Molecular characterisation of differentially expressed genes in the interaction of barley and *Rhynchosporium secalis*

Submitted by

Jafar Sheikh Jabbari, B.Sc., M.Sc.

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Discipline of Plant and Food Science

School of Agriculture, Food and Wine

Faculty of Sciences

University of Adelaide, Waite Campus

Glen Osmond, SA, 5064

Australia

STATEMENT OF AUTHORSHIP

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Jafar Sheikh Jabbari

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Publications

Conference Presentations:

- **Sheikh Jabbari**, J.; Langridge, P. and Oldach, K. (2006) Characterisation of a galactinol synthase and a Fiddlehead–like gene from barley. Proceedings of XV Federation of European Societies of Plant Biology Congress, Lyon/ France, 17-21st July 2006.

- **Sheikh Jabbari**, J.; Shirley, N.; Langridge, P. and Oldach, K. (2006) Characterisation of stress responsive genes from barley. Proceedings of the 8th International Congress of Plant Molecular Biology, Adelaide/Australia, August 20-25th 2006.

- **Sheikh Jabbari**, J.; Langridge, P. and Oldach, K. (2005). Characterization of differentially expressed genes in the interaction of barley and *Rhynchosporium secalis*. Proceedings of the 2nd ACPFG Annual Symposium, Clare Valley, SA/Australia 7-10th November 2005

List of Abbreviations

3'- or 5'-RACE	3' or 5' rapid amplification of cDNA ends
3'UTR	3' untranslated region
Avr	Avirulence gene
bp	Base pair(s)
BLAST	Basic Logical Alignment Search Tool
BSA	Bovine serum albumin
ºC	Degree Celsius
cDNA	Complementary DNA
Ci/mmol	Curies per millimole
CP	Cycling program
d	Day(s)
Da	Dalton
dCTP	Deoxycytidine triphosphate
DNA	Deoxyribose nucleic acid
DNase	DNA hydrolase
dNTP	Deoxynucleotide triphosphate
dsRNA	Double stranded RNA
DTT	Dithiothreitol
EDTA	Ethylene diamine tetra-acetic acid
E. coli	*Escherichia coli*
EST	Expressed sequence tag
Fdh	Fiddlehead
x g	Unit of relative centrifugal force
gDNA	Genomic DNA
G(s)OI	Gene(s) of interest
GolS	Galactinol synthase
GFP	Green fluorescent protein
Gus	β-glucuronidase gene
h	Hour(s)

hpi	Hours post-inoculation
HR	Hypersensitive response
INA	Iso-nicotinic acid
IPTG	Isopropyl-1-thio-b-D-galactosidase
ISR	Induced systemic resistance
JA	Jasmonic acid
kb	Kilobase pairs
KDa	Kilodalton(s)
l	Litre(s)
LB	Luria-Bertani
LBA	Luria-Bertani agar
LiBA	Lima bean agar
M	Molar
MCS	Multiple cloning site
ml	Milliliter
min	Minute(s)
mg	Milligram(s)
nm	Nanometer
MOPS	3-(N-morpholino) propanesulfonic acid
mRNA	Messenger RNA
Ni-NTA	Nickel-nitrilotriacetic acid
OD_{600}	Optical density at 600 nm
SDS-PAGE	SDS-Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
pI	Isoelectric point
Poly A^+	Polyadenylated
PR(s)	Pathogenesis related protein(s)
PVP	Polyvinyl pyrollidone
QTL	Quantitative trait loci
Q-PCR	Real-time Reverse Transcript PCR
RNA	Ribonucleic acid

RNase	RNA hydrolase
RNAi	RNA interference
rpm	Revolutions per minute
RT	Room temperature
RT	Reverse transcriptase
RT-PCR	Reverse transcription-polymerase chain reaction
SA	Salicylic acid
SAR	Systemic acquired resistance
Sarkosyl	N-lauroysarcosine
SDS	Sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
sec	Second(s)
TAE	Tris acetate EDTA
TE	Tris EDTA
T_m	Melting temperature
Tris	Tris[hydroxylmethyl] amino methane
Tris-HCl	Tris (hydroxymethyl) amino methane hydrochloride
µg	Micrograms(s)
µl	Microliter(s)
UV	Ultraviolet
v/v	Volume/volume
w/v	Weight/volume
x	Times
x-gal	5-bromo-4-chloro-3-indolyl-beta-D-galactopyrnoside

