VALUE ADDED WHEAT CRC

Stage 1
Second Year Review of
Value Added Wheat CRC Ltd

Presentations to Review Panel

Chris Hudson (Chair), Brian Hare, Doug Graham

21st & 22nd August 2003

Compiled by: Mary Foster

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STAGE 1
Second Year Review of Value Added Wheat CRC Ltd

Date: 21 and 22 August 2003

Venue: VAWCRC HQ, North Ryde

DAY 1
Thursday 21 August, 2003

TIMETABLE

Time	Subject	Presenter
9.30-10.00	**OPENING PRESENTATION** Overview of research being conducted in VAWCRC	Dr Bill Rathmell (Managing Director)
10.00-10.15	**Morning Tea**	
10.15- 12.00	**PROGRAM 1: Diagnostics** Overview of Program 1	Dr Neil Howes Manager Program 1 (SARDI)
	Project 1.1.1 Protein composition analysis	Dr Ian Batey Project Leader (Food Science Australia)
	Project 1.1.1 Development of a screening test for wheat protein quality	Anneleise Rittau CRC PhD Student
	Project 1.1.2 Antibody Based Diagnostics	Dr James Chin Project Leader (Ag NSW (EMAI))
	Project 1.1.2 Characterisation of polymorphic proteins for variety and quality traits	Michelle Powell CRC PhD student
	Project 1.1.2 Diagnostics for wheat varietal identification	Araluen Freeman CRC PhD student
	Project 1.2.3 Diagnostics Delivery	Mrs Felice Driver Project Leader (C-Qentec Diagnostics)
12.15 – 1.15	**Lunch / Student Posters**	**Panel Members** **Senior Management Group** **Project Leaders/Presenters** **Students**

DAY 1 continued.....

Time	Subject	Presenter
1.15 – 3.00	**PROGRAM 2: Products and Processing** Overview of Program 2	Ms Di Miskelly Manager Program 2 (Allied Mills)
	Project 2.1.1 Blending – Consequences of Wheat Breeding and achieving quality targets	Ms Di Miskelly
	Project 2.1.4 Optimisation of key stages of the baking process	Ms Di Miskelly
	Project 2.1.5 Australian wheat for the Sponge and Dough bread making process	Ms Di Miskelly
	Project 2.1.6 Strategies to replace flour chlorination as a treatment for cake flours	Ms Di Miskelly
	Project 2.1.7 Microbiological safety and stability of noodles, breadcrumbs and steamed breads made from Australian flour	Dr Yang Huang Project Leader (Food Science Australia)
	Project 2.1.8 Investigations on increasing the conditioning efficiency of wheat	Ms Di Miskelly
	Project 2.1.9 Gluten structure and modification for ingredient use	Dr Ian Batey Project Leader (Food Science Australia)
	Project 2.2.10 Analysis of starch lipid complexes	Ms Di Miskelly
	Project 2.3.11 Extended shelf life of bread and baked goods	Ms Di Miskelly
	Project 2.3.12 Wheat quality for starch and gluten production	Ms Di Miskelly
3.00-3.15	**Afternoon Tea**	
3.15 - 5.00	**PROGRAM 3: Genomics and Proteomics** Overview of Program 3	Dr Peter Sharp Manager Program 3 (USyd)
	Project 3.1.1 Markers and Mapping wheat quality traits	Dr Matthew Hayden (USyd)
	Project 3.3.4 Overview of Triticarte technology	Dr Eric Huttner Manager, (Triticarte P/L)
	DArT™ Microarrays for wheat	Brent Thomson CRC PhD Student
	Project 3.1.2 Wheat grain proteomics and bioinformatics	Dr Daniel Skylas Project Leader (APAF)
	Project 3.1.2 A proteomic approach to the characterisation of wheat proteins	Ms Yunxian Mak CRC PhD student
	Project 3.1.3 Targeted mutagenesis of wheat grain characteristics	Dr Chong Mei Dong (USyd)

STAGE 1
Second Year Review of Value Added Wheat CRC Ltd

Date: 21 and 22 August 2003
Venue: QWCRC HQ, North Ryde

DAY 2
FRIDAY 22 August, 2003

TIMETABLE

Time	Subject	Presenter
9.15-11.00	**PROGRAM 4: Germplasm and Varieties**	
	Overview of research being conducted in Program 4	Mr John Oliver Manager Program 4 (Ag NSW)
	Project 4.1.1 New genetic variation and markers	Dr Mathew Turner Project Leader (USyd)
	Project 4.1.1 Markers for seed dormancy	Dr Mui-Keng Tan (Ag NSW)
	Project 4.2.6 Marker selection of waxy wheat lines	Mohammed Shariflou (USyd)
	Project 4.1.1 Reconstitution studies: influence of protein and pentosans on pasta quality	Cindy Soh CRC PhD Student
	Project 4.3.9 Marker validation and identification for key quality attributes in WA adapted germplasm	Dr Michael Francki Project Leader (Ag WA)
	Project 4.3.9 Validation of molecular markers in wheat for flour colour quality traits.	Karon Ryan CRC PhD Student
	Project 4.1.2 Rapid breeding technologies – novel adapted wheats	Nizam Ahmed Project Leader (USyd)
	Project 4.1.3 Soft wheat program	Helen Allen Project Leader (Ag NSW)
	Project 4.3.8 Development of adapted germplasm and varieties with novel characters	Dr Akram Khan Project Leader (Ag NSW (Cobbitty))
11.00-11.15	**Morning Tea**	
11.15 – 11.45	**Sum Up**	Dr Bill Rathmell (Managing Director)
12.00 – 1.00	**Lunch**	Panel
1.00	**Panel to write-up report**	

STAFF ATTENDING THE REVIEW

VAWCRC SENIOR MANAGEMENT GROUP

Dr Bill Rathmell, Managing Director VAWCRC
Mr Peter Vaughan, Commercial Director, VAWCRC

Program Managers

Dr Neil Howes, Mgr Prog1, Diagnostics	(SARDI)
Ms Di Miskelly, Mgr Progr 2, Products & Processes	(Allied Mills)
Prof Peter Sharp, Mgr Prog 3, Genomics & Proteomics	(PBI, Uni Sydney)
Mr John Oliver, Mgr Prog 4, Germplasm & Varieties	(NSW Ag)
Ms Clare Johnson, Mgr Prog 5, Education & Technol. Adoption	(VAWCRC)

Extended SMG (advise across all programs)

Mrs Felice Driver	(C-Qentec Diagnostics)
Mr Andrew Kennett	(Arnotts)
Professor Don Marshall,	(GRDC)
Dr Michael Francki	(Agriculture WA)
Ms Naomi Hehir	(Goodman Fielder)

VAWCRC STUDENTS

Anneleise Rittau	(Food Science Australia)
Michelle Powell	(EMAI , NSW Ag)
Araluen Freeman	(EMAI ,NSW Ag)
Ms Yunxian Mak	(APAF)
Brent Thomson	(Triticarte P/L)
Cindy Soh	(NSW Ag)
Karon Ryan	(Agriculture WA)
Mary Tang	(Uni Sydney)

OTHERS PRESENTING

Dr Ian Batey	(Food Science Australia)
Dr James Chin	(EMAI, NSW Ag)
Dr Yang Huang	(Food Science Australia)
Dr Mathew Hayden	(PBI, Uni Sydney)
Dr Daniel Skylas	(APAF)
Dr Chong Mei Dong	(Uni Sydney)
Dr Eric Huttner	(Triticarte P/L)
Dr Matthew Turner	(PBI, Uni Syd,)
Dr Mui-Keng Tan	(NSW Ag)
Mr Mohammed Shariflou	(PBI, Uni Sydney)
Dr Nizam Ahmed	(PBI, Uni Sydney)
Mrs Helen Allen	(NSW Ag)
Dr Akram Khan	(NSW Ag)

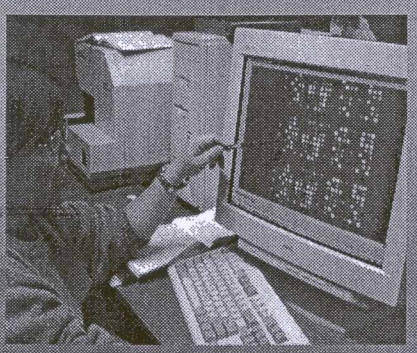
SUPPORT STAFF

Helen Warwick	VAWCRC Company Secretary
Mary Foster	VAWCRC Admin officer

Stage One

Second Year Review of Value Added Wheat CRC

21 – 22 August 2003



Stage 1

Second Year Review of Value Added Wheat CRC

21 & 22 August 2003

Dr Bill Rathmell
Managing Director

CRC criteria relating to science

- Science driven by the demands of the wheat industry
- High calibre, advanced science
- Intense collaboration between commercial and research laboratories/sites

The overall environment

- Revolutionary technological and scientific change has reached wheat
- The wheat industry in Australia (and worldwide) is in transition
  - Deregulation pressures, public to private sector (traders,breeding agencies)
  - Deteriorating terms of trade for producers and users

VAWCRC has developed a set of industry requirements ("outcomes") from research

- Market pull response to technology changes
- Revised targets from QWCRC – re-focussed

1. New diagnostic tools, quality specifications redefined
2. Consistent wheat supply, end-use flexibility and nutritional benefits
3. Wheat germplasm with novel and beneficial (profitable) properties
4. Better value in the domestic and export market (not "commodity" status)
5. Technology uptake and consumer education
6. Qualified experts for the industry

VAWCRC has a program integrating wheat research from science to retail ("outputs")

1. New quality diagnostic technologies
2. New growing, handling and processing systems
3. New wheat genetics and biochemical knowledge
4. New germplasm and new wheats with more added value
5. More trained scientists and technicians

VAWCRC is applying advanced science to the measurement of quality

1. Capillary electrophoresis for rapid variety identification
2. Advanced immunology techniques to create antibodies
3. Proteomics to find new quality determinants
4. "Back of the ute" test methods based on novel formats

Processing technology improvements have been achieved for the first time in VAWCRC

1. Dough processing module has novel quantifiable benefits
2. Microbiology studies on products and processes

VAWCRC is deploying advanced methods to identify and modify important quality genes

* Improved wheat genetics one of the most important routes to added value from science

1. Targeted mutation to investigate genes for quality.
2. Modern breeding techniques to incorporate in adapted material.

VAWCRC is introducing the quantitative use of molecular genetic markers for the first time in wheat

- Faster creation of wheat germplasm with profitable quality attributes

1. Marker associations with important quality traits
2. Application of new science to cost and throughput issues
3. World first low-cost, high-throughput, whole-genome marker service for wheat breeders

We have created a pipeline of germplasm and varieties with beneficial properties using the new science

1. Biscuit wheats with agronomy and processing benefits
2. Waxy wheat with product quality benefits and possible novel products
3. Germplasm with multiple resistances to disease and quality problems

The portfolio of VAWCRC is a spectrum of :

1. Advanced world leading science and applied technology
2. Projects with short and long term goals
3. Complex, more difficult (high risk) projects and more straightforward or feasible ones

VAWCRC has established collaborative links to progress research :

- Four principal types of collaborators (and commercialisation partners):

1. Close collaboration with Australian wheat breeders (Participants and others)
2. Commercialisation by Participants with expertise (eg diagnostics)
3. Technology uptake by growers and processors (eg microbiology)
4. Direct implementation - competitive commercial research with Participants (eg process control)

Organisation and emphasis

- Most VAWCRC projects have several sites; and scientists from commercial laboratories involved.
- There is a Senior Management Group to manage science (also with commercial members).
- There is a focus on added-value benefits from the research. We also produced (220) publications in 2001-2003.
- Intellectual property portfolio (patents, PBR, trademarks, copyright and know-how) is managed by the Commercial Director.

Postgradute fellowships, undergraduate vacation scholarships etc.

1. Postgraduates/undergraduates. An enhanced experience: integrated in program; collective exposure; multiple supervisors & laboratories, access to jobs
2. Postgraduates' research training: thirty sufficient for the industry

VAWCRC science is divided into four programs each containing world-standard research areas

Diagnostics

- Cereal Protein Chemistry
- Immunology – advanced techniques
- Novel immuno-detection and quantitation methods

VAWCRC science is divided into four programs each containing world-standard research areas

Products and Processing

- Process monitoring and control
- Microbiology of products and processes

VAWCRC science is divided into four programs each containing world-standard research areas

Genomics and Proteomics

- Two molecular marker technologies
- Targeted mutagenesis in wheat
- High technology proteomic analysis in wheat

VAWCRC science is divided into four programs each containing world-standard research areas

Germplasm and Varieties

- Sprout tolerance and quality genes located and tagged
- Application of doubled haploid/molecular marker techniques
- Pipeline of unique specialist wheat germplasm
- Application of genomic knowledge from other crops/countries

"He that will have a cake out of the wheat must needs tarry the grinding"

William Shakespeare

"If this is molecular genetics it must be Thursday"

Program 1: Diagnostics

- **Aim to develop tools and methods for wheat and wheat products**
- To measure quality traits such as starch properties and flour proteins
- Variety identification, grain purity and soundness
- Emphasis on development of on-the-spot tests for immediate decision making

Value Chain (increasing value)

- **Germplasm**
- **Varieties (Breeders)**
- **Seed increase (Seed growers)**
- **Production (Farmers)**
- **Grain Delivery (Grain handlers)**
- **Processors (Millers, Bakers)**
- **Delivery to Consumers (Retailers)**

Diagnostic Projects

- **1.1.1 Protein Composition Analysis**
  Leader: Ian Batey, CSIRO Food Science Australia
- **1.1.2 Antibody Diagnostics**
  Leader: James Chin, EMAI
- **1.2.3 Diagnostics Delivery**
  Leader: Felice Driver, C-Qentec

1.1.1 Protein Composition Analysis

- **Identify specific proteins in grain-genotyping (breeding, variety ID)**
- **Quantitative measurement of specific proteins or other compounds that are influenced by environment and affect end-use quality**

1.1.2 Antibody Diagnostics

- **Produce new antibodies to selected targets (inputs form 1.1.1 and 3.1.2)**
- **Develop new immunization strategies and optimize yield of monoclonal antibodies**
- **Evaluate phage-peptide technology**

1.2.3 Diagnostic Delivery

- **Wheat-Rite Format**
  (Rain-damage test: field, silo, processor): Marketed by C-Qentec
- **Breeders' Kits**
  LMA (Late Maturity Amylase)
  Wheat-Rye Translocations
  GBSS Null4A, Null7A Starch Quality

Value Chain (increasing value)

- **Germplasm**
  - ← GBSS,CE
- **Varieties (Breeders)**
- **Seed increase (Seed growers)**
- **Production (Farmers)**
  - ← WheatRite®, Variety ID
- **Grain Delivery (Grain handlers)**
  - ← CE, GBSS
- **Processors (Millers, Bakers)**
- **Delivery to Consumers (Retailers)**

Links to other Programs/Projects

Project 1.1.1:
- **Tools for breeding (Program 4)**
- **Identification of new quality targets (Program 2)**

Project 1.1.2:
- **Select new targets identified by proteomics (Project 3.1.2)**
- **Tools for selecting protein mutants following site-directed mutagenesis (Project 3.1.3)**

Project 1.2.3:
- **Breeder kits to assist selection (Program 4, core partners and others)**

Project 1.1.1
Protein composition analysis

AIMS:

- Develop methods for more efficient identification of variety and quality type
- Investigate methods deployable beyond the requirements of a central laboratory
- Identify specific protein markers indicative of genotype and/or end-use quality
- Technology transfer

Staff involved

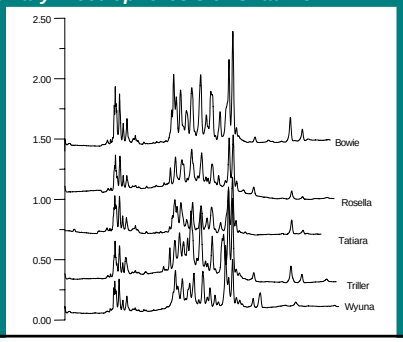
- Ian Batey (VAWCRC & FSA)
- Colin Wrigley (VAWCRC & FSA)
- Surjani Uthayakumaran (FSA) commenced February 2003
- Geoff Cornish (SARDI)
- Rebecca Tonkin (SARDI)
- Frank Bekes (consultant) commenced July 2003
- Anneliese Rittau (PhD student) commenced January 2003

