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http://www.plantphenomics.org.au/

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Phenotyping - the new bottleneck in plant science

- Genomics is accelerating gene discovery but how do we capitalise on these data sets to establish gene function and development of new genotypes?

- High throughput and high resolution analysis capacity now the factor limiting discovery of new traits and varieties

The technological opportunity

- Relieve phenotyping bottleneck with robotics, noninvasive imaging and analysis using powerful computing
- Provide “whole of lifecycle”, quantitative measurements of plant performance from the growth cabinet to the field
- Help deliver genomics advances to all plant science - e.g. model systems, cereals, grapevines, natural ecosystems
- **Accelerate** time from gene discovery to trait discovery and release of innovative new varieties

Why high throughput phenotyping?

- Phenotyping essential for
  - functional analysis of specific genes
  - forward and reverse genetic analyses
  - production of new plants with beneficial characteristics
- High throughput essential for phenotyping
  - in different growth conditions (e.g. under biotic or abiotic stress)
  - of many different lines (to discover the desirable line)
    - mutant populations
    - mapping populations
    - breeding populations
    - germplasm collections

Measuring systems and traits to be measured – model plants to crops

Established technology

- Colour images
  - Plant area, volume, mass, structure, phenology
  - Senescence, relative chlorophyll content, pathogenic lesions
  - Seed yield, agronomic traits
- Near IR imaging
  - Tissue water content
  - Soil water content
- Far IR imaging
  - Canopy / leaf temperature / water use
- Fluorescence imaging
  - Physiological state of photosynthetic machinery
- Hyperspectral imaging
  - Carbohydrates and protein

Future technologies

- X-ray computer tomographic images of roots in soil - Adelaide
- High resolution NMR-based imaging of roots in soil - Canberra
- Terahertz imaging of water content
- New software for new data mining

First paper from growth imaging system recently published:

Rajendran, K., Tester, M. &Roy, S.J. (2009) Quantifying the three main components of salinity tolerance in cereals. *Plant, Cell & Environment* **32,** 237-4

And a commentary in *Science* **325,** p 518

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Australian Plant Phenomics Facility – two nodes

Plant Accelerator Adelaide

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

High Resolution Plant Phenomics Centre Canberra

Bob Furbank (robert.furbank@csiro.au)

Plus $10M in Stimulus Package

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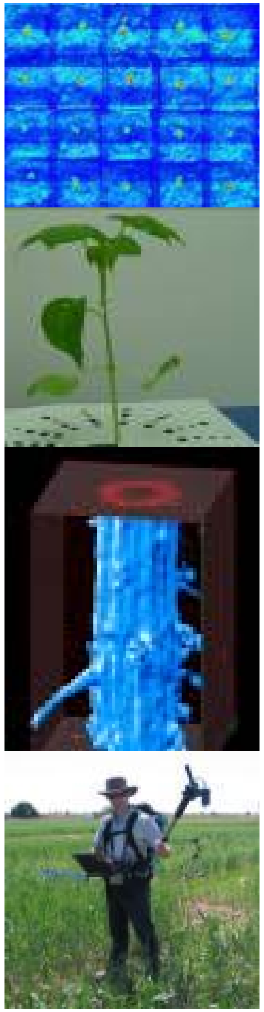
High Resolution Plant Phenomics Centre
From growth cabinet to the field



‘Deep phenotyping’ technology
- development, validation and deployment

- Model Plant Module (HTP)
- Crop Plant Shoot Module (MTP)
- Crop Plant Root Module (MTP)
- Crop Plant Field Module (HTP)

- 1500 m^2 lab space and ‘research hotel’
- Imaging modules interfaced with 245 m^2 greenhouse, 260 m^2 growth cabinets
- Large field site with distributed sensor networks portable ‘phenomobile’ and 15m imaging tower

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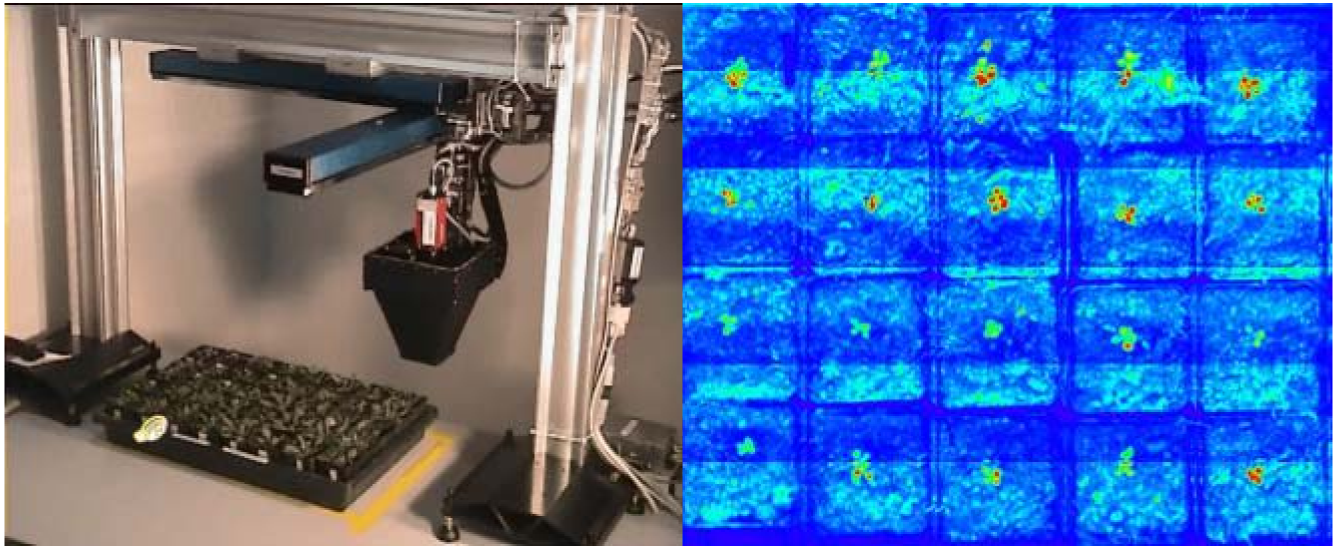
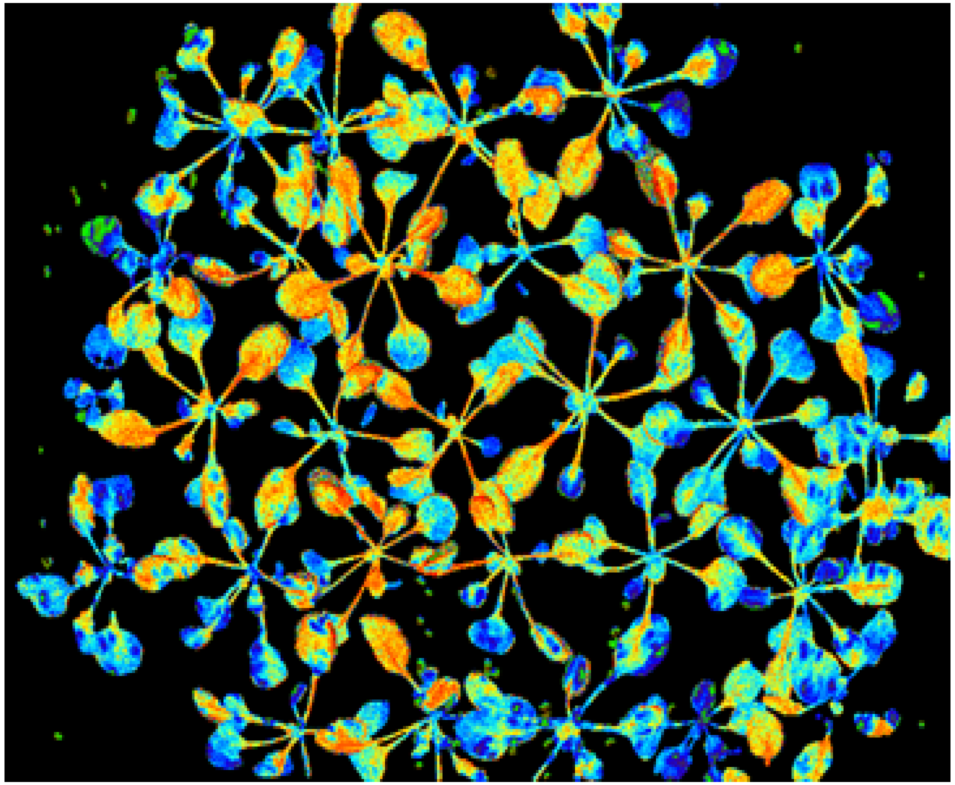
Model plant module
Imaging for growth cabinets and stress screens

Fluorogro-scan

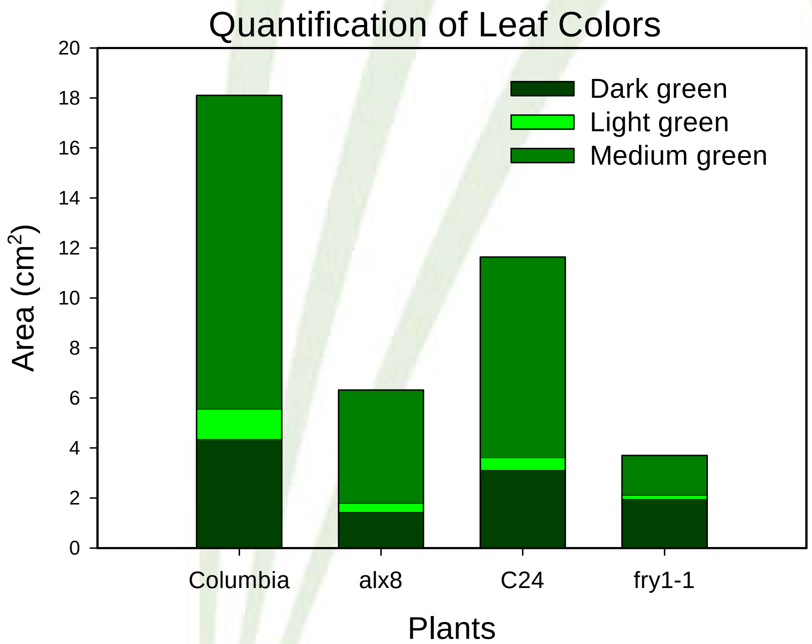


- Growth and morphology
- Photosynthetic performance (Chl Fluor) under defined conditions
- IR screening for leaf temperature
- Automated destructive sampling for metabolites, protein, DNA and RNA, delta^{13}C

