Draft Genome Resource of a Novel Virulent *Fusarium oxysporum* f. sp. *cubense* Race 1 Strain (VCG 0124) Infecting Cavendish (AAA) Group of Banana in India

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Abstract

Fusarium oxysporum f. sp. *cubense* is one of the most destructive soilborne fungi causing Fusarium wilt disease in banana. Generally, *F. oxysporum* f. sp. *cubense* race 1 (R1) severely affects most of the banana varieties, except Cavendish banana (AAA). Here, we present the draft genome of an isolate of VCG 0124, a novel virulent R1 strain that severely affects the Cavendish group of banana isolated from the Theni district of Tamil Nadu, India. The genome assembly of R1 comprises 61,471,473 bp with 88 contigs and 18,377 protein-coding regions. The genome contains homologs of *F. oxysporum* f. sp. *cubense* race-specific *secreted-in-xylem* (*SIX*) genes *SIX1*, *SIX5*, *SIX9*, and *SIX13*. The absence of *SIX4* and *SIX6* and deletion of a peptide in *SIX1* virulence factor genes in the R1 (VCG 0124) strain might be the contributing factor for strains infecting Cavendish banana in India.

Fusarium oxysporum f. sp. *cubense* is a serious and devastating soilborne fungus causing Fusarium wilt in banana (*Musa* spp.) classified into *F. oxysporum* f. sp. *cubense* race 1, race 2, and race 4 based on the pathogenic specificity to host cultivars (Ploetz 2006). *F. oxysporum* f. sp. *cubense* initially invades and colonizes the banana root system and then disrupts the water translocation in vascular tissues, leading to typical wilt symptoms, which include chlorosis and necrosis of foliage, drooping of leaves, and, finally, the death of banana plants (Stover 1962). As *F. oxysporum* f. sp. *cubense* can survive in the soil for decades, banana growers are finding difficulties in growing susceptible cultivars in the field is a menacing reality. In general, banana cultivars such as Gros Michel (AAA), Silk (AAB), Pisang Awak (ABB), and Bluggoe (ABB) are susceptible to *F. oxysporum* f. sp. *cubense* R1, while Cavendish banana are resistant. During 2009, Thangavelu and Mustaffa (2010) noticed an unusual wilt incidence on Grand Naine (AAA) banana in the Theni district of Tamil Nadu, India and reported that a novel virulent strain of *F. oxysporum* f. sp. *cubense* VCG 0124 was responsible for the cause of the wilt, which is the first report of the kind in Cavendish banana. Recently, the occurrence of the strain R1 (VCG 0124) was noticed by the senior author in important banana-growing states of India (namely, Gujarat, Maharashtra, Madhya Pradesh, and West Bengal), where it is attacking banana crops grown in more than 1,000,000 ha (personnel communication). In this context, a study was initiated to understand the genome of an *F. oxysporum* f. sp. *cubense* virulent strain in detail in comparison with the reference *F. oxysporum* f. sp. *lycopersici* 4287 (GCA_000149955.2) and R1 (GCA_000350345.1). Here, we report the draft genome of the *F. oxysporum* f. sp. *cubense* VCG 0124 and discuss

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Keywords

Cavendish banana, Foc R1, *Fusarium oxysporum* f. sp. *cubense* R1, Fusarium wilt, secreted in xylem, virulence-associated genes, whole-genome sequencing

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Table 1. Summary of genome characteristics and predicted features of reference and assembled genomes of *Fusarium oxysporum* f. sp. *lycopersici* 4287 and *F. oxysporum* f. sp. *cubense* race 1 (R1)

Characteristics	Genome assembly numbers (strain) ^a		
	GCA_000149955.2 (4287)	GCA_000350345.1 (R1)	GCA_011316005.3 (R1)
Genome size (bp)	61,386,934	47,657,417	61,471,473
Number of contig (count)	1,362	2,185	88
Contigs N ₅₀ (bp)	95,416	146,026	4,589,937
Maximum contig length	6,854,980	3,690,067	6,854,980
Minimum contig length	900	200	900
Average contig length	6,98,542	35,538	698,539
Median contig length	15,960	897	15,960
GC content	48.4	48.0	48.4
Number of protein coding genes	27,347	15,865	18,340
Total no. of genes	20,925	15,438	18,377
Gene BUSCO (%)	96.4	95	96.5

^a Genome assembly numbers of available in the NCBI database.

the *secreted-in-xylem* (SIX) genes of the isolate and the possible reason for the infection on Cavendish banana.

