Draft Genome of the Macadamia Husk Spot Pathogen, *Pseudocercospora macadamiae*

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Abstract

Pseudocercospora macadamiae causes husk spot in macadamia in Australia. Lack of genomic resources for this pathogen has restricted acquiring knowledge on the mechanism of disease development, spread, and its role in fruit abscission. To address this gap, we sequenced the genome of *P. macadamiae*. The sequence was de novo assembled into a draft genome of 40 Mb, which is comparable to closely related species in the family Mycosphaerellaceae. The draft genome comprises 212 scaffolds, of which 99 scaffolds are over 50 kb. The genome has a 49% GC content and is predicted to contain 15,430 protein-coding genes. This draft genome sequence is the first for *P. macadamiae* and represents a valuable resource for understanding genome evolution and plant disease resistance.

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Keywords

analytical plant pathology, mycology, whole genome sequencing

Genome Announcement

Macadamia is the most economically important tree nut crop in the family Proteaceae (Nock et al. 2014). Macadamias are cultivated for their edible cream-colored nut (kernel), which is enclosed in a very hard shell and husk (pericarp). The four species in the genus *Macadamia* (*M. integrifolia*, *M. tetraphylla*, *M. janssenii*, and *M. ternifolia*) originate from the tropical forest fringes of coastal southeast Queensland and northeast New South Wales in Australia (Gross and Weston 1992). *M. integrifolia*, *M. tetraphylla*, and their hybrids are cultivated in commercial orchards in several tropical and frost-free subtropical regions in Australia, South Africa, Kenya, Brazil, Hawaii, China, Vietnam, Guatemala, Mexico, Columbia, and Malawi. In Australia, *Pseudocercospora macadamiae* infects the macadamia fruit pericarp causing husk spot (Beilharz et al. 2003). Husk spot is a serious disease of macadamia, resulting in extensive premature fruit abscission, giving rise to nuts with low oil content (Akinsanmi et al. 2007; Beilharz et al. 2003). Infection occurring during the early phase of fruit development may have an incubation period up to 3 months before symptoms are expressed (Akinsanmi et al. 2007, 2016; Miles et al. 2010). Moreover, the biomass of *P. macadamiae* in infected tissue increases with symptom development (Ong et al. 2016). Husk spot symptoms appear in the fruit pericarp at near fruit maturity as chlorotic flecks and progress to necrotic lesions (Miles et al. 2010; Miles et al. 2009). Fruit abscission due to husk spot commences from the chlorotic-symptom stage (Akinsanmi et al. 2016); however, the mechanisms that trigger husk spot symptom expression and fruit abscission of diseased fruit are not well understood. In macadamia, high levels of abscisic acid (ABA) were associated with period of abscission of immature fruit and *P. macadamiae* was shown to produce ABA in vitro (McConchie and Salter 2005). Therefore, *P. macadamiae* possibly contributes to the induction of the fruit abscission process in macadamia (Akinsanmi et al. 2016; McConchie and Salter 2005).

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Table 1. Metrics of the draft genome of *Pseudocercospora macadamiae* (isolate BRIP 55526)

Features	Statistics
Estimated genome size (bp)	40,070,143
Number of scaffolds ≥ 2 kb	212
L50 scaffold (bp)	608,373
N50 scaffolds	19
Maximum scaffold length (bp)	3,067,473
Maximum contig length (bp)	3,067,473
Number of scaffolds >50 kb	99
GC content (%)	49%
Predicted protein-coding genes	15,430
Median protein length	321
Mean protein length	463.32
Complete BUSCOs (C)	1,287
Complete and single-copy BUSCOs (S)	1,285
Complete and duplicated BUSCOs (D)	2
Fragmented BUSCOs (F)	5
Missing BUSCOs (M)	23
Total BUSCO groups searched	1,315

Phylogenetic analysis of the 28S ribosomal RNA gene revealed that *P. macadamiae* falls within the *Pseudocercospora* genus complex (Ong et al. 2016). The *Pseudocercospora* genus complex accommodates most *Pseudocercospora* species, representing a wide range of host plant species, including those species of *Pseudocercospora* with publicly available genomes and known metabolic profiles including *P. fijiensis* (previously *M. fijiensis*), the causal agent of black Sigatoka disease of banana (Crous et al. 2012). Like *P. fijiensis*, and many other hemibiotrophic fungal pathogens, *P. macadamiae* may produce metabolites and proteins that kill the host tissue, which could influence the virulence and adaptation of the pathogen (Chang et al. 2016; Noar and Daub 2016). However, there is no information on the repertoire of effectors and toxic metabolites produced by *P. macadamiae* during host infection and disease development.

