Genome Resource for *Elsinoë batatas*, the Causal Agent of Stem and Foliage Scab Disease of Sweet Potato

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Abstract

Elsinoë batatas is a phytopathogenic fungus causing stem and foliage scab disease of sweet potato. At present, there is no reference genome available for *E. batatas*, limiting basic research for the pathogen. The present study applied the Nanopore single-molecule sequencing technology to sequence the *E. batatas* genome. This study reports the first high-quality genome sequence of *E. batatas*, with a total contig size of 26.49 Mb, 50.8% GC content, and an N₅₀ of 2,546,814 bp. The sequences obtained serve as a reference for analysis of *E. batatas* isolates and provide a resource to better understand the biology of stem and foliage scab disease of sweet potato.

Genome Announcement

The stem and foliage scab disease caused by *Elsinoë batatas* is one of the most prevalent and destructive diseases of sweet potato (Clark et al. 2013). The disease was first reported in Taiwan, China and currently is widely distributed throughout Southeast Asia, Japan, Brazil, and the Pacific Islands (Ames et al. 1997; Goto 1937; Jenkins and Viegas 1943). Infected leaves are often severely distorted and smaller. Lesions also occur on petioles and stems, causing distortion and twisting (Jenkins and Viegas 1943). The growing points of the vines may become necrotic. Yield losses due to stem and foliage scab can be over 50% (Ramsey et al. 1988). Of note, the disease is most severe in regions where rain, mist, and dews are prevalent. Most provinces in southern China record high rainfall levels in summer, which often exacerbate the *E. batatas* infection (Fang et al. 2004).

In 2019, *E. batatas* CRI-CJ2 was isolated from infected sweet potato stems in Guangzhou City, Guangdong Province, China. Subsequently, the pathogen was confirmed to be *E. batatas* using internal transcribed spacer and translation elongation factor 1- α gene sequencing analyses. Only a few genomic resources are available for *Elsinoë* spp. (Li et al. 2020; Shanmugam et al. 2020). In this study, the *E. batatas* genome was sequenced by Nanopore single-molecule technology based on long-read sequencing.

Mycelial tissue of *E. batatas* was grown on potato dextrose agar at 25°C for 14 days. Subsequently, mycelial tissue along with media was excised from plates and blended with potato dextrose broth (Difco) at 50% concentration. The mycelial tissue was grown with constant shaking (190 rpm) at 25°C for 5 days. Genomic DNA was extracted using a Qiagen genomic tip-100/G DNA isolation kit (Qiagen Inc., Hilden, Germany) and used for Nanopore and Illumina sequencing. The sequencing protocol was according to the instruction program of the PromethION DNA sequencer (Oxford Nanopore, Oxford, U.K.). DNA quality and concentration were assessed using 0.35% agarose gel electrophoresis and Qubit 3.0 fluorimeter (Invitrogen, Carlsbad, CA, U.S.A.). Large fragments of DNA were recycled by a BluePippin automatic nucleic acid recovery system. DNA libraries were constructed using the DNA-PCR Sequencing Kit

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Keywords

Elsinoë batatas, fungal pathogens, genome, genomics, microbe-genome sequencing, Nanopore single molecule sequencing, sweet potato scab

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Table 1. Genome statistics of *Elsinoë batatas* isolate CRI-CJ2

Feature	<i>E. batatas</i>
Depth of Nanopore sequence	166.42x
Depth of Illumina sequence	168.31x
Assembly size (bp)	26,493,939
Number of contigs (bp)	13
N ₅₀ length(bp)	2,546,814
N ₉₀ length (bp)	1,678,128
Contig maximum (bp)	3,686,497
GC content (%)	51
BUSCO completeness (%) ^a	97
Repeat rate (%)	15
Annotation	
Predicted protein-coding gene	9,521
Annotated nonredundant protein sequences	8,447
Secreted proteins	649
Secreted carbohydrate-active enzyme	506
Pathogen–host interaction proteins	2,594
GenBank accession number	JAESVG000000000

^a Benchmarking universal single-copy orthologs.

