Australasian Plant Pathology
https://doi.org/10.1007/s13313-025-01057-y

ORIGINAL RESEARCH ARTICLE

De novo long-read assembly and annotation for genomes of two cotton-associated *Fusarium oxysporum* isolates

Donald M. Gardiner[1] · Elizabeth A. B. Aitken[2] · Peter van Dam[3] · Duy P. Le[4] · Linda J. Smith[5] · Andrew Chen[2]

Received: 25 February 2025 / Accepted: 24 May 2025

Abstract
Fusarium wilt of cotton is a major production constraint worldwide caused by *Fusarium oxysporum* f. sp. *vasinfectum*. Strain variations contribute to differences in disease expression across different geographic locations. Genomes for two Australian isolates of *F. oxysporum* isolated from cotton are presented. The strains differ in their symptomatology on cotton plants and group in distinct lineages, different to those cotton infecting strains found overseas.

Keywords Fusarium wilt · Cotton · Fusarium oxysporum species complex

Introduction

In Australia, Fusarium wilt of cotton was first reported in Queensland during the 1992–1993 growing season (Kochman 1995). This production-limiting disease can be found in most cotton growing regions worldwide, however, in Australia, the strains of the causal agent, *Fusarium oxysporum* f. sp. *vasinfectum* are thought to have evolved locally (Wang et al. 2010). Four distinct genetic lineages of *F. oxysporum* f. sp. *vasinfectum* have been reported (Skovgaard et al. 2001) which, Wang et al. (2010) expanded to a fifth genetic lineage, which contains two different vegetative compatibility groups and some non-pathogenic *F. oxysporum* strains. Each lineage contains one or more races defined by their responses to a differential set of cotton cultivars and other non-host plants (Halpern et al. 2020). Although the Australian isolates behave similarly on cotton differentials in terms of pathogenicity to that of race 6 isolates found in South America, they do not share vegetative compatibility or genetic groupings (Davis et al. 1996; Wang et al. 2006, 2010).

The first full genome sequence of *F. oxysporum* f. sp. *vasinfectum* was for strain NRRL 25433, also known as BBA 69050 (DeIulio et al. 2018); a race 7 strain originally isolated in China (Skovgaard et al. 2001). Further sequencing of other races or genotypes of *F. oxysporum* f. sp. *vasinfectum* have also been reported (Seo et al. 2020). Early efforts were made to sequence an Australian isolate of *F. oxysporum* f. sp. *vasinfectum* (isolate 25400, VCG01111) based on Illumina short read technology which allowed assembly of the mitochondrial genome (Brankovics et al. 2017). Subsequently additional strains of *F. oxysporum* f. sp. *vasinfectum*, have been sequenced from a global collection. A suite of Australian strains originally isolated from cotton but without full demonstration of Koch's postulates are also publicly available (Achari et al. 2023). However, these Australian strains are based on short read sequencing technology and the corresponding protein annotations are not available in NCBI. Herein, we report the near-complete genome sequences, with protein coding annotations, for two strains of *Fusarium oxysporum* originally isolated from cotton, which differ in their pathogenicity towards cotton.

✉ Donald M. Gardiner
Donald.Gardiner@uq.edu.au

1 Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St Lucia, QLD, Australia

2 School of Agriculture and Food Sustainability, The University of Queensland, St Lucia, QLD, Australia

3 Genetwister Technologies B.V, Wageningen, Netherlands

4 New South Wales, Department of Primary Industries and Regional Development, Narrabri, NSW, Australia

5 Department of Primary Industries, Queensland Government, Dutton Park, QLD 4102, Australia

Published online: 04 June 2025

Materials and methods

Strains

Fusarium oxysporum f. sp. *vasinfectum* strain SG1 (BRIP 76769) and *F. oxysporum* BRF1 (BRIP 76768) are described elsewhere (Chen et al. 2024). Strains have been deposited in the Queensland Government Plant Pathology Herbarium with the accessions indicated in brackets in the previous sentence. *F. oxysporum* f. sp. *vasinfectum* SG1 is fully virulent on cotton whereas *F. oxysporum* BRF1 is weakly virulent.

Culture conditions and DNA extraction.

Monoconidial cultures of *F. oxysporum* f. sp. *vasinfectum* SG1 and *F. oxysporum* BRF1 were grown on plates containing half-strength potato dextrose agar (Rahway, NJ, USA). Under sterile conditions, four mycelial plugs (1 cm^2) were cut from each culture plate and used to inoculate 500 mL of minimal media broth, the recipe of which was described elsewhere (Solomon et al. 2004). After growth for 6 days at 26 °C, on an orbital shaker at 180 rpm, the culture was spun down in a centrifuge (5430R, Eppendorf, Hamburg, Germany) and washed twice in sterile distilled water. The mycelial pellets were uniformly ground into powder using a mortar and pestle under liquid nitrogen.

High molecular weight (HMW) DNA extraction was performed as previously described (Debler et al. 2020). The following modifications were made. Starting material consisted of 0.7 to 1.0 g of mycelia powder resuspended in 5 mL of the CTAB based lysis buffer. RNase A and Proteinase K were sourced from New England Biolabs (Ipswich, MA, USA). Following the second 80% ethanol wash, the tubes containing the DNA pellet were centrifuged again to collect any residual ethanol, which was subsequently removed manually using a pipette. Small fragment elimination using polyethylene glycol was not performed. The DNA pellet was resuspended in 0.1× TE buffer and was left to dissolve at 4°C overnight. DNA concentration was determined on a Nanodrop and a Qubit fluorometer (dsDNA broad-range assay, Thermo Fisher Scientific, Waltham, M.A. USA). HMW DNA (200 ng), was visually checked against a 1 kb ladder (N3232S, New England Biolabs, Ipswich, MA, USA) on a 1% agarose gel.

