THE BARLEY EXPANSIN FAMILY

Submitted by

Maria Lombardi, B. PharmMedBiotech., B.Sc. (Hons)

A thesis submitted in fulfilment of the requirements

for the degree of Doctor of Philosophy

School of Agriculture, Food and Wine

University of Adelaide, Waite Campus

Glen Osmond, SA 5064

Australia

December 2011

TABLE OF CONTENTS

TABLE OF FIGURES

ABSTRACT

Expansins are plant proteins that have been shown to induce cell wall extension and stress relaxation under acid pH conditions. The expansin gene family has been investigated in *Arabidopsis*, rice, maize, tomato and wheat. In barley (*Hordeum vulgare*), however, no systemic identification or characterisation of expansin genes has been reported. This study was undertaken to characterise the expansin family in barley and to investigate the mechanism of action of expansins in the cell wall via heterologous expression of barley expansin genes in *Escherichia coli*.

The expansins are usually encoded by a superfamily of genes. On the basis of phylogenetic sequence analysis, four sub-families of expansins are currently recognised in plants and are designated α-expansins (EXPA), β-expansins (EXPB), expansin-like A (EXLA) and expansin-like B (EXLB). In Chapter 2 the analysis of barley EST data deposited in the public databases is described. This resulted in the discovery of 34 partial or complete barley expansin genes (17 EXPB, 14 EXPA and 3 EXLA). Primers for mRNA transcript studies using quantitative PCR (Q-PCR) across a range of tissues were designed for genes for which 3' untranslated region (3'UTR) sequences were available. The Q-PCR results and barley Affymetrix data discussed in Chapter 3 show that the barley expansin genes are transcribed across a wide range of tissues and at various stages of cell wall development. This matches previously published information that expansins participate in a diverse range of developmental processes, including seed germination, fruit softening, root development, leaf growth and stem elongation. Their mechanism of action is yet to be determined unequivocally but is believed to involve the disruption of hydrogen bonds between cellulose microfibrils and "cross-linking" glycans in the cell wall; this in turn is believed to facilitate the wall extension and stress relaxation processes mentioned above.

In order to investigate the mode of action of expansins in the cell wall, an efficient expression system was required to produce biologically active recombinant expansin protein to characterise the function of the expansins. Complementary DNAs were used to build constructs that allowed expression of three full-length expansin genes in *E. coli.* The expression studies in which a number of approaches were used to obtain active protein are presented in Chapter 4.

Finally, the potential roles of expansins amongst a host of other proteins involved in cell wall-modification are discussed, along with functional assay results and proposed commercial applications.

STATEMENT OF AUTHORSHIP

I, Maria Lombardi certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university of 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.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Maria Lombardi

December 2011

ACKNOWLEDGMENTS

Many people have contributed to this thesis in a variety of ways. There are too many to name individually therefore I wish to express my gratitude to some of the key people and acknowledge the University of Adelaide for providing the scholarship that supported this work.

First and foremost I would like to thank my supervisors Professor Geoff Fincher and Associate Professor Rachel Burton for their generous support and encouragement. In particular, I would like to thank Geoff for his invaluable discussions, patience, extra funding that supported this research and for the endless proofreading of my work. I am also very grateful to Rachel for her scientific advice and knowledge and appreciate all her contributions of time and ideas. She was my primary resource for getting my science questions answered and provided the barley developmental cDNA library as part of the Q-PCR analysis. I am thankful for the excellent example she has provided as a successful female molecular biologist and Associate Professor.

I am grateful to Dr. Neil Shirley for his contribution in the quantitative analyses of the expansin transcripts and data for the co-transcription of the expansins with other cell wall-related genes. Dr. Qisen Zhang provided me help with the heterologous expression and binding assay protocol to which I am appreciative of his time. I would also like to acknowledge the help of Natalie Kibble in aspects of the heterologous expression work.

I would like to thank all the past and present members of the Fincher laboratory for their advice and technical help in particular aspects of this work. For their support, encouragement and friendship I wish to thank Dr. Damian Drew, Jessica Smith, Margaret Buchanan, Jillian Taylor, Mahima Krishnan, Julian Schwedt, Ashley Tan, Sie Chuong Wong and Dr. Helen Collins.

Finally to my mother, siblings, extended family and friends. These past several years have not been an easy ride, both academically and personally. I thank them all for their unwavering support, patience and understanding, even when I was grumpy and for whom the completion of this thesis will be as much as a relief as it is to myself.

ABBREVIATIONS

°C	Degrees centigrade
Å	Angstrom
A	Absorbance
AXAH	Arabinoxylan arabinofuranohydrolase
AGPs	Arabinogalactan proteins
ATPase	Adenosine Triphosphatase
BLAST	Basic local alignment search tool
bp	Base pairs
BSA	Bovine serum albumin
CBD	Cellulose Binding Domain
CBH	Cellobiohydrolase
CesA	Cellulose-synthase
cDNA	Complementary DNA
cm	Centimetre
Csl	Cellulose synthase-like
d	Days
Da	Daltons
dap	days after pollination
DEPC	Diethylpyrocarbonate
DMSO	Dimethy sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
E. coli	*Escherichia coli*
EDTA	Ethylene diamine tetra-acetic acid
EST	Expressed sequence tag
EXLA	expansin-like A
EXLB	expansin-like B
EXPA	α-expansin
EXPB	β-expansin
FAE	Ferulic acid esters
fra	kotanin-like protein
g	Gram
GH45	Glycoside Hydrolase family 45
GRP	Glycine-rich protein
h	Hour
H^+	Hydronium
HPLC	High-performance liquid chromatography
HRGP	Hydroxyproline-rich glycoprotein
HvEXP	Barley expansin
Hz	Hertz
IMAC	Immobilised metal affinity chromatography
IPTG	Isopropylthiogalactoside
kb	Kilobase
kDa	Kilo Dalton
LB	Luria-Bertani
M	Molar
MCS	Multiple cloning site
MES	2-(N-morpholino) ethanesulfonic acid
mg	Milligram
min	Minutes
mm	Millimeters
mRNA	Messenger RNA
NCBI	National Centre for Biotechnology Information
NEB	New England Biolabs
NF	Normalisation Factor
ng	Nanogram
Ni-NTA	Nickel-nitrilotriacetic acid
nm	Nanometers
nr	non-redundant
OD	Optical density
OH	Hydroxyl group
•OH	Hydroxyl radical
ORF	Open Reading Frame
PBS	Phosphate buffered saline
PMSF	Phenylmethylsulphonyl fluoride
PCR	Polymerase chain reaction
pH	Potential of Hydrogen
PRP	Proline-rich protein
Q-PCR	Quantitative PCR
RACE	Rapid Amplification of cDNA Ends
RG-I	Rhamnogalacturonan I

RG-II	Rhamnogalacturonan II
RNA	Ribonucleic acid
rpm	Revolutions per minute
RT	Room temperature (22°C)
SDB	Super Duper Buffer
SDS	Sodium dodecyl sulphate
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
s	Seconds
TA	Transcript Assemblies
TC	Tentative Contig
TEMED	Tetramethylethylene-diamine
TIGR	The Institute of Genomic Research
T_m	Melting temperature
Tris	Tris[hydroxymethyl] amino methane
U	Units
UTR	Untranslated region
UV	Ultraviolet
µ	micro
v/v	Volume for volume
w/v	Weight for volume
x g	Units of centrifugal force
XTH	Xyloglucan endotransglycosylases/ Hydrolases