Effector delivery and effector characterisation in Dothistroma needle blight of pines

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Abstract

The filamentous fungus *Dothistroma septosporum* causes a serious foliar disease, Dothistroma needle blight (DNB), on *Pinus radiata* in New Zealand and on many pine species worldwide. Potentially correlated to changes in climate, this disease has been on the rise for 20 to 30 years, and current countermeasures often struggle to contain the damage it causes. A molecular approach to combat DNB could be promising. Effectors are small proteins secreted by pathogens to promote host colonisation, and have been a major focus of plant pathologists in recent years. However, effector biology in pathogens of gymnosperms has received little research attention. Here, candidate effectors (CEs) were selected using a series of computational prediction tools, as well as RNAseq data from a compatible *D. septosporum*-–pine interaction. A shortlist of 55 highly *in planta* expressed CEs, predicted to be secreted to the apoplast, was characterised *in silico*. While almost half of them lacked a predicted function, none were exclusive to *D. septosporum*. Seventeen effector candidates of particular interest were taken forward for functional characterisation. Specifically, these proteins were screened for induction of plant defences in the form of cell death in the model plants *Nicotiana benthamiana* and *N. tabacum* using an *Agrobacterium* transient expression assay. Five CEs induced cell death in these plants, suggesting recognition by the plant defence machinery. Of those five, three are similar to previously described proteins. Effector screening methods are not available for pine, thus various approaches to achieve this were trialled. A high-throughput method to collect each protein in the apoplastic wash fluid of *N. benthamiana* was developed. This fluid was applied to *P. radiata* shoots raised from tissue culture to screen for a response, with promising results. Along with an array of *D. septosporum* CEs, these shoots may ultimately be used to screen for resistant pine genotypes. Selection of genotypes at this early stage could speed up DNB resistance screening for pine breeding and the protocol could be transferred to related pathosystems. This research also contributes to the molecular understanding of forest diseases and effectors that may be common among pathogens of distantly related hosts.

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Acronyms & units

aa	Amino acid
ATTA	*Agrobacterium* transient transformation assay
Avr	Avirulence factor
AWF	Apoplastic wash fluid
bp	Base pair(s)
cDNA	Complementary DNA
CE	Candidate effector
cm	Centimetre
d	Day(s)
dH_2O	Distilled water
DNA	Deoxyribonucleic acid
DNB	Dothistroma needle blight
dNTP	Deoxynucleotide triphosphates
dpi	Day(s) post inoculation
Ecp	Extracellular protein
fmol	femtomol
g	Gram(s)
h	Hour(s)
IP	Invasion pattern
kDa	Kilo-Dalton
kV	Kilovolt
l	Litre(s)
M	Molar (1 M = 1 mol per litre)
μg	Microgram(s)
μl	Microlitre(s)
μM	Micromolar
mg	Milligram(s)
ml	Millilitre(s)
mm	Millimetre(s)
mM	Millimolar
MQ	Milli-Q
ms	Millisecond(s)
min	Minute(s)
ng	Nanogram(s)
n/a	Not applicable
NR	Neutral red
nt	Nucleotides
OD	Optical density
opm	Oscillations per minute
pg	Picogram
PAMP	Pathogen-associated molecular pattern
PCR	Polymerase chain reaction
PRR	Pattern recognition receptor
qPCR	Quantitative PCR
RNA	Ribonucleic acid
RPMK	Reads per million per kilobase
s	Second(s)
SM	Secondary metabolite
v/v	Volume per volume (%)
w/v	Weight per volume (%)

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