DNA sequencing

Each isolate was sequenced on one fifth of a PacBio SMRT flow cell. Libraries were prepared by the Australian Genome Research Facility (AGRF, Melbourne, Australia) using a standard PacBio gDNA library preparation kit. Sequencing yielded approximately 1 million HiFi reads per isolate, with a median read length greater than 15 Kbp to yield over 15 Gbp of HiFi sequence data.

Read processing

Reads were downloaded from AGRF data server in bam format and converted to fastq format using SamToFastq (version 3.1.1.0) (Picard Toolkit 2019) in the Galaxy compute environment (usegalaxy.org.au).

Mitochondrial genome assembly

The complete sets of reads were used to independently assemble the mitochondrial genomes of both isolates using MitoHiFi (version 3) (Uliano-Silva et al. 2023). Assembly was performed in the Galaxy compute environment with default parameters, utilising the mitochondrial genome of *Fusarium oxysporum* strain 19–385, originally isolated from *Allium cepa* (GenBank accession number OR601176), as a reference.

Nuclear genome assembly

Reads identified by MitoHiFi as having blast hits to the *F. oxysporum* reference mitochondrial genome were removed from the total read pool using custom filters in Galaxy. HiFi Adapter Filter (2.0.0) (Sim et al. 2022) was used to remove any remaining adapter-containing reads, followed by genome assembly using hifiasm (version 0.1.12) (Cheng et al. 2021) as part of a public workflow (PacBio HiFi genome assembly using hifiasm v2.1). The assemblies were screened for contaminants using the NCBI Foreign Contaminant Screen (version 0.5.4) (Astashyn et al. 2024). This process led to the removal of a large contig from the *F. oxysporum* f. sp. *vasinfectum* SG1 assembly, identified as bacterial contamination. Genomes were analysed using BUSCO (Manni et al. 2021) (version 5.8.0) run in genome mode using the AUGUSTUS (Keller et al. 2011; Stanke et al. 2008; Stanke and Waack 2003) gene predictor with *Fusarium graminearum* as the species model for gene prediction against the Ascomycota reference lineage. Telomeres were detected with seqtk telo (version 1.4) (Li 2023) in Galaxy, with the minimum score threshold set to 100.

Comparison to *F. oxysporum* f. sp. *lycopersici* strain 4287

Alignments to the *F. oxysporum* f. sp. *lycopersici* strain 4287 genome was performed with nucmer (version 4.0.0) (Marçais et al. 2018), run with default parameters. The delta file parsed into the DotPrep python script (Aboukhalil 2018) followed by upload to the Dot website (Nattestad 2018) for

figure drawing. Screen shots were used to download the dotplots for final figure generation in Adobe Illustrator.

Gene structural annotation

Structural annotation of protein coding genes was performed using BRAKER3 (Gabriel et al. 2024) pipeline in Galaxy. RNAseq data (SRR14147748 from *F. oxysporum* f. sp. *cubense* tropical race 4) was used to train the pipeline. RNAseq reads were quality- and length-filtered using Trimmomatic 0.36.6 (Bolger et al. 2014), with the IlluminaClip step for TruSeq3 adapters, sliding window quality trimming using an average Phred score of 29 over 4 bases, and length trimming to retain reads of 40 bases or longer. Trimmed reads were aligned to the assemblies using HISAT2 (Kim et al. 2015) (version 2.2.1) with default parameters, except for setting the maximum intron length to 500 bases. Repeats in the genome were identified using RepeatModeler (Flynn et al. 2020) with default parameters (version 2.0.4) and masked using RepeatMasker (Smit et al. 2013) (version 4.1.5), utilising RepeatModeler's output as a custom repeat library. BRAKER3 (Gabriel et al. 2024) was run on the soft-masked genomes with the fungus option selected for the Genemark training step, alternative transcripts from evidence were disabled, and the output was set to GFF3 format. The BRAKER3 output was parsed through AGAT (Dainat et al. 2024) (version 1.4.0) to resolve overlapping genes, merging overlapping loci and retaining the longest transcript isoform where alternative transcripts resulted from merging loci.

Phylogenetic analysis

The genomes of 120 *F. oxysporum* strains, including special forms on various plant hosts, as well as that of a single isolate of *F. verticillioides* (isolate 7600), were used to determine the phylogenetic placement of the cotton-associated *F. oxysporum* strains sequenced in this study. Custom bash scripts were used to add strain information in the FASTA headers of the genome sequences to allow downstream merging of alignments.

All 123 genomes were loaded into the Galaxy computing environment (GalaxyCommunity 2024) and subject to *de novo* gene annotation using AUGUSTUS (Stanke et al. 2008; Keller et al. 2011; Stanke and Waack 2003) (version 3.4.0), with *Fusarium graminearum* splicing models applied and only genes without internal stop codons reported. The resulting coding sequences were analysed using BUSCO (Manni et al. 2021) (version 5.8.0), with the lineage set to Ascomycota. The tabular BUSCO output was downloaded to a local macOS computer, where a custom bash script was used to filter each genome's output, retaining only complete, single-copy conserved genes. FASTA sequences that were complete and single-copy across all 123 genomes were extracted using the seqkit grep command (Shen et al. 2024) (version 2.9.0), aligned using MAFFT (Katoh et al. 2002) (version 7.520) using default settings, refined by removing poorly aligned regions with trimAl (Capella-Gutiérrez et al. 2009) (version v1.5.rev0) using the gappyout setting. The edited alignments were then concatenated using the seqkit concat command. The custom bash script detailing the full processing workflow from BUSCO and AUGUSTUS outputs to the final alignment is provided in Supplementary File 1.

Phylogenetic reconstruction was performed using RAxML GUI version 2.0 (Edler et al. 2021). The best model, determined to be GTR+I+G4, was applied for the maximum likelihood tree search, with bootstrapping conducted using 100 replicates. *F. verticillioides* isolate 7600 was selected as the outgroup. The resulting phylogeny was imported into the interactive tree of life (Letunic and Bork 2024) where branches corresponding to three single isolates (two banana pathogens and one ginger pathogen) that are yet to be published were removed prior to export to Adobe Illustrator for final figure preparation.

