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MOLECULAR CLONING AND CHARACTERIZATION OF BARLEY β-GLUCANASE GENES

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A thesis submitted in fulfilment of the requirements for the degree of Master of Agricultural Science.

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STATEMENT OF AUTHORSHIP

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ABBREVIATIONS

ABA	abscisic acid
Amp	ampicillin
ATP	adenosine 5'-triphosphate
bp	base pair
BSA	bovine serum albumin
CAT	chloramphenicol acetyltransferase
cDNA	complementary DNA
dCTP	2'-deoxycytidine 5'-triphosphate
DEPC	diethyl pyrocarbonate
DNA	deoxyribonucleic acid
dNTPs	deoxyribonucleoside triphosphates
DTT	dithiothreitol
E. coli	*Escherichia coli*
EDTA	ethylenediaminetetraacetic acid
GA	gibberellic acid
GUS	β-glucuronidase
IPTG	isopropylthio-β-D-galactoside
kb	kilobases
kDa	kilodaltons
mRNA	messenger RNA
PCR	polymerase chain reaction
PEG	polyethylene glycol
pI	isoelectric point
RACE	rapid amplification of cDNA ends
RNA	ribonucleic acid
SDS	sodium dodecyl sulphate
SSC	sodium saline citrate
SSPE	sodium saline phosphate EDTA
Tris	tris(hydroxymethyl)aminomethane
TSP	transcription start point
X-gal	5-bromo-4-chloro-3-indoyl-β-D-galactoside

SUMMARY

Barley (1→3)-β-glucanases (EC 3.2.1.39) and (1→3,1→4)-β-glucanases (EC 3.2.1.73) are β-glucan endohydrolases encoded by a single super gene family. They share 44%-80% positional identities at the nucleotide sequence level. In this work, genes encoding (1→3)-β-glucanase isoenzyme GI and (1→3,1→4)-β-glucanase isoenzyme EII have been isolated from a barley genomic library with cDNA probes and specifically designed oligonucleotides.

The nucleotide sequence of a 3,327 bp genomic fragment for the (1→3)-β-glucanase isoenzyme GI gene has been determined. This DNA fragment encodes a mature protein of 310 amino acid residues. The molecular weight of the protein, deduced from the DNA sequence, is consistent with that of the purified enzyme reported previously. Nucleotide sequence analysis of the gene indicates that no targeting signals are present and that the initiating Met residue is removed from the precursor protein during post-translational processing. These observations suggest that the isoenzyme GI is a cytosolic enzyme. The mature enzyme-coding region of the gene has a high G+C content (68.9%) that is commonly found in other barley genes, and an extreme bias towards the use of G and C (99.4%) in the third position of codons. No intron-like sequence is detected in the 5' flanking region or coding region of the isoenzyme GI gene. Nucleotide sequence analysis of the promoter region of the isoenzyme GI gene reveals several *cis*-acting elements related to tissue-specific, hormonal and developmental regulation, which may be characterized by promoter deletion analysis in the future.

The genomic clone for the (1→3,1→4)-β-glucanase isoenzyme EII has been positively identified by the sequence of a 300 bp region that corresponds to the COOH-terminal encoding region of the mature enzyme and the 3' untranslated region of a previously characterized cDNA. Soon after the genomic clone for the barley (1→3,1→4)-β-glucanase was isolated in the present work, the full sequence of the gene was published elsewhere. Therefore, the complete sequence analysis of the gene was

abandoned in favour of characterizing the gene encoding the (1→3)-β-glucanase isoenzyme GI.

It is clear that (1→3,1→4)-β-glucanases function as hydrolytic enzymes during the degradation of cell walls that obstruct translocation of other hydrolytic enzymes during germination. The expression of the (1→3,1→4)-β-glucanase genes has been shown by other workers to be regulated in a tissue-specific manner, and in response to phytohormones. Expression of the isoenzyme EII gene is germination-specific, and is only detected in the aleurone of germinated grain. The expression of the isoenzyme EII gene is enhanced by gibberellic acid (GA_3) and suppressed by abscisic acid (ABA). The isolation of a genomic clone for the isoenzyme EII now allows promoter deletion analyses to be undertaken in order to define the sequences representing regulatory *cis*-elements that are responsible for the regulation of gene expression.

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