# Genotype X Environment influences on wheat quality: Trends and knowledge gaps

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# **Genotype X Environment influences on wheat quality:**
# Trends and knowledge gaps

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**CO-AUTHORS:**

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QAAFI,
University of Queensland

**Frank Békés,**
FBFD Pty Ltd

and **Di Miskelly**
Westcott Consultants

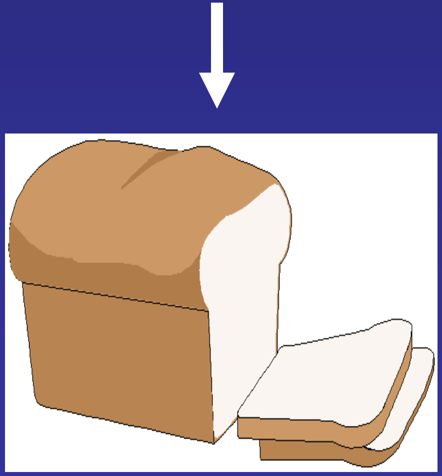

Wheat-based foods…require various dough properties
…and differences in grain hardness

# Only wheat dough can give extensibility like this!

# Start with the market needs

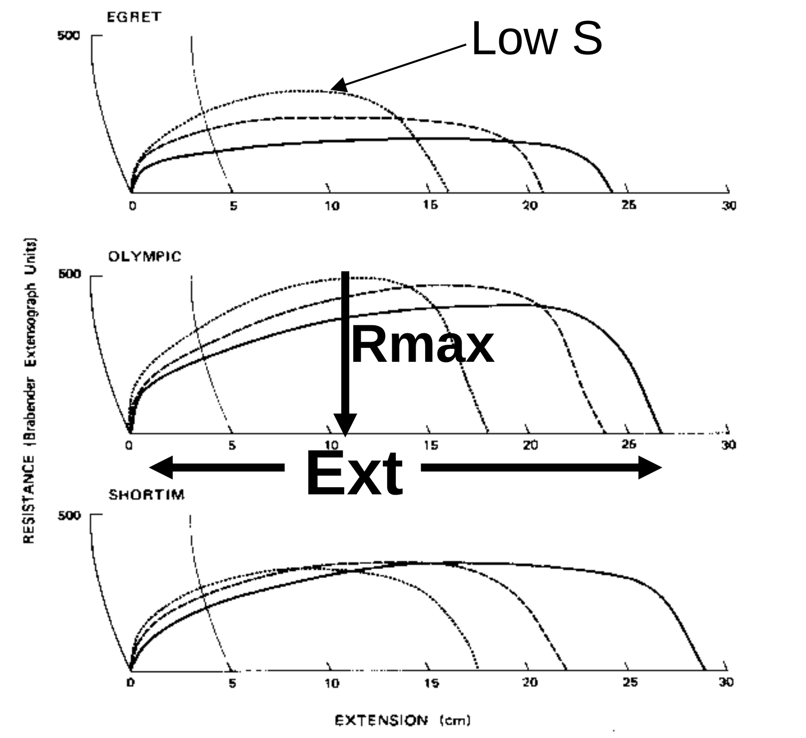
Anyone planning to sell a product must see what the market wants. ASK!!

## Some bakers' specifications:



| Flour for: | % Protein | Rmax | Ext |
|---|---|---|---|
| Bread | >11 | 350-400 | 19-23 |
| Bun | 13-14 | >350 | >20 |
| Cracker | 11-12 | 310-340 | 18-23 |

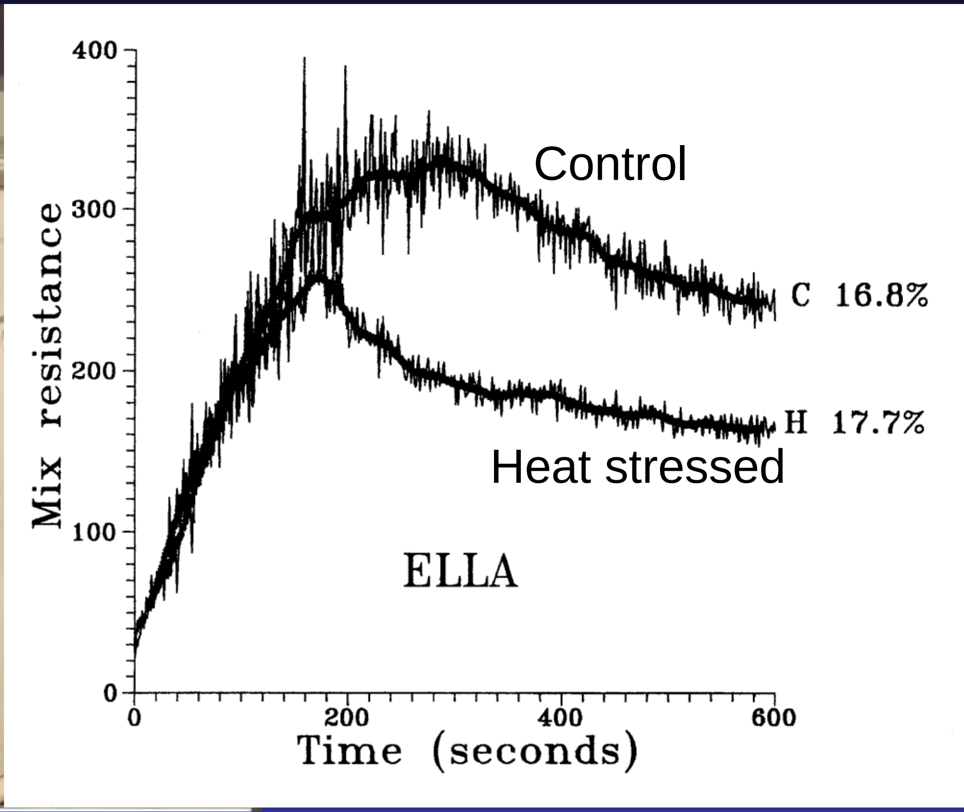
# To assign numbers to dough quality:
# The Extensograph


## G x Sulfur:

**Loss of extensibility due to sulfur deficiency for three varieties**

# To assign numbers to dough quality:
# The Mixograph




**G x Heat stress:**

**Heat stress (a few days >35ºC)**
- **Weaker dough**
- **Fewer B starch granules**

# The big picture

Genome

Growth Environment

Transcriptome (mRNA) → Proteome → Proteins

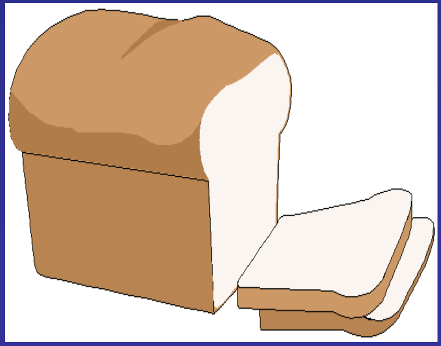
Grain comp'n ↓

Mill to flour ↓

Mix dough

Bake ↓

Bread, etc.



