QUALITY WHEAT CRC PROJECT REPORT

Program 1

Review of Program 1 New Wheats and Breeding Aids

Compiled by Clare Johnson

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Review of Program 1

New Wheats and Breeding Aids

18 November, 1999

compiled by Clare Johnson 20 December 1999

As there are 16 projects in program 1, not all could be presented. The following selection was reviewed:

• *Dr Lindsay O'Brien*	**Program Overview: New Wheats and Breeding Aids**
• *Dr Fred Stoddard*	**Improving the Quality of Wheat Starch**
• *Dr Daryl Mares*	**Yellow Colour of Asian Alkaline Noodles**
• *Mr Matthew Hayden*	**Developing New Microsatellite Markers**
• *Dr Peter Sharp*	**Markers for Quality Genes, and Developing Waxy Wheats**
• *Dr Lindsay O'Brien*	**Breeding New Soft Wheats**

This report consists of a summary and attachments pertinent to the discussion, followed by the collated overheads of the presentations made at the meeting. The summary should be read in conjunction with the overheads, as it contains predominantly clarification of information presented on them, and matters raised in discussion.

Appendix I Access to KEGG relational database: sequences/ maps/ pathways/ ligands.
Appendix II Abstracts on softness from AACC meeting Oct 31-Nov 3, 1999, Seattle
Appendix III Pedigrees of top Australian wheat varieties, C. Wrigley
Appendix IV *The genetic pool of Australian wheats*, chapter (in press) in "World Wheats" book, L. O'Brien, M. Morell, C. Wrigley, R. Appels.

Executive summary

In his overview, Lindsay O'Brien stated the aims of using new and natural sources to improve existing, or develop new, products and processes. Key issues are identification of suitable sources, and development and application of molecular markers for accelerated breeding and release of improved wheats.

Dr Fred Stoddard
Improving the Quality of Wheat Starch
Dr Stoddard opened by discussing starch quality and granule size. Nutritional and industrial characteristics depend on its structure, the gross distinction being between large *A* granules and small *B* granules. Small granules are lost in wet processes and waste water requires treatment. With their higher surface:volume ratio, small granules increase water absorption etc. in manufacturing processes. Effects on biscuit baking time are yet to be tested.

Granule size is known to be affected by the position of the grain on the plant, and the size of the grain. More B granules occur in florets 1 and 2, so this is used as the best case scenario in research.

Particle diameter was graphed *vs* percentage of starch for a number of domesticated and more primitive wheats, rye and triticale, producing biphasic profiles. Primitive wheats had smaller A granules and a predominance of B granules. A survey of B granule content in over 800 lines covering 20 species identified sources of high B and zero B granule content.

In experiments to domesticate wild wheats via cv Kewell x *Aegilops crassa*, *A. crassa* x *Triticum urartu* and *T. turgidum* x *A. tauschii* crosses (outcomes summarised on slides), Dr Stoddard observed normal segregation like a quantitative trait. There has been progress toward developing a marker.

Experiments are underway to domesticate Outlier 67, a soft, club, winter wheat released in the 70's, and high yielding in its own country, but performing very poorly in Australian conditions. The aim is to develop a soft wheat with low B granule content and good 5+10 and starch pasting characteristics. Lower water absorption and lower baking times are desirable.

Conclusions: We are observing complex inheritance including additive dominance and epistasis, that is, multiple genes are involved in producing the desired characteristics, so while it is possible, there will be problems harnessing the trait.
End use tests will follow. The MD requested speeding up to production of 2 populations per year.

One of Dr Stoddard's concluding remarks was that marker studies are under way to help identify the biochemical mechanisms behind the trait.

- **Ed. Note:** Some assistance in analysis may be obtained through use of the KEGG relational database at http://www.blast.genome.ad.jp/kegg/kegg2.html. This database relates sequence data to genomic maps, biochemical pathways and enzymological and ligand data. While only a few plant species are featured as yet, where genes belong to families present in species other than wheat, useful data may be extracted. Access and scope details are provided in Appendix I. **C.J.**

Dr Daryl Mares

Yellow Colour of Asian Alkaline Noodles

Colour is an important criterion for market acceptance of noodles. There is potential for improvement in both white salted noodles (WSN) and yellow alkaline noodles (YAN) for specific markets. The initial brightness of the noodle, particularly in YAN, which are often sold raw or uncooked, must be maintained over its 2-3 day lifespan. Xanthophylls are responsible for the creaminess in WSN, and together with flavonoids, which are colourless at neutral pH, but become yellow and darken as pH rises, are responsible for the yellow colour of YAN. Polyphenol oxidase (PPO) and other oxidases control the rate of darkening.

The proportions of the flavonoids, largely originating from the wheat germ, are variable. Colour from the 2 major flavone diglycosides (histogram 1), is quite stable, while a 3rd flavonoid is very unstable and auto-oxidizes. This latter flavonoid occurs in the bran, as well as the germ. The concentration of these flavonoids in flour is, to some extent, dependent on the milling process and the effects of different growing environments on milling quality, though there is always some carry-through.

Current wheat varieties and related species including *Triticum tauschii* and durum were examined to find new potential for variation in yellowness. The variety Sunco is low in PPO, while Tasman is high. When change in yellowness (b*) was graphed against time, line A charted even higher than Sunco, showing improved stability. This demonstrated good potential for development of novel YAN wheats from a high flavonoid x low PPO cross.

Xanthophylls are relatively unstable in WSN, but more stable in YAN. Another objective of the program is to recombine high xanthophyll levels x with low PPO in an "ingredient" wheat that could be used by customers to vary the final colour of YAN or WSN.
Colour is more stable in durum, but less in bread wheats because of lipoxygenase activity. (**Ed.:** A. Hill/ K. Gale at CSIRO PI are supervising a QWCRC summer studentship on development of antibodies to durum lipoxygenase this summer). Higher flavonoid / lower alkali may be a way of achieving the desired colour. Any effect alteration of these pigments may have on the plant and yield should also be studied.

Ed. Note: The KEGG relational database (Appendix I) may also be useful in this work. **C.J.**

Mr Matthew Hayden

Developing New Microsatellite Markers

A novel technique for developing microsatellite (SSR) markers was described. This technique, coined the Selectively Amplified Microsatellite (SAM) assay, provides a highly efficient approach for the development of SSR markers compared to traditional hybridisation-based techniques.

The key advantage of the SAM assay is the ability to utilise the amplified SSR-containing amplicons directly as genetic markers. This capacity permits the chromosomal location and map position of SAM amplicons to be determined prior to their conversion to SSR markers. The technique also facilitates several additional advantages that include compatibility with silver-staining, prevented redundant amplification of SAM amplicons, and the facilitated conversion of SAMs to SSR markers. These advantages result specifically from the design of the adapter system used in the SAM technique.

Preliminary investigations suggest that the SAM assay may be sampling a different class of SSR sequences than traditional techniques. This was indicated by the amplification of SAMs that contained predominantly short (n <15 dinucleotide repeat units) SSR sequences. These sequences are typically inaccessible to traditional techniques due to the minimum length of the hybridisation probe that is required for the reliable detection of SSR-containing clones. If short SSR sequences prove to be sufficiently polymorphic then the SAM assay could be used to develop SSR markers for a previously inaccessible, but extremely abundant class of SSR sequences. Results to date indicate that this is the case (average PIC 0.41).

The SAM technique is simple, rapid and inexpensive, and can provide a virtually unlimited supply of markers whose map locations and potential informativeness can be determined prior to SSR marker development. One limitation of the SAM technique is that its multiplex ratio is low, so while the SAM assay is useful for the development of SSRs, it should be complemented with other technologies.

There is some potential for commercialisation, such as kit provision.

GLOSSARY OF TERMS:

SSR	Microsatellite (Simple sequence repeat)
SAM	Selectively amplified microsatellite
SAMPL	Selectively amplified microsatellite polymorphic locus assay
SSP	Sequence-specific primer
AFLP	Amplified fragment length polymorphism
RFLP	Restriction fragment length polymorphism
PCR	Polymerase chain reaction
STM	Sequence-tagged microsatellite
PIC	Polymorphism information content

Dr Peter Sharp
Markers for Quality Genes
Dr Sharp reported on national and international collaborations with which he is involved. Targets of markers in development include quality targets of the GRDC National Wheat Molecular Marker Program, noodle quality, especially WSN, sprouting tolerance, late maturity α-amylase and waxy genes.

He summarised the development of a null 4A ELISA at CSIRO Plant Industry using a specific antibody probe. In the waxy locus of the B genome, 4 unique amino acids (A,D,N,Y) occurring within a 14 residue peptide enable presence/absence discrimination of Wx-B1 with antibodies raised. These do not recognise the A or D genome products. The importance of null4A in WSN quality parameters has been confirmed in a large number of crosses.

Data on a microsatellite linked to grain hardness was presented. Xwmc 233 (hardness) mapped close, but there are different hardness alleles. Progress has also been made on sprouting tolerance, mapping on chromosome 3D.

A QTL (Quantitative Trait Locus) important for RVA peak and final viscosity was found in the chromosome 7 group, and QTLs for flour swelling power have been examined in 2 crosses. Epistasis – allelic composition at other loci- is also important.

Developing Waxy Wheats

The 3 Wx null alleles are being backcrossed into 4 Australian cultivar backgrounds and molecular markers are being used to select progeny with null alleles and the desired background. (Further details are on the overheads). Over 3 tonnes of line TH7 grain has been produced for end product testing. Last season TH1 was produced in bulk. Although in that study most problems were experienced with starch and gluten separation and drying from line TH7 because of high arabinoxylan (fibre) content (QWCRC Report #30, 30/6/99), TH7 starch had the highest RVA peak viscosity, and being Tammin-derived, the line performed well agronomically.

Waxy starch granules are more fragile to rupture under pressure, swell at much lower temperature, and are more susceptible to rupture in processing.

* Drs Wrigley and O'Brien mentioned abstracts on softness seen recently at the AACC 84th Annual Meeting, Oct 31 to Nov 3, 1999, Seattle, USA. A copy of the relevant abstracts is provided (Appendix II).

Dr Lindsay O'Brien

Breeding New Soft Wheats

Dr O'Brien cited soft wheat breeding as a CRC success in collaboration and integrating technologies. The program was started in 1993 at the request of Arnotts because of freight costs sourcing suitable wheat. Successes include the development of the TAXT-2 analyser for use in dough extension testing, using mixograph testing for dough properties of soft wheats, and the recruitment of a former PhD student of the program by Arnotts.

The release of QAL 2000, a new soft wheat line resistant to three rust strains, was announced at a recent field day in Narrabri. It has a milling yield comparable to Tatiara, water absorption, mixing time, dough extensibility and resistance similar to Tincurrin, and produces good end product characteristics such as good packet length, baked dough weight and low biscuit fragility. QAL2000 is also high yielding in the field, being 15% higher yielding than the prime hard variety Sunstate. It is also superior to Tincurrin in the north. It is currently being tested at a variety of sites and regions.

Discussion

- Pedigree charts of top Australian and WA varieties were provided for this report by Dr Colin Wrigley – see Appendix III. A paper, currently in press, on the genetic pool of Australian wheats is also attached (Appendix IV)
- QWCRC will fund work to ensure early lines are fingerprinted.
- There is a need for agronomy packages to accompany release of new varieties, e.g. it is inappropriate to use the amount of nitrogen fertiliser required for prime hard on a soft wheat.
- ACAS (The Australian Crop Accreditation System) is now online at http://www.acas.on.net

Appendix I

Access to KEGG relational database: sequences/ maps/ pathways/ ligands.

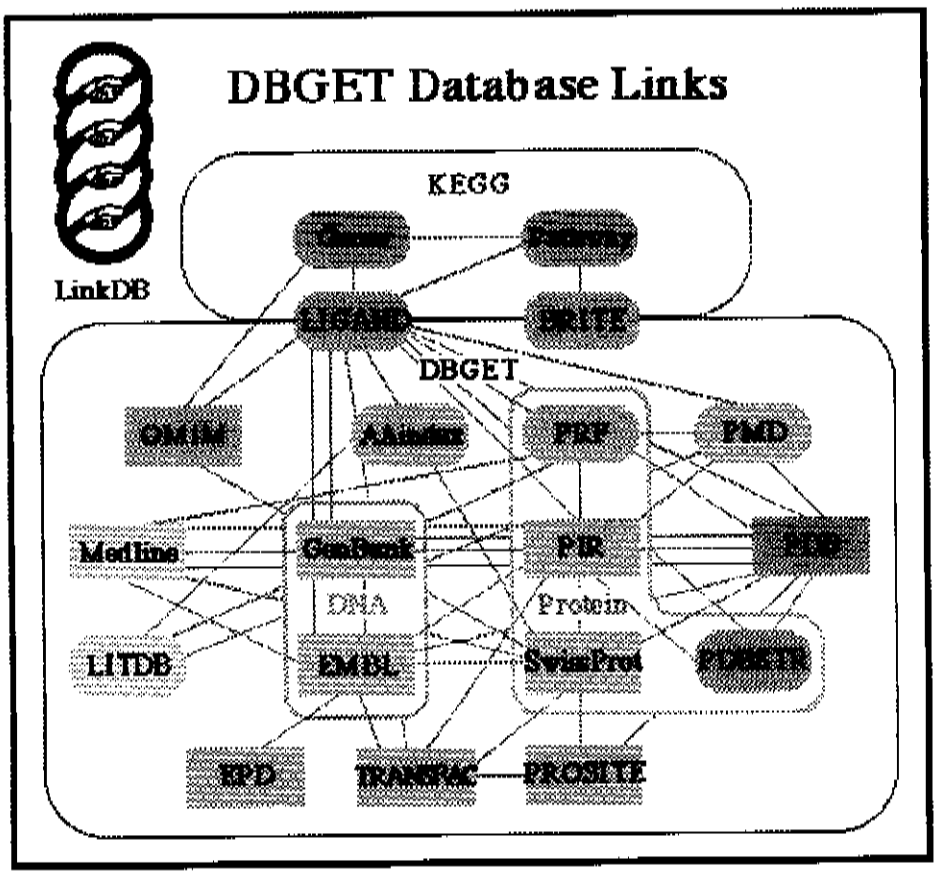
KEGG: Kyoto Encyclopedia of Genes and Genomes

Release 12.0, October 1999 (plus daily updates)

Kyoto Encyclopedia of Genes and Genomes (KEGG) is an effort to computerize current knowledge of molecular and cellular biology in terms of the information pathways that consist of interacting molecules or genes and to provide links from the gene catalogs produced by genome sequencing projects. The KEGG project is being undertaken in the Institute for Chemical Research, Kyoto University as part of the Japanese Human Genome Program.

- **Introduction**
- **Open KEGG**
- **Search and Compute with KEGG**
- **Links to Pathway and Other Databases**
- **KEGG Mirror Servers**
- **IDEAS Interface to DBGET/BLAST/FASTA**
- **Go to GenomeNet Home**

Last updated: October 15, 1999
www@genome.ad.jp



Click on the following:	to invoke the following:
Database name	Basic DBGET search
DBGET	Advanced DBGET search
KEGG	KEGG table of contents
LinkDB	LinkDB search

[**DB Release Info** | **DBGET Help** | **GenomeNet Home** | **IDEAS Interface**]

KEGG - Table of Contents

1. Pathway Information

1-1. Pathway Maps and Ortholog Tables

Category	Pathway Map Ortholog Table	Search & Compute	DBGET Search
Pathway	**Metabolic pathways**	Search objects in pathway maps Color objects in pathway maps Search or color genes in ortholog tables	PATHWAY GENES
	Regulatory pathways	Search similar sequences in pathway maps Search similar sequences in ortholog tables Generate possible reaction pathways	

1-2. Disease Catalogs, Cell Catalogs, and Molecule Catalogs

Category	Catalog		DBGET Search
Disease	ICD disease classification	OMIM gene map OMIM morbid map	OMIM
Cell	Cell lineage maps		
Enzyme	EC number classification	PIR superfamilies SCOP 3D-folds PROSITE motifs	LIGAND
Compound	Compound classification		
Element	Periodic table		

2. Genomic Information

2-1. Gene Catalogs

Category	Species	Com-pleted	Gene Catalog		DBGET Search
Human	Homo sapiens		KEGG Disease Map	LocusLink GDB	Hsa
Rodent	Mus musculus		KEGG	MGD	Mmu
Insect	Drosophila melanogaster		KEGG	FlyBase	Dme
Nematode	Caenorhabditis elegans	1998	KEGG		Cel

Higher plants	Arabidopsis thaliana				Ath
	Oryza sativa				Osa
	Zea mays				Zma
Protozoan	Plasmodium falciparum				Pfa
Cellular slime mold	Dictyostelium discoideum				Ddi
Fungi	Saccharomyces cerevisiae	1997	KEGG	SGD MIPS	Sce
	Schizosaccharomyces pombe				Spo
	Candida albicans				Cal
Proteobacteria	Escherichia coli	1997	KEGG Operons	Wisconsin Operons	Eco
	Haemophilus influenzae	1995	KEGG	TIGR	Hin
	Helicobacter pylori 26695	1997	KEGG	TIGR	Hpy
	Helicobacter pylori J99	1999	KEGG		Hpj
	Rickettsia prowazekii	1998	KEGG	Uppsala	Rpr
	Salmonella typhimurium				Sty
Gram-positive bacteria	Bacillus subtilis	1997	KEGG	BSORF	Bsu
	Mycoplasma genitalium	1995	KEGG	TIGR	Mge
	Mycoplasma pneumoniae	1996	KEGG	ZMBH	Mpn
	Mycobacterium tuberculosis	1998	KEGG	Sanger	Mtu
	Staphylococcus aureus				Sau
Chlamydia	Chlamydia trachomatis	1998	KEGG	Berkeley	Ctr
	Chlamydia pneumoniae	1999	KEGG	Berkeley	Cpn
Spirochete	Borrelia burgdorferi	1997	KEGG	TIGR	Bbu
	Treponema pallidum	1998	KEGG	TIGR	Tpa
Cyanobacteria	Synechocystis sp.	1996	KEGG	Kazusa	Syn
Hyperthermophilic bacteria	Aquifex aeolicus	1998	KEGG	Diversa	Aae
	Thermotoga maritima	1999	KEGG	TIGR	Tma
Archaea	Methanococcus jannaschii	1996	KEGG	TIGR	Mja
	Methanobacterium thermoautotrophicum	1997	KEGG	GTC	Mth
	Archaeoglobus fulgidus	1997	KEGG	TIGR	Afu
	Pyrococcus horikoshii	1998	KEGG	NITE	Pho
	Pyrococcus abyssi	1999	KEGG	Genoscope	Pab
	Aeropyrum pernix	1999	KEGG		Ape

2-2. Genome Maps

Category	Genome Map	Search & Analyze
Genome	Genome map browsers (Java) Genome map comparison (Java)	Search gene positions in genome map Color genes in genome map Identify gene clusters in two genomes Search homologous gene clusters

2-3. Gene Expression Profiles

Category	Expression Map	Search & Analyze
Expression	Expression map browser (Java) Expression clustering (Java)	Identify clusters of coregulated genes

3. Computational Tools

3-1. Search and Compute with KEGG

3-2. Sequence Similarity Searches

Server	Search GENES	Search GENOME
KEGG	Search GENES or its subset by FASTA Search GENES subset by BLAST	Search GENOME or its subset by FASTA Search GENOME subset by BLAST
GenomeNet	Search GENES by FASTA Search GENES by BLASTP Search GENES by BLASTX	

On-line Manuals
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- Compound Entries
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KEGG Release 12.0 (October 1999) plus daily updates

[KEGG Home | KEGG Help | KEGG Tools | KEGG Links | DBGET Links Diagram]

Appendix II

Abstracts on softness from AACC meeting Oct 31-Nov 3, 1999, Seattle

Abstracts from AACC 84th Annual Meeting
Oct 31 - Nov 3, 1999, Seattle USA.

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Puroindolines: The molecular-genetic basis for wheat grain hardness. C. F. MORRIS (1), M. J. Giroux (2), and M. Lillemo (3). (1) USDA, ARS, Western Wheat Quality Lab., Pullman, WA 99164; (2) Plant, Soil & Environ. Sci., Montana St. Univ., Bozeman, MT 59717; (3) Dept. Hort. & Crop Sci., Agric. Univ. Norway, N-1432 Aas, Norway.

A unique characteristic of wheat, as opposed to the other major cereal crops, is that it exists in 3 major hardness classes: soft and hard hexaploid and durum. The gene, *Hardness*, which controls the majority of variation among the three classes is the single most important quality gene in wheat. Grain hardness impacts nearly every aspect of milling, baking and utilization. With the report of friabilin in 1986, a new avenue opened to explore the molecular-genetic basis of grain hardness. After over a decade of research, puroindoline-the currently preferred name for friabilin provides an evocative model for the expression of the *Hardness* gene. Our research has described two highly-conserved mutations, *Pina-D1b* and *Pinb-D1b*, that are associated with hard grain texture in hexaploid wheat. The first, *Pina-D1b*, involves a complete loss of puroindoline a mRNA and protein. The second, *Pinb-D1b*, results from a single nucleotide mutation and causes a change from Glycine to Serine at position 46. The apparent loss of function of either puroindoline a or b causes a change from soft endosperm to hard. The complete absence of both puroindolines is associated with the very hard texture of durum. Results of genotypic surveys indicate that 93% of 42 N. American hard winter wheats possess the Serine-type mutation (*Pinb-D1b*). Among 75 hard spring wheats, 84% possess this mutation. Among a set of 314 hard wheats of mostly Scandinavian origin, 93% posses the Serine mutation. In addition, the results of a comparison of grain hardness, milling and baking quality among a set of *Pina-D1b* x *Pinb-D1b* HRS recombinant inbred lines will be presented.

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Analysis of waxy proteins of wheat and oats: Applications in breeding. R. A. GRAYBOSCH (1), and J. Skerritt. (3) USDA, ARS, University of Nebraska, Lincoln, NE, USA, (2) CSIRO Plant Industry, Canberra, Australia.

Waxy proteins, also known as the granule-bound starch synthase (GBSS), are responsible for the synthesis of amylose in developing endosperm cells. In hexaploid bread wheats, genes found on chromosomes 7A, 7D and 4A encode three isoforms of GBSS. Modified SDS-PAGE procedures have allowed the separation of these three isoforms, and the identification of null alleles, or genes that do not result in a detectable gene product. Through breeding, lines carrying various null alleles have been combined to develop both amylose-free (waxy) and reduced-amylose bread and durum wheats. Monoclonal antibodies raised against GBSS have been used to develop an ELISA protocol that can identify both waxy and reduced-amylose genotypes. Protein analytical techniques, rather than direct measurement of amylose, have proven a more precise tool in the identification of GBSS genotypes. Manipulation of the number of active GBSS-encoding genes has allowed the development of wheats with a range of amylose contents and starch pasting properties. Extension of the techniques developed in wheat to oats, another hexaploid crop, has demonstrated the existence of a similar gene system. The potential for the development of waxy and reduced amylose oats will be discussed.

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Waxy starch granules are more susceptible to mechanical damage than normal starch. A. D. BETTGE (1), M. J. Giroux (2), and C. F. Morris (1). (1) USDA, ARS Western Wheat Quality Lab, Pullman, WA 99164; (2) Dept. Plant, Soil & Environmental Sci, Montana St. Univ, Bozeman, MT 59717.

Waxy starch (<1% amylose) was isolated from bulked waxy wheat by water-washing. Starch granules were examined for physical integrity by crushing with a TA-XT2i at 0.5, 1, 2, 5, 10, 15 and 20 kg loads using a 1 cm round probe. Starch granules (2.5 µL of a 10 mg/mL suspension) were applied to a glass slide and spread to about 0.5 cm, covered with a glass slip and crushed, or allowed to dry and then crushed. After crushing, iodine stain was applied and percentage of crushed granules and intact granules counted. The results were compared to 'normal.

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Characterization of waxy wheat flours. GANG GUO (1), Robert Graybosch (2), and David R. Shelton (1). (1) Dept. of Agronomy, University of Nebraska–Lincoln, Lincoln, NE 68583-0915; (2) USDA, ARS, Lincoln, NE 68583-0915.

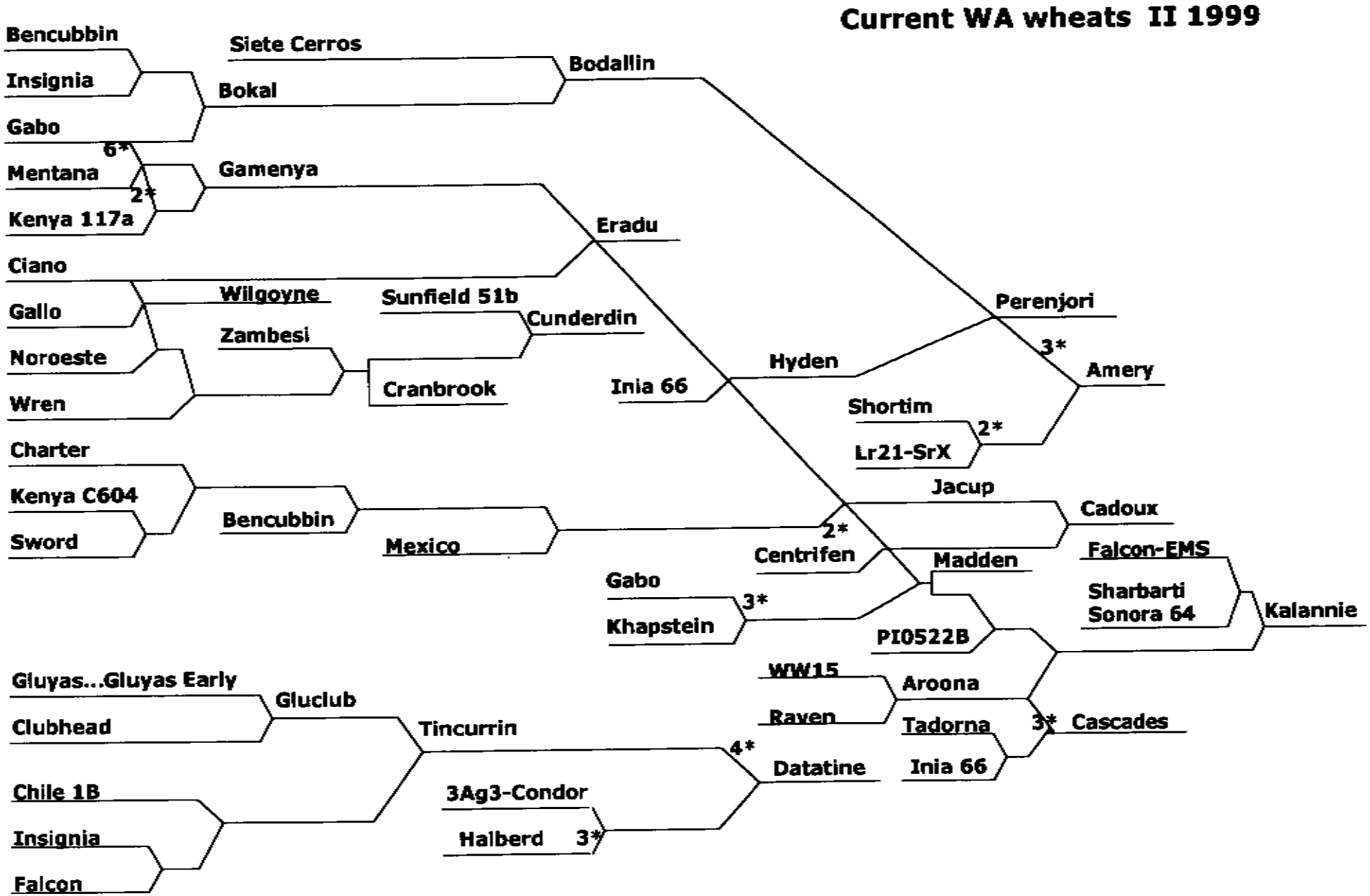
In this study, 41 waxy wheat samples were analyzed. Wheat alpha-amylase activities, kernel weights and hardness values, flour Rapid Visco Analyser (RVA) parameters, and falling number values were determined. These results were compared with those of the control wheats with non-waxy starch that were planted at the same location. The comparisons showed RVA parameters, such as peak time (temperature), holding strength (trough), breakdown, setback and final viscosity varied significantly between the waxy and control sample groups (p-value = 0.0001). The waxy wheats had much lower pasting (gelatinizing) temperatures, lower peak times and temperatures, lower holding strengths, lower setbacks and final viscosities when compared to those of the controls. Waxy wheats had much higher breakdown values. The falling number values of waxy wheats were much lower (about 70 seconds) than those of the controls (about 500 seconds). However, the alpha-amylase activities of waxy wheats and control samples were similar. These results indicated that the variations in pasting properties were mainly due to the waxy starch characteristics rather than other factors. No significant differences were found between the samples with different hardness values within the waxy lines. The quality parameters of the normal wild type and sprout damaged wheats were compared, and significant differences were found.