Detection of SIX gene homologs

The *Fusarium oxysporum* Effector Clustering (FoEC2) pipeline for the detection of the presence of SIX homologs was run using the parameters -g<genome_folder>and -e<query_effector_fastafile> (Brenes Guallar et al. 2022). The *Fusarium* genomes were downloaded from NCBI's genome portal and fourteen Fol-SIX nucleotide sequences taken from van Dam et al. (2016) were used as query. Clustering in the pipeline was performed using default settings: binary distance matrix and average distance calculation.

TBLASTN search of *F. oxysporum* f. sp. *lycopersici* SIX protein sequences against the genomes of SG1 and BRF1 was performed using the command line version of NCBI-BLAST+ (version v2.12.0) using an e-value cut-off of 1×10^{-10}.

Results and discussion

Mitochondrial genomes

The mitochondrial genomes were 48,054 bp for isolate *F. oxysporum* f. sp. *vasinfectum* SG1 and 51,696 bp for isolate *F. oxysporum* BRF1 (Fig. 1). *Fusarium oxysporum* mitochondrial genomes contain a highly conserved region and a large variable region, which exists in three major variants (Brankovics et al. 2017). The mitochondrial genome of *F. oxysporum* f. sp. *vasinfectum* SG1 closely resembled that of *F. oxysporum* f. sp. *vasinfectum* isolate 24500 (Brankovics

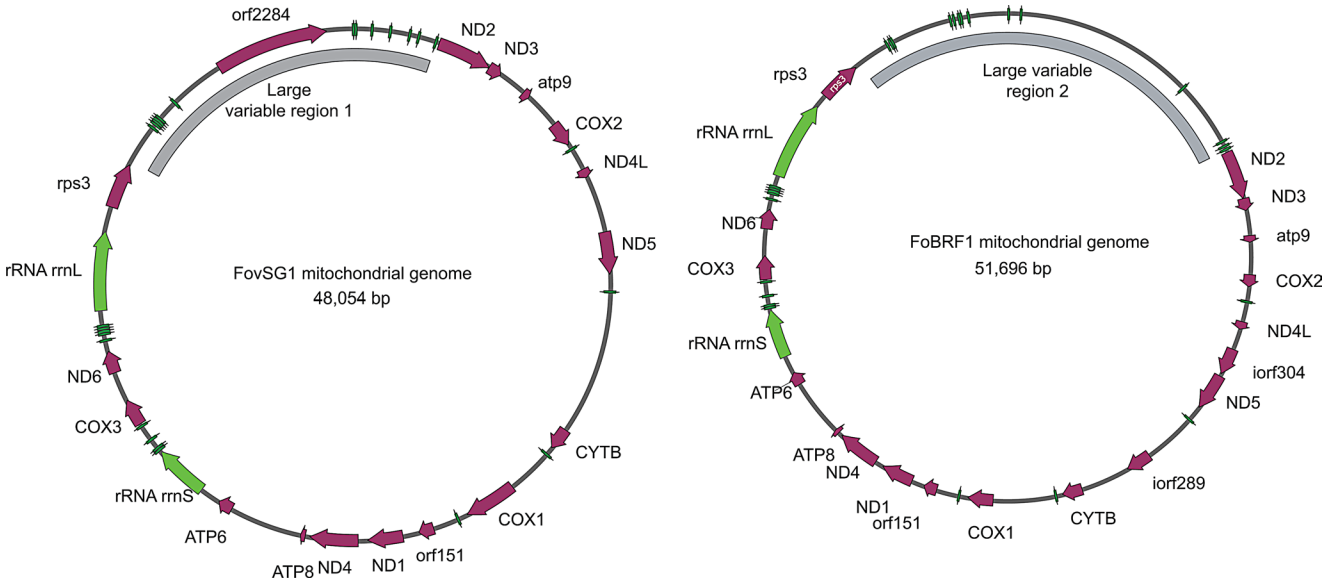

Fig. 1 Mitochondrial genomes of the two Australian cotton-derived *Fusarium oxysporum* isolates *F. oxysporum* f. sp. *vasinfectum* (FovSG1) and *F. oxysporum* (FoBRF1). Gene names were assigned by MitoHiFi with protein coding genes in purple and ribosomal RNA in light green. Dark green triangles that are unlabelled encode tRNAs

et al. 2017), an Australian isolate (Chakrabarti et al. 2011), which contains a region known as large variable region 1.

Unlike *F. oxysporum* f. sp. *vasinfectum* SG1, the mitochondrial sequence of isolate *F. oxysporum* BRF1 contains large variable region 2, making it most closely related to *F. oxysporum* f. sp. *conglutinans* strain NRRL 54008 (PHW808) (Brankovics et al. 2017). According to Brankovics et al. (2017), large variable region 2 includes additional open reading frames with characteristics consistent with endonucleases commonly round in fungal mitochondrial genomes (Megarioti and Kouvelis 2020).

Nuclear genomes

The nuclear genomes were assembled primarily into chromosome size contigs with N50 statistics consistent with chromosomes size pieces in the *F. oxysporum* species complex (Table 1). BUSCO analysis demonstrated both genomes were predicted to encode an almost complete set of conserved genes (compared to the Ascomycota). Most large contigs have telomeric repeats of 5'-TAACCC-3'. In both assemblies, a total of 23 regions were found with this repeat. In *F. oxysporum* f. sp. *vasinfectum* SG1, seven contigs had telomere sequences at both ends, while in the *F. oxysporum* BRF1 assembly, nine contigs had telomeric sequences at both ends.

