High throughput phenotyping for measuring drought tolerance

Bettina Berger, Mark Tester

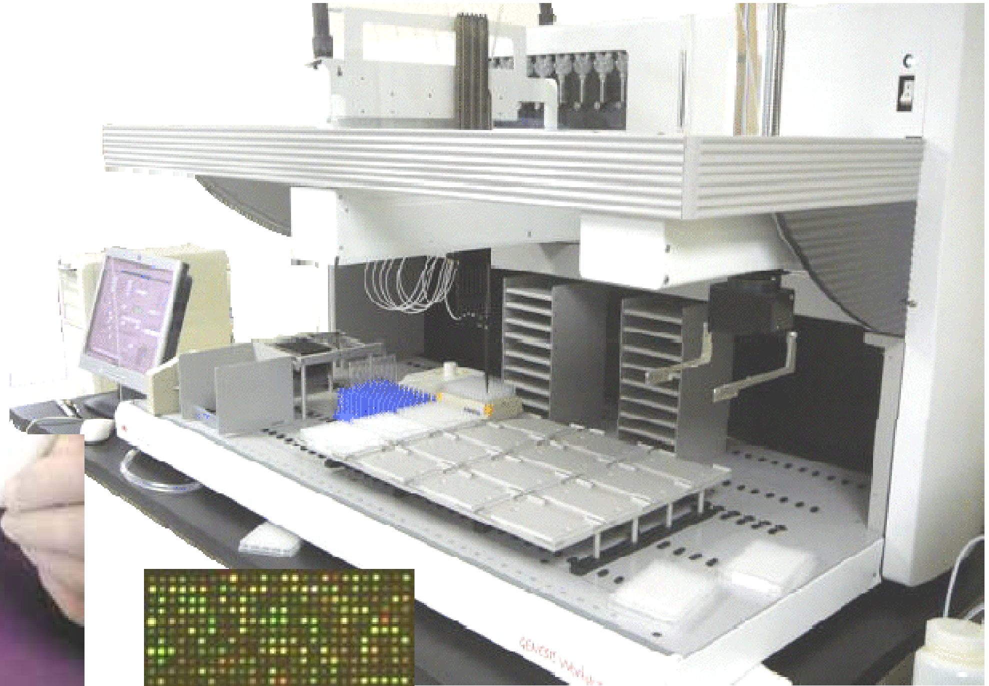
InterDrought III – Shanghai - October 2009

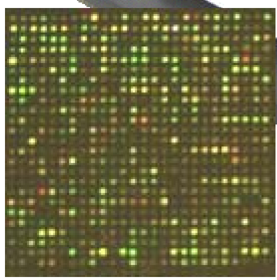
ACPFG
AUSTRALIAN CENTRE FOR PLANT FUNCTIONAL GENOMICS PTY LTD

Australian Plant Phenomics Facility

Why high throughput phenotyping?

- Genomics has accelerated gene discovery due to high throughput techniques and robotics





Why high throughput phenotyping?

- Physiological characterization of plants is still time consuming and labor intensive
- Phenotyping has become the new bottleneck in functional plant genomics

Why high throughput phenotyping?

- Phenotyping is essential for
  - functional analysis of specific genes
  - forward and reverse genetic analyses
  - production of new plants with beneficial characteristics
- High throughput is essential for phenotyping
  - in different growth conditions (e.g. watering regimes)
  - of many different lines
    - mutant populations
    - mapping populations
    - breeding populations
    - germplasm collections

Technological opportunity

- automated plant handling
- noninvasive imaging throughout the lifecycle
- powerful computing for automated data analysis

The Australian Plant Phenomics Facility

The Plant Accelerator Adelaide

Mark Tester (mark.tester@acpfg.com.au)

High Resolution Plant Phenomics Centre Canberra

Bob Furbank (robert.furbank@csiro.au)

