

Evaluation of physiological traits and identification  
of QTLs for drought tolerance in hexaploid wheat  
(*Triticum aestivum* L.)

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## List of abbreviations

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Acronyms & symbols	Definition
<b>AFLPs</b>	Amplified fragment length polymorphism
<b>ANOVA</b>	Analysis of variance
<b>BLUE</b>	Best linear unbiased estimator
<b>BLUP</b>	Best linear unbiased predictor
<b>CIM</b>	Composite interval mapping
<b>cm</b>	Centimeter
<b>cM</b>	centiMorgan
<b>Cre</b>	Crown Rot
<b>DArT</b>	Diversity Arrays Technology
<b>df</b>	Degrees of freedom
<b>DHLs</b>	Doubled haploid lines
<b>Eet</b>	Ear emergence time
<b>El</b>	Ear length
<b>Eps</b>	Earliness <i>per se</i>
<b>Fgw</b>	50-grain weight
<b>Flt</b>	Flowering time
<b>Gnu</b>	Grain number
<b>Gpp</b>	Green plant percentage
<b>Gw</b>	Grain weight
<b>Gwe</b>	Grain weight/ear
<b>h<sup>2</sup></b>	Heritability
<b>Ht</b>	Plant height
<b>ITMI</b>	International Triticeae Mapping Initiative
<b>Kpsm</b>	Kernel number per square metre
<b>Ler</b>	Leaf erectness
<b>LOD</b>	Logarithm of the Odds
<b>MAS</b>	Marker-assisted selection
<b>MIM</b>	Multiple interval mapping
<b>MQM</b>	Multiple QTL mapping

## List of abbreviations

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<b>Pa</b>	Pubescense
<b>PCR</b>	Polymerase Chain Reaction
<b>Pdl</b>	Peduncle length
<b>QTL</b>	Quantitative Trait Locus
<b>REML</b>	Residual maximum likelihood
<b>RILs</b>	Recombinant inbred lines
<b>SNP</b>	Single Nucleotide Polymorphism
<b>Spn</b>	Spikelet number per ear
<b>Spsm</b>	Spike per square meter
<b>SSR</b>	Single Sequence Repeat
<b>TDR</b>	Time Domain Reflectometry
<b>TGW</b>	Thousand Grain Weight
<b>Tn</b>	Tiller number per plant
<b>W</b>	Leaf waxiness
<b>Yld</b>	Grain yield

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## Summary

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This study comprised three major parts: a comparative physiological study of drought responses under controlled conditions; a genetic study to construct the skeleton map of a doubled haploid (DH) population; and a quantitative trait loci (QTL) analysis to identify QTLs associated with drought tolerance traits in the field.

In the first part (Chapter 3), three cultivars of wheat (*Triticum aestivum* L.) adapted to South Australian conditions were tested for drought tolerance under cyclic drought in growth rooms and glasshouse. Extensive physiological traits, including stomatal conductance, chlorophyll content and fluorescence, ABA content, water status traits (e.g. osmotic adjustment, RWC and leaf water potential), water soluble carbohydrates (WSC) and carbon isotope discrimination ( $\Delta^{13}\text{C}$ ) were measured during experiments. Through these experiments, the drought responses of the three cultivars were physiologically dissected and the likely processes contributing most to drought tolerance were identified.

In the South Australian wheatbelt, cyclic drought is a frequent event, represented by intermittent periods of rainfall which can occur around anthesis and post-anthesis in wheat. Three South Australian bread wheat cultivars, Excalibur, Kukri and RAC875, were evaluated in two growth room experiments under cyclic water-limiting conditions. In the first experiment, where plants were subjected to severe water stress, RAC875 and Excalibur (drought tolerant) showed significantly ( $P < 0.05$ ) higher grain yield under cyclic water availability compared to Kukri (drought susceptible), producing 44% and 18% more grain yield compared to Kukri, respectively. In the second growth room experiment, where plants were subjected to a milder drought stress, the differences between cultivars were less pronounced, with only RAC875 showing significantly higher grain yield under the cyclic water treatment. Grain number per spike and the percentage of aborted tillers were the major yield components that affected yield under cyclic water stress. Excalibur and RAC875 adopted different morpho-physiological traits and mechanisms to reduce water stress. Excalibur was most responsive to cyclic water availability and showed the highest level of osmotic adjustment (OA), highest stomatal conductance, lowest ABA content and most rapid recovery from stress under cyclic water stress. RAC875 was more 'conservative' in its responses, with moderate OA, high leaf waxiness, high chlorophyll content and slower recovery from stress.

Within this germplasm, the capacity for osmotic adjustment was the main physiological attribute associated with tolerance under cyclic water stress, which enabled plants to recover from water deficit.

In the second part (Chapter 4), the genetic linkage map of a DH population including 368 lines, which was developed from a cross between ‘RAC875’ and ‘Kukri’, was constructed. The genetic linkage map consisted of about 500 molecular markers including ~300 DArT (Diversity array technology) and ~200 SSR (Microsatellite markers).

In the third part (Chapter 5), Quantitative Trait Loci (QTLs) linked to plant phenology and production traits under irrigated and drought stress conditions were mapped by means of a DH population. To phenotype the population, 368 DH lines were cultivated in two replicates in five environments (three sites across South Australian wheatbelt in collaboration with Australian Grain Technology (AGT) in 2006, and two trials in Mexico in collaboration with CYMMIT, 2007). Data of grain yield, yield components, maturity related traits and some morpho-physiological traits such as leaf chlorophyll content, leaf waxiness, plant height, peduncle length, flag leaf and spike length were measured. Raw data were then analysed for spatial variation for each single trial using the REML procedure in GenStat (version 6). The DH lines showed significant variation for plant phenology, grain yield and yield components under irrigated and drought stress conditions. QTL analyses were performed using QTLCartographer and QTLNetwork for each trait in each site. Two major QTL for maturity traits were identified on chromosomes 2BS and 2DS corresponding to *Ppd-B1* and *Ppd-D1*, respectively. A region was identified on chromosome 7A that harbored major QTL for grain yield, number of grains per square meter, number of grain per spike and spike fertility under drought stress. For yield data in the irrigated trial, two major QTL were identified on chromosome 3B which were not detected in drought stress environments. By using different datasets in the QTL analysis (splitting the population into two subpopulation based on heading time and also adjusting the phenotypic data for heading time to eliminate heading time effect), a QTL for grain yield was consistently detected on chromosome 7A in drought-affected environments. The coincidence of a drought response index QTL on this chromosome indicated that it might be a QTL for yield response under drought. This study demonstrated that the region on the long arm of chromosome 7A identified for grain yield and yield components is a drought response

QTL which is closely linked to, but separate from, a heading time QTL. This QTL cluster on chromosome 7A could be used as a good target for positional cloning and gene isolation. However further work would be required to confirm and validate the identified QTLs in this preliminary QTL analysis.



## Declaration

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This thesis contains no material that 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 of the thesis.

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Ali Izanloo





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