Genome Sequence Resource of *Phytophthora vignae*, the Causal Agent of Stem and Root Rot of Cowpea

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Abstract

The causal agent of stem and root rot of cowpea, *Phytophthora vignae*, is a widely distributed species of the *Phytophthora* genus. Here, we generate a high-quality complete genome assembly of *P. vignae* PSY2020 (89.39 Mb, N₅₀ 2.99 Mb) from China, using Oxford Nanopore Technologies (ONT) sequencing. The genome assembly completeness as evaluated by benchmarking universal single-copy orthologs was 94.51% at the eukaryote level. We identified 42.54% as repeat sequences and a total of 20,536 protein-encoding genes, of which 15,184 genes could be annotated. And we also identified 924 candidate RXLR effectors in the genome assembly. The described genome sequence will provide a valuable resource for better understanding of pathogenicity mechanisms of *P. vignae* and help in uncovering phylogenetical classification of *Phytophthora* species.

Genome Announcement

Oomycete *Phytophthora* species are some of the most notorious plant pathogens that cause disease on hundreds of plant species, such as potato late blight disease caused by *P. infestans*, tobacco black shank caused by *P. nicotianae*, and *Vigna* species stem rot disease caused by *P. vignae*. Soil-borne *P. vignae*, first identified in 1957 (Purss 1957), produces non-papillate persistent sporangia and amphigynous antheridia (Stamps et al. 1990). Recent studies suggested that stem and root rot of cowpeas [*Vigna unguiculata* (L.) Walp. subsp. *unguiculata*] caused by this homothallic pathogen is emerging as one of the most serious soilborne diseases of cowpea worldwide (Dilantha Fernando and Linderman 1993; Kao and Leu 1982; Kitazawa et al. 1979; Purss 1957; Sivakadacham and Fernando 1991). It is estimated that *P. vignae* leads to significant losses in cowpea production under favorable environmental conditions (Davis et al. 1994; Purss 1957). Moreover, emergence of new pathogenic races and formae speciales of *P. vignae* possibly contribute to some reported resistant cultivars unavailable in disease management (Kondo et al. 2004; Purss 1957, 1972; Sun et al. 2021). Therefore, in-depth understanding of the molecular mechanisms underlying pathogenicity of *P. vignae* will be conducive to developing sustainable strategies for management of this disease. In this regard, a high-quality complete genome resource for the *P. vignae* strain will be helpful.

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The author(s) declare no conflict of interest.

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Table 1. Genome characteristics

Features	Strain PSY2020
Oxford Nanopore Technologies (ONT) long reads	6.83 Gb (about 76×)
ONT reads N ₅₀ (kb)	17.05
Illumina short reads	4.55Gb (about 51×)
Assembly size (Mb)	89.39
Contig number	58
Contig N ₅₀ (Mb)	2.99
Contig L ₅₀	12
Average contig length (Mb)	1.54
Maximum contig length (Mb)	6.62
GC content	55.12%
Repeat sequence	42.54%
BUSCO assessment in eukaryota (<i>n</i> = 255) ^a	94.51%
Protein-encoding genes	20,536
Candidate RXLR effectors	924
Genes annotated by Pfam ^b	12,993
Genes annotated by GO ^b	5,150
Genes annotated by KEGG ^b	8,453
Genes annotated by KOG ^b	14,080
Genes annotated by CAZY ^b	463
Total of protein-encoding genes annotated	15,184

^a Genome assembly completeness was assessed by benchmarking universal single-copy orthologs (BUSCO).

^b Pfam annotation conducted by InterProScan v5.47-82 while Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), EuKaryotic Orthologous Groups (KOG), and Carbohydrate Active Enzymes (CAZY) database annotations conducted by EggNogMapper v2.