Appendix III

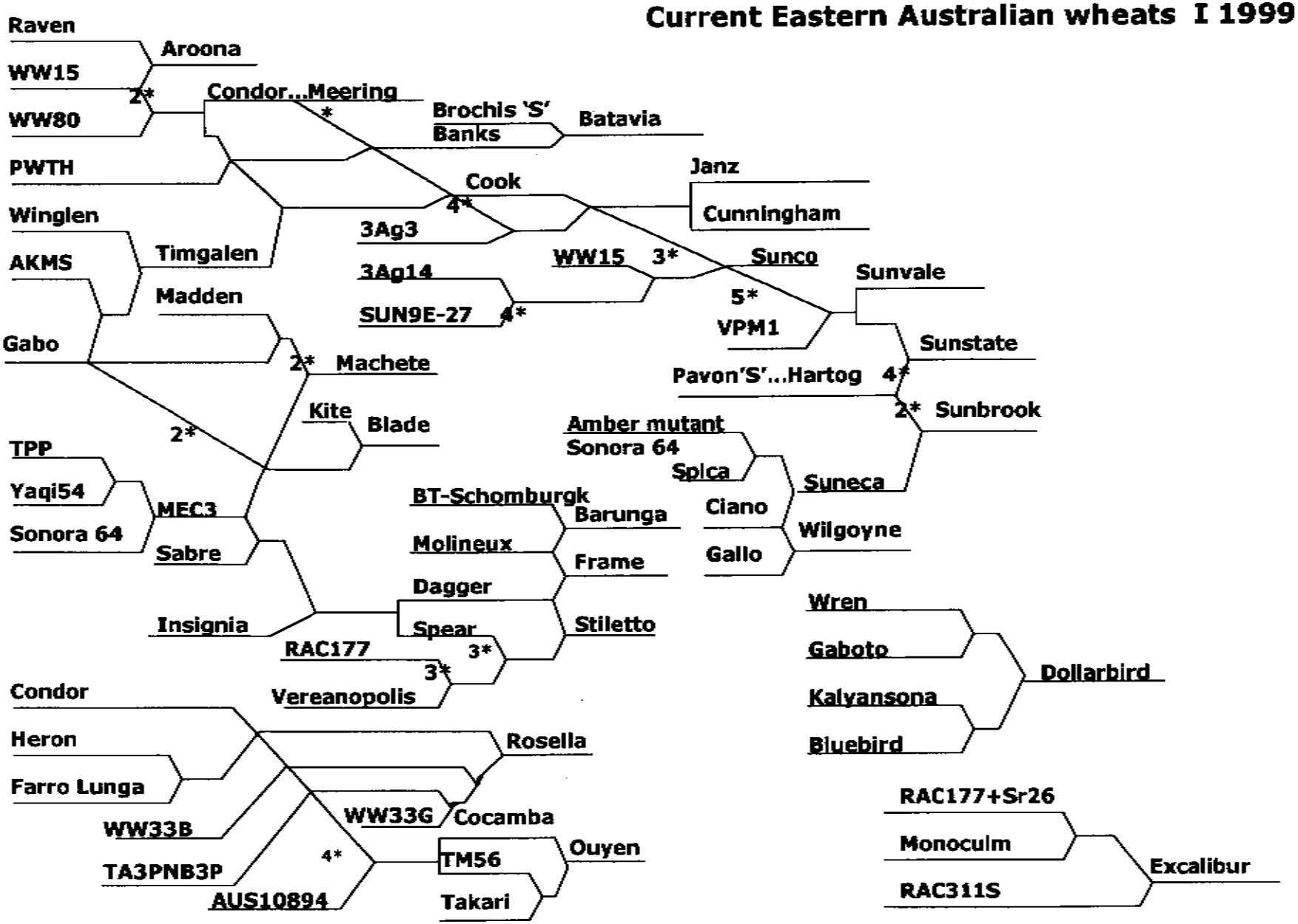
Pedigrees of top Australian wheat varieties

C. Wrigley

The following two pages provide pedigree charts for the thirty most popular wheat varieties based on recent receival statistics. (Statistics in confidence, AWB Ltd.*)**


Current WA wheats II 1999
Bencubbin
Insignia
Gabo
Siete Cerros
Bokal
Bodallin
6*
Mentana
2*
Kenya 117a
Gamenya
Eradu
Ciano
Gallo
Noroeste
Wren
Wilgoyne
Zambesi
Sunfield 51b
Cunderdin
Cranbrook
Inia 66
Hyden
Perenjori
3*
Amery
Shortim
2*
Lr21-SrX
Charter
Kenya C604
Sword
Bencubbin
Mexico
2*
Centrifen
Jacup
Cadoux
Madden
Gabo
3*
Khapstein
Falcon-EMS
Sharbarti
Sonora 64
Kalannie
PI0522B
WW15
Raven
Aroona
Tadorna
3*
Cascades
Inia 66
Gluyas...Gluyas Early
Clubhead
Gluclub
Tincurrin
4*
Datatine
3Ag3-Condor
Halberd
3*
Chile 1B
Insignia
Falcon

Current Eastern Australian wheats I 1999



Appendix IV

The genetic pool of Australian wheats

L. O'Brien, M. Morell, C. Wrigley, R. Appels

(in press) in "World Wheats" book.

Chapter (in press) in "World Wheats" book

The genetic pool of Australian wheats

L. O'Brien[1], M. Morell[2], C. Wrigley[2,3], and R. Appels[2]

[1]*University of Sydney, Plant Breeding Institute, I A Watson Grains Research Centre, Narrabri, NSW, Australia*

[2]*CSIRO Plant Industry, PO Box 1600, Canberra ACT 2601, and PO Box 7 Nth Ryde NSW 1670, Australia,*

[3]*Quality Wheat CRC, Locked Bag No 1345, PO North Ryde, NSW 1670, Australia*

Introduction

It is just over two centuries since the first wheat was grown in Australia. The first plot consisted of a few acres beside Farm Cove on Sydney Harbour and yielded little more than the amount of grain sown (1). At that time, the early colonists found themselves attempting to grow wheat under conditions that were completely different from anything that they had known, in a new country, in a new hemisphere. Today wheat is Australia's largest grain crop, with production averaging 20.5 million tonnes for the period 1995 to 1999 (2). Globally this ranked Australia in tenth position as a wheat producer and, with the majority of the crop being exported, in the top five wheat exporting countries.

Domestic uses for hexaploid wheats include bread making, starch-gluten manufacture, cake and biscuit production, grocery lines, a wide range of manufactured foods, and animal feed. In the case of durum wheats, a rapidly growing domestic market is in the area of pasta production. The 80% of the crop that is exported goes to Asian countries for many types of noodles and to Middle East countries for making a wide range of Arabic flat and pocket breads. Export uses also include the production of various other products such as Chinese steamed breads and conventional pan breads.

The great diversity of products that are made from Australian wheat poses a challenge for Australian breeders, with respect to selecting appropriate quality attributes. The diversity also poses challenges for AWB Limited (formerly the Australian Wheat Board - the body with sole export marketing responsibility) with respect to arranging segregation on the basis of grain quality to match the needs of specific markets. A feature of Australian wheat research in recent times has been the advances in knowledge of the quality requirements for this great diversity of products (3).

Overview of the main agro-ecological features of Australia

Australia has a great diversity in climatic and soil conditions, and this source of variation provides a challenge for the breeding and management of wheat, to achieve yield and quality targets.

Australian grain production areas largely follow the coast of south-west Western Australia and South Australia, and the regions inland from the Great Dividing Range in eastern Australia. Because grain production occurs over a latitude range from

tropical (22^0S) to temperate (30^0S to 38^0S), the rainfall distribution varies extensively over the wheat growing areas. A rapid decline in rainfall occurs in an east-west direction in eastern Australia, a south-west/north-east direction in western Victoria and in a west-east direction in South and Western Australia. Most areas receive less than 250mm of rainfall during the growing season (Fig. 1). Since many of the wheat production areas are away from the coast they can experience large diurnal temperature fluctuations due to the absence of cloud cover for much of the winter. The rapid heat loss at night under these conditions creates frosts that can be severe (down to -6°C) even as far north as central Queensland. The frosts occur during winter and late spring and their occurrence means wheat varieties with late flowering times are required to minimize the risk of frost damage to crops.

In eastern Australia, the northern grain-producing areas are located in sub-tropical to tropical latitudes and receive most of their annual rainfall in the spring/summer months between October and the end of March. Stored soil moisture can make a major contribution to final grain yield and protein content. Late summer rains and moisture conservation farming techniques generally provide the seed-bed moisture for planting. Best use is made of the available water by combining time of sowing with agronomic characteristics such as early vigour/establishment. The uncertain availability of moisture for planting, combined with the late spring frost risk requires wheat varieties that can be planted from late March to early April through to mid-July. A key requirement is that these varieties must flower at about the same time in the spring in order to achieve an optimum grain yield prior to the onset of high temperatures in late spring. With respect to disease stresses, the northern cropping region has generally dry and warm winters that do not favour the development of septoria tritici blotch (*Mycosphaerella graminicola*). The increased temperatures in spring, combined with increased likelihood and incidence of rainfall, provide ideal conditions for the three rust diseases, stem (*Puccinia graminis*), leaf (*Puccinia recondita*) and stripe rust (*Puccinia striiformis*). In addition, the transition to stubble retention farming has seen reductions in yield due to the foliar disease yellow leaf spot (caused by *Pyrenophora tritici-repentis*) and the crown and root disease, crown rot (caused by *Fusarium graminearum* Group I). Other diseases that can threaten production include common root rot (*Bipolaris sorokiniana*), the root lesion nematode (*Pratylenchus thornei*), and flag smut (*Urocystis agropyri*).

The southern cropping regions receive most of their rainfall in the winter months between June and September and so these regions are more temperate, Mediterranean-like. The annual rainfall patterns and a year round north to south temperature cline, combined with the low natural fertility of the soils, are the overriding environmental features (reviewed in 61) that determine the breeding objectives. In several areas water logging tolerance, is essential. Some of the acid soil environments require tolerance to aluminium and manganese whereas other regions require sodicity tolerance. The Mallee environment in the region requires tolerance to high boron and low levels of zinc and manganese. The cold and wet conditions during the winter months provide near optimum conditions for septoria tritici blotch. The increased temperatures in spring coincide with a decreased frequency and incidence of rainfall, and hence the incidence of stem rust is much reduced. Resistance and tolerance to cereal cyst nematode (*Heterodera avenae*) are essential. Common root rot (*Bipolaris sorokiniana*) can be a problem in parts of the region.

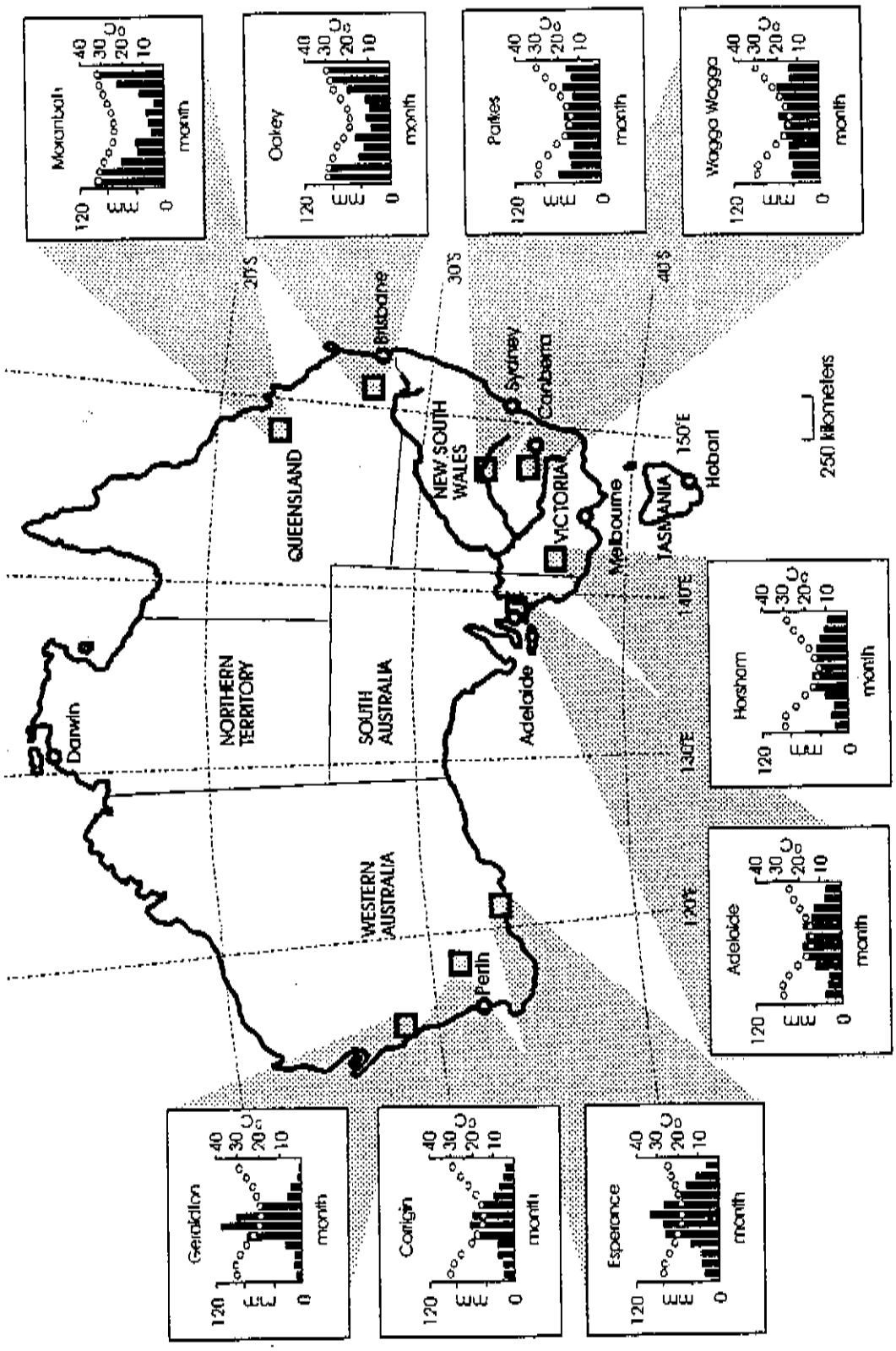


Fig. 1

Figure 1. The insets show the annual rainfall (black bars) **and temperatures** (average maximOnO as circles) figures for the respective Australian winter cereal production areas. In each inset the months are arranged from left to right in the order January, February ...December. The information shown was obtained from Bureau of Meteorology (Commonwealth of Australia, 1999, http://www.bom.gov.au).

In the western region, the majority of the soils are low in fertility, making the production of medium to high protein wheat difficult on other than the well-structured soil types. Hence, the major end-uses targeted are those which utilize wheat lines of low to intermediate protein content. Breeding is undertaken for acid soil tolerance as extensive areas of acid soils exist within the state. In some areas, the acid soil zone can occur at depth, so plant growth is relatively unaffected until its roots encounter the acid soil zone. Diseases of importance include septoria tritici blotch and septoria nodorum blotch (*Phaeosphaeria nodorum/Stagonospora nodorum*, 58). The rust diseases are spasmodic in occurrence so there is no major effort of breeding for resistance. Stripe rust has not yet been recorded in the state, but selection for resistance is made using the National Cereal Rust Control Program as a pre-breeding strategy to provide protection in the event of an introduction of the disease into the state.

Overview of current wheat production, average yield, cultivation techniques and end-uses

In the last five years the area sown to wheat in Australia has increased to more than 10 million ha and the variation in soil and climate result in annual average yields varying widely from 1.14 to 2.10 t/ha in the past decade (2). In 1994, drought effected much of eastern Australia and only 9 million tonnes were harvested nationally. The interest in wheat production was however maintained because of poor wool and beef prices, and this has seen production exceed 20 million tonnes when favorable weather conditions prevailed.

Farming systems have undergone major changes in the past 100 years (Fig. 2, reviewed in 61). The southern Mediterranean production regions pioneered management systems using annual medics and clovers in a wheat-sheep farming system (29). Such a structured approach has been absent, until recent years, in the northern cropping zones due to the highly variable climate, the lack of adapted pasture legumes and low prices for wool and beef. The practice of allowing fields to lie fallow has, in the past, incorporated repeated cultivations to control weed and crop re-growth. In present-day practice, the fallow involves the retention of stubble, with weed and crop re-growth controlled by herbicides (48, 49). The adoption of this strategy by farmers, in a country with old and fragile soils, is a major change in management practice and is expected to have long term beneficial effects on soil structure and fertility, and should substantially reduce wind and water erosion. A number of the key technological innovations and genetic improvements such as the introduction of semidwarf wheats to increase the amount of assimilate available for grain development as well as provide a shorter, stronger straw to improve lodging resistance, are summarized in Fig. 2.

A major portion of the Australian wheat crop is exported to markets where hard kernel textured wheats of protein contents of 11-14 % are used for the production of a wide range of products. Wheat is marketed using nine major grades, with protein content being a significant factor along with variety, moisture content and freedom from foreign matter (62). The minimum and maximum protein contents for each grade are summarised in Table 1.

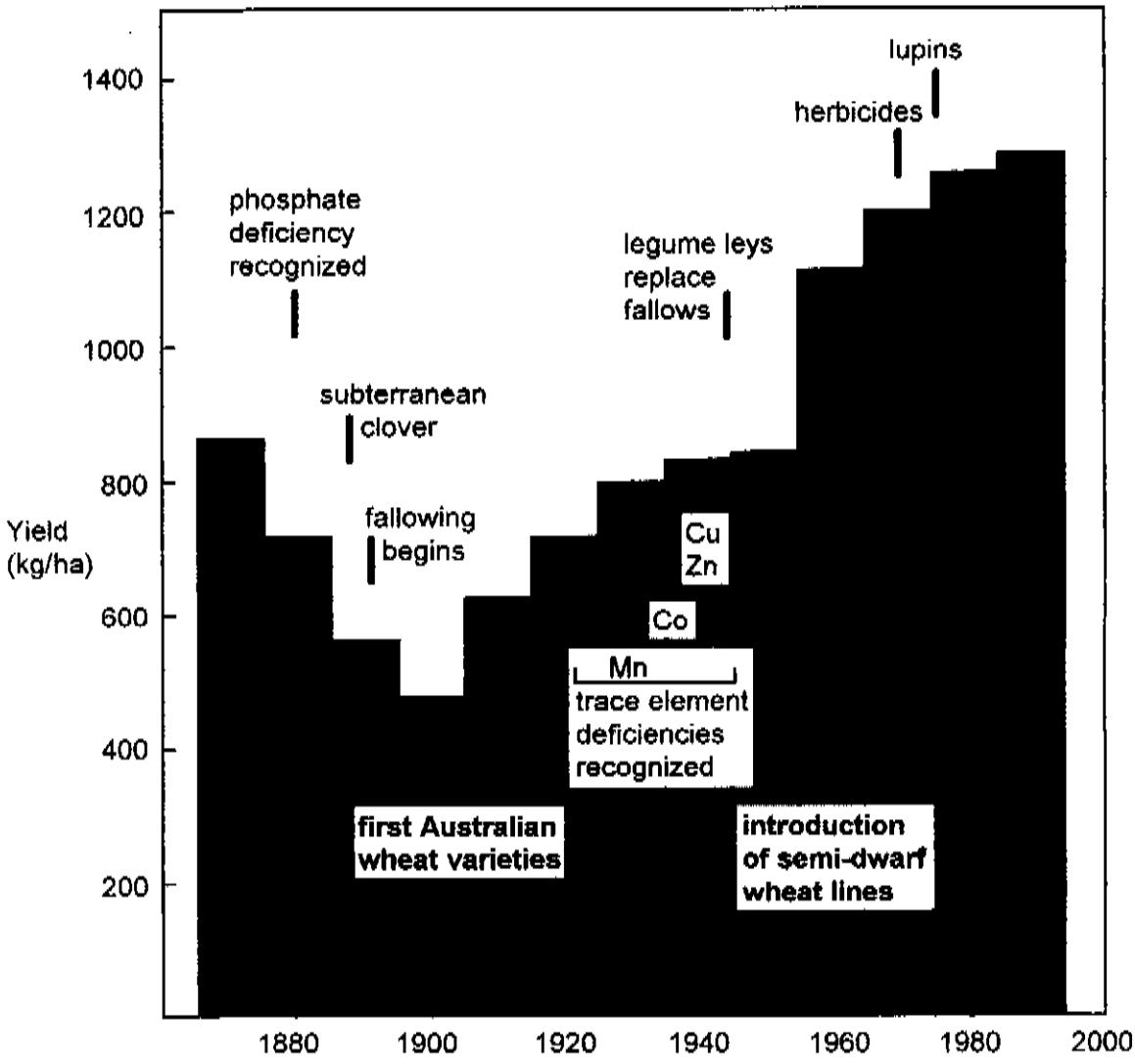


Figure 2. Ten year average wheat yields for Australia from 1870 to 1986 are indicated together with key technological innovations that interacted with the production of improved varieties (modified from 61, see also 64, 65). Double haploid technology is not indicated on the Figure because no current wheat variety has yet been produced using this technology. It is, however, progressively being used in Australia as a research tool underpinning efforts to develop specific populations for the genetic analysis of key traits. Doubled haploids have been routinely produced by SARDI, the University of Sydney Plant Breeding Institute and the QDPI Leslie Research Centre, Toowoomba using the wheat x maize system. SARDI currently offers a doubled haploid production as a commercial service.

Table 1. Minimum and maximum protein contents for receival into Australian wheat grades.

Prime Hard	Hard	Premium White	ASW[1]	Soft	Noodle	Durum	General Purpose	Feed
Min. 13.0%	Min. 11.5%	Min. 10.0%	Min. 9.0%	Max. 9.5%	Min. 9.0% Max. 11.5%	ADR1, Min. 13.0% ADR2, Min. 11.5% ADR3, Min. 10.0%	NS[2]	NS[2]

[1]ASW is Australian Standard White
[2]NS indicates that the protein content is not specified

Prime Hard. This is the top quality grade and is defined by a protein content of 13-14%, high milling quality and processing attributes. Flour is used to produce high protein yellow alkaline noodles with sought-after brightness, colour and eating quality. Wanton dumpling skins, high volume breads, flat breads, rotis and chapattis are also produced from this flour. This grade is also blended with lower protein wheats to produce flour suitable for a wide range of baked products.

Hard. This grade includes hard grained varieties with a minimum protein content of 11.5%. The grade is characterized by high milling quality and processing qualities that are suited to the production of European style pan, hearth and variety breads. This grade is also suited to the production of Middle Eastern flat breads and Chinese steamed products such as Mantou and Pao, as well as Chinese style yellow alkaline noodles.

Premium White. The grain marketed in this grade is a blend of wheat varieties selected to ensure consistently high milling performance and flour processing qualities. The protein content is a minimum of 10%. The grade is suitable for a wide range of products including Hokkien, instant and fresh noodles, Middle Eastern and Indian style breads, and Chinese steamed bread.

Australian Standard White (ASW). This grade is generally recognised as a highly versatile, medium to low protein white wheat. The milling quality and processing properties of this grade make it suitable for blending and for producing Middle Eastern, Indian and Iranian style flat breads, European style breads and rolls, and Chinese steamed bread.

Soft. Soft kernel textured wheats of 10-11 % protein and with good starch quality are used for the production of white salted noodles in Japan and Korea, whereas lower protein, soft, wheats are used for confectionary products such as cakes, pastries, biscuits, cakes, steamed buns and snack foods.

Noodle. The white salted noodle segregation is derived from soft-grained varieties grown in Western Australia to supply high quality wheat to the Japanese and South Korean markets. The small amount of wheat from Victoria that fits into this segregation consists of the variety Rosella. The yellow alkaline noodle segregation is derived from Prime Hard wheat grown in Queensland and New South Wales.

General Purpose. This category includes grain that did not meet the stringent standards for the above grades in criteria such as test weight, the presence of material that cannot be milled, high screenings, or defects such fungal stained, black point

effected, green and/or frost-damaged grain. The minimum falling number for wheat to be received into this grade is 250 seconds.

Feed. Although this category is usually high in protein, it has no minimum protein and falling number requirements and exists to accept grain with higher levels of the defects listed in the General Purpose category.

Durum. As indicated in Table 1, three grades are defined, based on protein content. The ADR1 grade is characterized by high yields of semolina with high water absorption and stable yellow pigment levels, suitable for a range of wet and dry pasta products.

Domestic wheat use in Australia is largely centered on pan bread production with lesser quantities used for confectionary products and the grocery flour market. A substantial amount of wheat (about 1 million tonnes annually) is used by the local starch and gluten industry.

Significant advances in wheat breeding over the past 100 years

The early lines of wheat grown in Australia after European settlement in 1788 were mixtures brought from England or those collected in Capetown (South Africa) *en route* to Australia (4). An awned wheat described as "Cape" was generally found to be unsuitable in the early years of settlement. The wheat lines most widely grown in the early 1800's, called "Common brown", were probably selections from the White and Red Lammas widely grown in England and Scotland (after their introduction from western Europe). A wheat line named "White Essex", similar to White Lammas except that it was early maturing, was widely grown in NSW after 1850.

The settlement of Victoria and South Australia provided a great stimulus to the development of wheat in Australia. Table 2 summarizes some of the key introductions into Australia in the 1860 – 1890 period.

Table 2. Significant introductions of wheat into Australia in the late 1800's.

Name of wheat line	Country of origin	Key persons involved
Purple straw, Tuscan	Italy/Scotland (introduced ca 1860)	J. Frame
Du Toits, Ward's Prolific, Gluyas	Sth Africa (introduced 1881)	R. Schomburgk, J. Ward, H.I. Gluyas
Fife	Canada (originally from Hungary)	W. Farrer
Etawah	India	W. Farrer
Muzaffar Nagar	India	W. Farrer
Summer Club	USA	W. Farrer

The selections made by growers for agronomic traits such as rust resistance provided lines that formed the basis for the deliberate cross-pollination experiments in the 1890's (4). The importance of these wheat lines in forming the basis of commercially significant wheat varieties grown in the past 100 years, is discussed below as the pedigrees are examined.

