Draft Genome Sequence Resource of Switchgrass Rust Pathogen, *Puccinia novopanici* Isolate Ard-01

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Funding

This work was supported by Noble Research Institute, LLC and, in part, by National Science Foundation's Experimental Program to Stimulate Competitive Research (EPS-0814361).

Abstract

Puccinia novopanici is an important biotrophic fungal pathogen that causes rust disease in switchgrass. Lack of genomic resources for *P. novopanici* has hampered the progress toward developing effective disease resistance against this pathogen. Therefore, we have sequenced the whole genome of *P. novopanici* and generated a framework to understand pathogenicity mechanisms and identify effectors, repeat element invasion, genome evolution, and comparative genomics among *Puccinia* spp. in the future. Long- and short-read sequences were generated from *P. novopanici* genomic DNA by PacBio and Illumina technologies, respectively, and assembled a 99.9-Mb genome. Transcripts of *P. novopanici* were predicted from assembled genome using MAKER and were further validated by RNAseq data. The genome sequence information of *P. novopanici* will be a valuable resource for researchers working on monocot rusts and plant disease resistance in general.

Keywords

mycology

Genome Announcement

Switchgrass rust caused by *Puccinia novopanici* is a significant disease of switchgrass (*Panicum virgatum* L.), an important biofuel and forage crop in the United States of America. Severe infestations of switchgrass rust under suitable environmental conditions have been reported in Oklahoma during past several years (Uppalapati et al. 2013). *Puccinia novopanici* was mistakenly reported as *P. emaculata* in previous publications (Demers et al. 2017; Gill et al. 2015, 2018; Uppalapati et al. 2013). However, sequence comparison of our fungal isolates with suggested *P. novopanici* sequences in recent reports (Kenaley et al. 2016; Orquera-Tornakian et al. 2017) confirmed our isolates as *P. novopanici*. *P. novopanici* is an obligate biotrophic fungal pathogen and has a mode of infection similar to other *Puccinia* spp. such as wheat and barley rust pathogens (Gill et al. 2015; Uppalapati et al. 2013). Variations in the degree of virulence in *P. novopanici* (reported as *P. emaculata*) have been previously shown in the United States (Frazier et al. 2013; Hirsch et al. 2010; Kenaley et al. 2016; Li et al. 2009; Uppalapati et al. 2013; Zale et al. 2008). These variations among isolates can be explained by differences at the genomic level. A recent population study using simple sequence repeat markers also suggested genomic differences among isolates collected from different locations in the United States (Orquera-Tornakian et al. 2017). Such heterogeneity in *P. novopanici* populations poses a risk to switchgrass cultivation due to development of new

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*The e-Xtra logo stands for "electronic extra" and indicates that one supplementary table is published online.

The author(s) declare no conflict of interest.

Accepted for publication 1 May 2019.

Table 1. Sequence data of genome and transcriptome of *Puccinia novopanici* isolate Ard-01

Parameters	Results
Genome size	99,934,463 bp
Number of contigs	11,088
Minimum contig length	2,034 bp
Maximum contig length	7,332 bp
N50 contig length	13,091 bp
N90 contig length	4,215 bp
Mean contig length	9,164 bp
GC content	44.9%
Transcripts	16,622

virulent races of the pathogen via recombination and mutagenesis. Therefore, efforts in our laboratory are directed toward understanding the pathogen biology and diversity via whole-genome sequencing and comparative genomics with other sequenced and well-studied *Puccinia* genomes: *P. tritici*, *P. striiformis* f. sp. *tritici*, *P. graminis* f. sp. *tritici*, and *P. sorghi*.