## Same picture for any grain

even for chocolate quality

**Genome**
(genes, DNA)

**Growth Environment**

→ **Transcriptome** (mRNA) → **Proteome**

↓

Proteins

Bean comp'n

↓

Crush

Ferment

Roast

Grind

Blend

Package

**Delicious food … Chocolate**

# Dough quality - Gluten

Genome

Growth Environment

Transcriptome → Proteome

(mRNA)

**Proteins**

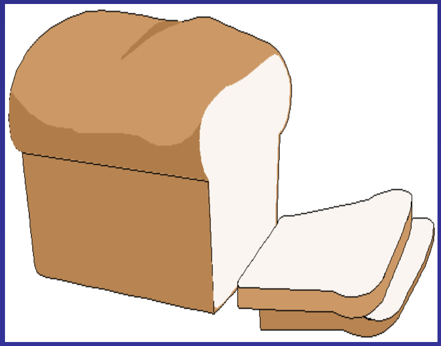
Grain comp'n

Mill to flour

Mix dough

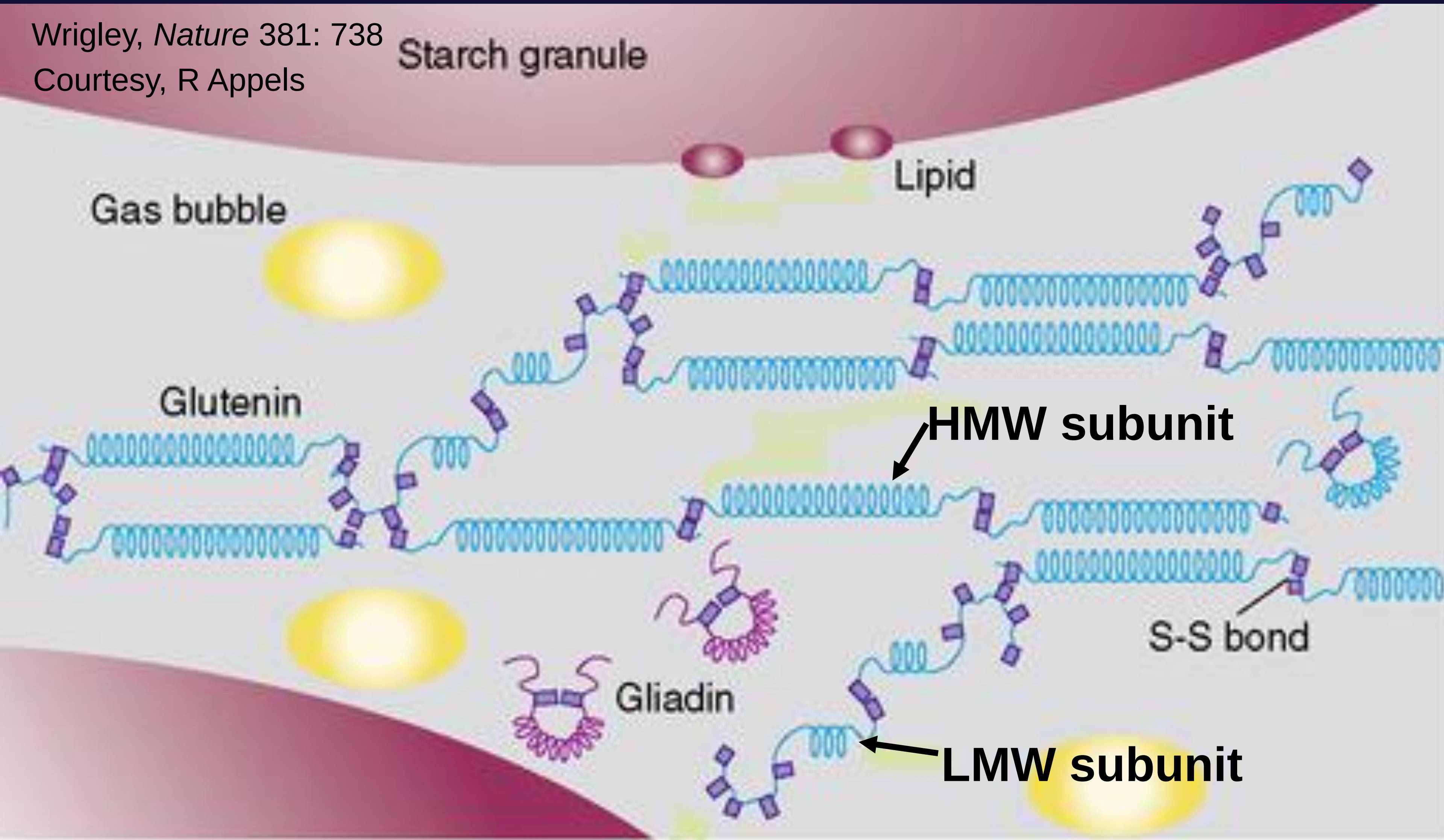
Bake

Bread, etc.