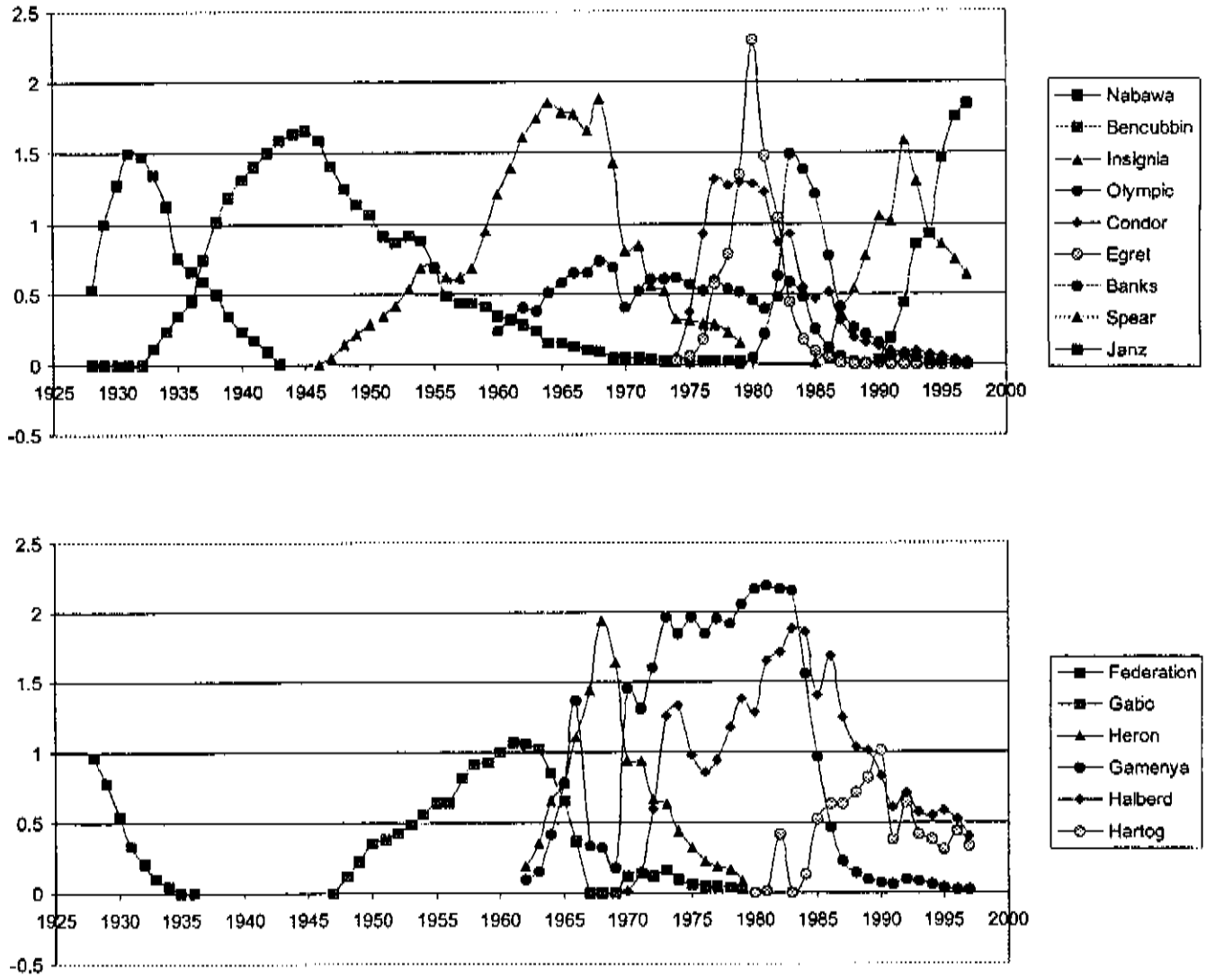


Fig. 3. Major wheat varieties grown in Australia over the past 100 years, illustrated by the areas of land devoted to growing each variety. Only varieties where plantings approached, or exceeded, one million hectares (nationally) are illustrated. Data from Wrigley and Rathjen (4), R. Williams, AWB Ltd (personal communication), and J. Brennan, NSW Agriculture (personal communication).

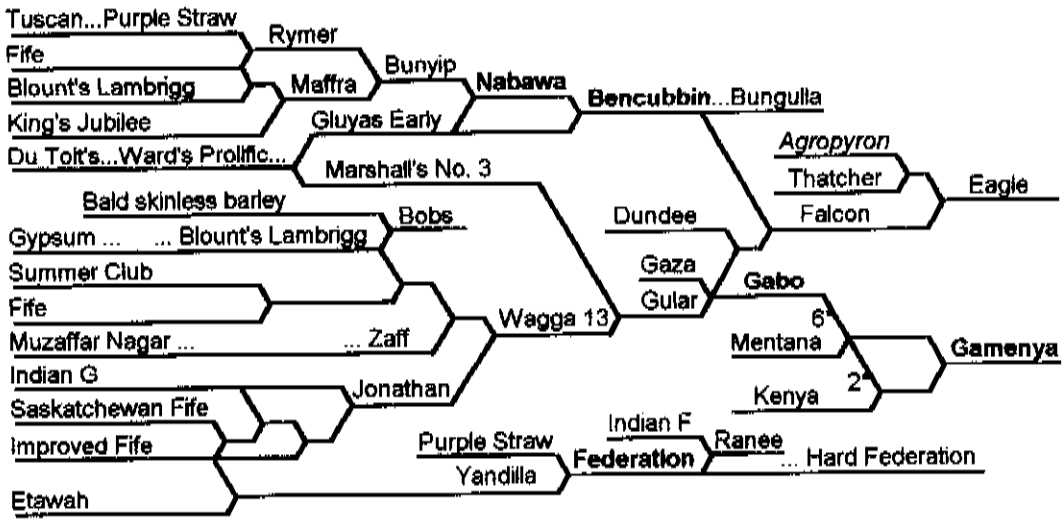


Fig. 4. Pedigree summarising the origins of Federation, Nabawa, Bencubbin, Gabo and Gamenya, The pedigrees are presented to emphasise the prominent lineages that have influenced modern wheat cultivars. This mode of presentation does not indicate the cytoplasm donors in crosses – a detailed study of the influence of cytoplasm donors in Australian wheat breeding needs consideration (M. Mackay, personal communication). Note the wide crosses that were carried out included a barley line (in the pedigree of Bobs, 57, 59) and a durum, Gaza (in the pedigree of Gabo). The Gabo that is listed here and elsewhere in the Figures is Gabo-Aus. The pedigree of Gabo has been extensively debated and it is considered likely that the single plant selection from Bobin 39 that gave rise to Gabo was in fact some Gular wheat mixed with the Bobin 39 grain (57, 63). This conclusion is based on glume colour (Gular and Gabo both have white glumes, whereas Bobin 39 has brown glumes) and gliadin protein analyses (5).

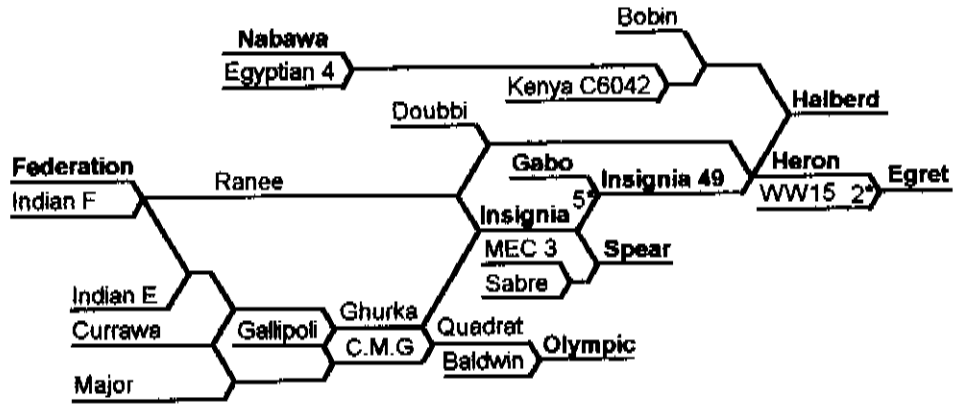


Fig. 5. Pedigree summarizing the origins of Insignia, Halberd, Spear, Heron, Olympic and Egret. As discussed in the legend to Fig. 4, the pedigrees have been presented to emphasize the prominent lineages that have influenced modern wheat cultivars.

Based on the graphs shown in Fig.3 it is evident that in the 1890 – 1998 period, wheat production was dominated by the varieties Federation, Nabawa, Bencubbin, Gabo, Gamenya, Insignia, Halberd, Spear, Heron, Olympic, Egret, Hartog, Banks, Condor and Janz. The pedigrees for Federation, Nabawa, Bencubbin, Gabo, Gamenya, Insignia, Halberd, Spear, Heron, Olympic and Egret (Figs. 4 and 5) show the very strong influence of germplasm generated through selection and crosses carried out at the turn of the century. The germplasm development focused on quality characteristics (from sources such as the Fife lines) combined with the agronomic

traits associated with the ability to grow in dry continents such as India (eg. Etawah, Indian F, Indian E).

In the 1960's a significant wave of new germplasm was introduced through the success of the CIMMYT (Mexico) program. The pedigree of Hartog (Fig. 6) clearly illustrates the impact of CIMMYT germplasm because this variety was the result of selecting suitable agronomic and quality phenotypes from the variety Pavon. Although Pavon originated in Mexico, the influence of the Australian variety Gabo is evident. The relationships in Fig. 6 also indicate the introduction of European germplasm (VPM1) as a source of disease resistance.

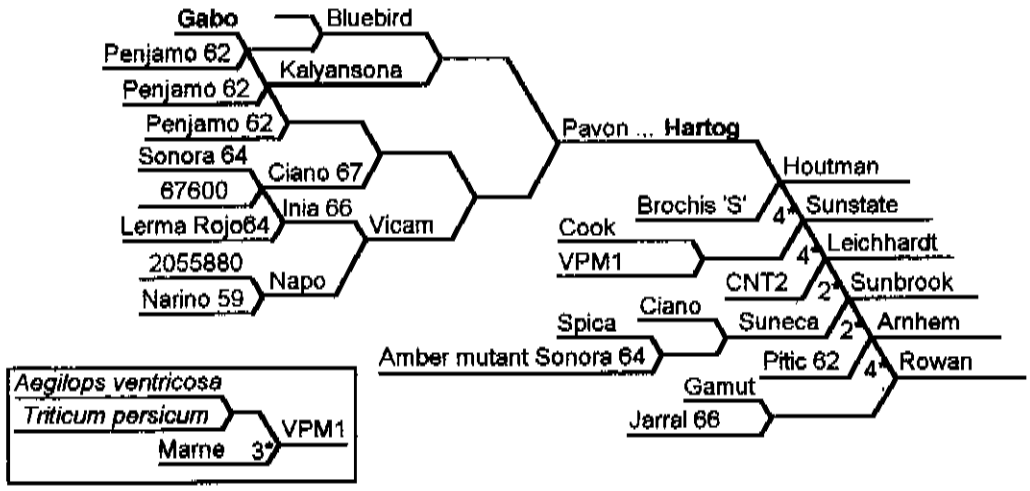


Fig. 6. Pedigree summarizing the origin of Hartog. As discussed in the legend to Fig. 4, the pedigrees have been presented to emphasize the prominent lineages that have influenced modern wheat cultivars.

The recent significant wheat varieties, Banks, Janz and Condor (Fig. 7), further demonstrate the influence of varieties such as Gabo and the importance of CIMMYT germplasm (the WW15, WW80 and Pavon selections) in Australian wheat improvement.

The influence of "alien" germplasm in the pedigrees is indicated by the presence of *Agropyron*, one of the wild relatives of wheat (6). The *Agropyron* chromosome segments (eg. 3Ag3, 3Ag14, Fig 7) have been used to provide a number of disease resistance genes (6,7,8,9, see also later in Fig. 11). In the case of VPM1 the alien chromatin is derived from *Aegilops ventricosa* and *Triticum persicum* (Fig. 6, see also 10) in a background of the French cultivar Marne. The rye segment 1RS from the short arm of chromosome 1 of rye has been introduced into Australian breeding programs as a 1BL.1RS translocation. The sticky dough quality defect associated with the presence of 1BL.1RS (50, 51) led to active selection against this chromosome segment in the early 1980's.

Alien germplasm that may contribute to future wheat varieties includes genes derived from *Aegilops tauschii*. In the late-1980's an extensive collection of *Ae. tauschii* (418 accessions, D genome donor to hexaploid wheat) was imported by CSIRO-Plant Industry. As a source of new germplasm the *A. tauschii* genome is particularly valuable because recombination with the natural D genome of wheat can occur (11).

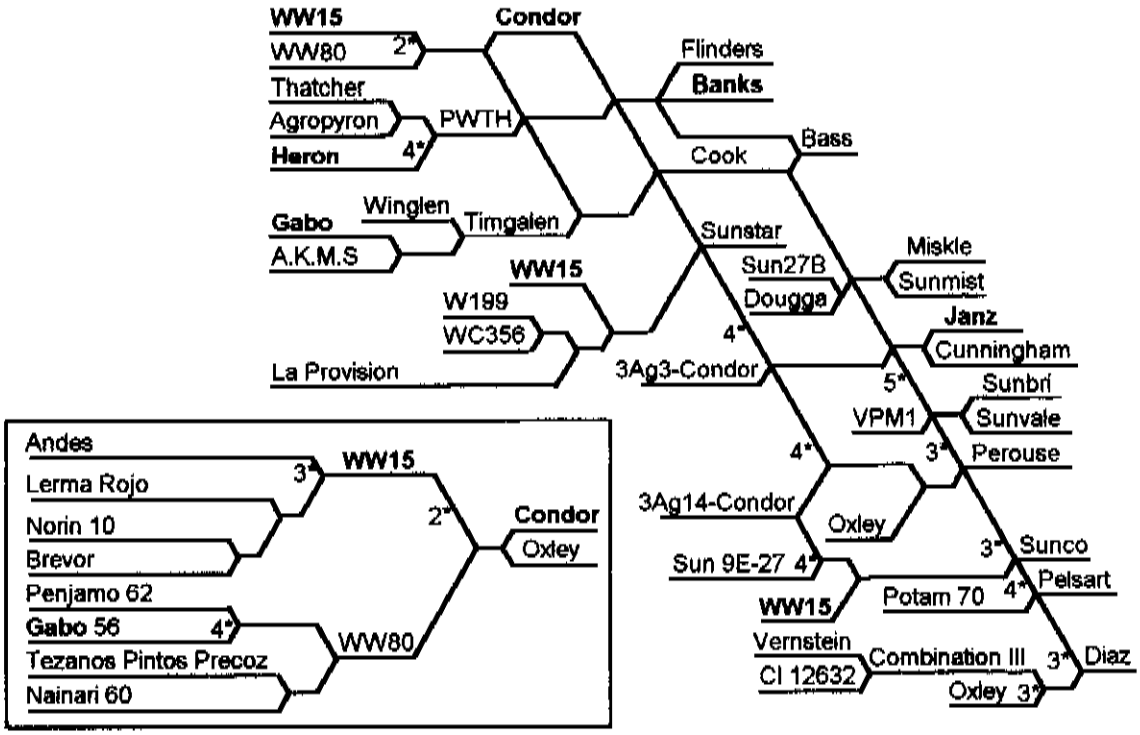


Fig. 7. Pedigree summarizing the origins of Banks, Janz and Condor. As discussed in the legend to Fig. 4 the pedigrees have been presented to emphasize the prominent lineages that have influenced modern wheat cultivars.

Direct crossing of the primitive wheat with hexaploid wheat has been established as a fast mechanism for introducing new variation (12,13). New germplasm carrying sources of cereal cyst nematode resistance (14, 41), root lesion nematode and crown rot resistance (E. Lagudah., R. Appels, J. Thompson, G, Wildermuth, P. Banks, I. Haak, unpublished, see also ref. 42) and septoria nodorum (44) has been identified. Many other disease resistance genes have been identified (45). A major program producing "synthetic" wheat lines by crossing durums (AABB) with *A. tauschii* (D) has been carried out by Dr M Kazi (CIMMYT) (39) and is providing new sources of variation for wheat breeding.

The molecular/biochemical analysis of Australian wheat germplasm (15, 16, 17, 18; X. Zhao, P. Sharp and C. Jenner, unpublished) has provided a large database from which the genetic relationships between the top 15 cultivars grown over the past 100 years can be examined. One of the dendrograms in Fig. 8 is based on the similarity between the cultivars as measured by assaying restriction fragment length polymorphisms (RFLPs) present in DNA isolated from the wheat lines. The dendrogram shows that the cultivars fall into three families, reflecting the four groups identified by Paull et al (18) in the analysis of a much larger sample of cultivars. The groupings are consistent with the pedigrees for these lines (Figs. 4 – 7, see also Fig. 8 for a dendrogram based on similarity indexes estimated from pedigrees). The relationship between certain lines within the two dendrograms shown in Fig 8 are consistent (eg the identification of family A) whereas in other instances they show some differences (eg the identification of family C). Detailed differences between the dendrograms presumably reflect the effects of selection for certain quality or agronomic traits. With the available database of DNA markers for wheat developing

quickly it should become possible to identify chromosome regions that are selected in breeding programs. To assess the feasibility of this, the current database was examined to map the RFLPs that showed a common polymorphism in both Federation and Janz, two wheat lines separated widely in both time (ie. when they were grown) and position within the dendrogram. The similarity between Federation and Janz at the RFLP polymophism level is 58%. This means that among the 1,968 polymorphisms assayed by 119 RFLP probes (18), just over half were identical between the two varieties. Since most of the RFLP probes have been assigned to chromosomal positions (19,20), the chromosomal distribution of the similarity between Federation and Janz can be estimated (lower portion of Fig 8). The preliminary mapping of the genetic loci defining the similarity between Federation and Janz indicates that they are spread across all chromosome groups (numbered 1 to 7 in Fig. 8) and not focused on particular chromosome segments. The trend in the maps shown in Fig. 8 for certain chromosome regions to be under-represented needs further investigation. The probes used in the study by Paull et al (18) were initially chosen for a relatively even distribution among chromosomes and if the trend indicated stands up to more detailed study it may help define chromosome regions of more general importance in breeding programs.

The analysis of wheat storage proteins in cultivars over many years (15; for review see 21) represents a detailed analysis of genetic loci on chromosome 1. The distribution of variation at the low molecular weight glutenin subunit loci (short arm of chromosome 1) and high molecular weight glutenin subunit loci (long arm of chromosome 1), as measured by variation in the protein products rather than RFLPs, is shown in Fig. 9. The proteins encoded by the *Glu-1* and *Glu-3* loci are central to determining the processing properties of wheat flour as certain alleles are clearly associated with properties such as dough strength (22). The information in Fig. 8 indicates that the alleles for the *Glu-1* and *Glu-3* loci in an older cultivar such as Federation have persisted in the major cultivars of more recent times. In some cases Federation introduced an allele (eg *Glu-D1d*) that is common in wheat lines at a world-wide level. In other cases, a cultivar such as Gabo introduced an allele (eg *Glu-B1i*) that is rare on a world-wide level (18) and its maintenance in breeding programs is presumably the result of favourable effects on quality attributes. A survey of *Glu-1* alleles in cultivars released in recent years by breeding programs across Australia (programs discussed in a later section) indicates 37% of the lines carry the *Glu-B1i* allele (data in ref. 23).

Another locus that is also important in determining flour processing quality encodes for granule bound starch synthase (GBSS). The 15 top cultivars in Fig. 8 have been examined for variation at the GBSS loci on chromosomes 4A, 7A and 7D (*Wx-B1*, *Wx-A1* and *Wx-D1*, respectively), a variant that is common is null for *Wx-B1* (loss of the entire GBSS gene, Table 3; see also 17). It is the *Wx-B1* null allele that accounts for a major portion of the variation in starch swelling properties of wheat cultivars grown in Western Australia and hence white salted noodle quality (17). When all three "normal" alleles are present and producing GBSS, the starch granules do not swell as much as when one of the loci (*Wx-B1*) has been lost.

It is significant that the *Wx-B1* null allele was common in Australian wheat cultivars from the turn of the century. The introduction of this important genetic locus into Australia has been investigated (X. Zhao, P. Sharp and C. Jenner, pers. comm.) and

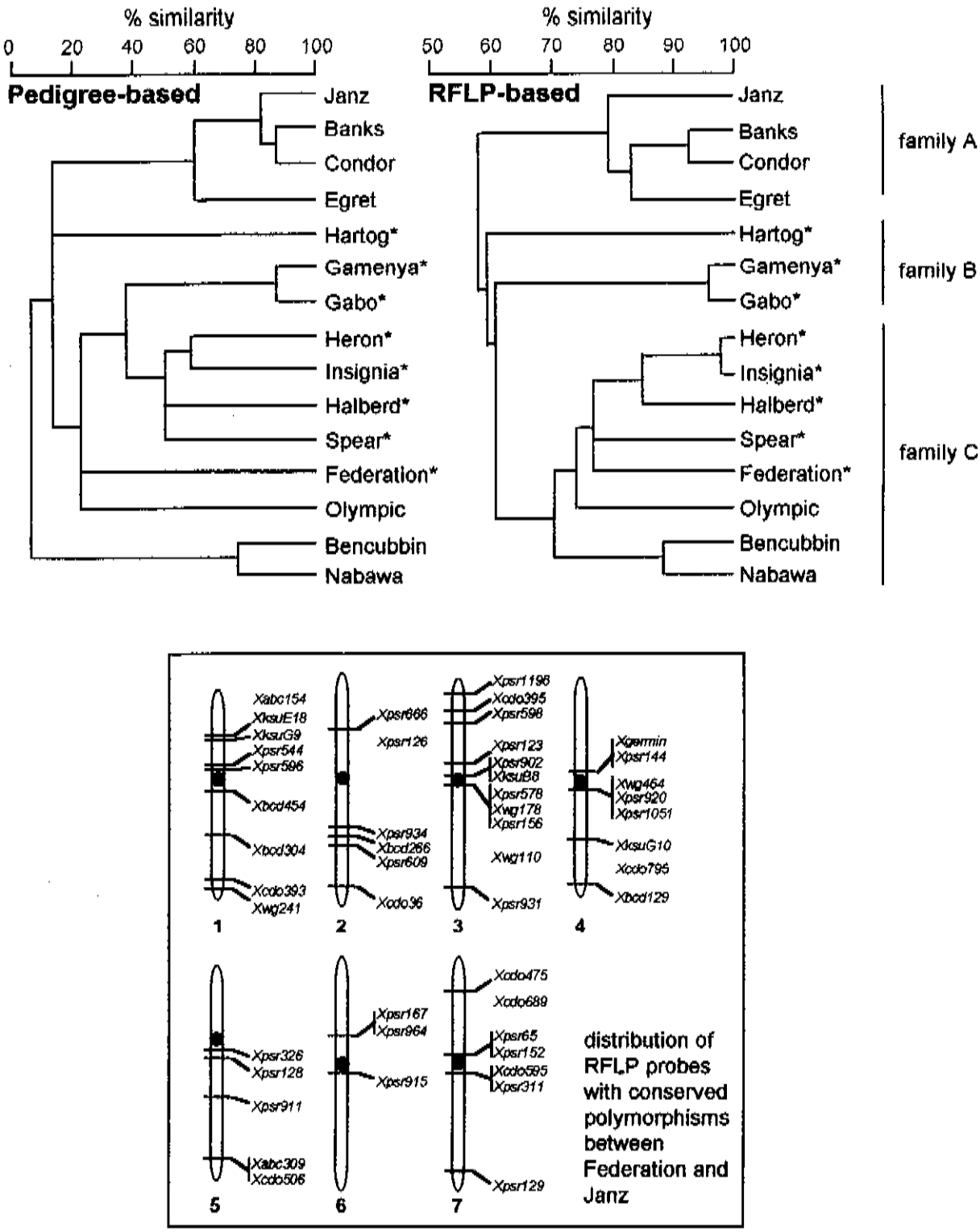


Fig. 8 The relationships between the major wheat cultivars grown in Australia over the past 100 years, based on RFLP-based DNA fingerprints (18) and pedigrees (40) as discussed in the text. The information for the RFLP-based dendrogram was kindly provided by Drs K. Chalmers and P. Langridge, University of Adelaide, South Australia. The cultivars with an * carry the *Wx-B1* allele, as discussed later in the text. The assignment of the chromosomal positions of the RFLP probes that define the similarity shared between Federation and Janz is shown in the lower half of the figure. This analysis is preliminary (R. Appels and P. Langridge, unpublished) and is based on 49 of 77 probes that could be assigned to unique map positions (within chromosome groups 1 - 7) using the ITMI database (19) or detailed published maps (20). The data is shown here to illustrate the value of databases of the type generated by Paull et al (18). The two dendrograms were produced from a similarity matrix of relationships between the cultivars and were optimized using the program PATMATCH (Dr. F. Bekes, CSIRO Plant Industry, Nth Ryde Sydney, NSW, Australia)

can be summarized by the following quote from Macindoe and Walkden-Brown (57), modified slightly by adding the *Wx-B1* allele status of the wheat lines mentioned.

Table 3. Variation at the *Wx* loci of Australian cultivars. Data from (18) and X. Zhao, P.Sharp and C. Jenner, unpublished.

Cultivar	*Wx-B1* allele	Cultivar	*Wx-B1* allele	Cultivar	*Wx-B1* allele
Federation	Null	Heron	null	Banks	normal
Nabawa	Normal	Olympic	normal	Halberd	null
Bencubbin	Normal	Condor	normal	Hartog	null
Gabo	Null	Egret	normal	Spear	null
Insignia	Null	Gamenya	null	Janz	normal

They indicated *"...in a row of Improved Fife **(normal Wx-B1)** sown at Lambrigg in 1894, Farrer observed an earlier maturing stranger with purple straw which he called 14A. This plant was probably a true Purple Straw wheat **(a mixture of normal and null Wx-B1 alleles)** ... crossed it the following year with Yandilla **(normal Wx-B1)** which has the parentage Improved Fife **(normal Wx-B1)** x Etawah **(Wx-B1 null)** (an Indian wheat) ... he kept this brown-eared selection because of its plump grain, and in 1901 decided to name the strain Federation **(Wx-B1 null)**"*. The additions in bold type indicate the status of the *Wx-B1* locus as far as could be ascertained from collections available today. The widespread occurrence of the *Wx-B1* null allele in breeding programs in Australia (see Fig. 8), provided the basis for selection over a long period of time (without knowledge of the importance of the *Wx-B1* locus) for white salted noodle quality, in collaboration with Japanese noodle producers (24,25). The selected wheat lines have allowed the relation between *Wx-B1* null and starch swelling/noodle quality to be established (17). A survey of *Wx-B1* alleles in cultivars released by Australian breeding programs in recent years, indicates 56% of the lines carry the *Wx-B1* null allele (data in ref. 23; X. Zhao, P. Sharp and C. Jenner, unpublished).

The origins of New Zealand wheat varieties contrasts with those of Australian wheat in that the New Zealand wheat derives from a much wider range of germplasm. Parental material used or introduced by New Zealand wheat breeders has come from countries such as Mexico (CIMMYT), the United Kingdom, Netherlands, South Africa, France and Portugal, and there are thus several distinct pedigree charts for New Zealand wheat varieties (39). Most New Zealand varieties are red grained, with the associated dormancy. Baking quality is focussed on the mechanical-dough-development technology (medium to high energy inputs).

The gene pools of regionally-based wheat-breeding programs

Crop breeding has been almost entirely conducted by State Departments of Agriculture/Primary Industries (43) with additional activity being undertaken by the Universities of Adelaide and Sydney, and the CSIRO. A privately funded programs such as the hybrid wheat improvement program conducted by SunPrime Research and Development Ltd, and companies with major breeding programs and international linkages, have also undertaken the introduction and evaluation of wheat breeding material in this country.