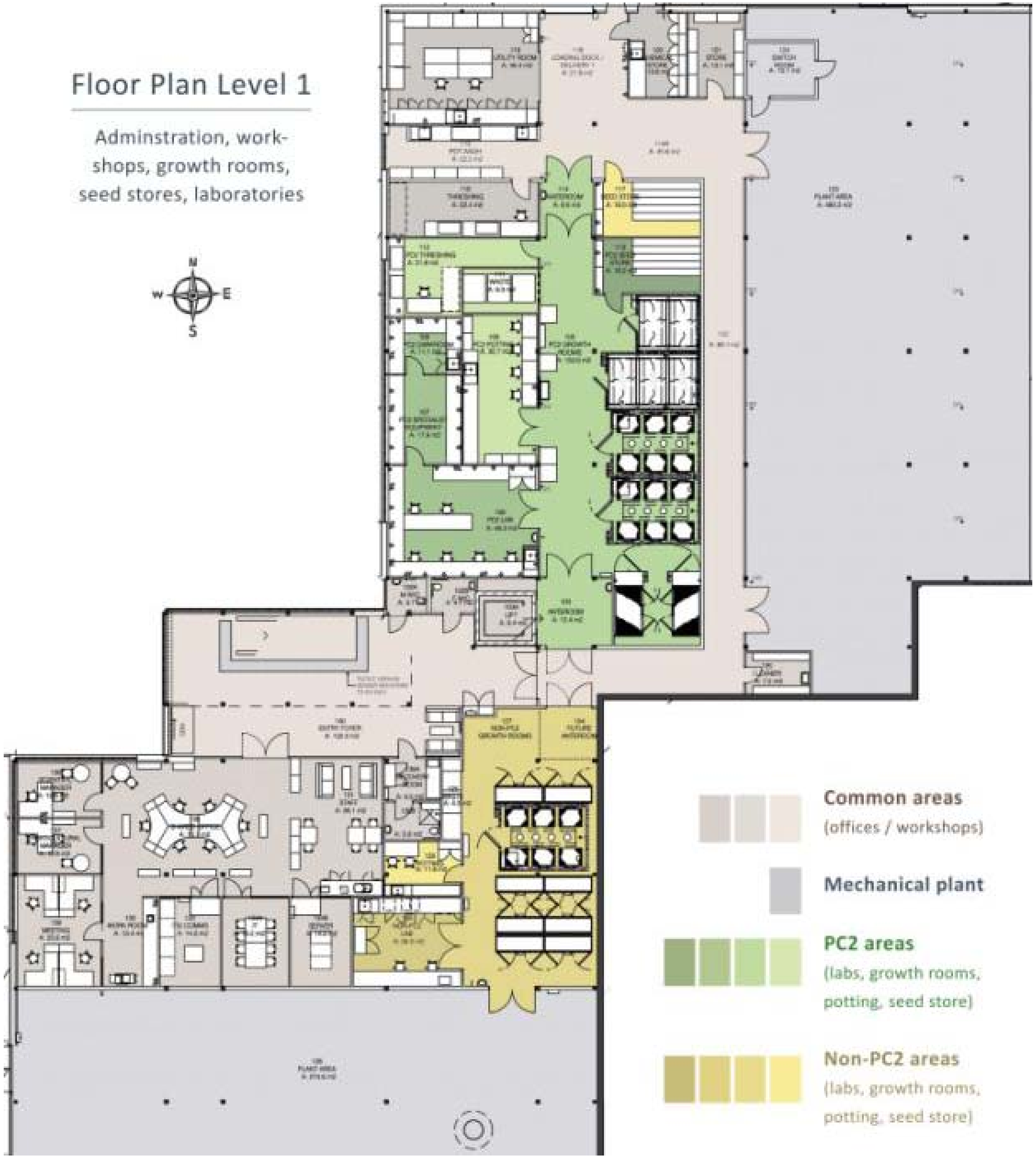

The Plant Accelerator
ACPFG

The Plant Accelerator

- 4,485 m^2 building, 2,340 m^2 of greenhouses, 250 m^2 for growth chambers
- Grow > 100,000 plants annually in a range of conditions
- 4 x 140 m^2 fully automated 'Smarthouses'
  - plants delivered on 1.2 km of conveyors to five sets of cameras
  - high capacity image capture and analysis equipment
  - regular, non-destructive measurements of growth, development, physiology
- First public sector facility of this type and scale in the world
  - Owned by University of Adelaide
  - Full GM and quarantine status
- UniSA and ACPFG established a Chair and Assoc Prof in Plant Phenomics and Bioinformatics

Floor Plan Level 1

Adminstration, work-
shops, growth rooms,
seed stores, laboratories



Common areas
(offices / workshops)

Mechanical plant

PC2 areas
(labs, growth rooms, potting, seed store)

Non-PC2 areas
(labs, growth rooms, potting, seed store)

Floor Plan Level 2

Greenhouses & Imaging

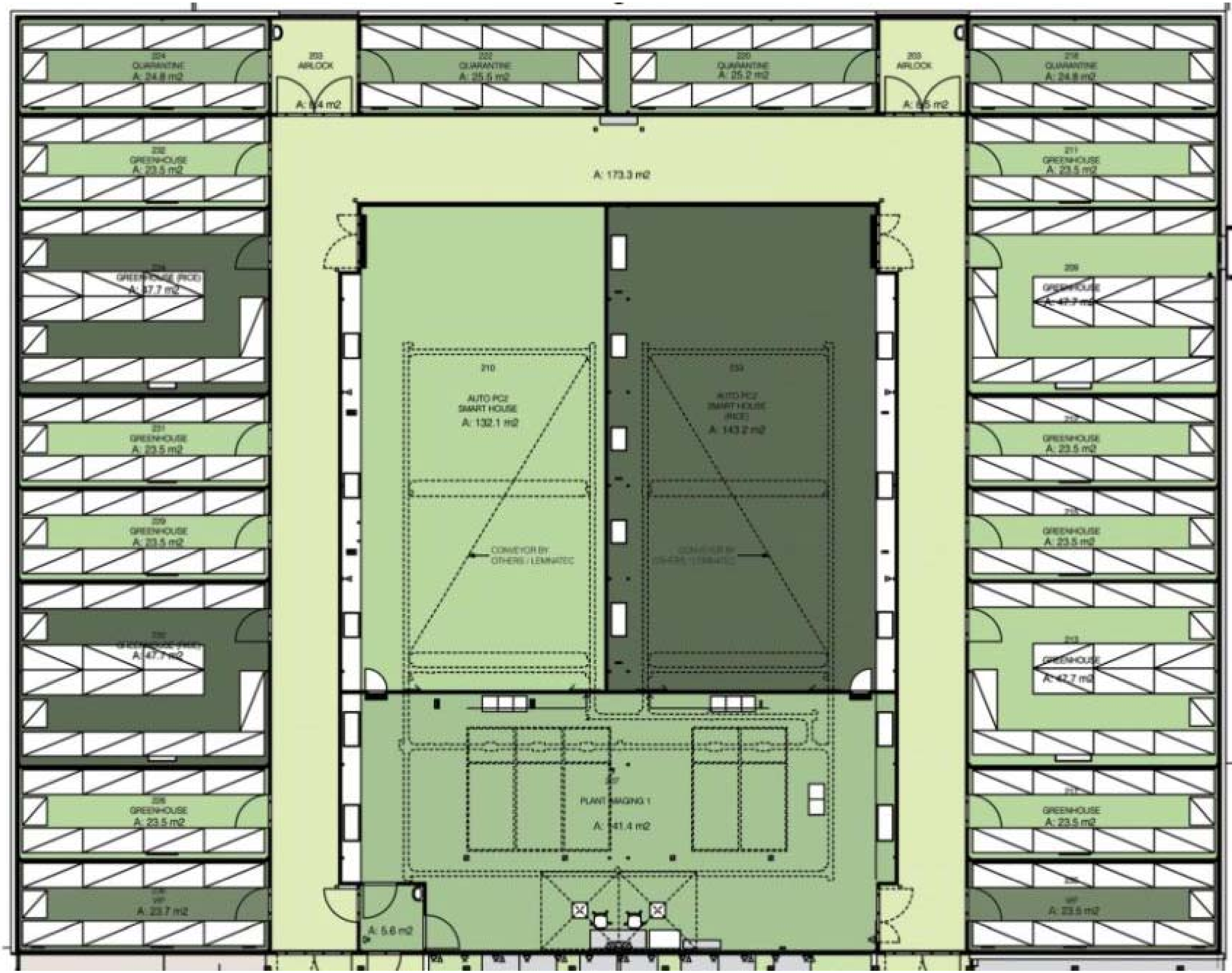
Common areas

PC2 areas

(Green- and Smart-houses: Rice, VIP, Quarantine, Standard)

