Cellulose, Stem Strength and the Endo-(1,4)-β-Glucanase Gene Family in Barley and Maize.

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DECLARATION

I, Margaret Buchanan, 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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ABSTRACT

Failure of the stem to support the grain head in cereals is a serious cause of economic loss to farmers worldwide and increasing the cellulose content of the cell wall has the potential to increase stem strength. The glycosyl hydrolase family 9 endo-(1,4)-β-glucanases are believed to have a role in cellulose synthesis. Plants containing mutants of the KORRIGAN-like endo-(1,4)-β-glucanase genes have perturbed cell walls and reduced cellulose. Also growth enhancement occurs in poplar when overexpressing a soluble endo-(1,4)-β-glucanase. However, little is known of the gene family in cereals. Examination of the GH9 endo-(1,4)-β-glucanase gene family in barley and maize was undertaken with exploration of the potential biological correlation between cellulase activity and stem strength.

A detailed phylogeny of the endo-(1,4)-β-glucanase family for barley, maize, sorghum, rice and *Arabidopsis* was performed using sequence data from various sources. Notwithstanding that the maize genome contains 29 endo-(1,4)-β-glucanase genes, including homoeologues and duplicates, the number of genes across the different species only varies between 23 and 25. The phylogenetic tree showed variations in clade structure between the cereals and *Arabidopsis* and indicated some differential gene loss and gain. Where information on map position was available, synteny between the cereals was examined and found to be strong. Along with comparative intron and exon gene structure studies, orthologous genes across barley, maize, sorghum and rice were identified. An evolutionary analysis provided evidence that sorghum was the donor genome in an allotetraploid event in maize that occurred 10-20 million years ago. It was also possible to differentiate between homoeologues resulting from the tetraploidisation of maize and gene duplications.

A transcript analysis for barley and maize was performed and indicated that some members of the endo-β-1,4-D-glucanase gene family are transcribed across a wide range of tissues while other genes are specific to one tissue. Furthermore, there are strong correlations between several members of the endo-(1,4)-β-glucanase family at the transcriptional level. Similar correlations were also shown to exist between members of the endo-(1,4)-β-glucanase family and other genes known to be involved in cell wall synthesis. This data also suggested that evolutionary conservation of transcription exists between orthologues in barley and maize.

Analysis of protein activity in barley was undertaken on plants that had been transformed with endo-(1,4)-β-glucanase genes using the 35s overexpression promoter. Stem strength of these transgenic plants was found to be positively correlated with cellulose levels. Plants of the T_1 generation showed strong phenotypic variation. A number of plants were dwarfed and had much reduced

levels of cellulose. It was speculated that gene inactivation of both transgene and the endogenous gene was producing „knockdown" plants. On the other hand, some overexpressing plants had higher levels of cellulose than the control plants, however, this was not strongly correlated with stronger stems.

Heterologous expression of barley endo-(1,4)-β-glucanases in microbial systems was attempted. In *E.coli* large amounts of protein were produced, but it was largely insoluble and inactive.

ABBREVIATIONS

AGRF	Australian Genome Research Facility
aa	Amino acid(s)
At	*Arabidopsis*
AX	arabinoxylan
BAC	bacterial artificial chromosome
BAC	bacterial artificial chromosome
BLAST	basic local alignment search tool
BMGY	Buffered glycerol complex medium
BMMY	buffered methanol complex medium
bp	base pairs
CAZy	carbohydrate active enzyme database
CBD	cellulose binding domain
CD	catalytic domain
cDNA	complementary deoxyribonucleic acid
CesA	cellulose synthase
cpm	cycles per minute
Cps	counts per second
Csl	cellulose synthase-like
DAP	days after pollination
DE	developing embryo
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleic acids: deoxyadenosine triphosphate, deoxycytosine triphosphate, deoxythymidine triphosphate and deoxyguanidine triphosphate
ECB	European corn borer
EDTA	ethylenediaminetetraacetic acid
ER	endoplasmic reticulum
ESTs	expressed sequence tags
GAX	galacturonoarabinoxylan
GH9	glycosyl hydrolase family 9
GSP	gene specific primer
GUS	B-glucuronidase
Hv	*Hordeum vulgare*
IPTG	isopropyl-thio-galactopyranoside
Ks	synonymous changes per synonymous site per year
LB	Luria broth
MLG	mixed linked glucans
MPSS	massively parallel signature sequencing
mRNA	messenger ribonucleic acid
mRNA	messenger RNA
Mya	million years ago

NCBI	National Centre for Biotechnology Information
Ni-NTA	Nickel-nitrilotriacetic acid
Ns	non-synonymous changes per synonymous site per year
NUP	nested universal primer
ORF	open reading frame
Os	*Oryza sativa*
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
PMSF	phenylmethylsulphonyl fluoride
ppm	parts per million
QPCR	real time quantitative polymerase chain reaction
RE	restriction enzyme
RNA	ribonucleic acid
RT	room temperature
Sb	*Sorghum bicolour*
SDS	sodium dodecyl sulphate
SIGNAL	Salk Institute Genomic analysis Laboratory
SMART RACE	switching mechanism at the 3‟ end of RNA transcript rapid amplification of cDNA ends
SSC	Sodium chloride/sodium citrate
TAE	Tris-Acetate-EDTA
TBS	Tris buffered saline
TE	Tris-EDTA
TIGR	The Institute of Genomic Research
tRNA	transfer RNA
UDP	uridine di-phosphate
UPM	universal primer mix
UTR	untranslated region
XET/XTH	xyloglucan endotransglycosylase/hydrolase
XG	xyloglucan
Zm	*Zea mays*