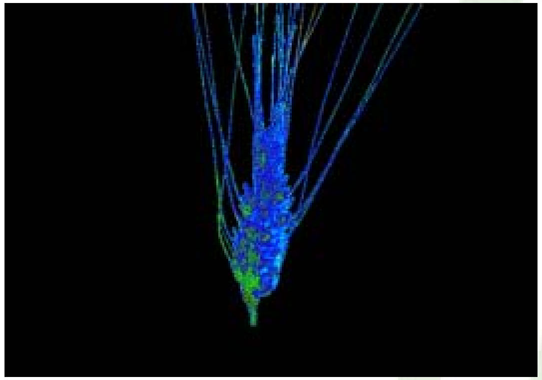
Data Analysis: non-destructive Growth Analysis and morphological clustering

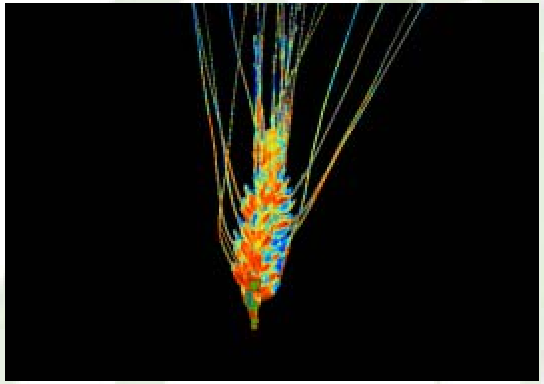


- Leaf area / growth analysis
- Lesions / pathogen attack
- Architecture / morphology
- Morphological clustering
- PODD phenotypic dBase



Crop Shoot Module :Growth imaging, 3D reconstruction and overlay of signals in controlled environments





- Whole of lifecycle photosynthesis and growth
- Dynamic growth and carbon allocation to plants organs
- Transpiration and water use
- Hyperspectral detection of leaf protein and CHO

Digital estimation of biomass validated for a range of species

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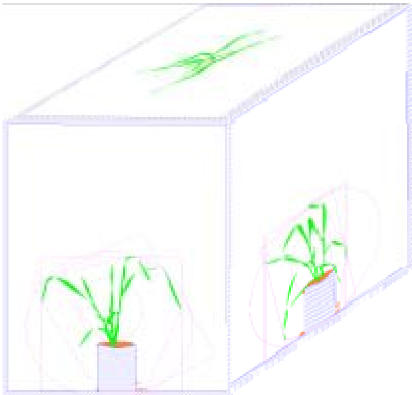
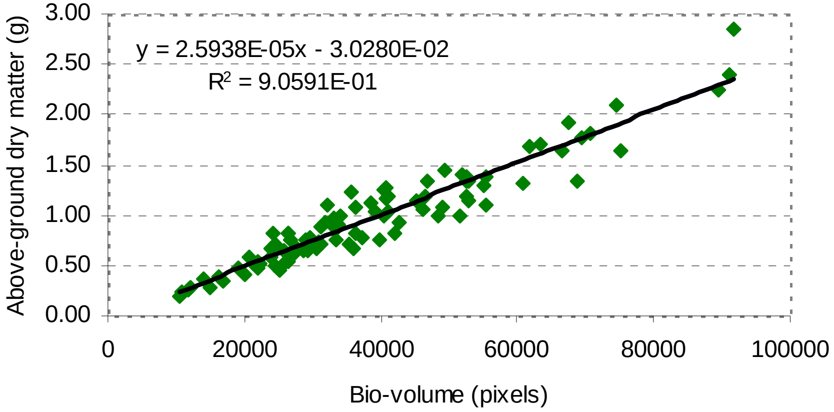
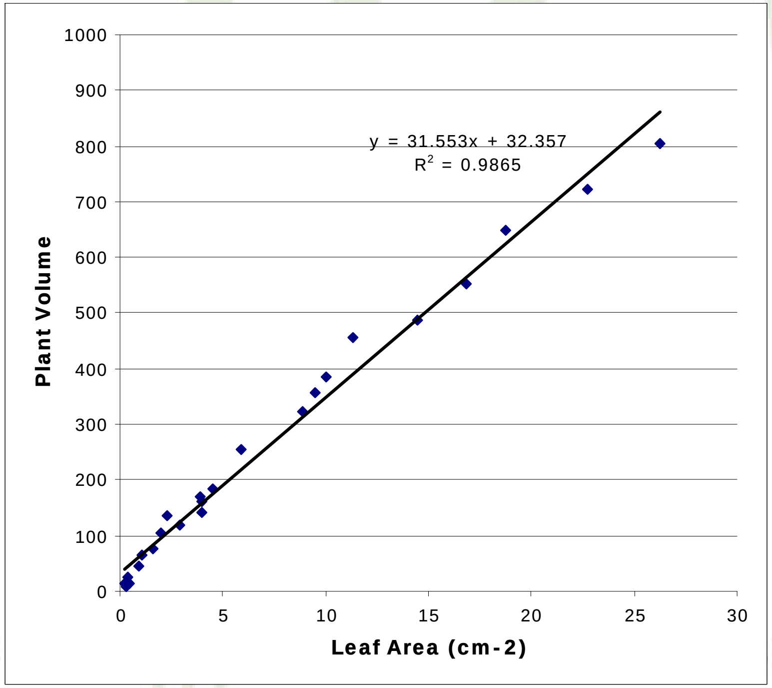
- Wheat
- Rice
- Cotton
- Chickpea
- Cowpea
- Flaveria
- Arabidopsis



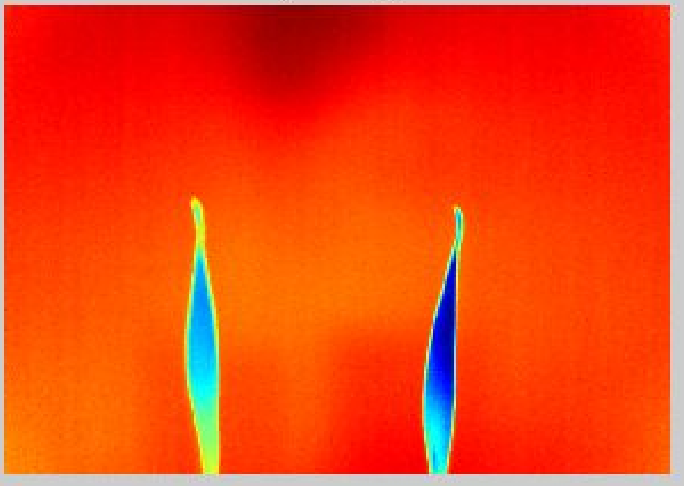
Relationship between biomass and bio-volume until late stem elongation stage





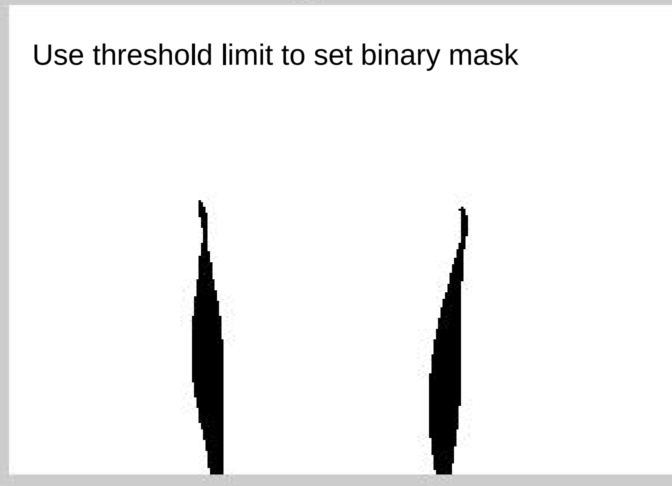
Seedling screens with IR thermography: osmotic stress tolerance

Input Image

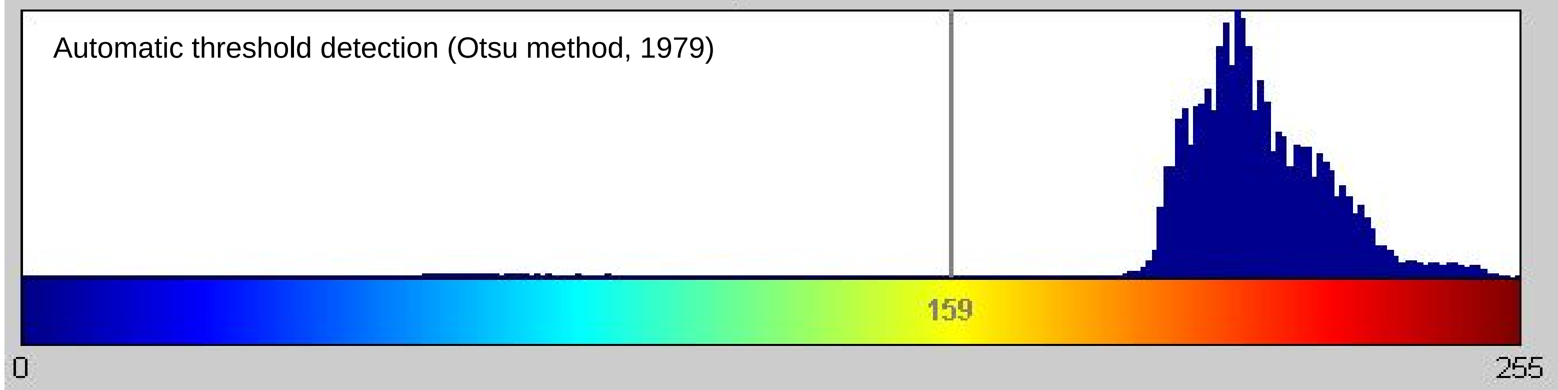


Thermograph: matrix of temperature [640x480]
(8-bit false colour image for visualisation)