Non-PC2 areas

(Green- and Smart-houses: Rice, VIP, Quarantine, Standard)



QUARANTINE
A: 24.8 m2
AIRLOCK
QUARANTINE
A: 25.5 m2
QUARANTINE
A: 25.2 m2
AIRLOCK
QUARANTINE
A: 24.8 m2
GREENHOUSE
A: 23.5 m2
A: 173.3 m2
GREENHOUSE
A: 23.5 m2
GREENHOUSE (RICE)
A: 47.7 m2
GREENHOUSE
A: 47.7 m2
AUTO PC2
SMART HOUSE
A: 132.1 m2
AUTO PC2
SMART HOUSE
(RICE)
A: 143.2 m2
GREENHOUSE
A: 23.5 m2
GREENHOUSE
A: 23.5 m2
GREENHOUSE
A: 23.5 m2
GREENHOUSE
A: 23.5 m2
CONVEYOR BY
OTHERS / LEMNATEC
GREENHOUSE
A: 47.7 m2
PLANT IMAGING 1
A: 141.4 m2
GREENHOUSE
A: 23.5 m2
GREENHOUSE
A: 23.5 m2
A: 23.7 m2
A: 5.6 m2
A: 23.5 m2

The site 5 months ago

Australian Plant Phenomics Facility

The site last week

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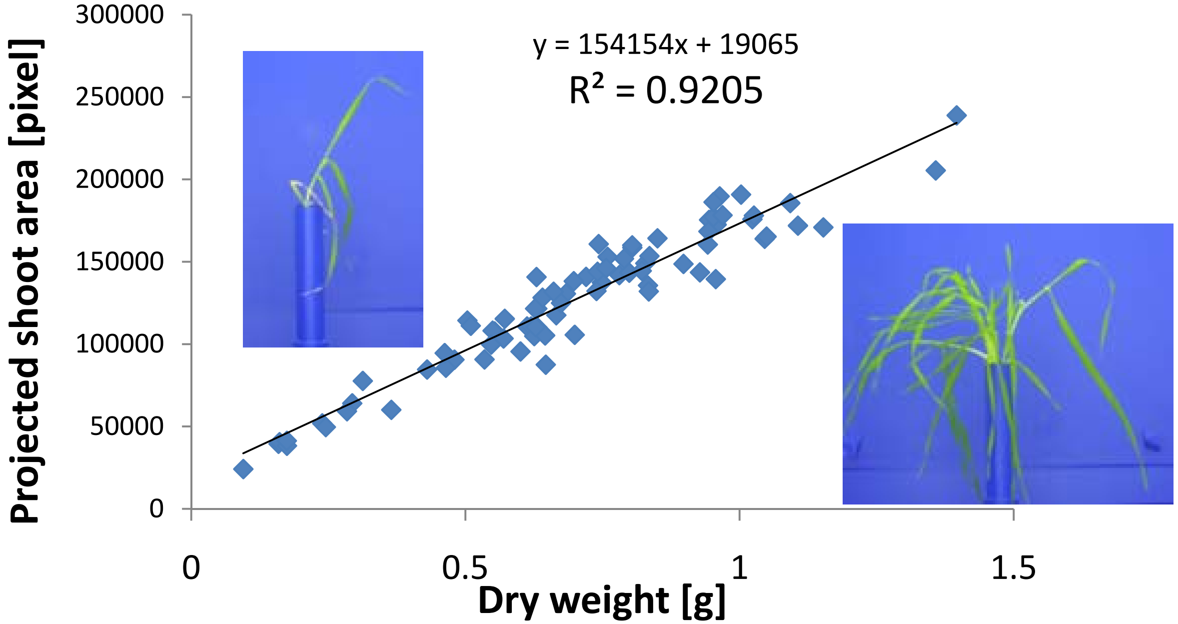
Measuring techniques relevant for drought research

- 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, control of drought conditions

