Abiotic Stress Tolerance in Cereals

Geoff Fincher
ABIC
Saskatoon, Canada
14 September 2010
1. The Australian Centre for Plant Functional Genomics

The Australian Centre for Plant Functional Genomics (ACPFG) is headquartered at The Waite Campus of the University of Adelaide. It also has partnerships with the University of Queensland and the University of Melbourne.
The ACPFG employs over 120 research scientists and associated staff...
Focus on Abiotic Stress

• Non biological stresses:
  – drought
  – salinity
  – frost
  – mineral deficiency or toxicity

• Major causes of yield and quality loss in cereal crops
Abiotic Stress: Saskatchewan 2010
Technology Platforms

- Genetics and mapping/mutant populations
- Germplasm collections
- Transcriptomics/robotics
- Protein analysis/structural biology
- Carbohydrate analysis
- Metabolite profiling
- Cereal transformation.
Delivery of Outcomes

- corporate structure to facilitate delivery
- interactions with private sector
- direct links with Australian and international wheat and barley breeding companies
- IP quarantining and management
- commercialization opportunities
- community and school education programs.
Two key ‘gene discovery’ strategies

**Forward genetics**

Discover and exploit naturally occurring variation
- screen landraces, mutant populations, breeding populations for desirable traits (phenotypes)
- non-biased approach
- useful for complex traits

**Reverse genetics**

Nominate candidate genes from other approaches, bioinformatics
- measure effects of altering levels and patterns of expression in candidate genes
- hypothesis-driven, thus biased by previous data
- can provide rapid improvements.
Drought

- Global crop losses $10 billion p.a.

- 1% increase in grain production due to better drought tolerance will generate $3-4 billion p.a. globally

- Obvious benefits in reduction of food shortages

- BUT drought is a very complex trait
  - Multiple genes
  - Multiple gene interactions
  - Progress has been slow in the past.

Program Leader: Peter Langridge
Dissecting the Trait

What is drought?

- Water stress
- Heat stress
- Excess irradiation (light intensity)
- Ancient soils (i.e. low organic matter, toxicity, poor structure)
- Pathogens/pests (e.g. nematodes)
Imposing Drought (water) Stress: Another Challenge

1.---------------------------------- Treatment 1 (Well watered)
   -------------------------- Treatment 2 (Low watered)
   ------------------ Treatment 3 (Restricted water)

2. VWC
   No watering
   Phenotype
   Soil capacity
   Sampling

Phenotype

Sampling

Phenotype

Sampling

Phenotype

Sampling

Sampling

Sampling

Sampling

Sampling

Sampling

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Sampling

Sampling

Sampling
Imposing Drought Stress: Another Challenge

3.

Rain

0% 100%

water availability

Critical developmental stages for yield

Booting Heading/Anthesis Grain filling Harvest

AUG SEP OCT NOV DEC
A Forward Genetics Approach

**RAINFALL**

- **Very dry**
- **Semi-dry**
- **No drought**

**Yield**

- **Stylet**
- **Excalibur** *
- **Kukri** *
- **RAC875** *

Majority of locations in SA

- **Mapping populations for QTL discovery:**
  - Excalibur x Kukri
  - RAC875 x Kukri

[Excalibur and Kukri show large differences in dry years]

[RAC875 and Kukri different in most years]
## QTL Table
(James Edwards)

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- **RAC851 and Excalibur traits here**
- **Complex stress: complex set of plant responses**
Drought Tolerance: reverse genetics with \textit{Rab17::TaDREB2} gene

<table>
<thead>
<tr>
<th>Control</th>
<th>Transgenic Line 3</th>
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- **2nd week of drought**
- **3rd week: recovery**

Sergiy Lopato
Salt tolerance: a complex trait with multiple mechanisms

- **Exclusion**
  - keep $\text{Na}^+$ out

- **Osmotic tolerance**
  - deal with low water potential

- **Tissue tolerance**
  - accumulate $\text{Na}^+$

Program Leader:
Mark Tester
Salt tolerance and pumping of Na$^+$ ions

- Tolerance correlated with exclusion from leaves

- In the outer part of the root:
  - Minimise Na$^+$ influx and/or maximise efflux

- In the inner part of the root:
  - Minimise Na$^+$ efflux and/or maximise influx to inner root cells

- Pump into sub-cellular compartments or out of leaf

Tester & Leigh (2001); Tester & Davenport (2003)
Forward genetics: Novel genes for salinity tolerance

Screen near-origin **landraces** for altered shoot \([\text{Na}^+]\)

Supported hydroponics
100 mM NaCl
10-d-old leaf 3, leaf 4

190 *T. aestivum*
179 *T. durum*
92 *T. monococcum*
22 *T. urartu*
17 *T. tauschii*
68 *H. vulgare*
50 *H. spontaneum*
(n = 4 per accession)

