.65.4.497-522.2001.

7 Edel-Hermann, V. and Lecomte, C. 2019. Current status of *Fusarium oxysporum* formae speciales and races. Phytopathology. 109(4):512-530. https://doi.org/10.1094/PHYTO-08-18-0320-RVW.

8 Flores-Rosa, F. R.; Luna, E.; Adame-García, J.; Iglesias-Andreu, L. G. and Luna-Rodríguez, M. 2018. Phylogenetic position and nucleotide diversity of *Fusarium oxysporum* f. sp. *vanillae* worldwide based on translation elongation factor 1# sequences. Plant Pathol. 67(6):1278-1285. https://doi.org/10.1111/ppa.12847.

9 Goloboff, P. A.; Farris, J. S. and Nixon, K. C. 2008. TNT, a free program for phylogenetic analysis. Cladistics. 24(5):774-786. https://doi.org/10.1111/j.1096-0031.2008.00217.x.

10 González-Oviedo, N.; Iglesias-Andreu, L. G.; Flores-Rosa, F. R.; Rivera-Fernández, A. and Luna-Rodríguez M. 2022. Genetic analysis of the fungicide resistance in *Fusarium oxysporum* associated to *Vanilla planifolia*. Mex. J. Phytopathol. 40(3):1-19. https://doi.org/10.18781/R.MEX.FIT.2203-3.

11 Gómez-Gómez, E.; Ruíz-Roldán, M. C.; Pietro, A.; Roncero, M. I. G. and Hera, C. 2002. Role in pathogenesis of two endo-#-1,4-xylanase genes from the vascular wilt fungus *Fusarium oxysporum*. Fungal Genet Biol. 35(3):213-222. https://doi.org/10.1006/ fgbi.2001.1318.

12 Gurjar, G.; Barve, M.; Giri, A. and Gupta, V. 2009. Identification of Indian pathogenic races of *Fusarium oxysporum* f. sp. *ciceris* with gene specific, ITS and random markers. Mycologia. 101(4):484-495. https://doi.org/10.3852/08-085.

13 Hall, T A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser. 41:95-98.

14 Hughes, A. L. and Friedman, R. 2008. Codon-based tests of positive selection, branch lengths, and the evolution of mammalian immune system genes. Immunogenetics. 60:495-506. https://doi.org/10.1007/s00251-008-0304-4.

15 Jorge, I.; Rosa, O.; Navas-Cortés, J. A.; Jiménez-Díaz, R. M and Tena, M. 2005. Extracellular xylanases from two pathogenic races of *Fusarium oxysporum* f. sp. *ciceris*:

DOI: https://doi.org/10.29312/remexca.v14i6.2711

enzyme production in culture and purification and characterization of a major isoform as an alkaline endo beta xylanase of low molecular weight. Antonie van Leeuwenhoek. 88:48-59. https://doi.org/10.1007/s10482-004-7584-y.

16 Kalluri, U. C.; Yin, H.; Yang, X. and Davison, B. H. 2014. Systems and synthetic biology approaches to alter plant cell walls and reduce biomass recalcitrance. Plant Biotechnol J. 12(9):1207-1216. https://doi.org/10.1111/pbi.12283.

17 Koyyappurath, S.; Conéjéro, G.; Dijoux, J. B.; Lapeyre-Montès, F.; Jade, K.; Chiroleu, F.; Gatineau, F.; Verdeil, J. L.; Besse, P. and Grisoni, M. 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.

18 Koyyappurath, S.; Atuahiva, T.; Le Guen, R.; Batina, H.; Le Squin, S.; Gautheron, N.; Edel-Hermann, V.; Peribe, J.; Jahiel, M.; Steinberg, C.; Liew, E. C. Y.; Alabouvette, C.; Besse, P.; Dron, M.; Sache, I.; Laval, V. and Grisoni, M. 2016. *Fusarium oxysporum* f. sp. *radicis-vanillae* is the causal agent of root and stem rot of vanilla. Plant Pathol. 65(4):612-625. https://doi.org/10.1111/ppa.12445.

19 Kumar, S.; Stecher, G. and Tamura, K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33(7):1870-1874. https://doi.org/10.1093/molbev/msw054.

20 Laurence, M. H.; Summerell, B. A. and Liew, E. C. Y. 2015. *Fusarium oxysporum* f. sp. *canariensis*: evidence for horizontal gene transfer of putative pathogenicity genes. Plant Pathol. 64(5):1068-75. https://doi.org/10.1111/ppa.12350.

