Genome Sequence Resource of *Phomopsis longicolla* YC2-1, a Fungal Pathogen Causing Phomopsis Stem Blight in Soybean

Xiaolin Zhao,^{1,2,3} Zhichao Zhang,^{1,2,3} Sujiao Zheng,^{1,2,3} Wenwu Ye,^{1,2,3,†}
Xiaobo Zheng,^{1,2,3} and Yuanchao Wang^{1,2,3}

¹ Department of Plant Pathology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China

² Key Laboratory of Plant Immunity, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China

³ Key Laboratory of Integrated Management of Crop Diseases and Pests (Ministry of Education), Nanjing, Jiangsu 210095, China

Abstract

Diaporthe-Phomopsis disease complex causes considerable yield losses in soybean production worldwide. As one of the major pathogens, *Phomopsis longicolla* T. W. Hobbs (syn. *Diaporthe longicolla*) is not only the primary agent of Phomopsis seed decay but is also one of the agents of Phomopsis pod and stem blight and Phomopsis stem canker. We performed both PacBio long-read sequencing and Illumina short-read sequencing and obtained a genome assembly for the strain *P. longicolla* YC2-1, which was isolated from soybean stem with Phomopsis stem blight disease. The 63.1 Mb genome assembly contains 87 scaffolds, with a minimum, maximum, and N₅₀ scaffold length of 20 kb, 4.6 Mb, and 1.5 Mb respectively, and a total of 17,407 protein-coding genes. The high-quality data expand the genomic resource of *P. longicolla* species and will provide a solid foundation for a better understanding of their genetic diversity and pathogenic mechanisms.

Genome Announcement

Soybean is one of the best sources of plant-based protein and an important oilseed and cash crop worldwide. During soybean production, members of the fungal genus *Diaporthe* and its anamorph *Phomopsis* can cause a *Diaporthe-Phomopsis* disease complex including seed decay, pod and stem blight, and stem canker, leading to considerable yield losses both quantitatively and qualitatively (Baird et al. 2001; Li et al. 2010; Zorrilla et al. 1994). Among these fungi, the seed-borne pathogen *Phomopsis longicolla* T. W. Hobbs (syn. *Diaporthe longicolla*) is not only the primary agent of seed decay (Sinclair 1993) but is also one of the agents of pod and stem blight (Zhang et al. 1998) and stem canker (Mena et al. 2020). However, the mechanisms of *P. longicolla* development and pathogenicity in soybean are largely unknown.

The first genome assemblies of *P. longicolla* were published, in 2015, for strains TWH P74 and MSPL10-6, which were isolated from field-grown soybean seeds in Ohio in 1983 and in Mississippi in 2010, respectively. Based on Illumina sequencing, the genome assembly size of TWH P74 was 64.7 Mb, with 986 scaffolds and an N₅₀ scaffold length of 213.1 kb (Li et al. 2015a), and the genome assembly size of MSPL10-6 was 66.7 Mb, with 108

Funding

This work was supported by grants from the National Key R&D Program of China (2018YFD0201000), the China Agriculture Research System (CARS-004-PS14), and the National Natural Science Foundation of China (31972250).

Keywords

Diaporthe, genome sequence, *Phomopsis longicolla*, soybean pathogen

Xiaolin Zhao and Zhichao Zhang contributed equally to this work.

†Corresponding author: W. Ye; yeww@njau.edu.cn

The author(s) declare no conflict of interest.

Accepted for publication 24 February 2021.

Copyright © 2021 The Author(s). This is an open access article distributed under the CC BY-NC-ND 4.0 International license.

scaffolds and an N_{50} scaffold length of 1.04 Mb (Li et al. 2015b). To expand the genomic resource and improve the quality of *P. longicolla* genomic data for a better understanding of the genetic diversity and pathogenic mechanisms, we sequenced the pathogenic strain *P. longicolla* YC2-1, which was isolated from a soybean stem with Phomopsis stem blight symptoms in Henan Province, China in 2019.

