



Data in Brief

Draft genome sequence of *Diaporthe aspalathi* isolate MS-SSC91, a fungus causing stem canker in soybeanShuxian Li ^{a,*}, Qijian Song ^b, Alexandre M. Martins ^b, Perry Cregan ^b^a United States Department of Agriculture, Agricultural Research Service (USDA-ARS), Crop Genetics Research Unit, Stoneville, MS 38776, USA^b USDA-ARS, Soybean Genomics and Improvement Lab, Beltsville Agriculture Research Center, Beltsville, MD 20705, USA

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ABSTRACT

Diaporthe aspalathi (Syn. *Diaporthe phaseolorum* var. *meridionalis*) is the causal agent of the southern stem canker (SSC) disease in soybean. This disease can kill plants from the middle to the end of the growing season resulting in severe yield loss. The mechanisms of SSC disease development and pathogen invasion of soybean are not fully understood. The genome sequence of *D. aspalathi* has not been described. In this article, we report the successful assembly of the draft genome sequence of a *D. aspalathi* isolate, designated MS-SSC91, that was isolated from the stem of a field-grown soybean plant in Mississippi, USA in 2006. This study represents the first reported genome sequence of *D. aspalathi* in the *Diaporthe-Phomopsis* complex. The whole genome shotgun sequence of the MS-SSC91 isolate has been deposited at DDBJ/EMBL/GenBank under the accession LJJS00000000 and the sequences could be found at the site http://www.ncbi.nlm.nih.gov/assembly/GCA_001447215.1/. The MS-SSC91 genome sequences will provide information on the genetic basis of fungal infection of the soybean stem. It is valuable for studying soybean-fungal interactions and developing new control strategies for this pathogen.

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Specifications

| | |
|---------------------------|---|
| Organism/cell line/tissue | <i>Diaporthe aspalathi</i> |
| Strain | MS-SSC91 |
| Sequencer or array type | Illumina HiSeq 2000 sequencer |
| Data format | Raw and processed |
| Experimental factors | DNA extracted from a field strain, no treatment |
| Experimental features | Genome sequencing |
| Consent | n/a |
| Sample source location | Soybean field in Stoneville, Mississippi, USA |

1. Direct link to deposited data

Deposited data can be found here: <http://www.ncbi.nlm.nih.gov/nuccore/LJJS00000000>.

2. Materials and methods

2.1. Pathogen isolation, identification, and pathogenicity test

Diaporthe aspalathi (Syn. *D. phaseolorum* var. *meridionalis*) is the causal agent of the southern stem canker (SSC) disease in soybean [1]. This disease can kill plants from the middle to the end of the

growing season resulting in severe yield loss [1]. It is one of the most economically important soybean diseases. Although SSC commonly occurs in the southern United States, *D. aspalathi* has been found in some of the northern states [2,3]. An isolate of *D. aspalathi*, MS-SSC91 was isolated from field-grown soybean stem in Stoneville, Mississippi, USA in 2006 using a modified seed plating procedure [4]. Briefly, stem samples with lesions were collected, cut into ca. 5-mm pieces, surface-disinfested with 0.5% NaOCl solution for 3 min, rinsed three times, and placed on acidified potato dextrose agar (APDA) medium (Difco Laboratories, Detroit, MI) adjusted to pH 4.8 with 25% lactic acid after autoclaving. Stem samples were placed on each Petri dish and incubated at 24 °C for 4–7 days. Colonies of interest were hyphal tipped, and examined under microscope. *D. aspalathi* was identified using morphological characteristics. Isolate of MS-SSC91 isolate were white, lanose, and turned tan with age as the typical *D. aspalathi* previously described [1]. Further identification was confirmed by analysis of the ITS region of rDNA amplified by PCR with primers ITS1, 5'-TCCGTAGGTGAACCTG CCG-3' and ITS4, 5'-TCCTCCGTTATTGATATGC-3' [5].

