

ANALYSIS OF SIGNAL PATHWAY PROTEIN-PROTEIN INTERACTIONS DURING BIOTIC AND ABIOTIC STRESS

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Table of Contents

Table of Contents.....	I
Abstract.....	VII
Statement of Authorship.....	IX
Acknowledgements.....	XI
Abbreviations.....	XIII
1 Chapter 1 – General Introduction.....	1
1.1 Introduction	2
1.2 <i>Rhynchosporium secalis</i>	3
1.2.1 Scald disease	3
1.2.2 Infection process	3
1.3 Barley - <i>R. secalis</i> interaction	5
1.3.1 Resistance of barley to scald.....	5
1.3.2 The NIP1/Rrs1 gene-for-gene based resistance.....	6
1.3.3 The defence response in compatible and incompatible interactions.....	7
1.4 Control of scald disease	8
1.4.1 Current treatment methods.....	8
1.4.2 Identifying new resistance targets.....	9
1.4.3 Targeting defence signalling.....	10
1.5 Candidate genes.....	11
1.5.1 14.3.3's.....	11
1.5.1.1 <i>14.3.3-protein interaction and specificity</i>	12
1.5.1.2 <i>Mode of action/function</i>	14
1.5.1.3 <i>Role of 14.3.3s in signal transduction</i>	16
1.5.1.4 <i>Role of 14.3.3s in plant stress response signalling</i>	16
1.5.1.5 <i>Hv14.3.3c</i>	18
1.5.2 MAP kinases	19
1.5.2.1 <i>MAP kinase families and MAPKK's</i>	19
1.5.2.2 <i>MAP kinase signalling cascades</i>	20
1.5.2.3 <i>Role of MAP kinases in plant stress response signalling</i>	20
1.5.2.4 <i>HvMAPKK1</i>	24
1.5.3 Immunophilins and FKBP's	25
1.5.3.1 <i>Function of immunophilins</i>	25
1.5.3.2 <i>Plant FKBP's</i>	26
1.5.3.3 <i>Role of FKPB type immunophilins in signalling</i>	27
1.5.3.4 <i>Role of FKPB type immunophilins in plant stress response signalling</i>	27
1.5.3.5 <i>HvFKBP41</i>	28
1.5.4 Interaction of candidate genes	29
1.6 Project aims	30

2 Chapter 2 – Isolation of Full Length cDNAs and Expression Analysis.....31

2.1	Introduction	32
2.2	Materials and methods	35
2.2.1	Materials	35
2.2.2	Plant material	35
2.2.3	Fungal material	35
2.2.4	Inoculation	36
2.2.4.1	<i>R. secalis</i>	36
2.2.4.2	<i>NIP1</i>	36
2.2.5	Oligodeoxyribonucleotides	36
2.2.6	Polymerase chain reaction (PCR)	36
2.2.6.1	<i>Standard – Taq polymerase</i>	36
2.2.6.2	<i>Elongase PCR</i>	37
2.2.6.3	<i>HiFi PCR</i>	37
2.2.6.4	<i>Nested PCR</i>	37
2.2.6.5	<i>Semi-nested PCR</i>	37
2.2.6.6	<i>Colony PCR</i>	37
2.2.7	Agarose gel electrophoresis	38
2.2.8	DNA and RNA quantification	38
2.2.9	Cloning of PCR Products.....	38
2.2.9.1	<i>DNA purification - Gel extraction and PCR purification</i>	38
2.2.9.2	<i>Restriction endonuclease digestion of DNA</i>	38
2.2.9.3	<i>PolyA tailing</i>	38
2.2.9.4	<i>Ligation</i>	39
2.2.9.5	<i>Transformation of E. coli by heat shock</i>	39
2.2.9.6	<i>Transformation of E. coli by electroporation</i>	39
2.2.9.7	<i>Glycerol stocks</i>	40
2.2.9.8	<i>Plasmid DNA mini-preparation</i>	40
2.2.10	DNA sequencing.....	40
2.2.11	Bioinformatics	41
2.2.12	3' RACE	41
2.2.13	5' RACE	41
2.2.13.1	<i>SMART RACE</i>	41
2.2.13.2	<i>RLM-RACE</i>	42
2.2.14	Genomic walking.....	42
2.2.15	RNA extraction	42
2.2.16	cDNA synthesis	43
2.2.16.1	<i>DNase treatment</i>	43
2.2.16.2	<i>First-Strand cDNA synthesis</i>	43
2.2.17	Quantitative (real time) PCR	44
2.3	Results and discussion.....	45
2.3.1	Cloning of a Hv14.3.3c cDNA fragment.....	45
2.3.1.1	<i>Determining unknown sequence</i>	45
2.3.1.2	<i>Amplification and cloning of full length Hv14.3.3c</i>	45
2.3.2	Cloning of a HvMAPKK1 cDNA fragment	45