Here, *P. vignae* PSY2020 was isolated from an infected cowpea collected from the Hainan Province in China and was incubated on V-8 agar media. High-quality genomic DNA of PSY2020 was extracted and purified, following relative DNA extraction kit protocol, for Oxford Nanopore Technologies (ONT) long reads sequencing and Illumina short reads sequencing. We got 6.83 Gb of ONT reads, with an N₅₀ read length of 17.05 Kb, and 4.55 Gb of paired-end Illumina reads, with an insert length of 350 bp (Table 1). The sequencing depth of ONT reads and Illumina reads represent 76× and 51× genome coverage, respectively (Table 1). To gain genome assembly, we deployed NextDenovo v2.3.1 to generate a draft assembly, using pure ONT reads, and NextPolish v1.3.1 to correct and generate a polished assembly, using both ONT reads and Illumina reads. Finally, we obtained an 89.39-Mb assembly, containing 58 contigs with 2.99 Mb contig N₅₀ and 12 contig L₅₀ (Table 1). The average contig length is 1.54 Mb and the largest contig length is 6.62 Mb, representing a very high continuous genome assembly of *P. vignae* (Table 1). GC content of PSY2020 is 55.12%, which is very close to isolates of *Phytophthora infestans* (Ye et al. 2016) (Table 1). The completeness of PSY2020 genome assembly was evaluated through benchmarking universal single-copy orthologs (BUSCO) v4.1.4 (Seppey et al. 2019) with 'eukaryota_odb10' (*n* = 255) as reference dataset. This analysis indicates that genome assembly of strain PSY2020 contains 94.51% (241 of 255) complete eukaryotic single-copy orthologs (Table 1; Fig. 1B). It is worth noting that proportion of repeat sequence in PSY202 genome is as high as 42.54%, suggesting the rapid amplification of repeat elements in *P. vignae* genome (Table 1; Fig. 1A).

Protein-encoding gene prediction was conducted through a combination of evidence-based prediction by BRAKER2 (Bruna et al. 2021), with *P. sojae* proteins as reference, and *ab initio* prediction by Fgenesh (Solovyev et al. 2006), with *Phytophthora* as training organism. A total of 20,536 protein-encoding genes were predicted (Table 1; Fig. 1A). To gain a functional gene annotation, we annotated whole-genome protein-encoding genes with Pfam, GO (Gene Ontology), KEGG (Kyoto Encyclopedia of Genes and Genomes), KOG (EuKaryotic Orthologous Groups), and CAZymes (carbohydrate active enzymes) databases, respectively. We identified a total of 12,993 proteins with Pfam domains, 5,150 genes with GO items, 8,453 genes involved in different KEGG pathways, 14,080 KOG genes, and 463 CAZymes (Table 1, Fig. 1A). Overall, we uncovered 1,910 tandem-duplicated genes that generate 797 gene clusters, by exploiting Orthofinder v2.4.1 (Emms and Kelly 2019) (Fig. 1A). To identify more candidate RXLR effectors, we scanned open reading frames containing signal peptide (SignalP v3.0) and RXLR motif but without any transmembrane helices

Fig. 1. A, Genome features of *Phytophthora vignae* PSY2020 were displayed with Circos (Krzywinski et al. 2009). The contigs more than 100 kb long were displayed in track a. The density of genes and transposable elements (TEs) calculated by sliding window analysis with 100-kb windows and 20-kb steps were shown in tracks b and c, respectively. The locations of genes with functional annotation, tandem duplicated genes, and RXLR effectors were displayed in tracks d, e, and f respectively. **B,** The completeness of genome assembly was assessed by BUSCO v4.1.4 with eukaryotic single-copy orthologs ($n = 255$) from OrthoDB v10. Raw = draft unpolished genome assembly of PSY2020, while ONT = polished assembly corrected with only Oxford Nanopore Technologies (ONT) long reads, and ONT+Illumina = final polished assembly corrected with both ONT long reads and Illumina short reads.

(TMHMM v2.0), following our previous study (PMID 33507115) and, finally, identified 924 candidate RXLR effectors in strain PSY2020 (Table 1; Fig. 1A).

Data Availability

The whole-genome sequence data reported in this paper have been deposited in the Genome Warehouse in the National Genomics Data Center, Beijing Institute of Genomics (China National Center for Bioinformation), Chinese Academy of Sciences, under accession number GWHAZIG00000000 (BioProject PRJCA004069, BioSample SAMC296216).

Author-Recommended Internet Resources

Circos: <http://circos.ca>

Denovo v2.3.1: <https://github.com/Nextomics/NextDenovo>

Genome Warehouse: <http://bigd.big.ac.cn/gwh>

NextPolish v1.3.1: <https://github.com/Nextomics/NextPolish>

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