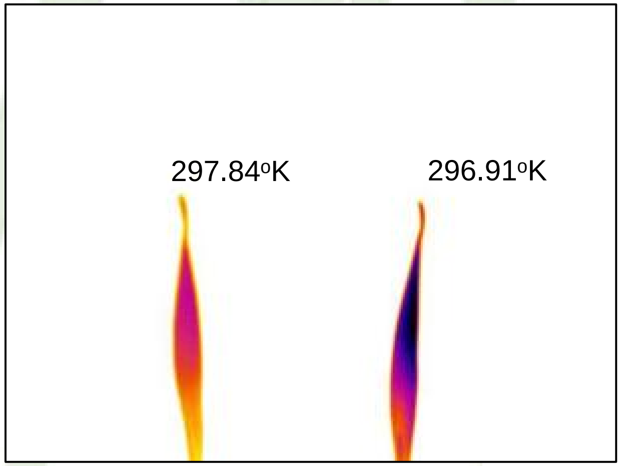
Segmented



Intensity Distribution



Array multiplication (element by element) to separate background from leaves and to apportion temperature data to leaf area



100 mM control

$\Delta = 0.93^oC$

Temperature data averaged for each plant and saved in EXCEL spreadsheet

Physiological consequences of osmotic stress

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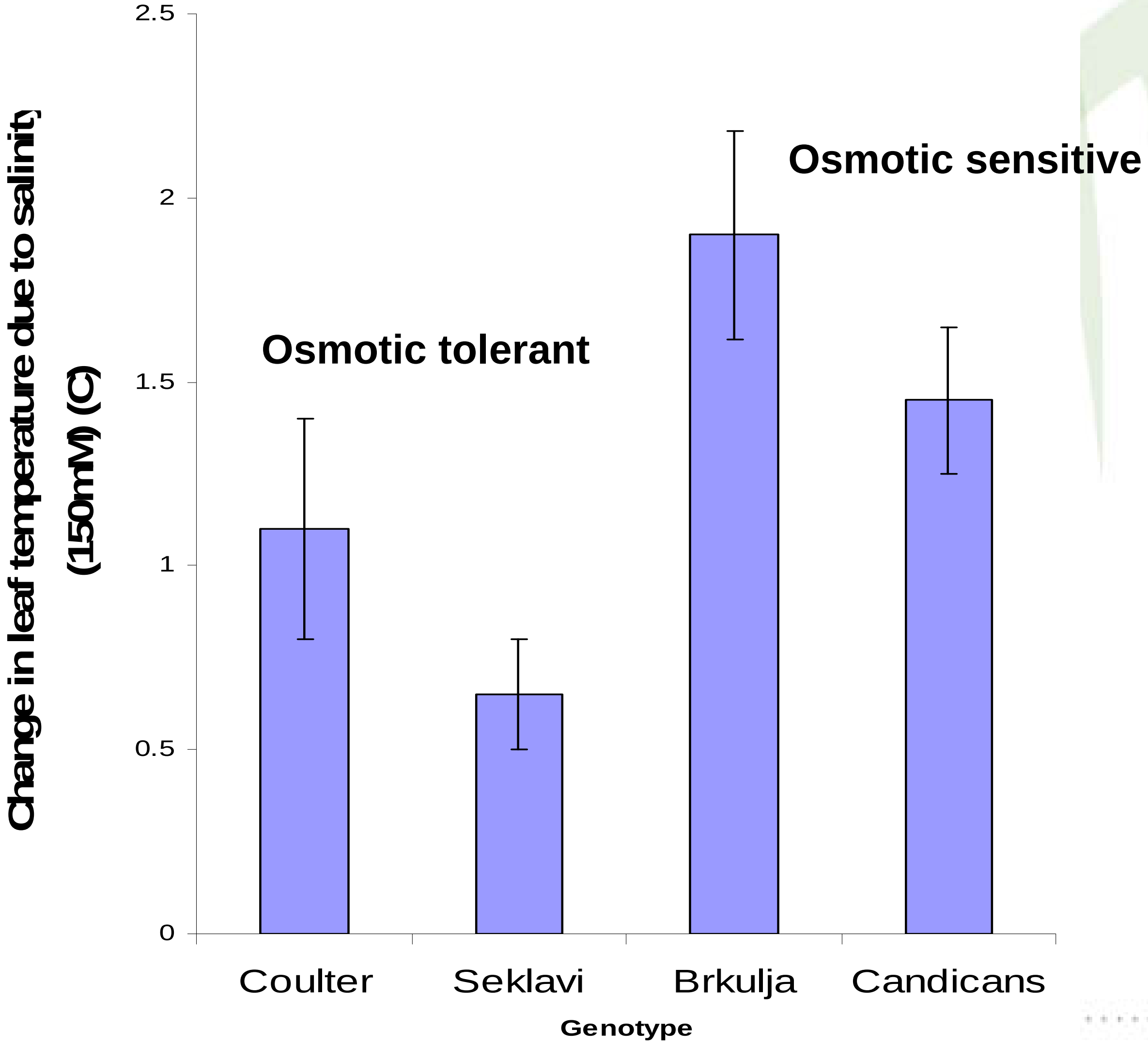
IR thermographic detection of leaf temperature is a powerful tool for screening for osmotic stress tolerance



Crop Plant Root Module : NIR imaging of soil moisture at TPA and HRPPC

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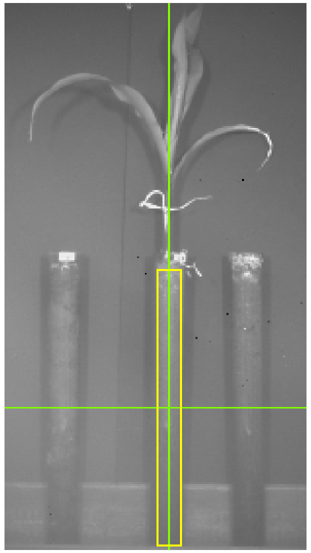
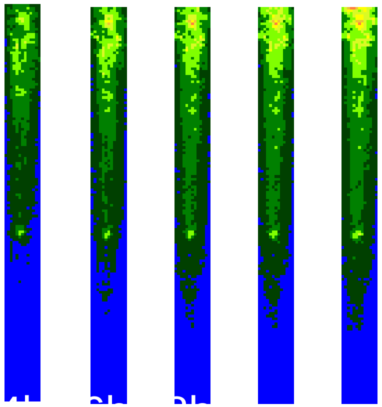
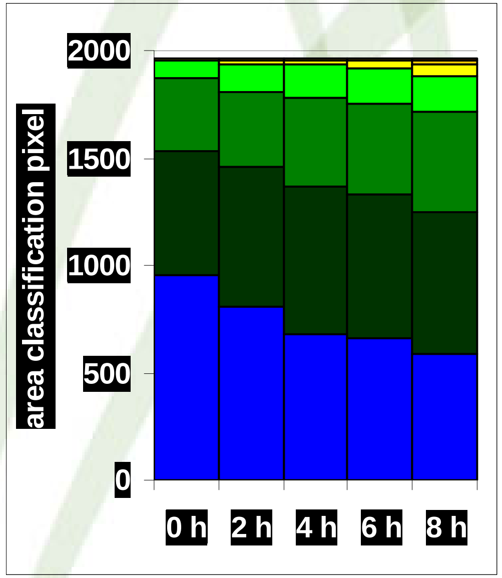


Corn plant grown in a transparent 8 cm polyacryl column



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



total development of drying over time

Data courtesy of Lemnatec

Rhizotron Shoot and Root Growth Imaging System HRPPC

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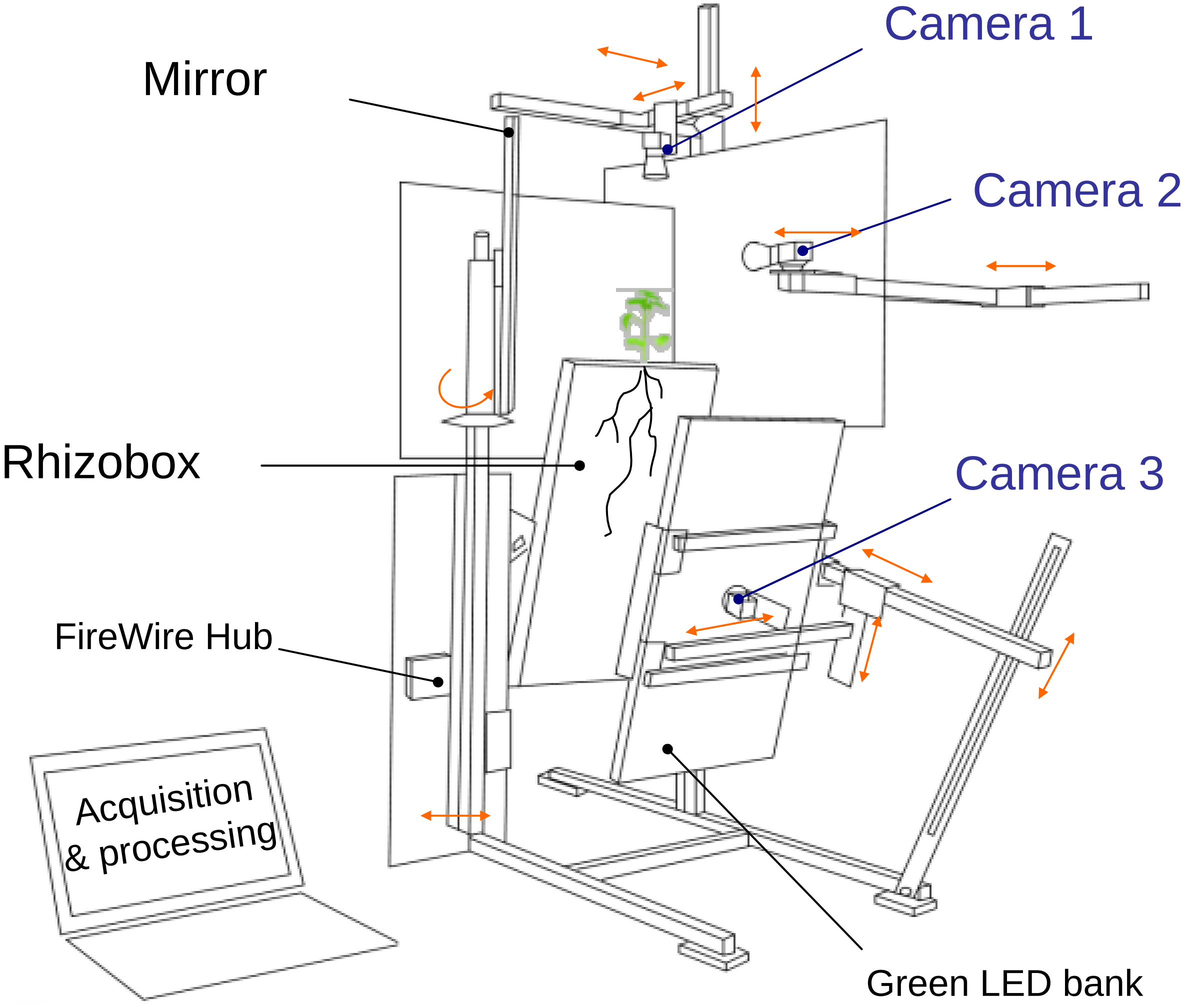
Top view