# Major genes for <u>dough quality</u> (*Glu-1* & *Glu-3*) High mol wt subunits + Low mol wt subunits

Wrigley, *Nature* 381: 738
Courtesy, R Appels



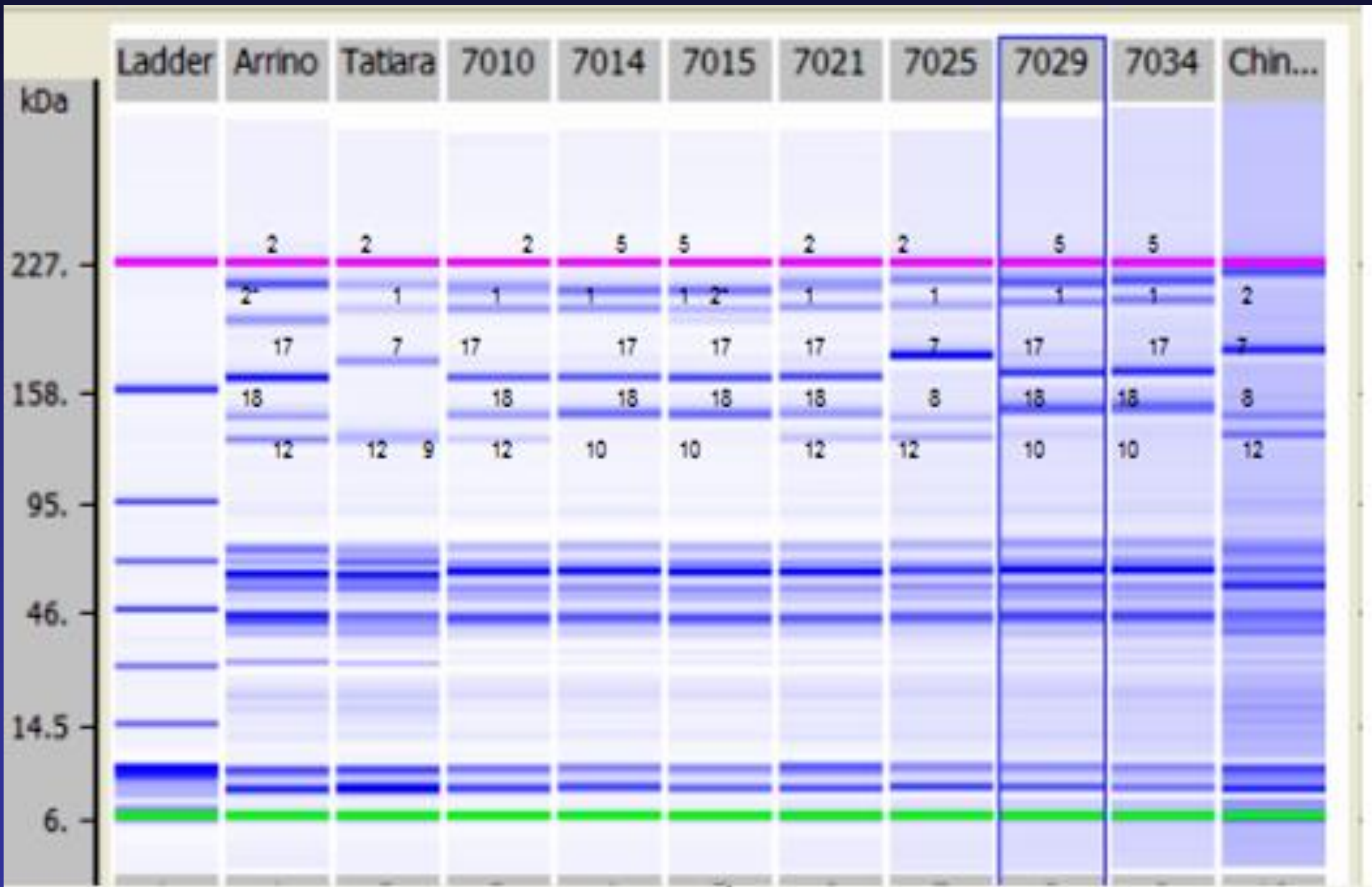
# Other genes for specific quality attributes, based on QTL analysis

Australian Molecular Marker Program, Aust J Agric Res 52, 1043-1423

- **Flour milling**
  - Chromosome 5BL
- **Grain hardness**
  - Chromosome 5DS for puroindoline, and 4B
- **Noodle colour**
  - Xanthophyll, 3B & 7A
  - Noodle brightness, 2D
  - polyphenol oxidase, 2D
- **Starch type**
  - Chromosome 4A (RVA)
  - 4B for A:B size ratio
- **Dough extensibility**
  - LMW subunits of glutenin,1B & 1D
- **Dormancy**
  - Chromosome arms 2AL, 2DL and 4AL

# Lab Chip micro-capillary electrophoresis for glutenins

# Lab Chip analysis (50 sec each) of HMW & LMW subunits of glutenin




HMW subunits

LMW subunits

# Subunits & alleles for glutenin

| HMW-GS | *Glu-A1* | *Glu-B1* | *Glu-D1* |
|---|---|---|---|
| Subunits | 1, 2*, Null, 3* | 7, 7+8, 7+9, 6+8 | 2+12, 5+10 |
| Alleles | *a*, *b*, *c*, … | *a*, *b*, *c*, … | *a*, *b*, *c*, *d* |
| | | | |
| LMW-GS | *Glu-A3* | *Glu-B3* | *Glu-D3* |
| Alleles | *a*, *b*, *c*, … | *a*, *b*, *c*, … | *a*, *b*, *c*, … |