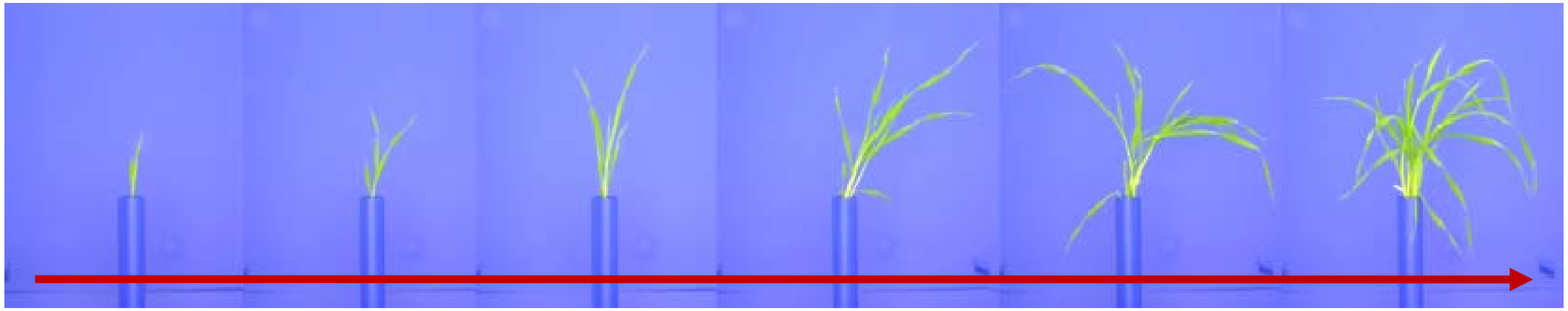
Plant imaging to estimate biomass

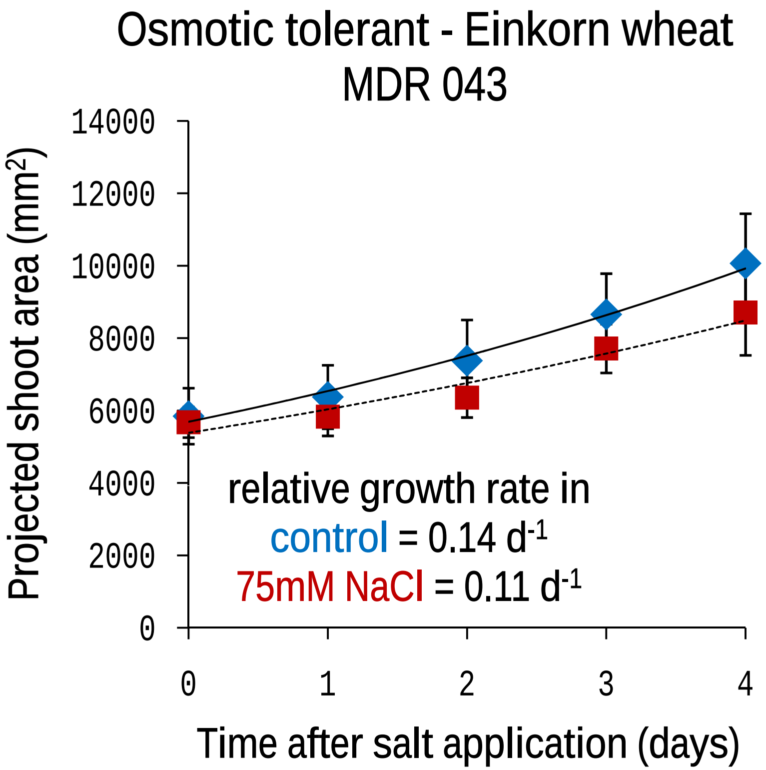
- The projected shoot area of the RBG images gives a good correlation with shoot fresh and dry weight
- Tested for various plant species
  - wheat, barley
  - rice
  - cotton
  - arabidopsis …

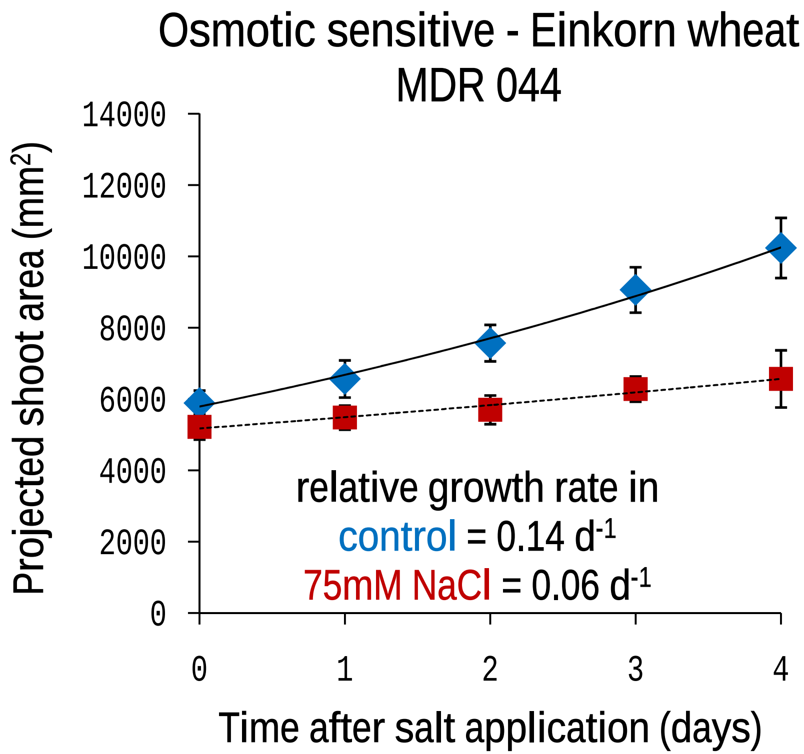


5w old barley plants, 8 cultivars

Measurement of plant growth







Rajendran et al. (2009) PCE, 32, 237-249

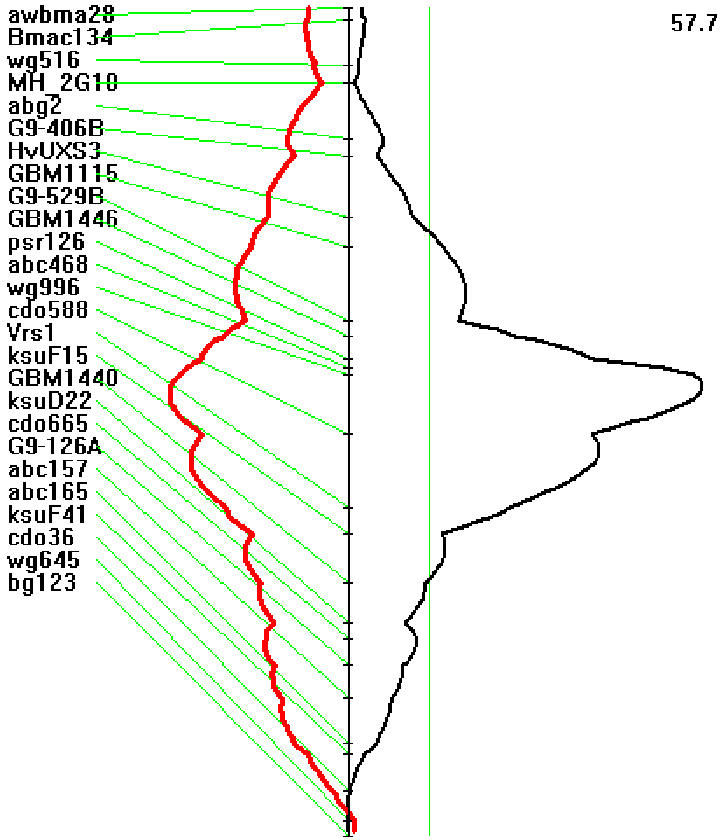
Use of colour information
e.g. boron toxicity screen