2 side views

Roots



Camera: FLEA2 – 2448 x 2048 pixels- 2/3'' CCD

Field Module: Non-destructive High Throughput Phenotyping from half plots to broadacre

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- Non-destructive estimate of biomass and crop structure pre and post-canopy closure
- Remote sensing of stress response, canopy water loss and photosynthetic response
- Remote sensing of chemical composition : CHO, protein N, pigments over entire lifecycle
- Application of distributed sensor networks for simultaneous continuous monitoring in the field (micromet plus low res versions of the above)
- Non-destructive detection of water and root biomass at depth in soil

Phenonet Distributed Sensor Network

- "Intelligent" sensors log via 3-G phone network
- Programmable to respond to data input (eg "if air T is <2C, log all ports every minute")
- Remote logging of canopy temp, micromet and RGB images for biomass and flowering time

40 cards ordered to be deployed 2009
Run indefinitely from 5cm X 5cm solar panel

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Phenomobile and imaging tower

15m tow behind tower

Gives $1m^2$ area coverage at 2M boom height

- **Variable span buggy (half to full plot span, 3M boom)**
- **IR Camera + Hyperspec Radiometer / camera**
- **Stereo camera / Lidar**
- **2cm Hi Res GPS registers all data**
- **Porometer / SPAD Licor 6400**
- **Fits on a trailer**

Nondestructive Estimation of CHO Pigments and Protein by Reflectance Chemometrics

ASD.Document 600540 Rev. F

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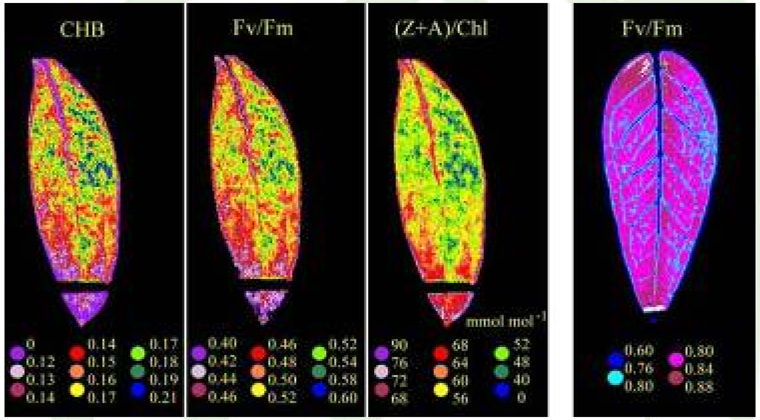
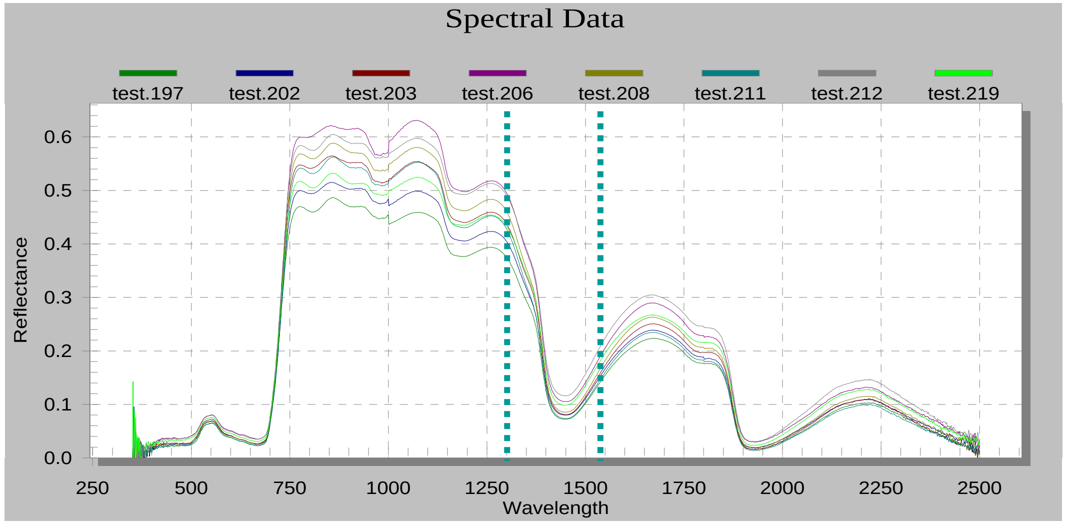
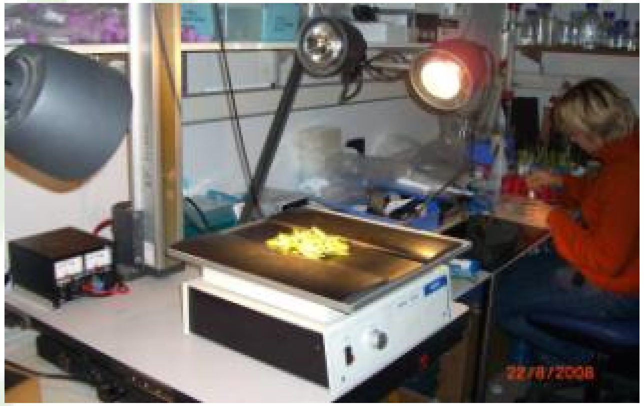
www.asdi.com







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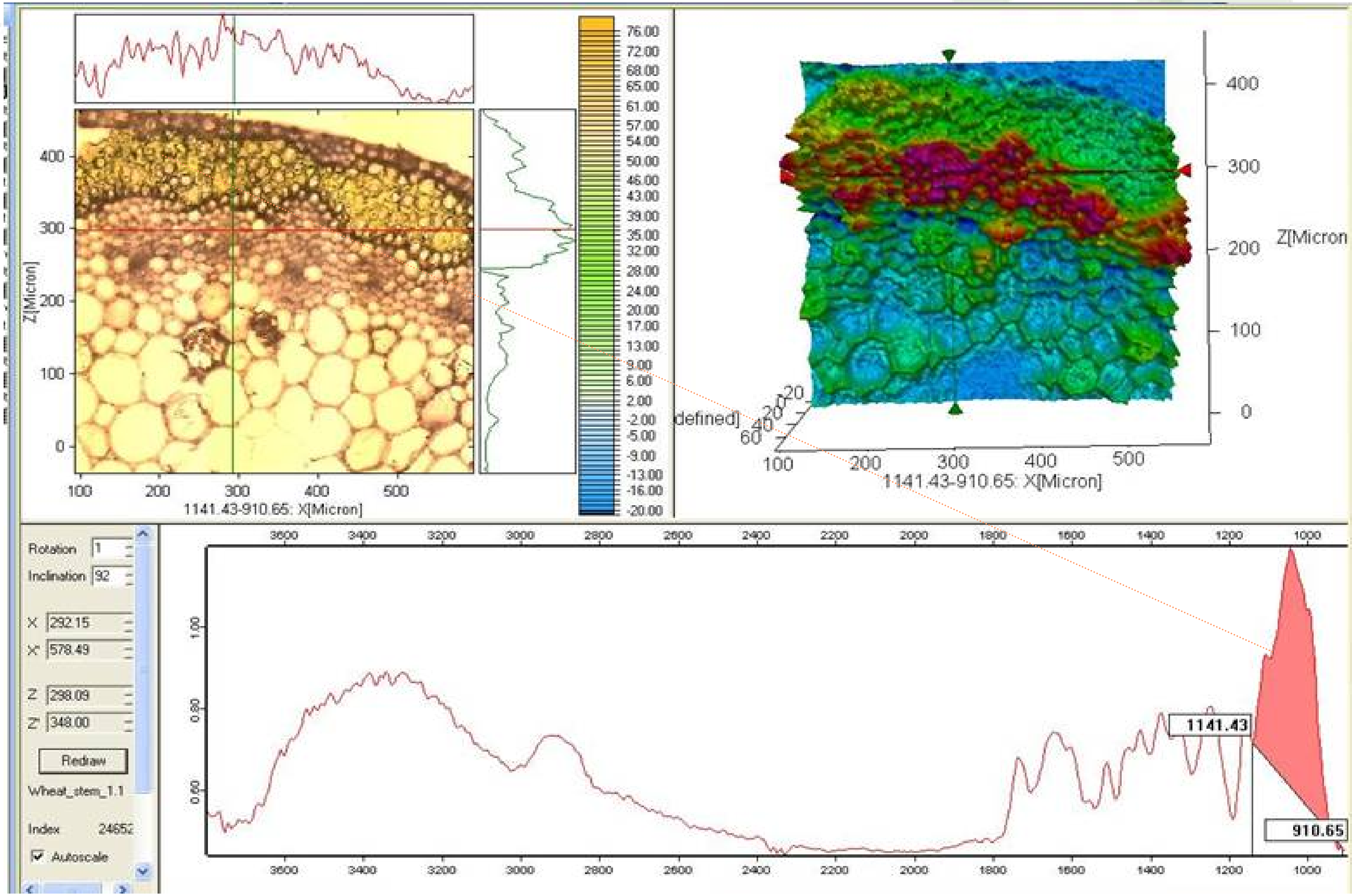
Micro module : Microimaging of Metabolites with FTIR Imaging



