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

Submitted by
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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

ACPFG	Australian Centre for Plant Functional Genomics
AGRF	Australian Genome Research Facility
bp	Base pairs
d	Days
DMSO	Dimethylsulphoxise
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] piperazidine-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 Whales
o/n	Overnight
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
QLD	Queensland
Q-PCR	Quantitative real-time PCR
RACE	Rapidly amplified cDNA ends
RLuc	*Renilla reniformis* 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
Tm	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-ß-D-galactopyranoside
YFP	Yellow fluorescence protein
YPD	Yeast potato dextrose
Y2H	Yeast two-hybrid
µl	Microlitres
µg	Micrograms
µM	Micromoles
Ω	Ohm