21 Metzger, K. J. and Thomas, M. A. 2010. Evidence of positive selection at codon sites localized in extracellular domains of mammalian CC motif chemokine receptor proteins. BMC Evol. Biol. 10(139):1-9. https://doi.org/10.1186/1471-2148-10-139.

22 Nei, M. and Gojobori, T. 1986. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol. Biol. Evol. 3(5):418-426. https://doi.org/10.1093/oxfordjournals.molbev.a040410.

23 O'Donnell, K.; Gueidan, C.; Sink, S.; Johnston, P. R.; Crous, P. W.; Glenn, A.; Riley, R.; Zitomer, N. C.; Colyer, P. and Waalwijk, C. 2009. A two-locus DNA sequence database for typing plant and human pathogens within the *Fusarium oxysporum* species complex. Fungal Gen. Biol. 46(12):936-948. https://doi.org/10.1016/j.fgb.2009.08.006.

24 Olivain, C.; Humbert, C.; Nahalkova, J.; Fatehi, J.; L'Haridon, F. and Alabouvette, C. 2006. Colonization of tomato root by pathogenic and nonpathogenic *Fusarium oxysporum* strains inoculated together and separately into the soil. Appl. Environ. Microbiol . 72(2):1523-1531. https://doi.org/10.1128/AEM.72.2.1523-1531.2006.

25 Pattathil, S.; Hahn, M. G.; Dale, B. E. and Chundawat, S. P. S. 2015. Insights into plant cell wall structure, architecture, and integrity using glycome profiling of native and AFEXTM-pretreated biomass. J. Exp. Bot. 66(14):4279-4294. https://doi.org/10.1093/jxb/ erv107.

26 Peña, M. J.; Kulkarni, A. R.; Backe, J. Boyd, M. O.; Neill, M. A. and York, W. S. 2016. Structural diversity of xylans in the cell walls of monocots. Planta. 244:589-606. https://doi.org/10.1007/s00425-016-2527-1.

27 Pinaria, A. G.; Liew, E. C. Y. and Burgess, L. W. 2010. *Fusarium* species associated with vanilla stem rot in Indonesia. Australasian Plant. Pathol. 39:176-183. https://doi.org/10.1071/AP09079.

28 Pinaria, A. G.; Laurence, M. H.; Burgess, L. W. and Liew, E. C. Y. 2015. Phylogeny and origin of *Fusarium oxysporum* f. sp. *vanillae* in indonesia. Plant Pathol. 64(6):1358-65. https://doi.org/10.1111/ppa.12365.

29 Ruiz-Roldan, M. C.; Di-Pietro, A.; Huertas-Gonzalez, M. D. and Roncero, M. I. 1999. Two xylanase genes of the vascular wilt pathogen *Fusarium oxysporum* are differentially

DOI: https://doi.org/10.29312/remexca.v14i6.2711

expressed during infection of tomato plants. Mol. Gen. Genet. 261:530-536. https://doi.org/10.1007/s004380050997.

30 Soto-Arenas, M. A y Solano-Gómez, A. R. 2007. Ficha técnica de *Vanilla planifolia*. En: información actualizada sobre las especies de orquídeas del PROY-NOM-059-ECOL-2000. Instituto Chinoin A.C. Herbario de la Asociación Mexicana de Orquideología AC. Proyecto No. W029. México. DF. 1-18 pp. http://www.conabio.gob.mx/conocimiento/ise/fichasnom/Vanillaplanifolia00.pdf.

31 Turrà, D.; Ghalid, M.; Rossi, F. and Pietro, A. 2015. Fungal pathogen uses sex pheromone receptor for chemotropic sensing of host plant signals. Nature. 527:521-524. https://doi.org/10.1038/nature15516.

32 Waweru, B.; Turoop, L.; Kahangi, E.; Coyne, D. and Dubois, T. 2014. Non-pathogenic *Fusarium oxysporum* endophytes provide field control of nematodes, improving yield of banana (*Musa* sp.). Biological Control. 74:82-88. https://doi.org/10.1016/j.biocontrol.2014.04.002.

33 Zhang, J.; Nielsen, R.; and Yang, Z. 2005. Evaluation of an improved branch-site likelihood method for detecting positive selection at the molecular level. Mol. Biol. Evol. 22(12):2472-2479. https://doi.org/10.1093/molbev/msi237.

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Detection of the *xyl3* gene in strains of *Fusarium oxysporum* f. sp. *vanillae*

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