(scale: red correlates to high, green to medium and blue to low carbohydrate concentration)

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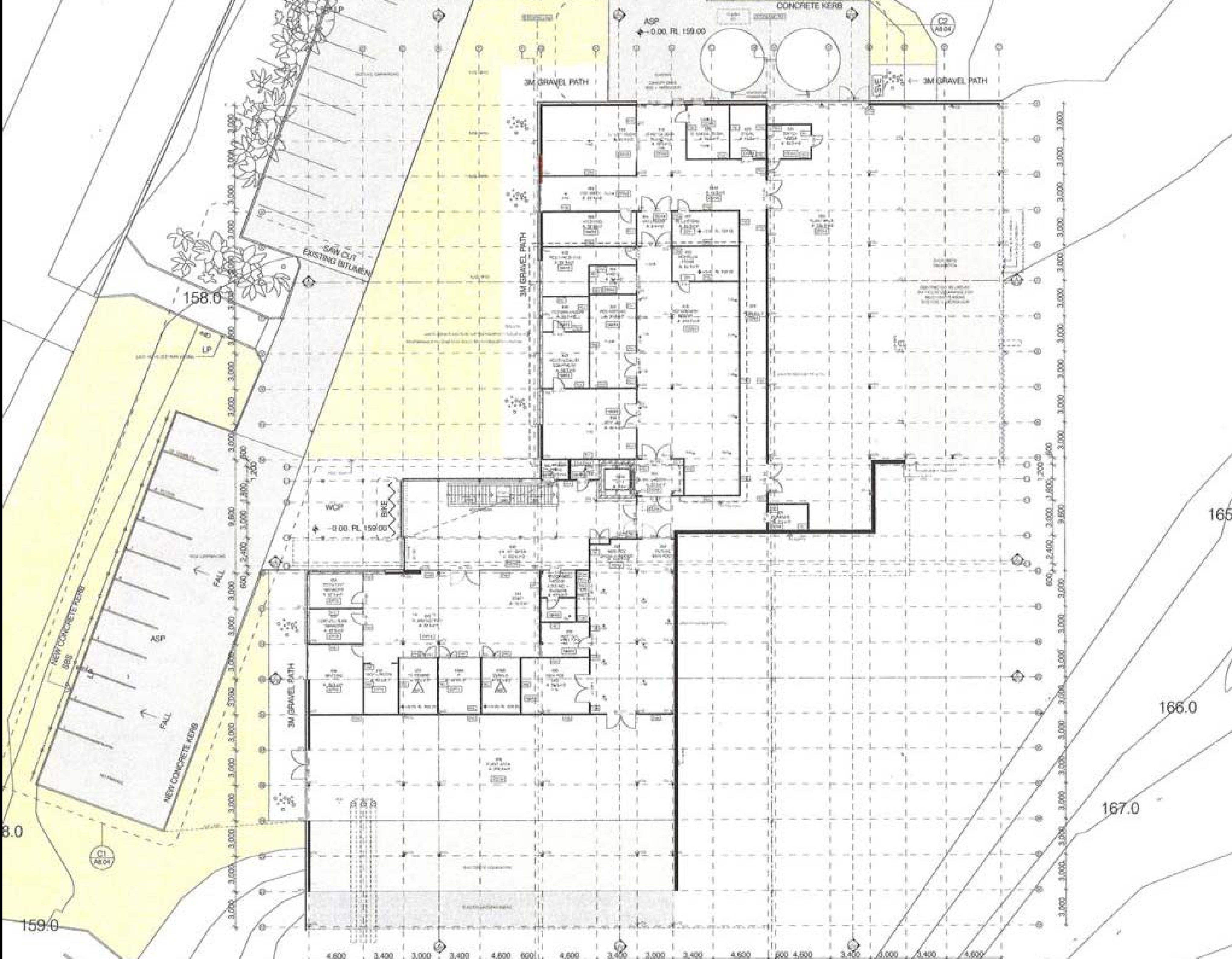
The Plant Accelerator

- 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
  - National facility to support Australian plant research
  - Full GM and quarantine status
- UniSA and ACPFG established a Chair and Assoc Prof in Plant Phenomics and Bioinformatics ($1.5m) – in School of Mathematics


CONCRETE KERB
ASP
0.00. RL 159.00
3M GRAVEL PATH
3M GRAVEL PATH
SAW CUT
EXISTING BITUMEN
158.0
3M GRAVEL PATH
LP
WCP
BIKE
0.00. RL 159.00
FALL
NEW CONCRETE KERB
ASP
SBS
FALL
NEW CONCRETE KERB
3M GRAVEL PATH
165
166.0
167.0
8.0
159.0
C1
A8.04
C2
A8.04
SVE
4,600
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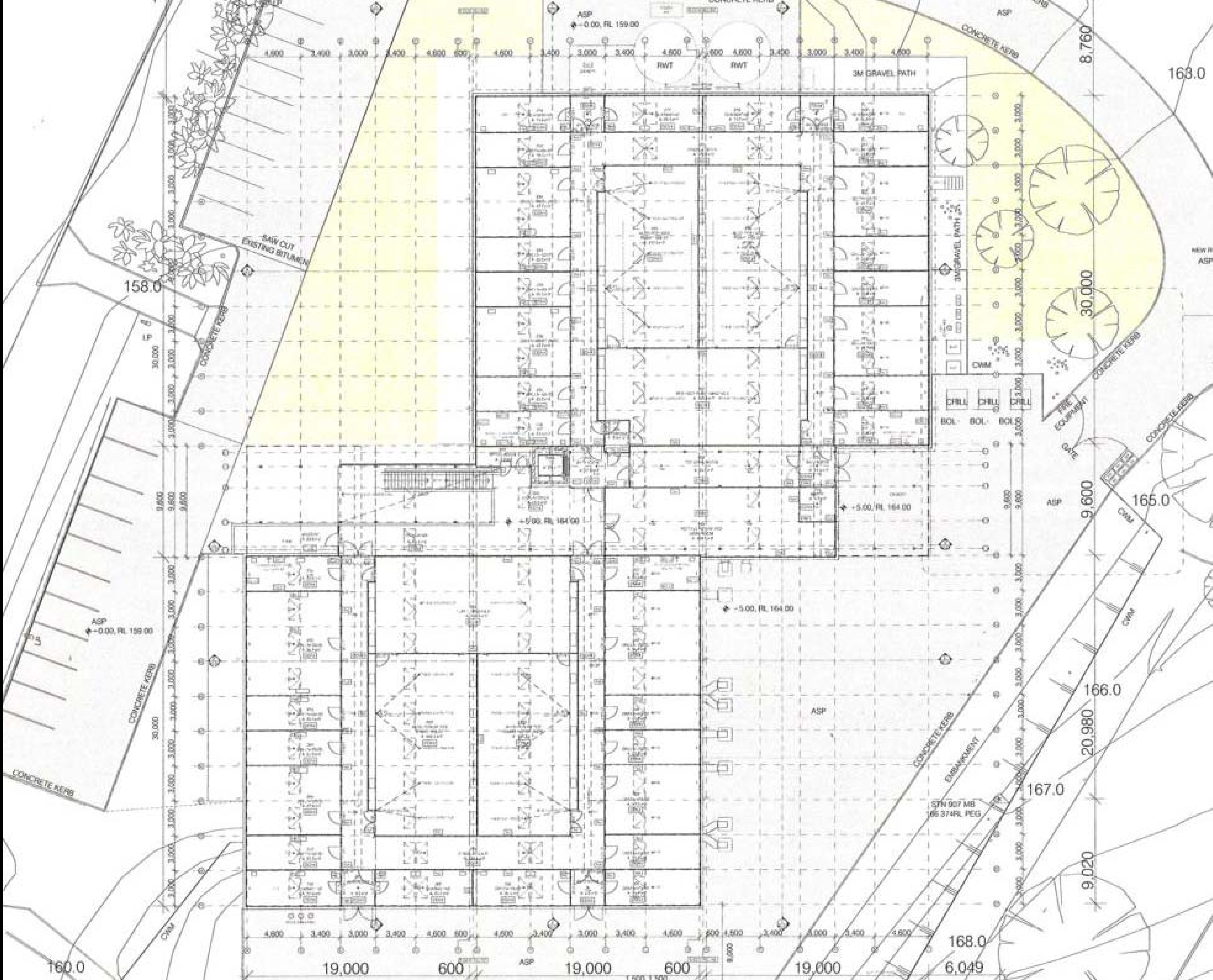

ASP
+0.00, RL 159.00
RWT
RWT
3M GRAVEL PATH
CONCRETE KERB
163.0
8,760
SAW CUT
EXISTING BITUMEN
158.0
LP
30,000
CONCRETE KERB
3M GRAVEL PATH
NEW R
ASP
30,000
CWM
GRILL
GRILL
GRILL
BOL
BOL
BOL
FIRE EQUIPMENT
GATE
CONCRETE KERB
9,600
ASP
+5.00, RL 164.00
+5.00, RL 164.00
165.0
9,600
CWM
+5.00, RL 164.00
ASP
+0.00, RL 159.00
CONCRETE KERB
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STN 907 MB
166.374RL PEG
CONCRETE KERB
9,020
CWM
4,600
3,400
3,000
3,400
4,600
600
168.0
160.0
19,000
600
ASP
19,000
600
19,000
6,049

