Genome Sequence Data of Three Formae Speciales of *Phytophthora vignae* Causing *Phytophthora* Stem Rot on Different *Vigna* Species

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Abstract

Phytophthora vignae is an important oomycete pathogen causing *Phytophthora* stem rot on some *Vigna* spp. Three *P. vignae* isolates obtained from mung bean, adzuki bean, and cowpea exhibited high similarities in morphology and physiology but are specialized to infect different hosts. Here, we report the first de novo assembly of the draft genomes of three *P. vignae* isolates, which were performed using the PacBio SMRT Sequel platform. This study will extend the genomic resource available for the *Phytophthora* genus and provide a good foundation for further research on comparative genomics of *Phytophthora* spp. and interaction mechanism between hosts and pathogens.

Genome Announcement

Phytophthora stem rot caused by *Phytophthora vignae* is an important soilborne disease on *Vigna* crops. The disease was first reported on cowpea (*Vigna unguiculata*) in Queensland, Australia and the causal agent was identified as *P. vignae* (Purss 1957). *P. vignae* had a narrow host range and initially was not regarded to infect leguminous plants other than cowpea. In 1978, Kitazawa et al. (1978) identified the pathogen inciting *Phytophthora* stem rot of adzuki bean (*V. angularis*) in Hokkaido, Japan as *P. vignae*, and found that it attacks only the three cultivars of adzuki bean used but did not affect another 14 cultivars of other crops tested. Subsequently, Kitazawa et al. (1979) found that the cowpea and adzuki bean isolates attack both cowpea and adzuki bean plants but the *P. vignae* isolates from cowpea were found to be more virulent to cowpea while the *P. vignae* isolates from adzuki bean were more virulent to adzuki bean plants. Tsuchiya et al. (1986) proposed two formae speciales, *P. vignae* f. sp. *vignae* and *P. vignae* f. sp. *adzukicola*, for the two organisms on the basis of their specific pathogenicity and host range. Zhu and Wang (2003) found that *P. vignae* f. sp. *adzukicola* isolates are not only virulent to adzuki bean but also infect individual cowpea and mung bean cultivars by using different inoculation methods. In recent years, *P. vignae* was also identified as the causal agent of stem rot on mung bean (*V. radiata*) in China. The pathogen showed similar morphology but host range was different from that of *P. vignae* f. sp. *vignae* and *P. vignae* f. sp. *adzukicola*. The *P. vignae* isolates from mung bean showed strong virulence to mung bean and relatively weak virulence to adzuki bean but no virulence to cowpea or other legume crops. Hence, these isolates were identified as a new forma specialis of *P. vignae*, *P. vignae* f. sp. *mungicola* (Sun et al. 2021). To date, the whole-genome sequences of many species in the genus

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Keywords

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Table 1. Isolate information and summary statistics of the draft genome assembly for the three formae speciales of *Phytophthora vignae*

Features	<i>P. vignae</i> isolates		
	PcV2	PaV1	PVMG4
Host	<i>Vigna unguiculata</i>	<i>V. angularis</i>	<i>V. radiata</i>
Source	Provided by Prof. Benjin Li	Gannan County, Heilongjiang	Anhui, Mingguang
Year	2017	2017	2017
Genome size (Mb)	84.5	84.0	89.9
Sequenced reads (Gb)	12.3	11.1	9.99
Number of contigs	227	203	268
Contig N ₅₀	1.14 Mb	1.04 Mb	567.8 Kb
Largest contig (bp)	3,085,209	2,857,727	3,246,594
Number of predicted genes	23,083	23,024	25,518
Secreted proteins	1,602	1,588	1,712
GC content (%)	54.9	55.0	54.8
BUSCO completeness (%)	100	99	98

Phytophthora have been published (Cui et al. 2019; Lee et al. 2020; Srivastava et al. 2020), providing novel insights into the molecular basis of plant disease, such as the identification of secreted RXLR and crinkler (CRN) effector proteins that are associated with plant–pathogen interactions (Kong et al. 2017; Stam et al. 2013; Tyler et al. 2006). Here, we sequenced and assembled the genomes of three isolates which represented different formae speciales of *P. vignae*, and expect that this will provide fundamental understanding of the genetic mechanism underlying host–pathogen interactions and reveal the evolutionary relationship among the three formae speciales of *P. vignae*.

