Analysis of Cellulose Synthase Genes and Regulatory Elements in Barley and Arabidopsis.

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for the degree of Doctor of Philosophy

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Abstract

Genetic manipulation of cellulose synthase genes (*CesA*) might be expected to increase cellulose content and improve the stem strength of cereals. Such a possibility would have beneficial applications in stem lodging and producing crops with higher cellulose content for biofuel feedstocks. Thus, an attempt to manipulate cellulose content was made via three approaches: (1) Driving barley *HvCesA* genes using the CaMV 35S constitutive promoter. (2) Driving barley secondary cell wall (SCW) *HvCesA* genes with specific promoters from barley and maize, and (3) Identifying novel transcription factors interacting with *CesA* promoters and over-expressing those candidate transcription factors in transgenic Arabidopsis lines.

In Chapter 2, attempts to drive *HvCesA* genes involved in both primary and secondary cell wall biosynthesis via the CaMV 35S promoter are described. Five sets of barley plants transformed with primary cell wall (PCW) and SCW *HvCesA* genes were analysed for transcript levels, phenotype, tissue morphology, cellulose content and crystalline cellulose distribution in cell walls. Only the lines transgenic for the SCW *HvCesA* genes exhibited aberrant phenotypes. One of the two constructs showed a dwarfed phenotype, where xylem cells in the vascular tissue were irregular in shape and partially collapsed. Transcript silencing, together with cellulose content reduction, were also observed in the dwarf plants and TEM images showed a significant decrease in cell wall thickness. Overall, there was no increase in either crystalline cellulose content or in stem strength.

In Chapter 3, experiments are described that further investigated if specific barley or maize promoters could ameliorate the aberrant phenotypes reported in Chapter 2. Promoter/gene combination constructs made up of either a barley *HvCesA4* or maize *ZmCesA10* (orthologue) promoters fused with either the *HvCesA4* or *HvCesA8* gene were used for transformation. Phenotyping, transcript profiling, stem strength measurements and cellulose content determination were conducted. There was no reduction in stature for any T_o transgenic plants, although other aberrant phenotypes were observed. One out of four promoter/gene combinations, namely $HvCesA4_{pro}$*:HvCesA4,* produced an increase in crystalline cellulose content and stem strength. *In situ* PCR results showed that the *HvCesA4* transgene transcripts in this line were concentrated in the vascular bundles of the stem.

Finally, an attempt to manipulate cellulose content was conducted by over-expressing candidate transcription factors shown to interact with the *CesA4* promoter, as described in Chapter 4. Candidate transcription factors in apple (*Malus x domestica*) were identified using the dual luciferase transient expression system in tobacco, with Arabidopsis and barley *CesA* promoters as bait. Transgenic Arabidopsis lines transformed with two candidate MYB transcription factors were analysed for phenotype, transcript abundance and cellulose content. Over-expression of these transcription factors did not increase crystalline cellulose content in Arabidopsis, despite an induction of SCW *AtCesA* transcript levels.

In conclusion, the results presented demonstrate the importance of controlled expression of *HvCesA* genes to increase cellulose content and stem strength as well as the existence of a finely balanced regulatory system that controls the cellulose biosynthetic process.

Statement of Authorship

I, Hwei-Ting Tan 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. In addition, I certify that no part of this work will, in the future, be used in a submission 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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Hwei-Ting Tan

May 2013

Dedicated with love to

My late grandmother, Kim-Moy Sim,沈金妹

Lilian Chiok-Hoon Kam

Li-Qun Ho

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Abbreviations

AIR	Alcohol Insoluble Residue
ANOVA	Analysis of variance
BAC	Bacterial Artificial Chromosome
BLAST	Basic Local Alignment Search Tool
bp	Base Pairs
BSA	Bovine Serum Albumin
CaMV	Cauliflower Mosaic Virus
CBM	Carbohydrate-Binding Module
cDNA	Complementary DNA
CDS	Coding DNA Sequence
CesA	Cellulose Synthase
ChIP	Chromatin Immunoprecipitation Analysis
cm	Centimetre
Cps	Counts Per Second
CSC	Cellulose Synthase Complex
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide Triphosphate
EAR	Ethylene-Responsive Element Binding Factor-associated Amphiphilic Repression
E.coli	*Escherichia coli*
EDTA	Ethylene Diamine Tetra-acetic Acid
EMSA	Electrophoretic Mobility Shift Assay
EST	Expressed Sequence Tag
FAA	Formalin–acetic acid–alcohol
g	Gram
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
GL	Gigalitre
GFP	Green Fluorescent Protein
GUS	beta-Glucuronidase
h	Hour
H^+	Hydronium
HPLC	High Performance Liquid Chromatography
Hv	*Hordeum vulgare*
In situ PCR	*In situ* Polymerase Chain Reaction
ISSA	Induced Somatic Sector Analysis
kb	Kilobase
LB	Luria-Bertani
LUC	Luciferase
M	Molar
Md	*Malus x domestica*
Mg	Milligram
min	Minutes
miRNA	MicroRNA
mm	Millimetre

ms	Millisecond
MS	Murashige and Skoog medium
Mya	Million years ago
n	Nano
N	Newton
NOS	Nopaline Synthase
NCBI	National Center for Biotechnology Information
ºC	Degree Centigrade
OD	Optical Density
ORF	Open Reading Frame
Os	*Oryza* sativa (rice)
PBS	Phosphate Buffered Saline
PCA	Principle Component Analysis
PCR	Polymerase Chain Reaction
PCW	Primary Cell Wall
PLACE	Plant Cis-acting Regulatory DNA elements
Ptd	*Populus trichocarpa*
PTGS	Post-Transcriptional Gene Silencing
QTL	Quantitative Trait Loci
RE	Restriction Enzymes
REN	*Renilla*
RNA	Ribonucleic Acid
RNAi	RNA Interference
rRNA	Ribosomal Ribonucleic Acid
rpm	Revolutions Per Minute
RT	Room Temperature
SCW	Secondary cell wall
St	*Solanum tuberosum* (Potato)
TAIR	The Arabidopsis Information Resource
SuSy	Sucrose Synthase
TF	Transcription Factor
TGS	Transcriptional Gene Silencing
TSS	Transcription Start Site
µ	Micro
µFd	MicroFarad
µM	Micrometer
UDP	Uridine diphosphate
UTR	Untranslated Region
XG	Xyloglucan
YFP	Yellow Fluorescent Protein
Zm	*Zea Mays* (maize)