A detailed description of the origin and isolation of the *F. oxysporum* f. sp. *cubense* strain, confirmation using *nit*-M testers of known vegetative compatibility groups, volatile odor production, and its pathogenicity were furnished by Thangavelu and Mustafa (2010). The genomic DNA was isolated from the single spore culture of late-log-phase growth (grown in the potato dextrose broth medium at 25 ± 2°C without shaking) by the cetyltrimethylammonium bromide method (Doyle and Doyle 1987) followed by proteinase K and RNase digestion (Roche Diagnostics). DNA purity was measured by the A260/A280 ratio using NanoDrop ND-1000 (Nanodrop) and agarose gel electrophoresis. The genomic library was prepared from a sheared DNA fraction of approximately 300 bp using Illumina paired-end TruSeq DNA sample preparation kits according to the manufacturer's instructions. Fungal libraries were sequenced using the Illumina NextSeq 500 system (M/s. Genotypic) for 150 × 2 cycles, generating approximately 2.6 million paired-ends reads amounting to approximately 17.16 million total raw reads. The short-read sequences of R1 were assembled using MaSuRCA v.3.2.4 (Zimin et al. 2013), mapped with *F. oxysporum* f. sp. *lycopersici* 4287 using BWA v.0.7.12 (Li and Durbin 2009), and annotated using Fungal Ensemble gene annotation system (<https://fungi.ensembl.org/index.html>). The draft genome sequence of R1 VCG 0124 was 61.47 Mb in length with 48.4% GC content (Table 1). The genome consisted of 88 contigs of 900 to 6,854,980 bp, with an average contig size of 698,539 bp. The completeness was estimated using BUSCO (v.3.0.2) with the Sordariomycetes data set (Waterhouse et al. 2018) and resulted in 96.5% (3,680) which occurred as intact and single copies out of 3,817 target genes. This is in accordance with Asai et al. (2019) and Warmington et al. (2019), who reported the whole-genome sequences of *F. oxysporum* f. sp. *cubense* using BUSCO. Functional annotation of AUGUSTUS (v.3.3)-predicted (Stanke et al. 2006) protein-coding genes was performed by combining InterProScan (Jones et al. 2014) and BLASTp searches against the UniProtKB Fungi protein database (ConsortiumUniProt 2018). In total, 18,370 genes were annotated from 18,377 protein-coding genes using the UniProt database with a cutoff E-value of 10⁻⁵ (Boutet et al. 2007). Based on the similarity search with the cluster of orthologous grouping (COG) database (<https://www.ncbi.nlm.nih.gov/COG/>), COG of the proteins was done with an E-value threshold of 10⁻⁵ (Conesa et al. 2005). Of the annotated proteins, 2,426 (13.2%) were associated with biological processes, 7,241 (39.4%) were associated with cellular processes and signaling, and 8,674 (47.2%) were associated with molecular functions. However, 37 (0.2%) protein-coding genes were not categorized into any of the COG classes and, thus, were considered to be proteins of uncharacterized functions or features. A further comparison with the reference R1 (GCA_000350345.1) revealed that there were 326 unaligned protein-coding sequences present in the studied genome, which is estimated to be approximately 3.7 Mb in size. Similar comparison with *F. oxysporum* I15 (GCA_000260195.2) revealed that there were 495 unaligned protein-coding sequences recognized in this genome, which is about 5.2 Mb in size. These are considered as unique regions present only in the genome of the study, which differentiates them from the reference

F. oxysporum f. sp. *cubense* R1 and R4 strains based on genome organization. To visualize the localization of blocks of collinearity, rearrangements, and inversions, MAUVE program v20150226 was used with a weight close to 5,000 (Darling et al. 2004). The results showed that there were 900 (4,60,874 bp) and 1,135 (5,20,324 bp) homologous regions of locally collinear blocks conserved in the genome of *F. oxysporum* f. sp. *lycopersici* 4287 and *F. oxysporum* II5, respectively, with R1.

Protein coding genes predicted in this study and subjected to Plant Host Interaction search (<http://www.phi-base.org>) showed that there were 1,042 genes in the genome of *F. oxysporum* f. sp. *cubense* R1 (VCG 0124), in which 30 are unique in comparison with reference R1. To characterize the isolate by the presence and absence of *SIX* protein genes (*SIX1* to *SIX14*), BLASTx analysis was performed. Among the *SIX* genes, *SIX1*, *SIX5*, *SIX9*, and *SIX13* were commonly found to present in both the reference (*F. oxysporum* f. sp. *lycopersici* 4287 and *F. oxysporum* f. sp. *cubense* R1) and R1 (VCG 0124) genomes, whereas *SIX4* and *SIX6* were absent in the genome of R1 (VCG 0124) infecting Cavendish banana when compared with reference *F. oxysporum* f. sp. *cubense* R1 and *F. oxysporum* f. sp. *lycopersici* 4287. Two homologous copies of *SIX1* and *SIX9* were present in chromosome 14 and *SIX13* in chromosome 6, whereas a single copy of *SIX5* was noticed in chromosome 14. The contig CM027182.1 of chromosome 14 between 2,04,004 and 2,04,365 nucleotides encoded a *SIX1* gene (alternatively, *AVR3*) has a sequence polymorphism at amino acid position 205 to 214 (by deletion). This is in corroboration with the report of Li et al. (2016), who found a deletion in the *SIX1* gene in *F. oxysporum* f. sp. *conglutinans* that enhanced the virulence in infecting cabbage. They also reported that the modification in *SIX1* is required for the compatible interaction with the other host cultivars. Further comparison of R1 with the genome of other *F. oxysporum* f. sp. *cubense* races may provide insights into why the strain is also attacking Cavendish banana, which will be explored in our future studies.

Nucleotide sequence accession numbers. The assembled short-read genome sequences of *F. oxysporum* f. sp. *cubense* race 1 (VCG 0124) infecting Cavendish banana has been deposited at DNA Data Bank of Japan/European Nucleotide Archive/GenBank under accession number GCA_011316005.3 (PRJNA552447), which is the third version.

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