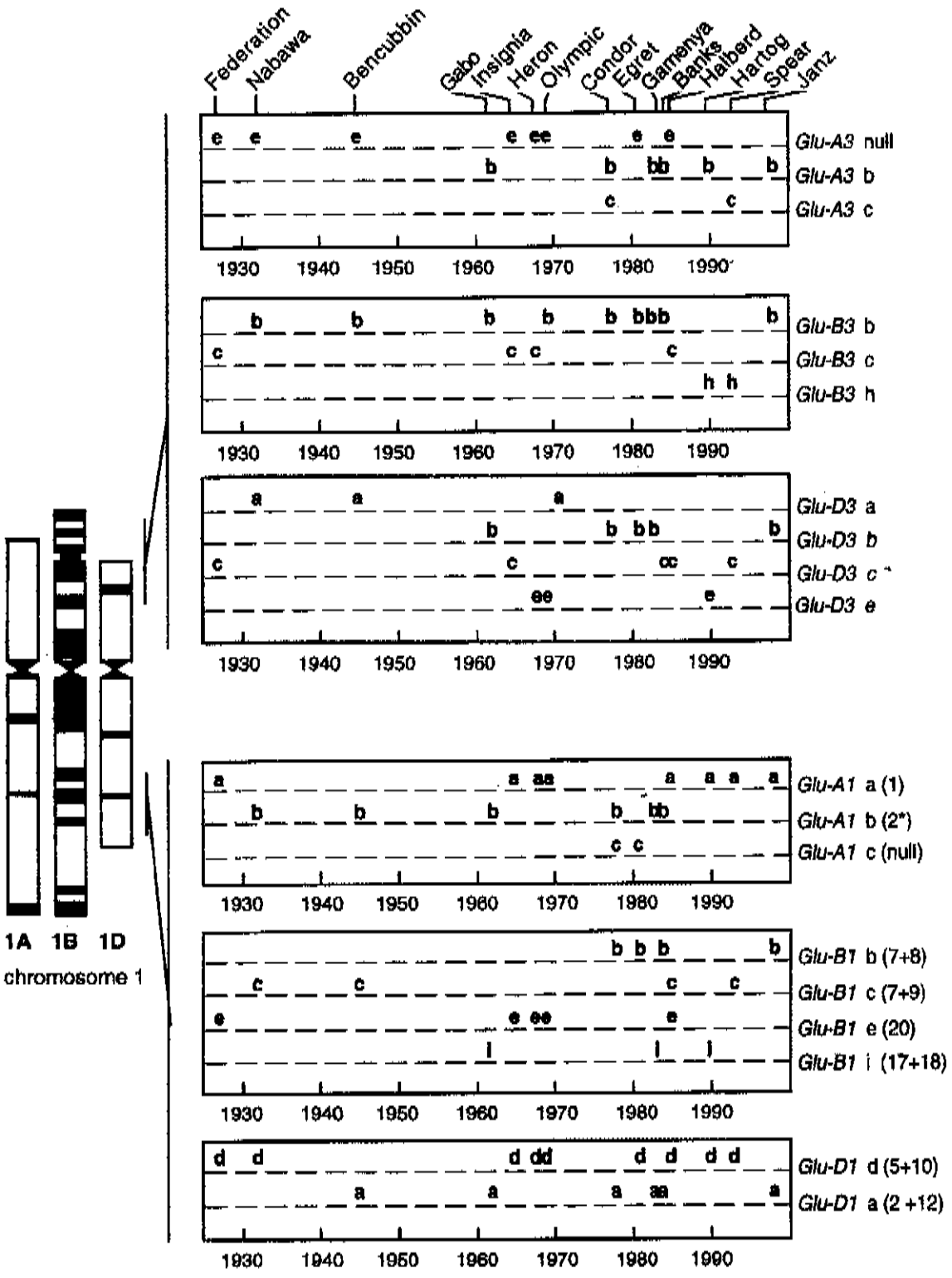


Fig. 9 The distribution of high and low molecular weight glutenin protein subunit alleles in the 15 major cultivars grown over the past 100 years (see Fig. 3).

In 1958 the Commonwealth Government introduced the Wheat Research Act which levied growers' production for the purposes of funding wheat research. Funds collected within a state were used to support research in that state, while a matching Commonwealth contribution supported research of national benefit. The new scheme resulted in the commissioning of three grower-initiated, and driven, research centers. The first was the University of Sydney at Narrabri in 1958, the second the Queensland Wheat Research Institute at Toowoomba in 1961 and the third the Victorian Department of Agriculture at Horsham in 1964. All three research centers continue to serve the industry to this day and operate on a mix of grower-contributed levies and host institution funds for their operation.

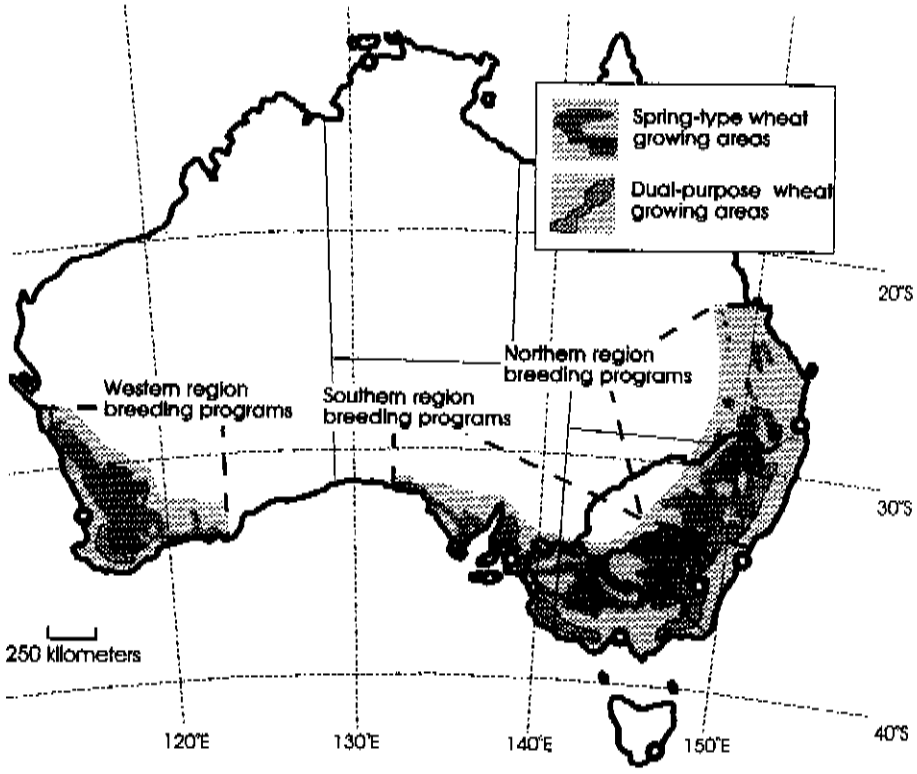


Fig. 10. Regional breeding areas defining the national wheat breeding strategies. The Northern Region is characterized by a tropical/sub-tropical climate and soils inherently high in soil fertility. Grain yield is highly dependent upon soil moisture stored from sub-tropical rainfall. The soils and climate provide conditions for both winter and summer grain crops to be grown and hence the farming enterprises are diverse. The Southern Region is characterized by a temperate climate and relatively infertile soils. Grain yield is dependent upon spring rainfall. Pasture ley farming forms a major part of the farming system. The Western Region is characterized by a Mediterranean climate and soils inherently low in fertility. Grain yield is dependent upon good winter rainfall as spring rainfall is unreliable.

The occurrence of droughts in Australia had the effect of rendering the funding of research associated with breeding programs, under the above scheme, highly variable because it was directly related to production. Programs highly dependent upon such funds suffered considerably after the droughts of 1967, 1976 and 1982 that severely reduced wheat production in eastern Australia. In order to minimize the impact of drought on grains research, the Grains Research and Development Corporation (GRDC) was established in 1991, with the mandate to manage funds collected from growers of 26 winter and summer grown grain crops. The GRDC brief was to distribute these funds, together with the matching Commonwealth funds, into national research programs. In 1992 the GRDC adopted a regional approach based on commonality of production environment (Fig. 10) for the national management of grains research.

The Northern Wheat Improvement Program (NWIP) is a GRDC funded initiative between the Queensland Department of Primary Industries (QDPI), the University of Sydney and NSW Agriculture. It primarily utilizes the QDPI resources at the Farming Systems Institute, Leslie Research Center (formerly the Queensland Wheat Research Institute), the University of Sydney, Plant Breeding Institute's I A Watson Grains Research Center at Narrabri, and the Plant Breeding Institute's Cobbitty campus. NSW Agriculture provides support to the program in the form of regional evaluation of advanced breeding lines.

A hybrid wheat breeding program (SunPrime Research and Development Ltd) targets Prime Hard quality wheats and achieves levels of heterosis that make the hybrids the highest yielding entries in collaborative yield trials conducted across the region. The program utilises a cytoplasmic male sterility system for the production of its hybrids.

The target breeding area extends from north-central NSW to central Queensland (see Fig. 10). The quality targets of the NWIP are high protein wheats of high milling quality, producing strong and extensible flours with starch quality, flour and colour characteristics that are suitable for a range of pan breads, Asian alkaline noodles, and starch and gluten processing. To meet the bread-processing needs of the domestic milling and baking industry wheat lines with a shorter dough development time are also selected. The top quality wheat lines grown in the northern region have traditionally set the standard for receivals into the premium Prime Hard grade. The characteristics of the Prime Hard grade were discussed earlier (see Table 1). Prevailing temperatures and the amount, and timing, of rainfall during grain filling have a major influence on final grain protein content. Grain of varieties that fail to meet the Prime Hard grade is received into the Australian Hard grade provided that it meets receival requirements for that grade (> 11.5% protein content). If lower protein results, growers still have options for receival in the Australian Premium White and Australian Standard White grades (see Table 1). Failure of grain to meet quality requirements is usually due to stresses encountered during grain filling.

Breeding programs therefore aim to complement the selection of quality characteristics of wheat lines with agronomic attributes, and resistance to the major biotic and abiotic stresses, suited to the production region. Abiotic challenges in the region include high temperature stress, drought, discoloration of the kernel (black-point, 52), and pre-harvest weather damage (sprouting). A major breeding effort is

Table 4

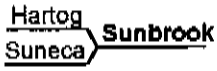
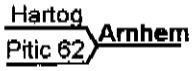
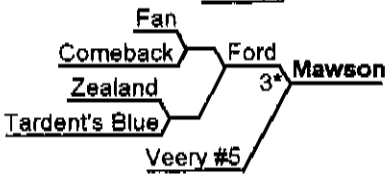
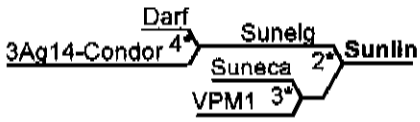
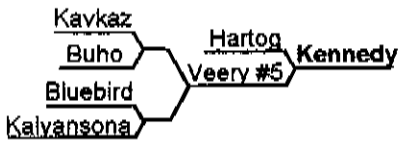
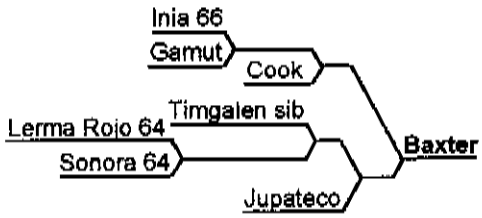
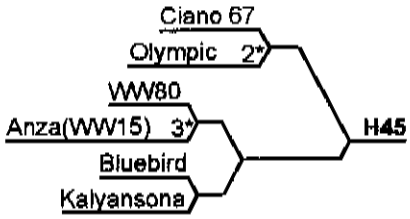
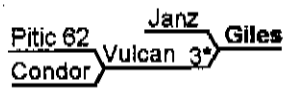
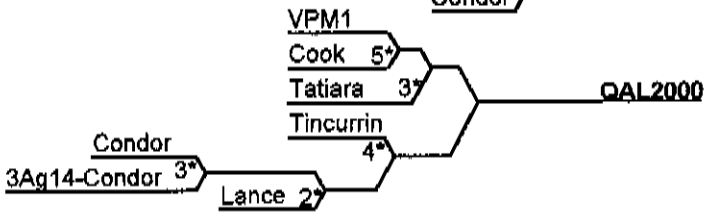
wheat variety	year	organisation	classification
	1996	QDPI	Hard
	1996	Sydney Uni	Prime Hard
	1997	QDPI	Hard
	1997	QDPI	hay wheat
	1997	Sydney Uni	Prime Hard
	1998	QDPI	Prime Hard
	1998	QDPI	Prime Hard
	1998	Sydney Uni	Soft
	1998	SunPrime R&D	Hard
	1999	QDPI	Prime Hard
	1999	Quality Wheat CRC	Biscuit

Table 4. Varieties released by the Northern Wheat Improvement Program since 1995. As discussed in the legend to Fig. 4, the pedigrees have been presented to emphasize the prominent lineages that have influenced modern wheat cultivars. The pedigrees in this table, and others following, give sufficient detail to cross-reference them to the IWIS database (40), for further information. Recent releases of hybrid wheat in the Prime Hard quality category include Hybrid Apollo and Hybrid Mercury and in the Hard category, Hybrid Gemini.

directed toward sprouting tolerance and, as a result, varieties with some tolerance are currently available to growers.

The gene pool underpinning the varieties developed in the above environment is defined by the pedigrees shown in Fig. 7. The lines released by the NWIP for the northern region since its commencement in 1995 and their quality grades are given in Table 4.

Three breeding programs comprise the Southern Wheat Improvement Program (SWIP). NSW Agriculture conducts breeding for southern and central NSW from the Agricultural Institute (Agriculture NSW, Wagga Wagga), while in Victoria, operations are centred on the Victorian Institute of Dryland Agriculture (VIDA) at Horsham. In South Australia, the University of Adelaide conducts breeding from both its Roseworthy and Adelaide campuses. The South Australian Research and Development Institute (SARDI) is integrated with the breeding programs through its role in regional yield testing, disease testing and quality evaluations.

The major quality targets are end-product based and include pan and flat breads, steamed breads, alkaline noodles, white salted and instant noodles and cakes, pastries and biscuits. The three programs have a collaborative yield trial system that involves the exchange of breeding lines on an annual basis. Lines then proceed on merit through the trialing system either within a particular agro-ecological environment or across the entire region. Six agro-ecological environments have been defined, namely, acid soil (southern NSW and north eastern Victoria, parts of central and south west Victoria and the lower south east of South Australia), red brown earth (mid-north of South Australia, parts of southern NSW), Mallee (south western NSW, northern Victoria and the northern parts of the Eyre and Yorke Peninsulas in South Australia), duplex soil (the lower Eyre Peninsula and the upper south east of South Australia), Wimmera (western, mid-Victoria), and irrigation (flanking the Murray and Murrumbidgee rivers) environments. The characteristics of the soils in these regions are reviewed in (61).

The agronomic attributes that provide for reliable production include lodging resistance, a range of maturities for flexibility in planting time and frost avoidance at flowering time, and enhanced establishment under minimum tillage farming. The environments of the region can be conducive to stripe rust, and stem and leaf rust and resistance to all three rust diseases is considered to be highly desirable.

The varieties released by the Southern Wheat Improvement Program since 1995, and their quality grade, are given in Table 5. The gene pool from which the varieties were selected is also indicated.

Wheat breeding in Western Australia is conducted by Agriculture Western Australia. The program is funded through a mix of state based and GRDC provided funds. Recently, a new Export Grain Center supported by the Council of Grain Growing Organisations, Agriculture Western Australia and the GRDC has been established to provide an enhanced export focus to grains research in the state. Growers pay an additional levy contribution to support the work of the Export Grains Center.

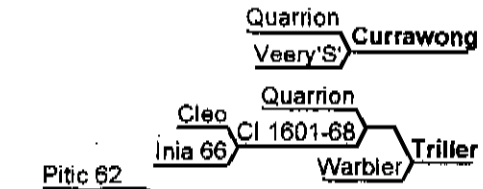
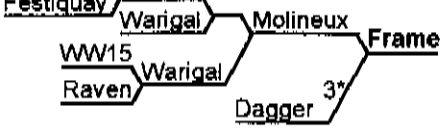
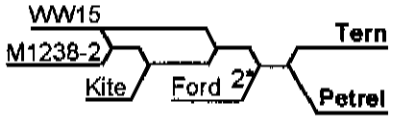
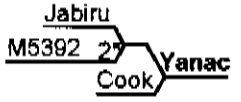
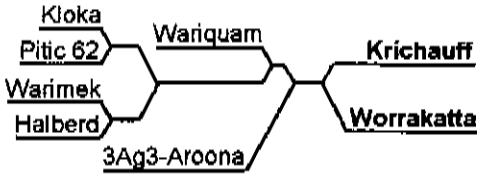
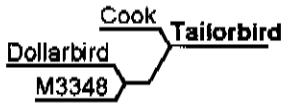
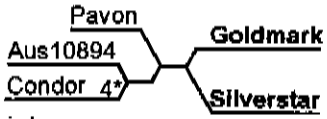
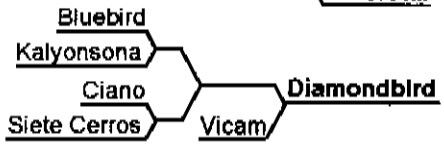
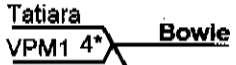
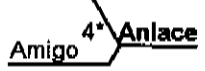
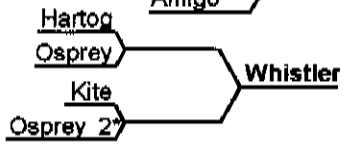
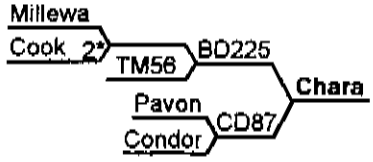
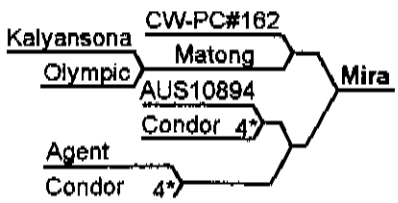
wheat variety	year	organisation	classification
	1995	NSW Ag	feed
	1995	NSW Ag	Soft
	1995	Uni Adelaide	Premium White
	1995	NSW Ag	hay
	1996	NSW Ag	hay
	1996	Ag Victoria	Premium White
	1997	Uni Adelaide	Premium White
	1997	Uni Adelaide	Premium White
	1997	NSW Ag	premium white
	1997	Ag Victoria	Premium White
	1997	Ag Victoria	Hard
	1997	NSW Ag	Hard
	1997	Uni Adelaide	Soft Biscuit
	1998	Uni Adelaide	Soft Biscuit
	1998	NSW Ag	Soft White
	1998	Ag Victoria	Prime Hard
	1998	Ag Victoria	Premium White

Table 5. Varieties released from the Southern Wheat Improvement Program since 1995. As discussed in the legend to Fig. 4, the pedigrees have been presented to emphasize the prominent lineages that have influenced modern wheat cultivars.

The wheat-producing area is extensive, and there is a distinct east to west rainfall gradient, with rainfall declining rapidly as distance from the coast increases (see Fig. 1). Western Australian wheat is largely destined for the export market, hence the quality objectives are almost entirely focussed on this market. High yielding soft club wheats, producing low protein grain (<9.0%), provide grain that can be used in many end-products. Soft wheats with the starch, flour and noodle colour properties required for white salted noodle (*udon*) production can be grown at the 10.0 - 10.5% protein content desired by markets. The balance of wheat varieties grown are hard grained varieties of intermediate protein content (10%) and are received into the Australian Premium White and ASW grades. The small domestic requirement of grain for pan breads and other uses are met because of the commonality of quality needs of export and domestic end-users.

A feature of the breeding program is the existence of special nurseries for the screening of resistance to the septoria diseases. Sources of new genetic resistance to septoria nodorum have been identified in *Aegilops tauschii* germplasm collections (44, 58)

The varieties released by the program since 1995 and their major attributes are given in Table 6. The pedigrees are shown to provide a summary of the gene pools that now underpin the program.

The gene pool of nationally focussed wheat breeding programs

The regional breeding programs have a long history of initiating and maintaining national cooperation for screening for yield, quality, disease resistance and other traits. In some cases this national cooperation takes the form of specific programs (discussed below) while in other cases it takes the form of a national network for screening for diseases at different locations; the Australian Septoria Observation Nursery (AUSEN), for example, provides a national network of septoria-screening locations.

National Cereal Rust Control Program (NCRCP)- Sydney University, Cobbitty, NSW

Cereal rust research in the University of Sydney started in the 1920's and was expanded in 1958 with the establishment of the Wheat Research Council. The National Cereal Rust Control Program (NCRCP) was established following a major stem rust epidemic in 1973, and was developed further after stripe rust first appeared in Australia (1979). Research in the program has included a back-crossing program to add resistance genes to current cultivars, the screening of early generation and advanced lines from all breeding programs, the provision of information and rust resistant germplasm, and a national survey of pathogenic variability. Molecular markers closely linked to rust resistance genes have also been identified.

NEXT PAGE:

Table 6. Varieties released by the Western region wheat breeding program since 1995. As discussed in the legend to Fig. 4 the pedigrees have been presented to emphasize the prominent lineages that have influenced modern wheat cultivars. The detailed pedigree of line 77W660 is Siete Cerros/XBVT223 (72W05-20)//(72W12-67) AWX11-6-48/XBVT221, where XBVT223=Chile 1B//Insignia/Falcon. XBVT221=Chile 1B/Insignia//Falcon, AXW11-6-48=M123/Mexico 120 and M123=Bencubbin/3/(M113) Charter//(M60)Sword/Kenya C6041.

Table 6

wheat variety	year	organisation	classification
WW15 / Raven / Aroona / Tadorna 3* / Inia 66 / **Cascades**	1995	WA Ag	Hard
Halberd / 3Ag3-Condor 3* / Tincurrin 4* / **Datatine**	1995	WA Ag	Soft
Gamenya / Siette Cerros / Bodallin / Bokal / Eradu sib. / Atlas 66 / **Tammin**	1995	WA Ag	Feed
Sunfield sib. (Sun 95H) / **Cunderdin** / Clano / Zambesi / Noroeste / Wren / Cranbrook	1997	WA Ag	Premium White
Gamenya / Inia 66 / Hyden / Bodallin / **Perenjori**	1997	WA Ag	Premium White
Bolsena selection 1CH (RAC529) / **Carnamah**	1997	WA Ag	Hard
Chile 1B / Siete Cerros / XBVT223 / Insignia / Falcon / 72W12-67 / Eradu / 77W660 / **Arrino**	1997	WA Ag	Noodle
Falcon-EMS / Sharbarti Sonora / PI 05228 / Madden sib. / Aroona / **Kalannie**	1997	WA Ag	Hard
Chino / Kulin / Reeves / **Calingiri**	1997	WA Ag	Noodle
Emblem / P1649 / 76W595 / Nuri 70 / Cranbrook / Torres / **Brookton**	1997	WA Ag	Premium White
Spica / Timgalen / Tosca / **Westonia** / Cranbrook / Bob White / Jacup 2*	1997	WA Ag	Premium White
3Ag3-Aroona / Condor / 3Ag14-Condor 4* / Jabiru / **Nyabing**	1997	WA Ag	Standard White
Sonora / Tezanos PP / Yaqui 54 / Gabo 2* / Kite / Kulin / Blade 2* / **Ajana**	1998	WA Ag	Standard White
Spear / **Camm** / VPM1 4* / Cook 5*	1998	WA Ag	Premium White

The information in Fig. 11 summarizes the genetic backgrounds of selected rust resistance genes used in Australian wheat breeding. It is evident from a study of the pedigrees of recent wheat cultivar releases (Tables 3 to 5; see also 23) that lines such as Kite (*Sr26*) and the white seeded recombinants derived from Sears' *Agropyron* chromosome 3 (3Ag) have been particularly valuable. In the latter case chromosome 3D translocations (6), 3Ag3 and 3Ag14 (*Sr24/Lr24*), are in use. The back-cross lines containing the VPM1 derived resistance genes (*Sr38/Lr37/Yr17*) have also been successful.

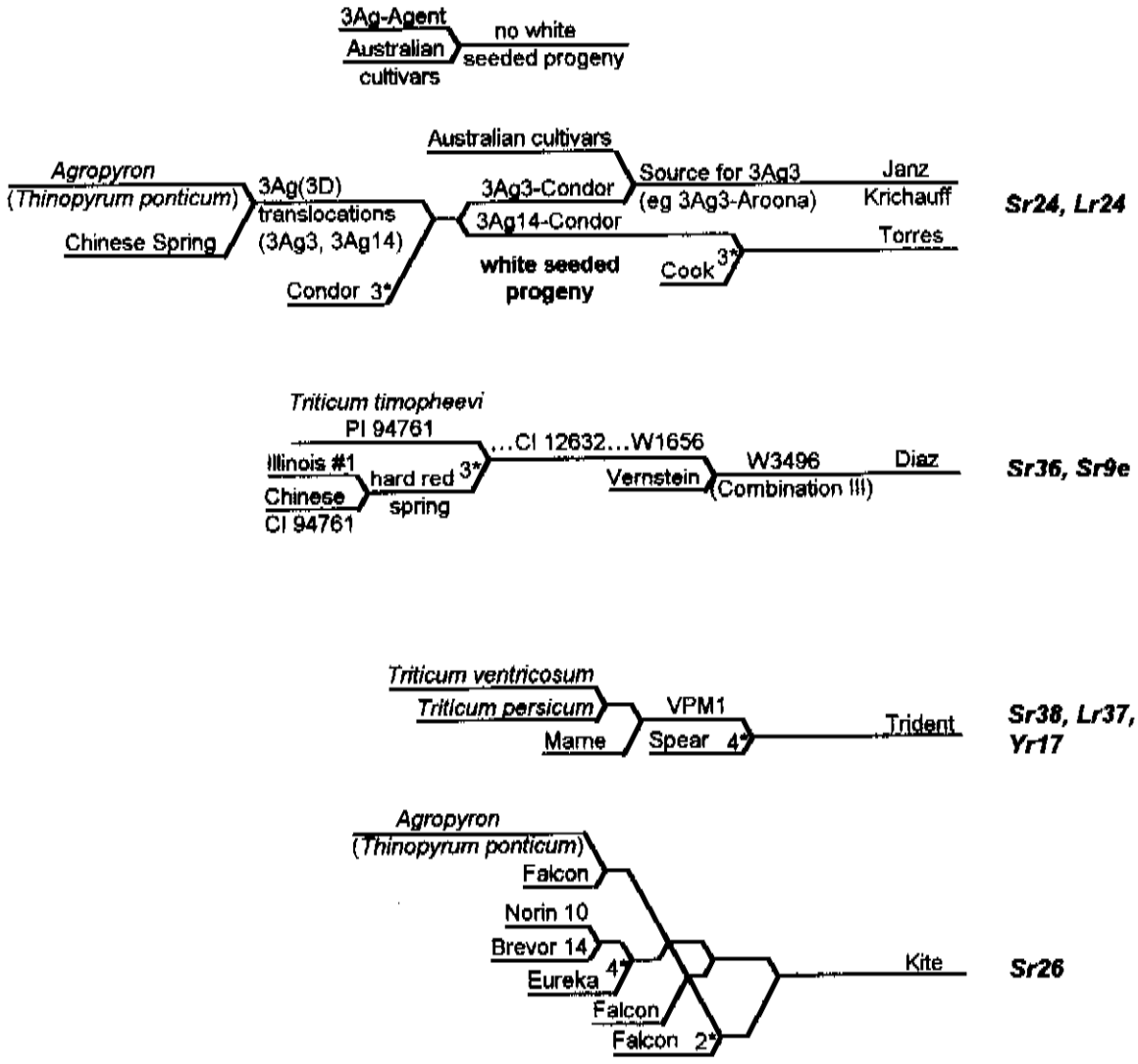


Fig. 11 Pedigrees of some rust resistance sources used in Australian breeding programs. As discussed in the legend to Fig. 4 the pedigrees have been presented to emphasize the prominent lineages that have influenced modern wheat cultivars. The varieties listed provide examples in which the respective rust resistance genes have been used.

CSIRO Plant Industry wheat improvement research program: dual-purpose wheats

The breeding of dual-purpose wheats for high rainfall areas nationally (see Fig. 10) is carried out at CSIRO Plant Industry, in partnership with AWB Limited and the GRDC. The target areas are generally non-traditional wheat growing areas, where the primary source of income for producers has been based on sheep and beef production. Soil types in these areas vary widely. The dual-purpose wheat lines are designed for early planting (February – March) to provide growth for grazing during the winter months. Following this grazing period two options are then available, the crop can either be completely grazed-out or allowed to produce grain for harvesting.

The program also targets the production of cultivars with improved performance and differentiated quality features. The first dual-purpose variety, Lawson, was released under contract with the Australian Wheat Board Ltd (AWB Ltd). Other varieties released since 1995 are summarized in Table 7. A dual-purpose wheat, a selection from the French cultivar Declic, was also released by Agriculture Victoria (VIDA) in 1996.

wheat variety	year	organisation	classification
Hobbit / Pitic 62 / Maris Huntsman → **Lawson**	1995	CSIRO, AWB	red feed
Hartog / Brock 2* → **Paterson**	1997	CSIRO, AWB	red feed
Hartog / Lawson 7* → **Gordon**	1997	CSIRO, AWB	red feed
Hartog / Mercia 2* → **Brennan**	1998	CSIRO AWB, and GRDC	white feed
Mardler / Hobbit / Pitic 62 / Hornet → **Tennant**	1998	CSIRO, AWB and GRDC	red feed

Table 7. Wheat varieties released by the CSIRO Plant Industry based breeding program.