Isolate *F. oxysporum* f. sp. *vasinfectum* SG1 had equivalents for 10 of the 15 chromosomes in *F. oxysporum* f. sp. *lycopersici* strain 4287, whereas *F. oxysporum* BRF1 had equivalents for 11 (Fig. 2). The difference was due to strain *F. oxysporum* f. sp. *vasinfectum* SG1 lacking a homologue of *F. oxysporum* f. sp. *lycopersici* strain 4287 chromosome 12 (Fig. 2A and B). Although chromosome 12 is considered part of the *F. oxysporum* core genome, it has been previously shown to be dispensable for vegetative growth (Vlaardingerbroek et al. 2016). Presumably the lack of chromosome 12 in *F. oxysporum* f. sp. *vasinfectum* SG1, which is highly pathogenic towards cotton, indicates it is also dispensable

Table 1 Nuclear genome statistics F.r the two F.sarium oxysporum isolates sequenced in this study. FovSG1, F. oxysporum F. Sp. Vasinfectum. FoBRF1, F. oxysporum BRF1

Isolate	Accession	Locus tag	Genome size (Mbp)	Count of large contigs (> 100 Kbp)	Gene count	BUSCO statistics	N50 (Mbp)
FovSG1	JBKAHI000000000	FOVSG1	54.5	14	15,560	98.9% complete 0.6% duplicated	4.3
FoBRF1	JBKAHJ000000000	FOBRF1	59.8	14	16,754	98.8% complete 0.9% duplicated	4.9

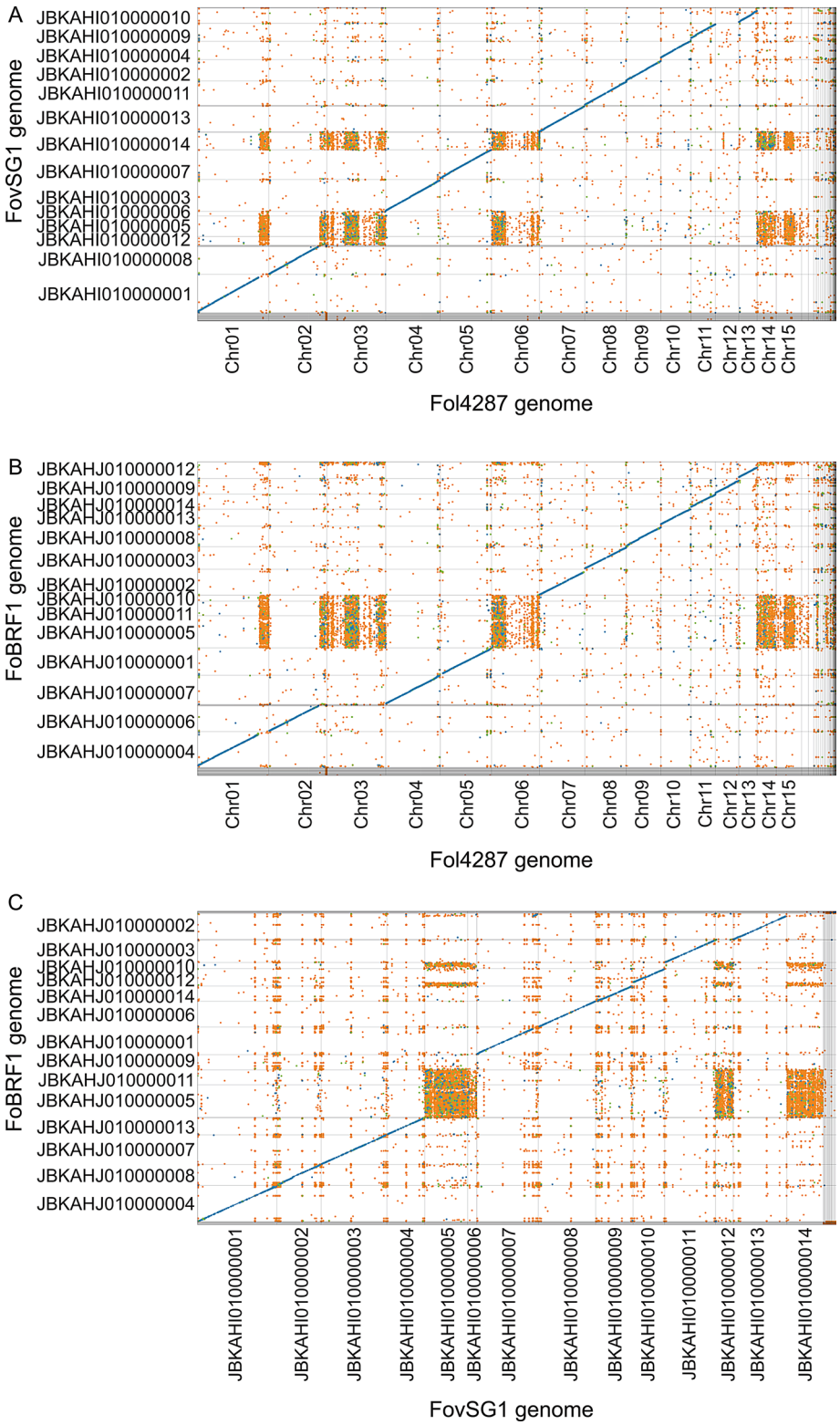

Fig. 2 Whole-genome contig alignment of (**A**) *Fusarium oxysporum* f. sp. *vasinfectum* isolate SG1 (FovSG1) and (**B**) *F. oxysporum* isolate BRF1 (FoBRF1) to each of the 15 chromosomes of the *F. oxysporum* f. sp. *lycopersici* strain 4287 (Fol4287) genome. (**C**) Alignment of FoBRF1 to FovSG1 with GenBank accessions indicated. Unique alignments are shown in blue (forward) and green (reverse complement). Repetitive alignments are shown in orange. Note the order of the FovSG1 and FoBRF1 contigs are not the same in each plot

for plant infection. Chromosome 12 has also been described as a 'fast-core' chromosome due to its characteristics of both core- and dispensable-chromosomes within the *F. oxysporum* species complex (Fokkens et al. 2018).

