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Genetic diversity and gene expression analysis of *Phytophthora pluvialis*, a foliar pathogen of conifers

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Abstract

Phytophthora pluvialis is the causal agent of red needle cast on *Pinus radiata* in New Zealand. It was first isolated in 2008 but had previously been recovered from tanoak (*Notholithocarpus densiflorus*) and Douglas fir (*Pseudotsuga menziesii*) trees in Oregon, USA in 2002. *Phytophthora pluvialis* was subsequently described as a new species in 2013 and classified as a clade 3 *Phytophthora* species. The aims of this study were to (1) gain a better understanding of the genetic diversity and population structure of *P. pluvialis* and (2) examine gene expression profiles of *P. pluvialis* from naturally infected *P. radiata* seedlings. Studying the genetic diversity and population structure of *P. pluvialis* provided insight into the mode of reproduction of this pathogen and helped determine if *P. pluvialis* was introduced into New Zealand. This information is also important for the development of management strategies for *P. pluvialis.* Twenty-seven single nucleotide polymorphism (SNP) markers were designed to genotype a total of 360 isolates of *P. pluvialis* collected from New Zealand and the USA. The genotypic data showed that the population in New Zealand has lower diversity than the USA population. A minimum spanning network (MSN) showed two unique clusters in the New Zealand population, suggesting there may have been two separate introductions of *P. pluvialis*. For the second study, samples were collected from 45 *P. radiata* grafted plants that were part of a field trial, with the aim of identifying genes that are highly expressed and may be important for virulence. Interestingly, *Phytophthora kernoviae* was found in more of the samples than *P. pluvialis.* Needle samples were collected, RNA was extracted and sequenced, and the normalised reads that mapped to the genome of *P. pluvialis* were compared to those from *P. pluvialis* grown in culture. Differentially expressed genes (DEGs) of *P. pluvialis* that showed higher expression in the field trial included potential orthologs of sugar transporter, GH12 and effector genes with known pathogenicity functions in other species. This is the first study to examine the genetic diversity of *P. pluvialis* in New Zealand and the USA., and to examine the gene expression of a *Phytophthora* forest pathogen in the field. The results from these studies provide useful tools for forest disease management. The SNP markers can be used to monitor the population of *P. pluvialis* in New Zealand. The highly expressed genes can be used to help identify resistance genes in *P. radiata* that can be incorporated into future breeding programs.

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Abbreviations

AFLP	Amplified fragment length polymorphism
AMOVA	Analysis of molecular variance
AA	Auxiliary activity
bp	Base pair
CBM	Carbohydrate binding module
CE	Carbohydrate esterase
CAzy	Carbohydrate-active enzymes
CRN	Crinkler effectors
DEG	Differentially expressed genes
He	Expected heterozygosity
FPKM	Fragments per kilobase per million reads
GO	Gene ontology
GBS	Genotype by sequencing
GH	Glycoside hydrolase
GT	Glycosyl transferase
HTHF	Healthy Trees Healthy Future MBIE research programme
Ha	Hectare
IGV	Integrated Genomics Viewer
ITS	Internal transcribed spacer
IBD	Isolation by distance
KAAS	KEGG automatic annotations
KEGG	Kyoto Encyclopedia of Genes and Genomes
µl	Microlitre
µM	Micromolar
ml	Millilitre
MSN	Minimum spanning network
min	Minutes
MLG	Multilocus genotype
Ho	Observed heterozygosity
PAMP	Pathogen-associated molecular pattern
PNB	Physiological needle blight
PTA	*Phytophthora agathidicida /* Phytophthora taxon agathis
PGPR	plant growth-promoting rhizobacteria
PL	Polysaccharide lyase
PCA	Principal component analysis
RNC	Red needle cast
RIN	RNA integrity number
RNA-Seq	RNA sequencing
SSR	Simple sequence repeats (microsatellite markers)
SNP	Single nucleotide polymorphism marker