Genetic analysis of host and phosphite mediated resistance to *Phytophthora cinnamomi* in *Arabidopsis thaliana*

by

Leila Eshraghi

B.Sc. (Plant Pathology), The University of Tabriz, Iran

M.Sc. (Plant Pathology), The University of Western Australia, Australia

This thesis is presented for the degree of Doctor of Philosophy of Murdoch University

2012

Declaration

The work described in this thesis was undertaken while I was an enrolled student for the degree of Doctor of Philosophy at Murdoch University, Western Australia. I declare that this thesis is my own account of my research and contains as its main content work which has not previously been submitted for a degree at any tertiary education institution.

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Leila Eshraghi
March 2012

Abstract

Phosphite (Phi), an analogue of phosphate (Pi) is highly effective for the control of *Phytophthora cinnamomi*, a devastating necrotrophic pathogen worldwide. This study describes the effect of phosphite (Phi) on the induction of defence responses in *Phytophthora cinnamomi*-infected *Arabidopsis thaliana* accessions Ler and Col-0, and mutants defective in salicylic acid (SA), jasmonic acid (JA), ethylene (ET), abscisic acid (ABA), phosphate starvation response (PSR) and auxin response signalling pathways. The inoculation of the resistant Col-0 with *P. cinnamomi* induced a rapid increase in callose deposition (by 6 h after inoculation) and hydrogen peroxide (H_2O_2) production (by 24 h after inoculation) whereas inoculation of susceptible Ler showed a delayed and reduced response. Treatment of Ler with Phi produced a response to *P. cinnamomi* inoculation similar to that observed in Col-0 in terms of timing and magnitude suggesting Phi primes the plant for a rapid and intense response to infection involving heightened activation of a range of defence responses.

A reliable method for measuring disease progression is important when evaluating susceptibility in host–pathogen interactions. A sensitive quantitative polymerase chain reaction (QPCR) assay was developed for the quantitative measurement of *P. cinnamomi* DNA (biomass) *in planta* that avoids problems caused by variation in DNA extraction efficiency and degradation of host DNA during host tissue necrosis. Purified plasmid DNA, containing the pScFvB1 mouse gene, was added during DNA extraction and the pathogen's biomass was normalized based on plasmid DNA rather than host DNA or sample fresh weight. It was demonstrated that normalization of pathogen DNA to sample fresh weight or host DNA in samples with varying degrees of necrosis led to an overestimation of the pathogen's biomass.

Inoculation of mutants in the SA, JA, and ET defence signalling pathways did not affect the resistance of Col-0 suggesting alternative pathways are involved. A high level

susceptibility was observed in the *aba2-4* mutant suggesting a role for ABA signalling in the induction of resistance to *P. cinnamomi*. Phi treatment of *aba2-4* increased resistance but not to the wild type levels indicating a possible role for ABA-dependent and ABA independent signalling in Phi mediated resistance. Application of Phi to non-inoculated *A. thaliana* seedlings elevated transcription of defence genes in the SA (*PR1* and *PR5*) and JA/ET (*THI2.1* and *PDF1.2*) pathways. Furthermore, analysis of gene expression in Col-0 revealed that either Phi or *P. cinnamomi* caused the down-regulation of the transcriptional level of *AtMYC2* (a positive regulator of ABA signalling which also negatively regulates JA-related genes) and increased the transcriptional abundance of *PDF1.2*. Together these results suggest that the resistance response of Col-0 and Phi treatment both act partially through an ABA dependent mechanism which is independent of the antagonism between ABA and elements of the JA/ET pathway such as *PDF1.2*.

Phosphite has been suggested to interfere with various plant processes including Pi homeostasis therefore the potential involvement of the Pi and auxin signalling pathways in resistance to *P. cinnamomi* was investigated using several PSR and auxin response pathway mutants. The mutants *tir1-1*, an auxin response mutant deficient in the auxin-stimulated SCF (Skp1−Cullin−F-Box) ubiquitination pathway and *phr1-1*, a mutant defective in response to Pi starvation were highly susceptible to *P. cinnamomi* compared to their parental background Col-0. Complementation restored resistance to the level observed in Col-0. Moreover, inhibition of auxin transporters by TIBA (2,3,5-triiodobenzoic acid) led to a significant increase in susceptibility of *Lupinus angustifolius* seedlings to *P. cinnamomi* supporting the importance of the auxin signalling pathway in *P. cinnamomi* resistance. The 26S proteasome subunits mutants; *rpn10-1* (Defective in ubiquitin/26S proteasome-mediated proteolysis) and *pbe1-1* (proteasome subunit beta type-5-A) were also susceptible to *P. cinnamomi*. The *rpn10-1*