For this study, *P. novopanici* isolates were collected from switchgrass plants grown in Ardmore, OK in the United States and confirmed by nuclear ribosomal DNA internal transcribed spacer (nuclear rDNA ITS) region amplification (Uppalapati et al. 2013). For whole-genome sequencing, urediniospores from isolate Ard-01 were inoculated on switchgrass cultivar Summer and a single pustule was selected for further rounds of inoculations on fresh, infection-free switchgrass plants to multiply urediniospores. All inoculations were performed in clean growth chambers at day and night temperatures of 29 and 22°C, respectively, with a 16-h photoperiod, 90% relative humidity, and photon flux density of 150 to 200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. For genomic DNA isolation, urediniospores were germinated in germination solution, as described previously (Gill et al. 2018). Genomic DNA was isolated by a Quick-DNA Fungal/Bacterial Kit (Zymo Research). PacBio (Pacific Biosciences) and Illumina (Illumina Inc.) chemistries were used to generate long- and short-read sequences, respectively, and to generate hybrid assembly. A PacBio RS II library was constructed with an average insert size of 20 kb and sequenced using P4-C2 chemistry to generate 21,880,460,804 bp of data (~22 GB), achieving 215× coverage for the genome. For Illumina sequencing, 2 × 300-bp reads were generated from inserts of 550 bp using Illumina MiSeq sequencer. PacBio and Illumina reads were assembled separately by using Canu (Koren et al. 2017) and Masurca (Zimin et al. 2013), respectively. De novo assembly of the processed data were performed using the Hybrid assembly method using CLC Genomics Workbench 11.0 (<https://www.qiagenbioinformatics.com/>). Genome assembly and transcript prediction results are presented in Table 1. The whole-genome assembly of *P. novopanici* resulted in a total genome size of 99.934 Mb assembled into 11,088 super scaffolding contigs, which is similar in size to its closest relative, *P. sorghi*, with genome size of 99.6 Mb (Rochi et al. 2018). Based on the Assemblathon (<http://assemblathon.org/>) statistics of the *P. novopanici* assembled genome, there were 11,088 predicted contigs in *P. novopanici* with average length of 9,164 bp. The assembled contigs are in line with the assemblies reported by Cantu et al. (2011) and Cuomo et al. (2017). The average N50 scaffold length was 13,091 bp, with shortest and longest scaffolds being 2,034 and 73,332 bp, respectively. The *P. novopanici* genome assembly was annotated using MAKER (Campbell et al. 2014; Cantarel et al. 2008). The input resource of MAKER comprised an in-house database consisting of six Illumina RNA-seq samples representing *P. novopanici* treated with different concentrations of phosphite (Gill et al. 2018). In addition, the protein sequences from the Uniprot database (<https://www.uniprot.org/>) and Joint Genome Institute phytozome (<https://phytozome.jgi.doe.gov/pz/portal.html>) annotation of *P. graminis*, *P. striiformis*, and *P. tritici* were included as input for MAKER. Repeat Mask (<http://repeatmasker.org>) was applied to remove retro-transposon sequences and low-complexity regions in the genome during gene model prediction in MAKER (Muszewski et al. 2011; Soliai et al. 2014). The MAKER pipeline was iteratively run three times to generate optimized 19,064-gene models. A final filtration step was performed to remove redundant duplicated gene models that resulted in 16,622 transcripts. Genome annotation was supported and further validated by available *P. novopanici* RNAseq data (Gill et al. 2018) wherein 76 to 81% of RNAseq reads were mapped to the finished hybrid *P. novopanici* assembly. Furthermore, the annotated contigs based on the RNAseq studies correlated with the predicted genes from genome sequence analysis. Blast

results of *P. novopanici* transcripts identified the maximum number of best hits from *P. sorghi* (76.1%) followed by *P. graminis* f. sp. *tritici* (15%) and *P. striiformis* f. sp. *tritici* (7.2%). Previous phylogenetic studies based on rDNA ITS sequences suggested *P. asparagi* as the closest relative of *P. novopanici* (Uppalapati et al. 2013). However, based on the assembled genome size and estimated homology, *P. novopanici* is relatively closer to *P. sorghi* (Rochi et al. 2018). Switchgrass, sorghum, and maize are C4 plants, hence suggesting more relatedness of *P. novopanici* with rust pathogens of C4 plants rather than C3 plants such as asparagus, wheat, and barley. Further investigations in the future by comparative genomics will reveal the diversity among these *Puccinia* spp.

The genome sequence of *P. novopanici* will provide an important resource for researchers working on *P. novopanici* detection, epidemiology, population genetics, and disease resistance. Comparative genomics with other worldwide economically important pathogens such as *P. graminis* f. sp. *tritici* that causes wheat stem rust and its closest relative based on the whole-genome sequence, *P. sorghi*, will help us better understand the evolution and diversity of *Puccinia* spp. in general. The sequence data of *P. novopanici* is available at the NCBI database under BioProject ID PRJNA507656; BioSample accession SAMN10502107. The raw data can be accessed at NCBI as SRA submissions (SRR8264864, SRR8264863, SRR8264866, SRR8264865, SRR8264868, and SRR8264867). Genome assembly of *P. novopanici* is made available at NCBI with the GenBank assembly accession number GCA_004348175.1. The annotated transcripts have been deposited at DDBJ/ENA/GenBank under the accession GHU000000000. The version described in this article is the first version, GHU01000000. In addition, the complete transcripts annotations along with the transcript IDs and the relevant NCBI accession numbers are presented in Supplementary Table S1.

Acknowledgments

We thank J. Gallaway for maintaining switchgrass plants in the greenhouse.

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