Approaches

- Workshops to identify industry needs
- Gliadin gel electrophoresis for variety ID
- Gliadin CE for variety ID
- Glutenin CE for HMW units
- "Lab-on-a-chip"
- Portable GC to analyse head-space gas

Variety Identification

Capillary Electrophoresis of Gliadins



Variety Identification

Mini Gels of Gliadins

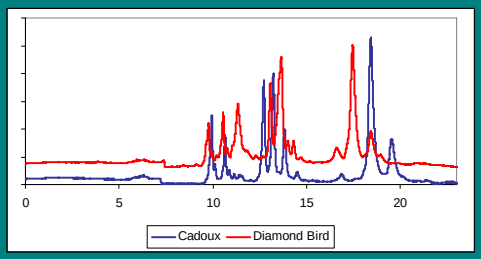
Variety Identification

Computer-assisted interpretation

- Program for converting desk-top scan of standard or mini electrophoresis gel to digital format for computer matching
- Application of PatMatch to scans, CE or HPLC data
- Working with Education/Technology Transfer

Glutenin Markers of Dough Quality

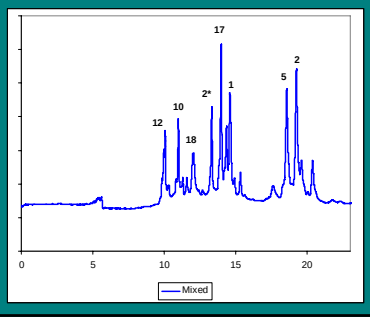
CE of HMW sub-units of glutenin



Project 1.1.1

Glutenin Markers

CE of HMW subunits



Project 1.1.1

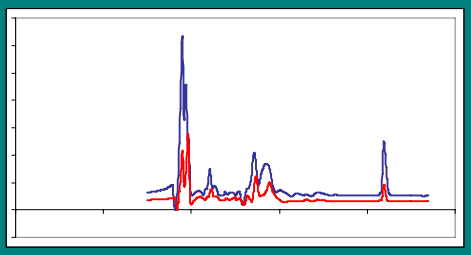
Other Research Approaches

Agilent Lab-on-a-chip (portable CE equipment)

Project 1.1.1

Other Research Approaches

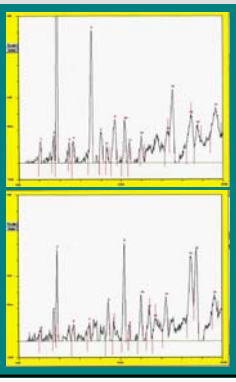
Agilent Lab-on-a-chip (gliadin profiles)



Project 1.1.1

Other Research Approaches

Rapid GC of headspace gas – rice varieties



Project 1.1.1

Other Research Approaches

Rapid GC of headspace gas

- **Initial differences observed**
- **In a larger sample set, differences between samples of the same variety were almost as great as differences between varieties**
- **Potential use for detecting defects and contaminants**

Project 1.1.1

Future Work

- Extend initial trial of “Lab on a chip”
- Other markers
  - GBSS
  - LMW glutenins
  - Other starch synthases
  - Serpins
- Computer-assisted interpretation
- Simple test for dough strength (%UPP)

Technology Transfer

- VAWCRC Reports
- Workshops
- Conference presentations
- Young scientists in training
- Further development of software (summer student)

Wheat Protein Quality

Anneliese Rittau (PhD student, started 1/2003)

Industry and academic co-supervisors -

- Dr Ian Batey, Value Added Wheat CRC
- Dr Colin Wrigley, Value Added Wheat CRC
- Prof. Les Copeland, University of Sydney
- Di Miskelly, Allied Mills Ltd

Experimental Approaches

A) **Examine a set of multi-null samples, to elucidate the consequences of allelic variation in high molecular weight glutenin subunits (HMW-GS).**

B) **Examine effects of climate conditions on protein quality of different wheat cultivars, using small scale quality tests**

C) **Investigate wheat parameters and the manner in which they affect rapid tests for quality, including SDS sedimentation**

- **In this talk I will focus on approach A**

Industrial Importance

- **Glutenins are 40% of flour protein**
- **Key quality factors in bread and biscuit manufacture**
- **HMW-GS play a major role, as they control dough viscoelasticity**
- **Potential to test wheats for bread and biscuit quality by elucidating HMW-GS allelic variation**

Rationale for research approach

- **The SDS sedimentation test**
  - **used for several years at Goodman Fielder**
  - **proved effective to select bread-making quality**
  - **is it effective to select biscuit-making quality?**
  - **does it indicate quality according to allelic variation in HMW-GS composition?**
- **Test a set of multi-null genotypes in a biscuit wheat, providing combinations of deletions of HMW-GS**

Grain samples

Grain samples studied:-

- **Cultivar Tincurrin crossed with the Gabo-Olympic set of multi-nulls by Dr Shah in a QWCRC project (Sydney University PBI, Narrabri).**
- **Multi-null lines available had HMW-GS alleles in :**

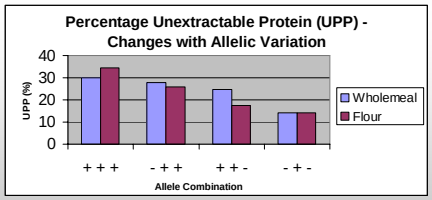
all 3 genomes	**+ + +**	**(2 samples)**
B & D genomes	**- + +**	**(2 samples)**
A & B genomes	**+ + -**	**(3 samples)**
B genome only	**- + -**	**(3 samples)**
Triple null	**- - -**	**(1 sample)**

Methods

- **Samples (>1 kg) were test-milled (Buhler lab scale)**
- **Small-scale tests were carried out to observe effects of the HMW-GS allele combinations:**
  - **SDS sedimentation (wholemeal)**
  - **SE-HPLC (flour and wholemeal)**
  - Zeleny sedimentation (flour)
  - Farinograph and extensograph
  - Solvent retention capacity

Results So Far

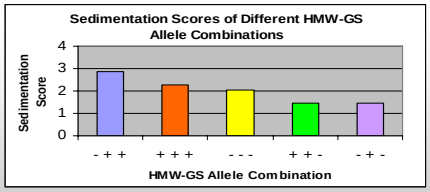
***SE-HPLC** - %UPP (large glutenin)*



- UPPs remain in same order for flour and wholemeal
- UPPs follow pattern expected for HMW-GS deletions
- Results show the importance of the D genome
- UPP is an important indicator of dough quality

Results So Far

SDS Sedimentation, corrected for % protein



- Significant differences between allele combinations
- The highest performing combination involves a null allele in the A genome
- Results do not follow expectations for deletion of HMW-GS

Significance of results

SDS sedimentation test

- effective to select for good dough properties for bread-making
- may be less effective indicating dough properties in biscuit wheat
- caution required in interpreting results for biscuit wheats
- reasons for this need more investigation

UPP test

- correlated with expected dough properties (relating to % of very large glutenin)
- a simple version of this should be valuable for industry
- research on a simple UPP-type test has started

Training

Completed

training with Allied Mills, including:

- plant inspections
- small-scale test methods (SDS and Zeleny sedimentation, Farinograph and Extensograph)

Planned

visit SARDI laboratories, Adelaide:

- gain expertise in SDS-PAGE for glutenin subunit identification
- observe breeding and selection for quality of wheats in southern states

visit Plant Breeding Institute, Narrabri:

- observe selection for quality of Prime Hard varieties grown in northern states

Future Directions

- Complete further testing of current sample set, including Farinograph and Extensograph tests.
- Expand sample sets to incorporate all combinations and testing of multi-null set.
- Test variation of allelic composition on different biscuit wheats e.g. Quarrion and Bindawarra

Project 1.1.2

Antibody based diagnostics

Location:	Elizabeth Macarthur Agricultural Institute
Project Leader:	Dr. James Chin
Research :	Dr. Thomas Giersch Ming-Jie Wu
Technical Support:	Louise Duncan Stephen McKay
Students:	Araluen Freeman (PhD) Michelle Powell (MSc)

Industry Needs

- **Rapid diagnostic test that is robust, simple and easy to perform at silo site**
- **Designer diagnostic kits, for breeders to match phenotype with genetic traits.**
- **Suite of lab/factory-based tests for more precise definition of quality traits**

Project Aim

To exploit the advantages of diagnostic antibodies for the development of commercial test kits that will facilitate the identification of specific wheat varieties and quality traits.

Objectives

1. **Analysis of protein polymorphisms in a selected panel of wheat varieties with a focus on starch granule-related proteins**
2. **Design and immunogenicity assessment of novel peptide structures for antibody production**
3. **Production of monoclonal antibodies against identified targets (Pipeline of antibody production)**

1. Protein polymorphisms in panel of wheat varieties; focus on starch granule-related proteins

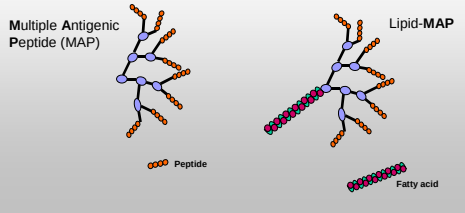
- **Optimise protein extraction techniques to detect new polymorphic proteins**
- **Sequence analysis and identification of new targets**
  - ➔ **in Collaboration with Program 3.1.2**

Some of this work is covered by Student presentations

2. Design and assessment of novel peptide structures for antibody production

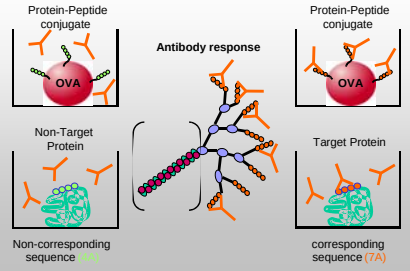
How to make a peptide immunogenic?

Assembling a unique target sequence in a lysine scaffold



2. Design and assessment of novel peptide structures for antibody production

Screening Strategies



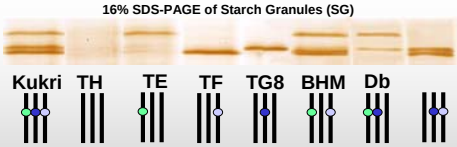
2. Design and assessment of novel peptide structures for antibody production

Proof of concept:
Granule Bound Starch synthase (GBSS 7A)

- Major enzyme in the starch biosynthesis pathway
- Sequences of the 3 alleles are known
- Present/absent in different wheat cultivars
- Demand by breeders for a quick method to identify isoforms

GBSS1-null wheat varieties (X. Zhao)

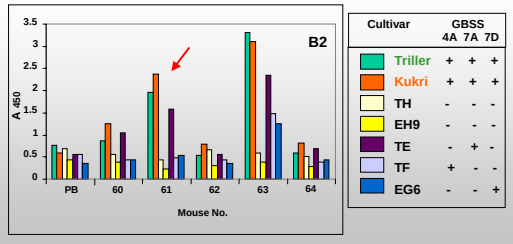
16% SDS-PAGE of Starch Granules (SG)



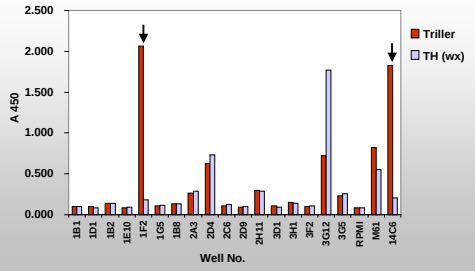
Kukri TH TE TF TG8 BHM Db

- *Wx-7A* - Chromosome 7, Genome A (7A)
- *Wx-7D* - Chromosome 7, Genome D (7D)
- *Wx-4A* – Translocated from Chromosome 7, Genome B (7B) to Chromosome 4, Genome A (4A)

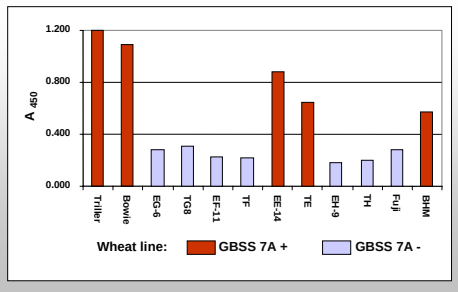
Reactivity of mouse B2 x 7A MAP against 8M urea SG extracts from GBSS-null lines



Primary screen of supernatants from F5 against 8M-urea extracts. from SG of Triller (4A+, 7A+, 7D+) & TH (Triple Null)



Can the F5-1F2 Mab detect GBSS 7A in 8M urea extracts from whole grains?



3. Production of monoclonal antibodies

What makes a "good" target?

- **Polymorphic between cultivars**
- **Quality trait or linked to quality**
- **Low sequence homology with related proteins**
- **Splits Australian wheats into distinct groups**

3. Production of monoclonal antibodies (Pipeline of antibody production)

Target	Function	Importance
GBSS 7A	Starch quality	Breeding Programs
GBSS 7D	Starch quality	Breeding Programs
Pan-GBSS	Starch quality	Test kit component
50kDa Protein	Linked to hardness?	Variety ID
Serpin 1a		Variety ID
Pan-Serpin		Test kit component
Branching Enz I	Starch quality	Breeding Programs
PinA	Related to hardness	Variety ID

3. Pipeline of antibody production Where are we now?

Student presentations

Michelle Powell:	How can different extraction methods help us to find protein polymorphisms?
Araluen Freeman:	Starch granules - Where are the proteins? Are there alternatives to antibodies?

Antibody-based diagnostics

Michelle Powell (Master's student)

- **Academic Supervisors:**
  - Dr. James Chin, *NSW Agriculture*
  - Dr. Elizabeth Hegedus, *University of Sydney*
- **Project Supervisor:**
  - Dr. Thomas Giersh, *NSW Agriculture*

- **Experimental approaches:**
  - Evaluate potential for differential extraction of starch granule proteins in hard and soft wheats, based on hydophobicity
  - Characterise polymorphic starch granule proteins from different cultivars using increasing concentrations of urea
  - Identify novel polymorphic peptides by mass spectrometry

Introduction

- **ELISA success depends on identification of polymorphic proteins in different varieties.**
- **Once identified, unique peptides can be used as targets to elicit specific antibodies.**

Hypothesis:

- **Most proteins in starch granules are held together by hydrophobic bonds.**
- **Increasing concentrations of urea will extract these proteins differentially.**

Methods

Preparation of Starch granules

Whole grain ground and soaked O/N in detergent solution

↓

Homogenised and filtered

↓

Centrifuge, aspirate supernatant

↓

Water wash (X2): resuspend pellet in water, centrifuge, aspirate supernatant

↓

Ethanol wash (X1): resuspend pellet in ethanol, centrifuge, aspirate supernatant

↓

Allow pellet to air dry

Sequential Urea Extraction

Starch granules

↓

1M urea for 30', centrifuge, collect supernatant

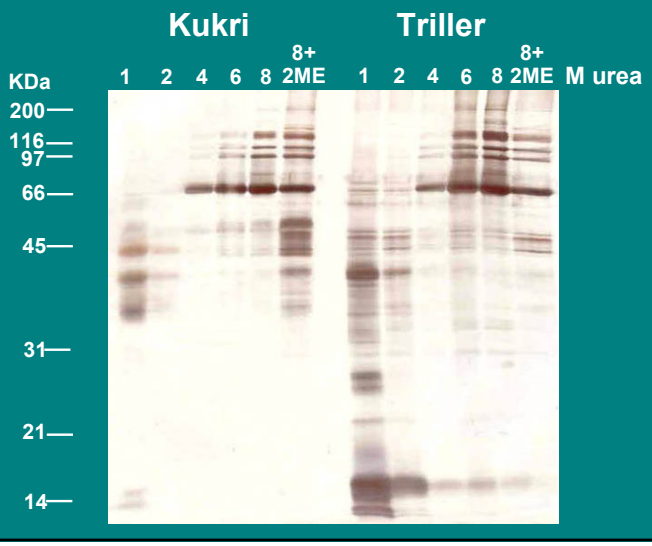
↓

Resuspend pellet in 2M urea for 30', centrifuge, collect supernatant

↓

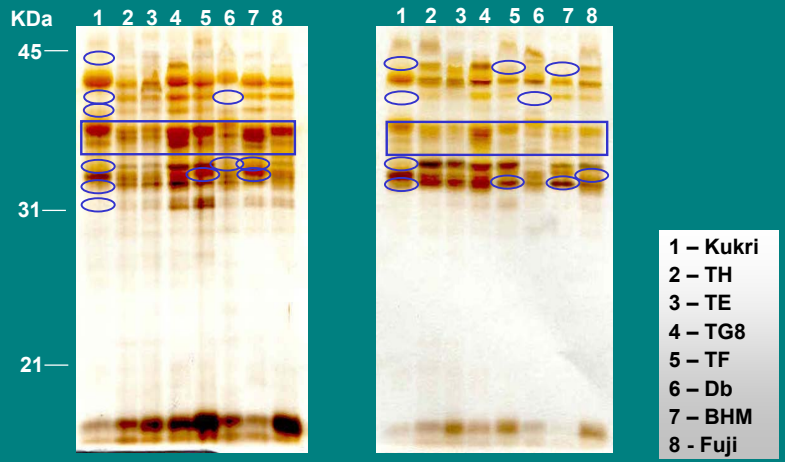
Repeat incubation for 30', centrifuge and collect supernatant for 4M, 6M, 8M and 8M+2-ME