mutant has also been associated with the auxin signalling pathway and the susceptibility of *rpn10-1* and *pbe1-1* indicates that the 26S proteasome and auxin signalling could play a role in resistance to *P. cinnamomi*. Given the apparent involvement of auxin and PSR signalling in the resistance to *P. cinnamomi*, the possible involvement of these pathways in Phi mediated resistance was also investigated. Application of Phi at both low and high concentrations attenuated some of the Pi starvation inducible genes such as *At4*, *AtACP5* and *AtPT2*. However, in phosphate sufficient plants, Phi treatment mimicked Pi starvation responses in terms of enhanced expression of *PHR1*, *AUX1*, *AXR1*, *AXR2* and *SGT1B*; suppression of primary root elongation, and increased root hair formation. Together, these results suggest that the auxin response pathway, particularly auxin sensitivity and transport, plays a role in the plant's resistance to *P. cinnamomi* and suggest that phosphite-mediated resistance may in some part be through its effect on stimulation of the auxin response pathway.

Statement of the contributions of jointly authored papers

The following manuscripts have either been published or have been prepared/submitted to scientific journals.

Chapter 2: Eshraghi, L., Anderson, J., Aryamanesh, N., Shearer, B., McComb, J., Hardy, G. E. S. and O'Brien, P. A. (2011) Phosphite primed defence responses and enhanced expression of defence genes in *Arabidopsis thaliana* infected with *Phytophthora cinnamomi*. *Plant Pathology*, 60, 1086-1095.

The contribution of work for this paper is 90% by the candidate of this thesis, Leila Eshraghi including the design, performance and analysis of experiments and writing of the manuscript, and 10% for all other authors in terms of advice on the experimental design, approach and revising the manuscript.

Chapter 3: Eshraghi, L., Aryamanesh, N., Anderson, J. P., McComb, J., Hardy, G. E. S., Shearer, B. and O'Brien, P. A. (2011) A quantitative PCR assay for accurate in planta quantification of the necrotrophic pathogen *Phytophthora cinnamomi*. *European Journal of Plant Pathology*, 131, 419-430.

The contribution of work for this paper is 90% by the candidate of this thesis, Leila Eshraghi including the design, performance and analysis of experiments and writing of the manuscript, and 10% for all other authors in terms of advice on the experimental design, approach and revising the manuscript.

Chapter 4: Eshraghi, L., Anderson, J., Aryamanesh, N., Shearer, B., McComb, J. and Hardy, G. E. S. (2012) Defence signalling pathways involved in plant resistance and phosphite-mediated control of *Phytophthora cinnamomi*. Submitted to *Planta* (March 2012).

The contribution of work for this paper is 90% by the candidate of this thesis, Leila Eshraghi including the design, performance and analysis of experiments and writing of the manuscript, and 10% for all other authors in terms of advice on the experimental design, approach and revising the manuscript.

Chapter 5: Eshraghi, L., Aryamanesh, N., Anderson, J. P., McComb, J., Shearer, B. and Hardy, G. E. S. (2012) Suppression of auxin response pathway enhances susceptibility to *Phytophthora cinnamomi* and phosphite stimulates *Arabidopsis* auxin signalling pathway. Prepared for *BMC Biology*.

The contribution of work for this paper is 90% by the candidate of this thesis, Leila Eshraghi including the design, performance and analysis of experiments and writing of the manuscript, and 10% for all other authors in terms of advice on the experimental design, approach and revising the manuscript.

Candidate, Leila Eshraghi
Signature: ……

Coordinating Supervisor, Professor Giles Hardy
Signature: ……

Conference publications pertaining to this thesis

Eshraghi L, Aryamanesh N, Anderson A J, McComb J, Hardy G E S, Shearer B & O'Brien P A (2011). Quantification of necrotrophic pathogen biomass using an internal control in a real-time PCR assay. In 'Asian Association of Societies for Plant Pathology (AASPP) and the Australasian Plant Pathology Society (APPS). 26-29 April 2011 Darwin, Australia.

Eshraghi L, Aryamanesh N, Anderson A J, McComb J, Hardy G E S, Shearer B & O'Brien P A (2011). Evaluating the role of defence pathways of *Arabidopsis thaliana* in resistance to *Phytophthora cinnamomi*. In 'Asian Association of Societies for Plant Pathology (AASPP) and the Australasian Plant Pathology Society (APPS). 26- 29 April 2011 Darwin, Australia.

Eshraghi L, McComb J, Hardy G E S & O'Brien P A (2008). The role of Phosphite in inducing resistance to *Phytophthora cinnamomi* in *Arabidopsis thaliana*. In '9th International Congress of Plant Pathology', 24–29 August 2008; Turin, Italy.

