Comparative Whole-Genome Sequence Analyses of Fusarium Wilt Pathogen (Foc R1, STR4 and TR4) Infecting Cavendish (AAA) Bananas in India, with a Special Emphasis on Pathogenicity Mechanisms

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Abstract: Fusarium wilt is caused by the fungus Fusarium oxysporum f. sp. cubense (Foc) and is the most serious disease affecting bananas (Musa spp.). The fungus is classified into Foc race 1 (R1), Foc race 2, and Foc race 4 based on host specificity. As the rate of spread and the ranges of the devastation of the Foc races exceed the centre of the banana’s origin, even in non-targeted cultivars, there is a possibility of variation in virulence-associated genes. Therefore, the present study investigates the genome assembly of Foc races that infect the Cavendish (AAA) banana group in India, specifically those of the vegetative compatibility group (VCG) 0124 (race 1), 0120 (subtropical race 4), and 01213/16 (tropical race 4). While comparing the general features of the genome sequences (e.g., RNAs, GO, SNPs, and InDels), the study also looked at transposable elements, phylogenetic relationships, and virulence-associated effector genes, and sought insights into race-specific molecular mechanisms of infection based on the presence of unique genes. The results of the analyses revealed variations in the organisation of genome assembly and virulence-associated genes, specifically secreted in xylem (SIX) genes, when compared to their respective reference genomes. The findings contributed to a better understanding of Indian Foc genomes, which will aid in the development of effective Fusarium wilt management techniques for various Foc VCGs in India and beyond.

Keywords: banana; fusarium wilt; pathogenicity genes; SIX genes; whole-genome sequencing

1. Introduction

India is the world’s largest producer of the fifth most vital horticultural fruit crop, the banana (Musa spp.) [1]. Although several diseases wreak havoc on banana production around the world, Fusarium wilt, caused by a soil-borne fungus Fusarium oxysporum f. sp. cubense (Foc), has received more attention due to its scientific and economic significance [2]. In India, production losses caused by the devastating wilt disease severely harm the national economy as well as the livelihoods of banana farmers [3]. To overcome economic hardship, rapid, accurate, and early diagnostic procedures, as well as the effective implementation of quarantine/control measures, are critical for the effective containment of the disease [4].

The first record of Fusarium wilt disease was reported in Java in the 18th century, in the most widely cultivated banana strain at the time, Gros Michel [5]. At present,
the disease has spread to many banana-growing countries, including a recent outbreak of the disease in the Cavendish banana in India involving *Foc* Race 1 (VCG0124), *Foc* subtropical race 4 (STR4, VCG01220) [6] and *Foc* tropical race 4 (TR4, VCG01213/16) [7,8]. The dangers of global banana monoculture were first highlighted by the spread of the harmful strain *Foc* R1 to the majority of the world’s banana producing countries, destroying the Gros Michel banana [9]. The recent outbreak of Fusarium wilt in the Cavendish banana is caused by a unique vegetative compatibility group, VCG01213/16, known as *Foc* TR4. This is the most destructive race because of its much broader host range (Cavendish banana and all cultivars sensitive to the *Foc* R1 and *Foc* R2) and widespread distribution far from its origin [7,10]. These *Foc* races have resulted in significant economic losses to commercial and subsistence banana producing areas of Australia, Cambodia, China, Colombia, India, Indonesia, Israel, Jordan, Laos, Lebanon, Malaysia, Mozambique, Myanmar, Oman, Pakistan, Taiwan, Thailand, the Philippines, Turkey, and Vietnam [3].

*Foc* produces dormant resting spores known as chlamydospores, which allow the pathogen to survive without a host for at least a few decades, preventing any susceptible banana cultivars from being grown in disease-infected fields in the future [5]. The disease cycle begins with an infection of the root system and colonises the vascular tissue, induces a reddish-brown discolouration in the rhizome and pseudostem, disrupts water and nutrient transportation, resulting in chlorosis, pseudostem splitting, and wilting [2,5], and eventually, results in plant death before fruit bunches are produced, resulting in a significant reduction in crop yield [11]. The disease incidence varies depending on banana cultivars/genotypes, environment, and *Foc* inoculum levels, but it can result in total crop loss in heavily affected fields [12]. In recent years, *Foc* TR4 and R1 have spread rapidly into Cavendish bananas in India and other regions, posing a threat to the global banana industry and drawing international attention to the future food security of banana-producing countries. Although the incidence of Fusarium wilt caused by *Foc* TR4 in India was initially estimated to be between 2% and 26.6%, a recent survey revealed that the incidence was more than 50% in both Bihar and Uttar Pradesh, where the Cavendish banana is grown on more than one lakh (100,000) hectares [3]. Unexpectedly, no single effective control method has been identified so far except prevention, through the use of pathogen-free tissue culture plants and the adaptation of quarantine strategies [13]. These sanitation practices, however, are mostly followed by commercial plantations and not by subsistence farmers in developing and underdeveloped banana-growing countries.

A better understanding of the fungal genome is required to elucidate the organization of pathogenicity-associated genes/pseudogenes and their virulence mechanisms to develop effective Fusarium wilt control measures. To discriminate the *Foc* races, various molecular methods such as random amplified polymorphic DNA markers [14], restriction fragment length polymorphisms [15], amplified fragment length polymorphism [16], race-specific duplex PCR marker [17], DNA sequence analyses [18], and transcriptomic approaches [19–22] have been employed. Recently, comparative genomic analysis has been employed to reveal the lineage-specific genomic regions that are responsible for the polyphyletic origin of host specificity [19,23]. Though studies have been established to demarcate each specific race and their molecular mechanisms [17,24], very little is known about the differences between *Foc* races, *Foc* R1, *Foc* STR4 and *Foc* TR4 at the genome levels [20,21,23,25–27], and specifically, Indian *Foc* races that have a serious impact on the Cavendish banana [28].

Therefore, the study aims to delineate the differences in the genomes of *Foc* R1, *Foc* STR4, and *Foc* TR4 infecting the Cavendish banana compared to their respective genome references (*Fol* 4287 and *Foc* races), and vice versa, based on putative virulence-associated genes. We believe that a better understanding of the genomes of the various Indian-origin races could aid in the development of race-specific control measures.
2. Materials and Methods

2.1. Collection of Foc Races

The VCGs of Indian Foc races used in the study, VCG0124 (hereafter Foc R1), VCG0120 (hereafter Foc STR4), and VCG01213/16 (hereafter Foc TR4), were obtained from the Plant Pathology Division, ICAR-National Research Centre for Banana, Tiruchirappalli. Foc R1 was originally collected from the Cumbum area, Theni District, Tamil Nadu (9°44′05" N 77°15′02" E; 482 m MSL), in 2009 by Thangavelu and Mustaffa [29]. Foc STR4 was collected from the Burhanpur district (21°19′58" N 76°12′46" E 256 m MSL) of Madhya Pradesh [6]. Foc TR4 was collected from Barari Village, Katikar District, Bihar (25°14′16" N 87°01′07″ E, 49 m MSL), in 2015 by Thangavelu et al. [8]. All these strains were isolated from the vascular strand of pseudostems in symptomatic Grand Naine cv. (Cavendish-AAA group). VCG analysis with nit-M testers, pathogenicity testing using micro-propagated Grand Naine plantlets, and molecular confirmation with race-specific PCR markers (e.g., [11,17,27]) were performed to reconfirm the virulence and race of the isolates as per standard procedures before the whole genome sequencing. Thangavelu et al. [8] provided a detailed description of these methods elsewhere.

2.2. DNA Extraction and Quantification

The genomic DNA of Foc strains was extracted from single spore cultures at the late log phase (5–7 days) grown in potato dextrose broth at 25 ± 2 °C under dark conditions without shaking [30]. Mycelia were collected in sterile filter paper and ground into a fine powder in liquid nitrogen with a sterilized pre-cooled pestle and mortar. DNA was extracted from the finely ground mycelia powder using the CTAB method [31] with the use of a phenol-chloroform-isoamyl alcohol mixture, followed by proteinase K and RNase digestion. DNA samples were suspended in TE buffer and used to check the purity as a ratio of A_{260}/A_{280} measured by NanoDrop® (M/s. NanoDrop, Wilmington, DE, USA) and agarose gel electrophoresis. The purified DNA was stored at −20 °C until further use.