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The Plant Accelerator - timeframe

- Business Manager and programmer in place
- Horticulturalist and electronics people start mid-year

- Construction started in early January 2009
- Conveyor and imaging installation starts in Sept 2009
- Fully commissioned by early-mid December 2009
- First tranche money ends June 2011

The phenotyping stations

LemnaTec
Imageprocessing in biology
Bildverarbeitung in der Biologie

Image capture complemented by metadata file compatible with, e.g., the Plant Ontology Consortium (www.plantontology.org)

System can quantify morphometric parameters e.g. canopy density, wilting

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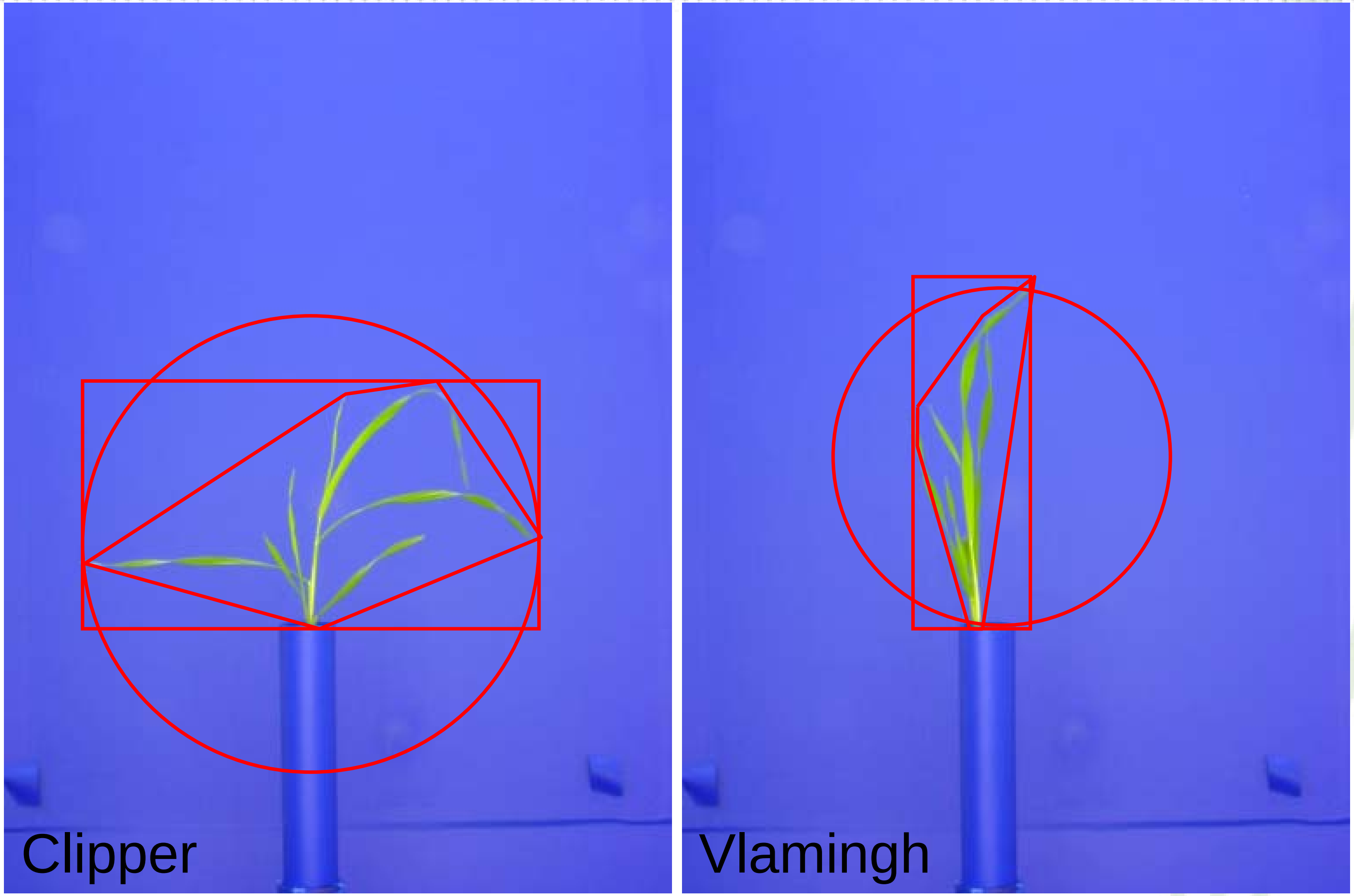


Object properties

- **minimum enclosing rectangle**
- **minimum enclosing circle**
- **convex hull**
- **compactness**

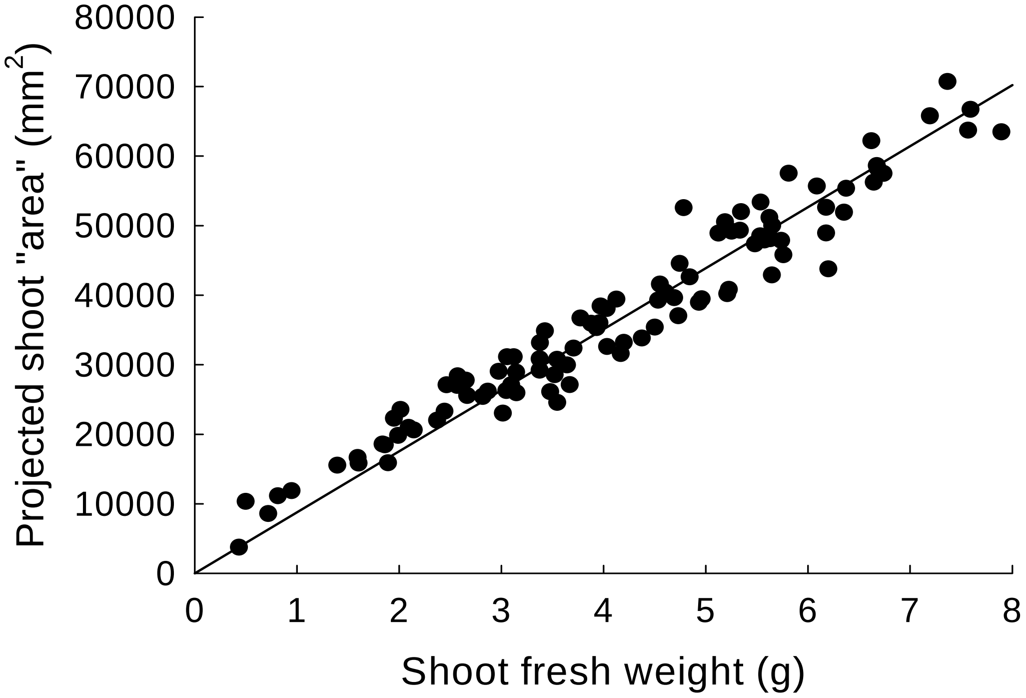
e.g. wilting:

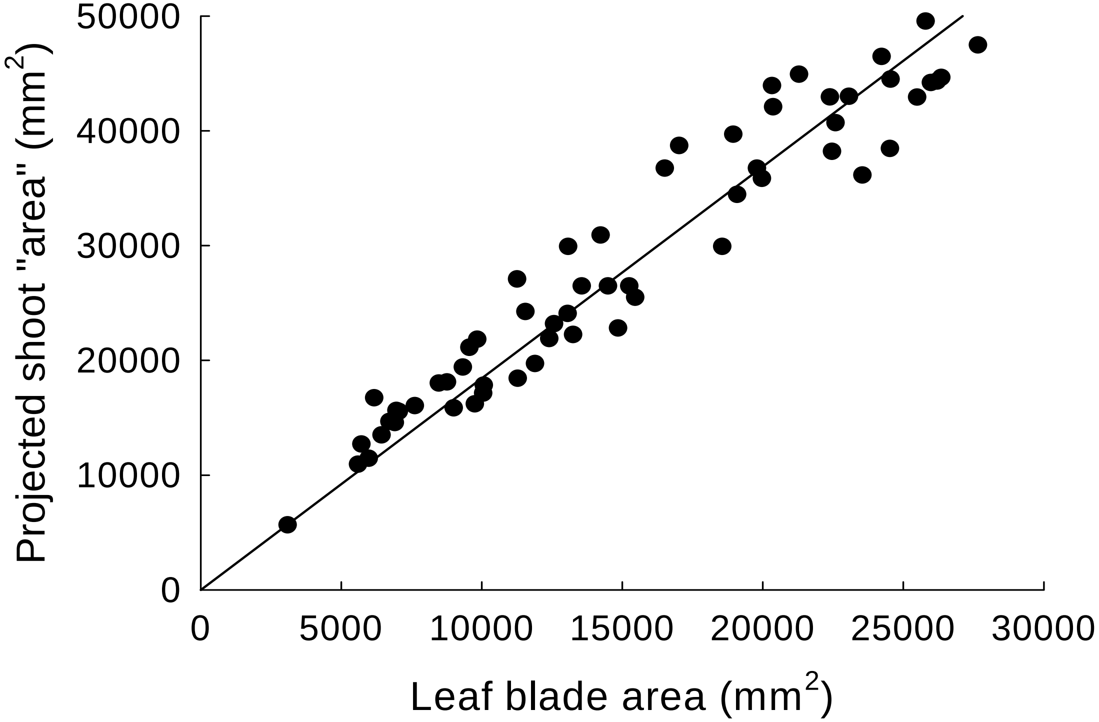
- Alters rectangle parameters

- Increases area below top of pot

- Increases the rotational moment

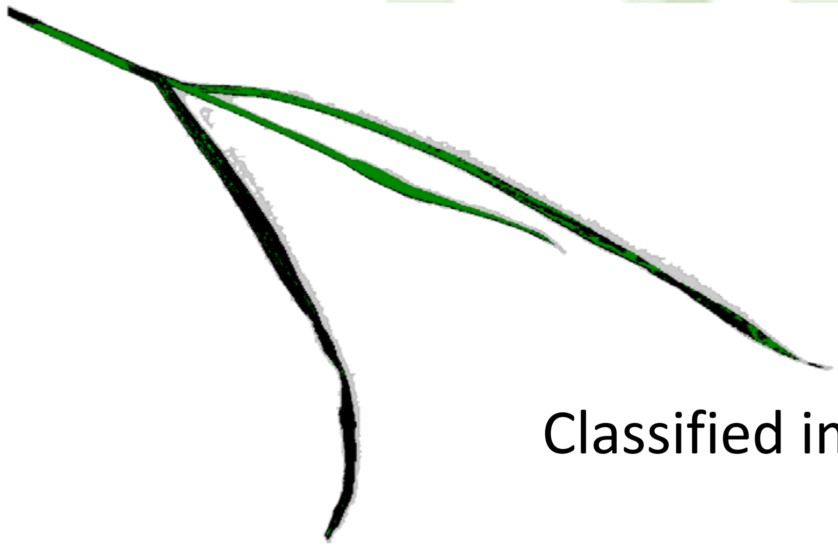
Digital imaging of growth in wheat





Rajendran *et al* (2009) *Plant Cell Environ* **32,** 237-249

Application of Colour Classification: Boron Toxicity Screens



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

Julie Hayes, Margie Pallotta and Tim Sutton

QTL for Ge tolerance identified using LemnaTec similar to QTL for B tolerance (1999)

