Using Advanced Wheat Genomic Resources and Metabolite Fingerprinting to Address Complex Traits

Raj Kishore Pasam

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Introduction

Several recent technological advances are accelerating our understanding of the genetic architecture and genic basis of trait variation in wheat and the rate of genetic gain in breeding programs

– Reduced costs for high density genotyping
  e.g. SNP chips, GBS
– More accurate, large-scale and simultaneous measure of multiple phenotypes
  e.g. digital phenotyping, molecular phenomics
– Improved assemblies and annotation of the wheat genome
  e.g. CSS, TAGC, NRGene

Our research is applying these technological advances to better understand the genetic basis of key breeding traits including yield stability and grain quality to assist breeding for varieties tailored for different end use and market classes

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Bread Wheat Diversity Panel

6,689 genetically and phenotypically diverse worldwide accessions comprising landraces, released varieties, synthetic derivatives and novel trait donor lines

– Genotyped using 90K SNP bead chip; total 65K SNP called
– Exome sequence for 1,000 accessions selected to maximally capture genetic diversity; total 9.5M SNPs
– Assessed for agronomic traits and grain quality across multiple seasons in replicated field trials grown under irrigated and rain-fed conditions
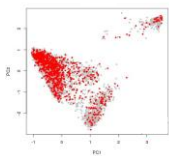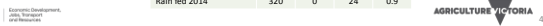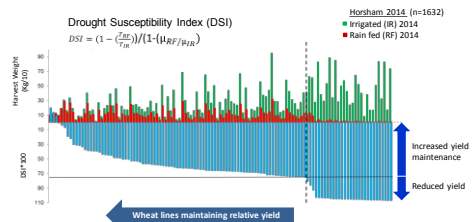

Genetic relatedness among wheat lines based on 90K SNPs. Red dots indicate exome sequenced wheat lines

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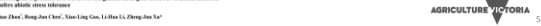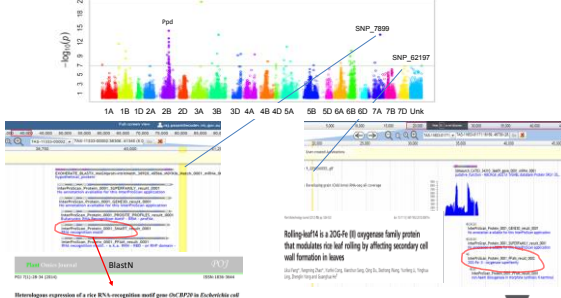
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Yield Stability

Identifying genomic regions and genes contributing to yield stability in water-limited environments in contrasts of irrigated *vs* rain-fed field trials
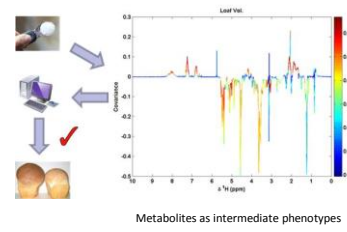
Drought Susceptibility Index (DSI)
$$DSI = (1 - (\tfrac{\gamma_{RF}}{\gamma_{IR}}))/(1-(\mu_{RF}/\mu_{IR}))$$

Increased yield maintenance

Reduced yield

Wheat lines maintaining relative yield

Plot harvest weight (kg)	Max	Min	Ave	SE Ave
Irrigated 2014	1353	18	296	4.2
Rain fed 2014	320	0	24	0.9

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Yield (DSI) QTL in 7A & 7D

GWAS of DSI for plot yield in Horsham 2014 field trials

Heterologous expression of a rice RNA-recognition motif gene *OsCBP20* in *Escherichia coli* confers abiotic stress tolerance

Rolling-leaf14 is a 2OG-Fe (II) oxygenase family protein that modulates rice leaf rolling by affecting secondary cell wall formation in leaves

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Molecular Phenomics

Several technology platforms for high throughput molecular phenomics are being used to investigate association of grain metabolites with grain quality and end use traits

Metabolites as intermediate phenotypes

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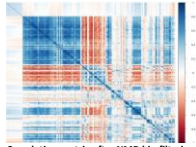
NMR Analysis of Grain Metabolites

NMR – Nuclear Magnetic Resonance

Flour samples from irrigated and rain-fed field trials were assayed for metabolite composition using several solvents

Approach for association mapping analysis
- NMR spectra split into bins
- Highly correlated ($r^2=0.95$) NMR bins removed
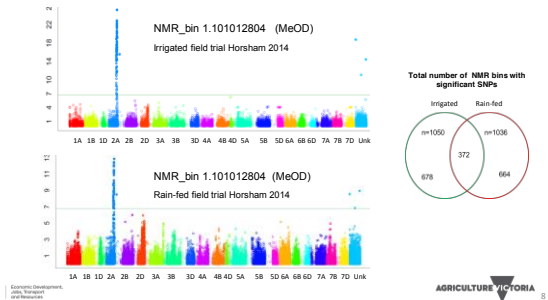- Average NMR bin absorbance used as trait values

Applications
- Biomarker discovery
- Quantitative metabolomics
- De novo structure elucidation
- 3D protein structure elucidation
- Dynamic studies

