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RESOURCE ANNOUNCEMENT

A Genome Resource for *Ciborinia camelliae*, the Causal Agent of Camellia Flower Blight

Irene Valenti,† Marco Saracchi, Luca Degradi, Andrea Kunova, Paolo Cortesi, and Matias Pasquali

Department of Food, Environmental and Nutritional Sciences, University of Milan, Milan, Italy

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camellia pathogen, long and short reads, plant-fungal interaction, Sclerotiniaceae

Abstract

Ciborinia camelliae Kohn is a camellia pathogen belonging to family Sclerotiniaceae, infecting only flowers of camellias. To better understand the virulence mechanism in this species, the draft genome sequence of the Italian strain of *C. camelliae* was obtained with a hybrid approach, combining Illumina HiSeq paired reads and MinIon Nanopore long-read sequencing. This combination improved significantly the existing National Center for Biotechnology Information reference genome. The assembly contiguity was implemented decreasing the contig number from 2,604 to 49. The N_{50} contig size increased from 31,803 to 2,726,972 bp and the completeness of assembly increased from 94.5 to 97.3% according to BUSCO analysis. This work is foundational to allow functional analysis of the infection process in this scarcely known floral pathogen.

Genome Announcement

Ciborinia camelliae Kohn, a member of the Sclerotiniaceae family, is a hemibiotrophic pathogen of camellia plants. This fungus affects only flowers by inducing a disease called camellia flower blight (Saracchi et al. 2019), responsible for economic losses in the floriculture industry (Denton-Giles et al. 2013). The infection shows a first asymptomatic biotrophic phase, shifting to necrotic lesions, spreading to the whole flower, imminent to fall. In a previous work, we explored the variability among Italian *Ciborinia camelliae* strains (Saracchi et al. 2022). Here, we present the draft genome of a representative Italian strain, contributing to improving the existing reference genome of the species (GenBank GCA_001247705.1).

The ITAC2 strain of *C. camelliae* was isolated from a sample collected in Oggebbio (Verbania, Italy). After the morphological and molecular characterization (Valenti et al. 2022), the strain was deposited in a public collection (DSMZ-German Collection of Microorganisms and Cell Cultures GmbH) with the accession number DSM 112729.

To obtain pure high–molecular weight DNA, conidia were collected from a 14-day-old colony grown on potato dextrose agar medium, and the DNeasy PowerSoil Pro kit (Qiagen, Hilden, Germany) was employed.

To sequence the ITAC2 genomic DNA, two different platforms were employed, namely, Oxford Nanopore Technologies (ONT, Oxford) and Illumina HiSeq, executed by Eurofins Genomics (Ebersberg, Germany). The MinION system (FLO-MIN-106 R9.4 flow-cell) was used to perform the ONT sequencing. The native barcoding protocol was carried out using

†Corresponding author: I. Valenti; irene.valenti@unimi.it

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Table 1. Genome assembly comparison between *Ciborinia camelliae* strains ITAC2 and ICMP 19812 (GCA_001247705.1)

Strain	*C. camelliae* ITAC2	*C. camelliae* ICMP 19812
Contigs	49	2,604
Largest contig (bp)	5,578,642	31,803
Genome size (Mb)	46.48	40.73
N_{50} (bp)	2,726,972	31,803
GC (%)	39.56	42.48
No. of mismatches	9,280	90,878
BUSCO completeness (%)	97.3	94.5

both EXP-NBD104 and EXPNBD114 in conjunction with the SQK-LSK109 kit and also the SQK-RAD004 sequencing kit (ONT).

The long Nanopore reads and short Illumina reads were analyzed using the European Galaxy web platform tools (Afgan et al. 2018) and Geneious Prime software, version 2021.1.1 (Biomatters, Auckland, New Zealand). The Illumina platform produced 10,324,028 paired-end reads 2 × 151 bp and ONT 641,071 single-molecule long reads with a mean length of 4,574 bases. The ONT sequencing data were analyzed, using the plotting script Nanoplot (W. De Coster) deposited in GitHub. FastQC 0.73+galaxy0 (S. Andrews) was used for the Illumina reads quality visualization and FastP 0.23.2+galaxy0 (Chen et al. 2018) for filtering out the bad-quality reads. The mean quality score for Nanopore and Illumina data was 9.9 and 30.8, respectively.