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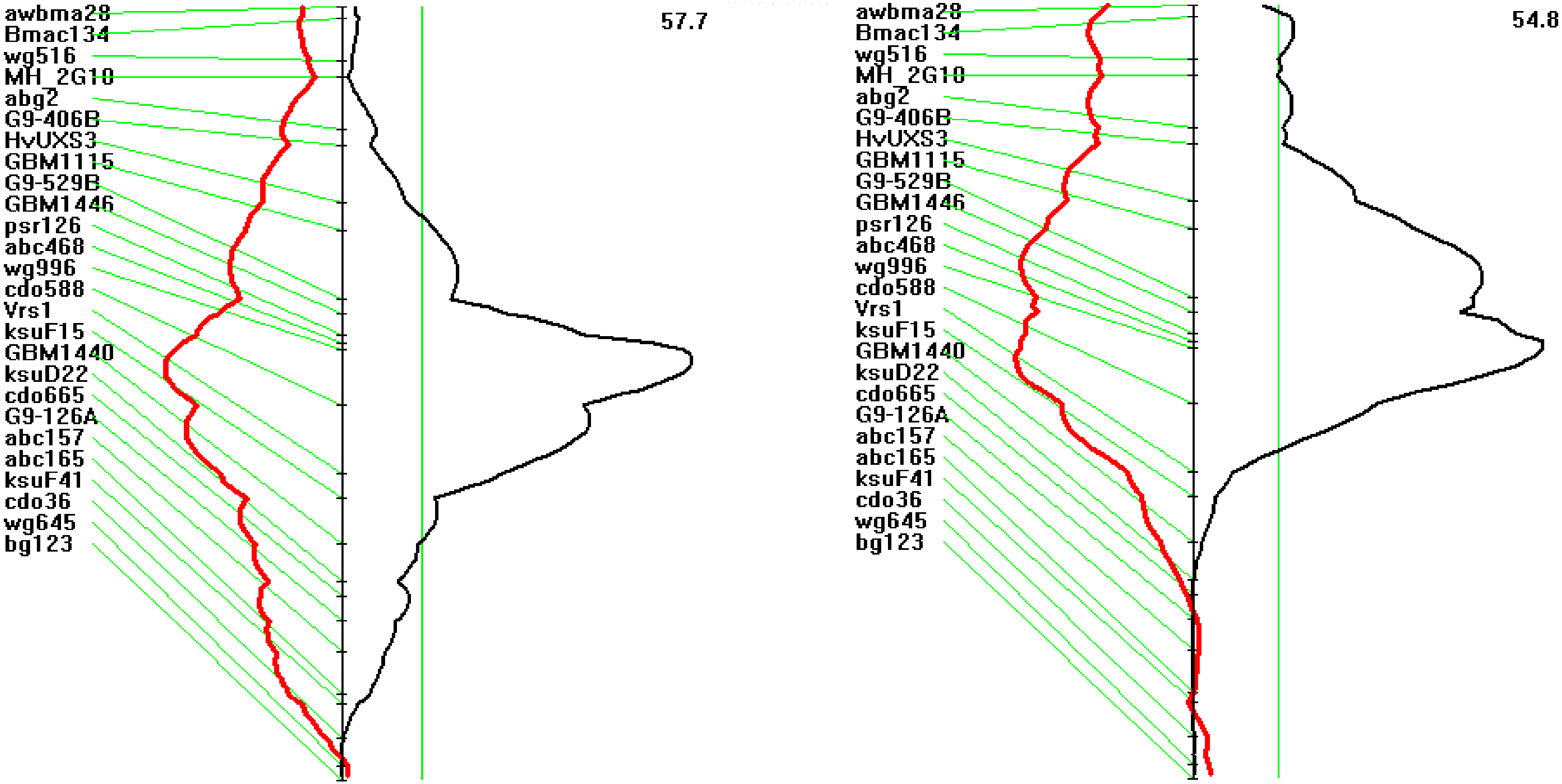


B toxicity - leaf symptoms | Ge toxicity - leaf symptoms

Jefferies et al. 1999. TAG 98, 1293-1303 | Hayes et al., unpubl., using LemnaTec

ACPFG
AUSTRALIAN CENTRE FOR PLANT FUNCTIONAL GENOMICS PTY LTD

Application to salinity tolerance

Three main strategies to maintain growth in high Na^+

Osmotic tolerance

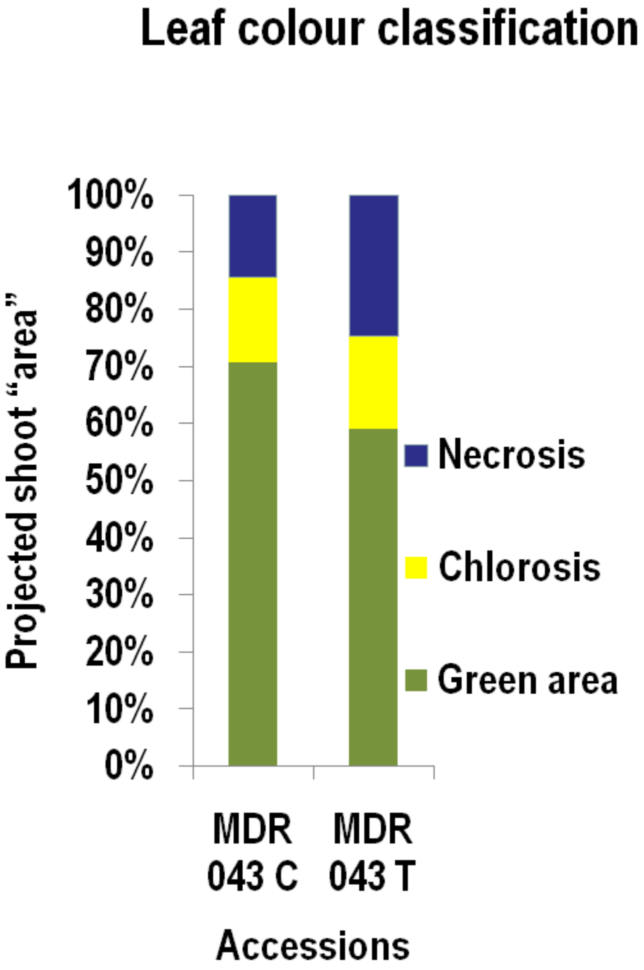
- Tolerate toxicity of osmotic component of NaCl

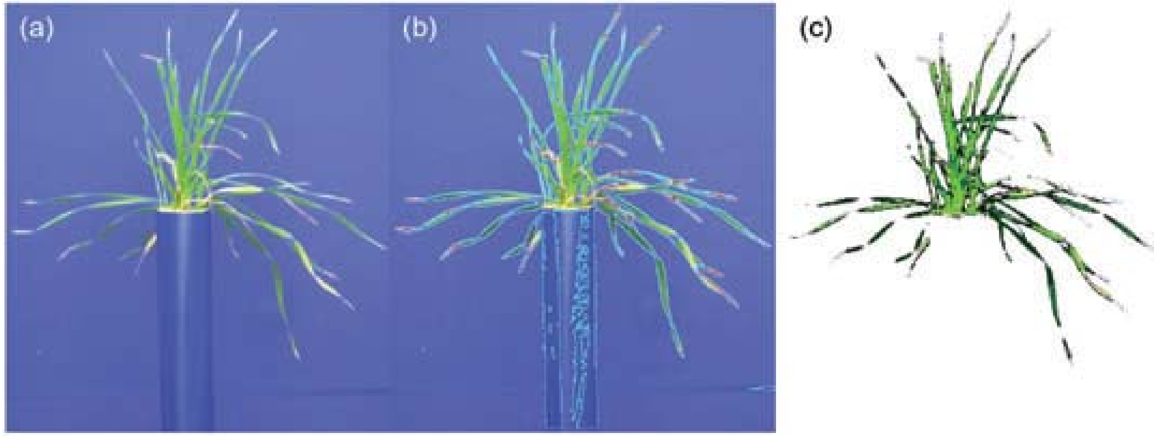
Ionic tolerance

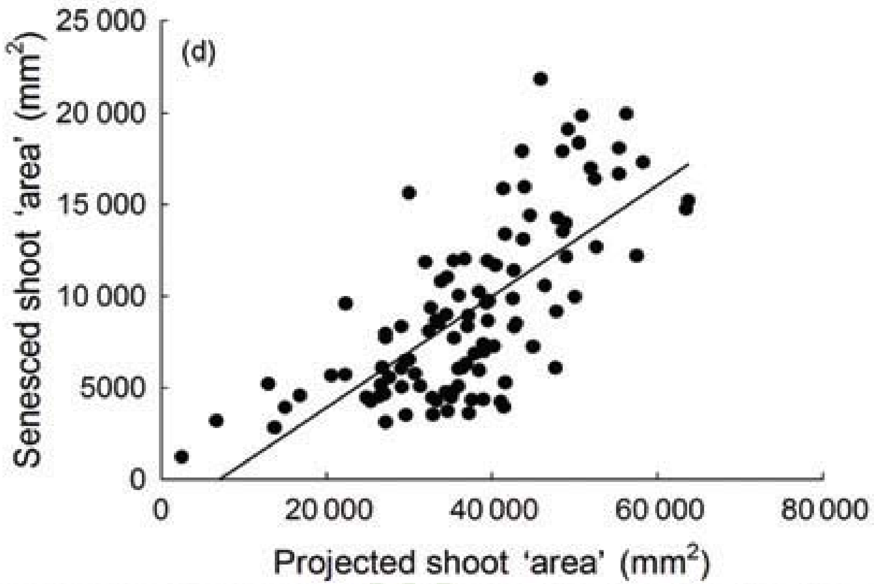
- Exclude Na^+ from leaves
- Tolerate Na^+ that cannot be kept out of leaves