CSIRO Plant Industry wheat improvement research program: altering physiological traits

The objectives of this program include the production of conventionally bred cultivars with novel physiological traits to improve yield and adaptation without sacrificing grain quality or disease resistance. The major traits of interest include transpiration efficiency, measured by carbon isotope discrimination (CID, 26), early vigour and long coleoptile length/establishment (27,28). The program utilizes genes for height reduction (*Rht*) and CID, salt tolerance, carbon and nitrogen partitioning in wheat, and for the control of leaf growth. A recent new development is the inclusion of transgenic cultivars that aims, in the future, to provide novel genes for tolerance to nutrient deficiencies, environmental stresses, unique grain quality features, higher yield potential, resistances to viruses, root pathogens and rusts, that are not currently available to wheat breeders. The program was the first in Australia to develop transgenic wheats (31). Field testing of these wheats began in 1996 under the

supervision of the Genetic Manipulation Advisory Committee (GMAC) for transgenic releases in the field. Approval has been given for a total of 89 plantings (wheat and barley).

With respect to the transpiration efficiency characteristic, the cultivar Quarrion (Fig. 12) has been found to give particularly low CID in a range of different environments. It is considered likely that WW33 (see Fig 12) is the origin of low CID values as judged from the analysis of pedigrees from other low CID wheat lines.

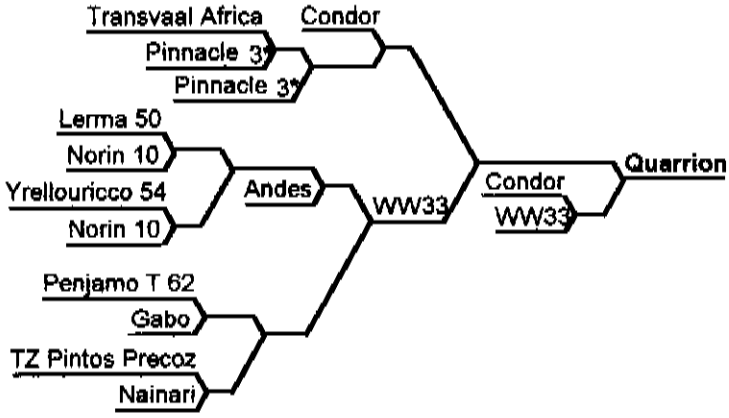


Fig. 12. Pedigree of Quarrion. As discussed in the legend to Fig. 4 the pedigrees have been presented to emphasize the prominent lineages that have influenced modern wheat cultivars. The pedigrees of a number of cultivars assessed for high transpiration efficiency (low delta) are given in (60).

Interest in the long coleoptile length/establishment phenotype of wheats has focused on the concept that the currently utilized dwarfing genes, *Rht1* and *Rht2*, not only reduce plant height but also coleoptile length. It has been considered possible that the short coleoptile lengths associated with *Rht1* and *Rht2* genotypes may be detrimental to plant establishment in dry environments and this has led to the investigation of alternate, "gibberellic acid sensitive" *Rht* genes (28) for reducing plant height. It is evident that the *Rht8* and *Rht9* genes can produce plants of reduced height without reducing coleoptile length because they do not suppress cell size and hence do not inhibit coleoptile cell elongation in the crucial establishment phase. Sources of *Rht8* and *Rht9* that have been investigated include Mara (*Rht8*/*Rht9*) and Chuan-Mai-18 (*Rht8*).

National Durum Wheat Improvement Program – Agriculture NSW, Tamworth

The durum breeding program produces varieties for a range of environments across Australia. Annual production in the past two years has been 4-600,000t, with approximately 70% produced in New South Wales and the remainder distributed between Queensland, Sth Australia and Western Australia. A harvest of 1 million tonnes is anticipated in 1999. Classification of the grain is based on protein content (see Table 1) and absence of defects such as sprouting, black point and screenings.

Germplasm stocks, for the breeding of lines suitable for the Australian environment, originate from ICARDA and CIMMYT, as well as from collections held in Morocco, Turkey, Italy, Canada, USA, France, Iran and Iraq. The wide germplasm base has provided genes for traits such as salt and drought tolerance, micronutrient uptake, yield, and crown rot resistance. Repeated cycling of advanced breeding lines,

interposed with the introduction of new germplasm and stringent selection (using a range of testing procedures) has resulted in the current high quality material. Quality attributes of the semolina that are targeted include its % extraction, protein and starch composition, protein content, and processing qualities monitored by the small-scale production of pasta. An overview of the pedigrees, and hence the gene pool, of the important cultivars are illustrated in Fig. 13.

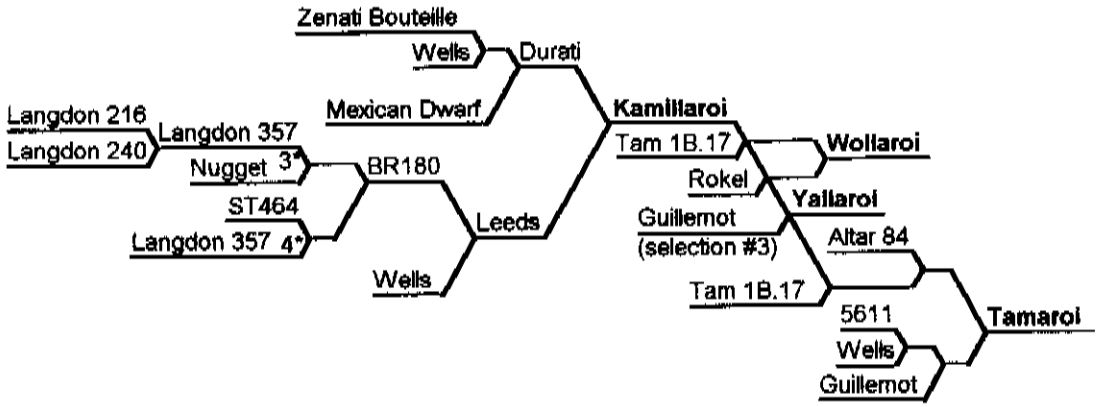


Fig. 13. Overview of the pedigrees of the major releases from the durum breeding program. As discussed in the legend to Fig. 4, the pedigrees have been presented to emphasize the prominent lineages that have influenced the modern cultivars. The durum Altar 84 is one of the durum lines used in the CIMMYT program (39) for producing "synthetic" wheat lines (AABBDD) by crossing durum (AABB) with *Aegilops tauschii* (DD)

National cooperation in targeting the gene pools of breeding programs to end-products

The Grains Research and Development Corporation (GRDC) has established the National Wheat Quality Evaluation Program to coordinate the progression of breeders lines from the near release stage to recommendations relating to classification into quality types (see Table 1). Domestic users, the Australian Wheat Board Ltd, the breeding programs and research groups involved in the cereal chemistry and molecular biology of flour quality participate in the program. The GRDC's Wheat Quality Objectives group has the responsibility for relating market needs to activities in breeding programs

Evaluation and release mechanisms

Breeding lines move through the yield and quality testing system on merit (43), with the decisions on lines primarily being made by the breeding programs. Quality evaluation is conducted using seed retained from the regional yield trials and sites are selected for use based on protein content (to cover the range of expected end-uses for the target quality class), test weight and freedom from weather damage.

Early-generation testing for grain quality is conducted in the cereal chemistry laboratories associated with the breeding programs. In the Toowoomba program later-generation testing of advanced lines (a year or two before release) is also carried out. Advanced breeding lines from the University of Sydney's program are quality tested by BRI Australia Ltd (formerly the Bread Research Institute) in Sydney, reflecting fifty years of ongoing collaboration in variety development between the two organizations.

Advanced lines from Western Australian breeding are tested in the Perth laboratories of Agriculture Western Australia. The Victorian center for quality testing is located at the Victorian Institute for Dryland Agriculture in Horsham. Testing of advanced South Australian lines for quality traits is performed by the South Australian Research and Development Institute (SARDI), situated at the Waite campus of the University of Adelaide. In Queensland, NSW and Victoria samples of potential varieties, prior to variety release, are also evaluated by the domestic milling and baking industry for their ability to suit local needs. The quality traits required for the export market are assessed by Agrifood Technology in Werribee, Victoria. Extensive end product testing is conducted at this stage, and grain samples covering a range of protein contents are selected for these evaluations.

Research to determine the fundamental characteristics of quality traits and their assessment using new small-scale tests is carried out in CSIRO Plant Industry laboratories (34, 35), co-located with BRI Australia Ltd. This unique environment continues to generate new technologies that integrate recent advances in molecular biology, the needs of the processing industry and the requirements of breeding programs.

The assessment of grain quality for the National Wheat Quality Evaluation Program is carried out by BRI Australia Ltd and Agrifood Technology. This program focuses extensively on end-product evaluation. The range of bread types produced include flat, steamed and pan breads. Pan breads are made by a range of baking methods and processing conditions are changed to assess their effects on product quality. The suitability of new wheats is also evaluated for a full range of noodle products, including yellow alkaline, white salted, Hokkien, and instant noodles. The analyses carried out on the incoming grain include:

- determination of hectolitre weight,
- determination of thousand kernel weight,
- measurement of protein (using NIR), % moisture (HE50), particle size index,
- establishing a single kernel analyser profile (using the SKCS 4100).
- milling a 0.8-5 kg sample, and weighing of individual milling streams (1bk, 2bk, 3bk, 1R, 2R, 3R, bran, pollard),
- blending of individual streams for "straight run" flour or 60% extraction,
- checking of increased extraction rate by returning bran and pollard to redresser,
- formation of dough from flour using Farinograph and Extensograph, and the determination of water absorption and mixing time.
- analysis flour for protein (Kjedahl), moisture (oven), ash, stirring number, maltose, starch damage, colour grade, yellow pigment, minolta colour, RVA, % bran (Branscan).
- determination of bakery water absorption, formation of pan breads (rapid, fermented), sponge and dough, sponge cakes,
- production of Asian noodles (white salted, yellow alkaline),
- production of flat bread, steamed bread, buns and biscuits.
- scoring of bread products for volume and crumb,
- determination of instron and minolta values on all products,
- evaluation of products by sensory panel.

DNA marker technology

Marker-assisted breeding

Research on DNA marker technologies for the wheat and barley industries has been carried out over the past 20 years. Two approaches have been followed. Firstly, a large suite of polymorphic markers to facilitate the development of high-density genetic maps has been established. These genetic maps allow the QTL analysis of key traits and the identification of closely linked markers (GRDC, National Wheat Molecular Marker Program, NWMMP). Secondly, DNA sequences within or near loci determining specific agronomic traits, quality attributes or disease resistance traits have been identified. For example specific DNA markers have been found for cereal cyst nematode (CCN) resistance (14, 37, 46, 55, 56), the *Agropyron* region introgressed for barley yellow dwarf virus resistance (9,7) and for the granule bound starch synthase (GBSS, 17,30,33) genes. A key strategy is to convert DNA markers to fast polymerase chain reaction (PCR)-based assays, as illustrated in Figure 14 for the PCR-based detection of the three GBSS genes from wheat.

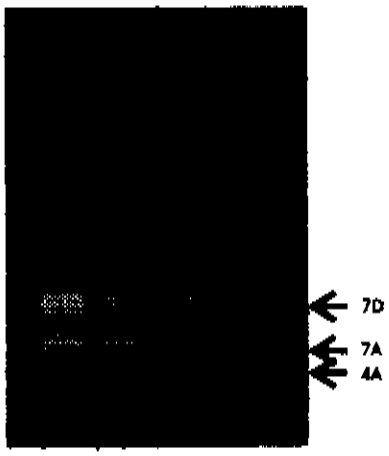


Fig. 14. The PCR-based detection of GBSS alleles. Primers designed on available DNA sequence information (30, 33) were used in PCR to amplify a polymorphic region of the GBSS gene from genomic DNA. The left lane shows the normal complement of three PCR products while the remaining lanes show the *Wx-B1* null genotype characteristic of wheat lines in the white salted noodle classification.

Two examples of the use of marker technology to assist in the breeding of new wheats in Australia are based on knowledge of the wheat granule bound starch synthase genes and the proteins encoded by these genes. It had been recognized that certain varieties grown in specific regions of Australia (principally Western Australia) were particularly well suited to the production of white salted noodles. The genetic basis for this observation was investigated and a strong correlation was drawn first between noodle quality and starch swelling power measurements (24) and secondly between starch swelling power and the absence of the GBSS gene on chromosome 4A (17). Methodologies for identifying the absence of the 4A gene at both the protein (17) and DNA levels (30) have been developed and these methods have been used to assist breeding programs to select for desired starch characteristics. In a second example, the protein and DNA analysis methods for the GBSS genes have been used to screen Australian and overseas wheats for mutations in each of the GBSS genes on chromosomes 4A, 7A and 7D. These mutations have been combined and a series of 8 genotypes containing the various possible combinations of GBSS genes produced

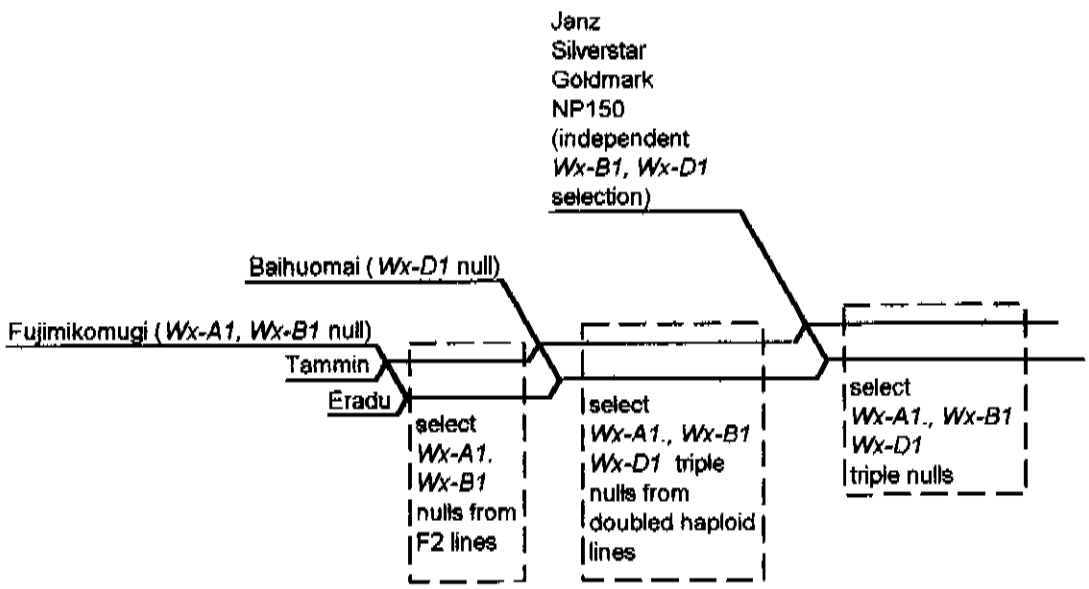


Fig. 15. Production of waxy wheats using GBSS molecular markers. The program to develop wheat lines with different combinations of *Wx* alleles is a project in the Quality Wheat Cooperative Research Centre (CRC), based at Sydney University.

(17). The development of waxy wheat cultivars is in progress based on the use of the marker technologies for GBSS (Fig. 15).

The application of DNA marker technologies into breeding programs has been supported by the GRDC through the establishment of DNA marker laboratories in each breeding program. Currently the focus is on the implementation of markers linked to specific key traits and there exists some marker assisted breeding activity to accelerate back-crossing programs (for example, see Fig.15).

Markers for wheat quality traits

The NWMMP has provided a focus for collaborative research between a wide range of research and breeding organization in Australia to establish doubled haploid populations from crosses between wheat lines of relevance to breeding programs. These populations provide the basis for detailed genetic maps that allow for the genetic analysis of key quality traits and the genes underlying those traits through QTL analysis. Six doubled haploid populations have been established for detailed studies. An important aspect of the phenotypic analyses being carried out is to assess consistent variation in the assay of complex traits, either in the laboratory or the field (spatial analysis of variation, H. Eagles, Victoria Institute of Dryland Agriculture, VIDA, Horsham, personal communication)

The molecular analysis of the populations is based on the application of RFLP, microsatellite and AFLP markers. The analysis of Australian wheats by RFLP technologies (18, 31,32) has been used to trace disease resistance genes as well as describe the genetic distance between cultivars grown in Australia. Microsatellite markers have been adapted from published literature (Fig. 16, see also 53) or developed by groups at CSIRO Plant Industry, Sydney University and Southern Cross University through an international collaboration coordinated by the company Agrogene (47). While the development of the framework molecular maps (Waite

campus of the University of Adelaide, South Australia) has been a challenging exercise, the measurement of phenotypic traits has been of equal importance to the development of linkages between traits and candidate genes. The development of improved methods for small-scale dough rheological measurements (34,35), accurate protein allele scoring (36) and starch pasting-properties (37), has provided enhanced resolution of the linkage data. Markers being developed by the program are being validated for use in breeding programs.

DNA-based variety identification

One outcome of the investment in DNA marker technology is the development of DNA based methods for variety identification (38). The rapid adoption of PBR by the cereal breeding programs and the progressive implementation of end-point royalties as a means of providing returns to breeding programs based on the adoption of specific varieties has lead to a need for objective methods of varietal identification. GRDC sponsored research has led to the development of the capacity to identify Australian wheat varieties using microsatellite markers.

DNA markers for specific physiological traits

The Australian dryland cropping environment imposes abiotic stresses on wheat production that significantly limit production and, because of the high degree of seasonal variation encountered, empirical selection for increased yield is a slow process. In response to these challenges, DNA markers closely linked to stress response genes are being targeted. Markers of interest include those linked to:

- boron tolerance,
- acid soil tolerance,
- salinity and sodicity tolerance,
- transpiration efficiency (see Fig. 12),
- improved emergence through increased coleoptile length (Fig. 16),
- increased early vigour through larger embryo, specific leaf area and large coleoptile,
- minimal expression of late maturity α-amylase.

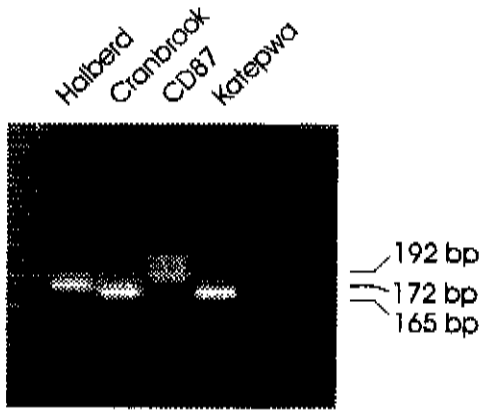


Fig. 16. The wms261 satellite screening for *Rht* alleles. The polymorphism in the wms261 microsatellite DNA has been used to map the DNA sequence and the very closely linked (0.6 cM) *Rht8* gene to chromosome 2D (53). Different alleles of the microsatellite can be assigned to different alleles of *Rht8* that vary in their adaptive significance (54). The photograph shows three alleles of wms261 in Australian wheat lines. The 192bp allele of the wms261 satellite links with the allele of *Rht8* that allows the long coleoptile phenotype to be expressed.

The use of physiological breeding, incorporating the use of DNA markers, is complementary to empirical breeding through providing a mechanism of overcoming the limitations imposed by seasonal variations in yield and G x E.

Genetically modified wheat lines using molecular biology

There are currently no commercial wheat varieties in Australia that have been developed using gene transfer technologies. The use of genetic engineering techniques is essentially a research tool that is being used in institutes such as CSIRO Plant Industry and the University of Adelaide, South Australia (Waite campus) to examine the roles of specific genes. This research includes genes determining disease resistances (rust, barley yellow dwarf, nematode) and quality traits (protein and starch composition) in wheat. As in many countries, there is currently considerable public debate concerning the future use of genetic manipulation technologies for producing new crops, particularly for the modification of the properties of staple food crops such as wheat. The use of gene technology for research purposes is administered by the Genetic Manipulation Advisory Committee (GMAC), a non-statutory body responsible for overseeing the development and use of novel genetic manipulation techniques in Australia. The Australian Government has announced the establishment of a new statutory body, the Gene Technology Office, to oversee the use of GMO technology from July 2001. Given the time that is required to select varieties suitable for release, it is unlikely that any significant areas of transgenic wheat crops will be grown in Australia before 2005.

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References

1. Campbell, W.S. (1936-1937). A historical sketch of William Farrer's work in connection with his improvements in wheats for Australian conditions. Australian Association for the Advancement of Science, Proceedings of Section G2, **13**, 525-536.
2. Australian Wheat Board Ltd. 1999. ABARE - Australian commodities forecasts and issues, **6**, 404
3. Wrigley, C.W. (1994) Developing better strategies to improve grain quality for wheat. Aust. J. Agric. Research, **45**:1- 17.
4. Wrigley, C.W., Rathjen, A. 1981. Wheat breeding in Australia. In Plants and Man in Australia (ed. D.J. and S.G.M. Carr) Academic Press, Sydney, New York, London, Toronto, San Francisco, pp 96- 135.
5. Wrigley, C.W., Shepherd, K.W. 1977. Pedigree investigation using biochemical markers: the wheat cultivar Gabo. Aust. J. Exp. Agric. Anim. Husb. **17**, 1028 – 1031.
6. Sears, E.R. 1981. Transfer of alien genetic material to wheat. In Wheat science –today and tomorrow (ed. L.T. Evans and W.J. Peacock). Cambridge University Press, Melbourne and Sydney, pp 75 – 89.
7. Sharma, H., Ohm, H., Goulart, L., Lister, R., Appels, R., Benlhabib, O. 1995. Introgression and characterisation of barley yellow dwarf virus resistance from *Agropyron intermedium* into wheat. Genome, **38**, 406 – 413
8. Banks, P.M., Larkin, P.J., Bariana, H.S., Lagudah, E.S., Appels, R., Waterhouse, P.M., Brettell, R.I.S., Chen, X., Xu, H.J., Xin, Z.Y., Qian, Y.T., Zhou, X.M., Cheng, Z.M., Zhou, G.H. 1994.

The use of cell culture for sub-chromosomal introgressions of BYD virus resistance from *Thinopyrum intermedium* to wheat. Genome, **38**, 395 - 405.

9. Larkin, P.J., Banks, P.M., Lagudah, E.S., Appels, R., Xiao, C., Xin, Z., Ohm, H.W. and McIntosh, R.A. 1995. Disomic alien addition lines in wheat representing chromosomes from *Thinopyrum intermedium* with barley yellow dwarf virus (BYDV) resistance and with rust resistance. Genome **38**, 385-394
10. Doussinault, G., Dosba, F., and Tanguy, A.M. 1981. Analyse Monosomique De La Résistance À La Rouille Jaune Du Géniteur Blé Tendre VPM1. Extrait du procés-verbal de la Séance du 14 Janvier 1981. Pp. 133-138. Cited in Cytogenetic studies in wheat. XV. Location of rust resistance genes in VPM1 and their genetic linkage with other disease resistance genes in chromosomes 2A[1]. H. S. Bariana[2] and R. A. McIntosh. Genome **36**, 476-482.
11. Lagudah, E.S., Appels, R., McNeil, D. and Schachtman, D.P. 1993. Exploiting the diploid D. genome chromatin for wheat improvement. Stadler Symposium **20**, 87 – 107.
12. Gill, B.S. and Raupp, W.J. 1987. Direct genetic transfers from *Aegilops squarrosa* L to hexaploid wheat. Crop Sci. **27**, 445 – 450.
13. Cox, T.S., Hatchett. J.H., Gill, B.S., Raupp, W.J., Sears, R.G. 1990. Agronomic performance of hexapoid wheat lines derived from direct crosses between wheat and *Aegilops squarrosa*. Plant Breed. **105**, 271 – 277.
14. Eastwood, R.F., Lagudah, E.S., and Appels, R. 1994. A directed search for DNA sequences tightly linked to cereal cyst nematode (CCN) resistance genes in *Triticum tauschii*. Genome **37**, 311 – 319.
15. Lawrence, G.J. 1986. The high-molecular-weight glutenin subunit composition of Australian wheat cultivars. Aust. J. Agric. Res. **37**, 125-133.
16. May, C.E. and Appels, R. 1992. The nucleolus organizer regions (*Nor* loci) of hexaploid wheat cultivars. Aust. J. Agric. Res. **43**, 889-906.
17. Zhao, X.C., Batey, I.L., Sharp, P.J., Crosbie, G., Barclay, I., Wilson, R., Morell, M.K., Appels, R. 1998. A single genetic locus associated with starch granule properties and noodle quality in wheat. J. Cer. Sci. **27**, 7-13.
18. Paull, J.G., Chalmers, K.J., Karakousis, A., Kretshmer, J.M., Manning, S., Langridge, P. 1998. Genetic diversity in Australian wheat varieties and breeding material based on RFLP data. Theor. Appl. Gen. **96**, 435 – 446.
19. International Triticeae Mapping Initiative (ITMI). Proceedings of workshops in 1995 and 1996. Report #18 (ed P.E. McGuire and C.O.Qualset). Genetic Resources Conservation program, Division of Agriculture and Natural Resources, University of California, USA. pp 1 – 195.
20. Boyko, E.V., Gill, K.S., Mickelson-Young, L., Nasuda, S., Raupp, W.J., Ziegle, J.N., Singh, S., Hassawi, D.S., Fritz, A.K., Namuth, D., Lapitan, N.L.V., Gill, B.S. 1999. A high-density genetic linkage map of *Aegilops tauschii*, the D-genome progenitor of bread wheat. Theor. Appl. Genet. **99**, 16 – 26.
21. Shewry, P.R., Miles, M.J., Tatham, A.S. 1994. The prolamin storage proteins of wheat and related cereals. Prog. Biophys Mol. Biol. **61**, 37-59.
22. Payne, P., Corfield, K.G., Holt, L.M., Blackman, J.A. 1981. Correlations between the inheritance of certain high-molecular-weigt subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. J. Sci. Food Agric. **32**, 51-60.
23. National Cereal Rust Control Program 1998. Circular #38, compiled by H.S. Bariana and J.A. Bell, Plant Breeding Institute, Sydney University, Cobbitty, NSW Australia.
24. Crosbie, G.B. 1991. The relationship between starch swelling properties, paste viscosity and boiled noodle quality in wheat flours. J Cer. Sci. **13**, 145-150.
25. Miura, H., Tanii, S. Endosperm starch properties in several wheat cultivars preferred for Japanese noodles. Euphytica **72**, 171-175.
26. Hall, AE., Richards, RA., Condon, AG., Wright, GC., Farguhar, GD. 1995. Carbon isotope discrimination and plant breeding. Plant Breeding Review, , 81-113.
27. Richards, RA. 1996. Defining selection criteria to improve yield under drought. Plant Growth Regulation **20**, 157 – 166.
28. Rebetzke, GJ., Richards, RA. 2000. Gibberellic acid-sensitive dwarfing genes reduce plant height to increase kernel number and grain yield of wheat. Aus. J. Ag. Res, **51**, in press
29. Heenan, DP, McGhie, WJ, Collins, D. 1998. Impact of lupins, grazed or ungrazed subterranean clover, stubble retention, and lime on soil nitrogen supply and wheat nitrogen uptake, grain yields, and grain protein. Aust. J. of Agr. Res. **49**, 487-494,