# Large database of dough-quality genotypes based on *Glu-1* & *Glu-3*

**Rmax and Ext predicted from HMW & LMW alleles for 8,000 wheats world-wide.**

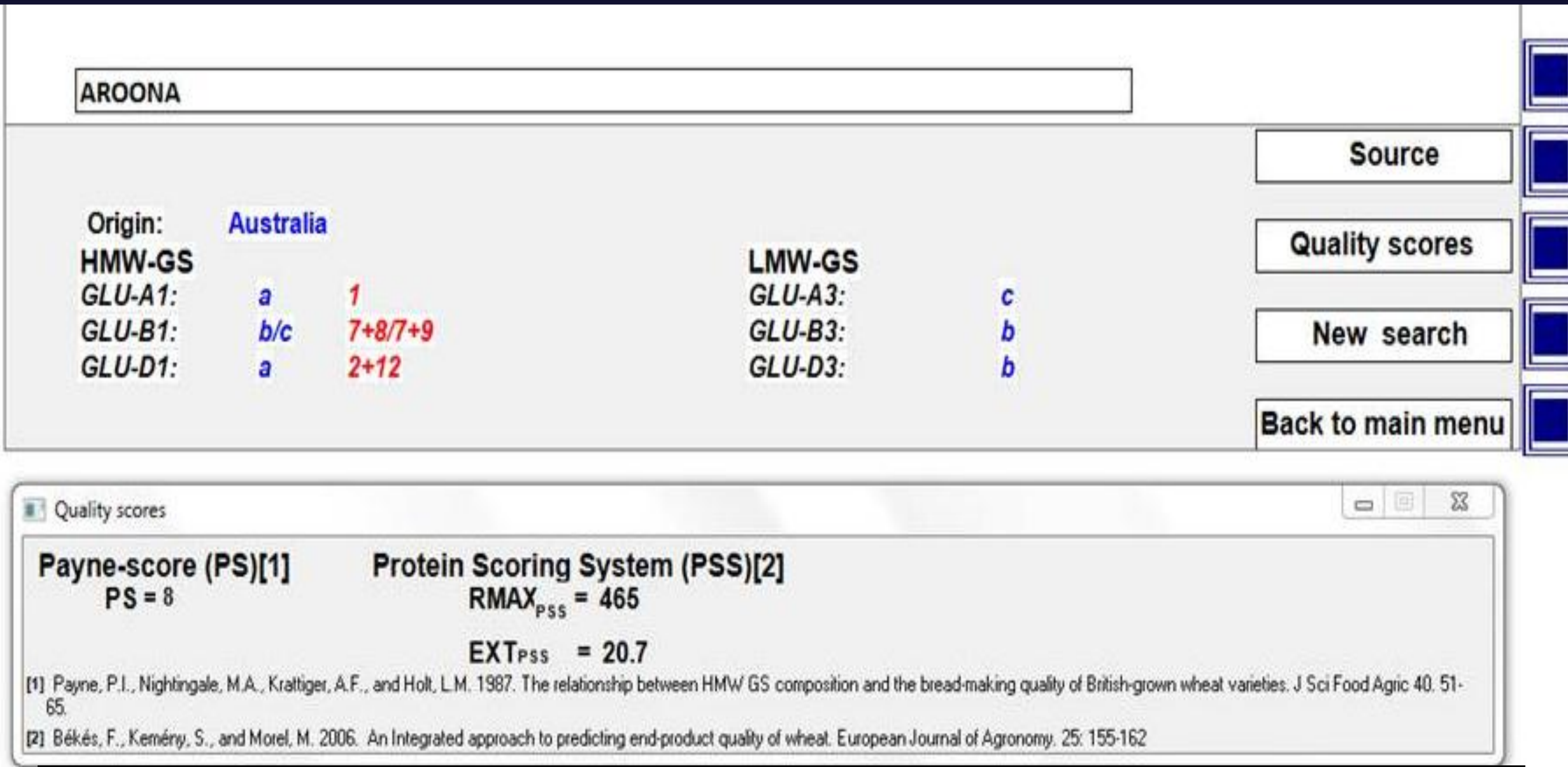
**Not recent Australian wheats**

Bekes & Wrigley, 2013. CFW 58 (6), 325-328.

**Gluten protein database on AACCI web site:**

http://www.aaccnet.org/initiatives/definitions/Pages/glutendatabase.aspx

# Gluten protein database on the AACCI web site:




**BUT… These predictions of genotype neglect growth environment!! …GAP**

# The big picture, including growth conditions

**Genome**

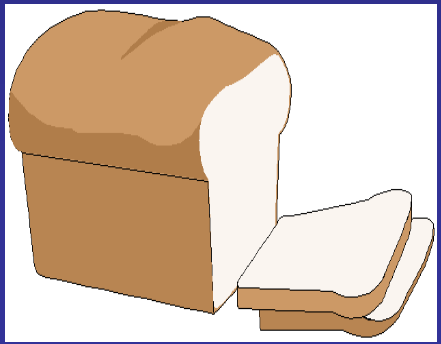
(*Glu* genes)

**Transcriptome** → **Proteome**

(mRNA)

**Growth Environment**

**Fertiliser**
**Heat**
**Sulfur**
**$CO_2$**



Proteins
Grain comp'n
↓
Mill flour
Mix dough
Bake
↓
**Bread, etc.**

# The transcriptome gap

**= Transcripts produced from genes under the environment at the time**

**First opportunity to see how E affects G**

**Showing:**

- **Which genes are transcribed** (mRNA)
- **How much of each gene product**

**Studies on transcripts on 31 wheats to relate mRNA sequences to quality (hardness, dough, etc) by Ravi Nirmal, QAAFI, University of Queensland**

# The transcriptome gap

**At the sub-genome level:**
**Identification of all alleles for a gene**

**e.g., Ravi has found the alleles in the sample wheats for *Pin a* and *Pin b* genes**

**The information facilitates MAS, as gene expression is indicated** (not just gene presence)

**Identification of highly similar expressed alleles is possible, + effects of growth environment**

**Ravi Nirmal, QAAFI, University of Queensland**

# The big picture, including growth conditions

**Genome**

(***Glu*** **genes**)

**Transcriptome** → **Proteome**

(mRNA)

(Polypeptides)

**Growth Environment**

**Fertiliser**
**Heat**
**Sulfur**
**$CO_2$**

↓

Functional proteins

Grain comp'n

↓

Mill flour

Mix dough

Bake

↓

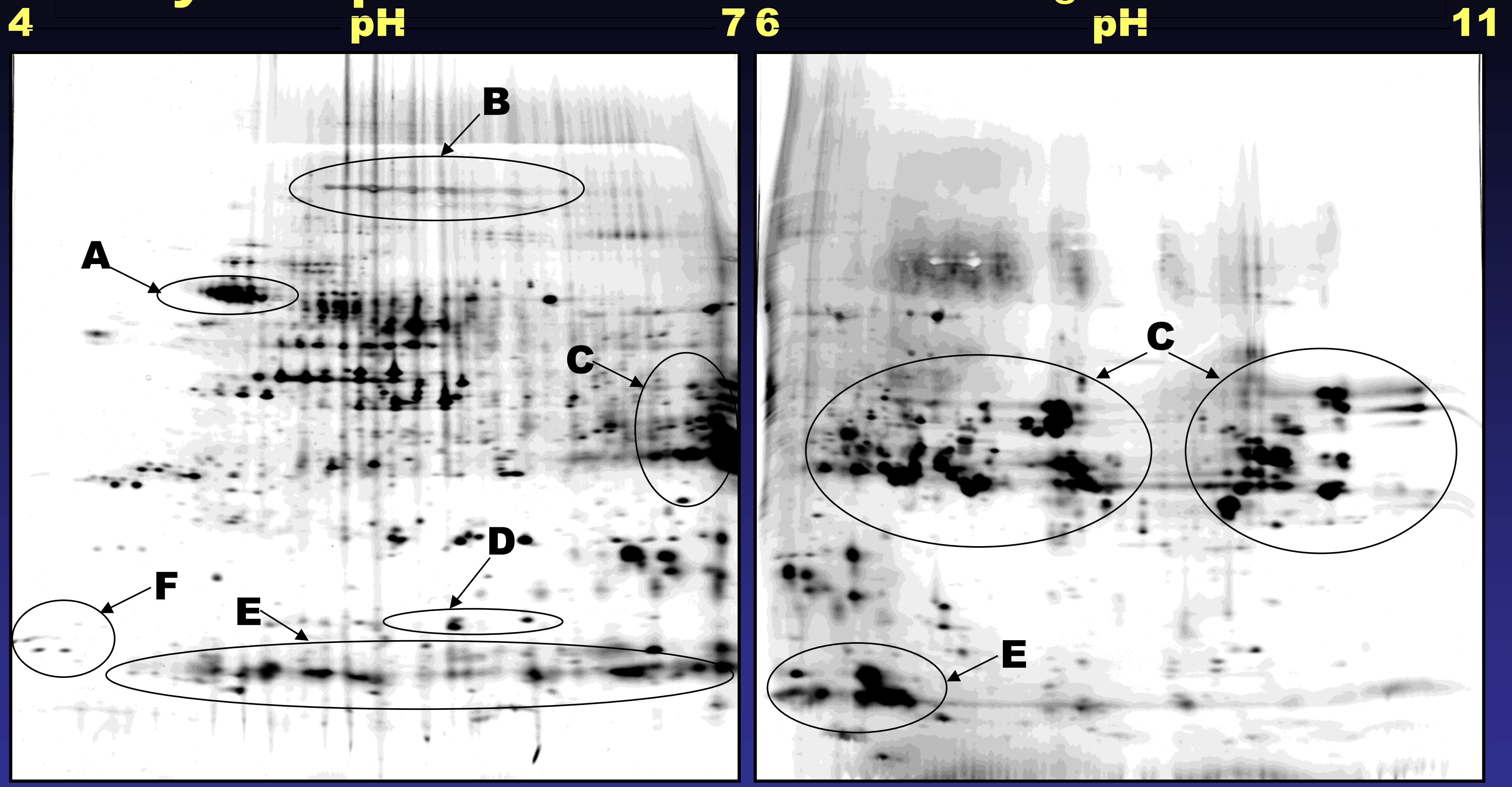
**Bread, etc.**

# The proteome gap

= Polypeptides synthesised (thousands):