2.3. Genome Sequencing and Assembly

The genomic library was prepared from a sheared DNA fraction of ~300 bp using Illumina paired-end sample preparation kits according to the manufacturer’s instructions. The prepared fungal libraries were sequenced using the Illumina NextSeq® 500 system (M/s. Genotypic, Bengaluru, Karnataka, India) for 150 × 2 cycles, generating ~2.6 million paired-end reads amounting to ~16.18, 29.63 and 17.4 million nucleotides for Foc R1, Foc STR4 and Foc TR4, respectively. The genome data were deposited at NCBI SRA under accession number SRP299372. The standard Illumina pipeline was used to filter the whole genome data. To remove low-quality reads and reads containing adaptor/primer contamination, FASTQ files were further subjected to stringent quality control using the NGS QC Toolkit (v.2.3). The short-read sequences were assembled using MaSuRCA v.3.2.4 [32] with the high-quality sequencing data using k-mer 23, and the gaps were filled using GapFiller44 v.1.11.

2.4. Mapping of Foc Genome

In addition to generating de novo assemblies, Illumina sequencing reads were mapped to the Fol 4287 assembly. Bowtie2 v.2.2.5 was used for the mapping of high-quality filtered reads of Foc R1 (GCA_011316005.3), Foc STR4 (GCA_016802205.1) and Foc TR4 (GCA_014228265.3) against the Fol 4287 genome (GCA_000149955.2). Subsequently, only uniquely aligned reads (with mapping quality ≥30 and minimum read depth 10) were considered in this study. Base quality score recalibration and individual variant calls were performed using Genome Analysis Toolkit (GATK) v3.5–0 with the Haplotype Caller module [33] following recommended practices [34].
2.5. Identification and Analysis of Variants in the Foc Genome

Genome-wide distribution of DNA polymorphisms was analysed by calculating the frequency of every 100 kb interval on each Fol 4287 chromosome. A Circos map (v.0.69–9) was created to visualize the distribution of the SNPs and INDELs related to the Fol 4287 chromosome [35]. To identify synonymous and non-synonymous SNPs, and large-effect SNPs and INDELs [36] between the reference and samples genome, samtools v.1.2 and bcftools v.1.2 tools were used and further annotated with SnpEff v.3.4, respectively.

2.6. Genome and Functional Annotation

RepeatModeler v.2.0.1, RepeatMasker v.4.0.6 and transposonPSI were used to identify transposable elements and repetitive and low complexity regions in the assembled genome using default settings. Gene prediction was performed on softmasked genomes using Augustus v.3.2, a pipeline for automated training and ab initio gene prediction [37]. The annotations of identified genes were performed with cut-off E-values of \( \leq 1 \times 10^{-5} \) and identity \( \geq 30\% \) using the BLAST against the NCBI nr database. Gene ontology (GO) analysis was carried out using UniProt and COG databases [38].

For pathway analysis, protein sequences of Foc races were subjected to KEGG database annotation using blastKOALA [39]. A total of 1105 predicted protein sequences were assigned KO identifiers. These assigned KO identifiers were used to map the KEGG database with the help of a KEGG mapper to identify the pathways. To identify the potential pathogenicity-related proteins, a BLASTP search was performed against the Pathogen-Host Interaction database (PHI-base) with a threshold E-value of \( \leq 1 \times 10^{-5} \) [40]. SIX genes in the genome of Indian Foc races were defined by using BLAST analysis to compare the assembled genome to SIX genes in the NCBI database [41,42].

2.7. Molecular Characterization of Indian VCGs for SIX genes

PCR-based molecular markers and BLAST techniques were used to confirm the presence of SIX genes in the VCGs of Indian Foc isolates and their counterparts. Initially, we employed published primers from Taylor et al. [43] for PCR-based diagnosis and found that they were not reproducible for Indian Foc VCGs, except for certain SIX genes, as they were intended for cepae and other f. sp. of different Fo VCGs (Supplementary Table S1). Therefore, we designed SIX primers specific to Foc based on sequences from different f. sp. of Fo available in the NCBI database, regardless of VCGs. To design Foc specific SIX primers, a total of 927 homologous SIX gene sequences from 25 f. sp. (Supplementary Table S2) were used, and were also subjected to primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast, accessed on 24 May 2020) against the target SIX sequences of Indian Foc genome assemblies using default parameters. Primers that exclusively hit with Foc and genome assemblies were subjected to PCR amplification using standard thermocycling conditions: one cycle of 2 min at 94 °C; 30 cycles of 45 s at 94 °C, 30 sec of annealing temperatures, and 1 min at 72 °C, followed by one cycle of 5 min at 72 °C. Following PCR confirmation, the presence/absence of SIX genes and subgroups was determined using genome BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 24 May 2020) against the genome assemblies of Indian VCGs and Fol 4287 using default parameters, with 927 sequences of SIX homologues used.

Given the relevance of SIX1 genes in defining the pathogenicity of VCGs and banana cultivars, as emphasized by Czislowski et al. [44] and Guo et al. [19], we further investigated the sequence similarity of SIX1a-i genes and their locations in genome assemblies. Further, the aligned SIX1 sequences with maximum similarity were extracted from the genome assemblies, compared and finally subjected to phylogenetic analysis to ascertain the variations between the Indian isolates infecting Cavendish banana and isolates not infecting (i.e., nonpathogenic to) Cavendish banana, e.g., Czislowski et al. [44] and Guo et al. [19]. The extracted and obtained sequences were aligned using the CLUSTAL-W method, and the consensus phylogenetic tree obtained through the maximum likelihood method with 1000 bootstrap resampling was visualized using MEGA-X (v.10.2.5).
3. Results and Discussion

The Indian-origin Foc VCGs are well-established virulent strains against the Cavendish banana, but they lack a contiguous genome assembly. We assembled the Foc genome into chromosome-scale contigs with unplaced and mitochondrial scaffold regions using high-coverage Illumina sequencing. Indian Foc races R1 (VCG 0124), STR4 (VCG 0120), and Foc TR4 (VCG 01213/16) were confirmed using VCG analysis and Foc specific PCR markers [7,11,31].

3.1. Genome Sequencing and General Features

The short sequences of Foc R1, Foc STR4, and Foc TR4 were assembled into 88, 85, and 88 scaffolds, respectively, with a total genome size of 61 to 63 Mb and ~48.5% GC (Table 1). Although the genome size of Foc TR4 was slightly larger (3.0%, 1.9 Mb) than the reference genome Fol 4287, the genome sizes of all three Indian genome assemblies were comparable. Interestingly, we observed a noticeable difference in the Foc genome of Indian races, specifically larger genome size, when compared to well-known Foc genome assemblies reported from other parts of the world. For instance, the genome of the novel Foc TR4 (VCG01213/16) of Indian origin contained 16.3 (Foc4_1.0; GCA_000350365.1)—26.4% (FO_IL5_V1; GCA_000260195.2) more nucleotides than controls, which translated into a 10.1—16.3 Mb genome size. Similarly, the genome assembly of Foc R1 had a 16.8 (Foc1_1.0; GCA_000350345.1)—22.5% (ASM593051v1; GCA_005930515.1) larger genome size i.e., 10.1 to 13.5 Mb. In the case of Foc STR4, the genome size was 23.9% (14.3 Mb) higher than the C1HIR_9889 (GCA_001696625.1). The difference in genome size between Indian Foc VCGs and other VCGs is likely attributable to the fact that we built libraries with larger inserts and obtained more mate-pair information from them, allowing us to connect contigs into scaffolds more easily [19]. Sequenced reads were aligned to the associated assemblies to check the assembly’s integrity; ~97–98% of reads could be aligned to the respective Foc assemblies. The high map ratio indicates that the assemblies covered the majority of the genome [19].