2.3.2.1	<i>Determining unknown sequence</i>	45
2.3.2.2	<i>3' RACE</i>	49
2.3.2.3	<i>5' RACE – SMART RACE</i>	51
2.3.2.4	<i>5' RACE – RLM-RACE</i>	51
2.3.2.5	<i>Genomic walking</i>	51
2.3.2.6	<i>Amplification and cloning of full length HvMAPKK</i>	54
2.3.3	<i>Cloning of a HvFKBP cDNA fragment</i>	59
2.3.3.1	<i>5' RACE</i>	59
2.3.3.2	<i>Genomic walking</i>	63
2.3.3.3	<i>Amplification and cloning of full length HvFKBP</i>	63
2.3.4	<i>Expression analysis</i>	63
2.3.4.1	<i>R. secalis infection</i>	68
2.3.4.2	<i>ABA</i>	71
2.3.4.3	<i>Frost</i>	73
2.3.4.4	<i>Drought</i>	76
2.3.4.5	<i>Salt</i>	78
2.4	Summary and conclusions	81

3 Chapter 3 – Identifying Interacting Proteins using Yeast Two-Hybrid Screening and Affinity Pulldowns83

3.1	Introduction	84
3.2	Materials and methods	87
3.2.1	<i>Materials</i>	87
3.2.2	<i>Polymerase chain reaction (PCR)</i>	87
3.2.3	<i>Cloning of PCR products</i>	87
3.2.3.1	<i>Restriction endonuclease digestion of DNA</i>	87
3.2.3.2	<i>Ligation</i>	88
3.2.4	<i>Generation of Y2H constructs</i>	88
3.2.5	<i>Generation of a Y2H prey library</i>	90
3.2.6	<i>Bait/prey vector swap</i>	90
3.2.7	<i>Yeast transformation</i>	92
3.2.8	<i>Screening for putative protein-protein interactions</i>	92
3.2.9	<i>Yeast DNA extraction</i>	93
3.2.9.1	<i>Zymolase treatment</i>	93
3.2.9.2	<i>Phenol/chloroform extraction</i>	93
3.2.10	<i>PCR amplification of interacting prey and identity determination</i>	94
3.2.11	<i>Large scale PCR purification</i>	94
3.2.12	<i>Transformation into E. coli</i>	95
3.2.13	<i>Self activation and co-transformation to confirm interactions</i>	95
3.2.14	<i>Generation of Hv14.3.3c E. coli expression construct</i>	95
3.2.15	<i>Expression induction in E. coli using IPTG</i>	95
3.2.16	<i>Purification of His-tagged protein</i>	96
3.2.17	<i>SDS-polyacrylamide gel electrophoresis (PAGE)</i>	96
3.2.18	<i>2 dimensional PAGE (2D PAGE)</i>	97
3.2.19	<i>Silver staining of proteins</i>	97