- In a specific tissue (e.g., endosperm)
- For a specific genotype
- Under specific growth conditions
- Thus reflecting G x E
- → Amino-acid sequences
- Identification of glutenin subunits

# Wyuna proteome – 17 DPA Region identities




**A-Protein disulphide isomerase (PDI isoforms)**
**B-HMW glutenin subunits**
**C-Gliadins**
**D-Small heat shock proteins**
**E-Alpha-amylase/Trypsin inhibitors**
**F-Acidic ribosomal proteins**

**All specific protein regions were assigned a**
**data from Skylas *et al.* (2000). Rregion D is a**
**small heat shock proteins based on Skylas**

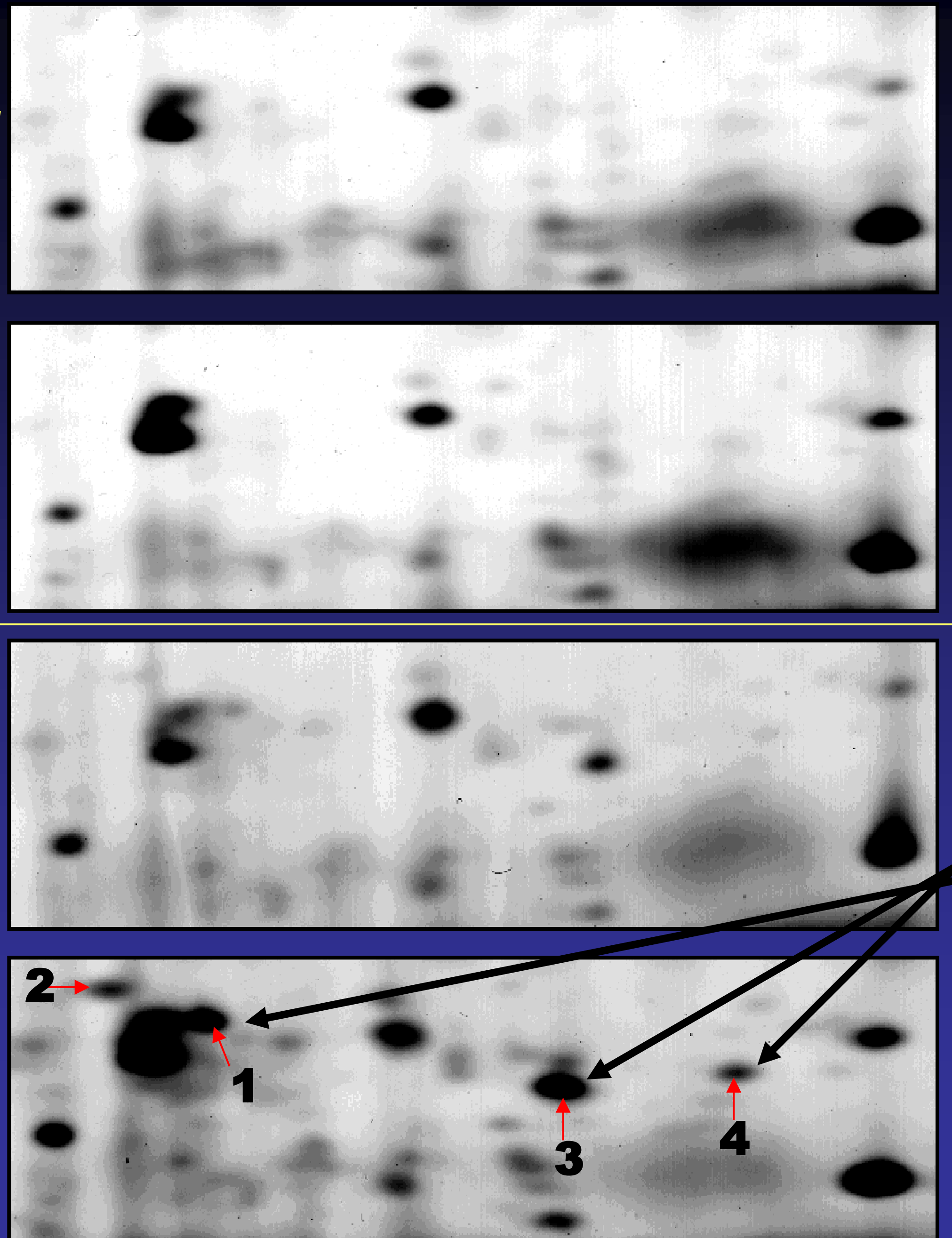
# Immature grain 17 DPA

**Wyuna control/ *susceptible***

**Wyuna heated**

**Fang control *tolerant***

**Fang heated**




**Spots 1, 3 & 4 are <u>heat-shock proteins</u>**

Potential markers of heat-tolerant genotypes to help in breeding.

**Spot 1 present at maturity**

# The big picture, including growth conditions

**Genome**
(***Glu*** **genes)**

**Transcriptome** → **Proteome**

(mRNA)

