Whole Genome Sequences of the Tea Leaf Spot Pathogen *Didymella segeticola*

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Abstract

The fungal pathogen *Didymella segeticola* (basionym *Phoma segeticola*) causes leaf spot on tea (*Camellia sinensis*), which leads to a loss in tea leaf production in Guizhou Province, China. *D. segeticola* isolate GZSQ-4 was sequenced using Illumina HiSeq and Pacific Biosciences (PacBio) RS technologies, and then assembled to approximately 33.4 Mbp with a scaffold N50 value of approximately 2.3 Mbp. In total, 10,893 genes were predicted using the Nonredundant, Gene Ontology, Clusters of Orthologous Groups, Kyoto Encyclopedia of Genes and Genomes, and SWISS-PROT databases. The whole-genome sequence of *D. segeticola* will provide a resource for future research on host–pathogen interactions, determination of trait-specific genes, pathogen evolution, and plant–host adaptation mechanisms.

Genome Announcement

Phoma spp. are significant phytopathogens and can cause diseases of several plant organs, including root, stem, and leaf, in a range of plant species. For instance, *Phoma sorghina* causes leaf spot of maize and wheat (Amaral et al. 2004; Perelló and Moreno 2005), *Phoma* sp. QC-2014b (LC 1633) causes leaf spot of the Tibetan thistle (*Cirsium segetum*) (Chen et al. 2015), and *P. bellidis* causes stem blight of *Eleocharis dulcis* and leaf spot of *Angelica dahurica* (Lv et al. 2011; Xu et al. 2016). According to the morphological characters of pycnidia, conidia, colonies, et cetera, *Phoma* spp. is known as a form genus. *Phoma* spp. comprises nine sections, which contain 223 species (Boerema et al. 2004). Nevertheless, Chen et al. (2017) classified 360 strains of the family *Didymellaceae* into 19 monophyletic generic clades by multilocus phylogenetic tree, and 9 sections in *Phoma* spp. were clustered into different monophyletic generic clades. *Phoma segeticola* was first reported by Chen et al. (2015) and can cause leaf spot on Tibetan thistle. *P. segeticola* was further clustered into monophyletic generic clades of *Didymella*. So, *P. segeticola* was renamed *Didymella segeticola* (Chen et al. 2017). Tea plants (*Camellia sinensis*) are widely grown in Guizhou Province, which has the greatest tea cultivation area (35 × 10⁴ ha) among all the provinces or cities in China (Li et al. 2019; Zhao et al. 2018). Tea leaf spot caused by *D. segeticola* widely occurs in the tea plantations of Guizhou Province, which lead to a huge loss of tea leaves (Li et al. 2019; Zhao et al. 2018). Presently, 14 genomes in the family *Didymellaceae* at NCBI are available for use in studying host–pathogen interactions, important traits, and pathogen evolution and plant–host, except the genome of *D. segeticola* being reported in our research group.

Fourteen isolates of *D. segeticola* var. *camelliae* were collected from Shiqian County in Guizhou Province from May 2016 to November 2017 (Zhao et al. 2018). These morphological

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Table 1. Genome assembly statistics of *Didymella segeticola* isolate GZSQ-4

Statistics	Isolate GZSQ-4
Assembly	
Number of all scaffolds	23
Bases in all scaffolds	33,448,292
Number of large scaffolds (>1,000 bp)	23
Bases in large scaffolds	33,448,292
Largest length	3,976,106
Scaffold N50	2,254,797
Scaffold N90	998,506
G+C content (%)	51.85
N rate (%)	
Gene prediction	0
Gene number	10,893
Gene total length (bp)	17,069,577
Gene average length (bp)	1,566
Gene density (kb)	0.325
GC content in gene region (%)	55.40
Gene/genome (%)	51.0

characteristics were similar with *D. segeticola* described by Chen et al. (2017). In the multilocus phylogenetic analysis, the strain GZSQ-4 was identical to reference strains *D. segeticola* CGMCC 3.17489, and the clade was supported by 99.89% bootstrap values. Based on Koch's postulate, these isolates proved to be the causal agents of tea leaf spot (Zhao et al. 2018). Genomic DNA was extracted from *D. segeticola* isolate GZSQ-4 using fungal miniprep kits (E.Z.N.A.; Omega Bio-Tek, Inc.). A DNA library with fragment sizes of approximately 300 to 500 bp was prepared using a TruSeq DNA Sample Prep Kit. Bridge-PCR, on the Illumina HiSeq platform, was conducted using a TruSeq PE Cluster Kit and the products were then sequenced using a TruSeq SBS Kit with the Illumina HiSeq PE technology, generating 53,530,422 raw reads and 8,029,563,300 raw bp. The Illumina reads were trimmed, corrected, and filtered. A total of 51,270,673 clean reads and 7,598,831,045 clean bp passed the quality control criteria and were used in subsequent analyses of *D. segeticola* isolate GZSQ-4. There was ~227× average coverage. Meanwhile, a Pacific Biosciences (PacBio) RS library, having fragment sizes of approximately 8,000 to 10,000 bp, was prepared using G-tubes and sequenced using single-molecule real-time cells, generating PacBio reads. PacBio reads were further filtered, generating PacBio subreads, with 1,489,032 total reads, and 11,821,890,861 total bp. The largest length was 63,621 bp, and the N50, N90, and average lengths were 10,453, 5,327, and 7,939 bp, respectively. The genome size was ~36.7 Mbp based on read sequence data being processed with the condition of 21-mers using the K-mer statistical method.

The raw sequence data were assembled using SOAPdenovo v2.04 (<http://www.genomics.cn/>) and then treated using GapCloser v1.12. The whole-genome assembly was ~33.4 Mbp, with a scaffold N50 value of ~2.3 Mbp (Table 1). The gene prediction was annotated using Non-redundant (Nr), Gene Ontology (GO), Clusters of Orthologous Genes (COG), Kyoto Encyclopedia of Genes and Genomes (KEGG), and SWISS-PROT databases using BLAST 2.2.28+, resulting in 10,893 annotated genes. For instance, the COG analysis was conducted using the eggNOG database v4.5.1 (<http://eggnogdb.embl.de/#/app/home>), and a KEGG enrichment analysis was also conducted (<https://www.genome.jp/kegg/>). The GO database was used to annotate genes at the cellular component, molecular function, and biological process levels (<http://geneontology.org/>). *D. segeticola* showed a high abundance of GO terms for catalytic activity (890), binding (628), transporter activity (113), and structural molecule activity (18).

The genome sequences of *D. segeticola* presented here provide a unique resource for studying pathogenicity determinants in this pathogen, understanding mechanisms of host adaptation, and designing specific disease control management strategies. The genome sequence has been deposited in GenBank under genome accession SDAV00000000 (BioProject PRJNA516041; BioSample SAMN10768991; and SRA accession SRR8492863).

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