Tagging Pathogenicity Genes in the Interaction of Barley and the Fungal Pathogen, *Rhynchosporium secalis*.

by

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Thesis Submitted for the Degree of Doctor of Philosophy

In the Discipline of Plant and Food Science,

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February, 2008

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Abstract

The purpose of this study was to identify pathogenicity genes in the fungal pathogen of cultivated barley, *Rhynchosporium secalis*. Pathogenicity genes are described as genes that are critical for the successful invasion and colonisation of the host plant but not necessary for life cycle completion in culture. To identify genes a pool of insertion mutants was generated.

Insertional mutants were generated by two methods, restriction enzyme-mediated integration (REMI) and *Agrobacterium tumefaciens*-mediated transformation (ATMT). A detailed REMI study showed circular pAN7-1 vector produced higher transformation efficiencies than linear vector at all enzyme levels tested. Fungal strain 5, in combination with 20 units of the restriction enzyme *Bam*HI produced the highest observed transformation efficiency with approximately 40% of these mutants producing simple, single integrations based on interpreted Southern data. The addition of *Bam*HI increased transformation efficiency at all enzyme levels tested with the exception of the highest enzyme concentration: 200 units of enzyme/transformation reaction. In comparison to REMI, the ATMT protocol proved more efficient than REMI and the binary vector backbone pPZP200 produced >50% simple single copy integrations, interpreted from Southern data. This study is the first ATMT protocol for *R. secalis* and was successfully adapted from other fungal species.

In total, 534 *Bam*HI and *Hind*III REMI mutants of *R. secalis* fungal strain UK7 (83) and strain 5 (453) were screened on the universally susceptible barley cultivar Sloop yielding 10 non-pathogenic mutants, eight from strain 5 and two from UK7, respectively.

During screening experiments strain 5 mutants failed to produce enough spores for a spore suspension to be prepared and inoculated. Strain 5 loses the ability to sporulate after four generations, or successive subculture steps. The inability to sporulate was not correlated to an observable, macroscopic loss in fungal biomass. Starvation experiments utilising carbon and nitrogen sources did not alter sporulation in the sporulating strain 5 sample or reverse the loss of sporulation. However, an overall trend was observed in the sporulation of strain UK7 where sporulation decreased with increasing nitrogen and increased with increasing carbon.

Genomic sequence flanking the integration site was isolated and analysed from six of the ten non-pathogenic mutants. Four putative genes were identified with integrations located in their putative promoter sequences. Sequence similarity searches showed three of these putative genes had similarities to amino acid permeases, cytochrome p450 and rhomboid-like genes. The two putative genes with similarities to amino acid permease and cytochrome p450 genes were selected for targeted gene disruption studies using homologous recombination (HR).

ATMT was used as the delivery system for the HR construct in an attempt to generate a disruption mutant and prove gene function. Over 200 mutants transformed with the two knock out vectors were screened. However, gene disruption experiments failed and could not be repeated due to a lack of resources and time.

In conclusion, this study has demonstrated that the REMI transformation technique is feasible for gene disruption studies in *R. secalis*. Furthermore, ATMT is a viable alternative transformation method that, for future studies, would be the preferable technique.

Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any University or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

Shae Yuill

October, 2008.

Acknowledgements

Firstly, to work under someone as world renowned in the field of plant-pathogen interactions as Dr. Wolfgang Knogge was a true blessing. I must thank Wolfgang for sharing his knowledge and his gentle and generous encouragement. To Dr. Peter Anderson and Dr. Carolyn Shultz, you have both had kind words and excellent insight whenever it was required. To all my supervisors, thank you so much for your editing, your advice and more than anything, your patience.

Help from our laboratory technician, Ms Annette Boettcher, was invaluable. It is my good fortune to have developed a lasting friendship with her and she is truly a scientist that deserves a higher title than technician. Dr. Angela Gierlich was generous in her provision of vectors for *Agrobacterium* studies and Dr. Slyvie Albert was responsible for the generation of UK7 REMI mutants used in this study. Thanks also to Dr. Steve Jefferies for Sloop seed supplies and Lindy Scott for use of her controlled environment rooms and access to *Rhynchosporium secalis* isolates.

Although not a single word is theirs, there are a number of people without whom a word would not have been written. I am most definitely one of the lucky one's to have the support of my family. My grandparents are a constant source of inspiration and remind me that when life is difficult, reflection on the World's recent history provides perspective on life. My parents have encouraged me in all aspects of my life and are responsible for sending me to university and have made a number of sacrifices to ensure that I would complete my studies. I am forever indebted to them for their support and encouragement.

My brother, Skye, was the first person to inspire me to greater heights in study. In fact, there has not been a better role model in my life to date. He and Matthew, my best friend for nearly 20 years, have always kept me laughing and shared in all my successes and failures.

If there was a single person that has been the most important in the completion of this thesis it is my wonderful partner, Amber. The one person who keeps me grounded and never fails to put a smile on my face when I truly need one.

Finally, to those who have not had a mention and deserve one my life is richer because of your presence.

Abbreviations

-wk	Week
-d	Day
h	Hour
min	Minute
sec	Second
ml	Millilitres
µl	Microlitres
g	Grams
mg	Milligrams
µg	Micrograms
(w/v)	Weight/volume
M	Molar
mM	Millimolar
mm	Millimetres
nm	Nanometres
UV	Ultraviolet
V	Volts
rpm	Revolutions per minute
REMI	Restriction enzyme-mediated integration
ATMT	*Agrobacterium tumefaciens*-mediated transformation
bp	Base pairs
n.d.	No data
NHEJ	Non-homologous end joining
PPS	Protruding single strand
ORF	Open Reading Frame
HR	Homologous recombination
RT-PCR	Reverse transcriptase-polymerase chain reaction
TAIL-PCR	Thermal asymmetric interlaced-polymerase chain reaction

PSS	Protruding single strand
BLAST	Basic logical alignment search tool
PDA	Pisatin demethylase
EST	Expressed sequence tag
GFP	Green florescent protein
gpdA	*gpdA* promoter
Amp	Ampicillin antibiotic resistance gene
hph	Hygromycin antibiotic resistance gene
Spec	Spectinomycin antibiotic resistance gene
Kan	Kanamycin antibiotic resistance gene
LB	Left border
RB	Right border
MAMPs	Molecular-associated molecular patterns
PAMPs	Pathogen-associated molecular patterns