Sequential urea extraction of starch granules



Comparison of urea-extracted peptides in different wheat lines

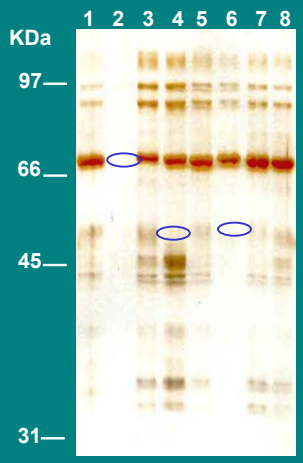
1M urea extraction *2M urea extraction*



1 – Kukri
2 – TH
3 – TE
4 – TG8
5 – TF
6 – Db
7 – BHM
8 - Fuji

Comparison of urea-extracted peptides in different wheat lines

8M urea extraction



1 – Kukri
2 – TH
3 – TE
4 – TG8
5 – TF
6 – Db
7 – BHM
8 - Fuji

Conclusions

- Different proteins extracted from Kukri (hard) and Triller (soft) wheat starch granules, using sequential urea extraction.
- Lower MW proteins extracted with low urea concentrations, and higher MW proteins extracted with increasing urea concentrations
- Observed proteins which are polymorphic between wheat lines, within a specific urea concentration

Future Directions

- Mass spectrometry to characterise polymorphic peptides identified by sequential urea extraction
- Immunogen design and synthesis, based on polymorphic peptide sequences, for antibody generation

Antibody based diagnostics

Araluen Freeman (PhD student)

- **Academic Supervisors:**
  - Dr. James Chin, *NSW Agriculture*
  - Prof. Cris dos Remedios, *University of Sydney*
- **Project Supervisor:**
  - Dr. Thomas Giersch, *NSW Agriculture*
- **Experimental Approaches:**
  - To challenge the concept that proteins are classifiable as 'surface' and 'integral' in the starch granule on basis of size
  - To obtain proof of concept that phage-displayed peptides can be used for specific identification of different wheat proteins

Accessibility of Starch Granule Protein

Surface proteins (●) (5-30 kDa)

Integral proteins (●) (59-145 kDa)

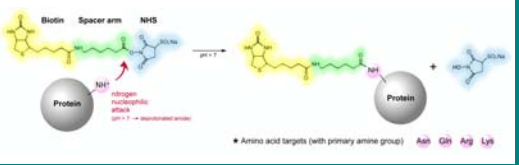
Starch granule

Extract protein

Total protein

Experimental Methods

Extract SG → **Label SG on ice** → **Wash** → **Extract protein** → **Analyse**



Accessibility of Starch Granule Protein

Surface proteins (●) (5-30 kDa)

Integral proteins (●) (59-145 kDa)

Biotin ○

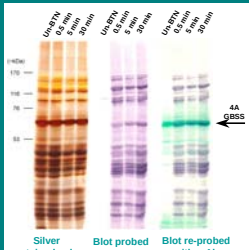
Starch granule

Extract protein

BTN surface proteins

Total protein

Accessibility of Starch Granule Protein



Silver stained gel | **Blot probed with streptav-AP** | **Blot re-probed with mAb (4A GBSS)**

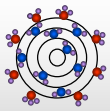
- **Accessible proteins of whole SG labelled via biotinylation**
- **Majority of SG-associated proteins labelled**
- **Extent of labelling unaffected by reaction time (30sec – 30min range)**

Accessibility of Starch Granule Protein

Surface proteins (●) (5-30 kDa)

Integral proteins (●) (59-145 kDa)

○ **Biotin**



Starch granule

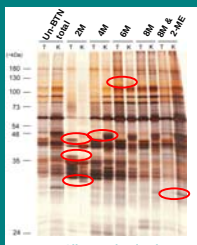
Extract protein
Analyse

BTN labelled protein

Total protein

- **Surface-accessible protein is not restricted to LMW protein**
- **Entire range of SG proteins accessible to solutes *in situ* including polymorphic protein**

Sequential Urea Extractions



Silver stained gel

Blot probed with
Streptavidin-AP/BCIP-NBT

- LMW proteins less tightly associated with SG
- Whole range of protein labelled
- Polymorphisms detectable in low concentration urea extractions

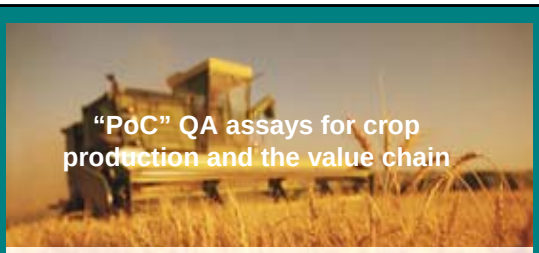
Future Directions

- TEM localisation of biotinylated SG proteins with monoclonal antibody to confirm location of specific protein
- Phage Peptide Display approach to purified and complex wheat antigens, to identify subsets of peptides with unique specificities
- Characterisation of phage peptides across different varieties to select discriminating peptides

DIAGNOSTICS DELIVERY
Project 1.2.3

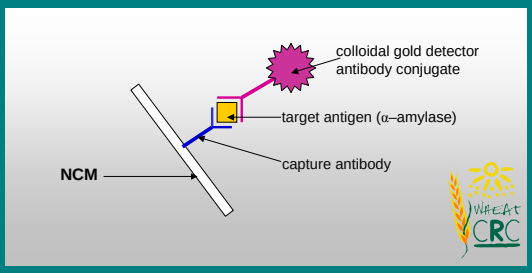
- Core Participant of VAW-CRC
- Commercialise the OUTPUTS of Programs 1 & 3
- Additional investment to provide a commercially acceptable test delivery platform and associated instrumentation

C-Qentec DIAGNOSTICS A subsidiary of Bayer CropScience *In partnership with* proteome systems integrated proteome technology *and* WHEAT CRC

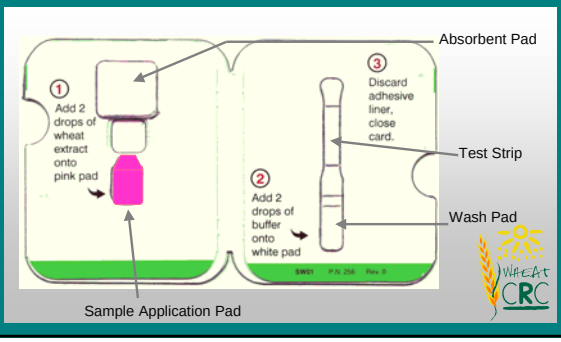


- Range of rapid immunoassays for PoC testing used as decision support tools
- Platform suitable for growers and the grain value chain
- Multi-screen tests: identification of variety and quality type in wheat
- Coordinated investment in development of electronic reader for objective test interpretation

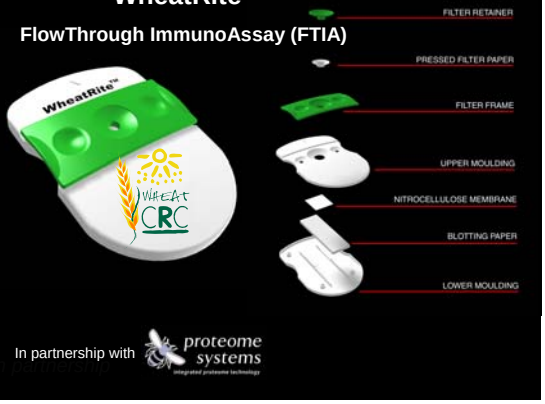
WheatRite® Test Principle
Double Antibody Sandwich Immunoassay



WheatRite®
Patented Folding Card (Binax, USA)

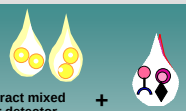


WheatRite®
FlowThrough ImmunoAssay (FTIA)



In partnership with proteome systems integrated proteome technology

Test Cassette, Incubation Chamber and Pre-Filter

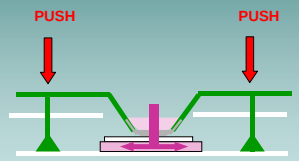


- Sample extract mixed with colour detector antibodies
- Detector antibody binds to α–amylase in pre-filter incubation well

- Detector antibody conjugate
- Procedural control conjugate
- α–amylase

Pre-filter in UP position provides controlled contact between membranes

- Reaction initiated by pushing down on removable pre-filter unit to bring membranes in contact
- Vertical flow-through of immunoassay reagents and antigens past capture antibodies



Detector antibody conjugate bound to antigen is captured by second recognition antibody printed on the membrane

Procedural control conjugate captured by its own recognition antibody printed to a separate site on the membrane

Band intensities depend on degree of occupancy of capture antibody sites by the immunoconjugate -antigen complex.

ReadRite ImmunoScanner:
Objective test interpretation

2002 MODEL

HAND-HELD UNIT

- **In-field use**
- **Modified optics, test band scanning**
- **Selectable menu for multiple test interpretation**
- **Quantitation and pattern recognition**

Breeders' Kits:
ELISA Plate Diagnostics

- **SARDI-assembled ELISA kits**
  - **Use cut and ground grain, 5 plates / kit**
- **1B/1RS wheat-rye translocations -12 kits (2400 lines tested)**
  - **Potential for CIMMYT(>10,000 lines)**
- **Late Maturing Amylase (LMA) - 92 kits equivalent (2,000 lines)**
  - **Supports national testing. Potential for CIMMYT (>10,000 lines)**
- **Granule Bound Starch Synthetase (GBSS) -2 kits (500 lines)**
  - **6 unsolicited enquiries (require IP to be resolved) - potential for >10,000 breeders' lines: USA, Japan, Canada, Australia**
  - **Need to develop test for silo and processors.**

Wheat Variety and Quality Type Diagnostic

- **Compatible sample preparation procedure**
- **Panel of antigens and immunoreagents, DAS format**
- **Monoclonal for conjugation to colloidal gold, with reactivities against GBSS 4A, 7A and 7D (GF-CSIRO IP)**
  - **Specific monoclonal antibodies to be striped to report 4A, and 7A (7D) in simultaneous display**
- **1RS/1BS initially trialled using peroxidase conjugate**
- **Blank cassettes provided for hand striping**
- **ReadRite Scanner for pattern recognition with diagnostic key for variety identification**

Delivery

- **Development linked to commercial platform**
- **Input from Proteome Systems**
  - **Cassette blanks**
  - **Colloidal gold conjugation**
  - **Technical training**
- **Further development and manufacturing at Proteome Systems**
- **Distribution into similar markets as WheatRite®**
  - **Processors: *quality type, variety exclusion***
  - **Farmers: *verify variety at seeding***
  - **Bulk handlers: *end point royalties for PBR***
- **Test format will be familiar and part of a suite of diagnostic products for grains industry**

Program 2
Products and Processing

Aims: to generate knowledge

- for enhancement of the processing performance of wheats

  and

- for the creation of new and improved products

Continuing

2.1.1 Blending – consequences for wheat breeding

2.1.4 Optimisation of key stages of the baking process

2.1.5 * Australian wheat for sponge and dough breadmaking process

2.1.6 * Strategies to replace flour chlorination as a treatment for cake flours

2.1.9 * Gluten structure and modification for ingredient use

2.2.10 Analysis of starch lipid complexes

New

2.3.11 Extended shelf life bread and baked goods

2.3.12 Wheat quality for starch and gluten production

Completed

2.1.8 Investigations on increasing the conditioning efficiency of wheat

2.1.7 Microbiological safety and stability of noodles, breadcrumbs and steamed breads

* GRDC funded

2.1.1 Blending
- consequences for wheat breeding

Leader: Geoff Cornish

Aims: apply blending models to

- breeding programs
- commercial flour blends for end products

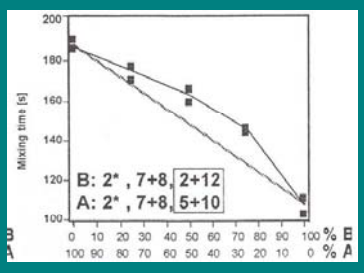
Collaborators: SARDI, VIDA, AM, Arnotts

Relevance to industry problem

- Varieties consisting of mixed biotypes
  - not desirable quality
  - use Bibrid© system to produce optimum varieties which are blends of two biotypes
- Processing industry needs to produce optimum blends of hard/soft wheats for some end products

Value to industry:
Annual return $7m if 1.4m tonnes wheat upgraded to APW

Past research -
non-linear interactions Glu-1 alleles

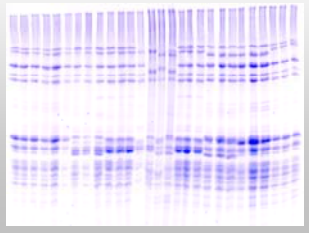


Past research

Germplasm

	Glu-1	Glu-3	Rmax
Kukri	1, 7+8, 5+10;	d, h, b	475
Janz	1, 7+8, 2+12;	b, b, b	225

- Kukri/Janz DH population field trial at Roseworthy in 2000.
- Glutenin alleles checked using SDS-PAGE.
- 3 lines of each biotype, 8 biotypes (+ parents), 2 reps



Outcomes to date

- SARDI 2000 Kukri/Janz doubled haploid lines tested.
  - Significant interaction for all dough strength measures found between Glu-1 and Glu-3 allelles
  - Over-expression of HMW glutenin allelle Glu-B1 (7+8*) detected in some lines = increased dough strength
    - found increases in Rmax of 130BU cf Janz
- industrial blending experiments of hard strong/weak and soft strong/ weak - dough and chemical tests, sweet biscuits, crackers, donuts

Future work

- Quality-test SARDI 2001 Kukri/Janz doubled haploid lines
- Complete industrial blending experiments

2.1.4 Optimisation of key stages of the baking process

Leader: Thomas Adamczak

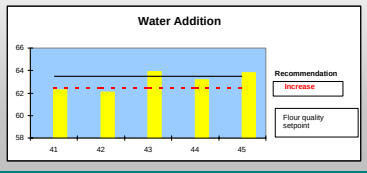
Aims:
- develop systems and equipment to optimise baking plants
- demonstrate benefits of new sensors and control systems
- develop systems for biscuit ovens

Collaborators: BRI, Arnotts, GF

Relevance to industry problem

- Integrated approach to creating uniformity at minimum production cost

Value to industry:
Potential $3M savings in yield and waste minimisation



Bakery advisory system (BAS)

Provides software, procedures and sensors to enable increase in process efficiency

- temperature probe
- stickiness sensor
- volume probe
- software

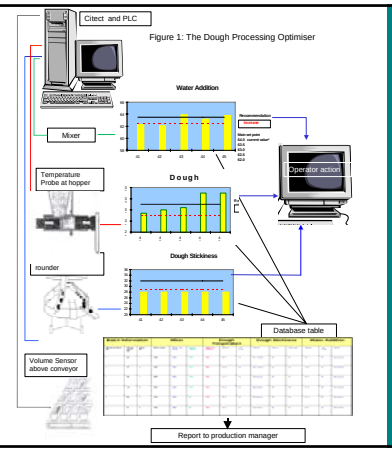


Figure 1: The Dough Processing Optimiser

Outcomes to date

- Trials of dough processing optimiser (DPO) completed at Gold Coast Bakery
- DPO hardware and software installed at Sydney bakery - commercial rollout trial
- Training manual and documentation completed (with Prog 5)
- Increased yields of up to 1% obtained in Sydney bakery
- Work commenced on “fingerprinting” biscuit ovens

Future work

- Modifications proposed by new bakery owners to be incorporated
- proceed to commercialisation of DPO
- implement cooler module
- optimise and control biscuit ovens

2.1.5 Australian wheat for the sponge and dough bread-making process

Leader: Ken Quail

Aims:

- Identify key wheat parameters and appropriate varieties for sponge and dough wheats

Collaborators: BRI, QDPI

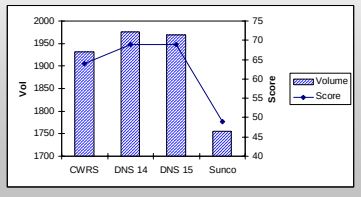
Relevance to industry problem

- Sponge and dough process used for breadmaking in N and SE Asia, but market considers PH wheat not suitable for process
- Emergence of non-traditional wheat exporters re-focussing export market on value markets

Value to industry: Market potential 6M tonnes

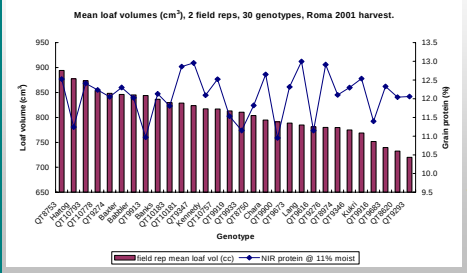
Outcomes to date

- Comparison of CWRS, DNS with Sunco
- 2001 Roma harvest samples tested at QDPI
- promising new QT entries with good bread volume



Outcomes to date cont.