Colour classified image

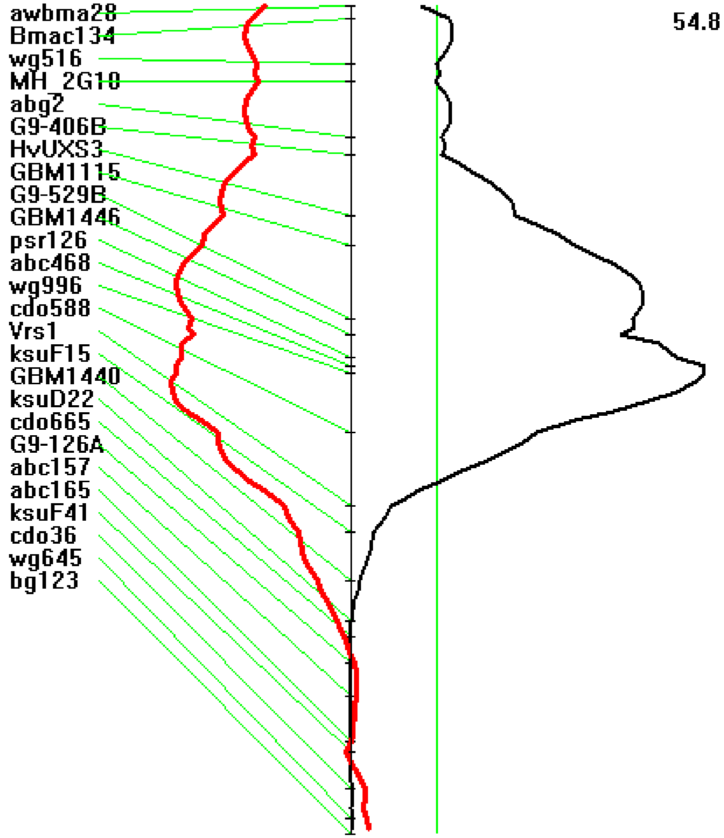
Line	Green area	Necrosis area	% Necrosis
Sahara	30739	4232	12%
Clipper	11640	15321	57%

Julie Hayes, Margie Pallotta and Tim Sutton, ACPFG

QTL for Ge tolerance identified using colour imaging overlaps QTL for B tolerance (1999)



B toxicity - leaf symptoms



Ge toxicity - leaf symptoms

Jefferies et al. 1999. TAG 98, 1293-1303

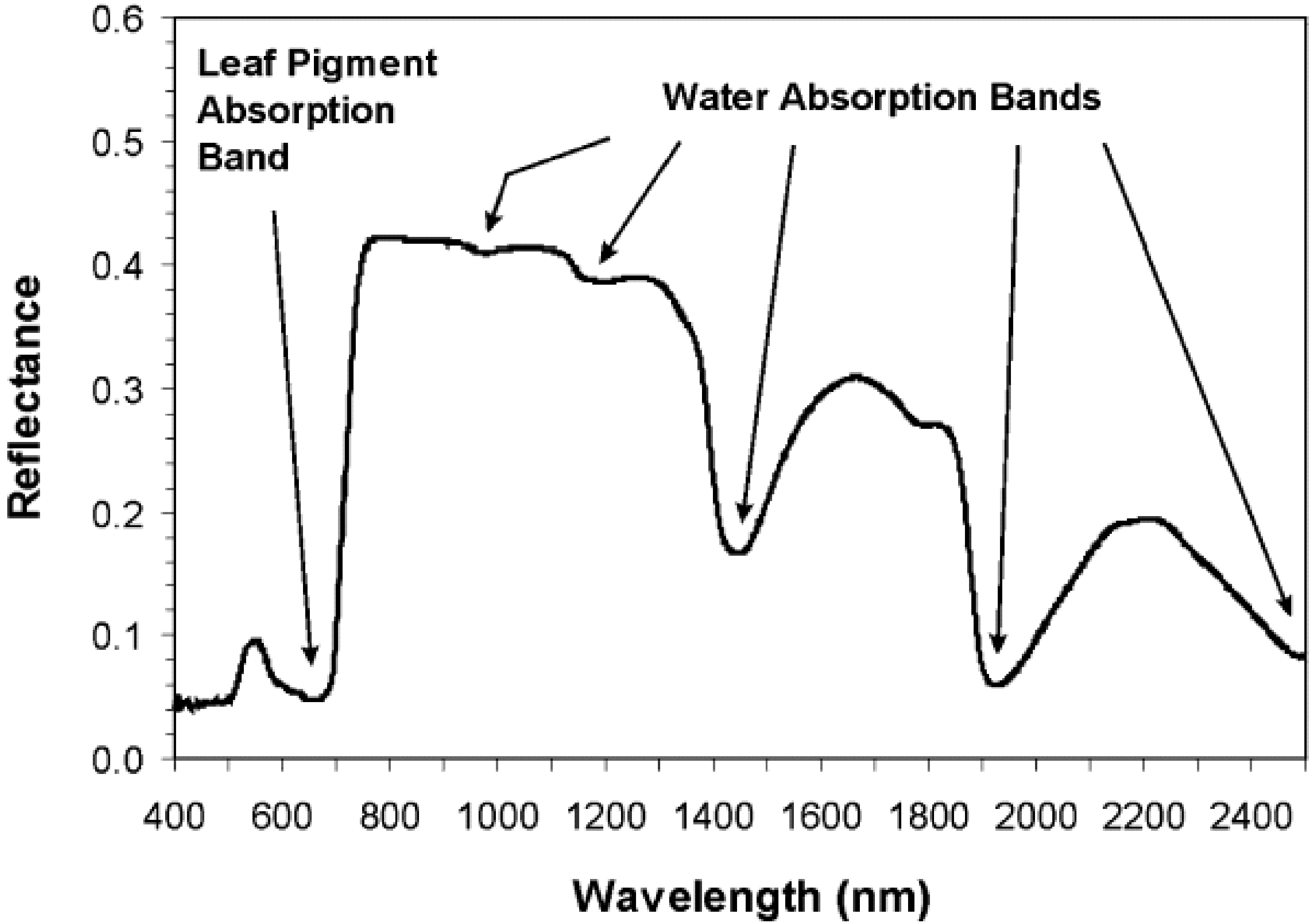
Hayes et al., unpubl., using LemnaTec

Advantage of imaging over visual assessment

- quantitative results
  - precise numeric values rather than score on arbitrary scale
- less subjective
  - selection of colour classes is still user driven but than applied to entire data set
- long term storage of images makes reanalysis at later stage possible
  - advancement in image analysis might improve quality of output
  - questions arising at later stage can be answered

