A Genome Sequence Resource of *Nigrospora sphaerica* Causing Fruit Dried-Shrink Disease in *Akebia trifoliata*

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Abstract

Nigrospora sphaerica is a worldwide plant pathogen causing fruit or leaf diseases on a variety of plant hosts such as *Citrullus lanatus*, *Vigna unguiculata*, *Hylocereus polyrhizus*, and *Akebia trifoliata* and other potential hosts. Here we report the first genome resource with high-quality assembly of the *N. sphaerica* strain ZJJ-C1, which causes fruit dried-shrink disease in *A. trifoliata* in China. The genome sequence of ZJJ-C1 will be useful for studying the evolution, host adaptation, and pathogenicity of *N. sphaerica*, which will be beneficial for a better understanding of the mechanisms of host-pathogen interaction during the endophytic period.

Genome Announcement

The genus *Nigrospora* includes several plant pathogens causing diseases on a variety of plant hosts. *Nigrospora sphaerica* is a damaging pathogen that causes leaf spots on *Citrullus lanatus* (Ismail and Abd Razak 2021), *Vigna unguiculata* (Deepika et al. 2021), *Arachis hypogaea* (Liu et al. 2020), and *Parthenium hysterophorus* (Zafri et al. 2021); it also causes fruit dried-shrink disease on *Hylocereus polyrhizus* (Kee et al. 2019) and *Akebia trifoliata* (Hong et al. 2021). In addition, *N. aurantiaca* and *N. oryzae* are the causal agents of leaf spot or wilt disease on *Hibiscus mutabilis* (Han et al. 2021), *Nicotiana tabacum* (Huang et al. 2021), and a number of other plant species.

N. sphaerica ZJJ-C1 was originally isolated from fruits of *Akebia trifoliata* at Zhangjiajie City, Hunan Province, China, and identified as a pathogen causing fruit dried-shrink disease on fruits (Hong et al. 2021). In the current study, this isolate was repurified employing single-spore isolation approach. This isolate was grown in sterile potato dextrose agar (PDA) at 25°C for 7 days before mycelia were harvested and ground with liquid nitrogen. Highly purified genomic DNA was extracted using a Cell Culture DNA Midi Kit (cat. no. 13343, Qiagen, Valencia, CA, U.S.A.) according to the manufacturer's protocol. DNA quality and concentration were assessed using 0.5% agarose gel electrophoresis and via Qubit fluorometer and Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Carlsbad, CA, U.S.A.). The A260/A280 ratio of 1.86 in the sample genomic DNA, and concentration of 163.0 ng/μl were recorded. Whole-genome sequencing data were obtained using the Oxford Nanopore Technology (ONT) PromethION platform (Oxford Nanopore, Oxford, UK) and short-read sequenced on a DNA Nano Ball Sequencing (DNBseq) platform.

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Keywords

Nigrospora sphaerica, genome sequence resource, fruit dried-shrink disease, *Akebia trifoliata*

Table 1. Genome statistics of *Nigrospora sphaerica* isolate ZJJ-C1

Features	Strain ZJJ_C1
Genome size (Mb)	51.75
BUSCO completeness (%)	99.3
Number of contigs	33
N50 (kb)	4.64
N90 (kb)	1.62
GC content (%)	56.09
Number of genes	11,518
Protein-coding genes	10,106
Repeat rate (%)	16.87%
GenBank accession no.	JAGVVL000000000