Table 1. Summary of genomic and predicted features of reference and assembled genomes of Fol 4287 and Indian Foc races.

| Characteristics                  | Fol 4287 | Foc R1 (VCG0124) | Foc STR4 (VCG0120) | Foc TR4 (VCG01213/16) |
|----------------------------------|----------|-----------------|-------------------|----------------------|
| Genome size (bp)                 | 61,386,934 | 61,471,473     | 61,380,681         | 63,220,715           |
| No. of scaffolds (Count)          | 114      | 88              | 85                | 88                   |
| No. of contig (Count)             | 1362     | 1371            | 1362              | 6560                 |
| Scaffold N50 (Mb)                 | 1,976,106 | 4,781,098       | 4,589,962          | 4,589,937            |
| Maximum contig length            | 6,854,980 | 8,732,082       | 6,853,100          | 7,450,211            |
| Minimum contig length            | 900      | 898             | 888               | 886                  |
| Average contig length            | 698,542  | 884,718         | 722,130            | 718,421              |
| Median contig length             | 15,960   | 17,299          | 14,354            | 15,960               |
| GC content                       | 48.4     | 48.5            | 48.4              | 48.5                 |
| Protein count                    | 27,347   | 22,151          | 18,946            | 19,651               |
| Total genes (Augustus)           | 20,925   | 21,842          | 17,118            | 17,745               |
| BUSCO (%)                        | 96.4     | 98.4            | 96.9              | 98.9                 |
| Secretome Genes                  | 1847     | 1949            | 1870              | 2330                 |
| tRNA                             | 320      | 524             | 304               | 358                  |
| rRNA                             | 125      | 223             | 121               | 134                  |
| ORF                              | 892,698  | 1,149,990       | 891,736           | 926,420              |
| Repeats (bp)                     | 10,770,810 | 10,755,517     | 10,901,072         | 8,844,756            |
| Total transposable elements (bp) | 2,446,574 | 346,254         | 342,987           | 273,767              |
| Retroelements                    | 433,505  | 87,052          | 85,124            | 56,365               |
| DNA transposons                  | 506,130  | 2527            | 2591              | 2391                 |
| Total interspersed repeats       | 89,579   | 87,787          | 58,756            |                      |
| Simple repeats                   | 342,368  | 110,011         | 99,987            |                      |
| Low complexity region            | 141,331  | 144,912         | 114,926           |                      |

Fol 4287: GCA_000149955; Foc R1 (TC1): GCA_013316005.3; Foc STR4: GCA_016802205.1; and Foc TR4 (BC2−4): GCA_014282265.3, are genome assembly numbers of the respective strains available in the NCBI database. † According to Ma et al. [45].
The relatedness and completeness of the three Foc genomes were determined using the orthologous genes from the Sordariomycetes data set through BUSCO (v.3.0.2). On average, Indian Foc VCGs had ~97% (3613) intact, single-copy and complete, 1.8% (65) duplicated, 0.9% (33) fragmented and 0.3% (10) missing orthologous genes out of 3725 target genes. The results showed that the genome assemblies of Indian Foc races were robust and complete, and the genome met or exceeded the BUSCO parameters for quality [46], which is in accordance with Asai et al., [23] and Warmington et al. [25].

3.2. Transposable Elements in the Foc Genomes

As the presence of DNA repeats and transposable elements (TEs) facilitates changes in genome size and gene expression, and thus phenotypic variation/pathogenicity in Foc isolates [45], the variations between the VCGs of Indian Foc races were compared with other Foc R1 and TR4 genomes. Transposable elements (TEs) were identified using ab initio prediction methods, and TEclass [47] categories were designated as DNA transposon, long interspersed nuclear element (LINE), short interspersed nuclear element (SINE), and retrotransposon with long terminal repeats (LTRs) [48]. Supplementary Table S3 furnishes a detailed summary of TEs, repeats, and their classification for all VGCs, as well as raw analysis data, which have been stored in the author’s repository [49]. The study accounts for approximately 0.27 to 0.35 Mb of TEs (0.43 and 0.56% of the assemblies) in the Indian Foc VCG assemblies (Table 1). When compared to Yingzi [21] and Ma [45], who reported TEs in Foc R1 (4.34–5.72%), R4 (4.0%), TR4 (5.22–8.63%) and Fol 4287 (3.98), TEs in the Indian isolates were comparatively lower in number and size. VCG0124 (5444) had the most TEs, followed by VCG0120 (5312) and VCG01213/16 (4582), although all were 55.4 to 62.5% less than Fol 4287, which had 12,216 TEs (Supplementary Table S3). Among individual chromosomes, Chr-3 (541–623) had the most TEs, followed by Chr-1 (497–573), Chr-6 (394–465), and Chr-2 (423–452), while Chr-11 (90–117), Chr-12 (64–94), and Chr-13 (64–94) had the least (55–71) TEs. It is interesting to observe that the highest number of TEs was found in lineage-specific Chr-3 and Chr-6, as well as in Chr-15 (250–288) and Chr-14 (68–219). The total composition of TEs included 20.6–25.1% retroelements, 0.73–0.87% DNA transposons, 21.5–25.9% total interspersed repeats, 32.0–36.5% simple repeats and 40.1–42.2% low complexity regions. The average percentages of deletion and insertion in Indian VCGs were 0.55–0.57% and 0.60–6.5%, respectively. Deletions were most abundant in Chr-3, 6, 14 and 15, while insertions were commonly richest in Chr-14 and 15, followed by Chr-3, Chr-8 and Chr-1. Previous studies indicated that the greater the number of insertions in the genome, the higher the amounts of chromosomal reorganization, altered expression, and the generation of new proteins [50] and thus variations in genome plasticity [51], pathogenicity [52], host range [53] and evolution [54]. The total number of repeated sequences in Indian VCGs ranged from 14% (VCG01213/16) to 17.8% (VCG0120), which was comparable to Fol 4287 (17.5%).

3.3. Gene Content in the Foc Genomes

Intact protein-coding genes were identified using a combination of homology-based and ab initio prediction methods. A total of 21,842 intact protein-coding genes were predicted from the consensus gene sets of the Foc R1 genome assembly, which was 4.4% (917) higher than the reference Fol 4287 in terms of number (Table 1). The total number of protein-coding regions in the reference Foc R1 strain NRRL 32,931 was 23,735, which was 8.0% (1893) higher than the Foc R1 VCG 0124 from India. The STR4 and TR4 genome assemblies contained 17,118 and 17,745 protein-coding regions, respectively, which were 18.2% and 15.2% less than the reference Fol 4287 genome assembly. Although the number of protein-coding regions differed between the gene predicted models of the respective races, ~66.9% of the protein-coding regions in the Foc genomes had at least three exons per gene, with an average exon length of 450 bp.

As the number of protein-coding genes across the genome varied, the distribution of the genes within the chromosomes of the different genomes was compared (Figure 1A).
Of the total genes, the largest proportions—38.4% (Foc STR4) to 42.8% (Foc R1)—of genes presented within the Chr-1, 2, 4, and 5 chromosomes, with the maximum of 2534 genes presenting in Chr-4 of Foc R1, while 1951 and 2137 genes presented in Chr-1 of STR4 and TR4, respectively. The lowest number of genes was typically observed in Chr-14, where Foc R1 encoded only 150, TR4 encoded 268 and STR4 encoded 357 genes. Apart from the 15 chromosomes, the other scaffold held 3.8% to 5.3% of genes of which STR4 had a maximum of 910 genes, followed by Foc R1 (820) and TR4 (671).