- imaging allows measurement of wavelength non-visible to human eye

The idea behind NIR imaging

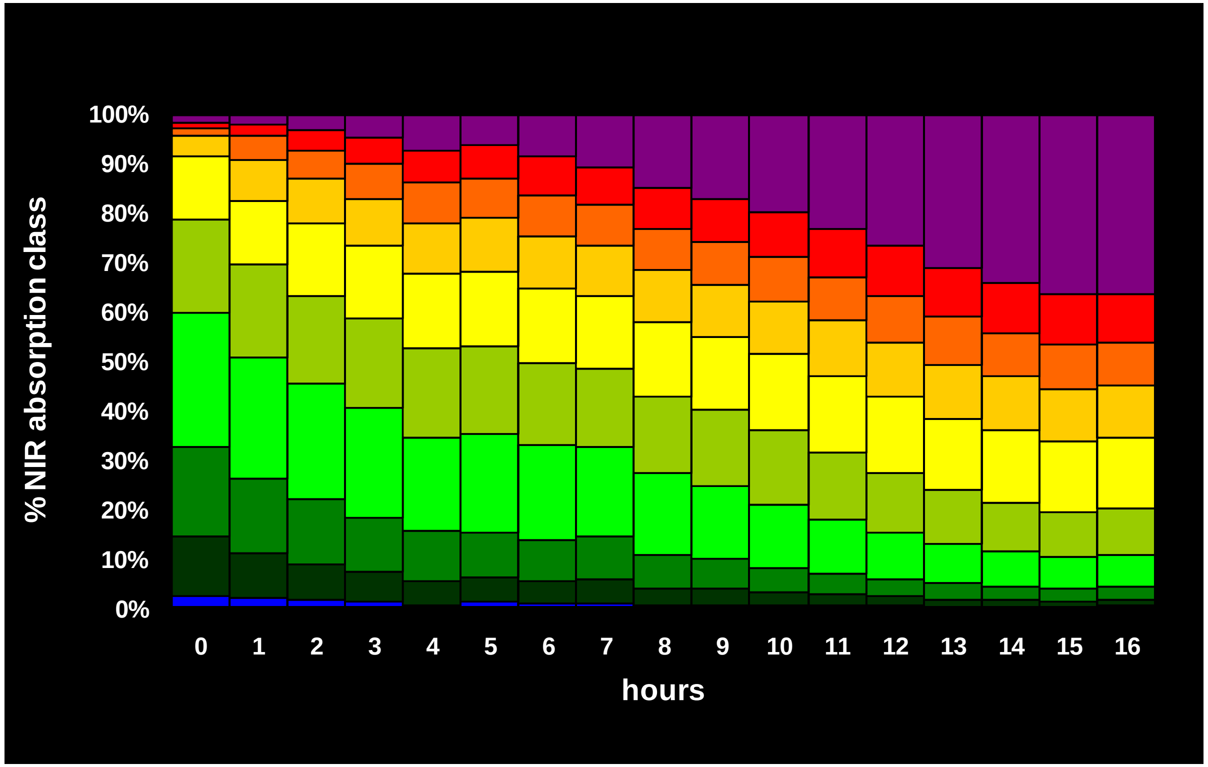


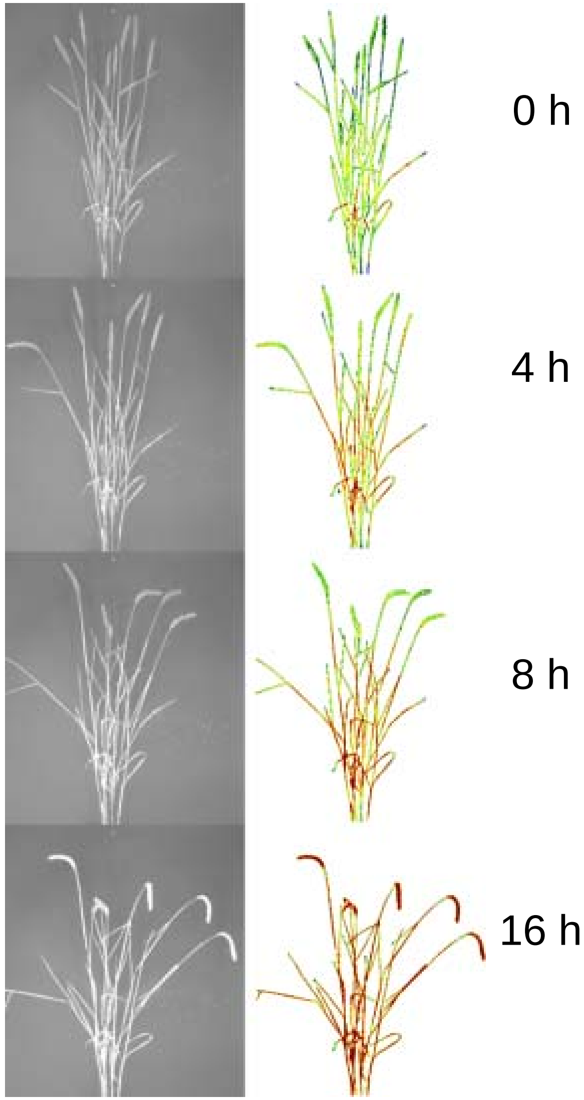
Reflectance spectrum of *S. lynise* leaf

Seelig et al. 2008, IJ Remote Sensing, 29,3701-13

NIR to monitor leaf water status

- The reflection in the NIR increases with decreasing water content in the leaves