30. Briney, A; Wilson, R; Potter, RH; Barclay, I; Crosbie, G; Appels, R; Jones, MGK. 1998. A PCR-based marker for selection of starch and potential noodle quality in wheat. Molecular Breeding, 4, 427-433.
31. Witrzens, B., Brettell, RIS., Murray, FR., McElroy, D., Li, Z., Dennis, ES. 1998. Comparison of three selectable marker genes for transformation of wheat by microprojectile bombardment. Aust. J. Plant Physiol., **25**, 39-44.
32. Shariflou, M., Sharp, P. 1999. A polymorphic microsatellite I the 3' end of the 'waxy genes of wheat, *Triticum aestivum*. 1999. Plant Breeding **118**, 275-277.
33. Yan, L., Bhave, M., Fairclough, R., Konik, C., Rahman, S., Appels, R. 1999. The genes encoding granule-bound starch synthase (GBSS) from the A, B and D progenitors of common wheat. Genome, in press
34. Gras, PW., Appels, R., Békés, F. 2000. Gluten protein functionality in wheat flour processing. J. Cereal Sci. In press.
35. Gras PW., Carpenter, HC., Anderson, R. 2000. Modelling the developmental rheology of wheat flour dough using extension tests. J. Cereal Sci., in press.
36. Cornish , G.B., Wrigley, C.W., Rathjen, A.J. and Hallamby, G.J. South Australian Wheat Families Cereals 1995. Proceedings 45th Australian Cereal Chemistry Conference, Pp. 104-107 Y. Williams and C.W Wrigley (eds) Melbourne: Royal Australian Chemical Institute.
Cornish, G.B., Lambe, W.J. and Wrigley, C.W. Western Australian Wheat Protein Families In: Cereals 1997. Proc. 47th Australian Cereal Chem. Conf. Pp. 217-224. Tarr, A.W., Ross, A.S., and Wrigley, C.W. (eds) Royal Australian Chem. Insit., Melbourne.
Cornish, G.B., Panozzo, J.F. and Wrigley, C.W. Victorian Wheat Protein Families In: Cereals 1998. Proc. 48th Australian Cereal Chem. Conf. Pp. 183-188. L.O'Brien, A.B. Blakeney, A.S. Ross and C.W. Wrigley)eds) Royal Aust. Chem. Inst., Melbourne.
Cornish, G.B., Griffin, W.B., and Wrigley, C.W. Glutenin alleles and their influences on the quality of New Zealand Wheats In: Cereals 1999. Proc. 49th Australian Cereal Chem. Conf. (in press) Royal Aust. Chem. Inst., Melbourne.
37. Williams, KJ; Fisher, JM; Langridge, P. 1996. Development of a PCR-based allele-specific assay from an RFLP probe linked to resistance to cereal cyst nematode in wheat. Genome, **39**, 798-801
38. Morell, M.K., Peakall, R., Appels, R., Preston, L.R., Lloyd, H.L. 1995. DNA profiling techniques for plant variety identification. Aust. J. Exp. Agriculture **35**, 807 - 819.
39. Mujeeb-kazi, A., USA, Wheat Program, CIMMYT. Http://198.93.240.203/index.htm
40. IWIS Database, Fox, P.N., Magaña, R.I., Lopez, C., Sanchez, H., Herrera, R., Vicarte, V., White, J.W., Skovmand, B and Mackay, M.C. 1997 International Wheat Information System (IWIS) Verison 2. Mexico, D.F., CIMMYT.
41 Eastwood, R.F., Lagudah, E.S., Appels, R., Hannah, M. ,Kollmorgen, J.F. 1991. *Triticum tauschii*: a novel source of resistance to cereal cyst nematode (*Heterodera avenae*). Aust. J. Agric. Res. **42**, 67-77.
42 Thompson, JP; Haak, MI. 1997. Resistance to root-lesion nematode (*Pratylenchus thornei*) in *Aegilops tauschii* Coss., the D-genome donor to wheat. Australian Journal of Agriculture Research, **48**, 553-559
43 Lazenby, A., Bartholomaeus, M., Boucher, B., Boyd, W.R., Campbell, A., Cracknell, R., Eagles, H., Lee, J., Lukey, G., Marshall, B. 1994. Trails and errors: a review of variety testing and release procedures in the Australian grains industry. Grains Research and Development Corporation (GRDC), Canberra.
44 Murphy, N. 1998. PhD thesis, Murdoch University, Western Australia
45 Kansas University, Wheat Germplasm Centre, www.ksu.edu/wgrc/
46 Barr, AR; Chalmers, KJ; Karakousis, A; Kretschmer, JM; Manning, S; Lance, RCM; Lewis, J; Jeffries, SP; Langridge, P. 1998. RFLP mapping of a new cereal cyst nematode resistance locus in barley. Plant Breeding, **117**, 185-187.
47 Peter Isaac email contact: pete.i@agrogene.com.
48 Latta, RA; Carter, ED. 1998. Increasing production of an annual medic-wheat rotation by grazing and grass removal with herbicides in the Victorian Mallee. Aust. J. of Exp. Agric. **38**, 211-217.
49 Jones, R; Medd, R. 1997. Economic analysis of integrated management of wild oats involving fallow, herbicide and crop rotational options. Aust. J. of Exp. Agric. **37**, 683-691.
50 Dhaliwal, AS., MacRitchie, F. 1990. Contributions of protein fractions to dough handling properties of wheat-rye translocation cultivars. J. Cereal Sci. **12**, 113-122.
51 Boggini, G; Tusa, P; Di Sivestro, S; Pogna, NE. 1998. Agronomical and quality characteristics of durum wheat lines containing the 1BL/1RS translocation. J. Genet. Breeding, **52**, 167-172, 1998

52 Williamson, PM. 1997. Black point of wheat: In vitro production of symptoms, enzymes involved, and association with *Alternaria alternata*. Aust. J. of Agric. Res., **48**, 13-19.
53 Korzun, V., Roder, MS., Ganal, MW., Worland, AJ., Law, CN. 1998. Genetic analysis of the dwarfing gene *Rht8* in wheat. Part I. Molecular mapping of *Rht8* on the short arm of chromosome 2D of bread wheat (*Triticum aesitum* L). Theor. Appl. Genet. **96**, 1104-1109.
54 Worland, AJ., Korzun, V., Roder, MS., Ganal, MW., Law, CN. 1998. Genetic analysis of the dwarfing gene *Rht8* in wheat. Part II. The distribution and adaptive significance of allelic variants at the *Rht8* locus of wheat as revealed by microsatellite screening. Theor. Appl. Genet. **96**, 1110-1120.
55 Taylor, C; Shepherd, KW; Langridge, P. 1998. A molecular genetic map of the long arm of chromosome 6R of rye incorporating the cereal cyst nematode resistance gene, *CreR*. Theor. Appl. Genet. **97**, 1000-1012.
56 Lagudah, ES; Moullet, O; Appels, R. 1997. Map-based cloning of a gene sequence encoding a nucleotide-binding domain and a leucine-rich region at the *Cre3* nematode resistance locus of wheat. Genome, **40**, 659-665.
57 Macindoe, SL., Walkden-Brown, C. 1968. Wheat breeding and varieties in Australia. Science bulletin #76, New South Wales Dept. of Agriculture, pp 1 – 254.
58 Murphy, NE., Loughman, R., Appels, R., Lagudah, ES., Jones, MGK. 1999. Genetic variability in a collection of *Stagonospora nodorum* isolates from Western Australia. Aust. J. Agric. Res. **50**, in press.
59 McMillan, JRA. 1933. Varieties of wheat in Australia. Council for Scientific and Industrial Research Bulletin #72.
60 Condon, AG., Richards, RA., Farquhar, GD. 1993. Relationships between carbon isotope discrimination, water use efficiency and transpiration efficiency for dryland wheat. Aust. J. Agric. Res. **44**, 1693 – 1711.
61 Perry, MW. 1992. Cereal and fallow/pasture systems in Australia. In Ecosystems of the World: Field Crop Ecosystems ed. CL. Pearson, Elsevier, Amsterdam, pp 451 – 483.
62 Crop report. 1998/99. Australian Wheat Board Ltd. pp 1 – 28.
63 Watson, IA., Luig, NH. 1961. Leaf rust on wheat in Australia: a systematic scheme for the classification of strains. Proc. Linean Society of NSW. **86**, 241-250.
64 Davidson, BR. 1985. The history of agriculture in temperate Australia. J. Aust. Inst. Agric. Sci. **51**, 159 – 168.
65 Donald, CM. 1982. Innovation in Australian agriculture. In Agriculture in the Australian Economy (ed DB. Williams), Sydney University Press, Sydney, Australia, pp 55-82.

Overview of Program 1

Dr Lindsay O'Brien

NEW WHEATS AND BREEDING AIDS

AIMS:

TO USE NEW NATURAL SOURCES OF GENETIC VARIATION THAT WILL IMPROVE EXISTING PRODUCTS AND PROCESSES AND LEAD TO THE DEVELOPMENT OF NEW PRODUCTS AND PROCESSES

NEW WHEATS AND BREEDING AIDS

METHODOLOGY:

IDENTIFY NEW, NATURAL, NOVEL SOURCES OF GENETIC VARIATION

DEVELOP MARKERS FOR THE RAPID INTROGRESSION OF THESE GENES INTO ADAPTED BREEDING LINES

APPLY THE MARKERS TO DEVELOP ADVANCED BREEDING LINES

RELEASE NEW WHEATS WITH THESE GENES FOR USE BY INDUSTRY

NEW WHEATS AND BREEDING AIDS

FOUR SUB-PROGRAMS TO DELIVER THESE OBJECTIVES.

1. **EXPANDING THE DIVERSITY OF WHEAT QUALITY**

2 **MOLECULAR MARKERS FOR WHEAT QUALITY**

2. **EFFICIENT BREEDING TECHNOLOGIES**

4. **NEW SOFT WHEATS AND NOVEL BREEDING LINES**

Improving the Quality of Wheat Starch

Dr Fred Stoddard

Improving the quality of wheat starch

Fred Stoddard

The University of Sydney
and
Quality Wheat CRC Ltd

Australia

- Starch requirements for end uses
  - Amylose
  - B granules
- Germplasm surveys
- Detailed genetic analysis of B-granule content
- Future genetic investigations & germplasm improvement

Starch

- α-1,4 linked glucose chains joined by α-1,6 branches
- represents 50-70% by weight of cereal grains
- represents 30-60% by energy value of human diets
- critically important for many industrial uses, e.g.,
  - carbon-free paper & other paper coatings
  - glues
  - sizing of textiles & papers
  - carrier for pharmaceuticals

Starch quality

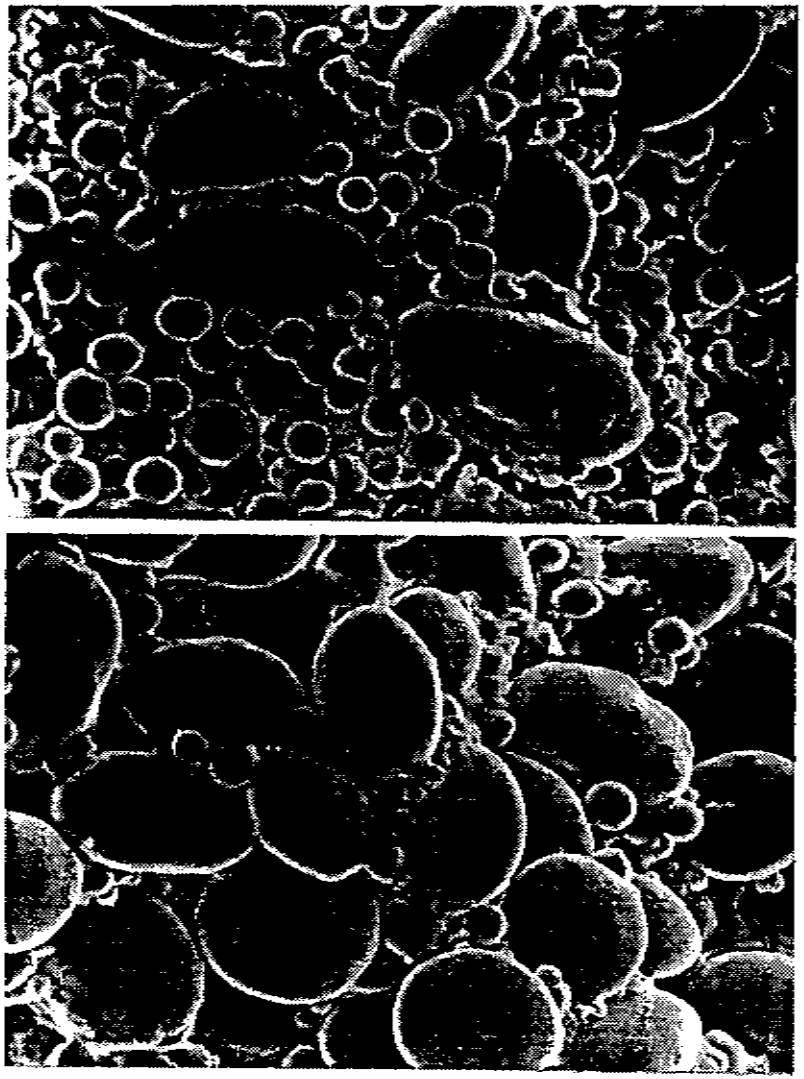
determined both chemically and physically

Amylose	Amylopectin
Little branching (<5)	Much α-1,6 branching
Long chains (~200)	Shorter chains (~25)
Lower MW (700 K)	High MW (to 100 M)
Forms firm, opaque gel	Softer, translucent paste

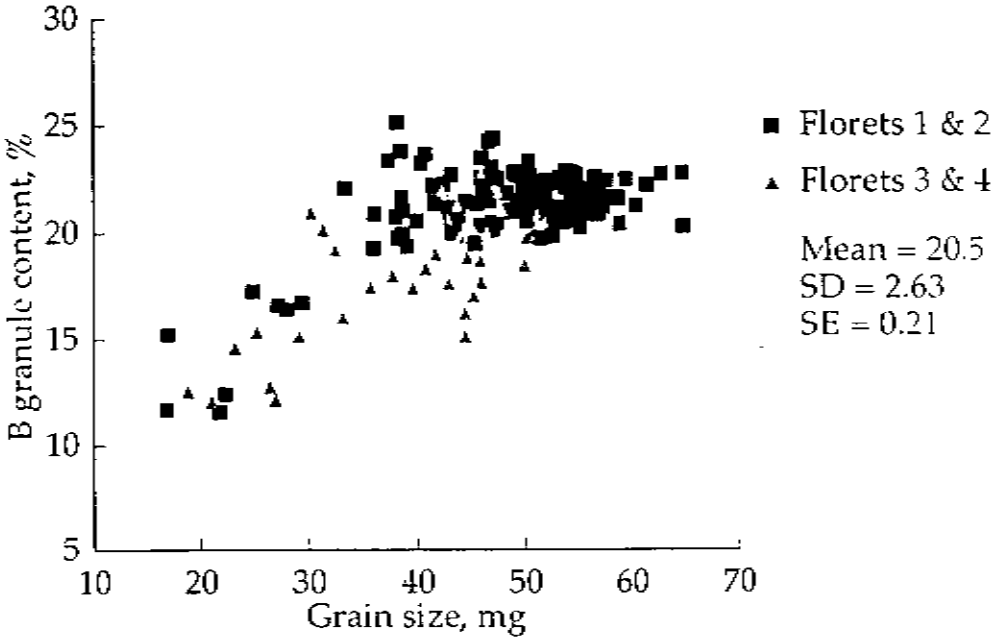
A granules	B granules
50-80% of starch mass	>90% of particles
One per amyloplast	Many
Initiated ~5 d after anthesis	Buds on edge of mature A granules
Lenticular, ~25 μm diam.	Spherical, ~5 μm diameter

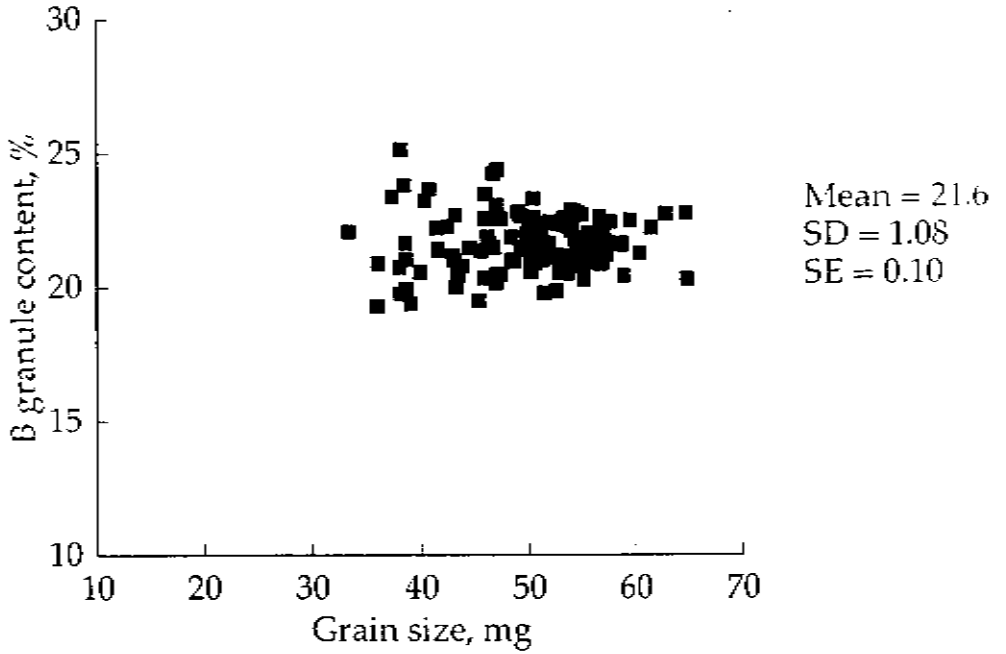
Importance of granule size

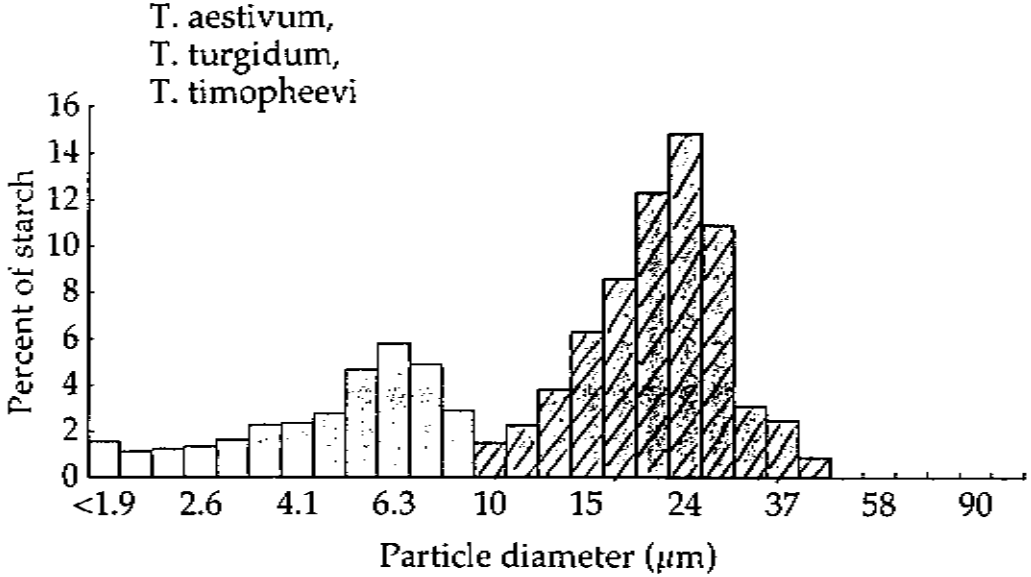
- Different industrial uses for specific sizes - 1, 5, 10, 25 μm
- Small granules precipitate from suspension more slowly than large ones, are wasted in a wet process such as starch/gluten manufacture
- Small granules have higher surface:volume ratio
  - increase water absorption
  - increase flour swelling volume
  - increase baking time
  - conflicting results on loaf height

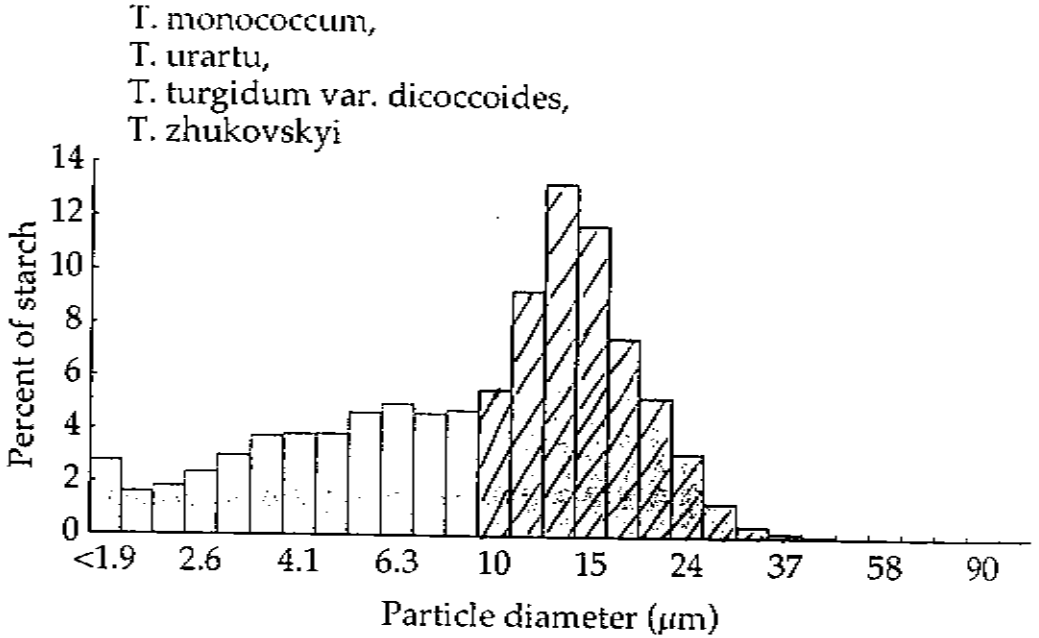


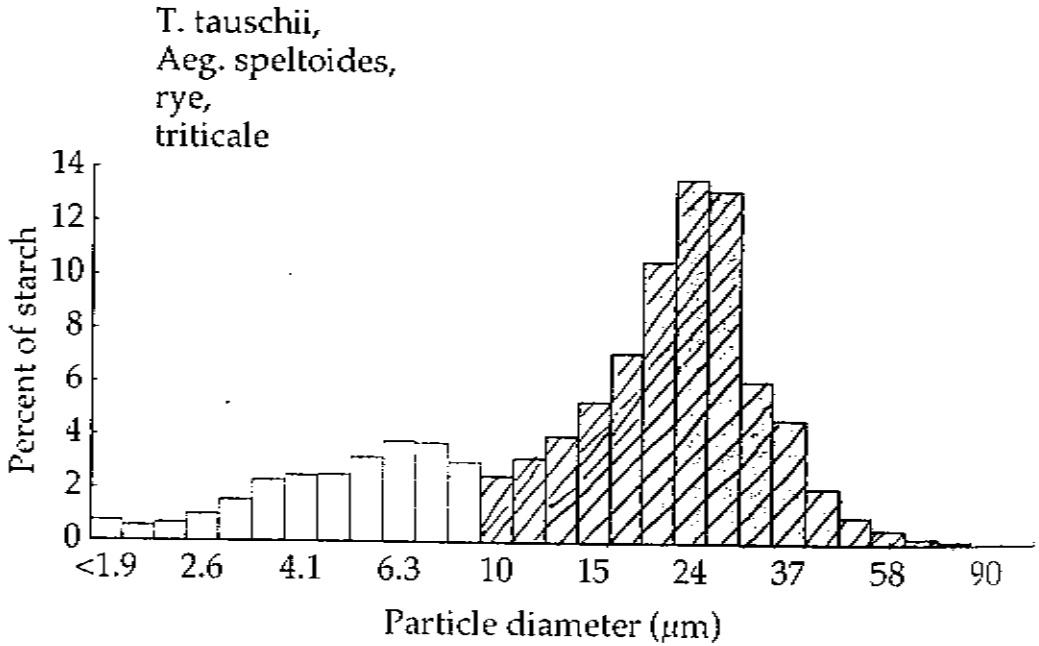
Electron micrographs of a split mature grain of wheat, showing large (A) granules and small (B) granules

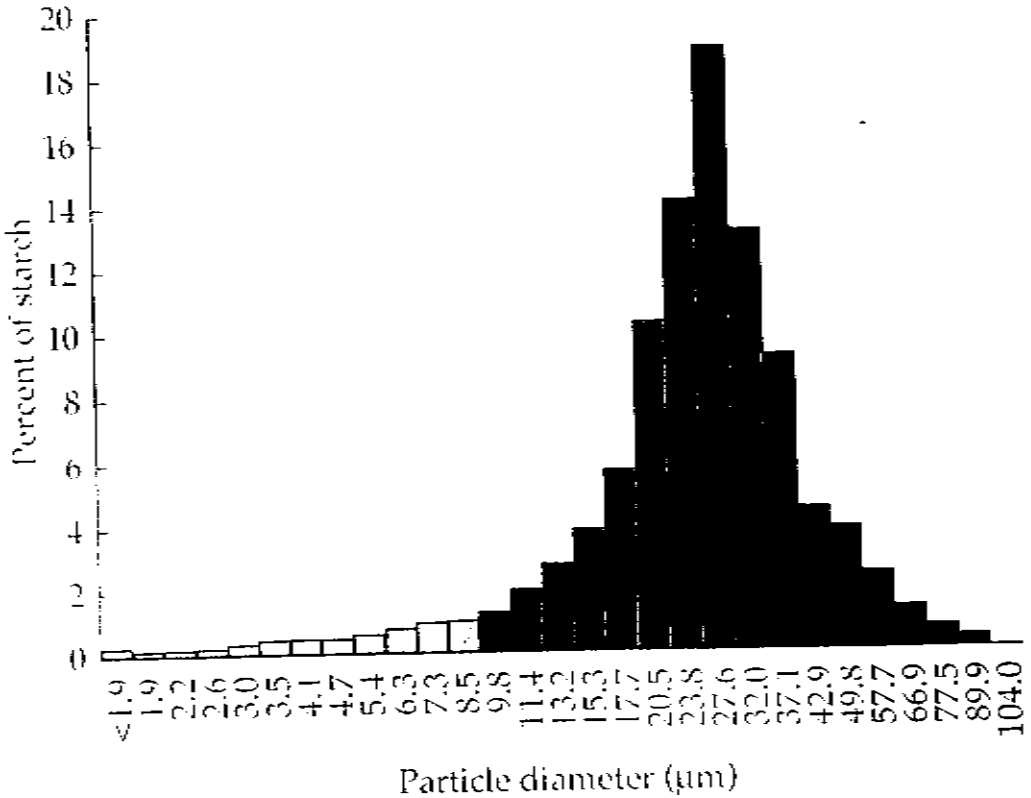





T. aestivum,
T. turgidum,
T. timopheevi
Percent of starch
0
2
4
6
8
10
12
14
16
<1.9
2.6
4.1
6.3
10
15
24
37
58
90
Particle diameter (μm)


T. monococcum,
T. urartu,
T. turgidum var. dicoccoides,
T. zhukovskyi
Percent of starch
0
2
4
6
8
10
12
14
<1.9
2.6
4.1
6.3
10
15
24
37
58
90
Particle diameter (μm)


T. tauschii,
Aeg. speltoides,
rye,
triticale
Percent of starch
0
2
4
6
8
10
12
14
<1.9
2.6
4.1
6.3
10
15
24
37
58
90
Particle diameter (μm)



Survey of available variation

Over 800 lines covering over 20 species

Domesticated species	B granule content
• Breadmaking wheats	17 - 50%
• Durum-type wheats	17 - 50%
• Ryes	20 - 40%
Ancestral wheats	
• T. monococcum types	23 - 60%
• T. speltoides types	10 - 20%
• T. tauschii	15 - 40%
Wild relatives	4 - 30%

Those with 4 - 8% have no 'peak' i.e. it appears that these 'B-granules' are the tail of the A-granule distribution.