In this study, we used a holotype isolate of *P. macadamiae* (BRIP 55526) that was collected from a husk spot lesion of the macadamia cultivar H2 (Hinde) obtained from a commercial orchard in the Glasshouse Mountains, Queensland, Australia. A monoconidial culture of the isolate was grown on half-strength potato dextrose agar (Difco Laboratories Inc., Sparks, MD) and the genomic DNA was extracted as described by Ong et al. (2016). Libraries were prepared according to the manufacturer's protocol using a Nextera DNA Flex Library Preparation Kit (20018705, Illumina, San Diego, CA). The library was prepared for sequencing on a NextSeq500 (Illumina) using NextSeq 500/550 High Output v2 2 $\times$ 150 bp paired-end chemistry at the Australian Centre for Ecogenomics according to the manufacturer's protocol. Raw paired-end reads were quality-checked and low-quality reads and adaptors were removed using Trimmomatic v0.36 (Bolger et al. 2014). Contigs with lengths less than 2,000 bp were removed using BBDMap (ver. 36.92) (Bushnell 2014) and the quality of the genome assembly was calculated using the Benchmarking Universal Single-Copy Orthologs tool (BUSCO v3.02) (Simão et al. 2015) with the Ascomycota lineage dataset. De novo assembly was performed using Spades v3.13 (Bankevich et al. 2012) from both the paired-end reads and resulted in 212 scaffolds with an average size of 608,373 bp. Ninety-nine scaffolds were over 50 kb. The whole-genome assembly resulted in a draft genome with an overall GC content of 49%, and the full genome size is estimated to be 40.0 Mb. Statistics of genome sequencing and assembly are summarized (Table 1). Most hits from the BLAST analysis correspond to sequences of the genera *Pseudocercospora* and *Passalora*. RepeatMasker v4.0.8 (Tarailo-Graovac and Chen 2009) was used to identify and mask repeat regions and GeneMark v4.38 (Besemer and Borodovsky 2005) was used to predict protein-coding genes within the masked scaffolds using the self-training and fungus algorithm options. Functional annotation of protein sequences was conducted using BLASTP within DIAMOND v0.9.22 (Buchfink et al. 2015). Protein sequences were aligned against Uniref100 (release 2018-3) (UniProt Consortium 2010) with an associated KEGG ID (Kanehisa et al. 2007) and Swissprot (release 2019-01) (Boeckmann et al. 2003) databases. In addition, eggNOG-mapper (Huerta-Cepas et al. 2017) was used to improve the functional annotation of the predicted proteins and 7,415 were assigned to orthologous group.

Gene prediction analysis yielded a total of 15,430 protein-coding genes. To calculate the average amino acid identity (AAI) between the predicted proteome and the species present in the UniProt database (UniProt Consortium 2017), we ran the predicted proteins through the

software AAI profiler (Medlar et al. 2018). The analysis revealed the three organisms with the greatest similarity to *P. macadamiae* were *P. fijiensis* (AAI = 81%), *P. eumusae* (AAI = 80%), and *P. musae* (AAI = 80%). RepeatMasker identified 6,120 repeats in the *P. macadamiae* genome, which included 5,643 simple repeats and 477 low complexity repeats, and TransposonPSI (<http://transposonpsi.sourceforge.net>) predicted 3,604 transposable elements that belonged to various transposon classes. The genome annotation completeness was assessed using BUSCO (Simão et al. 2015), which identified 1,285 complete benchmarking universal single-copy orthologs (BUSCOs) in the database of ascomycetes. SignalP v5.0 (Armenteros et al. 2019) predicted 389 secreted proteins, and EffectorP 2.0 (Sperschneider et al. 2018) predicted 66 of these as effectors. In addition, TMHMM 2.0 (Krogh et al. 2001) predicted that 2,955 of 15,430 (19.2% of all proteins) are transmembrane helices and out of the 66 proteins that were predicted as effectors, four were predicted to be transmembrane helices. The potential of *P. macadamiae* to degrade and metabolize a range of polysaccharides was evaluated with the automated carbohydrate active enzymes (CAZymes) annotation software dbCAN (Zhang et al. 2018), which predicted 676 putative CAZymes (HMMER, E-value < 1e-15, coverage > 0.35). Of the six major superfamilies, glycoside hydrolases (GH) is the most prevalent, representing 45.6% of the total predicted CAZyme, followed by auxiliary activities (18.9%), glycosyl-transferases (16.4%), carbohydrate esterase (15.7%), carbohydrate-binding module (2.1%), and polysaccharide lyases (1.3%). Similar percentages of GH was found in the CAZyme of other closely related *Pseudocercospora* species, *P. fijiensis* (47.2%), *P. musae* (45.3%), and *P. eumusae* (47.1%) (Chang et al. 2016). The genome sequence of *P. macadamiae* is an important resource for future comparative genomic analyses with other *Pseudocercospora* species and useful for improved understanding of the pathogen biology, epidemiology, and host–pathogen interaction.

Accession number. The assembled genome sequence of *P. macadamiae* isolate BRIP 55526 has been deposited in GenBank under the accession number WRNY00000000. The data for this experiment have been submitted to NCBI with SRA submission SUB6642804.

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