- 2001 Roma harvest samples tested at QDPI
- promising new QT entries with good bread volume



Future work

- complete testing of 2002 harvest
- test further North American samples

2.1.6 Strategies to replace flour chlorination as a treatment for cake flours

Leader: Ken Quail

Aims: develop alternative, cost effective strategies to replace flour chlorination

Collaborators: BRI, AM

Relevance to industry problem

- Chlorinated cake flours produce high quality cakes
- Treatment may be banned or withdrawn

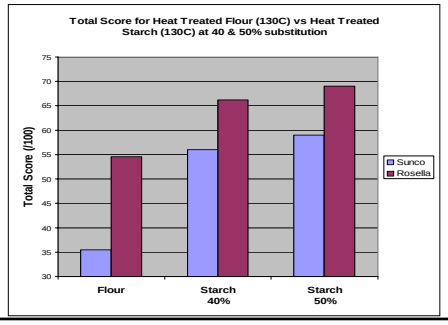
Value to industry:
150,000 tonnes cake flour produced pa. Also, export potential

Action of chlorine

- Bleach
- Lowers pH
- Protein
- Starch
- Lipids

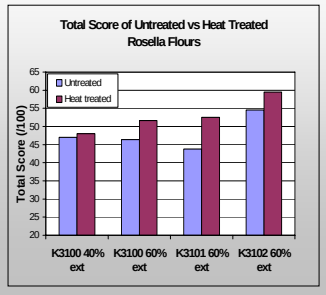
Outcomes to date

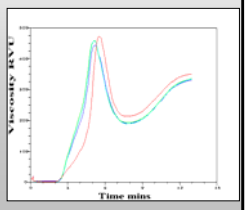
- commercial trial of flour fine grinding and fractionation
- optimal heat treatment defined
- other treatments



Varietal selection?

Rosella may offer advantages





Future work

- **continue investigation of starch replacement work and heat treatment of starches**
- **optimised formulation trials on commercial products**

2.1.7 Microbiological safety and stability of noodles, breadcrumbs and steamed breads

- **Presenter : Yang Huang**

2.1.8 Investigations on increasing the conditioning efficiency of wheat

Leader: **Michael Southan**

Aims:

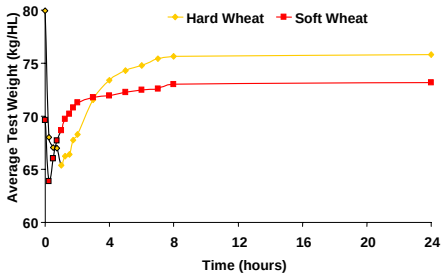
- **Monitor rate of water penetration into grain**
- **investigate chemical and biological methods to improve conditioning time and milling efficiency**

Collaborators: **BRI, AM**

Project completed 9/01

- **Conditioning: important step in preparation of wheat for milling**
- **Wheat is usually conditioned to a moisture content of 13.5 - 17.5%**
- **At this moisture content, maximum flour yield is obtained with minimal bran contamination**

Moisture penetration hard and soft wheat



Approaches

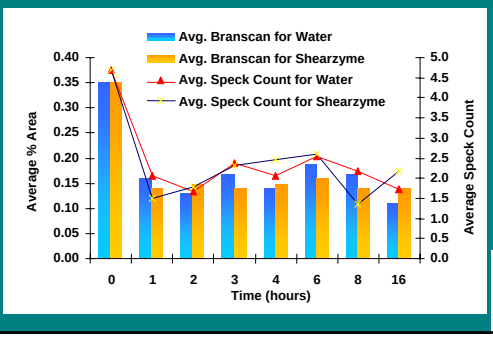
Chemical approach:

- **- 0.1M acetic acid**
- **0.1M sodium hydroxide**
- **1% SDS**
- **20% ethanol**

Biological approach - 3 enzymes:

- **Shearzyme 500L**
- **Pectinex SMASH**
- **Viscozyme L**

Branscan Average % and Average Bran Speck Count for Shearzyme



Main outcomes

- **Rate of moisture penetration into grain measured by hectolitre weight**
- **Use of chemicals and enzymes displayed a similar pattern to water**

2.1.9 Gluten structure and modification for ingredient use

Presentation : Ian Batey

2.2.10 Analysis of starch-lipid complexes

Leader: **Les Copeland**

Aims:

- **PhD project**
- **examine how starch lipid complexes can modify properties and behaviour of starches**

Collaborators: **SU**

Relevance to industry problem

- **Resistant starch important in promoting colonic health**
- **examine interactions in food systems between starch and lipids**

Value to industry:

Contribute knowledge of how lipids influence starch digestibility and viscoelastic behaviour of starch pastes

Outcomes to date

- Mary Tang appointed and commenced March 03
- Literature review in progress
- research plan and experimental protocol developed

Future work

- characterise mixtures of starch and various lipids eg saturated and unsaturated fatty acids and monoglycerides with different chain length

2.3.11 Extended shelf life - bread and baked goods

Leader: Ailsa Hocking

Aims:

- to extend shelf life of bread and baked goods by combinations of traditional means
- develop a predictive modelling tool for mould growth in MAP baked goods

Collaborators: CSIRO FSA, GF

Relevance to industry problem

- Bread-returns are a significant cost to industry
- Use combinations of preservatives, pH, a_w, enzyme systems
- Predictive model: allow development of high-moisture baked goods, using modified atmosphere packaging technology with no preservatives

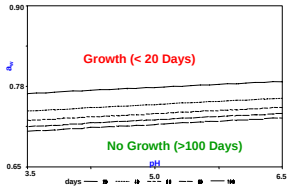
Value to industry:

- Decrease of 5% in bread returns = $5 million savings
- New market opportunities, domestic and export, for preservative-free baked goods with extended shelf life

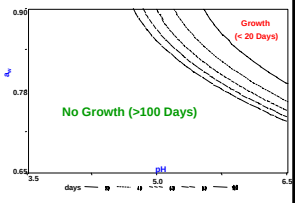
What is modelling?

- Finding equations to predict the response of microorganisms to key product parameters
- Not product-specific
- Create tools for 'virtual' product development:
  - safe/stable formulation options
  - screen ideas prior to challenge/shelf-life studies
  - reduce time to market!!
- Boundary mapping - specific type of modelling
  - describes limits of conditions that allow/do not allow growth
  - predicts Time to Growth (TTG)

E. rubrum , no preservatives



E. rubrum, 500 ppm Potassium Sorbate



Use active Excel spreadsheets to predict shelf life for product lines

(Example from previous project)

2.3.12 Wheat quality for starch and gluten production

Leader: Ian Batey

Aims:

- determine characteristics of good quality wheat for starch/gluten manufacturers
- identify effects of genotype, environment, storage and milling

Collaborators: CSIRO FSA, Allied Mills, Penford Aust

Relevance to industry problem

- Currently variability in performance of different flours in starch/gluten processing
- can have significant losses in effluent and products

Value to industry:

- over 500,000 t wheat processed into starch/gluten in Australia
- reduction of 1% in small granule starch = $1m additional profit

Prog 3: Genomics & Proteomics

Overall Aims

- Tools and knowledge for wheat breeders and end-users

Prog 3: Genomics & Proteomics

How?

- Use advanced bio-techniques in targeted ways in discovery
- Research in or with national / international centres
  - technology/expertise access
- Techniques:
  - Molecular Markers - 2 forms
  - Targeted Mutagenesis
  - Proteomics

Prog 3: Genomics & Proteomics

Delivery of outputs?

- Molecular Markers
  - through interaction in GRDC programs, and Triticarte service-provision to breeding programs
- Targeted Mutagenesis
  - development of technology; new mutants in quality genes
- Proteomics
  - transfer to Program 1 for diagnostic development; knowledge to breeders

Prog 3: Genomics & Proteomics

Progress

- Very good
- 6 talks
  - 2 marker development
  - 1 targeted mutagenesis
  - 1 proteomics
  - 1 proteomics PhD student
  - 1 marker PhD student

Project 3.1.1 Markers and Mapping Wheat Quality Traits

Dr Matt Hayden, Plant Breeding Institute - Cobbitty

Two main applications for molecular markers in wheat breeding:

1. **Marker assisted selection**
2. **Whole genome profiling**

Objectives

1. **To develop technologies to permit the low-cost, high-throughput assay of SSRs in wheat**
2. **To provide genotyping services in Triticarte Pty Ltd to facilitate the use of molecular markers in wheat breeding and the broader industry**

Triticarte Genotyping Services

- **Variety identity and/or purity**
- **Assay of trait-linked markers**
- **Skeletal genetic mapping**

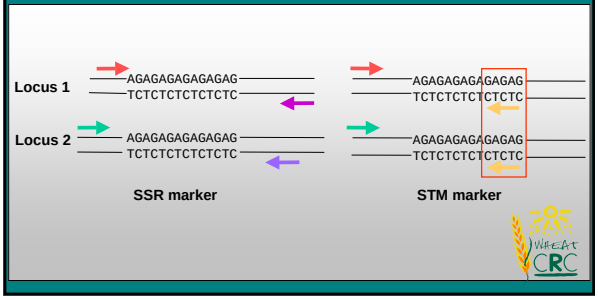
Technology Platform

Sequence tagged microsatellite profiling (STMP)

- **A VAWCRC proprietary technology that permits rapid and low-cost development of SSRs, which are amenable to high-throughput assays**

Advantages of STMs

1. Low cost and rapid marker development



Microsatellite Anchoring Primers

5'-anchoring primers for simple repeat sequences

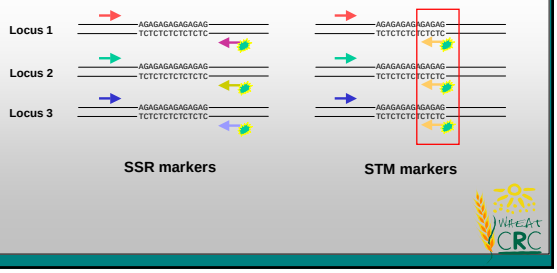
3' NNNNNNNNNNNNNNNNCTCTCTCTCTCTCTCTCTCTCTCTNNNNNNNNNNNNNNNN 5'
5' NNNNNNNNNNNNNNNNGAGAGAGAGAGAGAGAGAGAGAGAGNNNNNNNNNNNNNNNN 3'
CTCTCTCTCTCTRVRVRKK

junction anchoring primers for compound repeat sequences

3' NNNNNNNNNNNCACACACACACACACACTCTCTCTCTCTCTCTCTCTCTCTNNNN 5'
5' NNNNNNNNNNNGTGTGTGTGTGTGTGTGAGAGAGAGAGAGAGAGAGAGAGNNNN 3'
GTGTGTGTGAGAGAGAGAGAGA

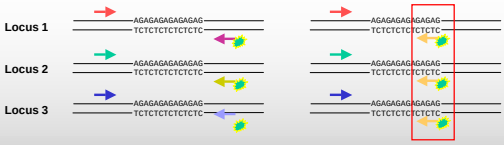
Advantages of STMs

2. Reduced cost for fluorescence-based detection



Advantages of STMs

3. High amenability to multiplex PCR



SSR markers
3-plex PCR = 6 primers
6-plex PCR = 12 primers

STM markers
3-plex PCR = 4 primers
6-plex PCR = 7 primers

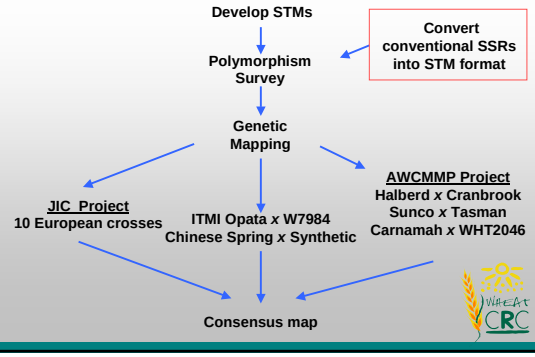
Marker Development and Mapping

Joint project between John Innes Center (JIC), AWCMMP & VAWCRC

- **AWCMMP and JIC to generate 1000 mapped STMs**

Outcome: **A consensus SSR map of bread wheat, relevant to current Australian and European germplasm, and fully integrated with published genetic maps**

Strategy



Progress – Library Development

	Total Tags	Unique Tags		Primers Synthesised	
$(Ag)_m(Ac)_n$	860	505	59%	394	78%
$(Tc)_m(Tg)_n$	965	383	40%	187	49%
$(Tc)_m(Ac)_n$	652	186	29%	82	44%
$(Tg)_m(Ag)_n$	695	196	28%	79	40%
$(Ac)_n$	2579	1230	48%	619	50%
Experimental	1103	433	39%	242	56%
Total	6854	2933	43%	1603	55%

Progress – Marker Development

Number of Primers Tested	984	
Primers Amplifying SSR(s)	628	64%
Polymorphic Markers	346	55%
Overall Efficiency	35%	

~50% of STMs are suitable for multiplex PCR

Progress – Genetic Mapping

Homoeologous Group	A-genome	B-genome	D-genome	Total
1	2	5	2	9
2	5	9	10	24
3	3	8	1	12
4	8	3	2	13
5	2	13	1	16
6	4	6	3	13
7	4	11	10	25
Total	28	55	29	**112**

large-scale genetic mapping currently in progress

Progress – Multiplex PCR

High multiplex levels are achievable

- **simple rules to construct primer bins**
- **no PCR optimisation required**
- **6-plex reactions easy to develop**
- **10-15¢ per data point**

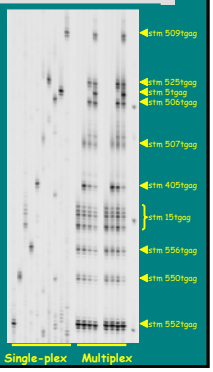
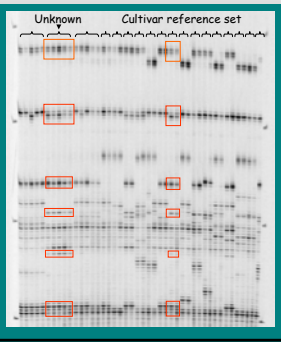


Figure. Multiplex STM amplification using 4-, 6-, 8- and 10-plex reactions

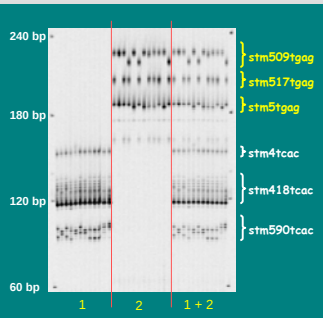
Progress – Genotyping Services

Variety identification and purity testing



Progress – Genotyping Services

Framework mapping



Progress – Sample Collection

- **Silica-based method for preserving leaf material**
- **High-throughput method to extract DNA from grain and leaf material**

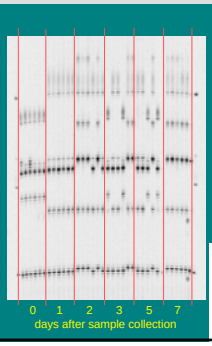


Figure. 6-plex STM amplification using DNA extracted from preserved leaf material

Diversity Arrays Technology for High-throughput Genotyping of Wheat

DArT: Novel markers for Molecular-Assisted Breeding

- **Genome-wide genotyping**
  - **Several hundred markers to provide a comprehensive 'genome scan'.**
- **High-throughput through parallel analysis**
  - **All markers are typed simultaneously in a single assay.**
- **Not reliant on DNA sequence information**
- **Cost reduction**
  - **Price per data point (= plant x marker) is ≥ 1 order of magnitude lower.**

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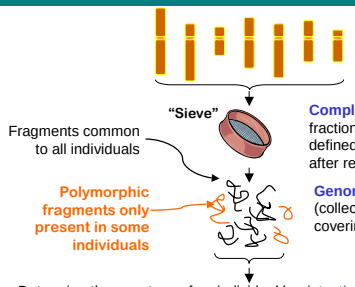
Principle of DArT



Complexity reduction: selecting a fraction of the genome based on defined criteria (e.g., size selection after restriction enzyme digestion)

Genomic representation (collection of DNA fragments covering 0.1 – 10 % of the genome)

Determine the genotype of an individual by detecting the presence/absence of the polymorphic fragments through hybridisation of the genomic representation to microarrays containing copies of the polymorphic sequences.