Yuri Shavrukov, Nenah Mackenzie, Robin Hosking, Jairus Bowne, Pat Warner
Forward genetics:
Huge variation in ancestral wheats

>50-fold variation in leaf 3 Na⁺ concentration in 84 *T. monococcum* vars

Now: introgress traits into commercial lines; test effects on yield; establish mapping populations; elucidate physiological basis for Na⁺ exclusion

Bowne, Shavrukov & Tester, unpubl.
Reverse genetics: Candidate gene studies

Hypothesis-driven

Examine salt tolerant and intolerant species
- Arabidopsis, rice, Physcomitrella, Lachnagrostis

Several candidate gene (families) identified
- HKT, AVP, SOS1, NHX, ENA, GLR, TFs

Over-expressed and silenced
- constitutively, inducibly, in specific cell types
- and/or in a range of species
- yeast, Physcomitrella, Xenopus, Arabidopsis, rice, barley

Andrew Jacobs, Julie Hayes, Olivier Cotsaftis, Damian Drew, Widodo, Deepa Jha, etc
Constitutive over-expression of genes can increase tolerance in cereals

- *AtAVP1* increases salinity tolerance of barley (vacuolar pyrophosphatase)

- In some cases, cell-specific expression of candidate genes is necessary (e.g. *HKT genes*)

- Field trials underway

Rhiannon Schilling *et al.* unpublished
2. Australian Plant Phenomics Facility

- Phenotyping is the new bottleneck in forward genetics (1,000s of lines)
- Genotyping is relatively fast; genome sequencing advancing!
- Physiological characterization of plants (phenotyping) is still time consuming and labor intensive (e.g. drought tolerance)
APPF has two nodes:
Commissioned January 2010

High Resolution Plant Phenomics Centre
Canberra
Bob Furbank and Murray Badger

The Plant Accelerator™
Adelaide
Mark Tester and Geoff Fincher

$20 m
$32 m
The Plant Accelerator

High throughput phenotyping of plant populations

- 4,485 m² building, 2,340 m² of greenhouses, 250 m² for growth chambers
- 4 x 140 m² fully automated ‘Smart-houses’
  - Plants delivered on 1.2 km of conveyors to four sets of cameras
  - High capacity state-of-the-art image capture and analysis equipment
- First public sector facility of this type and scale in the world
  - NCRIS national facility to support Australian plant research
  - Full GM and quarantine containment status.
LemnaTec Imaging system
Image capture

Side View  
Side View 90°  
Top view  

Barley cv. Sahara  
Bettina Berger
Non-destructive phenotyping for drought and salinity research

- Colour imaging
  - biomass, structure, phenology
  - leaf health (chlorosis, necrosis)
- Near infrared imaging
  - tissue water content
  - soil water content
- Far infrared imaging
  - canopy/leaf temperature
- Fluorescence imaging
  - physiological state of photosynthetic machinery
- Automated weighing and watering
  - water usage, imposing drought/salinity conditions
System can quantify morphometric parameters

Object properties
- minimum enclosing rectangle
- minimum enclosing circle
- convex hull
- compactness

E.g. wilting:
- Alters rectangle parameters
- Increases area below top of pot
- Increases the rotational moment
3. What’s next in sustainability??
ARC Centre in Plant Cell Wall Biology (2011-2017)

Research question:

• How do plants regulate the synthesis, assembly and re-modelling of their cell walls?

Mission:

• To advance fundamental scientific understanding of plant cell wall biology to enable sustainable biomass production for food security, human health, and energy biomass conversion.
1) key fundamental biological questions unanswered
   • cell walls of grasses particularly important

2) cell walls determine quality of most plant-based products
   • human nutrition and health
   • fibres for textiles
   • timber and paper products

3) major renewable biomass for liquid transport biofuels
   • 180 billion tonnes cellulose generated each year
   • lower plants (algae) and biodiesel production.
Research Programs

GENES  ➔  ENZYMES  ➔  POLYSACCHARIDES  ➔  CELL WALLS  ➔  BIOCOMPOSITES

Program 1: Molecular genetics ➔
   (Rachel Burton)

Program 2: Molecular architecture ➔
   (Mike Gidley)

Program 3: Cell biology/biochemistry ➔
   (Tony Bacic)
ARC Centre
(basic cell wall biology)

- Human Health (dietary fibre)
- Stalk Strength (lodging)
- Timber, Paper & Textiles
- Algal Biomass
- Syngas; Cellulosic Bioethanol Production
- Malting and Brewing
- Animal Feed
- Baking

Delivery of Research Outcomes

Approx. $30 million over 7 years
Final Comments

- plenty of work to be done on plant cell wall biology
- ARC Centre of Excellence in Plant Cell Wall Biology: jobs available 2011-2017
- fundamental science to support agricultural sustainability into the future.
Abiotic Stress Tolerance in Cereals

Geoff Fincher

ABIC

Saskatoon, Canada

14 September 2010