**Growth**

**Environment**

**Fertiliser**
**Heat**
**Sulfur**
**$CO_2$**

**Proteins**

Grain comp'n

Mill flour
Mix dough
Bake

**Bread, etc.**

# Glutenin polymers
## A gap in our knowledge

**Functional proteins are formed by folding and SS bonding of the polypeptides**

**Glutenin polymers are very large**

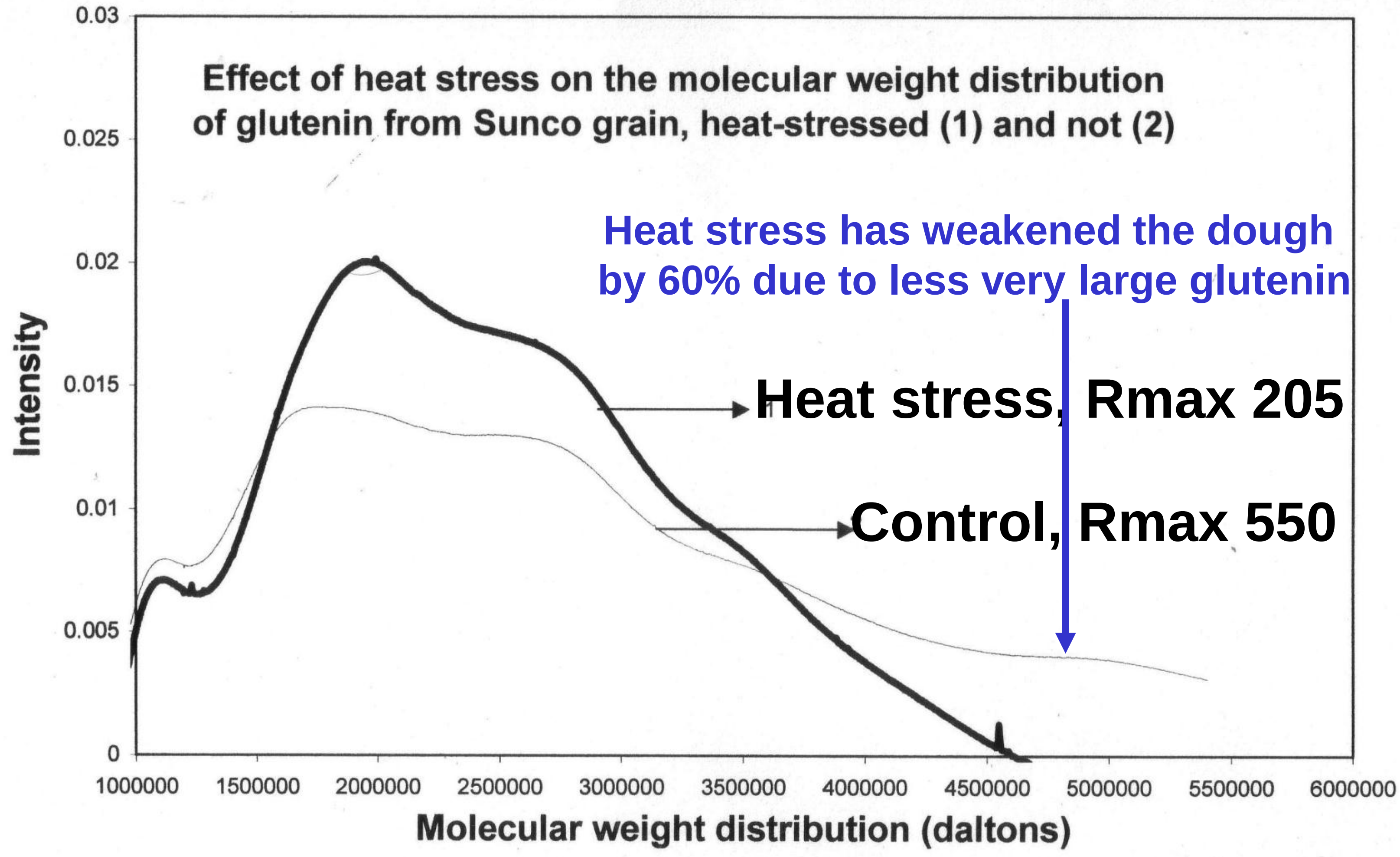
(Use FFF for Mol Wt distribution, up to 10M Daltons)

**Ignorance of how subunits polymerise**

**Importance of very large polymers** (heat stress)

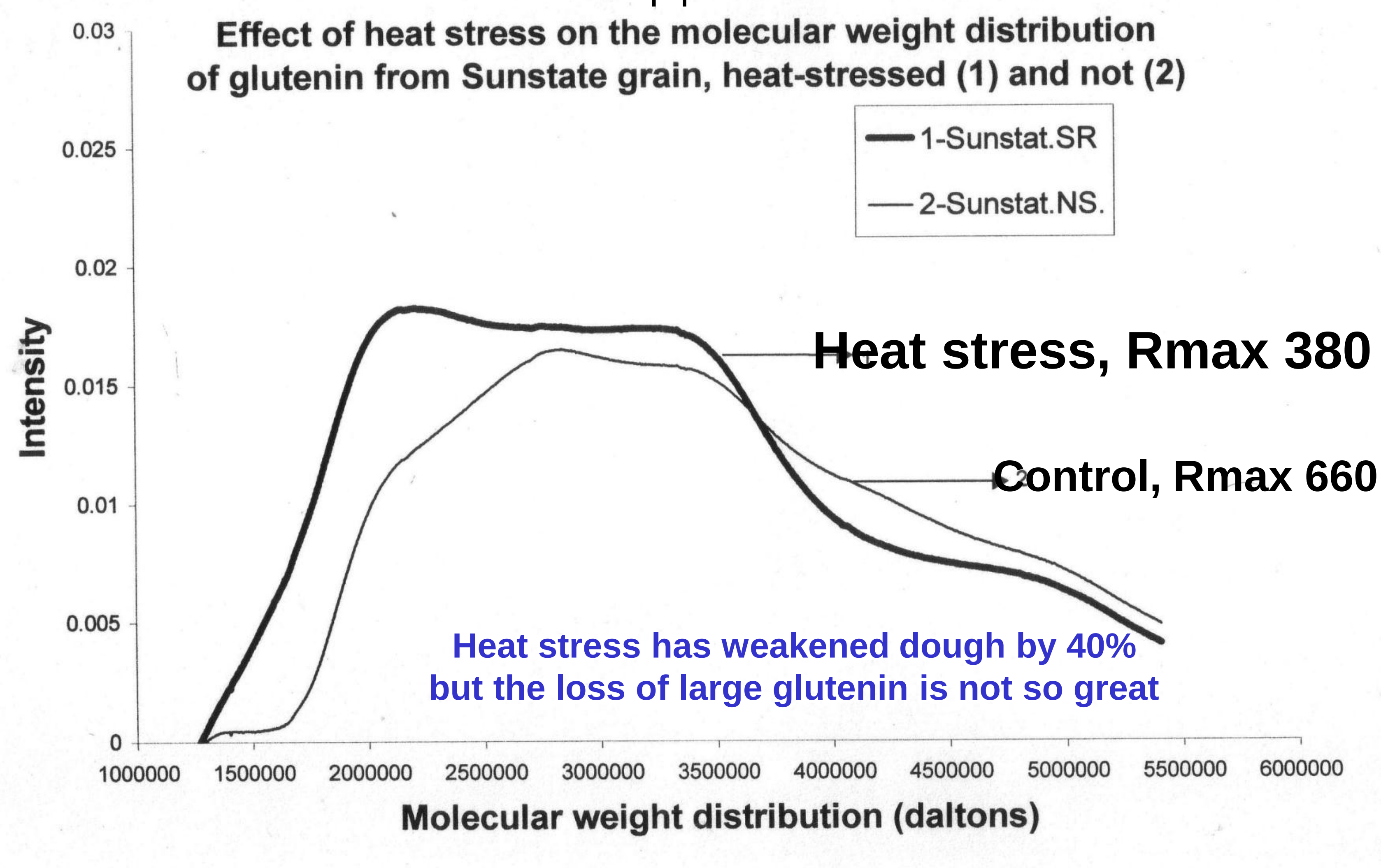
# Mol wt distribution of Sunco (aua) flour proteins by FFF

