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Assessing Genetic Diversity and DNA Polymorphism of *Fusarium oxysporum*

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ABSTRACT

***Fusarium oxysporum* is a globally distributed ascomycetous fungal pathogen associated with wilt disease in a wide range of host plants, especially tomato and chilli in Sri Lanka. This study aimed to determine the genetic diversity, DNA polymorphism, haplotype structure, and phylogenetic relationships of *F. oxysporum* using four gene regions: *tef1-α, rpb2, tub2,* and *cmdA*. The sequence data belonging to sixty-five strains of *F. oxysporum* were retrieved from NCBI GenBank. Multiple sequence alignment performed using MAFFT and adjusted manually using BioEdit. DNA polymorphism and diversity indices, including nucleotide diversity (π), haplotype diversity (hd), the number of segregating sites (S), and Tajima's D, were calculated using DnaSP v6.12. Haplotype networks were constructed using PopArt v1.7, and maximum likelihood phylogenetic analysis was performed using RAxML on the CIPRES platform. The *tef1-α* implied the highest nucleotide (π = 0.0181) and haplotype diversity (hd = 0.955), with a significant negative Tajima's D value (-1.8955), indicating purifying selection or recent population expansion, followed by *cmdA*, while *rpb2* and *tub2* displayed lower diversity and neutral evolution. Haplotype networks showed central haplotypes surrounded by subclusters, making a star-like topology, suggesting recent population expansion and gene flow. Phylogenetic analysis revealed distinct clades within *F. oxysporum*, supporting the presence of intraspecific structure. The key results provide new insights into the genetic diversity, population structure, and molecular taxonomy of the pathogenic fungus and a foundation for understanding pathogen behavior in further studies on *F. oxysporum* in Sri Lanka.**

KEYWORDS: DNA polymorphism, *Fusarium oxysporum*, Genetic diversity, Haplotype analysis, Multi-locus phylogeny

INTRODUCTION

Fungi are an important aspect of biogeochemical cycles and play crucial roles in several sectors (Blackwell *et al*., 2006), while some are pathogenic and cause diseases in humans, animals, and plants (Sun *et al*., 2020).

Fusarium oxysporum is a serious soil-borne fungal pathogen that can affect the production of several economically important crops worldwide (Crous *et al*., 2021; Zakaria, 2022). It is also a significant fungal pathogen in Sri Lanka, causing vascular wilt in tomato and chilli cultivations, wilt in brinjol cultivations, resulting in significant yield losses (Sandani and Weerahewa, 2018).

Members of the *Fusarium* genus are characterized by having falcate, hyaline, and multi-septate macroconidia, with foot-shaped basal cells and blunt to hooked apical cells. They also form ovoid to reniform, hyline, and septate microconidia, while the conidiophores are typically mononematous with monophialidic conidiogenous cells. The colonies commonly show cottony to woolly texture with white to shades of pink, purple, and yellow pigmentations (Crous *et al*., 2021).

As traditional morphological approaches are not revealing hidden genetic diversity and relationships, this study aimed to evaluate genetic diversity, haplotype structure, and phylogenetic relationships of *F. oxysporum* using multi-locus sequence data (*cmdA tef1-α, rpb1, rpb2, and tub2)*.

METHODOLOGY

Accessing DNA Sequences

The NCBI GenBank accession numbers for respective gene regions, including *tef1-α* (translation elongation factor 1-α), *rpb2* (RNA polymerase II second largest subunit), *tub2* (β-tubulin), and *cmdA* (Calmodulin), of *F. oxysporum* species representing diverse hosts from around the world, were retrieved from the latest publications: Xie *et al.* (2024), and Maryani *et al.* (2018). To ensure strong analysis, additional strains of *F. oxysporum* were added directly from GenBank including, 06603B, 06603C, 06603D, BRIP 64441, BRIP64449, BRIP64452, BRIP64454,

BRIP64455, CBS140424, CML 4291, CML4207, CML4288, CML4289, CML4293, CML4298, UM2003, UM2008, UM2235, UM2271, UM2279, UM991, XY1E208, ZSG18, ZSG29, ZSG4.

Determination of DNA Polymorphism and Genetic Diversity

The sequences for respective gene regions of 65 strains were downloaded from NCBI GenBank and aligned with MAFFT V.7 (Multiple sequence alignment program) (Katoh and Toh, 2008). The aligned sequences were edited and adjusted manually with Bioedit V.5 (Hall, 1999) when needed. The aligned sequences FASTA files were converted to PHYLIP files using ALTER (Alignment Transformation Environment) (Glez-Pena *et al*., 2010).

