

Genome-level studies on late maturity alpha amylase and boron tolerance in wheat



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Declaration and List of Papers Published

I declare that this thesis is my own account of my research and contains as its main content, work that has not previously been submitted for a degree at any tertiary education institution.

Papers that have been published from the research described in this thesis are:

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Abstract

Under certain environmental conditions, some varieties of wheat synthesize the enzyme alpha amylase late in grain ripening, even in the absence of rain or sprouting. The resulting grain has a sound appearance but can be unsuitable for end-product applications due to the presence of late maturity alpha amylase (LMA) activity. Reduction of LMA and the development of cultivars tolerant to boron toxic soils are high priority traits in the WA wheat breeding program and the use of molecular markers closely linked to these traits for marker assisted selection (MAS) is highly desirable. The aims of this study were to take a genomics approach to provide detailed structural information for the region on wheat chromosome 7BL in which quantitative trait loci (QTLs) for LMA and boron tolerance (Bo1) have been mapped. Once the structure had been determined, this then laid the foundation for further studies to investigate the function of putative candidate genes identified within this region. The research involved the use of bioinformatic tools and rice/wheat synteny to investigate the structure of this chromosome region, followed by the use of molecular probes to isolate genomic DNA clones (BAC clones) corresponding to this region.

A two-step bioinformatics strategy was used, involving (1) alignment of portions of the wheat and rice genomes, to identify rice genomic regions syntenic to wheat group 7L and (2) selection of candidate genes from those regions of the rice genome. The selected candidate genes included an anion transporter, as a candidate gene for boron tolerance, and *GAMYB-like* genes, as candidate genes for LMA. The *GAMYB* class of transcription factors identified were of particular interest because of published literature indicating its importance in controlling α -amylase levels in cereal grains. The key

phenotype of interest in this thesis is LMA and different levels of expression of α -amylase are a key feature of this phenotype.

Molecular markers and candidate genes were then used to screen two BAC libraries, one derived from the French cultivar, 'Renan' and the other derived from *Aegilops tauschii* (the source of the D genome of wheat). About 300 BAC clones corresponding to the chromosome region of interest were obtained. Of these, 8 BAC clones (6 chosen through hybridization to a GAMYB-like probe, and 2 from wheat ESTs anchored to the rice genome) were selected for sequencing, allowing for the development of new microsatellite and single-nucleotide polymorphism (SNP) markers and for the discovery of novel transposable elements that provide a rich source of polymorphism for the development of additional markers. Novel microsatellite and SNP markers that were identified from the BAC clone sequence were mapped on the Cranbrook/Halberd doubled haploid (DH) mapping population. Markers were located to chromosomes 7AL, 7BL and 7DL. New markers derived from the BAC sequence information were used to anchor the BAC clones to the genetic map and develop a framework physical-genetic map. An automated annotation pipeline has been established and was used to annotate selected contigs of the sequenced BAC clones.

A new marker assisted selection strategy, termed Multiplex Trait Signature (MuTs) analysis, was developed and tested on 39 wheat cultivars of known LMA phenotype. MuTs provides a graphical genotype of individuals for a particular chromosomal region and is a convenient tool for interrogating genetic similarity in the individuals surveyed. Based on assays of 22 markers (12 spanning the LMA QTL on chromosome 7BL and 10 spanning the LMA QTL on chromosome 3BS) on these 39 wheat cultivars, it was found

that the varieties can be grouped according to pedigree and provides a tool for interpreting LMA status for a variety. Validation of the 7BL LMA and boron tolerance (Bo1) QTL regions was achieved using a targeted mapping approach using the doubled haploid population Pastor/RAC891 using published molecular markers and markers developed in this thesis. The main outcome of this study is that the genomic organisation of this region on chromosome 7BL is complex, and that the identification of candidate genes in wheat controlling 1) tolerance of cultivars to boron toxic soils and 2) pathways regulating the expression of LMA, is likely to involve the interplay of a network of regulatory genes.

Abbreviations

ABA	Abscisic acid
ABF	ABA/stress inducible bZIP transcription factors
ABRE	abscisic acid responsive element
<i>abi</i>	abscisic insensitive mutant gene (Arabidopsis)
AFLP	Amplified fragment length polymorphisms
ANGIS	Australian National Genomic Information Service
ASW	Australian Standard White
AWB	Australian Wheat Board
BAC	Bacterial Artificial Chromosome
BLAST	Basic Local Alignment Search Tool
BPBF	barley prolamin-box binding factor
BSA	Bulked Segregant Analysis
CCG	Centre for Comparative Genomics
cDNA	complementary DNA
CIMMYT	International Maize and Wheat Improvement Centre, Mexico
CS	Chinese Spring
DAF	Days after flowering
DH	Doubled Haploid
DNA	Deoxyribonucleic acid
DOE	US, Department of Energy
DPA	Days post anthesis
EERE	Energy Efficiency and Renewable Energy (DOE)
EST	Expressed sequence tag(s)
Fl-cDNA	Full-length cDNA clone
FPC	Fingerprint contig software program
GA	Gibberellic acid
<i>gai</i>	<i>gibberellic acid-insensitive</i> gene
GAMYB	Gibberellin MYB transcription factor
GARC	GA response complex
GARE	GA response element
GMPOZ	GAMYB-associated POZ protein

HFN	Hagberg falling number
HMW-GS	High molecular weight glutenins
HRT	<i>Hordeum</i> repressor of transcription
HSP	high-scoring pair
INRA	National Institute for Agricultural Research, France
IRGSP	International Rice Genome Sequencing Project
ITMI	International Triticeae Mapping Initiative
JRGP	Japanese Rice Genome Project
KGM	Kinase associated with GAMYB
KOME	Knowledge-based Oryza Molecular biological Encyclopedia
LMA	Late maturity alpha amylase
LMEA	Late-maturity endosperm amylase
LMW-GS	Low molecular weight glutenins
MAS	Marker assisted selection
NCBI	National Center for Biotechnology Information
NSF	National Science Foundation
NT	Nullisomic-tetrasomic lines
ORF	open reading frame
PAC	Phage P1 artificial chromosome
PCR	Polymerase chain reaction
PHS	Preharvest sprouting
PMAA	Pre-maturity alpha-amylase activity
PrMS	Pre-maturity sprouting
QTL	Quantitative Trait Loci
RFLP	Restriction fragment length polymorphism
RGAs	<i>Resistance</i> gene analogs
Rht	<i>Reduced height</i> genes
RPAA	Retained pericarp alpha-amylase activity
SLNI	<i>Slender 1</i> gene
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats (or microsatellites)
STAT	Signal transducers and activators of transcription
TE	Transposable element
TFs	Transcription factors

TIGR	The Institute for Genomic Research
TREP	Triticeae Repeat Sequence Database
UC Davis	University of California, Davis
URGV	Unité de Recherche en Génomique Végétale
WA	Western Australia
wEST	wheat expressed sequence tag
WSN	White salted noodles
YAC	Yeast artificial chromosome

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