Genome Sequence Resource of a *Puccinia striiformis* Isolate Infecting Wheatgrass

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Abstract

Stripe rust caused by *Puccinia striiformis* is a disastrous disease of cereal crops and various grasses. To date, 14 stripe rust genomes are publicly available, including 13 *P. striiformis* f. sp. *tritici* and 1 *P. striiformis* f. sp. *hordei*. In this study, one isolate (11-281) of *P. striiformis* collected from wheatgrass (*Agropyron cristatum*), which is avirulent to most of standard differential genotypes of wheat and barley, was sequenced, assembled, and annotated. The sequences were assembled to a draft genome of 84.75 Mb, which is comparable with previously sequenced *P. striiformis* f. sp. *tritici* and *P. striiformis* f. sp. *hordei* isolates. The draft genome comprised 381 scaffolds and contained 1,829 predicted secreted proteins. The high-quality draft genome of the isolate is a valuable resource in shedding light on the evolution and pathogenicity of *P. striiformis*.

Puccinia striiformis, causing stripe (yellow) rust on wheat, barley, triticale, rye, and various grasses, is a dikaryotic and obligated biotroph belonging to the Basidiomycota (Chen and Kang 2017). Among all released genomes of *P. striiformis*, only two isolates (104E137A- and 93-210) of *P. striiformis* f. sp. *tritici* (the wheat stripe rust forma specialis) and one isolate (93TX-2) of *P. striiformis* f. sp. *hordei* (the barley stripe rust forma specialis) were sequenced by long-read sequencing platforms (Schwessinger et al. 2018; Xia et al. 2018a,b). Here, we present the genome of *P. striiformis* isolate 11-281, which was collected from wheatgrass (*Agropyron cristatum* (L.) Gaertn.), with virulence on only few genotypes among a large number of wheat and barley genotypes tested. Thus, the genome of this isolate is presumed to maintain more avirulence genes corresponding to resistance genes in both wheat and barley than previously sequenced isolates virulent on many wheat or barley genotypes with various resistance genes (Xia et al. 2018a,b). The genomic information of this isolate will advance the understanding of the evolution of host adaptation in *P. striiformis* and may enable the identification of potential avirulence genes in the pathogen.

Isolate 11-281 was originally collected from a wheatgrass plant in Lincoln County, ID in the United States in 2011. This isolate is virulent on wheat genotypes Lemhi, Michigan Amber, PI 478214, and PS 279 and barley genotypes Topper, Abed Binder 12, and Steptoe. However, the isolate was avirulent on Avocet S and the 18 Yr single-gene lines used to differentiate *P. striiformis* f. sp. *tritici* races and, thus, it was designated as race PSTv-18 of *P. striiformis* f. sp. *tritici* (Wan and Chen 2014). Among the 12 barley differentials for identifying *P. striiformis* f. sp.

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Table 1. Summary statistic of *Puccinia striiformis* genome 11-281

Feature	Statistics	
	Primary	Haplotype
Genome size (Mb)	84.75	60.09
Scaffolds	381	873
N50 scaffold length (kb)	431.12	116.17
Contigs	427	873
N50 contig length (kb)	385.55	116.17
GC (%)	44.37	44.37
Protein-coding genes	16,869	12,145
Total tRNAs:	667	496
rRNA	32	32
Transposable elements (Mb)	30.34	20.85
Transposable elements (%)	35.81	34.69
Annotation completeness (%)	94.16	79.40
Secreted protein genes	1,829	1,318