From L Daqiq PhD thesis



# Mol wt distribution of Sunstate (aid) flour proteins by FFF

From L Daqiq PhD thesis



# The big picture, including growth conditions

**Genome**

(*Glu* genes)

**Growth Environment**

**Fertiliser**
**Heat**
**Sulfur**
**$CO_2$**

**Transcriptome**

(mRNA)

**Proteome**

(Polypeptides)

Functional proteins

Grain comp'n

Mill flour

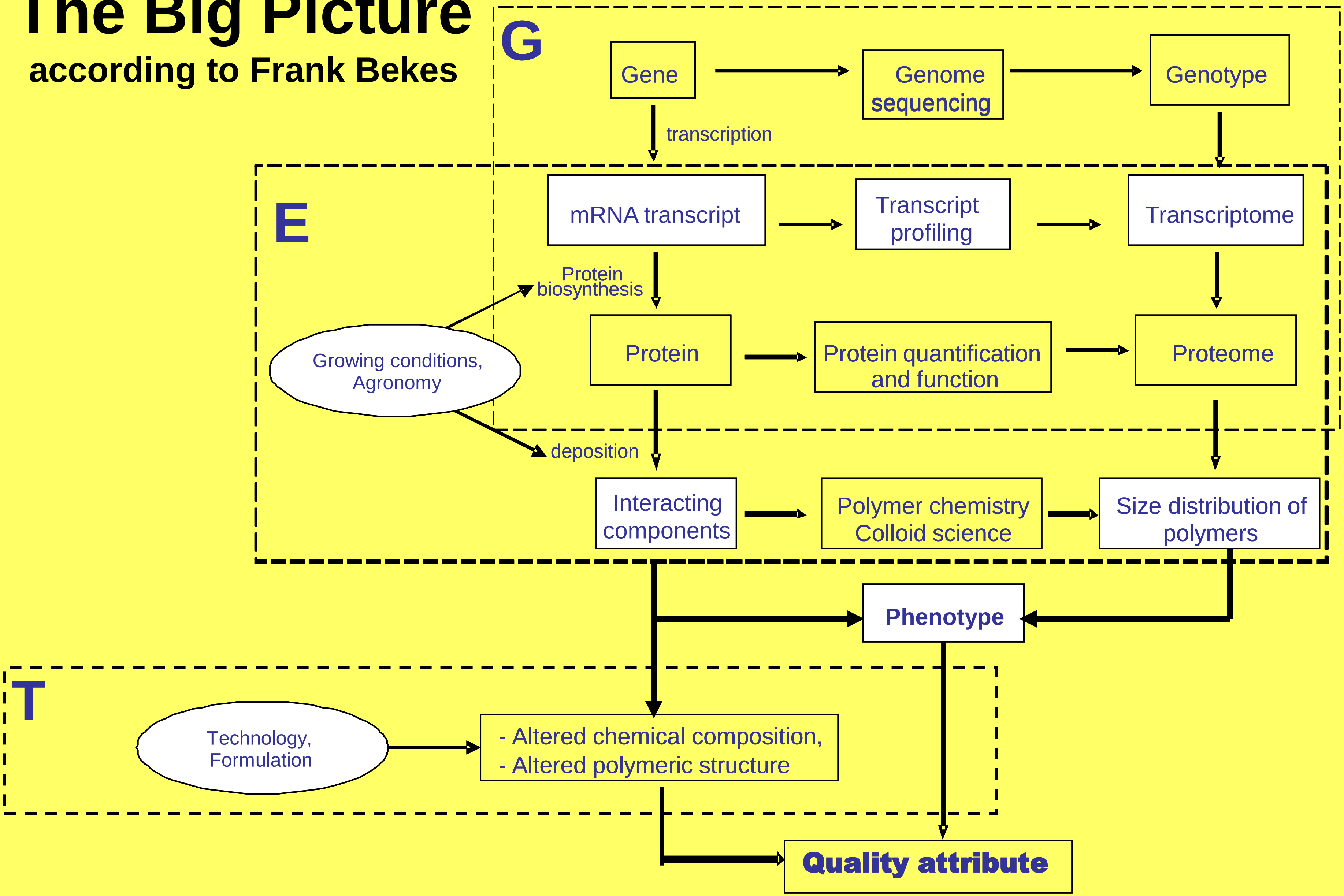
Mix dough

Bake

**Bread, etc.**

**Polymeric structure can be altered by processing technologies: additives, mixing and proofing**

# The Big Picture
## according to Frank Bekes

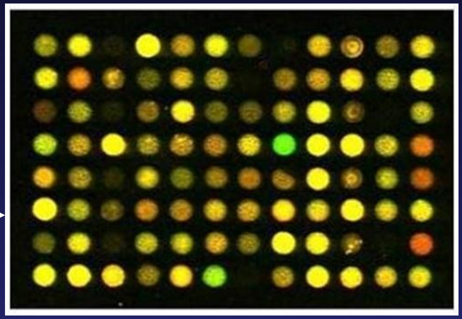


# Quality testing at grain receival

**Variety identification**

**On-the-spot, at silo: Lab-chip**

**Centrally: DArT Microarrays**



**Variety declaration =**
**'G' only, no 'E'**

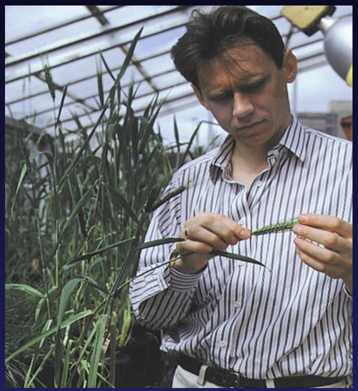
**Get load history for 'E'**

Quality data for each storage cell is valuable

# The future of receival testing

- More low-protein, weak wheat delivered?
  - ‘Cascade’ of APH → AH → lower grades?
- Need to maintain very good quality
  - Especially in APH and AH grades
  - We must exclude ‘borderline’ varieties
- 2013 harvest: 3 cvs = 70% deliveries
  - Most variety declarations were correct!
- Deregulation = more traders, co-mingling
  - less chance to define quality of grain lots
- Variety identification important for PBR
  - Cheap rapid methods still needed