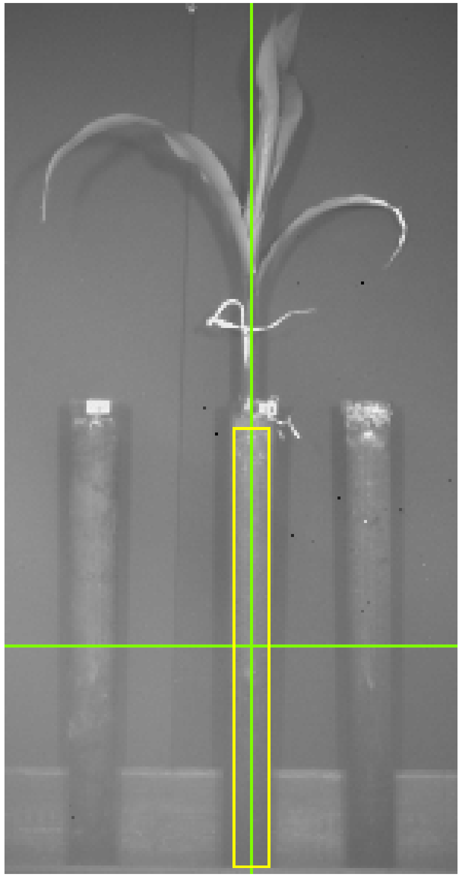


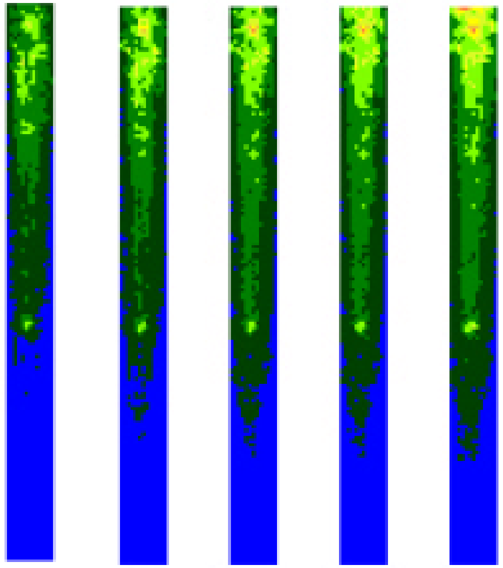
Data courtesy of Lemnatec

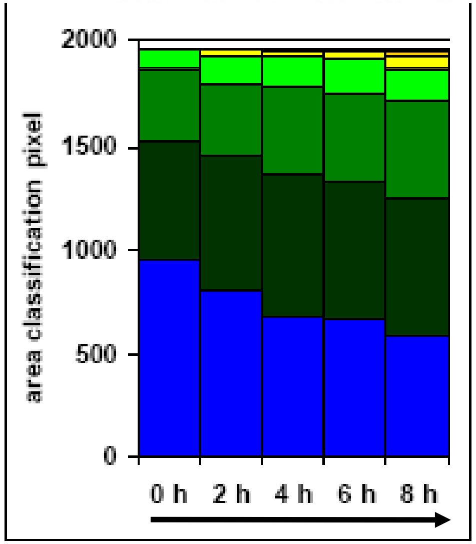
Root NIR to monitor water uptake

- Corn plant grown in a transparent 8 cm polyacryl column

- Results of NIR monitoring allow measurement of spatial distribution of water content in soil