The diversity indices were calculated for each gene region and the combined datasets, using DnaSP V.6.12 (Rozas, 2009). The haplotype diversity (hd), number of haplotypes (h), Wattersons's theta, number of segregation sites, nucleotide diversity, and Tajima's D, which is used to determine the potential departure from an equilibrium model of evolution, were calculated for each gene region and the combined data set.

Haplotype Analysis

The haplotype data of combined data sets, which were obtained from DnaSP V 6.12 software, were used to construct haplotype networks using PopArt V 1.7 (Leigh and Bryant, 2015). To understand the relationship among geographical areas and host ranges, the haplotype networks were constructed using the median joining method.

Phylogenetic Analysis Based on Multi-locus Sequencing

The sequences for respective gene regions of the species in *F. oxysporum* species complex obtained from Zhang *et al*. (2024) were downloaded from NCBI GenBank and aligned with MAFFT (Multiple sequence alignment program) (Katoh and Toh, 2008). The sequences were edited and adjusted manually with Bioedit V.5 (Hall, 1999) when needed. The combined gene sequences for *Fusarium* (*cmdA tef1-α, rpb1, rpb2, and tub2*) were prepared using Bioedit V.5.

The phylogenetic network was constructed using maximum likelihood (ML) in RAxML, for combined data sets, rooted with outgroup taxons of *F. udum* (CBS 177.31), and *F. foetens* (CBS 120665). The ML analysis was done using RAxML-HPC2 on ACCESS V.8.2.12 in the CIPRES Science Gateway Platform using the GTRGAMMA model of evolution with 1000 bootstrapping iterations.

RESULTS AND DISCUSSION

DNA Polymorphism and Genetic Diversity

According to the diversity results (Table 1), the *tef1-α* gene resulted in 39 segregating sites with the highest nucleotide diversity (π = 0.01816), suggesting greater diversity among all genes analyzed. The *tef1-α* also had the highest haplotype diversity (hd = 0.955), and with the highest nucleotide and haplotype diversity, making it a reliable marker for future diversity studies of *F. oxysporum*. Apart from that, almost all the gene regions showed relatively high haplotype diversity (hd >0.5), reflecting high genetic diversity within the population.

Notably, *cmdA* and *tef1-α* yielded significantly negative Tajima's D values (-1.99937 and -1.89738, respectively; P <0.05), which signifies excess of rare alleles, indicating strong evidence for presence of purifying selection or resent population expansion such as after a bottleneck or a selective sweep (Spies *et al*., 2021). The excess of rare alleles in those loci could be due to evolutionary pressure or environmental adaptation, both of which are relevant given the widespread pathogenicity of *F. oxysporum*. In contrast, the rpb2 displays a moderately high nucleotide diversity of 0.00572 with a non-significant positive D value (0.20315), indicating an excess of intermediate frequency alleles, balancing selection, or a decrease in population size (Spies *et al*., 2021), with neutral evolution (No selection). The combination of negative and strong signals of non-neutral evolution supports the hypothesis that *F. oxysporum* may be undergoing purifying selection or have experienced a bottleneck recently.

Haplotype Analysis

The presence of dominant haplotypes (haplotype 3, 7 and 9), widely shared among several countries and host range of the networks of *F. oxysporum* isolates (Figures 1 and 2) is consistent with several geographical regions and hosts, reflecting widespread dispersal and gene flow (Li *et al*., 2016). As haplotype 3, 9, and 22 branching off several closely related haplotypes with one to two mutations, creating a star-like topology, indicating recent population expansion (Delser *et al*., 2017). Overall, the haplotype network of *F. oxysporum* contains several clusters surrounded by more unique haplotypes and several mutational events, forming a web-like structure indicating high genetic diversity within the isolates.