Nanopore reads were assembled with two different assemblers, Flye v.2.8.3+galaxy0 (Lin et al. 2016) and Canu 2.1.1+galaxy0 (Koren et al. 2017) with default settings. The obtained assemblies were compared by Quast 5.0.2+galaxy5 (Mikheenko et al. 2018) and BUSCO 5.3.2+galaxy0 (Simão et al. 2015) tools to evaluate their quality and completeness, respectively. The Flye assembly was selected for further analysis, as it resulted in the best assembly. Medaka tool v.1.3.2+galaxy0 was performed for autopolishing, and minimap2 (Li 2018) was used for mapping the Illumina reads on the draft assembly to correct potential mistakes. The consensus sequence was obtained using Pilon v.1.20.1 (Walker et al. 2014). The assessment of genome completeness and quality was performed using Quast and BUSCO tools, respectively. The hybrid assembly resulted in a coverage of 67 and 63×, considering Illumina and Nanopore reads, respectively. Before genome annotation, the assembly was cleaned using both Funannotate assembly clean v.1.8.9+galaxy2 and Sort assembly v.1.8.9+galaxy2 tools (Palmer and Stajich 2020). The total length of the *C. camelliae* ITAC2 genome was 46.48 Mb. In comparison with the National Center for Biotechnology Information reference assembly of *C. camelliae* ICMP 19812 (GCA_001247705.1) from New Zealand (Table 1), the genome improved in contiguity, decreasing the contig number from 2,604 to 49. Instead, the N_{50} contig size increased from 31,803 to 2,726,972 bp and the completeness of assembly increased from 94.5 to 97.3%, according to BUSCO analysis.

The Funannotate predict annotation 1.8.9+galaxy2 tool (Palmer and Stajich 2020) was used to estimate genes, building a draft annotation. We employed EggNOG mapper 2.1.6+galaxy1 (Huerta-Cepas et al. 2016) and InterProScan 5.54-87.0+galaxy2 (Jones et al. 2014) to generate the functional annotation. The Funannotate functional annotation 1.8.9+galaxy2 tool provided conclusive annotation.

Moreover, from the sequencing data, the first fully annotated mitochondrial genome of *C. camelliae* (GenBank OK326902) was obtained, increasing the evolutionary knowledge of the Sclerotiniaceae pathogens (Valenti et al. 2022).

As previously reported (Aggarwal et al. 2019; Degradi et al. 2021; Li and Liu 2022), the hybrid assembly proved to enhance the genome quality and completeness, fundamental for future functional studies.

Data Availability

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JANPYM000000000. The version described in this paper is version JANPYM010000000.

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Author-Recommended Internet Resources

FastQC: http://www.bioinformatics.babraham.ac.uk/projects/fastqc
Galaxy Europe: https://usegalaxy.eu
Galaxy training materials: https://training.galaxyproject.org/training-material
NanoPlot script: https://github.com/wdecoster/NanoPlot
Medaka: https://github.com/nanoporetech/medaka

Literature Cited

Afgan, E., Baker, D., Batut, B., van den Beek, M., Bouvier, D., Cech, M., Chilton, J., Clements, D., Coraor, N., Grüning, B. A., Guerler, A., Hillman-Jackson, J., Hiltemann, S., Jalili, V., Rasche, H., Soranzo, N., Goecks, J., Taylor, J., Nekrutenko, A., and Blankenberg, D. 2018. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic Acids Res. 46:W537-W544.

Aggarwal, R., Sharma, S., Singh, K., Gurjar, M. S., Saharan, M. S., Gupta, S., Bashyal, B. M., and Gaikwad, K. 2019. First draft genome sequence of wheat spot blotch pathogen *Bipolaris sorokiniana* BS_112 from India, obtained using hybrid assembly. Microbiol. Resource Announc. 8: e00308-19.