(SQK-LSK 109), followed by sequencing by Biomarker Technologies (China). For functional annotation of predicted protein-coding genes, we compared their protein against the gene ontology (GO) (Ashburner et al. 2000), Kyoto Encyclopedia of Gene and Genomes (KEGG) (Kanehisa et al. 2004), eukaryotic orthologous groups (KOG) (Tatusov et al. 2000), GenBank nonredundant (nr) (Deng et al. 2006), and Swiss-Prot (Boeckmann et al. 2003) databases.

In total, 564,044 raw reads (4,760,199,650 bp) were generated, then filtered to remove short reads (length < 2 kb), reads with low-quality bases, and adapter sequences. Finally, 354,637 high-quality reads with 4,409,358,511 bp (4.41 G) of data representing 166-fold (166x) coverage of the genome were obtained and assembled using wtdbg v2.2 (Ruan and Li 2019). The assembled genome was further corrected using Pilon v1.22 (Walker et al. 2014) to obtain a more accurate genome. The assembled genome was approximately 26,493,939 bp (26.49 Mb) long in 13 scaffolds, with an overall GC content of 50.8%, The N₅₀ value was 2,546,814 bp, the N₉₀ was 1,678,128 bp, and the longest scaffold contained 3,686,497 bp (Table 1).

To assess the genome assembly quality, 2 × 350-bp Illumina paired-end sequencing was performed on an Illumina HiSeq2500 platform (Illumina, San Diego, CA, U.S.A.). Genomic DNA sequencing libraries were prepared using an SQK-LSK109 Ligation Sequencing Kit. Illumina reads representing 168-fold coverage of the genome were aligned using the Burrows-Wheeler alignment tool (Li and Durbin 2009). In total, 99.27% of the clean reads were mapped to the *E. batatas* genome assembly, indicating the accuracy of the Nanopore data in genome assembly.

Completeness of assembly was verified by benchmarking universal single-copy orthologs (BUSCO v5.2.2) (Simão et al. 2015), based on 3,786 conserved genes among members of class Dothideomycetes, and revealed 94.8% completeness, with 0.3% duplication and six fragmentations. Based on the constructed repeat sequence database, 3,848,344 bp (3.85 Mb) repeat sequences accounting for 14.53% of the genome assembly were detected by Repeat-Masker v4.0.6 (Tarailo-Graovac and Chen 2009).

In total, 9,521 protein-coding genes were predicted through ab initio-based, homolog-based, and RNA-sequencing-based methods integrated by EVM (version 1.1.1) (Haas et al. 2008) and modified by PASA (version 2.0.2) (Campbell et al. 2006). Among these genes, 4,896 (51%) were classified using the KOG database, 5,717 (60%) were mapped to the Swiss-Prot database, 3,286 (35%) were assigned GO terms, 3,123 (33%) were mapped to the KEGG database, 8,447 (89%) of the predicted genes were successfully annotated using NCBI's nr database by BLAST (version 2.2.31), and 2,594 genes were predicted using the pathogen–host interaction (PHI) database v4.8 (Urban et al. 2017). The putative secreted carbohydrate-active enzymes (CAZymes) were annotated using the HMMER package (Eddy 1998), revealing 506 genes encoding CAZymes in CRI-CJ2. Meanwhile, 858 proteins were predicted to encode secreted proteins using SignalP 4.0 (Petersen et al. 2011).

The draft genome sequence has been deposited at GenBank under BioProject PRJNA645248 and BioSample SAMN17204113. Meanwhile, the Whole-Genome Shotgun project has been deposited at the DNA Data Bank of GenBank under accession number JAESVG000000000. Data

has also been deposited in the Sequence Read Archive (SRA) database under SRA accession SRR13342287 and SRR13342288. The *E. batatas* isolate used in this study is available at the Agricultural Culture Collection of China (ACCC) in Beijing under the accession number ACCC 39729 and at the Crops Research Institute Guangdong Academy of Agricultural Sciences in Guangzhou, China.

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