

THE BARLEY EXPANSIN FAMILY

Submitted by

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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 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.

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Maria Lombardi

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ABBREVIATIONS

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

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