Genetic Factors and Genes Underpinning Drought Response in Wheat

By

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Declaration

Except where otherwise indicated, all work in this thesis is based on work carried out by me at the State Agricultural Biotechnology Centre (SABC) and the Australia China Centre for Wheat Improvement (ACCWI) Murdoch University, Australia. I declare the content of this thesis is my own account of my research and has not been previously submitted for a degree at any tertiary education centre. To the best of my knowledge, all work performed by others, published or unpublished, has been duly acknowledged.

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Abstract

Pollen fertility is one of the main factors limiting yield in crops such as rice and wheat, both highly inbreeding species (Wang *et al.,* 2003). Water deficit during reproductive stage growth can result in pollen sterility due to impaired carbohydrate supply (sucrose/glucose) to pollen and supporting tissues such as the tapetum. In cereal species, and monocotyledons in general, viable pollen development is accompanied by starch accumulation in amounts that are sufficient to support pollen germination and pollen tube growth (Franchi *et al.,* 1996). Cereal pollen grains rendered sterile by the down regulation of the sugar transport gene *IVR1* lack starch (Sheoran and Saini 1996) and have failed or impaired intine formation (Lalonde *et al.,* 1997). *IVR1* down regulation results in incomplete or total absence of sucrose cleavage to the hexose sugars glucose and fructose (the final energy substrates used in plant metabolism to support pollen development), resulting an accumulation of sucrose despite the high energy demand of the developing tissues (Dorion *et al.,* 1996).

The mechanisms underlying the sensitivity of pollen and the tapetum to abiotic stress (water deficit) provide a basis for developing molecular approaches aimed at increasing stress tolerance (Parish and Li 2010). This thesis has characterized the cell wall invertase gene family in detail in order to provide DNA sequence signatures that allow the expression of specific genes to be followed. The study demonstrated *IVR1.1-3B* expression was confined to leaves while *IVR1-4A* and *IVR1-5B* represented genes expressed during early head development. Two double haploid lines identified from a population of 225 lines derived from a cross between the varieties Westonia and Kauz showed significant differences in response to water deficit stress induced grain set reduction and expression of *IVR1* isoforms *IVR1-4A* and *IVR1-5B*. The differences in expression were investigated in more detail using a large-scale RNASeq study, which indicated there was a significant expression response to water deficit in a suite of genes involved in carbohydrate metabolism. In addition *IVR1* expression analysis was validated using SQ-PCR, which highlighted significant differences in response to

water deficit stress. Rice was used as a benchmark for selecting genes in this study.

The external phenotype, penultimate leaf internode auricle distance 5cm (Zadoks growth stages Z39/40), was validated as an indicator of pollen meiosis completion. A significant developmentally related drought escape QTL was identified on chromosomes 5B and 5D. High resolution mapping of the group 5 chromosomes (using a 90K SNP chip) enabled a high density of markers to be located across the chromosome 5B QTL in particular, and identified water deficit responsive UDP-glucose 6-dehydrogenase (from the KEGG carbohydrate metabolism pathway) as a gene of interest. A global analysis using RNASeq data identified a more extensive suite of anther-specific genes that were water deficit responsive. In turn this suit of genes provided the basis for defining a set of molecular markers to screen for variation in drought responsiveness in wheat varieties.

Although this work has focussed on a developmental stage specific water deficit response, it is evident the thesis contributes to the wider body of information becoming available for wheat, much of which now indicates it should be feasible to define a haplotype for wheat using the allelic variation in genes associated with drought tolerance in different environments. Results from this study have shown that reproductive stage water deficit tolerance is a complex quantitative trait and that it may be more useful to quantify a risk factor value using a suite of markers, rather than a plus/minus analysis of single markers. We propose the network of carbohydrate metabolism genes defined in this thesis would make significant contributions to risk factor analyses in early selection or backcrossing for sensitivity in particular environments.

