Investigating the synthesis and regulation of (1,3;1,4)-β-glucan biosynthesis

Submitted by

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A thesis submitted in fulfilment of the requirements

for the degree of Doctor of Philosophy

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February 2016

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Abstract

Cereals such as rice (*Oryza sativa* (*Os*)), barley (*Hordeum vulgare* (*Hv*)) and sorghum (*Sorghum bicolor* (*Sb*)) provide a considerable portion of our daily energy requirements. Their cell wall constituents, such as (1,3;1,4)-β-glucan, survive relatively intact through much of the upper human digestive system to reach the colon, where they are fermented by a range of commensal microorganisms. The products of this fermentation help reduce blood cholesterol levels and ameliorate diseases including coronary heart disease, type II diabetes and colorectal cancer. Efforts have therefore been directed toward understanding the regulation and mechanism of (1,3;1,4)-β-glucan biosynthesis to enhance the human health potential and industrial utility of cereal grain.

Numerous reports suggest that the *CELLULOSE SYNTHASE-LIKE F6* (*CslF6*) gene encodes the synthase responsible for producing the majority of (1,3;1,4)-β-glucan in cereals. These synthase genes contain species-specific polymorphisms that have been shown to influence the amount and structure of (1,3;1,4)-β-glucan produced when they are expressed heterologously in *Nicotiana benthamiana* and barley grain. Here, a chimeric approach exchanged sections of the barley (*Hv)* and sorghum (*Sb)* CSLF6 synthases to identify regions influencing (1,3;1,4)-β-glucan production and structure. Using this approach an 80 amino acid stretch, which contains the conserved TED and QxxRW motifs, was shown to be responsible for much of the difference in (1,3;1,4)-β-glucan production and structure between the barley and sorghum synthases. Of the six amino acid polymorphisms contained within this section, one affected polysaccharide structure whilst another dictated the amount of (1,3;1,4)-β-glucan.

Co-expression in *N. benthamiana* was used to investigate CSLF6 modulation and complex formation. Results from a variety of chimeric, truncated and mutated constructs suggest that a highly variable section of unknown function, termed the class-specific region (CSR), and the NH_2-terminal region of CSLF6 are separately able to mediate complex formation and increase (1,3;1,4)-β-glucan production. Expression of a construct missing the CSR indicated that the region was not structurally or functionally required for (1,3;1,4)-β-glucan

synthesis in *N. benthamiana*. A PilZ domain responsible for cofactor binding and cellulose synthase activation in bacteria was also identified at the COOH-terminal end of the NH_2-terminal region of CSLF6, and was shown to influence (1,3;1,4)-β-glucan production. Overall, the results presented here have furthered our understanding of the action of the CSLF6 isoform of the (1,3;1,4)-β-glucan synthase enzyme. This brings us closer to having the capacity to precisely control the synthase's function, and allowing the prospect of manipulating cereal tissues to contain the optimal amount of (1,3;1,4)-β-glucan with a defined structure for specific human health and industrial applications.

Declaration

I, George Dimitroff certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any 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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Chapter 2 has been presented as a publication in preparation. **Figure 2-1** and **Figure 2-2** contain work presented in my honours thesis (Dimitroff, 2011) and has been included here because it is a necessary contribution to the first publication in preparation.

George Dimitroff

February 2016

Acknowledgements

I would like to thank the following organisations and people for their roles in assisting me through my PhD project.

Thanks to the Australian Government, Department of Education and Training for providing an Australian Postgraduate Award, to the Grains Research and Development Corporation for providing a Grains Industry Research Scholarship, and to the ARC Centre of Excellence in Plant Cell Walls, University of Adelaide for providing an ARC COE Plant Cell Walls Scholarship and the facilities required to conduct the research.

To my supervisors Associate Professor Rachel Burton, Dr Alan Little and Professor Geoffrey Fincher, my most sincere gratitude for their guidance, mentorship and support throughout my studies. I would like to thank Professor Vincent Bulone, Professor Alison Roberts, Associate Professor Eric Roberts, Dr Matthew Tucker, Dr Natalie Betts, Dr Marilyn Hendersen, Dr Neil Shirley, Dr Yves Hsieh, Dr Rohan Singh, Mr Jelle Lahnstein, Mr Julian Schwerdt and Ms Shi Fang Khor for their technical knowledge, guidance and friendships. I would also like to thank all current and former members of the Plant Cell Wall group for their friendships and assistance; in particular Kuok Yap, Grace Ang, Ali Saleh Hassan, Christopher Hakachite, Ilaria Marcotuli, and Wai Li Lim for their endless laughter.

Thanks to all of my friends and family for their continued support, particularly my wife Sophia, mother Matoula and father Lio for his 'positive' criticism and weekly fishing trips.

Abbreviations

*As*GLO	*Avena sativa* globulin (oat, grain specific) promoter
BCSA	BACTERIAL CELLULOSE SYNTHASE SUBUNIT A
CesA	CELLULOSE SYNTHASE
CR	catalytic region
Csl	CELLULOSE SYNTHASE-LIKE
CSR	class specific region
DAP	days after pollination
DPI	days post infiltration
ExR	The CSLF6 'extra region'
F:R	firefly:*Renilla*
GFP	green-fluorescent protein from *Aequorea victoria*
GUS	beta-glucuronidase gene from *Escherichia coli*
HPLC	high performance liquid chromatography
Hv	*Hordeum vulgare*
Kb	kilobase
LarII	luciferase assay reagent II
LB	luria broth
OD_{600}	optical density at 600nm wavelength
Os	*Oryza sativa*

P-CR	plant-conserved region
Q-PCR	quantitative polymerase chain reaction
QTL	quantitative trait loci
Sb	*Sorghum bicolor*
S&GR	stop & glo reagent
TSS	transcriptional start site
35S	CamV35S constitutive promoter