3.2.20	Western blots	97
3.2.21	Antibody production	98
3.2.22	Antibody purification.....	98
3.2.23	Affinity Pull-downs using recombinant Hv14.3.3c	98
3.2.23.1	<i>Dynabeads</i>	98
3.2.23.2	<i>Sepharose column</i>	99
3.2.23.3	<i>Sepharose resin</i>	100
3.2.24	Affinity-pulldowns using anti-Hv14.3.3c antibody	100
3.2.25	Mass-spectrometry.....	100
3.3	Results and discussion.....	101
3.3.1	Y2H screens	101
3.3.2	Hv14.3.3c Y2H screen.....	101
3.3.2.1	<i>Identifying interactors</i>	101
3.3.2.2	<i>Confirming the interaction</i>	109
3.3.3	HvMAPKK1 Y2H screen	110
3.3.3.1	<i>Identifying interactors</i>	110
3.3.3.2	<i>Confirming the interaction</i>	110
3.3.3.3	<i>Repeat screen</i>	110
3.3.4	HvFKBP Y2H screen.....	115
3.3.4.1	<i>Identifying interactors</i>	115
3.3.4.2	<i>Confirming the interaction</i>	121
3.3.5	Affinity-pulldowns.....	122
3.3.6	Heterologous expression of Hv14.3.3c protein	122
3.3.7	Affinity-pulldowns using recombinant Hv14.3.3c	122
3.3.7.1	<i>Dynabeads</i>	122
3.3.7.2	<i>Sepharose column</i>	124
3.3.7.3	<i>Sepharose resin</i>	127
3.3.8	Anti-Hv14.3.3c antibody	130
3.3.9	Affinity-pulldowns using anti-Hv14.3.3c antibody	130
3.4	Summary and conclusions	136

4 Chapter 4 – Isolation of Interactor Full Length cDNAs, Expression Analysis and Confirming the Hv14.3.3c-HvEPSP Interaction139

4.1	Introduction	140
4.2	Materials and methods	143
4.2.1	Materials	143
4.2.2	Quantitative (real time) PCR	143
4.2.3	<i>In Vitro</i> Transcription/Translation using wheat germ extract.....	143
4.2.3.1	<i>In Vitro</i> transcription/translation.....	143
4.2.3.2	<i>Co-immunoprecipitation from wheat germ extract</i>	143
4.2.4	Western blot analysis	144
4.2.5	Generation of HvEPSP <i>E. coli</i> expression construct	144
4.2.6	Expression induction in <i>E.coil</i> using auto-induction media	145
4.2.7	Purification of His-tagged HvEPSP protein	145
4.2.8	Co-immunoprecipitation of recombinant Hv14.3.3c and HvEPSP	145

4.2.9	Generation of BRET expression constructs.....	146
4.3	Results and discussion.....	147
4.3.1	Cloning of a <i>HvEPSP</i> synthase cDNA fragment.....	147
4.3.1.1	<i>Determining unknown sequence</i>	147
4.3.1.2	<i>Genomic walking and 5' RACE</i>	147
4.3.1.3	<i>Amplification and cloning of full length mature HvEPSP</i>	155
4.3.2	Cloning of a <i>HvWound</i> cDNA fragment.....	155
4.3.2.1	<i>Determining unknown sequence</i>	155
4.3.2.2	<i>Amplification and cloning of full length HvWound</i>	159
4.3.3	Cloning of a <i>HvGTPase</i> cDNA fragment	159
4.3.3.1	<i>Sequence analysis</i>	159
4.3.4	Expression analysis	164
4.3.4.1	<i>R. secalis infection</i>	164
4.3.4.2	<i>ABA</i>	166
4.3.4.3	<i>Frost</i>	166
4.3.4.4	<i>Drought</i>	169
4.3.5	Co-immunoprecipitation from wheat germ transcription/translation	169
4.3.6	Heterologous expression of <i>HvEPSP</i>	171
4.3.7	Co-immunoprecipitation of recombinant <i>Hv14.3.3c</i> and <i>HvEPSP</i>	172
4.3.8	Bioluminescence resonance energy transfer (BRET).....	177
4.4	Summary and conclusions.....	179
5	Chapter 5 – Conclusions and Future Work	181
	Appendix.....	185
	References.....	189

Abstract

The overall objective of the work described in this thesis was to characterise the three genes *Hv14.3.3c*, *HvMAPKK1* and *HvFKBP41*, in terms of a role in defence and stress response signalling. These genes had previously been found to be differentially expressed in compatible versus incompatible interactions of barley with the fungus *Rhynchosporium secalis*, suggesting a possible role in the plant defence response, while current literature suggests these genes may also play a role in signal transduction, possibly under a broad range of stresses, including abiotic as well as biotic.

Two main approaches were undertaken to characterise gene function: expression analysis and the identification of protein-protein interactions.