Table 1: Polymorphism and genetic diversity of *Fusarium oxysporum*

Gene	n[a]	bp[b]	Theta-w (per sequence)	S[c]	h[d]	hd[e]	pi[f]	TD[g]
cmdA	25	549	1.324	5	5	0.300	0.0017	-1.9993 (P <0.05)
RPB2	28	809	4.369	17	11	0.831	0.0057	0.2031 (P >0.10)
Tef1	65	229	8.221	39	28	0.955	0.0181	-1.8955 (P <0.05)
TUB2	20	243	2.819	10	6	0.579	0.0061	-1.6294 (0.10>P>0.05)
Combine	65	229	8.221	39	28	0.955	0.0181	-1.8973 (P <0.05)

a- Sample size (n), b - Total number of sites (bp), c - Number of segregating sites (S), d - Number of alleles (nA), e - Haplotypic (allelic) diversity (hd), f - Average nucleotide diversity (pi), g Tajima's D (TD), (R) Estimate of R (Rm) minimum recombination events

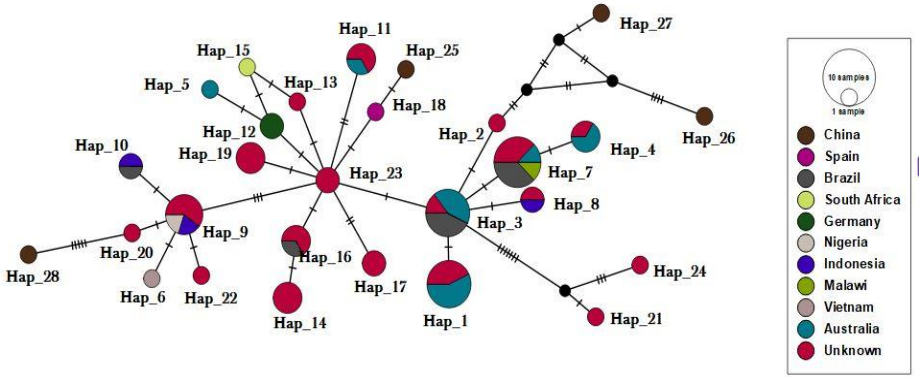

Figure 1: Haplotype network of the *Fusarium oxysporum* isolates with countries using PopArt v 1.7 *The size of the nodes is proportionate to the number of isolates and the number of polymorphic sites between individual haplotype is indicated by tick marks*

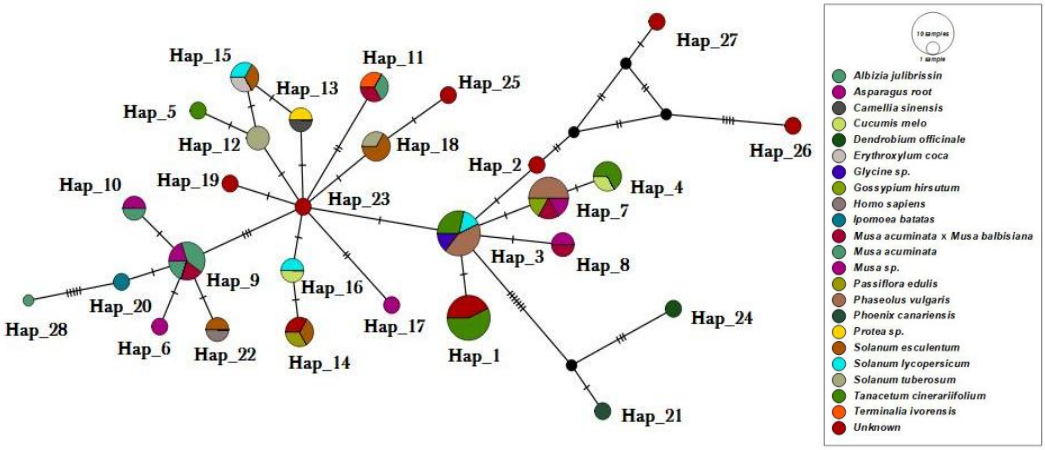

Figure 2: Haplotype network of the *Fusarium oxysporum* isolates with their hosts using PopArt v 1.7 *The size of the nodes is proportionate to the number of isolates and the number of polymorphic sites between individual haplotype is indicated by tick marks*

Phylogenetic Analysis Based on Multi-locus Sequencing

The ML phylogenetic tree (Figure 3) revealed distinct clustering of *F. oxysporum* isolates, with relevant bootstrap support, and clear separation from closely related species. In addition to that, several known species, *F. triseptatum, F. nirenbergiae, F. curvatum, F. trachichlamydosporum, F. duoseptatum, F. callistephi,* and *F. cugenangense* clustered with *F. oxysporum*. This questioned the speciation of those species, are they distinct individual species or genotypes of *F. oxysporum*.