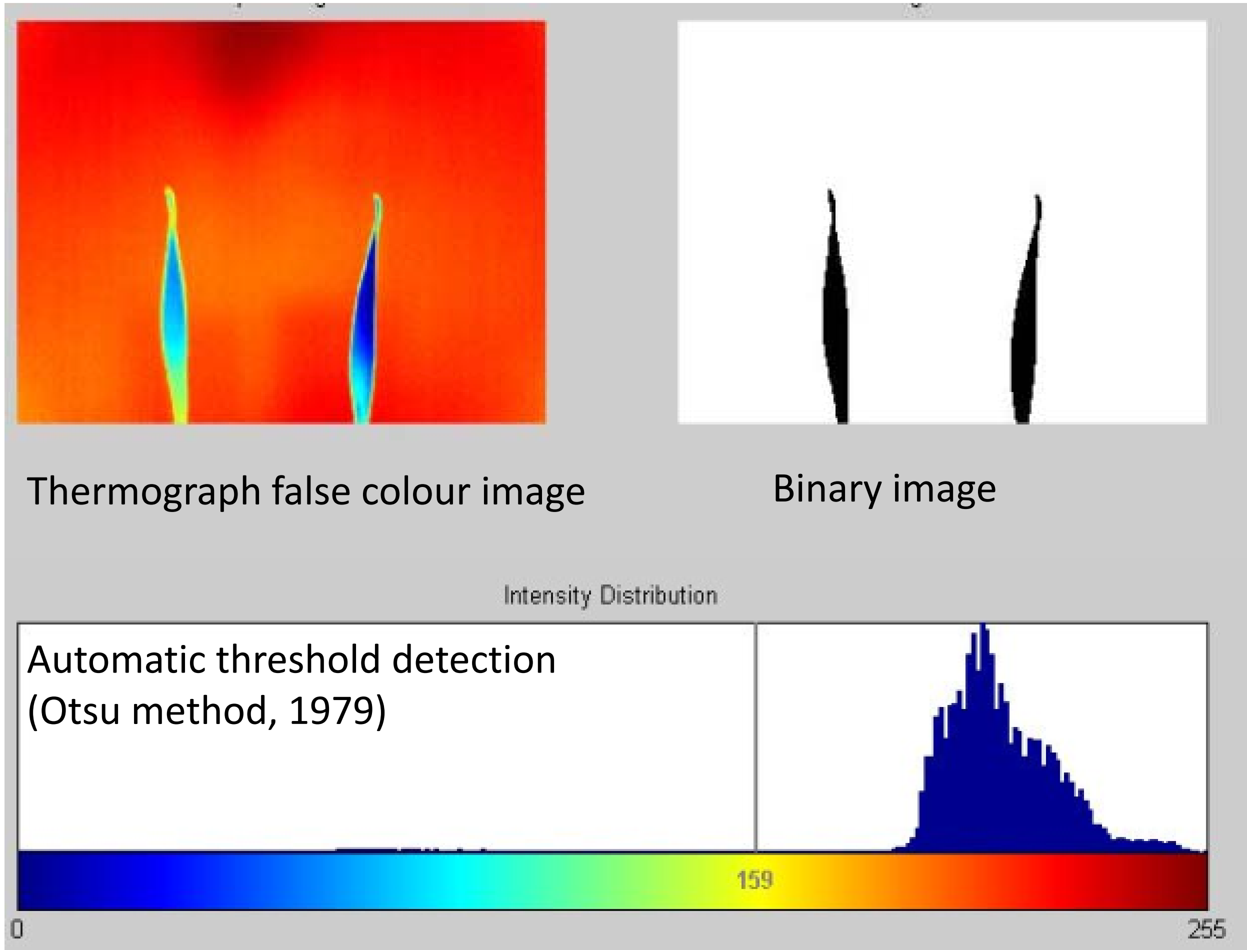


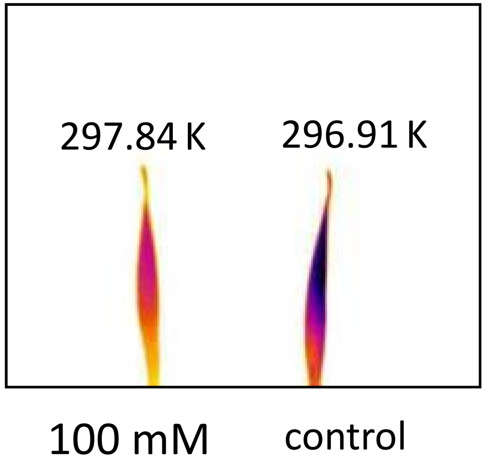




Data courtesy of Lemnatec

Far infrared to measure leaf temperature



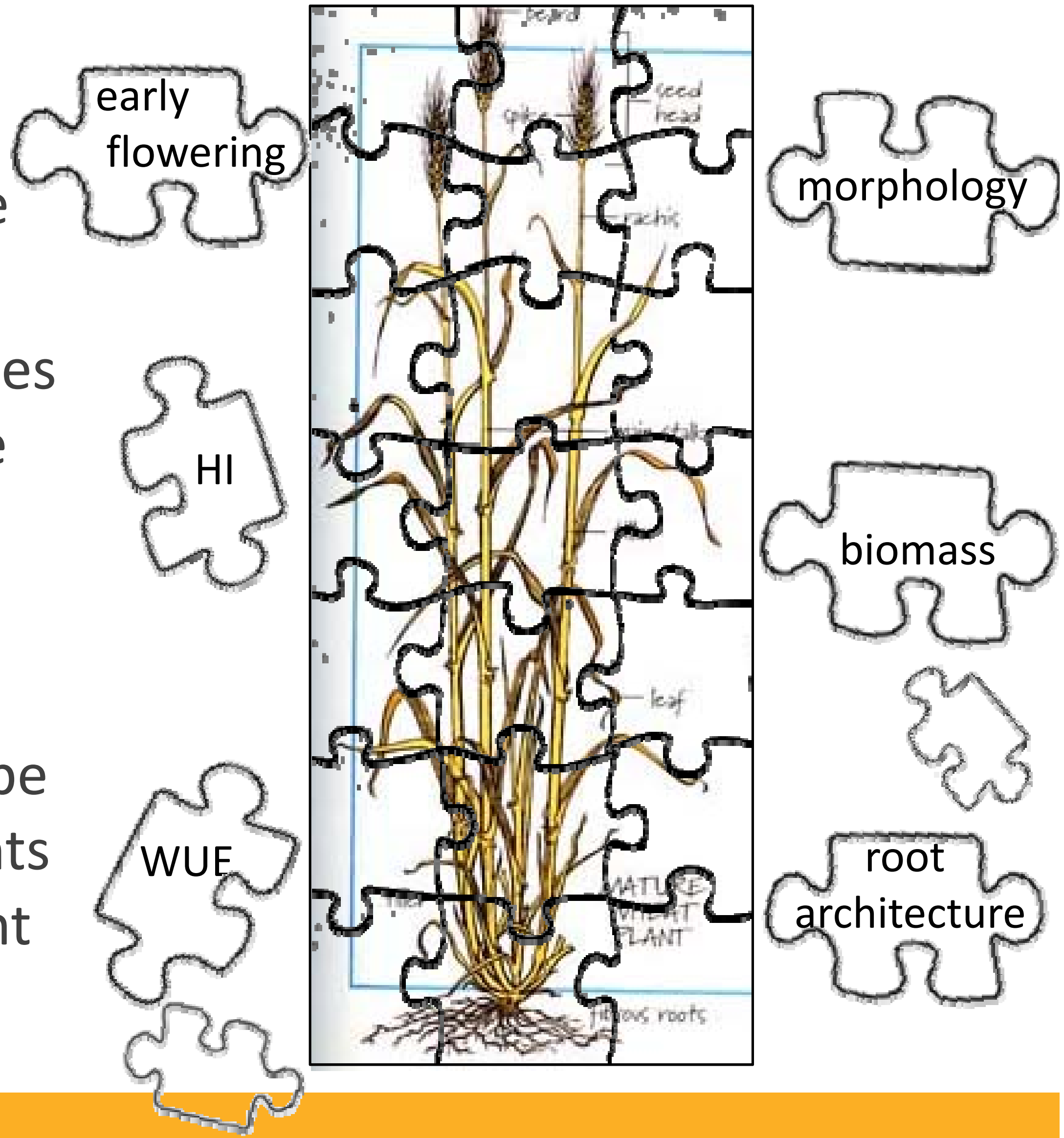


Δ = 0.93 K

Xavier Sirault & Bob Furbank, CSIRO, Canberra

Dissection of traits that contribute to drought tolerance

- Automated phenotyping allows screening for single traits
- High throughput techniques make forward and reverse genetic screens possible
- Gene discovery can be accelerated
- Genes for traits can then be combined to develop plants with tolerance for different environments



Challenges ahead

- optimize image analyses to obtain maximum information about plant structure – 3D plant modeling
- Data management and analysis
  - engage computer programmers, mathematicians, statisticians
- optimize image capture and analysis for nonvisible wavelengths (NIR, FIR, fluorescence)
- validation experiments:
  - to calibrate for leaf and soil water content
  - to test relevance of traits measured in controlled conditions to yield in the field

An invitation

- The Australian Plant Phenomics Facility is a research facility to be used by plant scientists
  - national and international researchers welcome
  - collaboration and provision of facilities and services
- A new opportunity to document germplasm collections in a standardised way, rapidly and reliably
- It provides a new opportunity to take a forward genetic approach to elucidate components that contribute to drought tolerance
  - bringing together physiology and genetics

The Accelerator team to date

- Mark Tester
- Geoff Fincher
- Helli Meinecke – business manager
- Bettina Berger – postdoctoral scientist
- James Eddes – computer programmer
- Robin Hosking – horticulturalist
- Richard Norrish – electrical engineer
- Karthika Rajendran – PhD student
- Brett Harris – Honours student
- Desmond Lun, Irene Hudson, Shalem Lee – UniSA collaborators