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RESOURCE ANNOUNCEMENT

Whole Genome Sequence Resource of the Asian Pear Scab Pathogen *Venturia nashicola*

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Abstract

Venturia nashicola, the cause of scab disease of Asian pears, is a host-specific, biotrophic fungus. It is restricted to Asia and is regarded as a quarantine threat outside this region. European pear displays nonhost resistance (NHR) to *V. nashicola* and Asian pears are nonhosts of *V. pyrina* (the cause of European pear scab disease). The host specificity of these two fungi is likely governed by differences in their effector arsenals, with a subset hypothesized to activate NHR. The *Pyrus-Venturia* pathosystem provides an opportunity to dissect the underlying genetics of nonhost interactions in this potentially more durable form of resistance. The *V. nashicola* genome will enable comparisons to other *Venturia* spp. genomes to identify effectors that potentially activate NHR in the pear scab pathosystem.

Genome Announcement

Venturia nashicola Tanaka et Yamamoto is restricted to causing scab disease on Japanese (*Pyrus pyrifolia* var. *culta*) and Chinese (*P. ussuriensis* and *P. bretschneideri*) pears (Abe et al. 2008; Ishii and Yanase 2000; Li et al. 2007; Tanaka and Yamamoto 1964). Despite a brief incursion in France (Le Cam et al. 2001), *V. nashicola* is limited to Asia and is regarded as a quarantine threat elsewhere (Jeger et al. 2017). *V. nashicola*, an ascomycete fungus, produces sexual fruiting bodies in dead leaf tissue, releasing ascospores in rain in spring. This primary inoculum results in biotrophic infections of the cuticle and subcuticular space of young leaves and fruit. Sporulating lesions release asexual conidia that burst through the cuticle, leading to deformed, cracked, unmarketable fruit and up to 30% yield loss (Eguchi and Yamagishi 2008). Multiple secondary infections produce conidia during the growing season until autumn, when the leaves fall and pseudothecia are then produced in the dead leaves over winter.

V. nashicola was previously regarded as a synonym of *V. pyrina* (syn. *V. pirina* [Rossman et al. 2018]), however Ishii and Yanase (2000) described *V. nashicola* as a distinct species based on morphology, sexual incompatibility, and pathogenicity with *V. pyrina*, which is restricted to infecting European pear (Abe et al. 2008; Bell 1990; Ishii et al. 2002; Ishii and

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Keywords

fungal effectors, fungus-plant interactions, genomics, mechanisms of pathogenicity, metabolomics, nonhost resistance, proteomics

Yanase 2000); species delineation was further supported with molecular analyses (Le Cam et al. 2001; Zhao et al. 2011). The incompatible pear scab interactions are regarded as examples of nonhost resistance (NHR) (Abe et al. 2000; Bus et al. 2013; Heath 2000; Park et al. 2000; Won et al. 2014). These fungal species are unable to hybridize; however, the host species can form viable hybrids, making the *Pyrus-Venturia* pathosystem excellent for dissecting the role of NHR in determining host-specificity (Won et al. 2014). We hypothesize that a subset of the pathogen effector arsenals (secreted proteins that govern virulence and pathogenicity) activate NHR in the *Pyrus-Venturia* pathosystem. Scab is currently controlled by 15 or more fungicide applications per year (Ishii and Yanase 2000; Ishii et al. 1985). However, resistance to fungicides (such as benzimidazoles and sterol demethylation inhibitors) is an increasing issue (Ishii 2012). There are currently no major commercial cultivars of Asian pears with resistance to *V. nashicola* (Ishii et al. 1992; Park et al. 2000); therefore, breeding for scab resistance is a high priority for Asian pear breeders. Very recently, an interspecific commercial pear cultivar, 'Yutaka', has been reported to be highly resistant to both scab and black spot (pathogen, *Alternaria alternata* Japanese pear pathotype) diseases (Ishii and Kimura 2018).

V. nashicola isolate Yasato 2-1-1 (MAFF 306981; NARO [National Agriculture and Food Research Organization] Genebank project, Japan) was sampled from 'Kousui' pear (*P. pyrifolia* var. *culta*) from Ibaraki, Japan (Ishii et al. 2002). Genomic DNA (gDNA) was extracted as per Kucheryava et al. (2008). A DNA library was generated using the Illumina Nextera mate-pair protocol, and a paired-end (PE) genomic library using the KAPA hyper prep kit. Libraries were sequenced in PE mode with 125-bp reads on the Illumina HiSeq1500.