The wheat project

Mona Akbari, Ilaria Catizone, Andrzej Kilian

- **Aims:**
  - **Proof of concept of DArT in durum and bread wheat.**
  - **Establish efficient complexity reduction methods.**
- **Materials: 13 cv of hexaploid wheat, 32 cv of durum wheat.**
- **Extensive testing of 4 representations**
  - **PstI fragments.**
  - **Less than about 1 kb.**
  - **Not containing a frequent cutter restriction site.**
- **Results**
  - **Hexaploid wheat: four libraries of about 1500 clones show ~4 – 10% polymorphism.**
  - **Durum: four libraries of about 1500 clones show ~3.5 – 9% polymorphism.**

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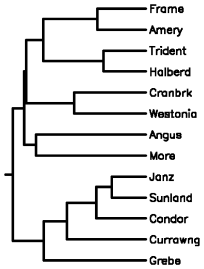
Example: wheat diversity analysis

- **PHYLIP UPGMA rooted tree with 291 polymorphic clones.**



Range of polymorphisms

Westonia - Grebe:	**154**
Sunland - Janz:	**32**
Average over 13 cv:	**112**

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Triticarte Pty Ltd

- **Delivery of high throughput genotyping to the wheat and barley industries.**
- **Two complementary platforms**
  - **DArT (Canberra)**
  - **STMP (Sydney)**
- **Services being established in 2003-2004**
  - **DNA to data**
  - **Diversity analysis of wheat and barley**
  - **Progeny testing, QTL mapping**
  - **Genotyping arrays**

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DArT Technology for Wheat

Brent Thomson, PhD student

Supervisors: Dr Andrzej Kilian (Triticarte), Prof Peter Sharp (USyd)

PhD Project Outline

- **Development of Diversity Array Technology (DArT) to aid wheat genome studies and plant breeding**

Focusing on

- **Miniature Inverted Repeat Transposable Element polymorphisms – genome polymorphisms and evolution**
- **DNA methylation variation – as a tool for analysis of epigenetic phenomena**

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Miniature Inverted Repeat Transposable Elements

MITEs

- Super-family of DNA-type transposons
- Abundant in eukaryotic genomes (thousands of copies)
- No obvious clustering in the genome
- Good genome coverage
- Recognised source of genetic variation (insertion/deletion events)
- Surrounded by low copy sequences
- Single primer amplifies both adjacent MITE regions
- Amenable for use with DA*r*T

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Experimental Approach

DArT technology applied to MITEs

- DA*r*T panels developed using DNA from two wheat cultivars - Janz and Westonia

Digest with restriction enzyme *Mse1* and ligate adapter

1st round amplification using DA*r*T F1 and stowaway primers

2nd round amplification using DA*r*T M13 and stowaway primers

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Experimental Approach

DArT technology applied to MITEs

- These fragments are cloned and amplified
- Arrayed onto glass microarray slides
- Targets prepared in the same way from the two wheat cultivars
- Targets fluorescently labelled and hybridised to the slides
- Slides scanned in a Microarray scanner to detect fluorescence

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Preliminary Results

Data generated within first 4 months

- Creation of a 1500 clone library representing Janz and Westonia
- 60 microarray slides printed, 16 of these tested
- Method 1 – targets prepared using F1 adapter
- Method 2 – targets prepared using F1b adapter

Results:

- Method 1 – 41 candidate polymorphisms (2.7%)
- Method 2 – 139 candidate polymorphisms (9.0%)

Plans for near future:

- Continued analysis of data
- Continued testing of technical aspects of MITE-DA*r*T
- Expand libraries with more wheat cultivars

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Project 3.1.2. Wheat Proteomics
(VAWCRC/APAF)

Summary of projects

- Proteomics of soft wheat cultivars (Bowie and Rosella)
  - Characterisation and identification of cultivar-specific proteins
- Serine Protease Inhibitor (serpin) polymorphism in Australian wheat cultivars
  - Extract and fractionate serpins by native-PAGE
  - Determine type and level of serpin polymorphism in over 70 Australian wheat cultivars
  - Localise serpin genes to chromosome/chromosome arm using Chinese Spring aneuploid series
  - Map serpin 3 polymorphism in an Egret/Sunstar doubled haploid population
- Proteome analysis of wheat Alien Segment Series

Proteomics of soft wheat cultivars
(Bowie and Rosella)

Investigate cultivar-specific proteins

- **AIM:** investigate, characterise and identify cultivar-specific proteins between Bowie and Rosella, for potential diagnostics
  - Wholemeal protein extracts
  - Starch granule protein extracts
  - Storage protein extracts (gliadins)
- Proteins separated using 2-D gel electrophoresis
- Cultivar-specific proteins characterised and identified via:
  - Mass spectrometry (PMF and MS/MS)
  - *N*-terminal amino acid sequencing
  - Interrogation of SWISS-PROT and TrEMBL databases

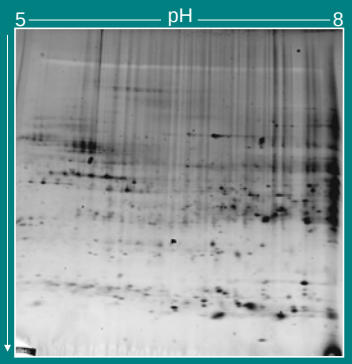
Proteomics of soft wheat cultivars (Bowie and Rosella)

Identification of cultivar-specific proteins

Summary

- For wholemeal samples, a total of 8 cultivar-specific proteins were analysed
  - Alpha-amylase/subtilisin inhibitor
  - Peroxiredoxin
  - Two alpha-gliadins
  - Remaining 4 spots not identified
- For starch granule samples:
  - Cultivars can be distinguished by presence/or absence of puroindoline-A Rosella (+) ; Bowie (-)
  - Therefore, cultivars can be distinguished from each other with anti-puroindoline-A antibody
- For storage protein samples:
  - 18 cultivar-specific gliadins characterised and identified
  - (8 from Bowie, 10 from Rosella)

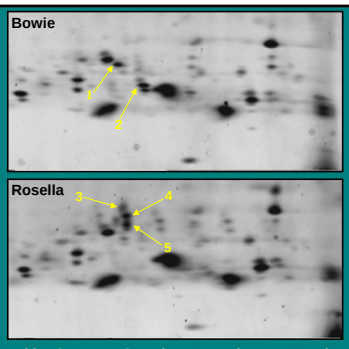
2-D gel electrophoresis



Bowie cultivar

Characterisation of target proteins
(wholemeal samples)

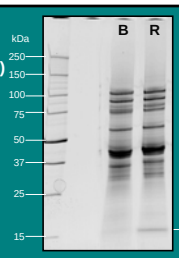
Example 1
(2-D PAGE)



Spot #	Matching protein	Peptide hits	Coverage (%)	Organism	Accession No.	Function
1	Endogenous alpha-amylase/ subtilisin inhibitor	5	30.6	Wheat	P16347 (SWISS-PROT)	Inhibitor of endogenous alpha-amylase and subtilisin

Characterisation of target proteins
(starch granule protein samples)

Example 2
(SDS-PAGE)



Distinctive protein band ~15 kDa

N-terminal sequence	Matching protein	Identity (%)	Organism	Accession No.
DVAGGGGAQQ	Puroindoline-A (precursor)	100% in 10 aa	Wheat	P33432

Comments on database match:
Our sequence commences at the *N*-terminus of the mature chain puroindoline-A (=residues 29-146), since the *N*-terminal tag starts at residue 29.
The precursor has a signal sequence = residues 1-19 and there is a propeptide sequence, residues 20-28.

Serpin polymorphism in Australian wheat cultivars

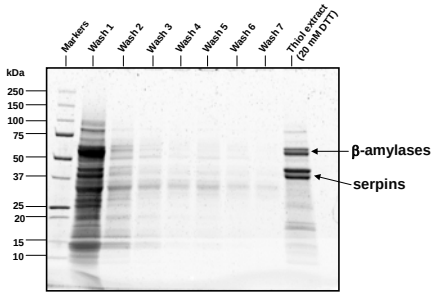
Background information

- Serpins found throughout the plant kingdom & studied in a variety of plants (e.g. wheat, barley, rye, oat and pumpkin)
- Six serpin forms identified in grains of hexaploid wheats
- Five of these serpins have been cloned and purified from *E.coli* (Danish research group)
- They are major albumins of wheat endosperm (~3-4 mg serpin/gram of grain)
- Their mobility on native-PAGE is reproducible and an excellent means of testing for polymorphisms

Collaboration with Dr Thomas Roberts (Biological Sciences, Macquarie University)

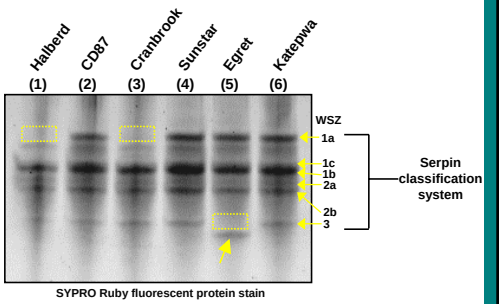
Extraction of serpins from Sunco wheat cultivar

(method from Østergaard *et al.*, 2000)



- **Washed with 0.1 M Tris/HCl pH 8.0 (30 min each wash)**
- **Added 20 mM DTT to washing buffer to extract serpins**

Screening of wheat serpins using native-PAGE

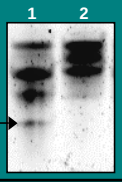


SYPRO Ruby fluorescent protein stain

Serpin polymorphism in Australian wheat cultivars

- 74 hexaploid wheats and 4 durum pasta wheats screened for serpin polymorphisms
  - 15 hexaploid wheats did not contain serpin 1a (~20% null)
  - 18 hexaploid wheats expressed a form of serpin 3 which had a higher mobility in native-PAGE
  - ALL 4 durum wheats contained serpin 1a but did not contain any form of serpin 3

(1) Lorikeet (biscuit wheat)
(2) Kamilaroi (pasta wheat)



Serpin 3 is detected in hexaploid wheats

Serpin polymorphism in Australian wheat cultivars

Chromosome localisation of serpin genes

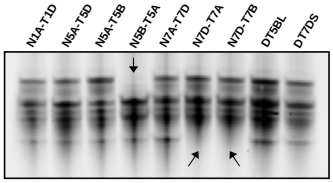
- Chinese Spring aneuploid and ditelocentric wheat lines screened to localise serpin genes to chromosomes and chromosome arms

Summary

- Serpin 1a was not detected in nulli5B-tetra5A wheat lines, indicating that the serpin 1a gene is located on chromosome 5B
- Further analysis of ditelocentric wheat lines indicated that the serpin 1a gene is located on the long arm of chromosome 5B
- Serpin 3 was not detected in nulli7D-tetra7A and nulli7D-tetra7B wheat lines, indicating that the serpin 3 gene is located on chromosome 7D
- Further analysis of ditelocentric wheat lines indicated that the serpin 3 gene is located on the short arm of chromosome 7D

Serpin polymorphism in Australian wheat cultivars

Chromosome localisation of serpin genes (1a and 3)

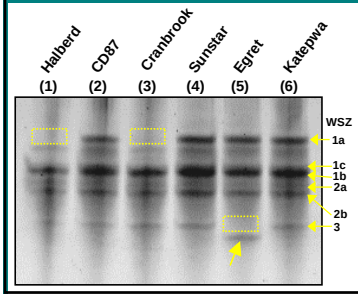


Chinese Spring

Note:
•Serpin 3 is located on the D genome
•so not detected in durum wheats
•can be used to distinguish between bread/durum wheats

Serpin polymorphism in Australian wheat cultivars

Mapping serpin 3 by screening Egret/Sunstar doubled haploid population



- **Serpins extracted from 157 doubled haploid samples**
- **Samples screened for normal or higher mobility serpin 3.**
- **Data set sent to Rudi Appels for mapping - mapped to 7D**
- **Confirms aneuploid results**

Acknowledgments

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Angela Connolly
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David Selby
Derek Van Dyk
Gary Cobon

Macquarie University
Thomas Roberts

EMAI
James Chin
Ming Wu
Thomas Giersch

VAW CRC
Peter Sharp
Neil Howes
Colin Wrigley
Yunxian Mak

A Proteomic Approach to the Characterisation of Wheat Proteins and the Investigation of Wheat Blackpoint

Candidate: Yunxian Mak[1,2,3]

Supervisors:

- **Professor Les Copeland[3]**
- **Professor Peter Sharp[1,3,4]**

Acknowledgements:

Di Miskelly [1,5], Dr Peter Williamson[6], Dr Daniel Skylas [1,2], Angela Connolly[2], Stuart Cordwell[2], Derek Van Dyk[2], Bernie McInerney[2], Liza Allen[2] Clare Johnson[1], Helen Warwick[1],

[1] Value Added Wheat CRC
[2] Australian Proteome Analysis Facility
[3] Faculty of Agriculture, Food and Natural Resource, University of Sydney
[4] Plant Breeding Institute, University of Sydney
[5] Allied Mills Technical Centre
[6] Leslie Research Centre

Aims of the Project

A: Proteomic characterisation of protein in wheat germ & bran tissues.

- **Existing proteomic technique for analysis of endosperm proteins**
- **Different type of proteins stored in different wheat grain tissues**
- **Developing proteomic methodology for wheat germ and bran tissues**

B: Proteomic analysis of blackpoint defect in mature wheat grains.

- **Blackpoint discolouration of germ and bran tissue causes significant downgrading and loss to Australian wheat industry**
- **Previously thought to result from saprophytic fungal infection (*Alternaria alternata, Bipolaris spp.* and *Fusarium spp.* etc.).**
- **Recent research indicates stressful environmental conditions (such as rain, high relative humidity and temperature) can induce black point symptoms.**

Proteomic Characterisation of *var.* Rosella Germ and Bran

Wheat samples: var. Rosella germ and bran
c/- Ms. Di Miskelly, Allied Mills Technical Centre

Proteins separated by 2-DE.
Spots excised and identified by mass spectrometry.

Germ tissue, pH 4-7 IPG strip, total 252 protein spots characterised.

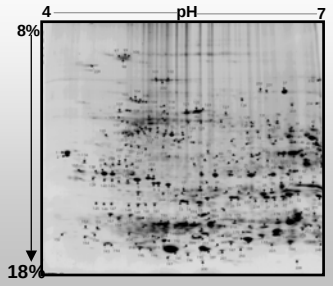


Figure 1. Proteome map of Rosella wheat germ

Bran tissue, pH 4-7 IPG strip, total 220 protein spots characterised.

