Mitochondrial Genome Resource of *Phomopsis longicolla*, a Fungus Causing Phomopsis Seed Decay in Soybean

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Abstract

Phomopsis seed decay is one of the most devastating seed diseases reducing soybean seed quality worldwide. This disease is caused primarily by a seed-borne fungus, *Phomopsis longicolla* (syn. *Diaporthe longicolla*). As part of a genome sequencing project for *P. longicolla*, we present the mitochondrial genome resource of the isolate MSPL 10-6, one of the most aggressive field isolates. The circular mitochondrial genome is 53,646 bp long with GC content of 34.27%, and it encodes 14 common protein genes, 23 tRNA and two rRNA genes, and 10 introns. Forty-five SNPs and InDels also were identified during comparative analyses with another isolate. The mitochondrial genome sequence provides a useful resource for developing molecular markers for pathogen detection and for improvement of control strategies for the disease.

Keywords

mitochondrial genome, *Phomopsis longicolla*, Phomopsis seed decay, soybean pathogen

Resource Announcement

Phomopsis longicolla T. W. Hobbs (syn. *Diaporthe longicolla*) is a seed-borne fungal pathogen and the primary cause of Phomopsis seed decay (PSD) in soybean, *Glycine max* (L.) Merrill (Hepperly and Sinclair 1978; Hobbs et al. 1985; Li 2011; Li et al. 2010, 2015a). PSD severely reduces seed viability, alters seed composition, and increases frequencies of moldy and/or split beans, resulting in poor soybean seed quality worldwide.

Mitochondria play essential roles in eukaryotic cells for energy production, respiratory metabolism, and many other indispensable cellular processes. The characteristics of small size, high copy number, and high mutation rate of the fungal mitochondrial genome make it a suitable candidate for species identification and marker development (Avisé et al. 1987; Deng et al. 2016). However, the mitochondrial genome of *P. longicolla* has not been well-studied. In this article we report the mitochondrial genome resource of *P. longicolla*.

P. longicolla isolate MSPL 10-6 was used in this study. We isolated this pathogen from field-grown soybean seed in Stoneville, Mississippi, U.S.A., in 2010 using the standard seed-plating procedure as previously reported (Li et al. 2011, 2015b, 2017a). It was first identified using morphological characteristics according to Hobbs et al. (1985). The identification was further confirmed by analysis of the internal transcribed spacer region of rDNA amplified by polymerase chain reaction with primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (Zhang et al. 1998). Sequences were analyzed using BLASTn at <https://blast.ncbi.nlm.nih.gov/Blast.cgi>. A cut-seedling inoculation method was used to test the pathogenicity of the pathogen (Li 2018). MSPL 10-6 is one of the most aggressive isolates causing PSD in soybean and has been used in previous studies to identify new sources of resistance to the disease (Li et al. 2011, 2015b, 2017a).

Genomic DNA was extracted using a Qiagen DNeasy Plant Mini kit (Qiagen, Valencia, CA) after growth of *P. longicolla* MSPL 10-6 isolate in potato dextrose broth (Difco Laboratories,

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Table 1. A list of single nucleotide polymorphisms and insertions/deletions identified in the mitochondrial genome of *Phomopsis longicolla* isolate MSPL 10-6

Reference accession ^a	MSPL 10-6 accession	Position (reference)	Nucleotide in reference	Nucleotide in MSPL 10-6	Position (MSPL 10-6)
KP137411.1	MT527962	8,592	G	T	7,122
KP137411.1	MT527962	9,065	.	TGAC	7,596
KP137411.1	MT527962	9,068	.	GTA	7,603
KP137411.1	MT527962	11,965	A	.	10,501
KP137411.1	MT527962	12,082	T	A	10,618
KP137411.1	MT527962	12,083	T	A	10,619
KP137411.1	MT527962	23,327	A	T	21,863
KP137411.1	MT527962	29,569	G	.	28,104
KP137411.1	MT527962	29,719	C	G	28,254
KP137411.1	MT527962	34,225	T	.	32,759
KP137411.1	MT527962	36,054	T	C	34,588
KP137411.1	MT527962	36,242	G	.	34,775
KP137411.1	MT527962	36,953	A	.	35,485
KP137411.1	MT527962	38,144	.	CAG	36,677
KP137411.1	MT527962	38,146	.	ACG	36,682
KP137411.1	MT527962	38,147	T	C	36,685
KP137411.1	MT527962	38,546	GGGGG	.	37,083
KP137411.1	MT527962	38,566	C	T	37,099
KP137411.1	MT527962	40,173	.	CTA	38,707
KP137411.1	MT527962	40,176	.	AGTC	38,713
KP137411.1	MT527962	40,502	T	C	39,042
KP137411.1	MT527962	40,503	T	G	39,043
KP137411.1	MT527962	40,505	.	T	39,046
KP137411.1	MT527962	40,507	C	A	39,048
KP137411.1	MT527962	40,511	.	GTA	39,053
KP137411.1	MT527962	40,533	.	CTA	39,078
KP137411.1	MT527962	40,536	.	AGTC	39,084
KP137411.1	MT527962	41,287	.	TGAC	39,839
KP137411.1	MT527962	41,290	.	GTA	39,846
KP137411.1	MT527962	41,336	.	CTA	39,895
KP137411.1	MT527962	41,339	.	AGTC	39,901
KP137411.1	MT527962	41,772	.	T	40,338
KP137411.1	MT527962	41,849	GGAGGCA	.	40,414
KP137411.1	MT527962	43,125	.	C	41,685
KP137411.1	MT527962	47,985	GGGGG	.	46,544
KP137411.1	MT527962	47,998	A	T	46,553
KP137411.1	MT527962	48,724	C	.	47,278
KP137411.1	MT527962	50,083	GCC	.	48,636
KP137411.1	MT527962	50,088	CGGC	.	48,638
KP137411.1	MT527962	51,537	.	A	50,086
KP137411.1	MT527962	52,297	C	A	52,162
KP137411.1	MT527962	52,300	G	A	52,165
KP137411.1	MT527962	53,083	.	GG	52,949
KP137411.1	MT527962	53,085	T	A	52,952
KP137411.1	MT527962	53,118	.	CCC	52,986