Genomic DNA of *P. longicolla* YC2-1 was extracted from a 2-day-old culture. Based on a library with fragment sizes of >10 kb, single molecule real time (SMRT) sequencing was performed on the Pacific Biosciences RSII sequencer (PacBio, Menlo Park, CA, U.S.A.), according to standard protocols (MagBead Standard Seq v2 loading, 1 × 180 min movie), using the P4-C2 chemistry. After an error correction of raw reads using MECAT v2, a total of 8.2 Gb (approximately 130× coverage) of clean PacBio long reads were obtained, with an average length of 6.2 kb, an N_{50} of 10.3 kb, and read number of 1,322,662 (Table 1). A short-read sequencing for the extracted DNA was also performed using an Illumina NovaSeq 6000 system with insert sizes of approximately 300 to 400 bp and a paired-end 150-bp strategy, and, finally, 6.1 Gb (approximately 92× coverage) of clean data with 40.4 million clean reads were generated. *k*-mer-based genome size estimation of the Illumina short reads, using GenomeScope v2.0 and KMC v3.1.1, revealed that the estimated genome size was 57.8 Mb, the proportion of repeat sequences was 6.2%, and the heterozygosity was extremely low (0.08%) (Table 1).

The clean PacBio long reads attained from SMRT sequencing were used for de novo assembly using MECAT v2. The preassembled genome was further corrected for random errors in the long reads by aligning the Illumina short reads using Pilon v1.23. After a correction of 7,588 bp (substitution: 603 bp, deletion: 1,640 bp, and insertion: 5,345 bp) on the pre-assembled genome, the final genome assembly size of *P. longicolla* YC2-1 was 63.1 Mb, consisting of 87 scaffolds, with an N_{50} scaffold length of 1.5 Mb, a maximum length of 4.6 Mb, a minimum length of 20.0 kb, and an average length of 0.7 Mb (Table 1). Compared with the two reported *P. longicolla* genome assemblies (TWH P74 and MSPL10-6), the assembly size was slightly reduced (1.6 to 3.6 Mb), the number of scaffolds was reduced by

Table 1. Genome features of *Phomopsis longicolla* YC2-1

Features	Result
PacBio long-read sequencing	
Total length of clean reads	8.2 Gb (130×)
N_{50} length of clean reads	10.3 kb
Mean length of clean reads	6.3 kb
Illumina short-read sequencing	
Total length of clean reads	6.1 Gb (92×)
<i>k</i> -mer estimated genome size	57.8 Mb
<i>k</i> -mer estimated repetitive content	6.2%
<i>k</i> -mer estimated heterozygosity	0.08%
Genome assembly	
Assembly size	63.1 Mb
Scaffold number	87
Scaffold N_{50} length	1.5 Mb
Scaffold L_{50}	12
Mean scaffold length	0.7 Mb
Maximum scaffold length	4.6 Mb
Minimum scaffold length	20.0 kb
GC content	48.7%
BUSCO assessment (fungi_db10; $n = 758$)	C:750, [S:746, D:4], F:3, M:5
Illumina reads mapping rate	
Overall	96.1%
Properly paired	93.2%
Genome annotation	
Proportion of repeat elements	21.9%
Protein-coding genes	17,407
Ribosomal RNAs	
28s_rRNA	5
8s_rRNA	35
18s_rRNA	5
Transfer RNAs	247

19.4 to 91.2%, and the scaffold N₅₀ length was increased to 1.4- to 7.0-fold. Based on a reference dataset with 758 fungal benchmarking universal single-copy orthologs (BUSCOs), BUSCO v4.14 (Simão et al. 2015) identified 750 (98.9%) complete BUSCOs, among which 746 were single-copy while only four were duplicated. The completeness of genome assembly was also evaluated by mapping Illumina short reads using HISAT2 v2.2.0, which showed that a total of 96.1% reads could be mapped and 93.2% reads were mapped and properly paired (Table 1).