Three isolates (*P. vignae* f. sp. *mungcola* PVMG4, *P. vignae* f. sp. *adzukicola* PaV1, and *P. vignae* f. sp. *vignae* PcV2) were grown on V8 juice agar at 25°C under a 12-h photoperiod for 7 to 10 days. Genomic DNA of the three isolates was extracted from mycelia using the cetyltrimethylammonium bromide method (Doyle 1987). The quality of the extracted DNA was tested using a NanoDrop One UV-Vis spectrophotometer (Thermo Fisher Scientific) and Qubit 3.0 Fluorometer (Invitrogen). Genome size of the three isolates was evaluated by paired-end sequencing with a 400-bp library on the Illumina HiSeq Xten platform. According to 17 K-mer statistics, *P. vignae* f. sp. *mungcola* isolate PVMG4 was estimated to be 106.6 Mb, with a heterozygosity of 0.5%. The genome size of the other two isolates, PaV1 and PcV2, was assessed to be 100.5 and 98.0 Mb, respectively. The GC-Depth showed that there were few contaminants in the sequenced reads. Moreover, preassembly of genomes of the three isolates by SOAPdenovo showed a lower integrity; thus, we used third-generation sequencing technology instead. For PacBio sequencing, SMRTbell libraries of a 20-kb-long insert fragment were constructed and sequenced on the PacBio Sequel platform (Pacific Biosciences) with one single-molecule real-time (SMRT) cell by Wuhan NextOmics (<https://www.grandomics.com/>) (Wuhan, China).

After filtering out the reads with low quality and trimming adapter sequences, clean sub-reads of 12.3, 11.1, and 9.99 Gb for PcV2, PaV1, and PVMG4, respectively, were used for de novo assembly by the FALCON v1.8.7 assembler with appropriate parameters (Chin et al. 2016) (Table 1). Arrow correction of the assembly results was performed using blasr 4.0 and bwa mem 0.7.12-r1039 with default parameters. The final assembled genome size of the three isolates ranged from 84.0 to 89.9 Mb, containing 203 to 268 contigs (Table 1). The contig N₅₀ for PVMG4, PaV1, and PcV2 was 567,800 bp, 1.04 Mb, and 1.14 Mb, respectively. Genome coverages were all more than 100× and GC content of the three isolates was approximately 55% (Table 1). The completeness of the draft genome assemblies was evaluated using Benchmarking Universal Single-Copy Orthologs (BUSCO) v 4.1.4 (Simão et al. 2015) by comparing 100 eukaryotic single-copy orthologs (SCOs) in the Stramenopiles dataset. The results showed that 100, 99, and 94% of SCO genes were found as a complete single copy for PcV2, PaV1, and PVMG4, respectively; 0, 0, and 4% were completely duplicated; 0, 1, and 2% were missing; and none were fragmented in all three genomes. The gene prediction was performed by Augustus 2.4 (Stanke and Waack 2003), GlimmHMM 3.0.4 (Majoros et al. 2004), GeneWise 2.4.1 (Birney and Durbin 2000), and PASA 2.3.3 (Haas et al. 2008), and the final gene sets were obtained by integrating all the above methods using EVM 1.1.1 (Haas et al. 2008) and, meanwhile, removing transposable elements using TransposonPSI (<http://transposonpsi>).

sourceforge.net) (Yagi et al. 2014). In total, 23,083, 23,024, and 25,518 genes were predicted in PcV2, PaV1, and PVMG4, respectively and the mean length of all coding sequences was approximately 1,400 bp. Of these genes, the annotated functional genes accounted for 97% in the three *P. vignae* isolates predicted by InterProScan v5.2-45.0. The gene numbers were similar to those of *P. sojae* in clade 7 (Sharma et al. 2015). To identify the secreted proteins, we used the software SignalP v5.0 to predict signal peptide and, meanwhile, removed the proteins with transmembrane domains predicted by TMHMM v2.0. In total, 1,712, 1,602, and 1,588 secreted proteins were predicted in the genomes of PVMG4, PcV2, and PaV1, respectively, which may contain conserved and virulence-related RXLR and CRN effectors.

We used OrthoMCL to conduct the gene family analysis in *P. vignae* and 121, 34, and 47 unique families were identified in *P. vignae* f. sp. *mungicola* PVMG4, *P. vignae* f. sp. *adzukiicola* PaV1, and *P. vignae* f. sp. *vignae* PcV2, respectively. Phylogenetic analysis based on SCOs of several sequenced *Phytophthora* spp. (*P. sojae*, *P. infestans*, and *P. parasitica*) and these three *P. vignae* isolates (PVMG4, PaV1, and PcV2) showed that *P. vignae* and *P. sojae* were clustered in a group and, at the same time, PVMG4, PaV1, and PcV2 were further divided into two branches. These data, coupled with the information about the plant–pathogen interaction in this study, will help further research on host specificity of the three formae speciales of *P. vignae* from different *Vigna* spp. (*V. unguiculata*, *V. angularis*, and *V. radiata*).

Data availability. The whole-genome shotgun projects of the three *P. vignae* isolates have been deposited at DNA Data Bank of Japan/European Nucleotide Archive/GenBank under the accession numbers JABJUM000000000 (PcV2), JABJUN000000000 (PaV1), and JABJXB000000000 (PVMG4). The versions described in this study are the first versions (JABJUM010000000, JABJUN010000000, and JABJXB010000000). The PacBio sequencing reads and genome assemblies for all three isolates have been deposited as an NCBI BioProject under the accession number PRJNA626502. All three isolates have been preserved in the Agricultural Culture Collection of China (ACCC) under ACCC39666 (PVMG4), ACCC39677 (PaV1), and ACCC39676 (PcV2) accessions.

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