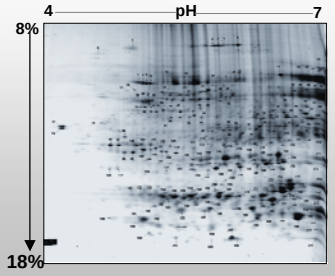


Figure 2. Proteome map of Rosella wheat bran

Proteomic Investigation of *var.* Rosella Germ and Bran

Comparison of wheat germ and endosperm: different classes of identified proteins.
(endosperm proteome data from Skylas et al., 2000. J. Cer Sci 32: 169-188)

	Wheat Germ Protein Identifications		Wheat Endosperm Protein Identifications	
	Numbers	%	Numbers	%
ENZYMES				
Oxidoreductases	10	4	11	3.4
(SOD*)	(2)	(0.8)	(8)	(2.5)
Transferases	14	5.6	0	0
Hydrolases	13	5.2	1	0.3
Lyases	4	1.6	0	0
Isomerases	7	2.8	15	4.7
(PDI *)	(4)	(1.6)	(14)	(4.4)
Ligases	3	1.2	0	0
PROTEINS				
Late Embryonic Proteins	12	4.8	0	0
Heat Shock Proteins	9	3.6	0	0
Regulatory Function Proteins	5	2.0	2	0.6
Factors	2	0.8	1	0.3
Transcription Proteins	2	0.8	0	0
Antioxidant Proteins	3	1.2	1	0.3
Transport Proteins	3	1.2	2	0.6
Ribosomal Proteins	2	0.8	5	1.6
Allergen Proteins	3	1.2	3	0.9
Plant Defense Proteins	1	0.4	1	0.3
Translationally Controlled Tumor Protein Homolog	3	1.2	3	0.9
Hypothetical Proteins	19	7.5	0	0
Other Proteins	52	20.5	1	0.3
α-amylase or α-amylase / trypsin Inhibitors	2	0.8	37	11.5
Storage Gliadins	0	0	85	26.5
Storage Glutenins	0	0	9	2.8
No Matched Proteins	83	32.8	55	17
No Sequence Proteins	0	0	89	28
Total Spots	**252**	**100**	**321**	**100**

*SOD: Superoxide dismutase; PDI: Protein disulfide isomerase.

Proteomic Analysis of Wheat Blackpoint

Wheat samples: Sun 239v

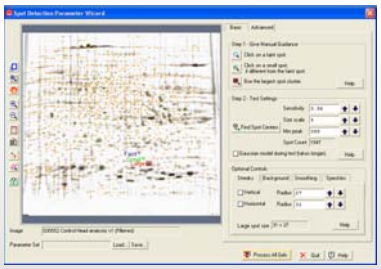
c/- Peter Williamson, Leslie Research Centre DPIQld

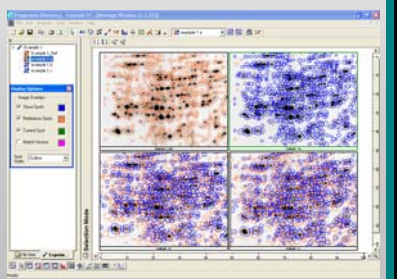
- **Blackpoint group - darkened germ end of grains; Control group - healthy grains**
- **Grains were dissected into 2 parts - germ and body**
- **6 replicate gels run from these 4 groups**
- **Spots detected using image analysis software PDQuest v7.1.1 (BioRad) and Progenesis v2002.01 (PerkinElmer)**
- **Will use MS (MALDI_TOF) and MS/MS (Q_TOF) to identify proteins**
- **Pathway-searching engines such as KEGG will be used to investigate identified protein functions in molecular pathways**

Proteomic Analysis of Wheat Blackpoint

Image Analysis Software Workflow

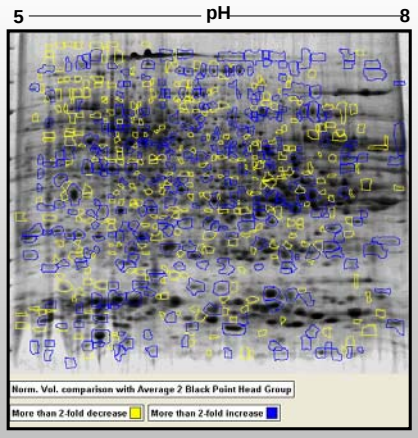
Acquire image
⇓
Image sizing and processing
⇓
Spot detection and editing
⇓
Spot comparison and matching
⇓
Data analysis
⇓
Report



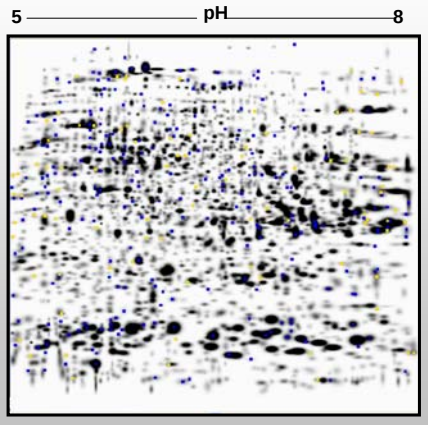


Two-fold changes in protein expression between control and blackpoint-affected germ tissue of Sun 239v

Progensis
Normalisation volume



PDQuest
Quantitative



Publication

submitted to Proteomics

Proteomic approach to characterising protein expression in wheat germ

Yunxian Mak[1,2,3*], Daniel J.Skylas[1,2],
Angela Connolly[1], Colin W. Wrigley[2,4],
Peter J.Sharp[2,3,5] and Les Copeland[3]

[1] Australian Proteome Analysis Facility, Macquarie University, NSW 2109, Australia
[2] Value Added Wheat CRC Ltd, North Ryde, NSW 2113, Australia
[3] Faculty of Agriculture, Food and Natural Resources, University of Sydney, NSW 2006, Australia
[4] Food Science Australia, North Ryde, NSW 1670, Australia
[5] Plant Breeding Institute, University of Sydney, Camden, NSW 2570, Australia

Training

Greatly enhanced efficiency

- **APAF 2-D course, March 2002: sample preparation, running 1st & 2nd dimensions, use of image analysis software.**
- **APAF Mass Spectrometry Course, March 2002: different types of mass spectrometer, use of MALDI-TOF and Q-TOF, analysis of MS and PTM data.**
- **Biolateral bioinformatic course, Dec. 2002: python language, bioinformatics applications, etc. enabling me to automate protein database searching, saving 2 months/year repetitive data entry.**

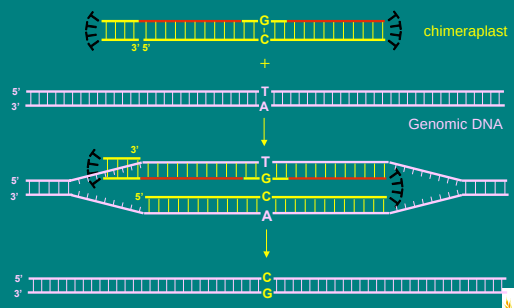
Future Directions

- **Identify protein expression related to wheat blackpoint.**
- **Search for function of the identified proteins using KEGG and other molecular pathway- searching engines.**
- **Publish this work.**
- **Induce blackpoint defect in susceptible seeds under controlled conditions**
- **Investigate the effect of the blackpoint defect on protein expression at different growth stages.**

3.1.3 Targeted mutagenesis of wheat grain characteristics

- Principle
- Proof-of-principle in wheat
- Tissue culture and selection strategy
- Future work

Principle

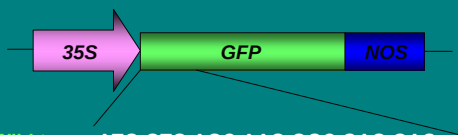


AIM

- Develop oligonucleotide-directed gene-targeting technology in wheat
- Produce wheat mutants with altered grain characteristics

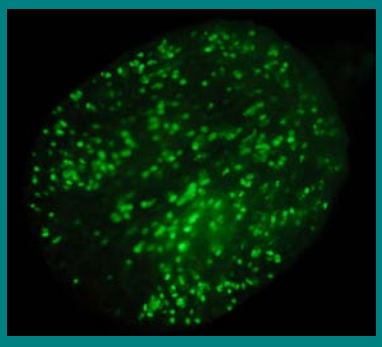
Proof-of-principle in wheat

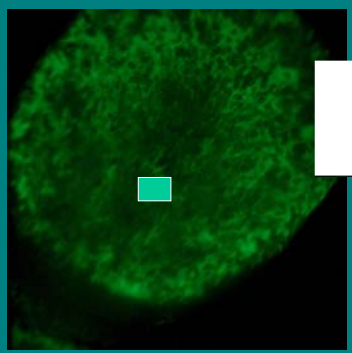
In vivo transient GFP restoration by gene repair



Wild type: ATG GTG AGC AAG GGC GAG GAG ….

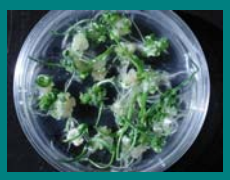
Δgfp: ATG GTG AGC AAG GGC AG GAG ….

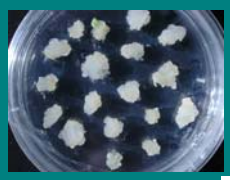




Wheat tissue culture

Regeneration on wheat scutella culture





Selection strategy

Herbicide resistance as a selection in tissue culture

- Acetohydroxyacid synthase (AHAS) gene
- Imidazolinone or sulfonylurea resistance by mutation of AHAS
- Double mutation will be the strategy for grain quality gene mutation

Wheat *AHAS* gene

Wheat AHAS gene and its expression has been studied

GCCTATGATC CCAAGCGGTG GTGCTTTCAA
GCCTATGATC CCAAGCGGTG GTGCTTTCAA
GCCTATGATC CCAAGCGGTG GTGCTTTCAA
GCCTATGATC CCAAGCGGTG GTGCTTTCAA
GCCTATGATC CCAAGCGGTG GTGCTTTCAA
GCCTATGATC CCAAGCGGTG GTGCTTTCAA
GCCTATGATC CCAAGCGGTG GTGCTTTTAA
GCCTATGATC CCAAGCGGTG GTGCTTTTAA

Future work

- *AHAS* gene mutation and efficient selection
- Haploid tissue targeting
  - Haploid scutellum culture
  - Microspore culture
- *Waxy* gene targeting

Program 4
Germplasm & Varieties

Program Manager: John Oliver

Program 4: Germplasm & Varieties

Target: Varieties with improved or novel traits

- **4.1.1 New genetic variation and markers for quality traits**
- **4.1.2 Rapid breeding technologies**
- **4.1.3 Soft wheat program**
- **4.1.4 Flexibility of wheat use**
- **4.1.5 Increasing the quantity of grains delivered to premium grades**
- **4.2.6 Waxy wheat program**
- **4.2.7 Commercialisation of sprouting tolerant germplasm**
- **4.3.8 Development of adapted germplasm and varieties**
- **4.3.9 Marker validation and identification for key quality attributes in WA adapted germplasm**

Clients: Arnott's, Allied Mills, Goodman Fielder, NSWAg, DAWA, SunPrime, Uni Sydney, SARDI, GrainCorp, GRDC

Program 4: Germplasm & Varieties

U Sydney, Cobbitty:

- **Matthew Turner (4.1.1, 4.3.8)**
- **Mohammad Shariflou (4.2.6)**
- **Nizam Ahmed (4.1.2)**
- **Akram Khan (4.1.3, 4.3.8)**

NSWA, Camden:

- **Mui-Keng Tan**

NSWA, Wagga Wagga

- **Helen Allen (4.1.3)**

DAWA, South Perth

- **Michael Francki (4.3.9)**
- **Karon Ryan (4.3.9)**

NSWA, Tamworth

- **Michael Sissons (4.1.1)**
- **Cindy Soh (4.1.1)**

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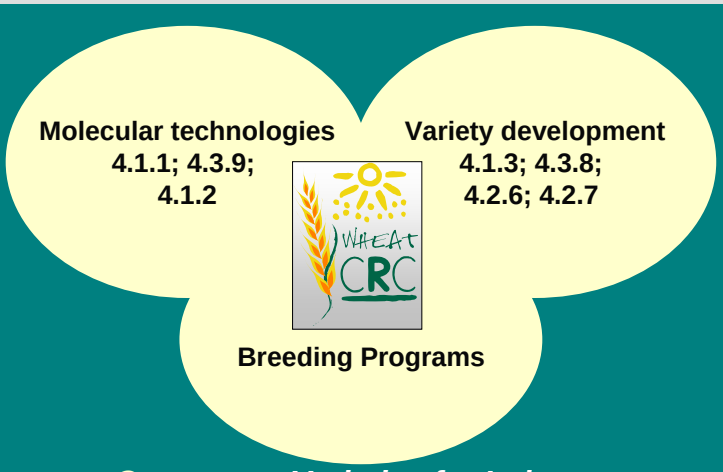
***Clients:** Arnott's, Allied Mills, Goodman Fielder, NSWAg, DAWA, SunPrime, Uni Sydney, SARDI, GrainCorp, GRDC*

4.2.7: Commercialisation of sprout-tolerant germplasm

Target: Varieties with improved agronomic traits

- **DM5637*B8:**
  - **PHS; LMA; low PPO; low blackpoint**
- **DH lines in H45 & Wyalkatchem backgrounds**
- **Pursuing molecular markers to assist selection**

Mission: Germplasm Enhancement



Outcomes: Varieties for Industry

Outputs

New variety; Novel germplasm; Molecular markers

- **QALBis**
- **Waxy wheat line**
- **Novel extremes in germplasm identified:**
  - **Null PPO**
  - **B-granule starch variants**
  - **High apparent amylose**
  - **Extremes in soluble pentosans**
  - **Large grain size**
- **Molecular markers PHS; LMA; 4A, 7A, 7D GBSS**

Project 4.1.1: New Genetic Variation and Markers for Quality Traits

Matthew Turner

Plant Breeding Institute, Cobbitty
The University of Sydney

New Sources of Variation

Targets

- **Low polyphenol oxidase (PPO) activity**
- **Extremes of starch granule size distribution**
- **High apparent amylose content**
- **Large round grain**
- **Extremes of soluble pentosan content**

Polyphenol oxidase activity

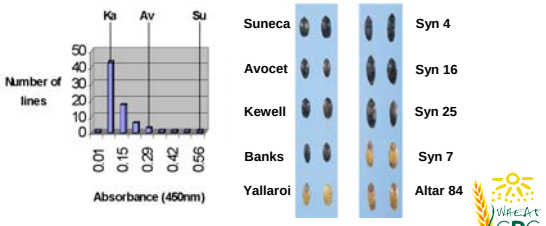
Introduction

- **Colour and brightness are important criteria by which wheat products are assessed**
- **Darkening associated with polyphenol oxidase (PPO) is an undesirable character**
- **Most hexaploid wheats have very high PPO activities**
- **The aim of this work is to identify low PPO sources and to develop low PPO wheat cultivars**

Achievements

A survey of PPO activity in synthetic wheats

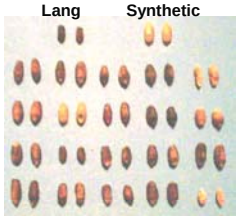
- **PPO activity in synthetic wheats**



Achievements

Inheritance of null PPO

Null PPO is conferred by three loci in Lang x Synthetic DH populations



Future Work

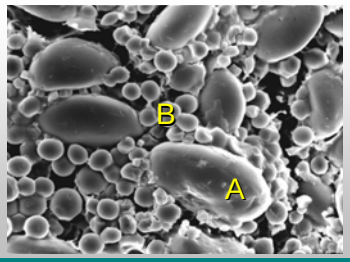
- **Identification of molecular markers for loci conferring null PPO**
- **Introgression into premium soft and hard wheats**

Starch Granule Size Distribution

Introduction

- Starch is stored in granules in endosperm of wheat grains
- Two types of starch granules designated A and B are classified by size and shape
- Little is known about variation in starch granule size distribution in wheat

Electron micrographs of endosperm

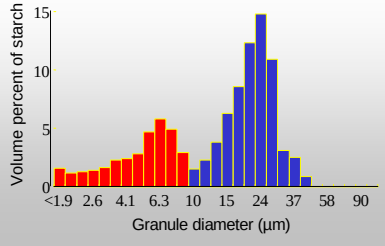


Rationale

- Starch granule size distribution influences processing and product quality
- We aim to identify germplasm with extremes of starch granule size distribution and to introgress them into elite wheats

Achievements

Survey results

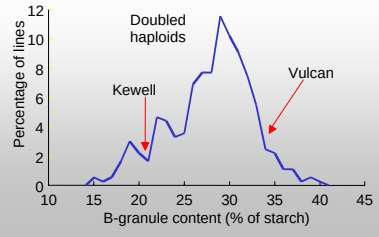


Achievements

- Identified an exotic winter wheat with approximately 10% B granules
- Identified Australian wheats with 20% (low) and 35% (high) B
- Doubled haploid populations generated:
  - low B and high B Australian wheats
  - low B exotic wheat and high B Australian wheat
- f5 lines generated from low B exotic wheat and elite biscuit wheat QAL2000

Achievements

Inheritance of B granule content



Future Work

- **Characterisation of the influence of starch granule size distribution on processing and product quality**
- **Generation of elite wheat cultivars with extreme starch granule size distributions**

Markers for seed dormancy in wheat

Mui-Keng Tan
EMAI, NSW Agriculture

Acknowledgements :