Pathogenicity tests were performed using a cut-seedling inoculation assay [6]. Soybean seed of a susceptible cultivar, Williams 82 was used in the tests. Mycelial plugs (4-mm in diameter) from the margin of a 10-day old culture on APDA were punched out with the large ends of disposable micropipette tips (200 µl). The micropipette tip containing the fungal mycelium was subsequently placed over a 3-week old cut soybean stem that was cut at just below the first trifoliolate node. Micropipette tips containing plugs of non-infested APDA were served

* Corresponding author.

E-mail address: shuxian.li@ars.usda.gov (S. Li).

as the negative control. Two days after inoculation, micropipette tips were removed. At 7 days after inoculation, the main stem length was measured from the soil line to the top of the plant, and the lesion on the stem was measured. The MS-SSC91 isolate has been used to evaluate soybean for resistance to stem canker as part of the USDA Uniform Soybean Tests (<http://www.ars.usda.gov/SP2UserFiles/Place/60661000/UniformSoybeanTests/2013SoyBook.pdf>).

2.2. DNA extraction, library construction, and sequencing

Genomic DNA of *D. aspalathi* MS-SSC91 isolate was extracted from a 4-day-old culture using a Qiagen DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA) and used to generate paired-end libraries with the TruSeq DNA PCR-Free Sample Preparation kit (Illumina San Diego, CA) according to the manufacturer's protocol. Libraries were sequenced in separate lanes on an Illumina HiSeq 2000 sequencer using a TruSeq SBS sequencing kit (version 3, Illumina) at the Genomics Core Facility, Purdue University, West Lafayette, IN.

2.3. Data analysis and results

A total of 131,083,049 paired-end 101 bp reads were generated. The total amount of sequence was 26,478,775,898 bp. After trimming or removing low quality reads or bases with the Trimmomatic [7] and/or fastx_clipper (http://hannonlab.cshl.edu/fastx_toolkit/) using the threshold of base Phred score greater than 20, a total of 126,051,864 paired-end reads and 4,778,811 un-paired reads were retained. The total remaining sequence was 24,801 Mb (approximately 330× coverage of the genome). The filtered sequence was assembled with ABySS de novo genome assembly software [8] at kmer = 80, and resulted in an assembly with 1873 scaffolds with a minimum size of 2000 bp (N50 = 86,905 bp). The largest scaffold in the assembly was 463,151 bp. The assembly contained 55,035,521 bases and the G + C content was 51%. Analysis of the completeness of the genome based on the analysis of the 248 ultra-conserved core eukaryotic gene datasets (CEGs) (http://korflab.ucdavis.edu/Datasets/genome_completeness/index.html#SCT2) was done with the CEGMA program [9]. The result showed 242 of the 248 proteins present in the genome sequence with two additional partial genes, indicating that the assembly was 97.6% complete.

Gene prediction analysis using the AUGUSTUS software [10] trained with the parameters of the fungal species *Fusarium graminearum* identified 14,962 genes. The average size of a gene was 1729 bp; the largest gene was 23 kb. Approximately 46% (25.8 Mb) of the whole genome sequence was contained in genes. Of the 25.8 Mb sequences within genes, 22.5 Mb were coding sequences. The *D. aspalathi* sequencing and assembly statistics were summarized in Table 1.

Table 1
Diaporthe aspalathi sequencing and assembly statistics.

| Assembly statistics | Scaffolds |
|---------------------------|-----------|
| Total assembly size | 55.0 Mb |
| Total assembled sequences | 1873 |
| Longest sequence length | 463.2 Mb |
| Average sequence length | 29.4 kb |
| N50 index | 185 |
| N50 length | 86.9 kb |
| N90 index | 787 |
| N90 length | 12.0 kb |

The draft genome of MS-SSC91 represents an important soybean fungal pathogen in the *Diaporthe-Phomopsis* complex.

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Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the United States Department of Agriculture. USDA is an equal opportunity provider and employer.

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