Summary

The barley scald pathogen (*Rhynchosporium secalis*) causes extensive economic losses, not only through lost product and quality, but also due to costs associated with chemical control. Economic and environmental impacts and the emerging resistance to fungicides and dominant resistance genes are reasons to understand molecular defence responses in order to develop new strategies to increase resistance of barley to this pathogen. In most pathosystems, defence gene expression in susceptible or resistant genotypes commonly differs quantitatively. Thus, differentially expressed genes between genotypes contrasting for response to infection by pathogens are considered candidate genes that have a role in resistance. This thesis presents functional analysis of a subset of genes isolated from a Suppression Subtractive Hybridisation library. The library was previously established and enriched for differentially expressed genes in epidermis of resistant and susceptible near-isogenic barley cultivars inoculated with *R. secalis*. Functional characterisation involved both investigating their putitative biochemical function as well as the genes' role(s) in biotic and abiotic stress responses.

Three cDNA clones from the library were selected based on the putative function of the encoded proteins and the full length of the clones and their homologues were isolated from cDNA and genomic DNA. One of the clones represented a member of the pathogenesis-related protein family 17 (PR-17). Southern hybridisation showed that a small multigene family encodes the barley PR-17 proteins. Three members were cloned with two of them being novel. The second clone was homologous to galactinol synthases (GolS) and Southern blot analysis indicated existence of two *GolS* genes in the barley genome and subsequently two *HvGolS* members were isolated. The last clone (a single gene) showed similarity to very long chain fatty acid elongases, which indicates its involvement in synthesis of cuticular waxes. A characterised Arabidopsis mutant named *fiddlehead* (*Atfdh*) was highly similar to this gene and it was named *HvFdh*.

Detailed expression analysis using Q-PCR, Northern blot analysis and publically available microarray data revealed that the isolated genes are regulated in response to a variety of abiotic and biotic stresses as well as different tissues during barley development. Under

some treatments expression patterns were consistent with their putative roles and in agreement with results of other studies. Nevertheless, in other treatments expression profiles were not in agreement with previous findings in other plants indicating potentially different stress adaptation mechanisms between species.

Further insight into the function of the encoded proteins was gained by their subcellular localisation using transient expression as GFP fusion proteins followed by confocal laser scanning microscopy. The results were in agreement with *in silico* predictions and their putative cellular function. In addition, a comprehensive list of homologous genes from other species was compiled for each gene by using public EST databases. Analyses of phylogenetic relationship and multiple sequence alignment of the homologues provided further clues to their function and conserved regions of the proteins.

HvPR-17 anti-fungal properties were investigated by heterologous protein expression in *E. coli* and subsequent *in vitro* bioassays using purified protein under different conditions against a number of phytopathogenic fungi. However, no anti-fungal activity was observed.

A construct with the *AtFdh* promoter driving the coding region of barley Fiddlehead was used for complementation of the Arabidopsis *fiddlehead* mutant to investigate functional orthology between these genes from dicots and monocots. The Arabidopsis *fiddlehead* mutant phenotype that shows contact-mediated organ fusion, germination of spore on epidermis and reduced number of trichomes was completely reverted by *HvFdh*.

Finally, more than fifty transgenic barley lines were regenerated over-expressing or suppressing one of the three genes. The analyses of the transgenic progeny exhibited some interesting developmental phenotypes and resistance to scald and drought tolerance. These lines are awaiting further experiments to investigate the effect of altered expression in conferring resistance to other pathogens and abiotic stress tolerance as well as biochemical analysis.

Collectively, in this work six barley genes were cloned and characterised by a variety of *in silico* techniques, temporal and transient expression analyses, subcellular localisation, *in vitro* bioassays and mutant complementation in Arabidopsis and loss- and gain-of-function

transgenic barley plants. This work has provided insight into the function of these gene families in barley. Furthermore, the data suggest that they are regulated by the defence response to pathogenic fungi as well as drought, salinity and frost in barley.