Phylogenetic analysis

A total of 123 genomes, including the two sequenced in this study, were used to construct a phylogeny. The analysis included a total of 1.37×10^6 nucleotide sites, with nearly 10% of sites showing variation across the alignment. *F. oxysporum* f. sp. *vasinfectum* SG1 grouped with several other isolates originally obtained from cotton plants in Australia, while *F. oxysporum* BRF1 clustered within a distinct lineage just outside a group of banana pathogens that includes strains classified as Race 1, Subtropical race 4 and Tropical Race 4 isolates of *F. oxysporum* f. sp. *cubense* (Fig. 3). Both the Australian isolates sequenced here, and other Australian isolates retrieved from NCBI, appear distinct to other isolates from cotton-associated isolates from elsewhere in the world, consistent with previous findings (Wang et al. 2010).

Presence of secreted in xylem effectors

.

The genomes of both *F. oxysporum* BRF1 and *F. oxysporum* f. sp. *vasinfectum* were analysed using the *F. oxysporum* effector clustering 2 pipeline (Brenes Guallar et al. 2022). Analysis was concentrated on the secreted in xylem (SIX) effectors originally isolated from *F. oxysporum* f. sp. *lycopersici*. *F. oxysporum* f. sp. *vasinfectum* isolate SG1 encoded two copies of both SIX6 and SIX11, and one copy of SIX13 and SIX14. There was also a weak tBLASTn hit to SIX4, only detected using tBLASTn and not the full clustering pipeline. All eight of these genes were encoded on a single contig (JBKAHI010000014.1), which is a repeat rich contig absent from *F. oxysporum* BRF1 (Fig. 2C). *F. oxysporum* BRF1 was not predicted to encode any of the SIX effectors. Analysing the presence/absence of SIX genes encoded by two genomes sequenced here in the context of a suite of other *F. oxysporum* isolates revealed that the profile of *SIX* genes in *F. oxysporum* f. sp. *vasinfectum* SG1 was shared across all of the Australian cotton derived isolates (with the exception of BRF1), but highly distinct to other cotton infecting isolates (Fig. 4). The absence of SIX gene homologues in *F. oxysporum* BRF1 was similar to another non-plant-pathogenic isolates (Fo47), the clinical isolate (NRRL 32931) and to the tomato crown root pathogen *F. oxysporum* f. sp. *radicis-lycoperscici* (26381) known to be devoid of SIX gene homologues (Jelinski et al. 2017). Another strain from cotton (LA3B) (Seo et al. 2020) also lacked any SIX genes but it is unclear if this strain has been demonstrated to be pathogenic towards cotton in artificial inoculation experiments. The groupings based on the SIX gene profiles are incongruent with the broader phylogeny (Fig. 3) as expected for gene involved in host interactions in the *F. oxysporum* species complex where host specificity is known to have polyphyletic origins.

Conclusion

The sequence data presented here represent a foundational resource for studying cotton-associated *F. oxysporum* from Australia which are distinct compared to those from other geographic regions both in terms of basal genome and effector profile. More importantly, future comparisons between the two isolates here, which differ drastically in their ability to cause classical Fusarium wilt symptoms (Chen et al. 2024), will be crucial for understanding of *Fusarium* wilt in cotton.

