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:


..........................................................  
Meredith Diane Carter
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

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ABA</td>
<td>Abscisic acid</td>
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<tr>
<td>ABF</td>
<td>ABA/stress inducible bZIP transcription factors</td>
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<td>ABRE</td>
<td>abscisic acid responsive element</td>
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<tr>
<td>abi</td>
<td>abscisic insensitive mutant gene (Arabidopsis)</td>
</tr>
<tr>
<td>AFLP</td>
<td>Amplified fragment length polymorphisms</td>
</tr>
<tr>
<td>ANGIS</td>
<td>Australian National Genomic Information Service</td>
</tr>
<tr>
<td>ASW</td>
<td>Australian Standard White</td>
</tr>
<tr>
<td>AWB</td>
<td>Australian Wheat Board</td>
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<tr>
<td>BAC</td>
<td>Bacterial Artificial Chromosome</td>
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<tr>
<td>BLAST</td>
<td>Basic Local Alignment Search Tool</td>
</tr>
<tr>
<td>BPBF</td>
<td>barley prolamin-box binding factor</td>
</tr>
<tr>
<td>BSA</td>
<td>Bulked Segregant Analysis</td>
</tr>
<tr>
<td>CCG</td>
<td>Centre for Comparative Genomics</td>
</tr>
<tr>
<td>cDNA</td>
<td>complementry DNA</td>
</tr>
<tr>
<td>CIMMYT</td>
<td>International Maize and Wheat Improvement Centre, Mexico</td>
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<tr>
<td>CS</td>
<td>Chinese Spring</td>
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<tr>
<td>DAF</td>
<td>Days after flowering</td>
</tr>
<tr>
<td>DH</td>
<td>Doubled Haploid</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DOE</td>
<td>US, Department of Energy</td>
</tr>
<tr>
<td>DPA</td>
<td>Days post anthesis</td>
</tr>
<tr>
<td>EERE</td>
<td>Energy Efficiency and Renewable Energy (DOE)</td>
</tr>
<tr>
<td>EST</td>
<td>Expressed sequence tag(s)</td>
</tr>
<tr>
<td>Fl-cDNA</td>
<td>Full-length cDNA clone</td>
</tr>
<tr>
<td>FPC</td>
<td>Fingerprint contig software program</td>
</tr>
<tr>
<td>GA</td>
<td>Gibberellic acid</td>
</tr>
<tr>
<td>gai</td>
<td>gibberellic acid-insensitive gene</td>
</tr>
<tr>
<td>GAMYB</td>
<td>Gibberellin MYB transcription factor</td>
</tr>
<tr>
<td>GARC</td>
<td>GA response complex</td>
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<tr>
<td>GARE</td>
<td>GA response element</td>
</tr>
<tr>
<td>GMPOZ</td>
<td>GAMYB-associated POZ protein</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<td>--------------</td>
<td>-----------</td>
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<tr>
<td>HFN</td>
<td>Hagberg falling number</td>
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<tr>
<td>HMW-GS</td>
<td>High molecular weight glutenins</td>
</tr>
<tr>
<td>HRT</td>
<td><em>Hordeum</em> repressor of transcription</td>
</tr>
<tr>
<td>HSP</td>
<td>high-scoring pair</td>
</tr>
<tr>
<td>INRA</td>
<td>National Institute for Agricultural Research, France</td>
</tr>
<tr>
<td>IRGSP</td>
<td>International Rice Genome Sequencing Project</td>
</tr>
<tr>
<td>ITMI</td>
<td>International Triticeae Mapping Initiative</td>
</tr>
<tr>
<td>JRGBP</td>
<td>Japanese Rice Genome Project</td>
</tr>
<tr>
<td>KGM</td>
<td>Kinase associated with GAMYB</td>
</tr>
<tr>
<td>KOME</td>
<td>Knowledge-based Oryza Molecular biological Encyclopedia</td>
</tr>
<tr>
<td>LMA</td>
<td>Late maturity alpha amylase</td>
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<tr>
<td>LMEA</td>
<td>Late-maturity endosperm amylase</td>
</tr>
<tr>
<td>LMW-GS</td>
<td>Low molecular weight glutenins</td>
</tr>
<tr>
<td>MAS</td>
<td>Marker assisted selection</td>
</tr>
<tr>
<td>NCBI</td>
<td>National Center for Biotechnology Information</td>
</tr>
<tr>
<td>NSF</td>
<td>National Science Foundation</td>
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<tr>
<td>NT</td>
<td>Nullisomic-tetrasomic lines</td>
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<tr>
<td>ORF</td>
<td>open reading frame</td>
</tr>
<tr>
<td>PAC</td>
<td>Phage P1 artificial chromosome</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PHS</td>
<td>Preharvest sprouting</td>
</tr>
<tr>
<td>PMAA</td>
<td>Pre-maturity alpha-amylase activity</td>
</tr>
<tr>
<td>PrMS</td>
<td>Pre-maturity sprouting</td>
</tr>
<tr>
<td>QTL</td>
<td>Quantitative Trait Loci</td>
</tr>
<tr>
<td>RFLP</td>
<td>Restriction fragment length polymorphism</td>
</tr>
<tr>
<td>RGAs</td>
<td><em>Resistance</em> gene analogs</td>
</tr>
<tr>
<td>Rht</td>
<td><em>Reduced height</em> genes</td>
</tr>
<tr>
<td>RPAA</td>
<td>Retained pericarp alpha-amylase activity</td>
</tr>
<tr>
<td>SLN1</td>
<td><em>Slender 1</em> gene</td>
</tr>
<tr>
<td>SNP</td>
<td>Single nucleotide polymorphism</td>
</tr>
<tr>
<td>SSR</td>
<td>Simple sequence repeats (or microsatellites)</td>
</tr>
<tr>
<td>STAT</td>
<td>Signal transducers and activators of transcription</td>
</tr>
<tr>
<td>TE</td>
<td>Transposable element</td>
</tr>
<tr>
<td>TFs</td>
<td>Transcription factors</td>
</tr>
<tr>
<td>Acronym</td>
<td>Full Form</td>
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<td>---------</td>
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</tr>
<tr>
<td>TIGR</td>
<td>The Institute for Genomic Research</td>
</tr>
<tr>
<td>TREP</td>
<td>Triticeae Repeat Sequence Database</td>
</tr>
<tr>
<td>UC Davis</td>
<td>University of California, Davis</td>
</tr>
<tr>
<td>URGV</td>
<td>Unité de Recherche en Génomique Végétale</td>
</tr>
<tr>
<td>WA</td>
<td>Western Australia</td>
</tr>
<tr>
<td>wEST</td>
<td>wheat expressed sequence tag</td>
</tr>
<tr>
<td>WSN</td>
<td>White salted noodles</td>
</tr>
<tr>
<td>YAC</td>
<td>Yeast artificial chromosome</td>
</tr>
</tbody>
</table>
Acknowledgements

