Regulation of (1,3;1,4)-beta-glucan synthesis

in

barley (*Hordeum vulgare* L.) endosperm and leaf tissues

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Adelaide

By

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List of Abbreviations

ANOVA	Analysis of variance
bp	Base pair
cDNA	Complementary DNA
CDS	Coding DNA sequence
Csl	Cellulose synthase like
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleotide triphosphate
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
HPLC	High performance liquid chromatography
kb	Kilobase
M	Molar
OD	Optical density
PCR	Polymerase chain reaction
QTL	Quantitative trait locus
RNA	Ribonucleic acid
rpm	Revolutions per minute
SNP	Single nucleotide polymorphism

Table of Contents

List of Tables

List of Figures

List of Appendices

Abstract

This project was carried out to explore the regulatory mechanism for (1,3;1,4)-β-glucan synthesis in barley (*Hordeum vulgare* L.) in both endosperm and leaf tissues.

For the grain (1,3;1,4)-β-glucan content regulation study, transcriptional profiles of candidate genes involved in (1,3;1,4)-β-glucan synthesis and degradation, from the developing barley endosperm, were compared across parental lines that had been previously used for grain (1,3;1,4)-β-glucan QTL studies. Correspondence between differences in transcript levels of selected genes and the QTL detected in parental lines was analysed. In the high (1,3;1,4)-β-glucan parent of a mapping population with a grain (1,3;1,4)-β-glucan QTL near/at the location of the *HvCslF6* gene, *HvCSLF6* transcript levels increased sharply late in endosperm development. In contrast, *HvCslF6* transcript levels did not differ between parents of a mapping population in which no grain (1,3;1,4)-β-glucan QTL had been mapped near/at the *HvCslF6* gene. Co-transcription of a β-glucan exo-glucanase gene, *HvExoIV* gene with *HvCslF9* early in endosperm development and with *HvCslF6* late in endosperm development indicated that HvEXOIV could be involved in (1,3;1,4)-β-glucan synthesis.

It has been reported that leaf (1,3;1,4)-β-glucan is degraded when plants are incubated in the dark for prolonged periods and is re-synthesized upon re-exposure to a normal day/night cycle. Thus, to investigate the regulation of leaf (1,3;1,4)-β-glucan, the transcript levels of (1,3;1,4)-β-glucan synthase genes and related genes were profiled during (1,3;1,4)-β-glucan mobilization upon dark incubation. Some of the genes that responded to prolonged dark incubation showed diurnal transcription patterns, even in continuous darkness. Among the (1,3;1,4)-β-glucan synthase candidate genes, only *HvCslH1* was up-regulated upon dark incubation. Its transcripts quickly returned to control levels upon re-exposure to the normal day/night cycle. None of the (1,3;1,4)-β-glucan synthase genes were up-regulated upon re-

exposure to normal day/night cycles. Consistent with what was observed for *HvExoIV*, *HvCslF6* and *HvCslF9* in developing endosperm, *HvExoIV* seemed to exhibit co-transcription gene with the *HvCslH1*.

Declaration

I 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 whose due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in submission for any other degree or diploma in university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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