- **Daryl Mares**
- **Ruijun Li**
- **Renu Srivastava**
- **Peter Sharp**

Sprouted Grains from Liverpool Plains in 2000

Differences in wheat genotype for PHS

Those with PHS tolerance include

- **red wheats**
- **Aus1408**

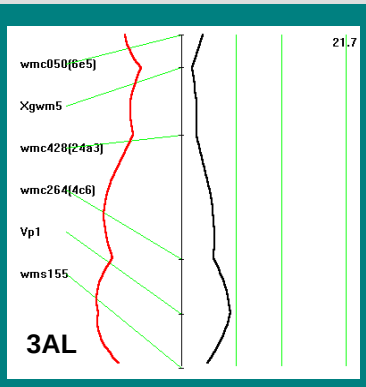
Reference points for start of project:

- **red alleles in wheat**
- **taVp1 gene**
- **3DL chromosome**

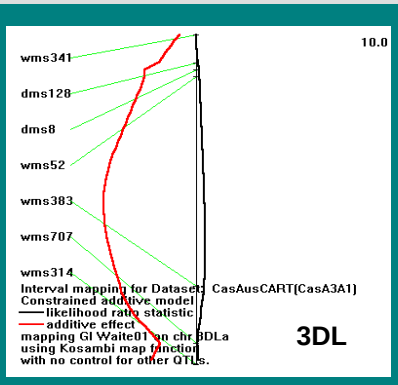
Mapping population:
DH Cascade X Aus1408 (83 lines)

- **174 microsatellite markers**
- **4 sets of phenotype data (D. Mares)**

No significant QTL found on Group 3



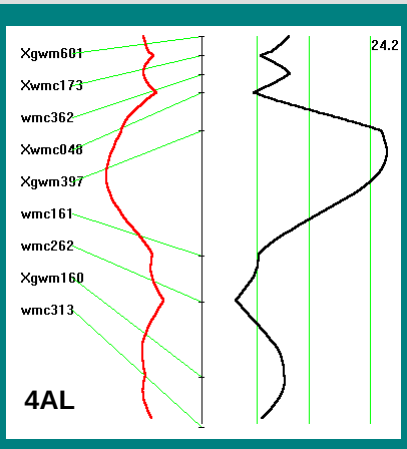
Interval mapping of germination data on 3AL



Interval mapping of germination data on 3DL

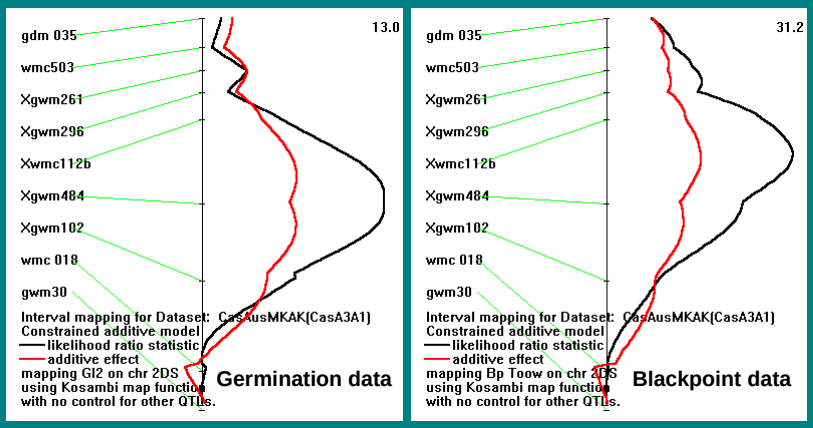
★ **None on 3B**

A major QTL linked to seed dormancy on 4AL(p<0.001) in Aus 1408



A major QTL on 4AL has been reported in a red wheat, AC Domain

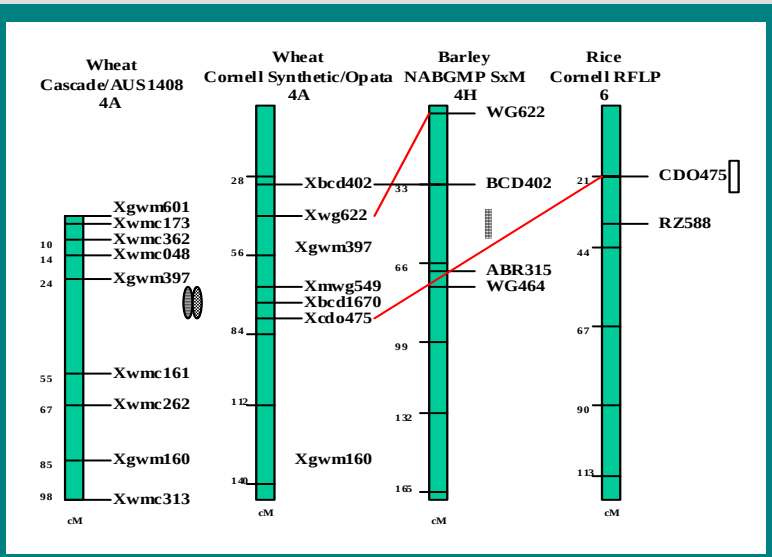
Contributions to PHS from blackpoint expression



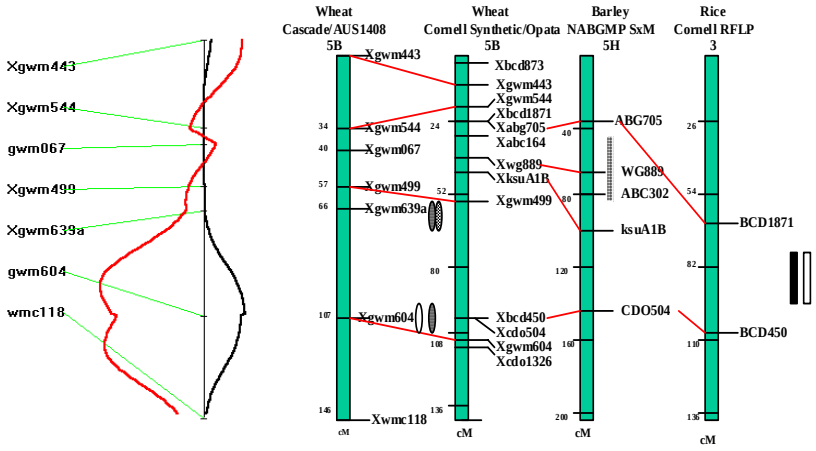
Chromosome 2DS

Blackpoint expression from Cascade confounds the measurement of germination data for QTL analysis in three environments.

QTL on 4AL comparatively maps to QTL in barley and rice



QTL on 5BL-comparatively maps to major QTLs in barley and rice



Validation

Some selected wheat lines from the following crosses have been obtained for validation:

- **Janz 3*/Aus1408**
- **Rosella2*/Aus1408**
- **Seri82/Aus1408**
- **2*HTG/Vasco///Aus1408**
- **2*URES/Jun/KAUZ//Au1408**
- **Sunco/2*QT7475**

Validation of putative QTLs for seed dormancy on 4AL and 5BL

		Aus1408 as dormancy source		QT7475 as dormancy source	
		Ratio: Aus1408: non- Aus1408 alleles		Ratio: QT7475: non-QT7475 alleles	
chromosome	locus	Tolerant (50 lines)	Non-Tolerant (14 lines)	Tolerant (20 lines)	Non-Tolerant (20 lines)
1A	gwm164	0 : 52	1 : 11	No polymorphism	
2AL	gwm312	31 : 16	2 : 12	14 : 5	12 : 7
2AL	gwm356	No polymorphism		15 : 5	13 : 7
3AL	*Vp1*	26 : 24	2 : 12	5 : 15	7 : 13
4AL	gwm397	**48: 2**	**0 : 14**	**10 : 10**	**3: 17**
4AL	wmc161	**49 : 1**	**0: 14**	No polymorphism	
5BL	gwm604	**49 : 1**	**0 : 14**	**0 : 20**	**0 : 20**
3BL	gwm108	21 : 29	0 : 14	No polymorphism	

Tolerant: GI <0.15

Non-Tolerant: GI>0.4

Papers submitted for approval to publish:

- **Mapping quantitative trait loci associated with grain dormancy in white wheat**
  Tan M.K., Mares, D. J. and Sharp P. J.

- **Molecular markers for a genetic determinant of late maturity alpha-amylase (LMA) expression in wheat cultivars Spica and Cranbrook**
  M. K. Tan, K. Mrva, D. J. Mares, M. Pallotta, P. J. Sharp

Marker assisted selection waxy wheat breeding

Mohammad Shariflou and Peter Sharp

University of Sydney

Search for DNA markers

- **Perfect markers from within the genes**
  - A $(TA)_n$ repeat at the 3' end of the waxy genes
  - Deletion-based polymorphism within the waxy genes
- **Tightly linked markers from outside the genes**
  - **Screening a large number of SSR markers from different sources**
    - **International collaborations**
      - Wheat Microsatellite Consortium (WMC)
      - Expressed Sequence Tags (EST) SSRs
    - **Access to other groups**
      - French groups
      - Roder et al 1998
      - USDA

Populations used to search for DNA markers

F_2 waxy populations for detecting DNA markers

- Fixed for null alleles at the three waxy loci
- Segregating for other loci in the genome
- Powerful for detecting linked DNA markers
- PCR analysis of bulked DNA and parents

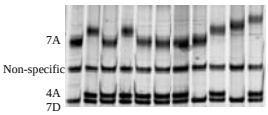
Mapping populations for locating DNA markers

- Halberd x Cranbrook
- Egret x Sunstar
- Sunco x Tasman
- CD87 x Katepwa

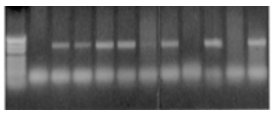
Properties of PCR primers and detected loci

Primer	Primer sequence	Ta	PCR products size (bp)	Locus designation
Sun1F1 Sun1R1	CGCTCCCTGAAGAGAGAAAGAA ATAGGCACAACCCCTAAC	56	256 in CS 204 in CS	*Xsun1-7A* (SSR) *Xsun1-7D* (STS)
Sun1F3 Sun1R1	TGCCAAGAACTGGGAGGA ATAGGCACAACCCCTAAC	56	312 in CS 260 in CS 264 in CS	*Xsun1-7A* (SSR) *Xsun1-7D* (STS) *Xsun1-4A* (STS)
Sun1F5 Sun1R5	CTGCCATTACAAGTGACAACTG CACCATGAATGTTGAGACG	55	377 in Cs	*Xsun1-4A* (STS)
Sun4F Sun4R	ACAGGATCTCTCCTGGAAG GCAAGGAAAATAGTGAAGC	55	840 in Janz (normal) 260 in DHWx12 (null)	*Xsun4-7D* (STS)
WMC262F WMC262R	GCTTTAACAAAGATCCAAGTGGCAT GTAAACATCCAAACAAAGTCGAACG	61	198 in CS	*Xwmc262-4A* (SSR)

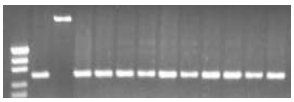
DNA markers from within the waxy loci



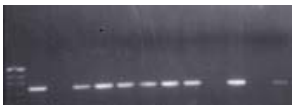
Codominant SSR *Xsun1-7A* detecting the *Wx-A1* locus and dominant STS *Xsun1-4A* (*Wx-B1)* and *Xsun1-7D* (*Wx-D1*)



Dominant STS *Xsun1-4A* (*Wx-B1*)

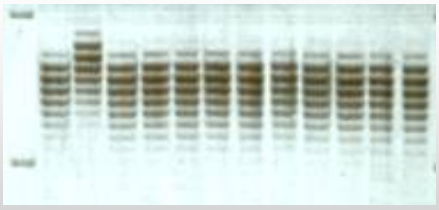


Codominant STS *Xsun4-7D* detecting null and normal *Wx-D1* alleles



Dominant STS *Xsun4-7D* detecting two different null alleles at the *Wx-D1* locus

Tightly linked SSR marker for the *Wx-B1* locus

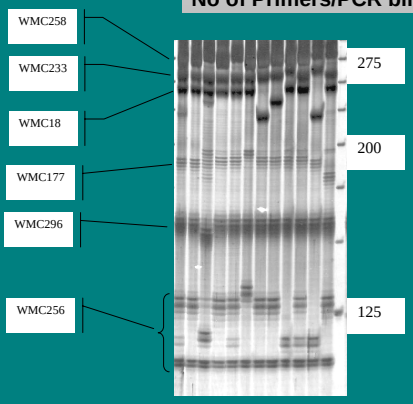


A linked pattern for the *Xwmc262-4A* and the *Wx-B1* loci

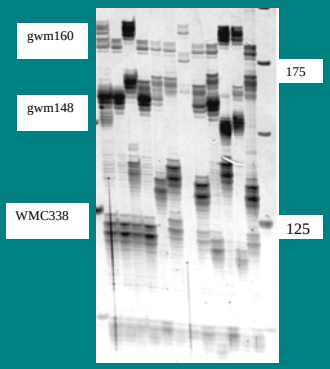
A partial linkage group on chromosome 4AL showing the *Wx-B1* and *Xwmc262* loci

Development of multiplex PCR

Total number of primers assayed:	270
Accepted for multiplex assay:	59
No of multiplexable primers:	40
No of PCR bins:	13
No of Primers/PCR bin:	2-6 (mostly 3-4)



6-plex PCR



3-plex PCR

MAS waxy wheat breeding strategy

Donor Parent x Recurrent Parent

Grow BC_x in 5 cm pots in the green house

Rapid DNA prep

MAS of waxy genes and background genotype

DH production — Further backcrossing

Confirmation with DNA markers and/or staining

Field trials for plant type and rust selections

Multiple site yield trials — Pure seed increase

Selection of pure lines based on yield performance

Confirmation of waxiness with DNA markers and staining — Rust test

Commercial scale seed increase

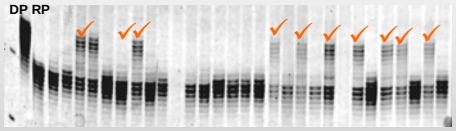
Small and large scale quality tests during the program

Applied marker assisted selection (MAS)

- **Growing large numbers of BC plants in 5 cm pots**
- **Rapid DNA prep from seedlings**
- **PCR for MAS of the waxy genes with linked DNA markers**

Xwmc262 **detecting the** ***Wx-B1*** **gene**

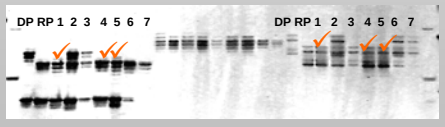
(selection of heterozygotes)



- **PCR for MAS of background genotype with genome wide SSRs**

SSRs detecting the background genotype

(Selection of recurrent parent type)



Field experiments

Marker assisted selected BC_2 plants in the greenhouse

Field trials for selection of plant type and rusts

Yield trials for further selections and seed increase

Pure seed increase

Commercial scale seed increase

Expected yield of #100 tones waxy wheat this year

Achievements

- **Well set up waxy wheat breeding program with the following features**
  - **A full set of DNA markers for selection of the three waxy loci**
  - **Large numbers of SSR markers for selection of background genotype**
  - **Practical application of DNA markers in this program**
- **Commercial results**
  - **Production of advanced lines in shorter time with MAS**
  - **Commercial production of waxy wheat**
- **Adaptability of the program**
  - **The MAS waxy wheat breeding program is optimized**
  - **It can be adapted as a model in other breeding programs**

Reconstitution studies: Influence of Protein and Pentosans on Pasta Quality

Presented by: Cindy Soh (PhD student)

Supervisors: Dr. M. J. Sissons

Dr. M. A. Turner

Influence of Proteins and Pentosans on Pasta Quality

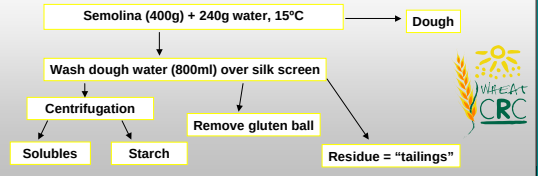
Aims and Objectives

- **To determine the influence of protein fraction (HMW and LMW glutenins, soluble proteins, gliadins) on pasta quality, using a reconstitution model.**
- **To investigate the influence of starch granule size distribution on pasta quality**
- **To determine the influence of pentosans on pasta quality.**

Reconstitution System

-building of a dough from its components: starch, lipids, soluble material and gluten, and varying the content of component to be tested, while all other components remain identical

Fractionation of Components



Review of Extraction Methods

Isolation Methods

- **Review of all published methods for the isolation of glutenin (glu) and gliadins (gli) and the isolation of HMW-GS and LMW-GS**
- **Most published methods are based on common wheat.**