![Figure 1. Cont.](image-url)
Similarly, the presence and distribution pattern of rRNAs and tRNAs in the chromosome of each genome was related (Figure 1B,C). The results revealed that the Foc R1 contained a maximum of 524 tRNAs and 223 rRNAs, with the highest number of tRNAs present in Chr-12 (100), followed by Chr-11 (64) and Chr-1 (52), and the highest number of rRNAs present in Chr-2 (91). TR4 contained a maximum of 358 tRNAs and 134 rRNAs, with the highest number of tRNAs present in Chr-12 (50) followed by Chr-2 (43) and Chr-11 (40), and the highest number of rRNAs present in Chr-2 (70), Chr-11 (13) and Chr-12 (10). STR4 had a total of 304 tRNAs and 121 rRNAs in the genome, with the highest number of tRNAs in Chr-12 (48) followed by Chr-1 (34), Chr-11 (34) and Chr-2 (33), and the highest number of rRNAs in Chr-2 (38) followed by Chr-11 (9), Chr-12 (9), and Chr-5 (9). We were unable to detect any tRNA or rRNA in Chr-3, Chr-14, or Chr-15 in this study. Surprisingly, the absence of tRNA and rRNA has been linked to the most important lineage-specific chromosomes of the Foc, namely Chr-3, Chr-14, or Chr-15 in this study. Surprisingly, the absence of tRNA and rRNA has been linked to the most important lineage-specific chromosomes of the Foc, namely Chr-3, Chr-14, or Chr-15, which encode the majority of the virulence-associated genes that determine the pathogenicity of the Foc strains [45].

3.4. GO and KEGG in the Foc Genome

A total of 8756 (39.5%) of 22,151 protein-coding genes in Foc R1, 5384 (28.4%) of 18,946 protein-coding genes in STR4 and 11,220 (57.1%) of 19,651 protein-coding genes from TR4 were annotated based on the Gene Ontology database (http://www.ncbi.nlm.nih.gov/COG/, accessed on 16 June 2020) with a cutoff E-value of $10^{-5}$. The results are depicted in Figure 2. Of the total of 39.5% annotated proteins in Foc R1, 1952 (22.3%) were associated with biological processes, 2969 (33.9%) were associated with cellular processes and signalling, and 3835 (43.8%) were associated with molecular functions (Figure 2A). In the case of TR4, of the 57.1% of proteins annotated, 2626 (23.4%) were associated with biological processes, 4096 (36.5%) with cellular processes and signalling, and 4498 (40.1%) with molecular functions (Figure 2A). In the case of STR4, only 28.4% of proteins were annotated, of which 1325 (24.6%) were associated with biological processes, 1704 (31.6%) with cellular processes and signalling, and 2355 (43.7%) with molecular functions.
This study also recorded a large number of protein-coding genes, ranging from 42.9% (TR4) to 71.6% (STR4), which are uncategorized into any of the GO classes and thus considered proteins of uncharacterised functions/features, or potentially genes undergoing rapid evolution and thus displaying high variation that did not match the BLAST result for the given criteria [19]. If we assume that a large number of genes are evolving rapidly, predicting the virulence and pathogenicity of Indian Foc TR4 and Foc STR4 will be beyond our current or anticipated disease trends, which is an immense reality. This is a concerning result for the banana farmers of northern India because it is a major banana-growing region of the country, accounting for 20.1% of total banana production and 57% of Cavendish banana cultivation. A similar analysis was carried out using the KEGG and Interpro databases to identify the protein-encoding genes involved in the various pathways (Figure 2B). According to the findings, the highest number of 570 genes was identified in the Foc TR4 genome, followed by Foc R1 (390) and Foc STR4 (330). Among the pathway categories, the largest numbers of 116 (STR4) to 202 (TR4) genes belong to the metabolic pathway, followed by the biosynthesis of secondary metabolites (52–105) and biosynthesis of antibiotics (48–87) pathways. The lowest numbers of genes were involved in the fatty acid degradation (9–10), propanoate metabolism (5–8) and tyrosine metabolism (6–8) pathways.

![Figure 2](image-url)

**Figure 2.** Functional annotation of the Indian VCGs of Foc genome assemblies based on COG (A) and KEGG (B). The colour in the bar represents the respective functional groups in the y-axis and value represents the number of genes identified from the respective functional groups.
3.5. SNPs, InDels and Phylogenetic Relationship

The assembled genome of Indian Foc VCGs was scanned with Fol 4287 as the reference and a total of 4866 high-quality SNPs were found, with 1502 in Foc R1, 1844 in STR4 and 1520 in TR4 (Supplementary Figure S1A; Supplementary Table S4) while a total of 139,023 InDels (88,532 insertions and 50,491 deletions) was found of which 53,602 were in Foc R1, 33,298 in STR4 and 52,123 in TR4 (Supplementary Figure S1B; Supplementary Table S4). The variations in type and positions of the SNPs and InDels between the genomes are presented as a circular Circos plot in Figure 3A. The results indicated that the genes were distributed more densely on longer scaffolds than on shorter ones, which could be attributed to scaffold integrity. SNPs were more common and evenly distributed than InDels. It is worth noting that InDels and SNPs were abundant in lineage-specific chromosomes, viz., Chr-3, Chr-6, Chr-14 and Chr-15.

Based on the orthologous genes from the Sordariomycetes data, single-copy genes (3613) from each species were concatenated into a supergene to infer the phylogeny tree, comparing nine published Fusarium oxysporum formae speciales genomes. As shown in Figure 3B, the tree indicated a close relationship between the known reference Fol, Foc and Fo genomes, suggesting that they may have descended from a common ancestor. The results showed that the strains of different formae speciales clustered into two clades: clade-1 had four Fo members, i.e., pisi, apii, melonis and sesami, and clade-2 had Fol 4287, Foc race-1, TR4 and STR4 with Fo f. sp. physali, indicating the close phylogenetic relationships among the different formae speciales. Specifically, the four strains of Foc were clustered in the same subclade. This hierarchical relationship was in accordance with the race types of Foc, as the Foc TR4 strains VCG01213/16 and Foc NRRL54006 belonged to TR4 and the Foc race with VCG0124 belonged to Foc race 1, while strain VCG0120 grouped with Foc. The divergence of Foc races might be caused by several significant race-specific genes [55].
3.6. Virulent Genes and Their Functions in the Genome of Foc

To find potential virulence-associated genes, whole-genome BLAST analysis was carried out against the Pathogen-Host Interactions (PHI) gene database (http://www.phi-base.org/, accessed on 16 June 2020), a collection of genes proven to affect the outcome of pathogen-host interactions from fungi, oomycetes and bacteria [41]. After removing the genes that were not related to pathogenicity, we identified 1738 (Foc R1) to 2809 (Foc STR4) putative virulence-associated genes (Figure 4B). Of the total PHI genes identified in the Foc R1, 35.7% (620) were ascribed to biological functions, 22.2% (386) to cellular processes and signalling functions and 42.1% (732) to molecular functions. For Foc STR4, 31.8% (892) were ascribed to biological functions, 22.6% (636) to cellular processes and signalling functions and 45.6% (1281) to molecular functions. For Foc TR4, 37.3% (755) were ascribed to biological functions, 17.1% (347) to cellular processes and signalling functions and 45.6% (924) to molecular functions.

To identify the unique PHI genes, the functions of virulence-associated genes were compared (Figure 4A). The results revealed that there were 243, 418 and 351 putative virulence-associated genes in Foc R1, TR4 and STR4, respectively (Supplementary Table S3). The total number of virulence genes reported from this study was comparable to Foc R1 and Foc TR4, which contained a total of 347 and 348 putative virulence genes, respectively [19]. A total of 46 putative genes were common among the three genomes, while 72 and 30 genes were common between R1 and TR4 and STR4, respectively. Similarly, TR4 shared 16 virulent genes with STR4. A total of 94, 283 and 258 virulence genes were unique to the Foc R1, TR4 and STR4 genomes, respectively. The identified PHI genes were subjected to analysis of secreted protein (http://www.cbs.dtu.dk/services/SecretomeP, accessed on 16 June 2020) followed by effector genes (http://www.cbs.dtu.dk/services/SignalP, accessed on 16 June 2020) and were functionally annotated based on BLAST results. The number of effector genes encoded by the genome varied, in which the minimum was 17 genes present in TR4, followed by 20 genes in Foc R1 and a maximum of 28 genes in STR4. Similarly, the number of genes involved in increased virulence was 13, 23 and 27 in TR4, STR4 and Foc R1, respectively (Figure 4B).