I would like to thank my supervisors Professors Mike Jones and Rudi Appels for their constant support, mentoring and guidance throughout my PhD. I would especially like to thank Mike for initially giving me the opportunity to ‘swap sides’ from the world of medical research to agricultural research. I am also grateful to Rudi for the opportunities he has given me during my PhD, and for not simply treating me as ‘just another PhD student’, but giving me the respect and trust of a research colleague which has enabled me to believe in myself as a scientist. I would also like to thank Dr Boulos Chalhoub and Prof Jan Dvorak for the opportunity to work in their labs for a period of time. This was invaluable to my PhD and for my development as a research scientist.

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4.9 Graphical display of sequence information for Renan BACs selected with BE637817 probe (GAMYB-gp7).

4.10 CLUSTALW alignment of nucleotide sequences for BAC clone 192J22, Cranbrook BE637817F2/R2 genomic amplicon and wEST BE637817.

4.11 Amino acid alignment of FGENESH predicted protein from the Ren_192J22 BAC clone and translated sequence of wheat EST BE637817.

4.12 Pseudomolecule for BAC Ren_192J22 assembled using PHRED/PHRAP.

4.13 Observed number of microsatellites with di-, tri-, and tetraneucleotide motifs in the 6 GAMYB-like BAC sequences using the SSRIT program.

4.14 Genotyping of the Cranbrook x Halberd DH population with microsatellite marker developed from sequence of wheat BAC clone Ren_147P05.

4.15 Autoradiographs of Cranbrook/Halberd lines segregated into LMA and non-LMA lines (28 DNA samples each), and probed with $[^{32}P]$-labelled wEST probes.
4.16 Southern hybridization of *Aegilops tauschii* mapping parents DNA digested with 15 different restriction enzymes and probed with BE637817 (GAMYB-gp7) probe.

4.17 Genetic map of *Ae. tauschii* F2 population.

**Chapter 5**

5.1 Screening of Pastor x RAC891 and Cranbrook x Halberd DH populations for boron tolerance.

5.2 Genotyping of BAC SSRs **A)** Ren_435M3, alleles from chromosomes 7AL, 7BL and 7DL are indicated by arrows and **B)** Ren_935N19 on Cranbrook/Halberd DH population.

5.3 Genetic maps of chromosomes 7A and 7B from Cranbrook/Halberd DH population.

5.4 Example of rating system used to score plants for tolerance to boron based on length of root.

5.5 Frequency distributions of Boron tolerance for individual lines of DH populations derived from (A) Cranbrook/Halberd and (B) Pastor/RAC891; and for Pastor/RAC891 DH population split into two populations C) Population A (47 lines) and D) Population B (42 lines).

5.6 Frequency distribution of LMA tolerance for individual lines of A) Pastor/RAC891 DH population (58 lines) and Pastor/RAC891 DH Population split into two populations B) Population A of 33 lines, and C) Population B of 25 lines.

5.7 Genotyping Pastor x RAC891 DH population with microsatellite marker gwm577.

5.8 QTL linkage analysis on the long arm of chromosome 7B associated with A) boron tolerance and B) LMA in Pastor x RAC891 DH population B.

5.9 QTL linkage analysis on the long arm of chromosome 7B associated with LMA in Cranbrook x Halberd DH population B (161 lines).

5.10 Two-dimensional representation of the distribution of alleles from 19 SSRs across 39 wheat varieties of known LMA phenotype.

5.11 Cluster dendrogram of 39 varieties genotyped with SSR markers mapped to LMA QTLs on chromosomes 3B (A) and 7BL (B).
5.12 Haplotype profiles for the CIMMYT-derived material (Veery 1,2,4,7,8, Super Seri 1,2, Pastor, Kennedy) for 7BL and 3B microsatellite data located near LMA QTLs.

Chapter 6

6.1 Two models for boron efflux as the basis of B tolerance in barley.

6.2 Model of GA regulation of High pI α-amylase expression in cereal aleurone cells.

6.3 Proposed roles of OsMYBs in the GA hormone regulation of α-amylase gene expression in cereals.

6.4 Hypothetical model of the signal transduction pathway and a mechanism of gene regulation for high pI α-amylase in LMA-prone cultivars.

6.5 Framework genetic-physical map for chromosome group 7L targeted to the terminal end of 7L containing QTLs for LMA and boron tolerance (Bo1)