# Grain Supply Chain



Breeder

Seed merchant

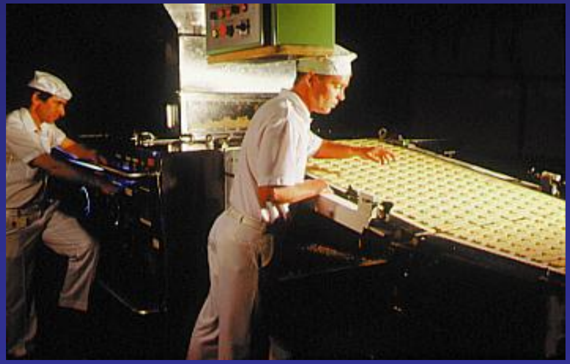
Grower

Transport & Storage

Marketing

Miller

Secondary processor



Food manufacturer

Retail

Consumer

**Breed for G x** e

# Selection for G x e?

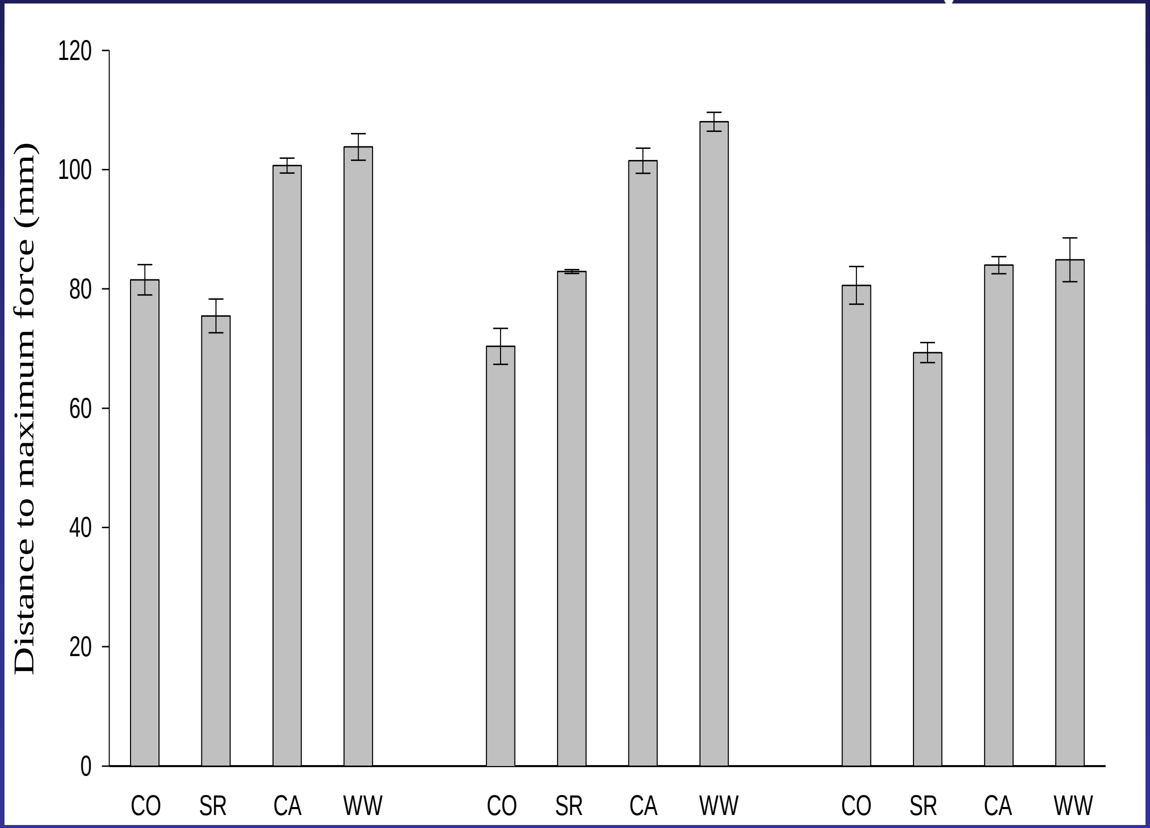
**These three varieties grown in 4 diverse sites**
**Guardian showed less quality variation**

| | Janz | EGA Gregory | LongReach Guardian |
|---|---|---|---|
| ***Glu-1* alleles** | a, b/u, **a** | a, u, **a** | a, u, **d** |
| **HMW-GS subunits** | 1, 7+8/7*+8, **2+12** | 1, 7*+8, **2+12** | 1, 7*+8, **5+10** |
| **LMW-GS *Glu-3* alleles** | b b b | c b c | b, b, b |

**Uthayakumaran, S., Tanner, R.I, Dai, S., Qi, F., Newberry, M., Wrigley, C., and Copeland, L. 2012. J. Agric. Sci. 4, (7) 41-50.**

# Is it possible to breed and select for G x e?

**Janz  Gregory  Guardian**




**Small extension tester**
**Distance at *Fmax***

**Guardian (5+10) showed uniformity of dough properties *vs* the others (2+12)**

# The big picture

**Genome**

**Growth Environment**

**Transcriptome** → **Proteome**

(mRNA)

Proteins

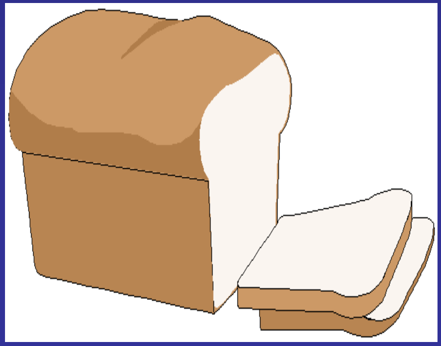
Grain comp'n

**Mill to flour**

Mix dough

Bake

**Bread, etc.**



# **GAPS** - SUMMARY

- **Transcriptomics and proteomics to identify quality markers**
- **HMW & LMW alleles for recent Australian wheats**
- **Understanding how subunits form into very large glutenin polymers**
- **Analysis of quality type at grain receival**
- **Breed for G x** e

# References


Bekes, F., and Wrigley, C.W. 2013. Gluten alleles and predicted dough-quality for wheat varieties world-wide: A great resource – free on the AACC International website. Cereal Foods World 58 (6), 325-328.

Bekes, F., and Wrigley, C.W. 2013. Gluten protein database.

http://www.aaccnet.org/initiatives/definitions/Pages/glutendatabase.aspx

Uthayakumaran, S., Tanner, R.I, Dai, S., Qi, F., Newberry, M., Wrigley, C., and Copeland, L. 2012. Genotype-based stability of dough quality in wheat from different growth environments. J. Agric. Sci. 4, (7) 41-50.

Uthayakumaran, S., Tanner, R.I., Dai, S.-C., Qi, F., and Wrigley, C.W. 2014. Relationships between traditional and fundamental dough-testing methods. Cereal Research Communications 42 (2), (in press)

Juhasz, A., Bekes, F., and Wrigley, C.W. 2014. Wheat proteins. In: Applied Food Protein Chemistry, to be published by Wiley-Blackwell. Zey Ustunol, Editor. (in press).