Figure 4. (A) Venn diagram of the putative virulence-associated genes identified in the comparisons of Foc R1 vs. Foc STR4 vs. Foc TR4. (B) Number and functions of PHI genes present in the Indian Foc races.
To identify the unique PHI genes, the functions of virulence-associate genes were compared (Figure 4A). The results revealed that there were 243, 418 and 351 putative virulence-associated genes in Foc R1, TR4 and STR4, respectively (Supplementary Table S3). The total number of virulence genes reported from this study was comparable to Foc R1 and Foc TR4, which contained a total of 347 and 348 putative virulence genes, respectively [19]. A total of 46 putative genes were common among the three genomes, while 72 and 30 genes were common between R1 and TR4 and STR4, respectively. Similarly, TR4 shared 16 virulent genes with STR4. A total of 94, 283 and 258 virulence genes were unique to the Foc R1, TR4 and STR4 genomes, respectively. The identified PHI genes were subjected to analysis of secreted protein (http://www.cbs.dtu.dk/services/SecretomeP, accessed on 16 June 2020) followed by effector genes (http://www.cbs.dtu.dk/services/SignalP, accessed on 16 June 2020) and were functionally annotated based on BLAST results. The number of effector genes encoded by the genome varied, in which the minimum was 17 genes present in TR4, followed by 20 genes in Foc R1 and a maximum of 28 genes in STR4. Similarly, the number of genes involved in increased virulence was 13, 23 and 27 in TR4, STR4 and Foc R1, respectively (Figure 4B).

Among the virulence-associated genes, ABC1, kdpB, acrB, qoxA & B and pstB were cellular transporter protein-encoding unigenes present in the Foc genome (Supplementary Table S3), which are essential for the import of nutrients and export of secondary metabolites [56]. ABC is one of the largest gene families in the ATP-binding cassette transporters superfamily, which hydrolyse ATP to transport a wide range of substrates across biological membranes. ABC genes can be either importers or exporters in the cell membrane depending on the direction of transportation relative to the cytoplasm [57], and have been divided into seven families (A–G) in fungi [58]. Transgenic expression of ABC genes in Arabidopsis thaliana indicated that the ABC transporter gene is required for its transportation of salicylic acid (SA), fungicide resistance, mycelial growth and pathogenicity. SA is a critical plant defense hormone, which contributes to plant defense against a wide range of pathogens with biotrophic and hemibiotrophic lifestyles [59]. A study estimated that Fusarium sp. contained ~45–61 ABC transporter, which was found to provide tolerance to a wide array of antifungal compounds generated by a broad host range [45].

kdpB is part of the high-affinity P-type ATP-driven transporter which catalyses the hydrolysis of ATP coupled with the electrogenic transport of potassium into the cytoplasm. Expression of kdp in Staphylococcus aureus, Yersinia pestis, mycobacteria etc. [60,61] has been found to connect with the regulation of several virulence genes during pathogenesis and also contributes to survival under a variety of stressful conditions [62]. AcrB is a resistance-nodulation-cell division family of efflux pumps that plays a major role in a multidrug efflux system by facilitating intrinsic resistance mechanisms. Experimental results demonstrated that the presence of AcrB and overexpression of an efflux system significantly increases virulence in Caenorhabditis elegans [63] and Klebsiella pneumoniae through the expression of OqxA and OqxB efflux pump [64]. It is hypothesised that an increase in mutation frequency enables the rapid evolution of high-level resistance via the accumulation of point mutations.

The Pst (phosphate transport) system is involved in transporting Pi, which is typically composed of four types. Specifically, cytosolic PstB energizes the release of free Pi in the cytoplasm through ATP hydrolysis function in the transmembrane channel. Peirs et al. [65] reported that Pst systems are important for intracellular survival in the host and play a crucial role in virulence [66].

Additionally, in the Foc TR4 genome, Chr-4 encodes an intracellular signalling system, known as two-component regulatory systems (PXO_04659), which regulate pathogenesis and biological processes as very similar in Xanthomonas axonopodis pv. citri was identified. A study showed that a knockout strain lacking the PXO_04659 gene exhibited a drastic reduction in virulence relative to the wild-type [67]. The enhanced virulence generally observed in Foc TR4 may be attributed to detoxifying regulator mechanisms involved in
increasing extracellular polysaccharide synthesis, tolerance to reactive oxygen species, and iron homeostasis, either alone or in cooperation with other regulatory factors [67].

3.7. The Organisation of Secreted in Xylem Protein-Encoding Genes in Foc Genome

BLASTx analysis and PCR based primers were employed to characterise the presence or absence of secreted in xylem SIX genes (SIX1-SIX14) in Indian Foc isolates [42]. The presence of SIX genes and their subgroups was recognized in this study if the VCG showed both PCR amplification to a molecular marker and a BLAST hit with >80% similarity with Foc/Fol; otherwise, it was regarded as absent (Supplementary Table S1). The difference in PCR results with BLAST hits or previously reported primers (e.g., [42], [44]) may, however, be attributed to sequence variation in the genome or optimal annealing temperature of the primer [28]. As we manually checked the sequencing depth and synteny relationship near the SIX regions, we ruled out assembly errors and assumed that the difference was most likely due to strain variations and other unknown factors [19].

Genome BLAST analysis including 927 SIX query sequences of different formae speciales reveals (Supplementary Table S3) that VCG0120 (1039) had the highest number of hits followed by VCG01213/16 (929) and VCG0124 (653). Foc VCG0124 had the highest number of hits with Fo f. sp canariensis (152), followed by Foc (91) and Fo f. sp. pisi (42). Foc VCG0120 had the highest number of hits with Foc (201), Fo f. sp. canariensis (152) and Fo f. sp. palmarum (73). In the case of Foc VCG01213/16, the maximum number of BLAST hits registered with Foc (153), Fo f. sp. canariensis (152), and Fo f. sp. palmarum (73). Similarly, the maximum number of hits was compared based on the number of SIX hits. Foc VCG0124 had a maximum number of hits with SIX1 (135) followed by SIX7 (102) and SIX6 genes, while VCG0120 recorded a maximum of 284 hits with SIX8 followed by SIX1 (136), SIX13 (110), SIX7 (102) and SIX6 (99). Foc TR4 had a maximum number of hits with SIX8 (223) followed by SIX1 (139), SIX6 (115) and SIX7 (102). Although the analysis resulted in SIX genes that were not specific or reported previously in Foc, this study only considered the presence of the SIX genes and subgroups if the PCR result was positive. Therefore, the following discussion on the presence and absence of SIX genes is based on the concurrent results of both PCR and BLAST.

Based on the variation in the SIX gene profiles of Foc races reported previously [17,42], it was found in the present study that SIX11 and SIX14 were absent in all three Indian Foc genomes (Table 2). Among the SIX genes, SIX1, SIX4, SIX6 and SIX13 were present in the Foc R1 genome whereas SIX8 and SIX9 were absent. We found differential results with SIX2, SIX3, SIX5, SIX7, SIX10 and SIX12 between BLASTx and PCR markers. The study identified that two homologous copies of SIX1 and SIX13 and a single copy of all the remaining SIX genes were present in Chr-14 and Chr-13. A copy of the SIX1 gene (alternatively AVR3) present in Chr-14 had sequence polymorphism at amino acid positions 205–214 (by deletion), which could explain why Foc R1 infects Cavendish banana so unusually in India [68]. This corroborates the findings of Li et al., [69], who found that deletion and complementation of SIX1 in Fusarium oxysporum f. sp. conglutinans resulted in increased virulence against cabbage.