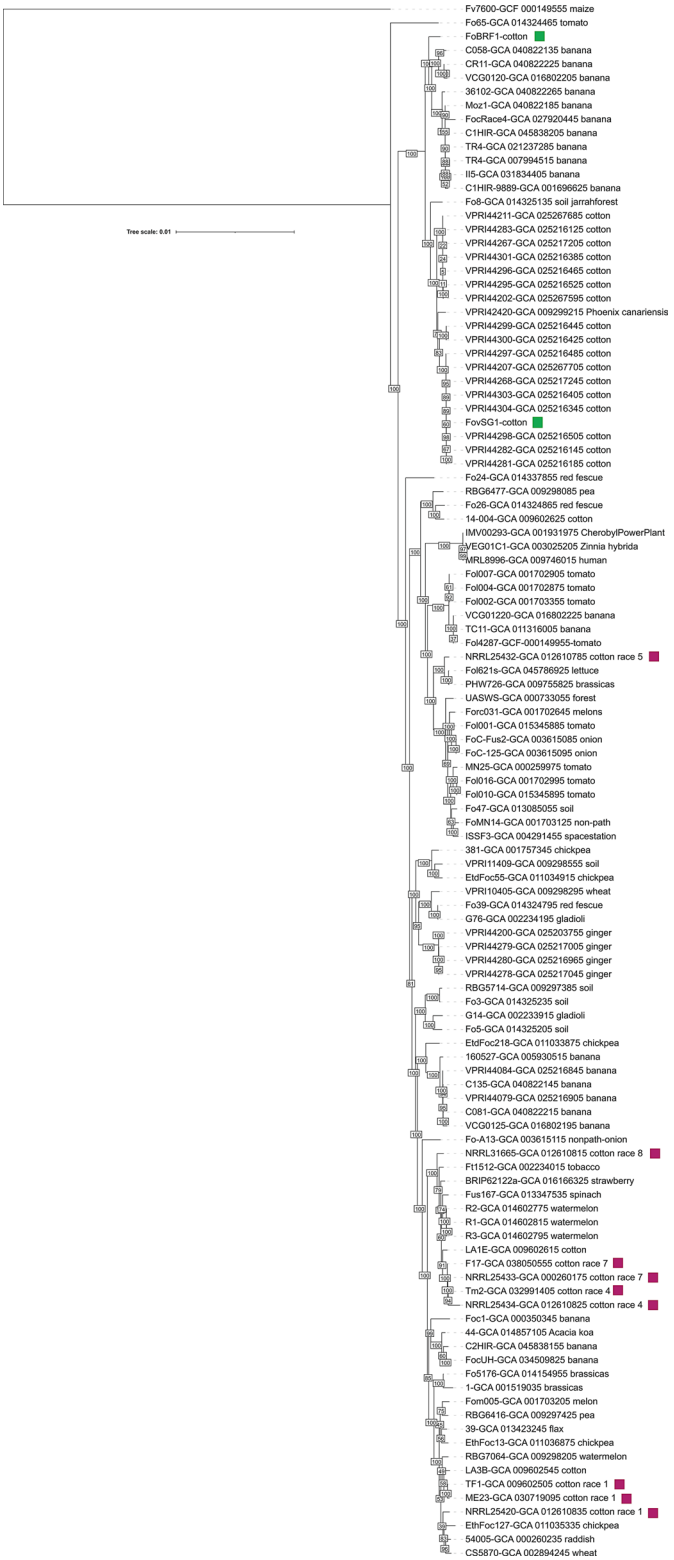

Fig. 3 Phylogenetic placement of the two isolates of *Fusarium oxysporum* isolated from cotton (marked with green boxes). The phylogeny was based upon the aligned nucleotide sequence of 810 conserved genes. Where available, the host of origin, is indicated, along with the race designations for cotton isolates (marked with magenta boxes) where this information could be found in databases or publications. Branch support based on bootstrap analysis (percentage) is indicated by boxed numbers

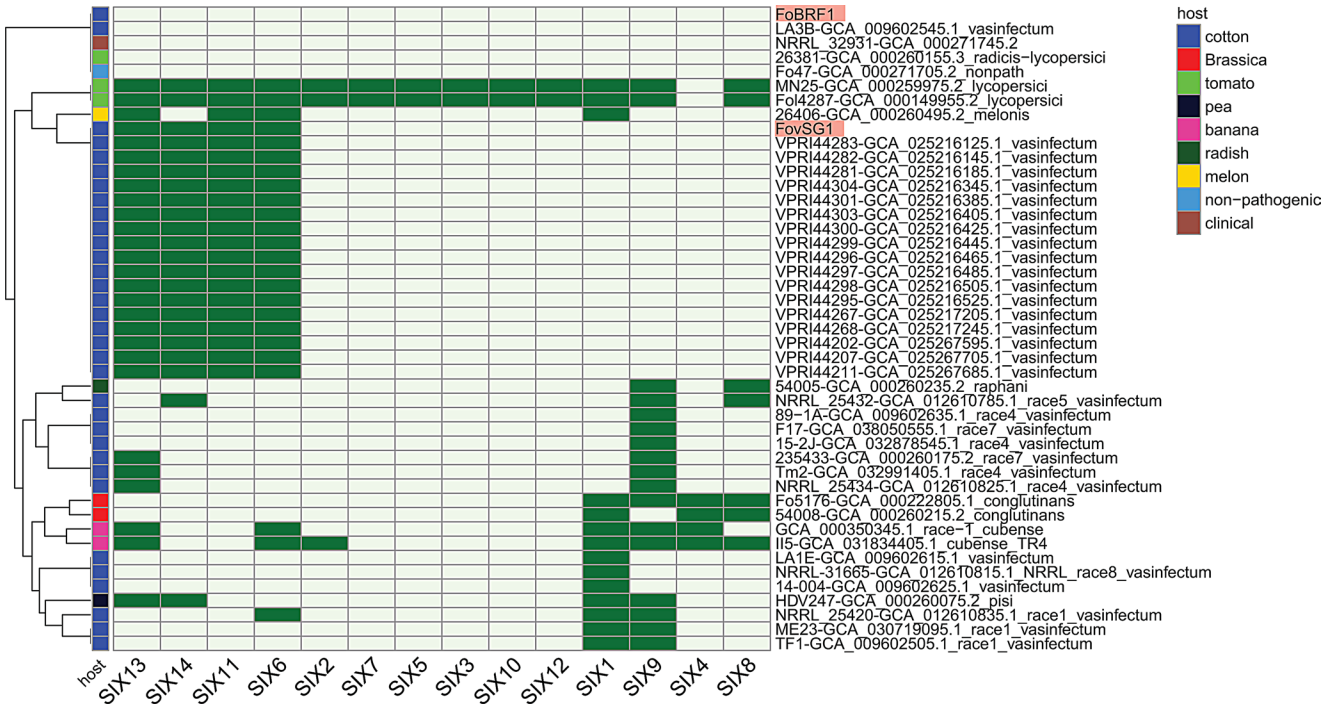

Fig. 4 Secreted in xylem (SIX) gene profile in 45 selected *Fusarium oxysporum* genomes. The analysis was performed using the *Fusarium oxysporum* effector clustering pipeline version 2 (Brenes Guallar et al. 2022). The two isolates sequenced here are highlighted. The selection of isolates was biased to include mostly those derived from cotton with the addition of a number of key reference isolates. The Australian *F. oxysporum* f. sp. *vasinfectum* isolates have a very similar effector profiles that differs from non-Australian isolates of *F. oxysporum* f. sp. *vasinfectum*. The tree structure on the groups the isolates based on the similarity of their SIX gene profile

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13313-025-01057-y.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions
This work is supported by Galaxy Australia, a service provided by Australian BioCommons and its partners. The service receives NCRIS funding through Bioplatforms Australia, as well as The University of Melbourne and Queensland Government RICF funding. This work was supported by Australian Research Council Research Hub for Sustainable Crop Protection (IH190100022) funded by the Australian Government.

Data availability Data associated with this project are available at NCBI under the BioProject PRJNA1198635. Strains are available upon request or via the Queensland Plant Pathology Herbarium under the accessions BRIP 76768 and BRIP 76769 For *F. oxysporum* BRF1 and *Fusarium oxysporum* f. sp. *vasinfectum* SG1, respectively.

Declarations

Ethical approval Not applicable.

Competing interests The authors competing interests to declare..