hordei races, the isolate was virulent only on differentials Topper and Abed Binder 12 and, thus, designated as PSH-33 (Wan and Chen 2012). Genomic DNA was extracted from dried urediniospores using the cetyltrimethylammonium bromide method, with modifications (Chen et al. 1993). The genomic DNA was sequenced using both Illumina Hiseq2000 sequencing by Novogene Corporation (Sacramento, CA, U.S.A.) and Pacific BioSciences (PacBio) RS II sequencing by the Genomics Laboratory in Washington State University (Pullman, WA, U.S.A.). Illumina sequencing yielded 6,656,674,305 bp of sequencing data, an estimated 79× average coverage. Raw paired-end Illumina sequencing reads were quality checked using FastQC v0.11.6, and low-quality reads were trimmed using Trimmomatic v0.36 (Bolger et al. 2014). Eleven single-molecule real-time cells were used in PacBio sequencing, which yielded 9,366,231,501 bp of sequencing data, representing an estimated average coverage of 111×. The raw PacBio sequence reads were corrected with short Illumina reads using proovread-2.14.0 (Hackl et al. 2014). The error-corrected reads were then assembled to contigs using Falcon, Falcon-unzip, and Canu v1.7 (Chin et al. 2016; Koren et al. 2017). The mapping coverage of every assembled contig was calculated using Qualimap v2.2.1 by mapping Illumina reads to PacBio reads (Okonechnikov et al. 2015). Contigs with a mean coverage greater than 1,000× were considered as mitochondrial contigs and were excluded from the assembled genomic sequences. The draft assembly contigs were polished using Illumina reads and Pilon-1.22 (Walker et al. 2014). The preassembled contigs were scaffolded by SSPACE-STANDARD-3.0 and GapFiller v1-10 using paired-ends Illumina reads (Boetzer et al. 2010). The sequences assembled by Falcon-unzip and Canu assembler were merged to form a single superior sequence using Metassembler v1.5 (Wences and Schatz 2015). The merged sequences were then aligned to those of the three *P. striiformis* isolates sequenced by PacBio using the nucmer script in the MUMmer 3.0 package (Kurtz et al. 2004). The contigs in alignment with the reference scaffold were manually merged into one scaffold. The assembled *P. striiformis* 11-281 genome consists of 381 primary and 873 haplotype scaffolds, with the genome size of 84.75 and 60.09 Mb, respectively (Table 1). The genome size of primary scaffold is comparable with those of isolates 104E137A- (83.36 Mb) (Schwessinger et al. 2018), 93-210 (84.63 Mb), and 93TX-2 (77.39 Mb) (Xia et al. 2018a). The average nucleotide identity (ANI) was calculated between isolate 11-281 and each of the three reference genomes (Jain et al. 2018). All ANI values were above 98.8%, indicating the high genomic similarities of different *P. striiformis* isolates.

Total RNA was extracted separately from urediniospores, germinated urediniospores, and leaves 24 h, 48 h, 7 days, and 14 days after inoculation on susceptible and resistant wheat cultivars using TRIzol Reagent with the PureLink RNA Mini Kit (Invitrogen, San Diego, CA, U.S.A.). Equal amounts of the 10 RNA samples were mixed for mRNA sequencing by Illumina Hiseq2000 sequencing (Novogene Corporation, Sacramento, CA, U.S.A.). Error-corrected mRNA sequencing reads were aligned to the assembled genome using HISAT2 v2.1.0 (Kim et al. 2015). The alignments were assembled into transcripts by StringTie v1.3.4 (Pertea et al. 2015). Gene predictions by the Ab initio method were conducted using Augustus v3.2.3, GeneMark v3.54, and SNAP (Korf 2004; Ter-Hovhannisyan et al. 2008). Repeats were masked using RepeatMasker v4.0.7 and RepeatModeler v1.0.9. tRNAs were predicted using tRNAscan v2.0 (Lowe and Chan 2016). All predicted genes were incorporated into the annotation pipeline MAKER2 v2.31.10 (Holt and Yandell 2011). The assembled transcripts by StringTie were used in the analysis with MAKER2 to improve the quality of genome annotation. In total, 16,869 and

12,145 protein-coding genes were identified from the primary and haplotype scaffolds, respectively (Table 1). Of these genes, 8,951 (53.06%) and 6,897 (56.79%) were validated by the assembled transcripts using Gffcompare v0.10.6 and StringTie v1.3.4, respectively. The genome annotation completeness was assessed by benchmark universal single-copy orthologs (BUSCO v2.0.1) (Simão et al. 2015). The 1,298 complete and fragmented BUSCOs from the primary scaffolds and haplotigs were detected from 1,335 BUSCOs in the dataset of basidiomycetes, with the overall completeness of 97.22%. Of the 16,869 protein-coding genes in the primary scaffold, 1,957 and 1,415 signal peptides were identified by SignalP 4.1 from the primary set and the haplotype set of protein-coding genes (Nielsen 2017), respectively, and 1,829 with signal peptides and 1,318 proteins with excluded transmembrane helices were predicted to be secreted proteins (Table 1).

Because grasses harbor a higher diversity of genetic variation among isolates than wheat and barley hosts, they are reservoirs of genetic variation of *P. striiformis* (Cheng et al. 2016). Some *P. striiformis* isolates infecting wheatgrasses could be the origin of different formae speciales. The genome sequence of isolate 11-281 presented here is an important resource, which will make it possible to identify forma specialis-specific genes and avirulence genes in the future by comparable genomics analyses with other *P. striiformis* isolates that have different virulence spectra.

Accession Number

The assembled genome sequence of *P. striiformis* isolate 11-281 has been deposited at GenBank under the accession number SBIN000000000. The version described in this article is version SBIN01000000. The raw data for this experiment have been submitted to NCBI with SRA study SRR8446446. More information of annotation files and protein sequences are available on GitHub at https://github.com/yuxiang-li/Puccinia_striiformis_genome_11_281.

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