The genetic diversity of *F. oxysporum* revealed through this study highlights the adaptive potential among several hosts and geographical regions of the species.

Furthermore, this study was conducted using publicly available gene sequences from NCBI GenBank, based on the most recent and peer-reviewed publications.

Due to the limited availability of the sequences in public repositories and the unequal gene region coverage, the *tef1-α* had the highest number of usable sequences, which influenced the diversity induced in both single and combined gene analysis. Also, it identifies the *tef1-α* as a barcode gene for *Fusarium* genetics.

Although the dataset was not perfectly balanced across all gene regions, the results gave meaningful insights into the genetic diversity, haplotype structure, and the evolutionary patterns of selected strains among the gene regions. However, that does limit some comparative power among loci, and a more balanced dataset would be ideal for future analyses. Though *F. oxysporum* is considered a major pathogen in Sri Lanka, this study used internationally sourced strains due to the lack of availability of sequence data for local isolates. The patterns here serve as a reference point for future comparison of Sri Lankan isolates, which could reveal region-specific diversity.

CONCLUSIONS

This multi-locus sequence analysis revealed high genetic diversity within the population of *F. oxysporum*, with the highest genetic diversity accounted with *tef1-α*. The *tef1-α* and the *cmdA* provided strong evidence for the presence of purifying selection or recent population expansion, such as after a bottleneck or a selective sweep.

The haplotype networks supported the idea of wide dispersal of the species and high gene flow. As the observed value for Watterson's theta (8.195) in *tef1-α* is relatively high, it suggests that it could be a different species from known *Fusarium* species. However, this study demonstrates the usage of multi-locus data in revealing the hidden diversity of *F. oxysporum* isolates, emphasizes *tef1-α* as the barcode gene for *Fusarium*, and highlights the need for regional isolates to assess local diversity and evolution in future work.

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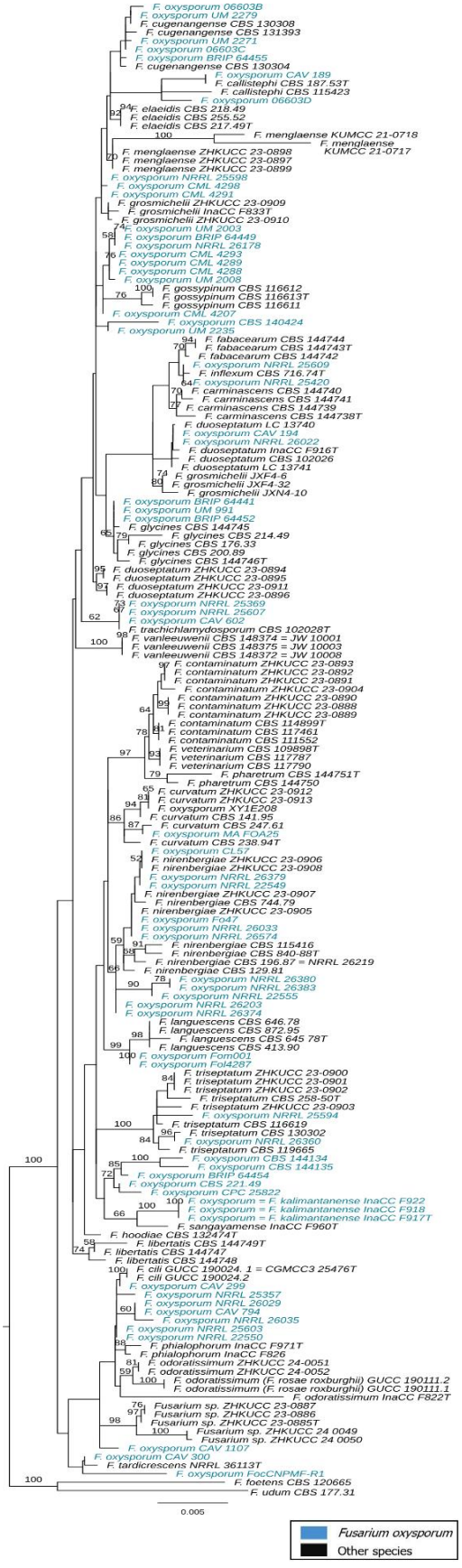

Figure 2: Phylogenetic tree of the combined data (*cmdA, tef1-α, rpb1, rpb2*, and *tub2*)
Bootstrap support values ≥50% for maximum likelihood are shown on the branches

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