To facilitate expression analysis, full length cDNA fragments of each gene were first obtained using bioinformatics, RACE and genomic walking techniques. Expression was then investigated using quantitative real-time RT-PCR. The results of the expression analysis confirmed that the candidate genes were in fact differentially expressed during infection, suggesting a role in the defence response of barley against *R. secalis*. Analysing their expression in the context of other stresses and treatments, namely frost, drought and ABA, indicated their role may not be limited only to biotic stress, but include abiotic stress as well.

To investigate the possibility that these genes are involved in signalling during the defence response, protein-protein interaction techniques such as yeast two-hybrid and affinity pulldowns were used to identify interacting proteins in an attempt to place the genes within a known signalling network and build and extend on these networks.

Y2H screening was used successfully to identify two putative interactors of *Hv14.3.3c*; an EPSP (5-enolpyruvylshikimate-3-phosphate) synthase and a putative wound-induced protein, and two interactors of *HvFKBP41*; a Rab-type GTPase and the same wound-induced protein. From what is known about the function of these genes in the literature, they fit well with a role in stress response signalling and the potential to be involved in signalling networks with the candidate gene products and also with each other.

Through the trial of many different affinity pulldown techniques, a method for identifying interacting proteins from plant extracts was successfully established, however, issues with protein identification meant that interacting proteins were not identified using this technique.

Steps were then made towards confirming the interactions identified using the Y2H system. Full length cDNA sequences of the identified interactors were obtained and

expression analysis performed, in the aim of investigating co-expression patterns between the genes encoding the interacting proteins and the three candidate genes, to support a potential interaction.

To confirm the Hv14.3.3c-HvEPSP interaction, co-immunoprecipitation and BRET were then used, however confirmation was unsuccessful due to issues with non-specific binding in co-immunoprecipitation and technical issues trying to establish the BRET analysis system in barley.

In summary, the results of this study place the candidate genes *Hv14.3.3c*, *HvMAPKK1* and *HvFKBP41* as players in signal transduction during the plant defence/stress response. With the identification of previously uncharacterised protein interactions, some progress has also been made towards placing these genes within known signalling networks and identifying potential downstream genes that could possibly play a more specific role in defence response signalling and therefore be potential targets for the generation of resistant or stress tolerant plants.

Statement of Authorship

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

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Abbreviations

ACPFPG	Australian Centre for Plant Functional Genomics
AGRF	Australian Genome Research Facility
bp	Base pairs
d	Days
DMSO	Dimethylsulphoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide
DTT	Dithiothreitol
EDTA	Ethylene diamine tetra-acetic acid
EST	Expressed sequence tag
Fig	Figure
g	Grams
g	Units of relative centrifugal force
Gal4	Yeast transcriptional activator protein
GTP	Guanosine triphosphate
h	Hour
HEPES	(N-[2-hydroxyethyl] piperazine-N'-[2-ethanesulphonic acid])
HPLC	High performance liquid chromatography
IgG	Immunoglobulin G
IMVS	Institute of Medical and Veterinary Science
IPTG	Isopropylthiogalactoside
Kb	Kilo base
kDa	Kilo Dalton
kV	Kilo Volts
LB	Luria-Bertani
Luc	Luciferase
M	Molar
Min	Minutes
ml	Millilitre
mm	Millimetre
mM	Millimolar
mg	Milligrams
mRNA	Messenger RNA
ng	Nanograms
nm	nano metres
NSW	New South Wales
o/n	Overnight
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
QLD	Queensland
Q-PCR	Quantitative real-time PCR
RACE	Rapidly amplified cDNA ends
RLuc	<i>Renilla reniformis</i> Luciferase
RNA	Ribonucleic acid
rpm	Revolutions per minute
SARDI	South Australian Research and Development Institute

SD	Synthetic dropout
SDS	Sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
sec	Seconds
SSH	Suppressive subtractive hybridisation
SA	South Australia
TBS	Tris buffered saline
T _m	Melting temperature
Tris	Tris [hydroxymethyl] amino methane
U	Units
V	Volts
VIC	Victoria
v/v	Volume per volume
w/v	Weight per volume
X- α -Gal	5-Bromo-4-chloro-3-indolyl- β -D-galactopyranoside
YFP	Yellow fluorescence protein
YPD	Yeast potato dextrose
Y2H	Yeast two-hybrid
μ l	Microlitres
μ g	Micrograms
μ M	Micromoles
Ω	Ohm