Chen, S., Zhou, Y., Chen, Y., and Gu, J. 2018. fastp: An ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34:i884-i890.

Degradi, L., Tava, V., Kunova, A., Cortesi, P., Saracchi, M., and Pasquali, M. 2021. Telomere to telomere genome assembly of *Fusarium musae* F31, causal agent of crown rot disease of banana. Mol. Plant-Microbe Interact. 34: 1455-1457.

Denton-Giles, M., Bradshaw, R. E., and Dijkwel, P. P. 2013. *Ciborinia camelliae* (Sclerotiniaceae) induces variable plant resistance responses in selected species of *Camellia*. Phytopathology 103:725-732.

Huerta-Cepas, J., Szklarczyk, D., Forslund, K., Cook, H., Heller, D., Walter, M. C., Rattei, T., Mende, D. R., Sunagawa, S., Kuhn, M., Jensen, L. J., von Mering, C., and Bork, P. 2016. EgNOG 4.5: A hierarchical orthology framework with improved functional annotations for eukaryotic, prokaryotic and viral sequences. Nucleic Acids Res. 44:D286-D293.

Jones, P., Binns, D., Chang, H. Y., Fraser, M., Li, W., McAnulla, C., McWilliam, H., Maslen, J., Mitchell, A., Nuka, G., Pesseat, S., Quinn, A. F., Sangrador-Vegas, A., Scheremetjew, M., Yong, S. Y., Lopez, R., and Hunter, S. 2014. InterProScan 5: Genome-scale protein function classification. Bioinformatics 30:1236-1240.

Koren, S., Walenz, B. P., Berlin, K., Miller, J. R., Bergman, N. H., and Phillippy, A. M. 2017. Canu: Scalable and accurate long-read assembly via adaptive-mer weighting and repeat separation. Genome Res. 27:722-736.

Li, H. 2018. Minimap2: Pairwise alignment for nucleotide sequences. Bioinformatics 34:3094-3100.

Li, Z., and Liu, T. 2022. High-quality genome sequence data of *Trichoderma gracile* HK011-1, a fungal antagonistic agent against plant pathogens. Plant Dis. 10:1035-1038.

Lin, Y., Yuan, J., Kolmogorov, M., Shen, M. W., Chaisson, M., and Pevzner, P. A. 2016. Assembly of long error-prone reads using de Bruijn graphs. Proc. Natl. Acad. Sci. U.S.A. 113:E8396-E8405.

Mikheenko, A., Prjibelski, A., Saveliev, V., Antipov, D., and Gurevich, A. 2018. Versatile genome assembly evaluation with QUAST-LG. Bioinformatics 34:i142-i150.

Palmer, J. M., and Stajich, J. 2020. Funannotate v1.8.1: Eukaryotic genome annotation. Zenodo, Geneva.

Saracchi, M., Colombo, E. M., Locati, D., Valenti, l., Corneo, A., Cortesi, P., Kunova, A., and Pasquali, M. 2022. Morphotypes of *Ciborinia camelliae* Kohn infecting camellias in Italy. J. Plant Pathol. 104:761-768.

Saracchi, S., Locati, D., Colombo, E. M., and Pasquali, M. 2019. Updates on *Ciborinia camelliae*, the causal agent of camellia flower blight. J. Plant Pathol.101:215-223.

Simão, F. A., Waterhouse, R. M., Ioannidis, P., Kriventseva, E. V., and Zdobnov, E. M. 2015. BUSCO: Assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics 31:3210-3212.

Valenti, I., Degradi, L., Kunova, A., Cortesi, P., Pasquali., M., and Saracchi, M. 2022. The first mitochondrial genome of *Ciborinia camelliae* and its position in the Sclerotiniaceae family. Front. Fungal Biol. 2:802511.

Walker, B. J., Abeel, T., Shea, T., Priest, M., Abouelliel, A., Sakthikumar, S., Cuomo, C. A., Zeng, Q., Wortman, J., Young, S. K., and Earl, A. M. 2014. Pilon: An integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963.