Table 2. Profile and inter-comparison of SIX genes in the reference Fol 4287 and assembled Foc races of Indian origin.

| Accession          | SIX1 | SIX2 | SIX3 | SIX4 | SIX5 | SIX6 | SIX7 | SIX8 | SIX9 | SIX10 | SIX11 | SIX12 | SIX13 | SIX14 |
|--------------------|------|------|------|------|------|------|------|------|------|-------|-------|-------|-------|-------|
| Fol 4287           | +    | +    | +    | –    | +    | +    | +    | +    | –    | +     | –     | +     | –     | –     |
| Foc VCG0124 (TC1 ‡)| +    | –    | –    | –    | +    | –    | –    | –    | –    | –     | –     | –     | –     | –     |
| Foc VCG0124 (TC1 *)| ++   | +    | +    | –    | –    | –    | –    | +    | –    | +     | –     | +     | +     | –     |
| Foc VCG0120 STR4 ‡ | +    | +    | –    | –    | +    | +    | +    | +    | –    | –     | –     | –     | –     | –     |
| Foc VCG0120 STR4 * | ++   | +    | +    | –    | –    | +    | +    | +    | –    | –     | –     | –     | –     | –     |
| Foc VCG01213/16 (BC2-4 ‡)| +    | +    | –    | –    | +    | +    | +    | +    | –    | –     | –     | –     | –     | –     |
| Foc VCG01213/16 (BC2-4 *)| +    | +    | +    | +    | +    | +    | +    | +    | +    | –     | –     | –     | –     | –     |

* Represents SIX in the genome of Foc; where +: present; –: absent in both PCR and BLAST; ++: presence of SIX other than Foc f. sp. genome where Foc TC1: GCA_011316005.3; Foc STR4: GCA_016802205.1; and Foc BC2-4: GCA_014282265.3. ‡ Results of SIX specific PCR marker.
In the case of STR4, SIX1, SIX2, SIX7, SIX8, SIX9 and SIX13 were present and SIX12 was absent. The presence of SIX3, SIX4, SIX5, SIX6, and SIX10 was detected by genome BLASTx but not by PCR markers. The genome contained two homologous copies of SIX1 and SIX9 in Chr-14, as well as 13 homologous copies of SIX8 distributed across Chr-2 (1), Chr-3 (3), Chr-6 (2), Chr-7 (1) and Chr-14 (6) and a copy of SIX8b in Chr-14. The strain of STR4 possessed a homolog of SIX-6, an effector gene located in the Chr-14 between 4258 and 4987 nt which facilitates colonisation of the host, specifically by suppressing I-2-mediated cell death [70].

SIX1, SIX2, SIX8, SIX9 and SIX13 have been found to present in the *Foc* TR4 genome alongside the SIX8a, which is specific to TR4 [44]. Our BLASTx analysis revealed the presence of SIX3, SIX4, SIX5, SIX6, SIX7, SIX10, and SIX12 in the genome, but PCR primers failed to generate amplicons. Similarly to *Foc* R1 and STR4, TR4 has two homologous SIX1 in Chr-14 while 13 homologous copies of SIX8 are distributed in Chr-2, Chr-3, Chr-6, and Chr-14 without any sequence variations, which corroborates the report of Czislowski et al. [44] especially in the VCG 01213/16. They reported that the presence of multiple copies of *Foc* TR4 specific SIX homologues is usually acquired through a horizontal transfer event from other *Foc* formae speciales. In general, SIX8a is present in all race 4 isolates, whereas SIX8b is present in all subtropical race 4 isolates, but has yet to be detected in *Foc* TR4 isolates [71]. However, copies of two SIX8 homologues, SIX8a and SIX8b, were found in the genomes of *Foc* TR4 (in Chr-14) without sequence variation, which is a major difference noted in this study. As a result of the SIX8b effector gene, the *Foc* TR4 of India can infect Cavendish bananas grown in the subtropical region as well, and this confirms the fact that the *Foc* TR4 was isolated from the Katihar district of Bihar which is within the subtropical climate zone of India.

Genome BLASTx analysis revealed that all SIX1a-i homologues (compared to Czislowski et al. [32] and Guo et al. [33]) were present in the pathogenic lineage of Chr-14 in VCG0124 (CM027182.2), VCG0120 (CM028826.1), and VCG01213/16 (CM026317.2) with 82% to 84% similarity, 79 to 92 bp mismatches, and 12 to 15 bp gaps (Supplementary Table S3). Even though the SIX1 sequences of Elizabeth and Guo differ slightly, we were able to obtain a maximum of >80% similarity with the genome of Indian *Foc* VCGs infecting the Cavendish banana. According to Czislowski et al. [44], SIX1a, d, f-i have been found to present in VCG0124, 0120 and 01213/16, of which SIX1d and f belong to VCG0124, SIX1g belongs to VCG0120, and SIX1a, h and i are found in VCG01213/16, alongside SIX1b in VCG0121. In contrast, the BLAST results of 927 SIX gene sequences showed that Indian VCGs had the best match with SIX1a, b, and f, where SIX1a and SIX1b are from *F. sp. physali*, and SIX1f is from *Foc*. The study emphasis that describing the presence of SIX1 subgroups in VCGs or lineage/horizontal gene transfer mechanisms between race/*f. sp. is very difficult to comprehend solely on sequence similarity without PCR confirmation, as we do not have VCG/SIX1 specific primers to investigate at the moment. Multiple sequence alignment of extracted SIX1 gene segments revealed significant variations between the VCGs of Indian isolates and those isolated by Czislowski et al. [44] and Guo et al. [19], specifically aligned positions 207–230 and 535–552 (Figure 5A). This variation could be interpreted as the principal difference of Cavendish infecting *Foc* VCGs of India. Furthermore, maximum likelihood phylogenetic analysis revealed that Indian *Foc* isolates were found to be a distinct lineage with a separate clade (Figure 5B) when compared to the SIX1a-i sequences of Czislowski et al. [44] and Guo et al. [19]. This finding supports our hypothesis that *Foc* VCGs of Indian races evolved as a separate lineage with various combinations of SIX genes and subgroups or effector genes.
4. Conclusions

When compared to *Foc* genome assemblies reported from other parts of the world, this comparative whole-genome sequence analysis revealed significant differences in Indian *Foc* races in terms of genome size and protein-coding regions. Furthermore, annotation of the...
TR4 and STR4 genomes revealed that the genome contains 42.9–71.6% protein-coding genes with unknown functions/features or genes undergoing rapid evolution, emphasizing the importance of early detection methods. The current study also explained the mechanisms of the major pathogenicity-related protein families that are involved in increasing the pathogenicity and virulence of organisms. Moreover, the study established the presence of both SIX8a and SIX8b with multiple copies of SIX1, distinguishing the examined TR4 genome from other references. Based on our findings, we believe that a new accurate and early diagnosis procedure is required to identify rapidly evolving Foc races of India to sustain the country’s banana production in these regions. In addition, the variable sequence/genome regions discovered in the Foc TR4 of India will be useful in future to develop race-specific molecular markers targeting SIX genes and TEs.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/jof7090717/s1, Supplementary Table S1: SIX primers of Fusarium oxysporum f. sp. cepae and Fusarium oxysporum f. sp. cubense characterised in the study. Supplementary Table S2: A list of 927 SIX gene databases of different Fo f. sp used in the study for genome-BLAST and VCG based results. Supplementary Table S3: Comparison of transposable elements and repeatable elements in the VCGs of Indian isolates. Supplementary Table S4: SNPs in the Foc race infecting the Cavendish banana in India. Supplementary Table S5: InDels in the Foc race infecting the Cavendish banana in India. Supplementary Table S6: PHI genes in the Foc race infecting the Cavendish banana in India, Supplementary Figure S1: Comparison of SNPs and InDels in the Indian Foc races.

Author Contributions: T.R. conceived the project, planned experiments, oversaw the experiments, compiled data and wrote the manuscript; E.E.R. performed the experiments, data analyses and wrote the paper; S.K., S.R., G.M., P.P., M.N. and P.M. performed the experiments; and M.L. and U.S. wrote the paper. T.R. and E.E.R. contributed equally. All authors have read and agreed to the published version of the manuscript.