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List of abbreviations

Abbreviation	Description
ABA	Abscisic acid
ABARE	Australian Bureau of Agricultural and Resource Economics
ABS	Australian Bureau of Statistics
AC	Anthesis commenced
ACCWI	Australia China Centre for Wheat Improvement
AD	Auricle distance
AFV	Awns first visible
AMS	Aborted microspore
ANOVA	Analysis of variance
APW	Australian prime hard
AT	Ambient temperature
AUD	Australian dollar
BAC	Bacterial artificial chromosome
BBCH	Biologische Bundesanstalt Bundessortment Scale
BP	Bicellular pollen
BS	Boot swollen
C-INV	Cytoplasmic invertase
CCG	Centre for Comparative Genomics
CDS	Coding deoxyribonucleic acid sequence
CIMMYT	International Maize and Wheat Improvement Centre
cM	Centimorgan
CNV	Copy number variation
CPM	Counts per million
CW-INV	Cell wall invertase
DAFWA	Department of Agriculture and Food Western Australia
DAPI	4',6-diamidino-2-phenylindole
DAS	Days after sowing
DEPI	Department of Environment and Primary Industry (Victoria)
DF	Degrees of freedom
DH	Double haploid
DNA	Deoxyribonucleic acid
EC	Enzyme commission number
ER	Endoplasmic reticulum
EST	Expressed sequence tag
ET	Evapotranspiration
FAO	Food and Agriculture Organization of the United Nations
FDR	False discovery rate
FEH	Fructan exohydrolase
FHB	Fusarium head blight
fl-cDNA	Full length cDNA
FPC	Fingerprint contig

Fru	Fructose
GO	Gene ontology
GSS	Genome survey sequence
GWAS	Genome wide association study
HCS	High confidence score
HICF	High information content fingerprinting
HXK	Hexokinase
ICIM	Inclusive composite interval mapping
ID	Identity description
IPCC	Intergovernmental Panel on Climate Change
IRGSP	International Rice Genome Sequencing Project
IWGSC	International Wheat Genome Sequencing Consortium
JCVI	J. Craig Ventur Institute
KEGG	Kyoto Encyclopaedia of Genes and Genomes
LAI	Leaf area index
LB	Lysogeny broth
LCS	Low confidence score
LEA	Late embryogenesis abundant
LOC	Locus
LOD	Logarithm of edits
LTC	Linear topological contig
MAB	Marker assisted backcross
MAGIC	Multi-parent advanced generation inter-cross
MAS	Marker assisted selection
Mbp	Mega base pairs
MDS	Multi dimensional scaling
MIPS	Munich Information Centre for Protein Sequence
MMC	Microspore mother cell
MP	Mature pollen
MS	Mean sums of squares
NA	Not applicable
NB	nota bene/take note
NCBI	National Centre for Biotechnology Information
NSW	New South Wales
PCA	Principal component analysis
PCD	Programmed cell death
PCP	Principal component biplot
PCR	Polymerase chain reaction
PDB	Protein data bank
PPP	Pentose phosphate pathway
PH	Plant height
Ppd	Photoperiod
PVE	Phenotypic variation explained
PWP	Permanent wilting point
QC	Quality control

QLD	Queensland
QTL	Quantitative trait loci
REML	Restricted maximum likelihood
RH	Relative humidity
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RSWD	Reproductive stage water deficit stress
RT	Reverse transcription
SABC	State Agricultural Biotechnology Centre
SCW	Seedling cuticle waxiness
SNP	Single nucleotide polymorphism
SQ-PCR	Semi quantitative polymerase chain reaction
SS	Sums of squares
SSR	Simple sequence repeat
Suc	Sucrose
SucTP	Sucrose transporter
SVWC	Soil volumetric water content
TD-PCR	Touch down polymerase chain reaction
TDR	Tapetal degradation retardation
THT	The hordeum toolbox
TMM	Trimmed means of m
UGPase	Glucose-1-phosphate
UN	United Nations
USA	United States of America
USD	United States of America dollar
UV	Ultra violet
V-INV	Vacuolar invertase
Vrn	Vernalization
WxK	Westonia x Kauz
Z	Zadoks growth stage scale

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