Correlation matrix after NMR bin filtering
Sorted by NMR bin order

Solvent	Total NMR bins	Bins after filtering
DMSO	3,555	1,232
MeOD	7,628	5,588

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Metabolite GWAS

Association mapping using NMR spectral bins as trait values identifies genomic regions influencing grain metabolite content
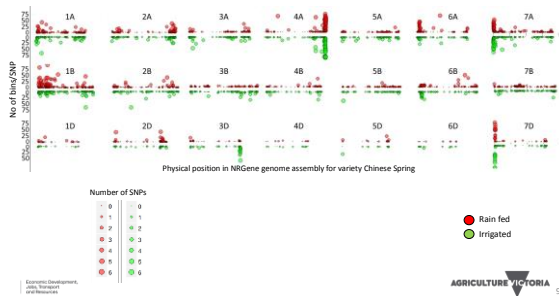

NMR_bin 1.101012804 (MeOD)
Irrigated field trial Horsham 2014

NMR_bin 1.101012804 (MeOD)
Rain-fed field trial Horsham 2014

Total number of NMR bins with significant SNPs

Irrigated: n=1050, Rain-fed: n=1036, 372, 678, 664

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Metabolite GWAS

Genomic distribution of SNPs significantly associated with NMR bins (MeOD solvent) in Horsham 2014 irrigated and rain-fed field trials
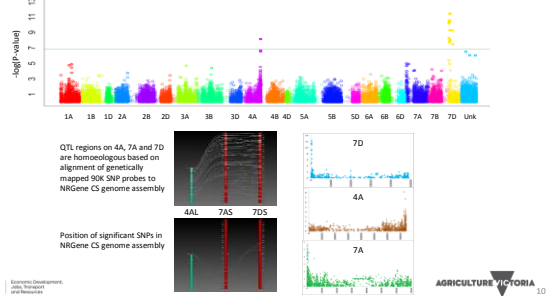

Physical position in NRGene genome assembly for variety Chinese Spring

Number of SNPs

Rain fed
Irrigated

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Metabolite GWAS – Grain Fructan

Wet-chemistry was used to correlate NMR data with grain fructan content. GWAS with correlated NMR bin identified genetic variation for grain fructan content


QTL regions on 4A, 7A and 7D are homoeologous based on alignment of genetically mapped 90K SNP probes to NRGene CS genome assembly

Position of significant SNPs in NRGene CS genome assembly

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Metabolite GWAS – Grain Fructan

Most significant SNPs from GWAS are physically located in two chromosome 7A scaffolds that contain clusters of fructan biosynthesis genes
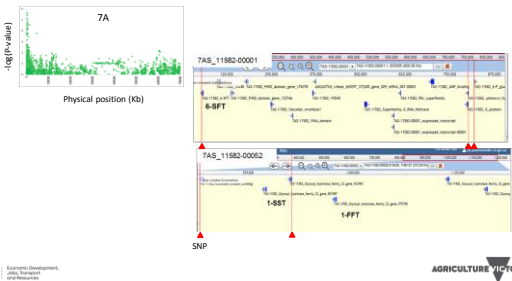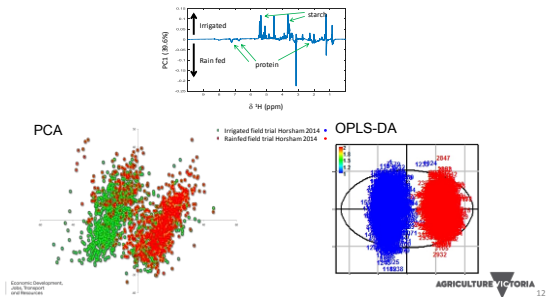

Physical position (Kb)

SNP

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Drought Responsive Metabolites

NMR data shows differential metabolite accumulation in grain samples harvested from irrigated and rain-fed field trials


PCA

OPLS-DA

Irrigated field trial Horsham 2014
Rainfed field trial Horsham 2014

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Drought Responsive Metabolites

Genomic distribution of significant SNPs in a GWAS using NMR bins showing $\geq\log_2$-fold value change between irrigated and rain-fed environments

Physical position in NRGene genome assembly for variety Chinese Spring

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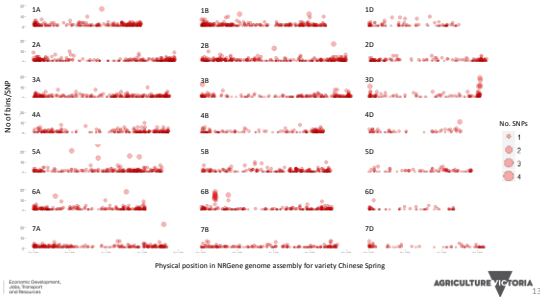
Summary

- Metabolite GWAS is providing insights into the architecture of metabolite variation in wheat grain grown in different environments

- Connecting the grain metabolome to the underlying genome may allow identification of causative genetic loci that are less influenced by environment to assist breeding for varieties with consistent grain quality or tailored end use

- A connection between grain metabolites and yield is yet to be established but could potentially provide novel biomarkers, and ultimately new phenotyping methods to help breeding for yield stability

- Metabolite QTL correlated with grain yield and grain quality traits can be used as biological priors to improve accuracy for genomic prediction; e.g. BayesRC

- Availability of high quality wheat genome assemblies and gene annotations are facilitating our understanding for the genetic basis of complex traits

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