Funding: This paper was made possible by a grant from the Indian Council of Agricultural Research (ICAR), New Delhi, for an extramural project named “Integrated Management of Fusarium wilt-Tropical Race-4: A Devastating Strain on Banana”.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data on transposable elements [49] and gene structure annotations and sequences used for genome BLAST and phylogenetic analysis [48] have been deposited in the Mendeley Data repository. The assembled short-read genome sequences of Foc Race 1VCG0124, Foc STR4 VCG0120 and Foc TR4 (VCG 01213/16) infesting Cavendish banana have been deposited at DDBJ/EMBL/GenBank under accession number GCA_011316005.3, GCA_016802205.1 and GCA_014282265.3 respectively. Raw Illumina sequencing data of Indian Foc races are available in the NCBI Sequence Reads Archive (SRA) under the accession number SRP299372. All the data generated or analysed during this study are included in this published article.

Acknowledgments: We acknowledge the financial support provided for this study through extramural projects rendered by the ICAR, New Delhi and the facilities provided by the Director. We appreciate the contribution from the CGIAR Research Program on Roots, Tubers and Bananas (RTB) and the CGIAR Fund Donors for covering the open access fee.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References
1. Sen, C.; Mishra, H.; Srivastav, P. Modified atmosphere packaging and active packaging of banana (Musa spp.): A review on control of ripening and extension of shelf life. J. Stored Prod. Postharvest Res. 2012, 3, 122–132. [CrossRef]
2. Ploetz, R.C. Fusarium wilt of banana. Phytopathology 2015, 105, 1512–1521. [CrossRef]
3. Thangavelu, R.; Loganathan, M.; Artheem, R.; Prabakaran, M.; Uma, S. Fusarium wilt: A threat to banana cultivation and its management. Cab Rev. 2020, 15, 24. [CrossRef]
4. Thangavelu, R.; Muthukathan, G.; Pushpakanth, P.; Murugan, L.; Edwin, R.E.; Marimuthu, N.; Uma, S. First report of Fusarium oxysporum f. sp. cubense VCG 0125 and VCG 01220 of Race 1 infecting Cavendish bananas (Musa sp. AAA) in India. Plant Dis. 2020, 105. [CrossRef]

5. Stover, R.H. Fusarial Wilt (Panama Disease) of Bananas and Other Musa Species; Commonwealth Mycological Institute: London, UK, 1962.

6. ICAR National Research Centre for Banana. Anonymous. Annual Report 2018–19; ICAR-National Research Centre for Banana: Tiruchirappalli, India, 2019; Available online: http://nrcb.res.in (accessed on 11 May 2020).

7. Mostert, D.; Molina, A.B.; Daniells, J.; Fourie, G.; Hermanto, C.; Chao, C.P.; Fabregar, E.; Sinohin, V.G.; Masdek, N.; Thangavelu, R.; et al. The distribution and host range of the banana Fusarium wilt fungus, Fusarium oxysporum F. Sp. Cubense, in Asia. PLoS ONE 2017, 12, e0181630. [CrossRef]

8. Thangavelu, R.; Mostert, D.; Gopi, M.; Devi, P.G.; Padmanaban, B.; Molina, A.B.; Viljoen, A. Cavendish banana in India First detection of Fusarium oxysporum f. sp. cubense tropical race 4 (TR4) on Cavendish banana in India. Eur. J. Plant Pathol. 2019, 154, 777–786. [CrossRef]

9. Ordoñez, N.; García-Bastidas, F.; Laghari, H.B.; Akkary, M.Y.; Harfouche, E.N.; al Awar, B.N.; Kema, G.H.J. First Report of Fusarium oxysporum f. sp. cubense Tropical Race 4 Causing Panama Disease in Cavendish Bananas in Pakistan and Lebanon. Plant Dis. 2016, 100, 209. [CrossRef]

10. Maryni, N.; Lombard, L.; Poerba, Y.S.; Subandiyah, S.; Crous, P.W.; Kema, G.H.J. Phylogeny and genetic diversity of the banana Fusarium wilt pathogen Fusarium oxysporum f. sp. cubense in the Indonesian centre of origin. Stud. Mycol. 2019, 92, 155–194. [CrossRef] [PubMed]

11. Dita, M.A.; Waalwijk, C.; Buddenhagen, I.W.; Souza Jr, M.T.; Kema, G.H.J. A molecular diagnostic for tropical race 4 of the banana fusarium wilt pathogen. Plant Pathol. 2010, 59, 348–357. [CrossRef]

12. Moore, N.; Hargreaves, P.; Pegg, K.; Irwin, J. Characterisation of Strains of Fusarium oxysporum f. sp. cubense by Production of Volatiles. Aust. J. Bot. 1991, 39, 161. [CrossRef]

13. Ploetz, R.C. Panama disease: Return of the first banana menace. Int. J. Pest Manag. 1994, 40, 326–336. [CrossRef]

14. Bentley, S.; Pegg, K.G.; Dale, J.L. Genetic variation among a world-wide collection of isolates of Fusarium oxysporum f. sp. cubense analysed by RAPD-PCR fingerprinting. Mycol. Res. 1995, 99, 1378–1384. [CrossRef]

15. Koenig, R.L.; Ploetz, R.C.; Kistler, H.C. Fusarium oxysporum f. sp. cubense strain consists of a small number of divergent and globally distributed clonal lineages. Phytopathology 1997, 87, 915–923. [CrossRef]

16. Groenewald, S.; Van Den Berg, N.; Marasas, W.F.O.; Viljoen, A. The application of high-throughput AFLP’s in assessing genetic diversity in Fusarium oxysporum f. sp. cubense. Mycol. Res. 2006, 110, 297–305. [CrossRef] [PubMed]

17. Carvalhais, L.C.; Henderson, J.; Rincon-Florez, V.A.; O’Dwyer, C.; Czisloowski, E.; Aitken, E.A.B.; Drenth, A. Molecular Diagnostics of Banana Fusarium Wilt Targeting Secreted-in-Xylem Genes. Front. Plant Sci. 2019, 10, 547. [CrossRef]

18. O’Donnell, K.; Kistler, H.C.; Cigelnic, E.; Ploetz, R.C. Multiple evolutionary origins of the fungus causing panama disease of banana: Concordant evidence from nuclear and mitochondrial gene genealogies. Proc. Natl. Acad. Sci. USA 1998, 95, 2044–2049. [CrossRef] [PubMed]

19. Guo, L.; Han, L.; Yang, L.; Zeng, H.; Fan, D.; Zhu, Y.; Feng, Y.; Wang, G.; Peng, C.; Jiang, X.; et al. Genome and Transcriptome Analysis of the Fungal Pathogen Fusarium oxysporum f. sp. cubense Causing Banana Vascular Wilt Disease. Fungal Genet. Biol. 2019, 128, 1–10. [CrossRef] [PubMed]

20. Qin, S.; Ji, C.; Li, Y.; Wang, Z. Comparative transcriptomic analysis of race 1 and race 4 of Fusarium oxysporum f. sp. cubense induced with different carbon sources. G3 Genes Genomes Genet. 2017, 7, 2125–2138. [CrossRef]

21. Yun, Y.; Song, A.; Bao, J.D.; Chen, S.; Lu, S.; Cheng, C.; Zheng, W.; Wang, Z.; Zhang, L. Genome data of Fusarium oxysporum f. sp. cubense race 1 and tropical race 4 isolates using long-read sequencing. Mol. Plant-Microbe Interact. 2019, 32, 1270–1272. [CrossRef] [PubMed]

22. Poon, N.K.; Tee, C.H.; Othman, R.Y. Differential gene expression analysis of Secreted in Xylem (SIX) genes from Fusarium oxysporum f. sp. cubense tropical race 4 in Musa acuminate cv. Berangan and potential application for early detection of infection. J. Gen. Plant Pathol. 2020, 86, 13–23. [CrossRef]