Glu and Gli Separation Methods

Method	Principles	Results
MacRitchie (1989) (J. Cereal Sci 6: 259)	Acid Fractionation	Starting material: gluten Poor fractionation obtained
Fu & Sapirstein modified (1996) (Cereal Chem 73:143)	50% propan-1-ol (gli) then to 70% propan-1-ol (glu)	Starting material: gluten Gli ≈ 86% pure Glu ≈ 50%
Fu, Sapirstein & Bushuk (1996) (J. Cereal Sci 24: 241)	Initial 0.5M NaCl washing, Glu and Gli separated by subsequent water washing	Starting material: semolina Poor fractionation obtained
Fu & Kovacs modified (1999) (J. Cereal Sci 29: 113)	0.3 M NaI, 7.5% propan-1-ol	Starting material: gluten Gli ≈ 86% pure; Glu ≈ 50%, NaI residue in fraction

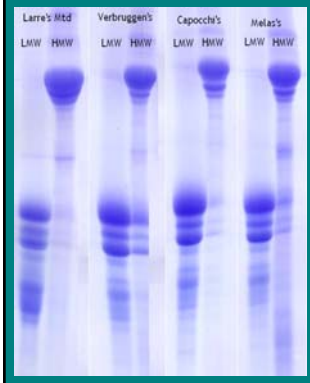
Review of Extraction Methods

Isolation Methods

HMW-GS and LMW-GS Isolation Methods:

Method	Principle	Comments
Melas et. al. (1994) (Cereal Chem 71:234)	50% iso-propanol with 0.08M Tris-HCl, pH 8.0, precipitation of HMW-GS and LMW-GS with 40% and 80% acetone respectively	Pure LMW fractions; but HMW fractions contain some LMW
Larre et. al. (1997) (J. Cereal Sci 25: 143)	50% propan-1-ol with 1% DTT, HMW precipitated with 65% propan-1-ol with 1% DTT	Starting material glutenin. Good purity of both fractions obtained
Verbruggen et. al. (1999) (J. Cereal Sci 28: 25)	50% propan-1-ol with 1% DTT at 60°C; HMW precipitated with 60% propan-1-ol with 1% DTT at 7°C	Pure LMW fractions but impure HMW fractions
Capocchi et. al. (2000) (Cereal Chem 77: 105)	Initial extraction with 0.5M NaCl, 50% Propan-1-ol with 1% DTT 60°C; HMW precipitated with 60% propan-1-ol at 4°C	Both HMW and LMW relatively pure

Information to be presented as a poster at the forthcoming Australian Cereal Chem conference



Conclusion from review of extraction methods:

- **Based on fraction purity and yield data, the methods of Fu & Sapirstein (1996) and Larre et al. (1997) have been selected for glu/ gli separation and for HMW-GS/ LMW-GS isolation, respectively.**

Other Experiments to Date

Protein and Starch Granule Influence

- **Influence of HMW-GS on Pasta Quality**
  - being assessed using a set of lines with common LMW-GS but varying HMW-GS [Glu A1 (null, 1, 2*) and Glu B1 (7+16, 13+16, 17+18)].
  - gluten isolated and placed in a reconstitution model
- **Influence of LMW-GS on Pasta Quality**
  - assessed using a set of lines with a common HMW-GS but varying LMW-GS [varying cfa, caa, caa/dab, or bba]
- **Small scale extensograph method developed for durum wheat. Method verified at large scale**
  (to be presented as a poster at Australian Cereal Chem conference)
- **Influence of starch granule distribution on pasta quality**
  - starch with various A/ B granule ratios to be isolated and used in reconstitution model

Proposed Experiments

- **Effect of altering total percent of soluble protein**
- **Development of method for isolation of water extractable pentosans (WEP)**
  - **determine possible effects of WEP on pasta quality, particularly water absorption, by adding isolated WEP to base flour.**
- **Target completion of these experiments: Jul 2004**

Validation of molecular markers for flour colour in WA adapted germplasm

Karon Ryan (PhD student)

Supervisors:

Dr Michael Francki
Prof Rudi Appels
Prof Mike Jones

Flour colour

- **Determines the quality and use of end products**
- **Determined by:**
  - **ease of separation and**
  - **colour of starchy endosperm**
- **White (no pigment) to yellow (xanthophylls)**
- **Identify markers for steps in the pathway leading to xanthophyll accumulation**

Experimental approaches

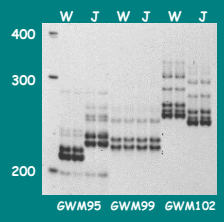
A. **Grain quality attributes and genetic inheritance**
- **Identification of QTLs for flour colour/xanthophyll content**
- **Comparison of alleles**

B. **Whole-genome scans for new class of markers for flour colour/ xanthophyll content**
- **Xanthophyll pathway**
- **Correlation of genetic and physical maps**

C. **Correlation of new marker class and phenotypes**

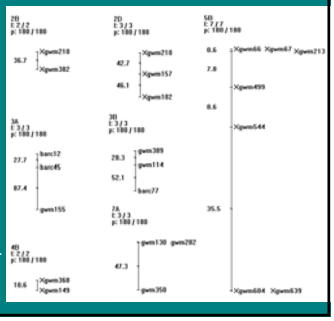
Identification of QTLs for flour colour

Polymorphic screening



333 markers screened, 40% polymorphic
72 markers for mapping

Structural framework maps



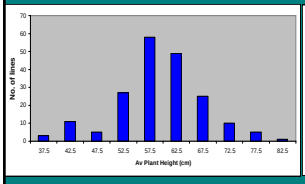
Field trials and phenotyping

Wongan Hills 2002 trial

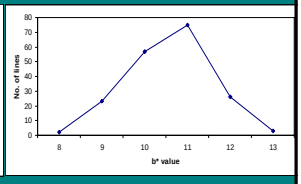
Westonia*2/Janz DH Population

- **DH Field trials**
  - **2002 – Wongan Hills and Katanning**
  - **2003 – Wongan Hills, Katanning and Merridan**
- **Phenotyping**
  - **Flour colour (b*)**
  - **Xanthophyll content**
  - **Grain size**

W*2/J Wongan Hills 2002 trial

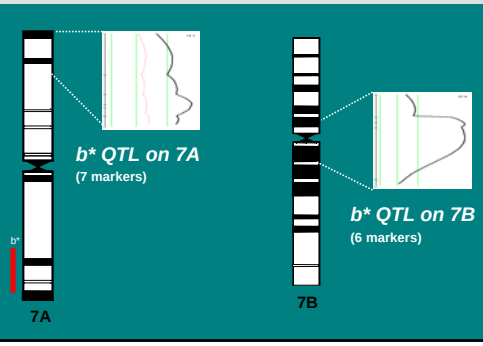


Av. Plant height



Flour colour (b*)

QTL analysis – Carnamah / WAWHT2046



b* QTL on 7A
(7 markers)

b* QTL on 7B
(6 markers)

Whole-genome scans for new molecular markers

Flour colour

- **Strategy:**
  - **Identify wheat ESTs involved in flour colour and xanthophyll content (rice-wheat synteny)**
  - **subsequent cloning and sequence analysis of new marker class**

Targeting new classes of markers for b* and xanthophyll content on 3BS

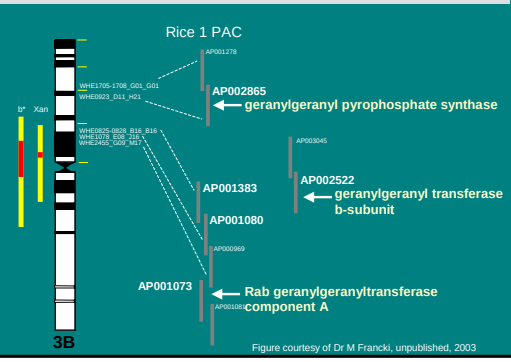
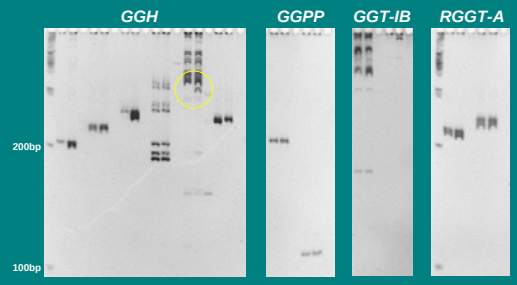


Figure courtesy of Dr M Francki, unpublished, 2003

Assessment of new marker class in Sunco & Tasman



Future Directions

Is there variation in WA germplasm?

- **Alternative variation**
  - **Flour colour (b*) QTL on 7AS and 7BS/7BL**
- **Whole-genome scan using rice to develop new markers in region of interest**
- **Further development and validation of markers across populations**

Project 4.1.2: Rapid Breeding Technologies in Wheat

Staff members

Dr. Nizam U Ahmed
Mr. Wade McKechnie

Aims and Background

Aims

- To improve the efficiency of DH technique
- To produce DH lines for VAWCRC breeders on routine basis

Background

- In recent years, emphasis has been given to reducing time for developing new varieties
- This can be achieved by the use of doubled-haploids (DH)
- With DH system, pure breeding plants can be obtained in a single generation

Principle of DH production (Wheat x Maize system)

Wheat (2n) X Maize (2n)

Gametes n n

Zygote (n + n)

Embryo n (wheat)

Haploid wheat

Colchicine 2n (doubled haploid) → DH seed

Production Method

Measures to increase efficiency of the technique

- Commercial production of DH depends on maximising efficiency of the technique
- Since joining in July 2001, I have been refining the technique to increase efficiency
- Developed ways to
  - grow very healthy wheat, maize, haploids and doubled-haploid plants
  - grow embryos
  - apply 2,4-D
- Technique now of highest efficiency

Effectiveness of this technique

- Embryo production 7-8 per spike
- Embryo germination 70-80%
- Survival of haploids upon transfer to soil nearly 100%
- Survival of plants after colchicine treatment 100%
- Chromosome doubling rate more than 90%
- Frequency of seed formation in DH line 5-150

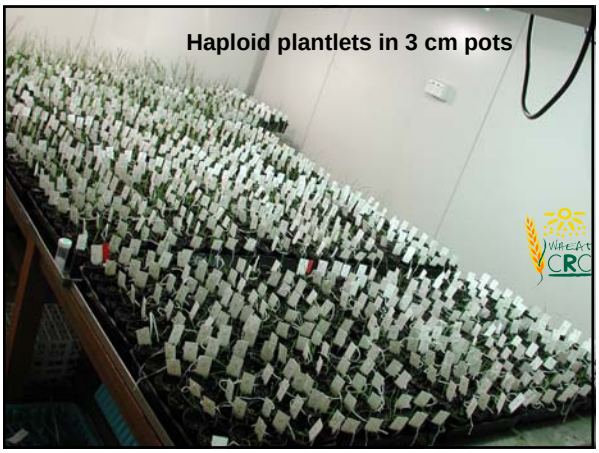
Achievement

Last year (2002-2003)

Requested : 2,500
Produced : 3,500

Current year (2003-2004)

Target :10,000
No.of crosses sown : 54
No. of crosses done : 25
No. haploids obtained : 6,000 (5,400 expected DH)



4.1.3 Soft Biscuit Wheat Program

Helen Allen[1], Andrew Kennett[3], Akram Khan[2] and Helen Pan[1]

[1]NSW Agriculture - Wagga Wagga
[2]NSW Agriculture - Cobbitty
[3]Arnotts Australia Ltd.

Soft Wheat Trials at NSWA Wagga Wagga 2002

Pure Seed and Rust

EMAI and Cobbitty

- **Pure seed plots: 110 at EMAI**
- **Rust screening: 5000 plots**
- **Doubled Haploid: 1400**
- ***Septoria tritici* test: 436 lines at WWAI**
- **WAITE - Adelaide University**
  - Blackpoint: 11 advanced lines
  - LMA: 11 advanced lines

Series 1 trials 2003

Sown sites and date

- **Sown at Wagga Wagga and Narrabri**
- **158 lines in total + controls**
- **Sown June 11 and June 16**
- **Establishment completed at Wagga Wagga**

Advanced Lines Series 3 - 2003

Trial Sites Sown - 2003

- **Narrabri, Trangie, Wagga Wagga, Benerembah, Coleambally**
- **Promoted lines - 29**
- **Sowing dates between May 2 and June 8**
- **Establishment and Zadok (Trangie only)**
  Wagga Wagga, Trangie, Coleambally, Benerembah, Narrabri
  (will be completed on Aug 28)

Soft Wheat Trial Series 3 at Trangie, June 18, 2003

Soft Wheat Plants in Fields

Quality Results, 2002 Harvest

Protein for Advanced Lines

Narrabri 1 (02ayt)	**8.1-13.3% WP**
Narrabri 2	**11.4-15.5% WP**
Wagga Wagga	**10.4-12.7% WP**
Coleambally	**(not harvested - drought)**
Condobolin	**(not harvested - drought)**
Wagga Wagga (WS)	**10.3-12.5% WP**
Benerembah	**9.7- 12.0% WP**
Coleambally	**8.5-10.8% WP**
Leeton	**9.0-11.4% WP**
Yenda	**9.1-12.4% WP**

Helen Pan determining protein on the 2002-2003 harvest samples using the Scanning NIR

Quality Assessment 2002 harvest

Tests completed

- **Wagga Wagga and Benerembah**
  - Quadrumat milling with full statistical analysis of the data
  - Flour colour is in progress
- **Coleambally**
  - Buhler milling, RVA completed
  - PDT in progress
- **Narabbri**
  - Buhler milling in progress

Data Management

Data Management System

- **Long term data storage**
  - **Agrobase for early crop series 1 (S1) and 2 (S2)**
  - **Heron database for advanced crop series 3 (S3)**
- **Short term or in progress data store**
  - **Excel**

Introduction

Dr Akram Khan

- Plant breeder
- NSW Agriculture
- VAWCRC 100%
- Located at PBI Cobbitty

4.1.3 Soft Wheat Program

Soft wheat 60%

- Create genetic variability
- Pyramid rust resistance genes
- Promising line to Wagga for trials & quality
- Produce pure seeds
- PBR trials

4.1.3 Soft Wheat Program

Current Status

- Last target - Prime Hard wheats
- Mostly Hard lines - EGA
- Soft lines for VAWCRC to Wagga
- VAWCRC start year – 2003
  New crosses planned

4.3.8 Novel characteristics

Novel characters 40%

Dr Matthew Turner & Dr Akram Khan

- Matthew - novel character hunter
- Assist Matthew & other CRC Programs
- Seed multiplication of novel sources
- Populations for markers
- Gift wrapping novel & others characters in high-yielding disease-resistant lines

Breeding techniques

Past System-1

- Developed for limited resources
- Target - accumulate desirable genes in selected genotypes

Resulted in:

- New wheat varieties
- Acid soil tolerance
- Small plot equipment

Breeding techniques

Past System-2

- 1993 Cross in field Diamondbird / VPM Sunfield
- 1994 F1 summer glasshouse
- 1995 F2 field-selected single heads bulked - plot
- 1997 / Janz
- 1998 / H45
- 1999 /Prime Hard varieties

Breeding techniques

VAWCRC System -1

- Essential team:
  breeder, doubled haploid and marker personnel

Example – improve QAL2000

- 2003 - field
- Cross 1
  QAL2000 (Yr17) / Avocet Yr15+Yr24
  (DH)
- Cross 2
  QAL2000 (Sp sus) / (Sp Tolerant)

Breeding techniques

VAWCRC System -2

- 2004
  Cross 1 / Cross 2 (summer)
  DH glasshouse - winter
  Marker-assisted selections for
  grain hardness, Yr15, Sp tolerance &
  general glasshouse rusts screening
- 2005 - Field plots from selected plants
- 2006 - Field plots
- 2007 - Regional testing & quality

Breeding techniques

VAWCRC System -3

- 2003 Current crosses:
  Diamondbird/VPMSunfield//Janz/3/H45/4/PH lines &
  Tincurrin*2//Suneca/Cook
  DH
  Field transplant – rusts, agronomy
- 2004 Field plots
- 2005 Regional field testing
- Further improve the technique - four PhD students

Breeding Program VAWCRC

Summary

- Located at PBI and EMAI, using novel techniques

Through this program we will:

- Breed biscuit wheat
- Evaluate novel characters
- Gift-wrap novel characters
- Develop efficient breeding techniques
- Train plant breeders using modern tools
- Serve as genetic resource centre