References

Aboukhalil R (2018) DotPrep.py. https://github.com/MariaNattestad/dot/blob/master/DotPrep.py. 2025

Achari SR, Mann RC, Sharma M, Edwards J (2023) Diagnosis of *Fusarium oxysporum* f. sp. *ciceris* causing *Fusarium* wilt of chickpea using loop-mediated isothermal amplification (LAMP) and conventional end-point PCR. Scientific Reports 13 (1):2640. https://doi.org/10.1038/s41598-023-29730-6

Astashyn A, Tvedte ES, Sweeney D, Sapojnikov V, Bouk N, Joukov V, Mozes E, Strope PK, Sylla PM, Wagner L, Bidwell SL, Brown LC, Clark K, Davis EW, Smith-White B, Hlavina W, Pruitt KD, Schneider VA, Murphy TD (2024) Rapid and sensitive detection of genome contamination at scale with FCS-GX. Genome Biol 25(1):60. https://doi.org/10.1186/s13059-024-03198-7

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for illumina sequence data. Bioinformatics 30(15):2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Brankovics B, van Dam P, Rep M, de Hoog GS, van der Lee J, Waalwijk TA, van Diepeningen C AD (2017) Mitochondrial genomes reveal recombination in the presumed asexual *Fusarium*

oxysporum species complex. BMC Genomics 18(1):735. https://doi.org/10.1186/s12864-017-4116-5

Brenes Guallar MA, Fokkens L, Rep M, Berke L, van Dam P (2022) *Fusarium oxysporum* effector clustering version 2: an updated pipeline to infer host range. Front Plant Sci 13. https://doi.org/10.3389/fpls.2022.1012688

Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) TrimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics 25(15):1972–1973. https://doi.org/10.1093/bioinformatics/btp348

Chakrabarti A, Rep M, Wang B, Ashton A, Dodds P, Ellis J (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(2):232–243. https://doi.org/10.1111/j.1365-3059.2010.02363.x

Chen A, Le DP, Smith LJ, Kafle D, Aitken EAB, Gardiner DM (2024) Assessing the pathogenicity of Berkeleyomyces rouxiae and F.sarium oxysporum F. Sp. vasinfectum on cotton (Gossypium hirsutum) using a rapid and robust seedling screening method. J Fungi 10(10):715. https://doi.org/10.3390/jof10100715

Cheng H, Concepcion GT, Feng X, Zhang H, Li H (2021) Haplotype-resolved de Novo assembly using phased assembly graphs with hifiasm. Nat Methods 18(2):170–175. https://doi.org/10.1038/s41592-020-01056-5

Dainat J, Hereñú D, Murray KD, Davis E, Urgin I, Crouch K, Sol L, Agostinho N, pascal-git, Zollman Z, tayyrocv (2024) NBISweden/AGAT: AGAT-v1.4.1. https://doi.org/10.5281/zenodo.13799920

Davis R, Moore N, Kochman J (1996) Characterisation of a population of *Fusarium oxysporum* F.sp. *Vasinfectum* causing wilt of cotton in Australia. Aust J Agric Res 47(7):1143–1156. https://doi.org/10.1071/AR9961143

Debler JW, Jones A, Nagar R, Sharp A, Schwessinger B (2020) High-molecular weight DNA extraction and small fragment removal of Ascochyta lentis. Protocolsio. https://doi.org/10.17504/protocols.io.bgw5jxg6

DeIulio GA, Guo L, Zhang Y, Goldberg JM, Kistler HC, Ma L-J (2018) Kinome expansion in the *Fusarium oxysporum* species complex driven by accessory chromosomes. mSphere 3(3):10. https://doi.org/10.1128/msphere.00231-18. 1128/msphere.00231–00218

Edler D, Klein J, Antonelli A, Silvestro D (2021) RaxmlGUI 2.0: A graphical interface and toolkit for phylogenetic analyses using RAxML. Methods Ecol Evol 12(2):373–377. https://doi.org/10.1111/2041-210X.13512

Flynn JM, Hubley R, Goubert C, Rosen J, Clark AG, Feschotte C, Smit AF (2020) RepeatModeler2 for automated genomic discovery of transposable element families. Proc Natl Acad Sci 117(17):9451–9457. https://doi.org/10.1073/pnas.1921046117

Fokkens L, Shahi S, Connolly LR, Stam R, Schmidt SM, Smith KM, Freitag M, Rep M (2018) The multi-speed genome of *Fusarium oxysporum* reveals association of histone modifications with sequence divergence and footprints of past horizontal chromosome transfer events. bioRxiv:465070. https://doi.org/10.1101/465070

Gabriel L, Brůna T, Hoff KJ, Ebel M, Lomsadze A, Borodovsky M, Stanke M (2024) BRAKER3: fully automated genome annotation using RNA-seq and protein evidence with GeneMark-ETP, AUGUSTUS, and TSEBRA. Genome Res 34(5):769–777. https://doi.org/10.1101/gr.278090.123

GalaxyCommunity (2024) The galaxy platform for accessible, reproducible, and collaborative data analyses: 2024 update. Nucleic Acids Res 52(W1):W83–W94. https://doi.org/10.1093/nar/gkae410

Halpern HC, Qi P, Kemerait RC, Brewer MT (2020) Genetic diversity and population structure of faces of *Fusarium oxysporum* causing cotton wilt. G3 genes|genomes|genetics| genes|genomes|genetics| genes|genomes|genetics 10. 93261–3269. https://doi.org/10.1534/g3.120.401187

Jelinski NA, Broz K, Jonkers W, Ma L-J, Kistler HC (2017) Effector gene suites in some soil isolates of *Fusarium oxysporum* are not sufficient predictors of vascular wilt in tomato. Phytopathology® 107(7):842–851. https://doi.org/10.1094/phyto-12-16-0437-r

Katoh K, Misawa K, Kuma Ki, Miyata T (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast fourier transform. Nucleic Acids Res 30(14):3059–3066. https://doi.org/10.1093/nar/gkf436