^a Type strain of *P. longicolla* ATCC 60325.

^b Period (.) indicates absent or deletion.

Detroit, MI) for 4 days under 12-h light-and-dark cycles at 24°C. The quality of DNA was assessed by 1% agarose gel electrophoresis, and DNA was quantified using a Nanodrop 1000 and a Qubit version 2.0 fluorometer. Two sequencing libraries were generated. The no-gel mate-pair libraries were made with the Nextera Mate-Pair Sample Preparation kit (Illumina, San Diego, CA), and the paired-end libraries were generated with the TruSeq DNA PCR-Free Sample Preparation kit (Illumina) as described by the manufacturer. Sequencing of the libraries was performed in separate lanes on an Illumina HiSeq 2500 sequencer using a TruSeq SBS sequencing kit (version 3, Illumina) in the Genomics Core Facility, Purdue University, West Lafayette, Indiana, as reported previously (Li et al. 2017b). The mate-pair library generated 72,216,734 reads with a total of 8.2 billion bp and average length of 113 bp, representing 128-

fold coverage. The paired-end library produced 63,763,666 reads with a total of 6.2 billion and average length of 97 bp, representing 97-fold coverage.

The mitochondrial genome of *P. longicolla* was assembled using the program Velvet 1.2.03 (Zerbino and Birney 2008). Protein-coding and rRNA genes as well as introns were annotated automatically using the online program MFannot (<http://megasun.bch.umontreal.ca/cgi-bin/mfannot/mfannotInterface.pl>) with the mold genetic code. The types of intron-encoded open reading frames were identified by searching the National Center for Biotechnology Information (NCBI) conserved domain database (Deng et al. 2018). The dispersed and inverted repeat sequences in the mitochondrial DNA were identified by performing self-BLAST through BLASTn search ($E < 10^{-10}$). Analysis of single nucleotide polymorphisms (SNPs) and insertions/deletions (InDels) was conducted using MUMmer 4.x (Marçais et al. 2018). A SNP detection script was written using the “nucmer”, “delta-filter”, and “show-snp” components of MUMmer.

The circular mitochondrial genome of *P. longicolla* isolate MSPL 10-6 is 53,646 bp long with GC content of 34.27%. It encodes 14 common protein genes, 23 tRNA genes, and two rRNA genes. Ten introns were identified: four introns in *cox1* and one intron in each *cox2*, *nad5*, *atp6*, *rnl*, *nad1*, and *cob*. BLAST analysis against available sequence data at NCBI showed that the mitochondrial genome sequence of *P. longicolla* isolate MSPL 10-6 is 99.81% identical to the *P. longicolla* type strain ATCC 60325 (accession KP137411.1) and 95.88 and 95.75% identical to *Sordaria macrospora* k-hell (accession XM 003342344) and *Diaporthe phaseolorum* (accession AY779295), respectively. By comparing two identical mitochondrial genome sequences of *P. longicolla* (accessions KP137411.1 and NC_027509) available at GenBank, 45 SNPs and InDels were identified (Table 1).

The mitochondrial genome sequence of *P. longicolla* isolate MSPL 10-6 will be useful for further investigation of the genetic basis of seed-borne pathogens causing seed disease in soybean, and it provides a valuable resource for molecular marker development, which will aid in detection of the pathogen, disease monitoring, and improvement of control strategies for PSD.

The mitochondrial genome of *P. longicolla* isolate MSPL 10-6 has been deposited in the NCBI GenBank database under accession number MT527962.

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