Transcripts from *V. pyrina* isolates ICMP 11032 and P35.2 (New Zealand isolates from European pear cultivar Winter Nelis in Havelock North, Hawke's Bay, New Zealand) were used to assist *V. nashicola* Yasato 2-1-1 gene prediction. Transcripts were generated from *V. pyrina* isolate ICMP 11032 grown in vitro on cellophane as per Kucheryava et al. (2008). RNA was extracted following the method described by Chang et al. (1993). RNA-seq libraries were prepared using an Illumina Truseq stranded mRNA library preparation kit (Illumina) and were sequenced in PE mode as 125-bp reads on an Illumina HiSeq1500 at La Trobe University. Transcripts produced during infection were generated as follows: conidial suspension of about 2×10^5 conidia/ml from *V. pyrina* isolate P35.2 was used to inoculate grafted clones of two interspecific hybrids of *Pyrus* (PEAR1 and PEAR2), previously described by Won et al. (2014), and two of their progeny (BAG 6305 and BAG 6379), along with *Malus* × *domestica* ('Royal Gala'). Leaf samples were collected at 3 and 10 days postinoculation, each with three biological replicates. RNA was extracted from ground leaf tissue using the RNA plant spectrum kit (Sigma Aldrich), following manufacturer protocol. RNA extracts of high quality were checked using an Agilent2100 Bioanalyzer (Agilent Technologies). RNA from the 60 samples was sequenced in PE mode as 125-bp reads at the Australian Genome Research Facility Melbourne, on the HiSeq2500, across four lanes of a flow cell.

Initially, the *V. pyrina* mRNA sequences were mapped to the *V. pyrina* genome (Cooke et al. 2014) to retrain AUGUSTUS (Stanke et al. 2006) and build a parameter profile for *Venturia* spp. Evaluation of the retraining using the combined set of RNA-seq data showed a nucleotide, exon, and gene-level sensitivity and specificity of 0.984/0.992, 0.91/0.92, and 0.834/0.857, respectively. A total of 1,834,814 *V. pyrina* mRNA reads and 10,993 venturiales-identical protein groups were mapped to the *V. nashicola* genome. With these two sets of alignments as hints and applying the parameter profile trained for *Venturia* spp., we predicted genes on the repeat-masked *V. nashicola* genome with AUGUSTUS (Stanke et al. 2006) via the BRAKER2 pipeline (Hoff et al. 2016; Stanke et al. 2006).

Sequence quality of raw mate pair (MP) reads were checked with FastQC v0.11.2 (S. Andrews). The PCR duplication levels in sequencing data were assessed and genome size was estimated using string graph assembler (SGA) v0.10.13/PreQC (Simpson and Durbin 2014), and the reads were separated into multiple categories: PE reads with short insert size, MP reads with large inserts, and single reads. The PE sequences were cleaned using Trim Galore v0.4.3, where five bases from the 5′ end were trimmed and adapters were culled with a quality cut-off of 28. Overlapping reads in the cleaned PE data were merged, using PEAR v0.9.10 (Zhang et al. 2014), to build longer reads. Gene expression data from the closely related species *V. pyrina* as well as all venturiales proteins extracted from the National Center for Biotechnology Information (NCBI) Identical Protein Groups database (2004, cited 2018 03 01).

MEGAHIT v1.1.1 (Li et al. 2015) was then used to assemble the merged long reads with the rest of the PE data. Resulting contigs were assembled into scaffolds using the MP

Table 1. Genome scaffolding statistics

Metric	Megahit_scaffolded
N	40,800 bp
SUM	45,407,238 bp
N50	69,684 bp
BUSCO	
Complete	96.9%
Missing	2.4%
Fragmented	0.7%
Genes	11,094
Predicted secreted proteins[a]	1,232
Predicted effector proteins[b]	273

[a] Proteins harboring predicted signal sequence as determined by SignalP v4.0 (Petersen et al. 2011).
[b] Secreted proteins predicted to be effectors as determined by EffectorP v2.0 (Sperschneider et al. 2018).

data with SSPACE-LongRead v2.0 (Boetzer and Pirovano 2014). Repeats were identified using RepeatModeler v1.0.8 (A.F.A. Smit and R. Hubley 2008-2015) and masked using RepeatMasker v4.0.5 (A.F.S. Smit, R. Hubley, and P. Green 2013-2015) resulting in a whole-genome shotgun (WGS) sequence of 45 Mb (scaffolds ranged from 200 to 552,647 bp, N_{50} of 69,684 bp). The genome completeness level was assessed based on the coverage of representative genes in the BUSCO v2.0 (Simão et al. 2015) gene sets.