Domesticating wild species

Conventional wheat (cv Kewell) x *Aegilops crassa*

B-granule content of F1 13%, between parental values, although grains poorly filled

now up to third back-cross

Aegilops crassa x *Triticum urartu*

F1 hybrid grains develop well, potential for embryo rescue

potential "bridging" cross with enough similarity to conventional wheat

Triticum turgidum x *Aegilops tauschii*

Resynthesise common wheat

Synthetics have higher B-granule contents than either parent

Potential to develop new wheats with small A granules, more B granules

Genetics of B-granule content in conventional or durum wheats

Choose parents

Cross P1 x P2 => F1 and P2 x P1 => RF1

Grow P1, P2, F1, RF1 in controlled environment (18°/13°, well fed and watered)

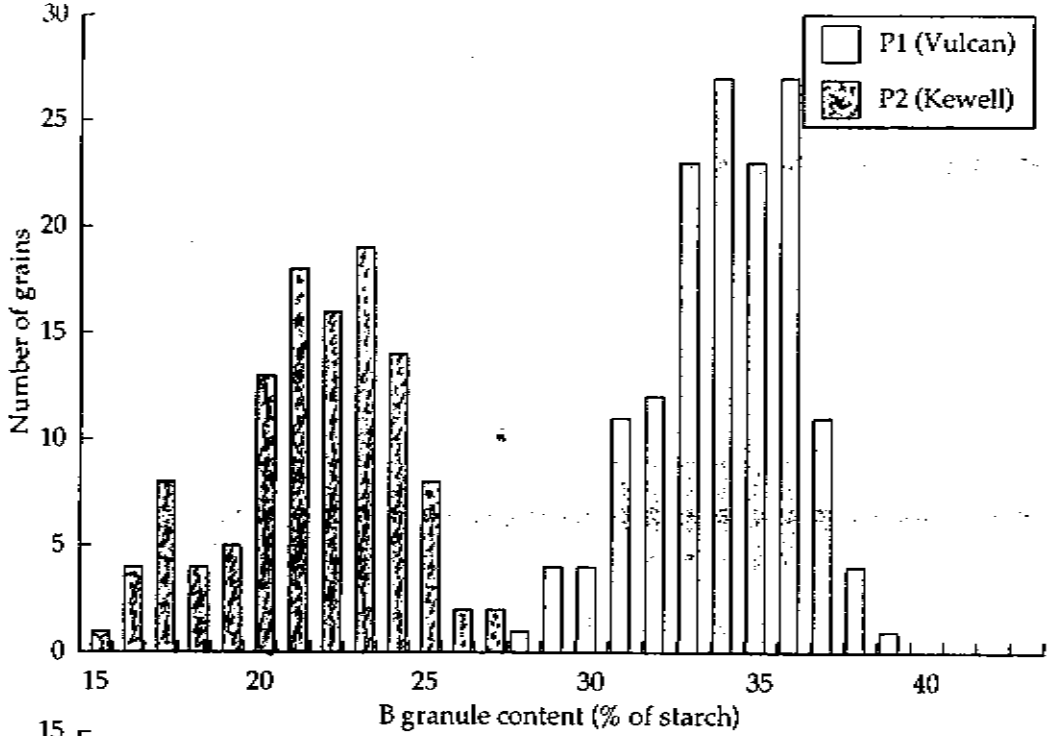
Prepare fresh F1 and RF1

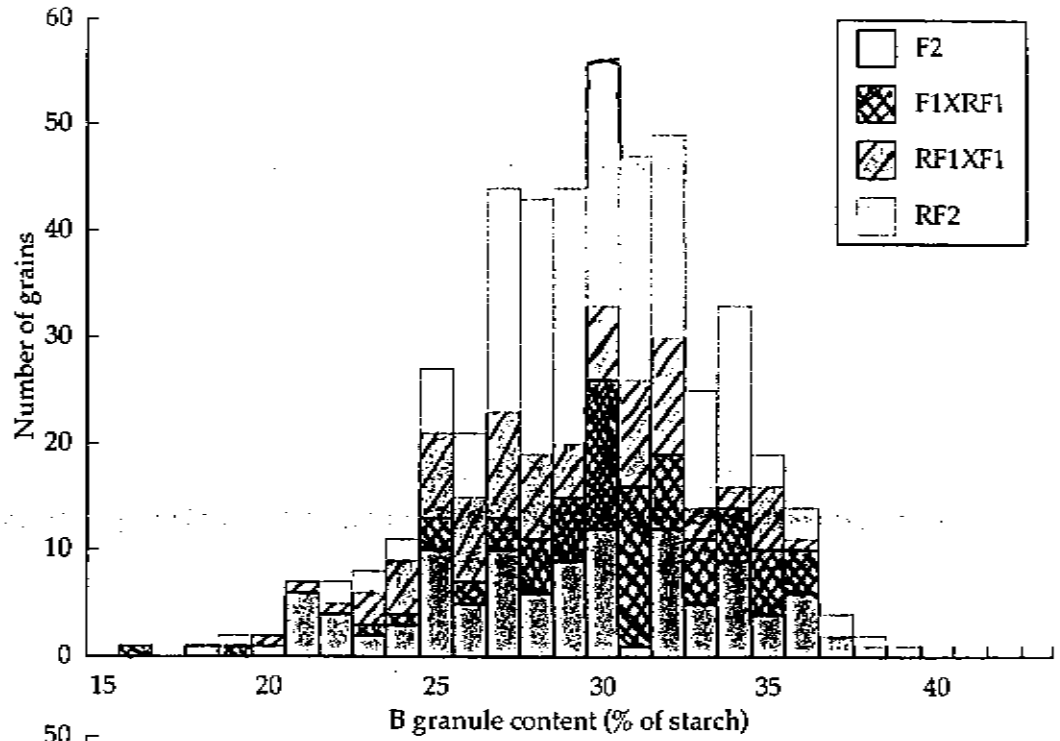
Prepare F1 x P1, RF1 x P1, P1 x F1, P1 x RF1 : B1

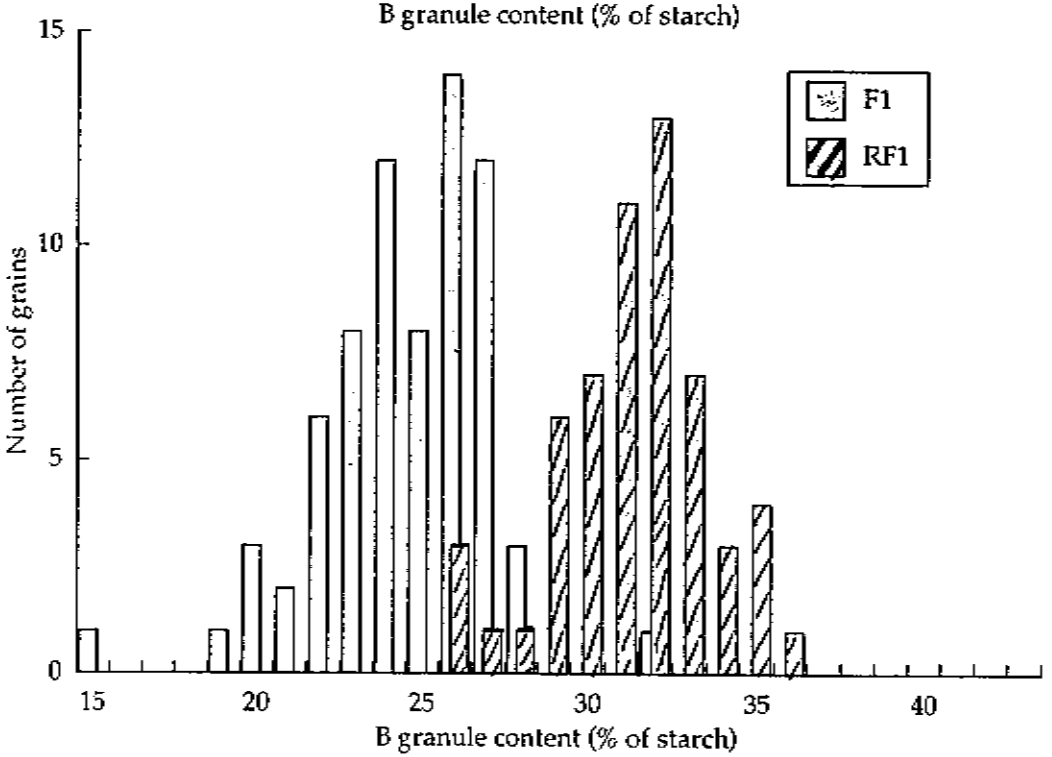
Prepare F1 x P2, RF1 x P2, P2 x F1, P2 x RF1 : B2

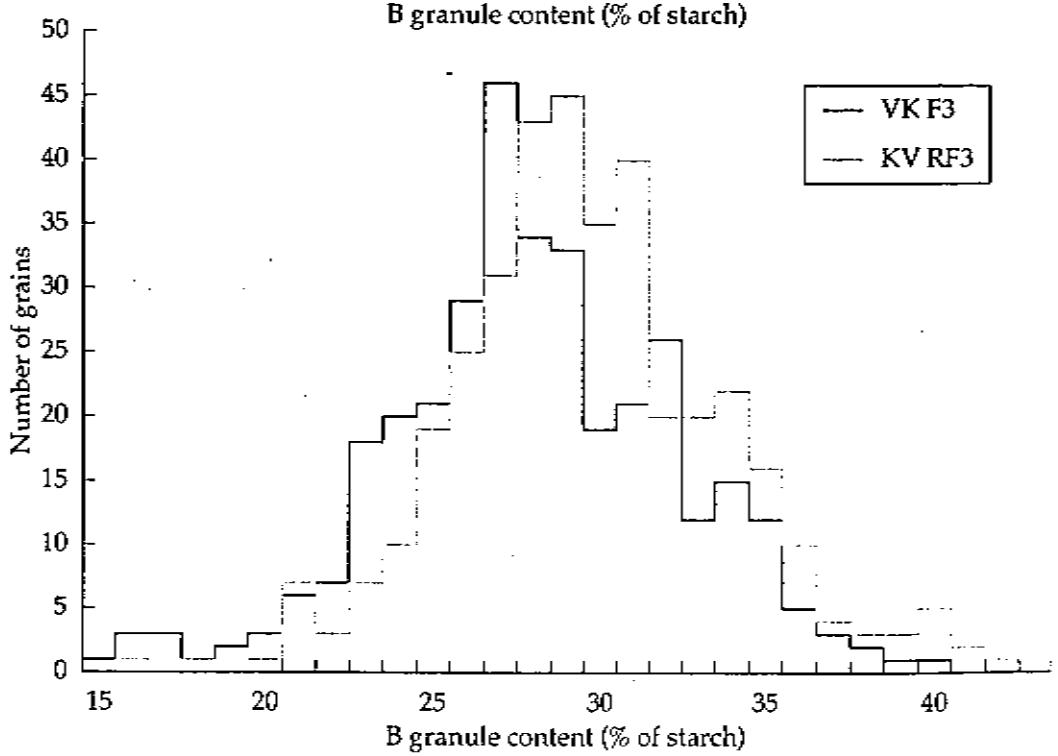
Determine B-granule content in 50-200 mature grains of P1, P2, F1, RF1, F2, RF2, B1 and B2

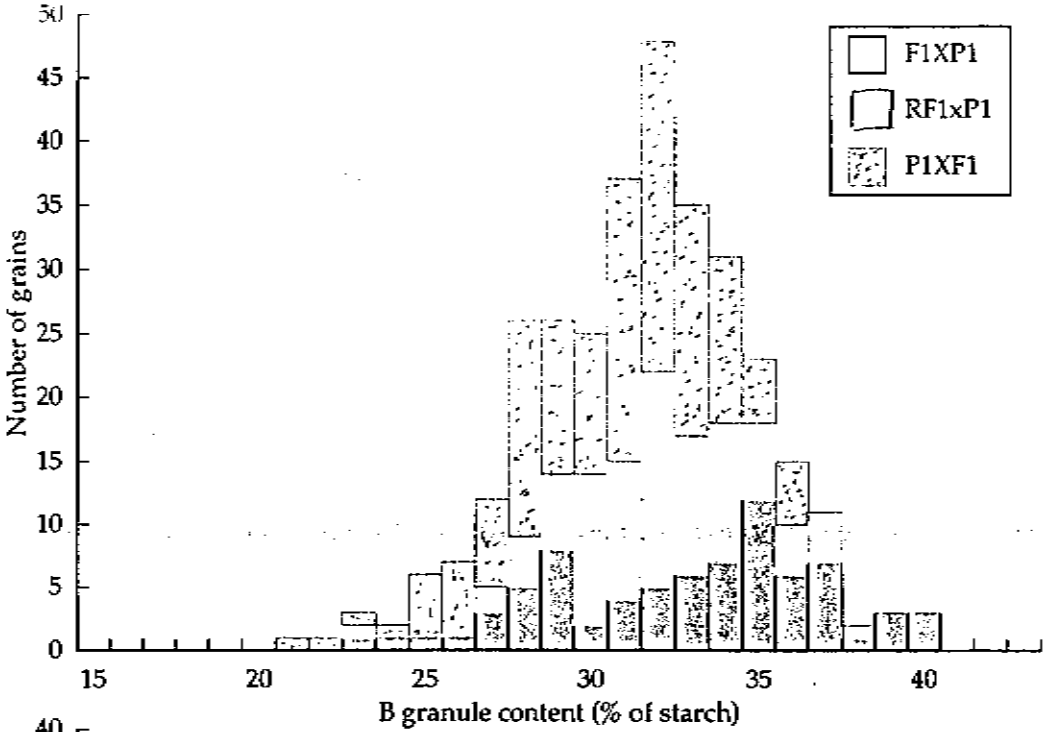
Analyse results

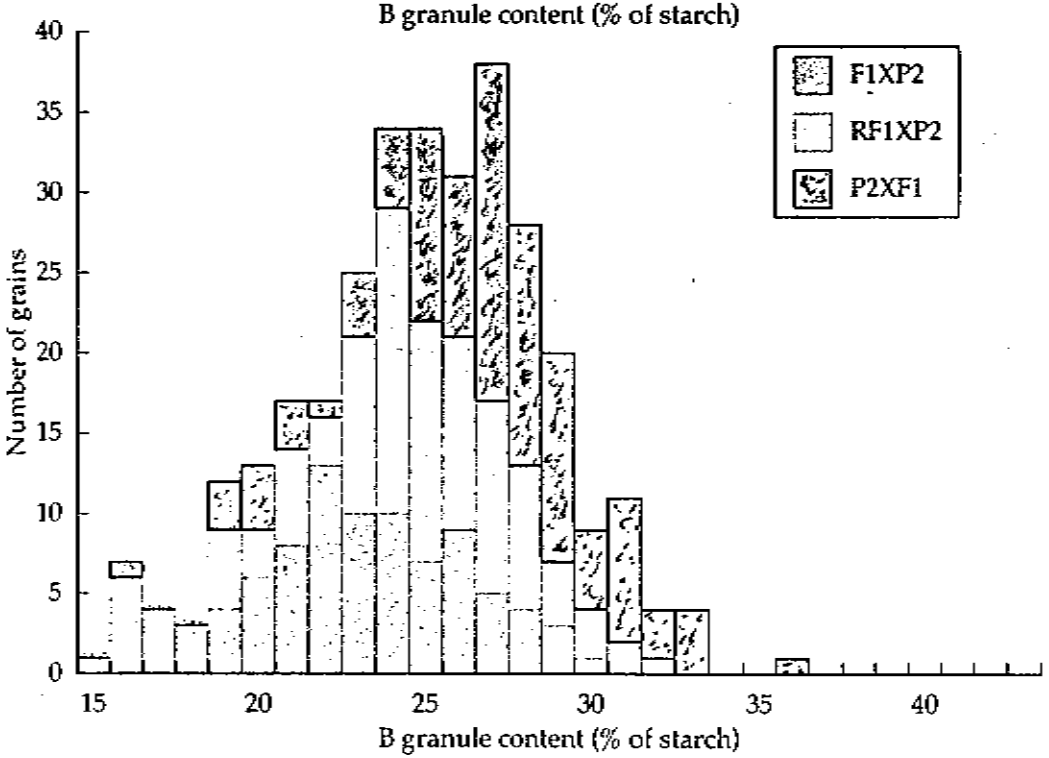

P1 (Vulcan)
P2 (Kewell)
Number of grains
B granule content (% of starch)


F2
F1XRF1
RF1XF1
RF2
Number of grains
B granule content (% of starch)


F1
RF1
Number of grains
B granule content (% of starch)

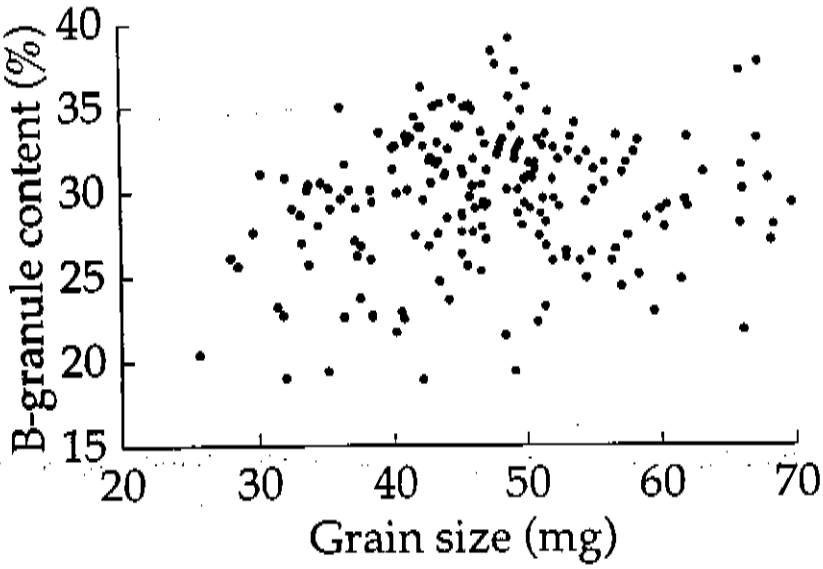

VK F3
KV RF3
Number of grains
B granule content (% of starch)

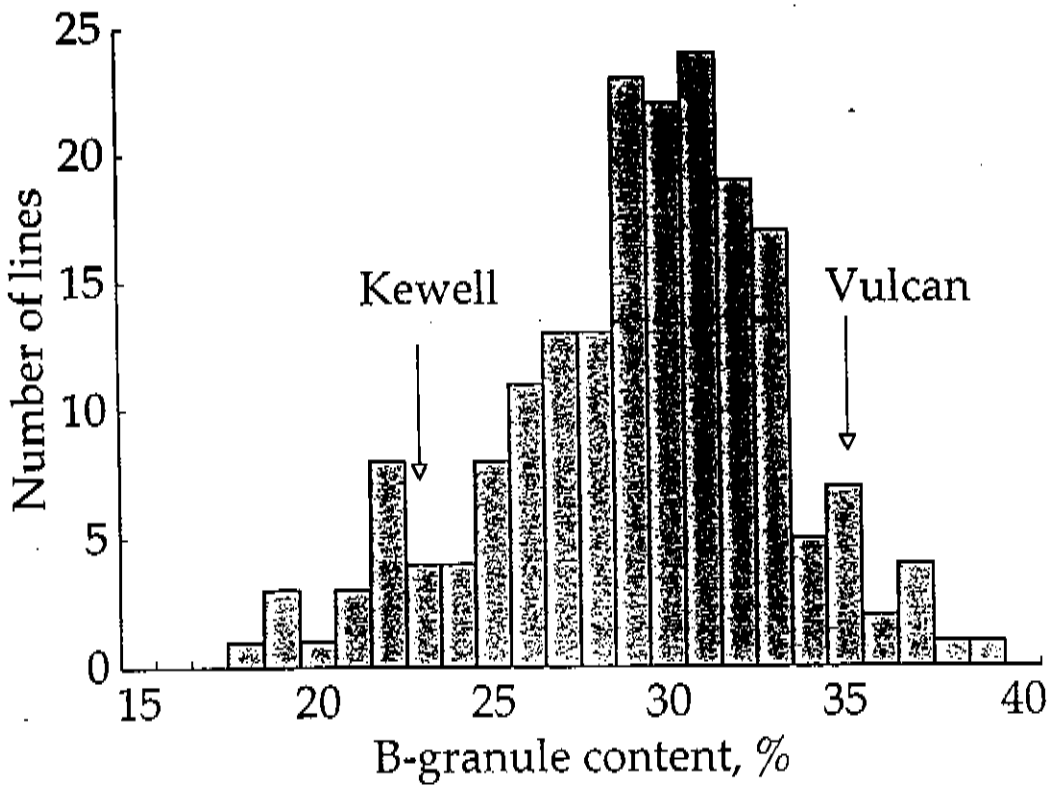

F1XP1
RF1xP1
P1XF1
Number of grains
B granule content (% of starch)


F1XP2
RF1XP2
P2XF1
Number of grains
B granule content (% of starch)

Genetic analysis of B-granule content in Vulcan (33%) x Kewell (20%)

Term		Value	SE
Mid-parent value	m	29.81	0.31
Effect of crossing		-0.45	0.21
Additive genetic effect	a	6.21	0.15
Dominance genetic effect (BBb genotype)	d1	-6.24	1.12
Dominance genetic effect (Bbb genotype)	d2		
Epistasis effects	aa	-3.55	0.35
	ad1	2.89	0.74
	ad2	-3.92	0.94
	d1d1	-2.74	1.02
	d2d2	1.58	0.68


B-granule content (%)
40
35
30
25
20
15
20
30
40
50
60
70
Grain size (mg)


Number of lines
25
20
15
10
5
0
Kewell
Vulcan
15
20
25
30
35
40
B-granule content, %

Doubled haploid population for further genetic analysis

450 lines of Vulcan x Kewell

2 x 200 grown in controlled environment chamber, starch analysed

Top 15 & bottom 15 from each 200 selected

Growing in 3 replicates in controlled environment chamber

Top 12 & bottom 12 to be chosen for further work

Top 10 & bottom 10 from first 200

AFLP analysis in bulked segregants

20 potential molecular markers

to be confirmed in larger population

Domesticating "Outlier 67"

Soft, club, winter, long season, 5+10, ca. 10% B granules

Crossed with

Janz (wide adaptation, hard, ~~high~~ low starch paste viscosity)

Sunstate (hard, ~~low~~ high starch paste viscosity, different gene pool)

QAL 2000 (prime soft quality biscuit wheat)

F1 hybrid seeds mature, ready to plant 1 December

Large F2 populations to eliminate individuals requiring chilling & long days

Initial screen for B-granule content, backcross to adapted parents

Soft, low-B, 5+10, high paste viscosity : starch-gluten

Soft, low-B, 2+12, ??? : potential for improved biscuit wheat

Conclusions

Survey has shown sources of higher & lower B-granule content

Genetic studies have demonstrated inheritance of the trait

Marker studies are under way to help us

- find new variants in the trait
- follow the trait in breeding populations
- identify the biochemical mechanisms behind the trait

Breeding is under way to capitalise on these developments

Yellow Colour of Asian Alkaline Noodles

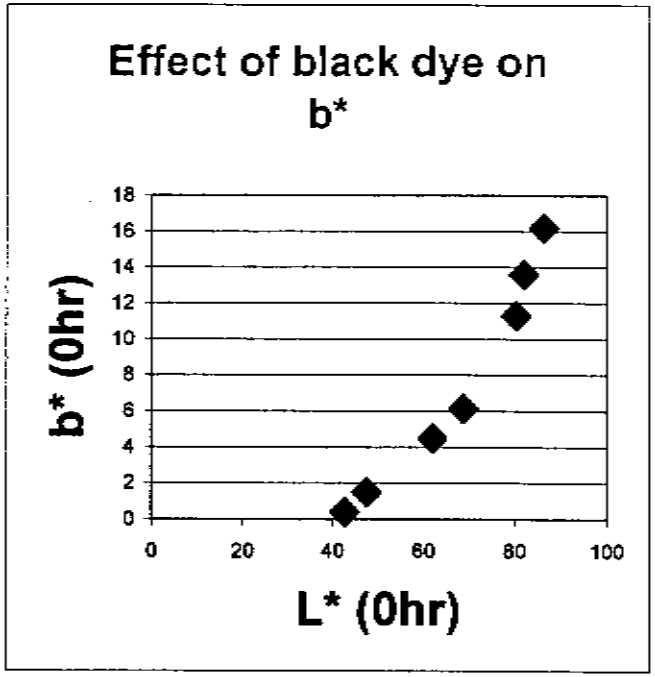
Dr Daryl Mares

COMPONENTS OF ALKALINE NOODLE COLOUR

- **Initial brightness: L***
- **Speckiness (bran contamination)**
- **Rate of darkening with time (PPO and other oxidases): ΔL***
- **Yellow colour: b***
  a) xanthophylls
  b) flavonoids

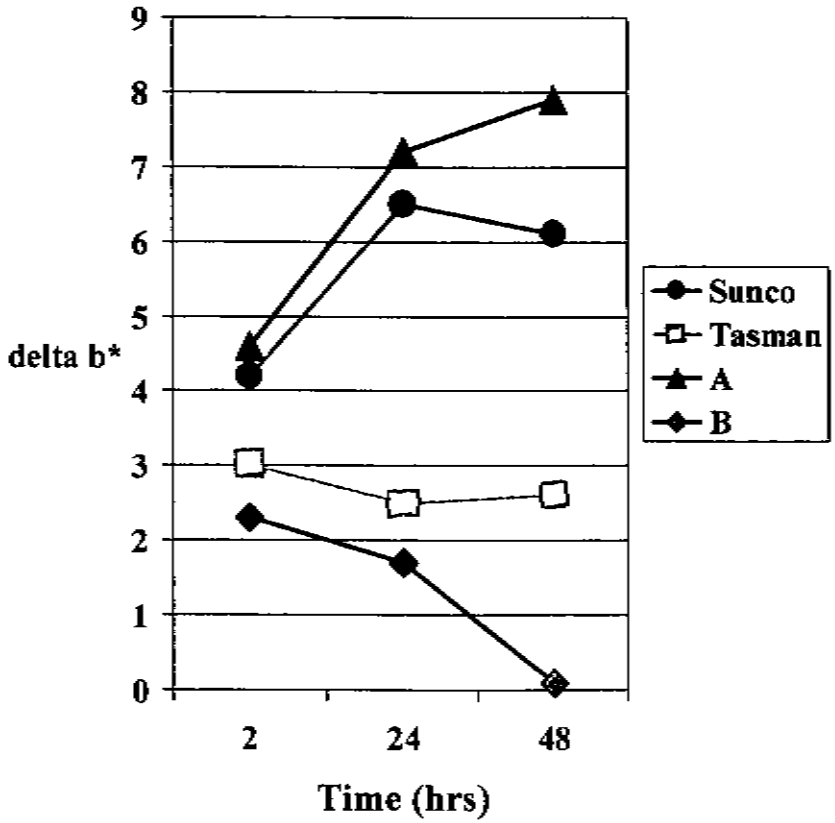
Colour of Asian Noodles: Aims

- **Components of colour and colour stability**
- **Biochemistry and genetics of grain/flour constituents involved in colour**
- **Chemical structure/enzymes/ tissue location**
- **Extent of genetic variation**
- **Efficient screening techniques**
- **Role of the environment and the milling process**



Variation in Δb*

(Change in yellowness)



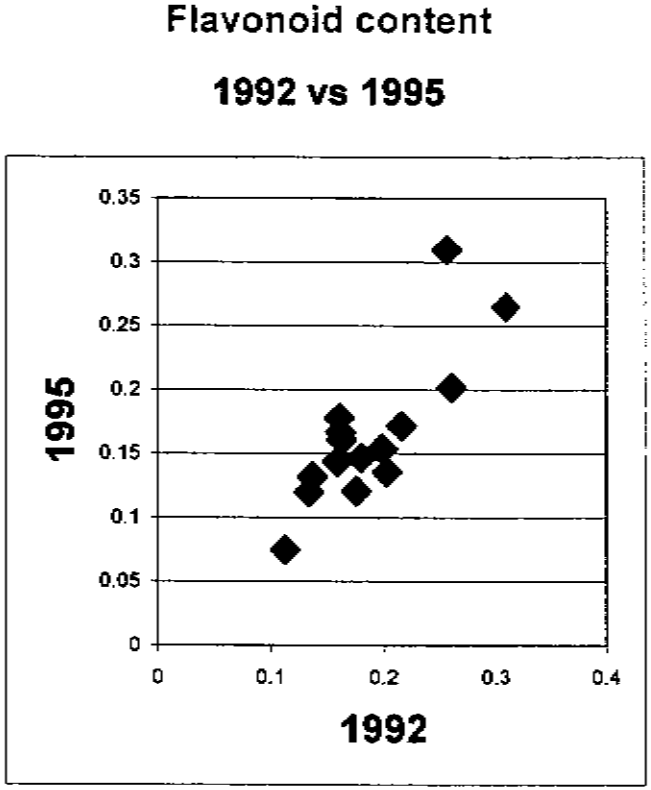
Colour of Asian Noodles: Xanthophylls

- **Genetic variation**
- **Changes during grain development and in noodles**
- **Optimum levels**
- **High levels recombined with low PPO**
- **Stability**