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Acknowledgments

I would like to convey my sincere gratitude to my supervisors Professor Giles Hardy, Prof. Jen McComb, Adjunct Prof. Dr Bryan Shearer and Dr Jonathan Anderson. I am grateful for their continual advice, motivation, knowledge and encouragement, which enabled me to push through the hard times during my PhD research.

This project would not have been accomplished without the generous support and guidance of Dr Nader Aryamanesh from the University of Western Australia at every step of the journey.

To the many friends and colleagues who have enriched my life, I offer my gratitude. Particular thanks go to my closest friends Miles, Janja, Rebecca, Stephan, Papori, Tina, Vanessa and Julie for creating such a great atmosphere in the School of Biological Sciences and made this time memorable for me.

I would like to thank A/Prof. Carolyn Jones, A/Prof. Graham O'Hara, Prof. Bernard Dell, Mrs Karen Olkowski and many members of Murdoch University for their assistance and support throughout my research. I would like also to thank Dr Philip O'Brien, Mr Gordon Thomson and Dr Wayne Reeve from School of Biological Sciences and Biotechnology at Murdoch University, Prof. Hans Lambers from School of Plant Biology at UWA, Ms Ricarda Fenske and Prof. Ian Small from ARC Centre of Excellence in Plant Energy Biology, Dr Dave Berryman and Frances Brigg from the State Agriculture Biotechnology Centre (SABC) and Prof. Karam Singh from CSIRO Plant Industry for their assistance in my project and the use of their facilities.

And most importantly, thanks to my brothers Omid and Vahid for their unconditional love and support in my entire life. You are the best companions I could wish for and I could not have achieved this without your love.

Dedicated to

MY PARENTS

for their ongoing support and

keen interest in my study and

teaching me the values of hard work

and discipline.

List of abbreviations

Δct	Change in threshold cycle
ABA	Abscisic acid
ANOVA	Analysis of variance
AGI	*Arabidopsis* Genome Initiative
ARG	Auxin responsive genes
ARF	Auxin response factor
ASK	*Arabidopsis* SKP1-like
AUX/IAA	Auxin/indole-3-acetic-acid
AXR	Auxin resistant
COI	Coronitine insensitive
COP	Constitutive photomorphogenic
CTR	Constitutive triple response
Ct	Threshold cycle
CUL	Cullin
CV	Coefficient of variation
E1	Enzyme 1 (same as UBA, ubiquitin activating enzyme)
E2	Enzyme 2 (same as UBC, ubiquitin conjugating enzyme)
E3	Enzyme 3 (same as ubiquitin protein ligase)
EIN	Ethylene insensitive
ET	Ethylene
ETI	Effector-triggered immunity
ETR	Ethylene receptor
ERF	Ethylene response factor
FBX2	F-box protein 2
GFP	Green fluorescent protein

GUS	Beta-glucuronidase
GMO	Genetically Modified Organisms
HR	Hypersensitive response
IAA	Indole-3-acetic acid
ISI	Induces systemic resistance
JA	Jasmonic acid
LRR	Leucine-rich repeat
MAPK	Mitogen-activated protein kinase
MES	2-morpholinoethanesulfonic acid
Myc	Epitope tag from c-Myc protein
NIM	Non-inducible immunity
NO	Nitric oxide
NPR	Non-expressor of pathogenesis-related genes
PAMP	Pathogen associated molecular pattern
Pc	*Phytophthora cinnamomi*
PCR	Polymerase chain reaction
PCD	Programmed cell death
PDF	Plant defensin
Pi	Phosphate
Phi	Phosphite
PHR1	Phosphate starvation response 1
PIN	Pin-formed
PPCK1	Phosphoenolpyruvate carboxylase kinase 1
PR	Pathogenesis-related
PSR	Phosphate starvation response
PTI	PAMP-triggered immunity

QPCR	Quantitative polymerase change reaction
R gene/protein	Resistance gene/protein
RAR	Required for MlA12 resistance
RBX	RING-box protein, same as ROC1 and Hrt1p
RING	Really interesting new gene protein domain
RNA	Ribonucleic acid
ROS	Reaction oxygen species
ROC	Regulator of cullins
RT	Reverse transcription
RNAase	Ribonuclease
SA	Salicylic acid
SAR	Systemic acquired resistance
SE	Standard error
SCF	Skp1-Cullin1-F-box
SGT	Suppressor of G2 allele of skp1
SKP	S phase kinase-associated protein
SON	Suppressor of nim1-1
T-DNA	Transfer DNA
THI	thionin
TIBA	2,3,5-triiodobenzoic acid
TIR1	Transport inhibitor response1
UBA	Ubiquitin activating enzyme
UBC	Ubiquitin conjugating enzyme
U-box	UFD2-homology domain
UPP	Ubiquitin proteasome pathway