Improved Whole-Genome Sequence of *Phytophthora capsici* Generated by Long-Read Sequencing

Jinxia Shi,¹ Wenwu Ye,² Dongfang Ma,³ Junliang Yin,³ Zhichao Zhang,² Yuanchao Wang,² and Yongli Qiao^{1,†}

¹ Shanghai Key Laboratory of Plant Molecular Sciences, College of Life Sciences, Shanghai Normal University, Shanghai 200234, China

² Department of Plant Pathology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China

³ College of Agriculture, Yangtze University, Jingzhou 434025, China

Abstract

The soilborne oomycete *Phytophthora capsici* is the most destructive pathogen of vegetable crops and is responsible for substantial economic losses worldwide. Here, we present an improved genome assembly of *P. capsici* generated by Oxford Nanopore long-read sequencing (for de novo assembly) and Illumina short-read sequencing (for polishing). The genome of *P. capsici* is 100.5 Mb in length (GC content = 50.8%) and contains 26,069 predicted protein-coding genes. The whole genome of *P. capsici* is assembled into 194 scaffolds, 90% of which are larger than 300 kb. The N₅₀ scaffold length and maximum scaffold length are 1.0 and 4.1 Mb, respectively. The whole-genome sequence of *P. capsici* will broaden our knowledge of this pathogen and enhance our understanding of the molecular basis of its pathogenicity, which will facilitate the development of effective management strategies.

Genome Announcement

Phytophthora capsici is an important oomycete phytopathogen that causes foliar blight, damping-off, wilting, and root, stem, and fruit rot in many vegetable crops such as lima bean (*Phaseolus lunatus*), tomato (*Solanum lycopersicum*), snap bean (*P. vulgaris*), eggplant (*S. melongena*), cucurbits, and pepper (*Capsicum annuum*) (Lamour et al. 2012a). *Phytophthora capsici* was first reported in New Mexico by Fabián García, then systematically described and isolated from chile pepper in New Mexico by Leon H. Leonian in 1922 (Barchenger et al. 2018; Granke et al. 2012). Since its discovery, *P. capsici* has caused severe disease epidemics in North, Central, and South America as well as in Europe and Asia, resulting in serious economic losses worldwide. Although diseases caused by *P. capsici* represent a significant threat to the commercial cultivation of vegetable crops, current management strategies are based on the implementation of cultural practices, application of chemicals, and development of resistant cultivars (Hausbeck and Lamour 2004). More importantly, *P. capsici* is difficult to control because its thick-walled oospores remain dormant for a long period of time. Moreover, *P. capsici* shows rapid adaptation to fungicides and new hosts because of the significant mitotic loss of heterozygosity and many sexual progeny in field isolates

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Keywords

genome assembly, long-read sequencing, *Phytophthora capsici*, plant pathogen

[†]Corresponding author: Y. Qiao; qyl588@gmail.com

J. Shi, W. Ye, and D. Ma contributed equally to this work.

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Table 1. Overview of the genome assembly statistics of nine *Phytophthora capsici* isolates^a

Genomic features	SD33	LT263	LT1534	CPV-219	CPV-262	CPV-267	CPV-270	CPV-277	CPV-302
Genome size (Mb)	100.5	95.2	64.0	58.9	40.8	52.2	58.3	53.4	56.9
Number of scaffolds	194	424	917	7,580	11,754	11,832	8,363	9,341	9,277
N ₅₀ length (kb)	1,013.4	313.0	706.0	14.1	4.6	6.6	11.6	9.4	10.0
GC content (%)	50.8	ND	51.0	49.9	50.0	49.9	49.9	49.9	49.9
BUSCO-completeness (%)	94.0	97.0	91.0	91.5	72.6	82.9	89.8	91.0	93.2
Number of protein-coding genes	26,069	19,391	19,805	25,102	19,275	23,579	25,004	23,404	24,322
Number of predicted RxLR effectors	640	357	ND	379	263	341	371	350	353
Number of predicted CRN effectors	59 + 170	29 + 70	ND	230	121	183	213	181	187
Source of strain	<i>Capsicum annuum</i>	<i>Cucurbita pepo</i>	<i>Cucurbita pepo</i>	<i>Capsicum pubescens</i>	<i>Cucurbita pepo</i>	<i>Cucurbita pepo</i>	<i>Capsicum annuum</i>	<i>Capsicum annuum</i>	<i>Capsicum chinense</i>
Reference	This study	Cui et al. 2019	Lamour et al. 2012a	Reyes-Tena et al. 2019	Reyes-Tena et al. 2019	Reyes-Tena et al. 2019	Reyes-Tena et al. 2019	Reyes-Tena et al. 2019	Reyes-Tena et al. 2019

^a ND = not described.

(Hulvey et al. 2010; Lamour et al. 2012b; Lyon et al. 2016; Zhu et al. 2020). Compared with other *Phytophthora* spp. (Wang et al. 2011; Wang et al. 2019; Whisson et al. 2016), the molecular mechanisms underlying the pathogenicity of *P. capsici* and its interactions with host plants remain poorly described and characterized.

Several different versions of the *P. capsici* genome sequence have been recently released publicly, based on two isolates collected from the United States (Lamour et al. 2012a; Cui et al. 2019) and six isolates collected from Mexico (Reyes-Tena et al. 2019). However, given its complex genome and life history, the genome assembly of *P. capsici* remains challenging, and a high-quality reference genome of *P. capsici* is unavailable. In addition, no genome sequence has been documented for the *P. capsici* strain isolated from China. To broaden our current knowledge of *P. capsici* and to manipulate the molecular basis of its rapid adaptation and high pathogenicity, additional genome resources are needed. These resources will also help to control *Phytophthora* blight in pepper. Here, we report an improved whole-genome sequence of *P. capsici* strain SD33 with fewer contigs.