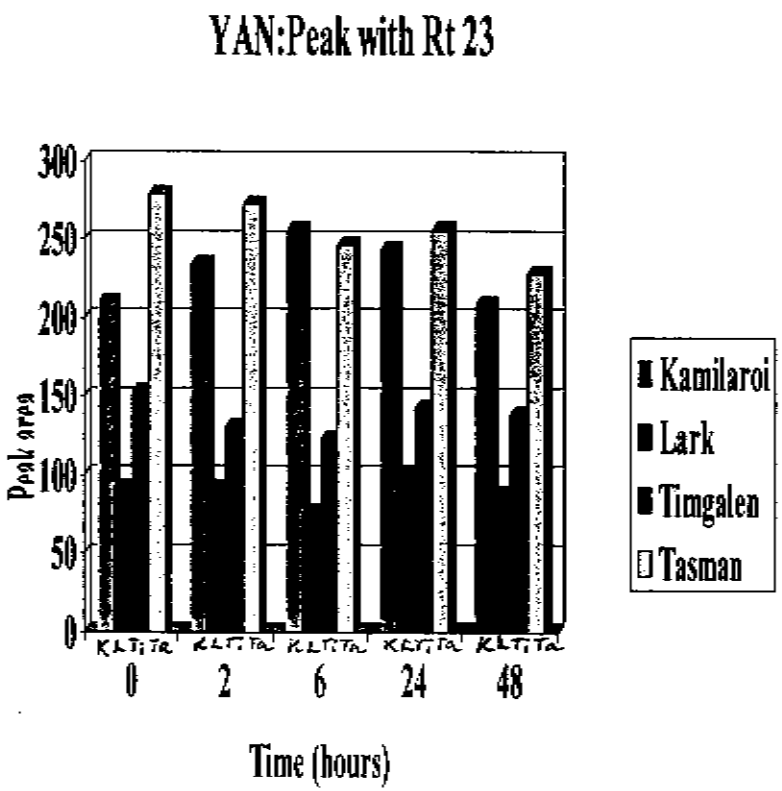
Colour of Asian Noodles: Flavonoids

- pH dependence
- Chemical structure and tissue location
- Stability in noodles
- Genetic variation - bread wheats, durums, T.tauschii, synthetics
- Screening techniques

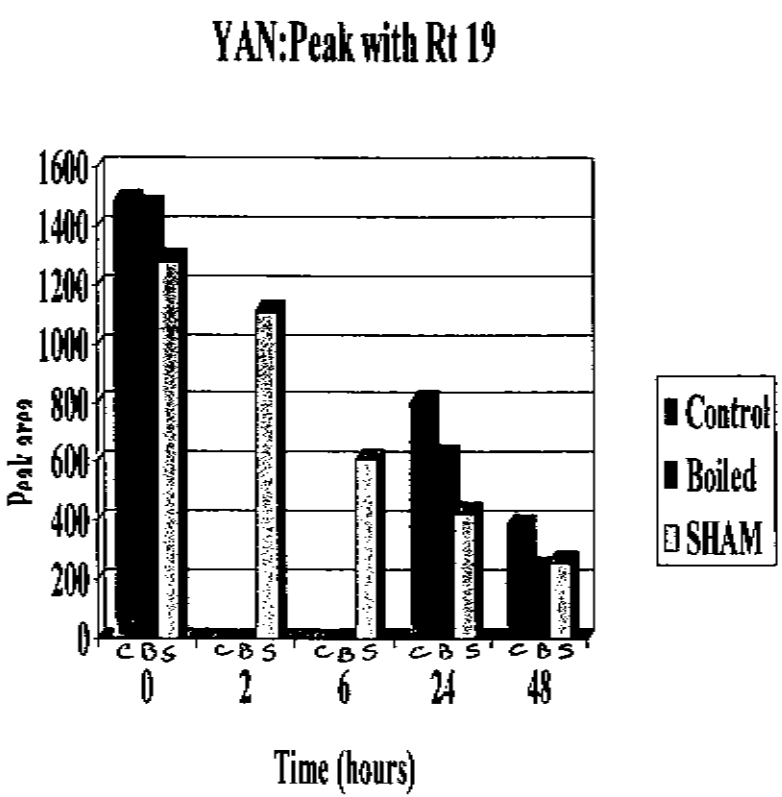
  Genetic and molecular manipulation



YAN: Change in peak area for Rf=23



YAN: Change in peak area for Rf=19



Developing New Microsatellite Markers

Mr Matthew Hayden

A novel technique for developing SSR markers

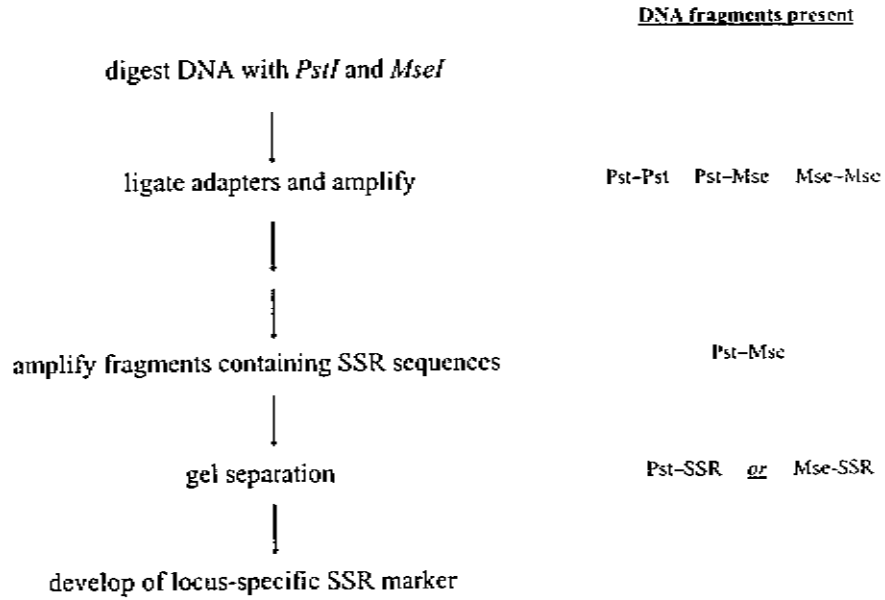
Disadvantages of traditional methods

- time consuming
- high level of sequence redundancy
- no prior knowledge of SSR informativeness

Selectively Amplified Microsatellite (SAM) technique

- builds upon the AFLP technology
- combines characteristics of AFLP markers with properties of SSRs
- no requirement of DNA sequence information
- multiplexed reaction
- similar to SAMPL assay but uses a different mechanism

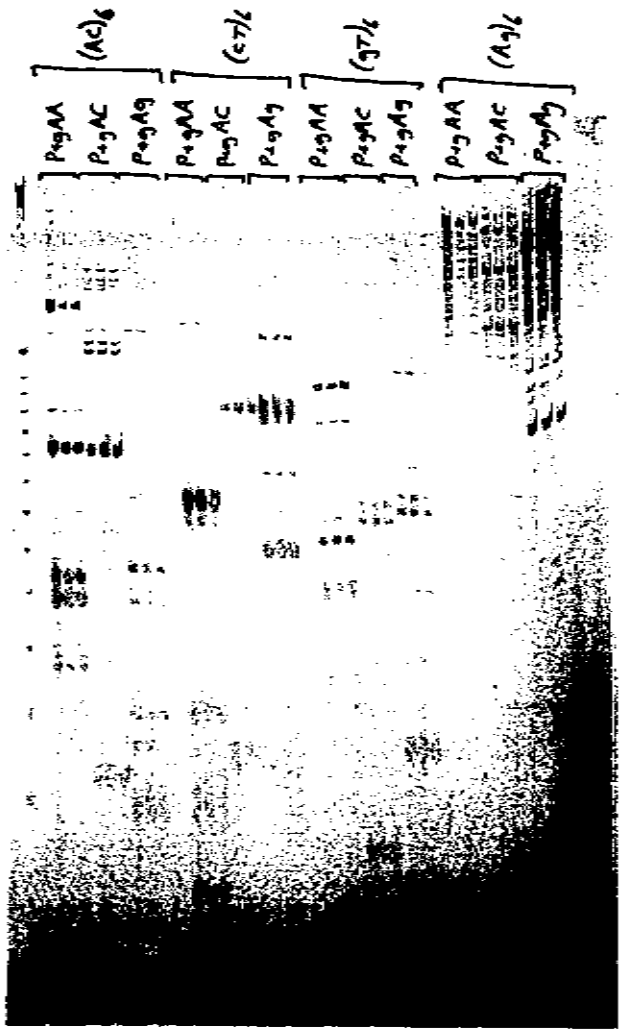
Overview of the SAM assay



Functions of the SAM adapters:

- provide compatibility with silver-staining
- prevent amplification of redundant SAMs
- facilitate the conversion of SAMs to SSR markers

EXPERIMENT 1: Effect of changing primer combination on SAM fingerprint pattern.



Experiment 2: Inheritance of SAMs

- 72 polymorphic SAM markers scored
- Mendelian inheritance observed
- 58 SAMs mapped on a framework map composed of RFLP markers

Chromosomal distribution of SAMs mapped in a subset of the ITMI mapping population.

Homoeologous Group	A-genome	B-genome	D-genome	Total
1	3	5	2	10
2	4	3	3	10
3	2	3	1	6
4	2	1	3	6
5	1	6	5	12
6	4	1	4	9
7	4	–	1	5
Total	20	19	19	58

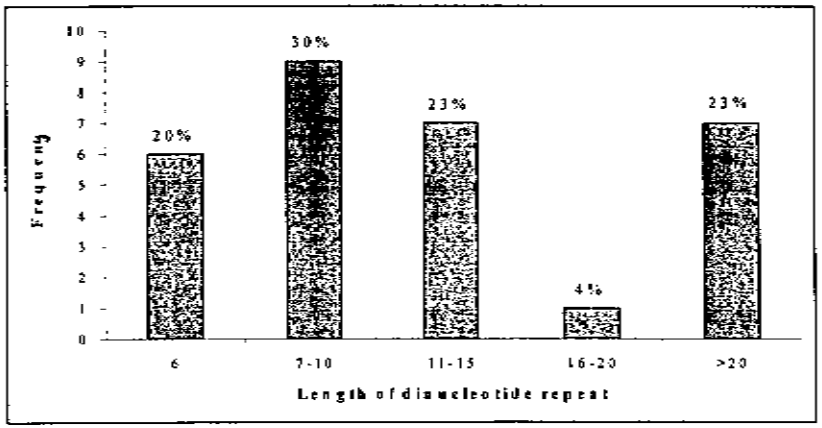
Experiment 3: Sequencing of SAMs

- 40 polymorphic SAMs were directly sequenced
- sequence data quality was sufficient to exclude a cloning step

Characteristics of sequenced SAMs

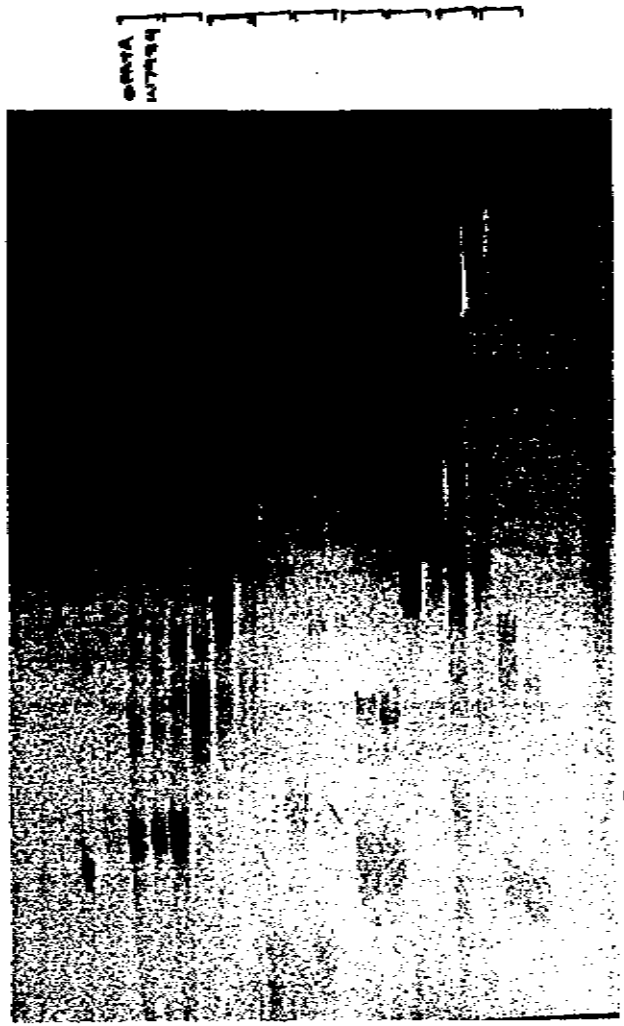
Number of SAMs successfully sequenced	36
Unique sequences	26 (72%)
Redundant sequences	4 (11%)
Allelic sequences	6 (17%)
Types of SSR arrays in unique sequences:	
Perfect	13 (50%)
Imperfect	3 (12%)
Compound	5 (19%)
Cryptic	5 (19%)

Frequency distribution of the total length of the SSR arrays in SAMs



EXPERIMENT 4: Conversion of SAMs to SSR markers.

- 21 SAMs converted to SSR markers
- 19 SSRs were functional



Generating STMs

sequence SAM

↓

design primer (ssp)

(Ag) adapter ssr

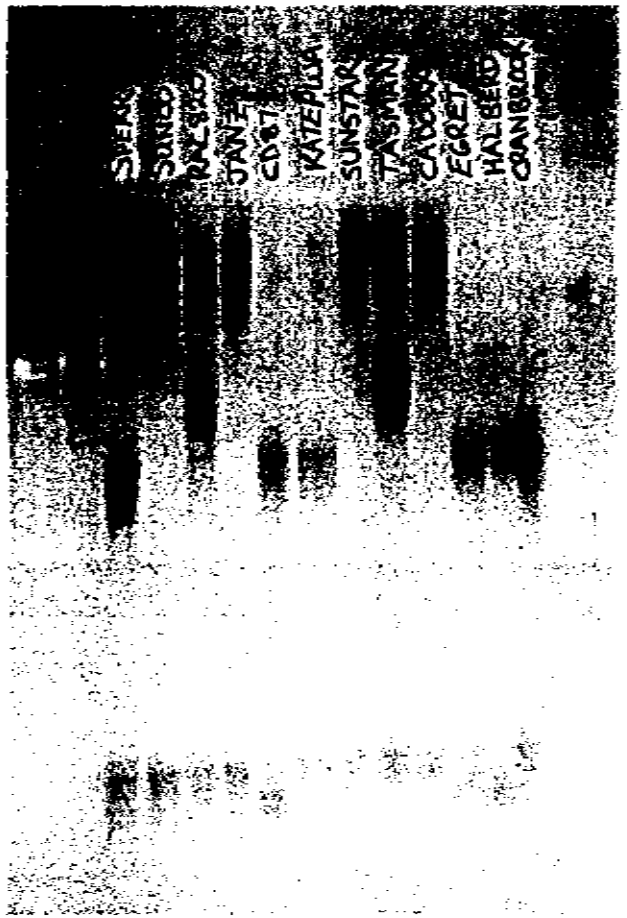
↓

amplify genomic DNA with ssp and SSR primers.

DNA (Ag)n

EXPERIMENT 5: Informativeness of short SSR sequences.

- 13 STMs tested; 6 were polymorphic
- PICs 0·15 – 0·68 ; average 0·41
- average number of alleles per locus was 2·2



Polymorphism revealed by ms. L.
↳ SSR array in SAM was $(CACG)_3(AG)_6$

Conclusions

Characteristics of the SAM technique

- simple and rapid to perform
- inexpensive compared to traditional methods
- virtually unlimited supply of markers
- map location of SAMs can be determined prior to SSR development
- potential informativeness of SAMs can be assessed before conversion to SSR markers
- a tool to complement existing marker technologies

Potential Applications

- development of SSR markers for population genetic studies
- targeted development of SSRs for specific chromosomal regions by
    - selecting mapped SAMs located in regions of interest
    - screening of aneuploid or deletion stocks for unique SAMs
    - bulked segregant analysis

Markers for Quality Genes, & Developing Waxy Wheats

Dr Peter Sharp

The University of Sydney

PLANT BREEDING INSTITUTE

Markers for Quality Genes

&

Developing Waxy Wheats

Quality Wheat CRC

CRC

Peter Sharp

The University of Sydney

PLANT BREEDING INSTITUTE

MARKERS

work in cooperation with the GRDC National Wheat Molecular Marker Program

collaborate in international efforts to develop a large set of wheat microsatellites

have a joint project with the NWMMP that is validating wheat molecular markers

contribute to the International Triticeae EST Collaboration to isolate ~40,000 expressed sequence tags

Quality Wheat CRC

CRC

PLANT INDUSTRY

Cross	Milling yield	Starch	Protein	Colour	Dough properties
Cranbrook x Halberd	■	■	■		■
CD 87 x Katepwa	■		■		■
Sunco xTasman				■	
RAC820 x Janz	■		■		■
Egret x Sunstar (HMW, LMW glutenin virtually identical)	■	■	■ (Proteins virtually identical)	■	■
Cadoux x Cascades		■		■	

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MARKERS FOR WHAT?

quality targets of *N* WMMP

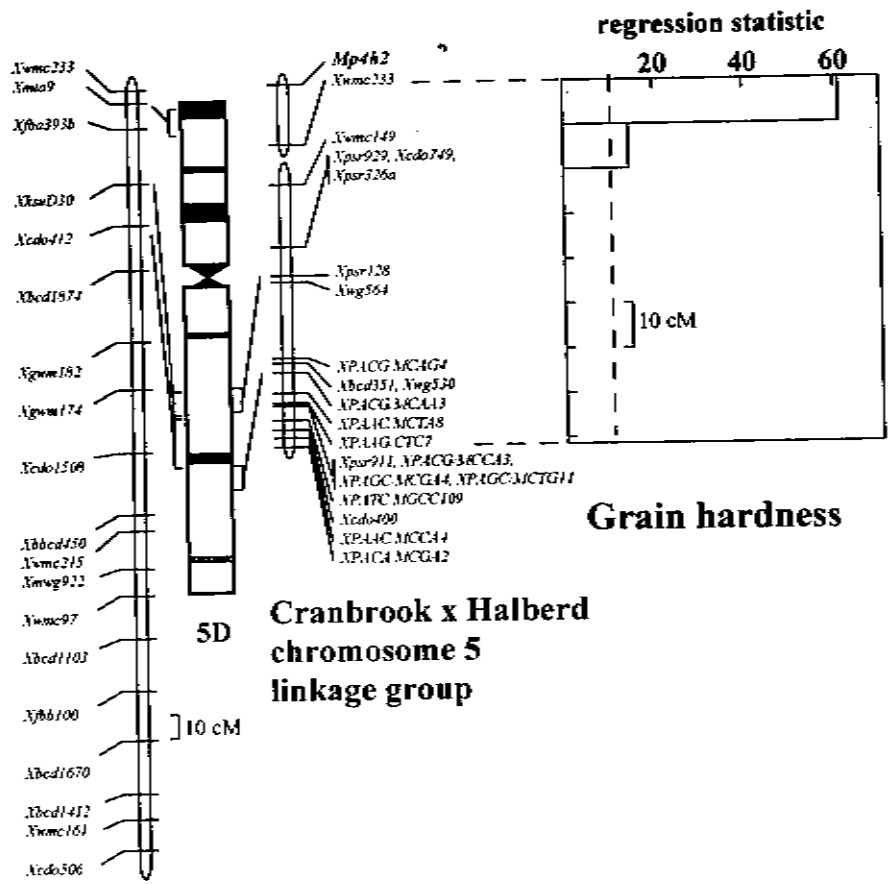
noodle quality, especially for White Salted Noodles

sprouting tolerance

late maturity α-amylase

waxy genes for the waxy breeding program

PLANT INDUSTRY



Cranbrook x Halberd
chromosome 5
linkage group

Grain hardness

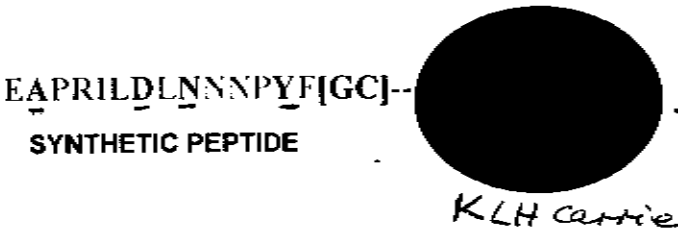
Synthetic x Opata
chromosome 5D
linkage group

CRC

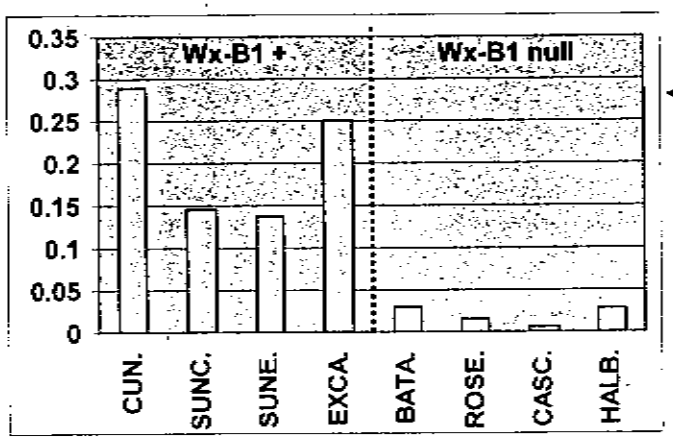
PLANT INDUSTRY

Development of a Wx-B1 (GBSS-4A) - Specific Antibody Probe

	201	240
'D' GENOME:	LEVPRILNLDNNPYFSGPYGEDVVFVCNDWHTGLLACYLK	
'B' GENOME:	LEAPRILDLNNNPYFSGPYGEDVVFVCNDWHTGLLACYLK	
'A' GENOME	LEVPRILDLNNNPHFSGPYGEDVVFVCNDWHTGLLACYLK	



TEST SERUM IN ELISA



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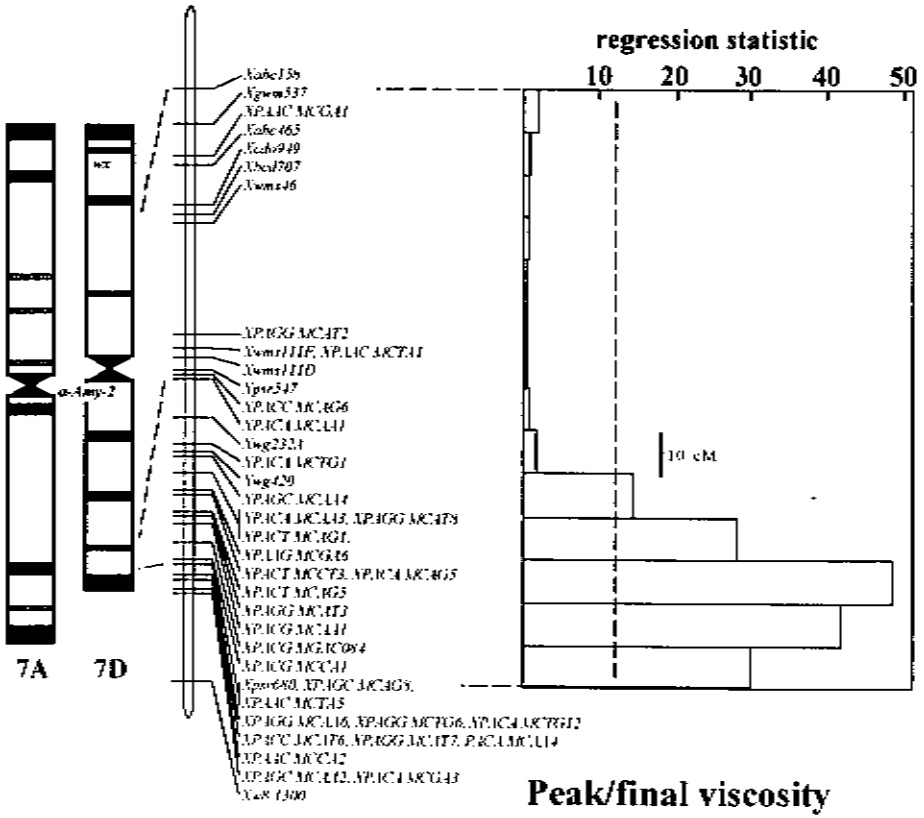
PROGRESS

confirmation in a number of crosses of the large effect of the null4A gene on WSN quality
- peak & final RVA viscosity
- flour & starch swelling power

identification of a microsatellite completely linked to grain hardness

confirmation that genes controlling aspects sprouting tolerance of AUS1804 are on chromosome 3D, and preliminary indications of map position

PLANT INDUSTRY



Peak/final viscosity (no silver)

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PROGRESS

identification of a QTL of large effect on RVA peak & final viscosity on a group 7 chromosome

QTLs for flour swelling power in 2 crosses (Halberd x Stiletto; Halberd x Cranbrook)

- Glu-A1, Glu-B3, Gli-D2
- chromosome 3A
- Wx-B1, Wx-D1
- epistasis is important

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PROGRESS IN WAXY BREEDING

backcrossing the three Wx null alleles into four Australian cv backgrounds (Janz, Silverstar, Goldmark, NP150)

using molecular markers to

select progeny with null alleles
select progeny with highest % cv genes

have produced large amounts of waxy grain for testing by CRC partners and others

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PROGRESS IN WAXY BREEDING

selections from Janz F5 lines made
- to summer increase, then plots 2000

15 NP150 backcross 2 doubled haploids
- made last summer, increasing now
- to summer increase, then plots 2000

are in middle of producing doubled haploids from selected backcross 3 lines
- aiming for 100s of DH for each of the four backgrounds
- plots in 2001

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CRC

PROGRESS IN WAXY BREEDING

production of waxy material 1999 for partner and other testing 2000

- 3-4 t of another line
- 100s of kg amounts of 6 other lines

Breeding New Soft Wheats

Dr Lindsay O'Brien

NEW SOFT WHEATS

QUALITY OF QAL2000

MILLING YIELD COMPARABLE WITH TATIARA

WATER ABSORPTION COMPARABLE WITH TINCURRIN

MIXING TIME AND DOUGH EXTENSIBILITY AND RESISTANCE COMPARABLE WITH TINCURRIN

GOOD BISCUIT PACKET LENGTH, BAKED DOUGH WEIGHT AND HARDNESS

NEW SOFT WHEATS

COMPARATIVE YIELD OF QAL2000

BASED ON 16 SITES OVER 3 YEARS, EXTENDING FROM NORTH STAR TO NUMURKAH

	t/ha	%SMY
QAL2000	3.28	116
SUNSTATE	2.82	100
SMY	2.82	100

(site mean yield)

NEW SOFT WHEATS

HOT OFF THE HEADER DATA FROM 1999

	NORTH STAR (t/ha)	%SMY	Nbi t/ha	%SMY
QAL2000	3.58	134	3.97	124
BOWIE TATIARA	2.00	75	2.67	74
SUNSTATE	3.39	126	3.11	89
SMY	2.67		3.32	

(site mean yield)