23. Asai, S.; Ayukawa, Y.; Gan, P.; Masuda, S.; Komatsu, K.; Shirasu, K.; Arie, T. High-Quality Draft Genome Sequence of Fusarium oxysporum f. sp. cubense Strain 160527, a Causal Agent of Panama Disease. Microbiol. Resour. Announc. 2019, 8, e00654-19. [CrossRef] [PubMed]

24. Czisloowski, E.; Zeil-Rofe, I.; Aitken, E.A.B. Effector Profiles of Endophytic Fusarium Associated with Asymptomatic Banana (Musa sp.) Hosts. Int. J. Mol. Sci. 2021, 22, 2508. [CrossRef] [PubMed]

25. Warwington, R.J.; Kay, W.; Jeffries, A.; O’Neill, P.; Farbos, A.; Moore, K.; Bebber, D.P.; Studholme, D.J. High-Quality Draft Genome Sequence of the Causal Agent of the Current Panama Disease Epidemic. Microbiol. Resour. Announc. 2019, 8, e00904-19. [CrossRef] [PubMed]

26. Urbaniak, C.; Massa, G.; Hummerick, M.; Khodadad, C.; Schuerger, A.; Venkateswaran, K. Draft genome sequences of two Fusarium oxysporum isolates cultured from infected Zinnia hybrida plants grown on the International Space Station. Genome Announc. 2018, 6, e00326-18. [CrossRef]
53. Yoshida, K.; Saunders, D.G.O.; Mitsuoka, C.; Natsume, S.; Kosugi, S.; Saitoh, H.; Inoue, Y.; Chuma, I.; Tosa, Y.; Cano, L.M.; et al. Host specialization of the blast fungus Magnaporthe oryzae is associated with dynamic gain and loss of genes linked to transposable elements. *BMC Genom.* 2016, 17, 370. [CrossRef]

54. Grandauber, J.; Balessdent, M.-H.; Rouxel, T. Evolutionary and Adaptive Role of Transposable Elements in Fungal Genomes. In *Advances in Botanical Research*; Elsevier: Oxford, UK, 2014; Volume 70, pp. 79–107.

55. Liu, X.; Xing, M.; Kong, C.; Fang, Z.; Yang, L.; Zhang, Y.; Wang, Y.; Ling, J.; Yang, Y.; Lv, H. Genetic diversity, virulence, race profiling, and comparative genomic analysis of the fusarium oxysporum f. sp. Conglutinans Strains infecting cabbages in China. *Front. Microbiol.* 2019, 10, 1373. [CrossRef]

56. Sharma, M.; Sengupta, A.; Ghosh, R.; Agarwal, G.; Tarafdar, A.; Nagavardhini, A.; Pande, S.; Varshney, R.K. Genome wide transcriptome profiling of Fusarium oxysporum f sp. ciceris conidial germination reveals new insights into infection-related genes. *Sci. Rep.* 2016, 6, 37353. [CrossRef] [PubMed]

57. Dassa, E.; Bouige, P. The ABC of ABCs: A phylogenetic and functional classification of ABC systems in living organisms. *Res. Microbiol.* 2001, 152, 211–229. [CrossRef]

58. Becher, R.; Weihmann, F.; Deising, H.B.; Wirsel, S.G. Development of a novel multiplex DNA microarray for Fusarium graminearum and analysis of azole fungicide responses. *BMC Genom.* 2011, 12, 52. [CrossRef] [PubMed]

59. Glazebrook, J. Contrasting Mechanisms of Defense Against Biotrophic and Necrotrophic Pathogens. *Annu. Rev. Phytopathol.* 2005, 43, 205–227. [CrossRef] [PubMed]

60. Hansen, L.H.; Johannesen, E.; Burmølle, M.; Sørensen, A.H.; Sørensen, S.J. Plasmid-encoded multidrug efflux pump conferring resistance to olaquindox in Escherichia coli. *Antimicrob. Agents Chemother.* 2004, 48, 3332–3337. [CrossRef] [PubMed]

61. Sørensen, A.H.; Hansen, L.H.; Johannesen, E.; Sørensen, S.J. Conjugative plasmid conferring resistance to olaquindox. *Antimicrob. Agents Chemother.* 2003, 47, 798–799. [CrossRef]

62. Sørensen, A.H.; Hansen, L.H.; Johannesen, E.; Sørensen, S.J. Conjugative plasmid conferring resistance to olaquindox. *Antimicrob. Agents Chemother.* 2003, 47, 798–799. [CrossRef]

63. Dani, P.; Ujaoney, A.K.; Apte, S.K.; Basu, B. Regulation of potassium dependent ATPase (kdp) operon of Deinococcus radiodurans. *PLoS ONE* 2017, 12, e0188998. [CrossRef] [PubMed]

64. Bialek-Davenet, S.; Lavigne, J.P.; Guyot, K.; Mayer, N.; Tournebize, R.; Brisse, S.; Leflon-Guibout, V.; Nicolas-Chanoine, M.H. Membrane efflux and influx modulate both multidrug resistance and virulence of Klebsiella pneumoniae in a Caenorhabditis elegans model. *Antimicrob. Agents Chemother.* 2010, 54, 4373–4378. [CrossRef]

65. Bialek, S.; Lavigne, J.; Chevalier, J. Membrane efflux and influx modulate both multidrug resistance and virulence of Klebsiella pneumoniae in a Caenorhabditis elegans model. *Antimicrob. Agents Chemother.* 2010, 54, 4373–4378. [CrossRef]

66. Peirs, P.; Leefvère, P.; Boarbí, S.; Wang, X.-M.; Denis, O.; Braibant, M.; Pethe, K.; Locht, C.; Huygen, K.; Content, J. Mycobacterium tuberculosis with Disruption in Genes Encoding the Phosphate Binding Proteins PstS1 and PstS2 Is Deficient in Phosphate Uptake and Demonstrates Reduced In Vivo Virulence. *Infect. Immun.* 2005, 73, 1898–1902. [CrossRef]

67. Soni, D.K.; Dubey, S.K.; Bhatnagar, R. ATP-binding cassette (ABC) import systems of Mycobacterium tuberculosis: Target for drug and vaccine development. *Emerg. Microbes Infect.* 2020, 9, 207–220. [CrossRef]

68. Nguyen, M.-P.; Park, J.; Cho, M.-H.; Lee, S.-W. Role of DetR in defence is critical for virulence of X anthomonas oryzae pv. oryzae. *Mol. Plant. Pathol.* 2016, 17, 601–613. [CrossRef] [PubMed]

69. Widinugraheni, S.; Niño-Sánchez, J.; Van Der Does, H.C.; Van Dam, P.; García-Bastidas, F.A.; Subandiyah, S.; Meijer, H.J.G.; Kistler, H.C.; Kema, G.H.J.; Rep, M. A SIX1 homolog in Fusarium oxysporum f.sp. cubense tropical race 4 contributes to virulence towards Cavendish banana. *PLoS ONE* 2016, 11, e0152273. [CrossRef]

70. Li, E.; Wang, G.; Xiao, J.; Ling, J.; Yang, Y.; Xie, B. A SIX1 homolog in Fusarium oxysporum f. sp. conglutinans is required for full virulence on cabbage. *PLoS ONE* 2016, 11, e0152273. [CrossRef]

71. Gawehns, F.; Houterman, P.M.; Ichou, F.A.; Michielse, C.B.; Hijdra, M.; Cornelissen, B.J.C.; Rep, M.; Takken, F.L.W. The fusarium oxysporum effector six6 contributes to virulence and suppresses i-2-mediated cell death. *Mol. Plant Microbe Interact.* 2014, 27, 336–348. [CrossRef] [PubMed]

72. Fraser-Smith, S.; Czislofiski, E.; Meldrum, R.; Zander, M.; O’Neill, W.; Balali, G.; Aitken, E. Sequence variation in the putative effector gene SIX8 facilitates molecular differentiation of Fusarium oxysporum f. sp. cubense. *Plant. Pathol.* 2014, 63, 1044–1052. [CrossRef] [PubMed]