Keller O, Kollmar M, Stanke M, Waack S (2011) A novel hybrid gene prediction method employing protein multiple sequence alignments. Bioinformatics 27(6):757–763. https://doi.org/10.1093/bioinformatics/btr010

Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. Nat Methods 12(4):357–360. https://doi.org/10.1038/nmeth.3317

Kochman JK (1995) *Fusarium* wilt in cotton — a new record in Australia. Australas Plant Pathol 24(1):74–74. https://doi.org/10.1071/APP9950074

Letunic I, Bork P (2024) Interactive tree of life (iTOL) v6: recent updates to the phylogenetic tree display and annotation tool. Nucleic Acids Res 52(W1):W78–W82. https://doi.org/10.1093/nar/gkae268

Li H (2023) seqtk. https://github.com/lh3/seqtk

Manni M, Berkeley MR, Seppey M, Simão FA, Zdobnov EM (2021) BUSCO update: novel and streamlined workflows along with broader and deeper phylogenetic coverage for scoring of eukaryotic, prokaryotic, and viral genomes. Mol Biol Evol 38(10):4647–4654. https://doi.org/10.1093/molbev/msab199

Marçais G, Delcher AL, Phillippy AM, Coston R, Salzberg SL, Zimin A (2018) MUMmer4: A fast and versatile genome alignment system. PLoS Comput Biol 14(1):e1005944. https://doi.org/10.1371/journal.pcbi.1005944

Megarioti AH, Kouvelis VN (2020) The Coevolution of fungal mitochondrial introns and their homing endonucleases (GIY-YIG and LAGLIDADG). Genome Biol Evol 12(8):1337–1354. https://doi.org/10.1093/gbe/evaa126

Nattestad M (2018) Dot. https://dot.sandbox.bio/

Picard Toolkit (2019) Broad Institute. https://broadinstitute.github.io/picard/

Seo S, Pokhrel A, Coleman JJ (2020) The genome sequence of F.ve genotypes of *Fusarium oxysporum* F. Sp. *vasinfectum*: a resource F.r studies on *Fusarium* wilt of cotton. Mol Plant-Microbe Interactions® 33(2):138–140. https://doi.org/10.1094/mpmi-07-19-0197-a

Shen W, Sipos B, Zhao L (2024) SeqKit2: A Swiss army knife for sequence and alignment processing. iMeta 3(3):e191. https://doi.org/10.1002/imt2.191

Sim SB, Corpuz RL, Simmonds TJ, Geib SM (2022) HiFiAdapterFilt, a memory efficient read processing pipeline, prevents occurrence of adapter sequence in PacBio HiFi reads and their negative impacts on genome assembly. BMC Genomics 23(1):157. https://doi.org/10.1186/s12864-022-08375-1

Skovgaard K, Nirenberg HI, O'Donnell K, Rosendahl S (2001) Evolution of *Fusarium oxysporum* f. sp. *vasinfectum* races inferred from multigene genealogies. Phytopathology® 91 (12):1231–1237. https://doi.org/10.1094/phyto.2001.91.12.1231

Smit AF, Hubley R, Green P (2013) RepeatMasker Open-4.0. http://www.repeatmasker.org

Solomon PS, Lee RC, Wilson TJG, Oliver RP (2004) Pathogenicity of *Stagonospora nodorum* requires malate synthase. Mol Microbiol 53(4):1065–1073. https://doi.org/10.1111/j.1365-2958.2004.04178.x

Stanke M, Waack S (2003) Gene prediction with a hidden Markov model and a new intron submodel. Bioinformatics

19(suppl2):ii215–ii225. https://doi.org/10.1093/bioinformatics/btg1080

Stanke M, Diekhans M, Baertsch R, Haussler D (2008) Using native and syntenically mapped cDNA alignments to improve *de Novo* gene finding. Bioinformatics 24(5):637–644. https://doi.org/10.1093/bioinformatics/btn013

Uliano-Silva M, Ferreira JGRN, Krasheninnikova K, Blaxter M, Mieszkowska N, Hall N, Holland P, Durbin R, Richards T, Kersey P, Hollingsworth P, Wilson W, Twyford A, Gaya E, Lawniczak M, Lewis O, Broad G, Martin F, Hart M, Barnes I, Formenti G, Abueg L, Torrance J, Myers EW, Durbin R, Blaxter M, McCarthy SA Darwin tree of life C (2023) MitoHiFi: a python pipeline for mitochondrial genome assembly from PacBio high fidelity reads. BMC Bioinformatics 24 (1):288. https://doi.org/10.1186/s12859-023-05385-y

van Dam P, Fokkens L, Schmidt SM, Linmans JHJ, Kistler HC, Ma L-J, Rep M (2016) Effector profiles distinguish *formae speciales* of *Fusarium oxysporum*. Environ Microbiol 18(11):4087–4102. https://doi.org/10.1111/1462-2920.13445

Vlaardingerbroek I, Beerens B, Schmidt SM, Cornelissen BJC, Rep M (2016) Dispensable chromosomes in *Fusarium oxysporum* F. Sp. *lycopersici*. Mol Plant Pathol 17(9):1455–1466. https://doi.org/10.1111/mpp.12440

Wang B, Brubaker CL, Tate W, Woods MJ, Matheson BA, Burdon JJ (2006) Genetic variation and population structure of *Fusarium oxysporum* F.sp. *Vasinfectum* in Australia. Plant Pathol 55(6):746–755. https://doi.org/10.1111/j.1365-3059.2006.01445.x

Wang B, Brubaker CL, Summerell BA, Thrall PH, Burdon JJ (2010) Local origin of two vegetative compatibility groups of *Fusarium oxysporum* F. sp. Vasinfectum Australia Evolutionary Appl 3(5–6):505–524. https://doi.org/10.1111/j.1752-4571.2010.00139.x