All annotated predicted proteins were analyzed with SignalP v4.0 (Petersen et al. 2011) to predict the presence of N-terminal secretion signals. Predicted secreted proteins were then analyzed with EffectorP v2.0 (Sperschneider et al. 2018) to determine the abundance of predicted effector proteins. Gene annotation completeness was assessed using BUSCO (version 3.0) (Simão et al. 2015) to determine the presence of conserved, single-copy orthologs from the ascomycota lineage.

The draft WGS assembly of *V. nashicola* isolate Yasato 2-1-1 is a fragmented genome. Despite this fragmentation, BUSCO completeness analyses of the assembled genome revealed 96.9% of core fungal genes are represented in this assembly (Table 1). There were 11,094 predicted genes, which is similar to the 11,960 predicted for *V. pyrina* (Deng et al. 2017). Prediction of isoforms was permitted if supported by evidence, but little alternative splicing was predicted, with 10,977 genes with one predicted isoform, 111 with two, and six with three. Overall, 37% of protein-coding gene models are supported with evidence from *V. pyrina* protein or mRNA evidence, or both, along an average of 34% of the length of the transcript. BUSCO analysis was also used to assess the completeness of the predicted gene set. When compared with the "fungi" BUSCO set, 285 of 290 orthologs (98.3%) were complete and single copy (with one duplicated, three fragmented, and one missing). When compared with the "ascomycota" BUSCO set, 1,292 of 1,315 (97.7%) of orthologs were complete and single copy (with three duplicated, 21 fragmented, and six missing). This shows an improvement of the level of completeness of the total gene set following gene prediction. Therefore, we conclude that this gene set represents an accurate and mostly complete representation of *V. nashicola* genes. A total of 1,232 proteins were predicted to have a putative secretion signal and approximately 22% are predicted effectors (Table 1).

This draft WGS of *V. nashicola* will enable the identification of candidate effectors and genes involved in fungicide resistance and contributes to the current genomic resources for *Venturia* spp., specifically *V. pyrina* (Cooke et al. 2014), *Fusicladium effusum* (pecan scab fungus) (Bock et al. 2016), *V. inaequalis* (apple scab fungus) (Deng et al. 2017), and *V. carpophila* (peach scab fungus) (Chen et al. 2017). These resources will facilitate the identification of effectors involved in host specificity, both within Asian pears and, also, the broader NHR of European pear. Effector-assisted breeding will be used to screen for durable disease resistance in pear breeding programs. The WGS will also be useful for identifying essential pathogenicity factors, for example, genes governing specific host-pathogen interactions, and the identification of fungicide-targeted protein genes, which will likely be involved in fungicide resistance. Comparative genomics of the pear scab pathogens will also provide an opportunity to identify unique targets for molecular diagnostics of the quarantine pathogen *V. nashicola* from the other *Venturia* species.

All sequences for this genome sequencing project have been deposited to the NCBI Short Read Archive under the accession number SRX4051481. The BioProject designation for this project is PRJNA439019. This WGS project has been deposited at DDBJ/ENA/GenBank under the accession VCHV00000000, BioProject PRJNA439019, BioSample SAMN08741156. The version described in this paper is version VCHV01000000.

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Author-Recommended Internet Resources

FastQC v0.11.2: http://www.bioinformatics.babraham.ac.uk/projects/fastqc
NCBI Identical Protein Group website: https://www.ncbi.nlm.nih.gov/ipg
RepeatMasker v4.0.5 and RepeatModeler v1.0.8: http://www.repeatmasker.org
Trim Galore v0.4.3: https://www.bioinformatics.babraham.ac.uk/projects/trim_galore

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