All the raw data of about 43.39 Gb were trimmed by SOAPnuke (version 1.5.6) (Chen et al. 2017) (<https://github.com/BGI-flexlab/SOAPnuke>). High-quality reads were de novo assembled using CANU (version 2.1) (Koren et al. 2017), Samtools (Li and Durbin 2009), GATK (version 3.5) (McKenna et al. 2010), and Racon (Vaser et al. 2017) software. The nuclear genome of *N. sphaerica* consists of 33 contigs with a total assembly length of 51,752,671 bp (N50 = 4.64 Mbp), 56.09% GC content. Genome completeness was assessed through the presence of conserved single-copy fungal genes using Benchmarking Universal Single-Copy Orthologs (BUSCO) (version 3.0) (Simão et al. 2015), and its BUSCO completeness was 99.3% (753/758). The completeness of the assembly about gene set was verified with BUSCO (version 3.0) (Simão et al. 2015) software, which is based on a set of 746 common fungal genes. About 98.4% (746/758) of the BUSCOs in full length genes were found in the assembly. Assembled contigs with lengths less than 300 bp were discarded in the following analysis.

We constructed a de novo repeat library using Piler (Edgar and Myers 2005) (<http://www.drive5.com/piler/>), RepeatScout (<https://bix.ucsd.edu/repeatscout/>), and RepeatModeler (<http://www.repeatmasker.org/RepeatModeler/>) software. The library was searched as the reference database, and repeats were predicted using RepeatMasker (version 4.1.0). A total of 8,728,703 bp of repeat sequences that accounted for 16.87% of the assembly, including Retrotransposon, DNA transposon, Tandem Repeat sequence, and other transposon, were detected in the genome assembly. A summary of the genome assemblies and gene prediction is shown in Table 1.

To obtain functional information, the protein sequences were aligned against the NCBI nonredundant protein sequences (Nr) (<https://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/>) database, Swiss-Prot (<https://www.uniprot.org/uniprot/?query=reviewed:yes>) database, Gene Ontology (GO) (<http://geneontology.org/>) database, the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (<https://www.genome.jp/kegg/>), Clusters of Orthologous Groups (COG) database (<https://www.ncbi.nlm.nih.gov/research/cog-project/>), Evolutionary Genealogy of Genes: Nonsupervised Orthologous Groups (eggNOG) (version 5.0.0) (<http://egglog5.embl.de/>) database, and Protein family (Pfam) (<http://pfam.xfam.org/>) database by DIAMOND (Buchfink et al. 2015) with an e-value cutoff of $1e^{-5}$. The result of the functional annotation indicated that 10,096 (87.65%), 7,220 (62.68%), 3,107 (26.98%), 4,001 (34.74%), 8,313 (72.17%), 8,313 (72.17%), and 7,534 (65.41%) of the genes were annotated in the database of Nr, Swiss-Prot, GO, KEGG, COG, eggNOG, and Pfam, respectively. There are 10,106 genes with functional annotations, accounting for 87.74% of total predicted genes. Of the protein-coding gene models, 65.41% (7,535) contained PFAM domains annotated by InterProScan 5.2-45.0 (threshold e-value of $1e^{-5}$) (Ohm et al. 2012). Meanwhile 1,118 proteins were predicted with signal peptide by using SignalP v4.1 (Petersen et al. 2011). Among the 1,118 proteins, 908 were computationally predicted to localize in the extracellular space as secreted proteins based on results from WoLF PSORT v0.2 (Horton et al. 2007). In addition, genes coding for the putative chitinase and hydrolase, the main proteases of fungal involving host invasion, such as *NSph01G001130*, *NSph01G023940*, *NSph09G001450*, *NSph17G006420*, and *NSph01G001130*, have been validated as genes derived from *N. sphaerica* genome by PCR amplification and Sanger-sequencing.

In summary, this study reports a high-quality genome sequence resource of *N. sphaerica* ZJJ-C1, a strain causing fruit dried-shrink disease of *Akebia trifoliata* in China, which will allow further analysis of species diversity and evolutionary mechanisms and may serve as a

foundation for understanding the interacting mechanisms between plants and fungal pathogens. The assembled whole genome of ZJJ-C1 has been deposited at GenBank under the accession number of JAGVVL000000000 (https://www.ncbi.nlm.nih.gov/nuccore/JAGVVL000000000.1/).

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