P. capsici strain SD33, the predominant stain in Shandong (one of the main chili pepper production areas in China), was originally isolated in 2006 from lesions on the leaves of chili pepper plants in Shandong province. Strain SD33 was maintained on rye agar medium supplemented with 2% sucrose, and genomic DNA was extracted from hyphae using DNeasy Plant Mini Kit (Qiagen). The genome of SD33 was sequenced by Oxford Nanopore sequencing at Biomarker Technologies Co., Ltd. (Beijing, China). The genome of strain SD33 was sequenced at 116× coverage, generating 11.6 Gb of high-quality long-read sequences (clean reads), with a mean length of 36.5 kb and N₅₀ length of 27.7 kb. The reads were de novo assembled using Canu v1.5 (Koren et al. 2017) and polished using Pilon v1.22 (Walker et al. 2014) by mapping Nanopore clean reads and Illumina short reads (obtained in parallel; 6.9 Gb, 150-bp paired-end reads) on to the preassembled genome. The final genome assembly was 100.5 Mb in length and contained 194 scaffolds, 90% of which were larger than 300 kb in size. The N₅₀ scaffold length and maximum scaffold length were 1.0 and 4.1 Mb, respectively, and the GC content was 50.8%. Compared with the most recently released *P. capsici* LD263 genome assembly, the *P. capsici* SD33 genome assembly was larger in size by 5.6% (100.5 versus 95.2 Mb) (Table 1) (Lamour et al. 2012a). Additionally, compared with the LD263 genome assembly, the number of contigs in the SD33 genome assembly was reduced by 45.8% (194 versus 424), and the N₅₀ scaffold length was increased by 3.2-fold (1,013.4 versus 313.0 kb) (Lamour et al. 2012a). The completeness of the SD33 assembly was calculated against the “alveolata_stramenophiles_ensembl” database using benchmarking universal single-copy orthologs (BUSCO) v2.0 (Simão et al. 2015). Of 234 complete BUSCOs, 220 (94.0%) were identified (single-copy = 168, 71.8%

Fig. 1. Gene synteny between the SD33 and LT1534 assemblies was analyzed using MCscan (Python version) (Tang et al. 2008). **A**, Landscape of all scaffolds which contain any genes showing collinearity (98.4% of SD33 assemblies) and **B** and **C**, two examples of the largest scaffolds in SD33 assemblies.

and duplicate = 52, 22.2%), with only two fragmented (0.9%) and 12 missing (5.1%) BUSCOs. The assembly completeness was also evaluated by mapping with Illumina short reads, which showed that 93.1% of all reads were mapped accurately.

Repetitive elements identified by RepeatMasker v4.0.6 (Tarailo-Graovac and Chen 2009) accounted for 36.2% of the whole-genome sequence. Among these repetitive elements, 27.3% were class I (retrotransposons), and 6.4% were class II (DNA transposons). Protein-coding genes were identified by ab initio methods using Augustus v2.4 (Stanke and Waack 2003), Genescan v1.0 (Burge and Karlin 1997), GlimmerHMM v3.0.4 (Majoros et al. 2004), GeneID v1.4 (Alioto et al. 2018), and SNAP v2006-7-28 (Korf 2004), and by homology-based methods using GeMoMa v1.3.1 (Keilwagen et al. 2016). Then, the data obtained were integrated with EVM v1.1.1 (Haas et al. 2008). In total, 26,069 protein-coding genes were identified, with a mean gene length and coding sequence length of 1,762.7 and 1,385.1 bp, respectively, and an average intron number of 1.74 per gene. Among the protein-coding genes, 25,889 (99.3%) could be annotated using the Pfam (15,268, 58.6%), Gene Ontology (11,261, 43.2%), Kyoto Encyclopedia of Genes and Genomes (6,547, 25.1%), euKaryotic Orthologous Groups (9,863, 37.8%), Swiss-Prot (9,487, 36.4%), TrEMBL (13,991, 53.7%), or NCBI nonredundant protein (25,885, 99.3%) databases. In addition, analysis using SignalP v3.0 (Bendtsen et al. 2004) and TMHMM v2.0 (Krogh et al. 2001) showed that 2,352 proteins (9.0%) containing a signal peptide but no transmembrane helix composed the candidate secretome. In addition, 640 RxLR and 59 CRN (170 others may code CRN-like proteins or pseudogenes) effector-encoding genes were predicted. Macrosynteny and local synteny were accomplished by MCscan (Python version) (Tang et al. 2008). A preliminary synteny analysis revealed an overall gene collinearity between the SD33 and LT1534 assemblies, and showed an expansion in SD33 assemblies with more continuous and detailed genomic sequences. These sequences may include those previously unassembled using short sequencing reads (e.g., repetitive elements, and tandem repeated genes or genome fragments) and those absent in the LT1534 assemblies (Fig. 1).

Overall, the *P. capsici* strain SD33 genome sequence assembly presented in this study will serve as a valuable resource for investigating blight, crown and root rot, and fruit rot diseases caused by *P. capsici*, identifying its novel virulence factors and effectors, and elucidating the molecular mechanisms underlying its high pathogenicity and rapid adaptation. Moreover, this new genomic resource will facilitate comparative genomic analysis of plant pathogens.

Data Availability

The whole-genome sequence data reported in this article have been deposited in the Genome Warehouse in the National Genomics Data Center (National Genomics Data Center Members and Partners et al. 2020), Beijing Institute of Genomics (China National Center for Bioinformation), Chinese Academy of Sciences, under accession number GWHZID000000000, and are publicly accessible online.

Author-Recommended Internet Resource

Accession number GWHZID000000000: <https://ngdc.cncb.ac.cn/gwh>

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