Noncoding ribosome RNAs (rRNAs) prediction was carried out using RNAmmer (28s_rRNA = 5; 8s_rRNA = 35; 18s_rRNA = 5) and transfer RNAs (tRNAs) were identified by tRNAscan-SE ($n = 247$). Repetitive elements identified by RepeatMasker v4.1.1 accounted for 21.9% of the genomic sequences (DNA transposons, 21.1%; retrotransposons, 0.8%). Repeat-masked genome assembly was applied for protein-coding gene prediction using GeneMark-ES v4.35, and 17,407 protein-coding genes were predicted (Table 1). Functional annotation of these genes was conducted using the National Center for Biotechnology Information nonredundant protein (16,279 genes, 93.5%), Kyoto Encyclopedia of Genes and Genomes (14,496 genes, 83.3%), Pfam (11,257 genes, 64.7%), Gene Ontology (8,314 genes, 47.8%), EuKaryotic Orthologous Groups (6,064 genes, 34.8%), Pathogen Host Interactions (4,559 genes, 26.2%), and Carbohydrate-Active EnZYme (2,766 genes, 15.9%) databases. SignalP v4.0 predicted 2,089 proteins containing signal peptide (12.0%), and antiSMASH v4.1.0 identified 1,315 genes (7.6%) in 104 predicted secondary metabolic gene clusters (Table 1).

Data Availability

The genome sequencing raw data, assembly, and annotated genes reported in this paper have been deposited in the Genome Sequence Archive database (accession number sub-CRA004856) and the Genome Warehouse database (accession number GWHASJP00000000) in the National Genomics Data Center of China.

Author-Recommended Internet Resources

Kyoto Encyclopedia of Genes and Genomes database: <https://www.genome.jp/kegg>
SignalP: <http://www.cbs.dtu.dk/services/SignalP>
antiSMASH: <https://antismash.secondarymetabolites.org>
Carbohydrate-Active EnZYme database: <http://www.cazy.org>
Pathogen Host Interactions database: <http://www.phi-base.org>

Literature Cited

- Baird, R. E., Abney, T. S., and Mullinix, B. G. 2001. Fungi associated with pods and seeds during the R6 and R8 stages of four soybean cultivars in southwestern Indiana. *Phytoprotection* 82:1-11.
- Li, S., Darwish, O., Alkharouf, N., Matthews, B., Ji, P., Domier, L. L., Zhang, N., and Bluhm, B. H. 2015b. Draft genome sequence of *Phomopsis longicolla* isolate MSPL 10-6. *Genom. Data* 3:55-56.
- Li, S., Hartman, G. L., and Boykin, D. L. 2010. Aggressiveness of *Phomopsis longicolla* and other *Phomopsis* spp. on soybean. *Plant Dis.* 94: 1035-1040.
- Li, S., Song, Q., Ji, P., and Cregan, P. 2015a. Draft genome sequence of *Phomopsis longicolla* type strain TWH P74, a fungus causing Phomopsis seed decay in soybean. *Genome Announc.* 3:e00010-15.
- Mena, E., Stewart, S., Montesano, M., and Ponce de León, I. 2020. Soybean stem canker caused by *Diaporthe caulivora*; pathogen diversity, colonization process, and plant defense activation. *Front. Plant Sci.* 10:1733.
- Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., and Zdobnov, E. M. 2015. BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics* 31:3210-3212.
- Sinclair, J. B. 1993. Phomopsis seed decay of soybeans—A prototype for studying seed disease. *Plant Dis.* 77:329-334.
- Zhang, A. W., Riccioni, L., Pedersen, W. L., Kolipara, K. P., and Hartman, G. L. 1998. Molecular identification and phylogenetic grouping of *Diaporthe phaseolorum* and *Phomopsis longicolla* isolates from soybean. *Phytopathology* 88:1306-1314.
- Zorrilla, G., Knapp, A. D., and McGee, D. C. 1994. Severity of Phomopsis seed decay, seed quality evaluation, and field performance of soybean. *Crop Sci.* 34:172-177.