Munns & Tester (2008) *Annu Rev Plant Biol* **59:** 651-681

Colour classification: Tissue tolerance index in monococcum wheat







Rajendran *et al* (2009) *Plant Cell Environ* **32,** 237-249

Plant tolerance index and QTL analysis for salinity

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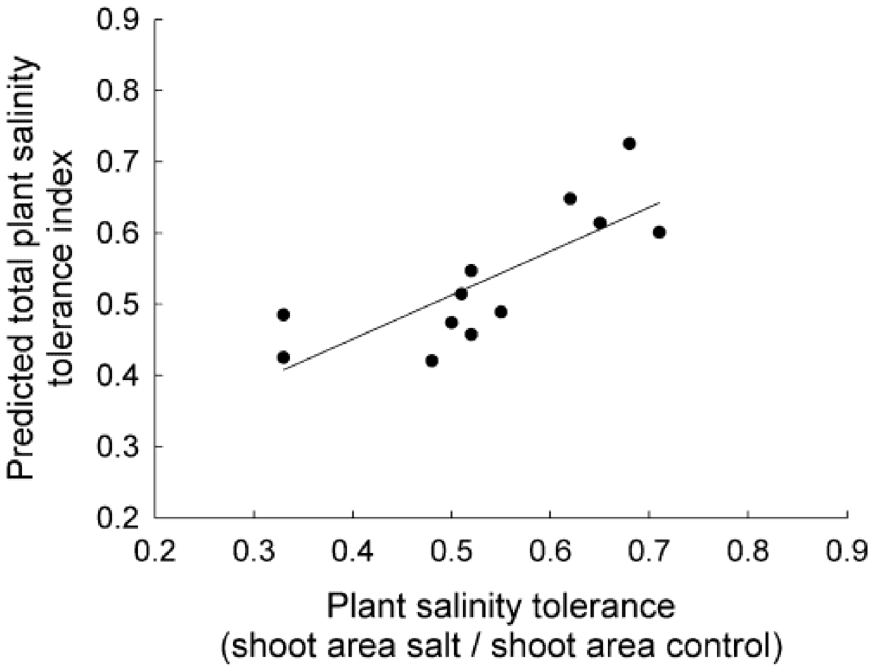

Predicted total plant tolerance =
(0.5 x Na^+ exclusion index) + (0.14 x osmotic tolerance index) + (0.38 x tissue tolerance index) + 0.06

Rajendran *et al* (2009) *Plant Cell Environ* **32,** 237-249

QTLs for components of salinity tolerance are being determined for

- Krichauff x Berkut, 154 DH lines from Hugh Wallwork
- Significant QTL for osmotic tolerance on 1D
- Strategy : Map, then fine map, monococcum and bread wheat loci for osmotic and tissue tolerance

The future with the APPF

- Looking forward to the APPF to:
  - Accelerate work
  - Improve indices of tolerance components by incorporating NIR, FIR, fluorescence and more sophisticated analyses of visible images developed by HRPPC

- A new opportunity to take a forward genetic approach to elucidate more components that contribute to salinity tolerance (and drought tolerance, nutrient deficiencies and toxicities, disease responses….)

- And relating our components of tolerance to field measurements, particularly of yield
  - TEST hypotheses, not just make hypotheses

- With latest investment, aim to
  - consolidate facilities at Adelaide (growth rooms, cheaper energy, bioinformatics)
  - extend capability at Canberra for climate change research ($CO_{2,}$ temperature)

Finkel, 2009: *Science* **325:** 380-1

24 July 2009

IMAGING

With 'Phenomics,' Plant Scientists Hope to Shift Breeding Into Overdrive

MELBOURNE, AUSTRALIA—Last May, a scrawny grass, *Brachypodium distachyon*, joined the exclusive club of plants whose genomes have been sequenced. *Brachypodium* may look unassuming, but under the hood it is a geneticist's dream. It has a short life cycle and a small genome with one pair of chromosomes (wheat, for instance, has three pairs) that readily reveals the effects of genetic modification. In short, the temperate grass is a superb model organism for cereals like wheat and rice and for biofuels like switchgrass. But *Brachypodium*'s genome alone cannot revolutionize plant breeding.

Enter plant phenomics. Borrowing imaging techniques from medicine, phenomics offers plant scientists new windows into the inner workings of living plants: infrared cameras to scan temperature profiles, spectroscopes to measure photosynthetic rates, lidar to gauge growth rates, and MRI to reveal root physiology. "Phenomics will give plant scientists the tools to unlock the information coded in genomes," says Mark Tester, director of the Australian Plant Phenomics Facility (APPF), a new $40 million venture with headquarters in Adelaide.

Institutes worldwide are racing to build facilities with instrument arrays that can scan thousands of plants a day in an approach to science akin to high-throughput DNA sequencing. "This will allow plant physiology to 'catch up' with genomics," says Tester. APPF, the first national lab of its kind in the world, consists of two nodes. One is a High Resolution Plant Phenomics Centre (HRPPC) in Canberra, which opened last week. A debut project of the center is an international collaboration to screen *Brachypodium* variants for drought-tolerance and for less lignin in their cell walls. The second node is the Plant Accelerator in Adelaide, a screening facility that Tester will run and aims to get online by December.

"Australia is leading the way," says David Kramer, a spectroscopist at the Institute of Biological Chemistry at Washington State University, Pullman. But other countries are ramping up fast. In Germany, for example, the Institute for Phytosphere Research (IPR)

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Access and Pricing: High Res Centre

OPEN TO ALL AUSTRALIAN RESEARCHERS AT MARGINAL COST

- 3 PDF level staff and 1 TO working to develop screening systems with customers and analyse data (informatics and data storage as for Accelerator)
- *We need help from your hands to carry out the project in the "research hotel" (NCRIS won't fund the research component)*

- **Pricing model is marginal cost recovery**
  - **3 levels of charging** :
  - ***"Member"*** : $15k p.a. for a project leader to encompass a suite of related projects (plus plant culture charges if Centre grows plants).
  - ***"Non-member":*** Fixed charge out for equipment use and time of staff
  - ***"Industry":*** Full cost recovery by negotiation

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Access and Pricing High Res Centre: a costing example for Arabidopsis

7 different mutant lines, 2 WT control lines
10 reps of each line, 15 reps controls
4 week growth and imaging experiment
Imaged every 3 days

Project establishment fee	$220
HRPPC staff cost to conduct experiment ($50/hour /assume 2 hours every 3 days)	$1,000
Scanalyser	$1,000
Fluoro-gro-scan	$500
Growth cabinet (1x A1000AR at $200/week for 4 weeks)	$800
Plant raising and management (100 plants, ie $2 per plant)	$200
Potting mix (20L at $0.50/L)	$10
Image analysis ($110 per hour)	$220
TOTAL	**$3,950**

Access and pricing
The Accelerator

- Available for use by all Australian plant science community

Base costs for Scanalyzer use	**$1.25/pot/day**
Commercial customers in Australia	**$5/pot/day**

e.g. 100 DHs, 3 reps for 3 wks in Smarthouse, 3 mnth in g'house will cost $10k

- Base costs include: watering, energy, pots, soil, waste disposal, pest control, horticultural support and standard informatics services
- Standard Informatics Services include:
  - Consultation on experimental design, data processing, expected analysis outcomes
  - Routine processing of raw measurement data (RGB, FIR, NIR, Fluorescence images) including algorithm design, colour classification, area measurement, morphological measurement, basic statistics, graphing of curves
  - Collation and presentation of processed data within 2 weeks following the date of the last acquired measurement
  - Confidential access and archival storage of raw measurement and processed data for six months from the date of the last acquired measurement
  - Provision of raw and processed data on appropriate media; placement of data in the public domain unless requested otherwise

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Bursaries, top-ups and training

- **4 PhD top-ups of $5k p.a. for students based in the Centre**

- **Travel bursaries and support for researchers and students to visit the facility from Australia and abroad (competitive "innovators program")**

- **Project design workshop: How CAN The Plant Accelerator address biological problems? 19 November, 2009**

- **Technical training component : Phenomics Techniques Workshop, Feb each year more specific workshops will follow**

- ***International Plant Phenomics Symposium held biannually via International Plant Phenomics Initiative (next is 2011 Juelich Germany).***

Australian Plant Phenomics Facility

The Australian Plant Phenomics team

Adelaide

Mark Tester
Geoff Fincher

Helli Meinecke – manager
Bettina Berger – postdoc
James Eddes – bioinformatics
Richard Norrish – electronics
Robin Hosking – horticulturalist

Canberra

Bob Furbank
Jeremy Burdon
Murray Badger

Chris Buller – manager
Xavier Sirault – postdocs
Dave Deery
Xueqin Wang
Scott Berry- TO

Australian Plant Phenomics Facility

The Australian Plant Phenomics Facility: Your Facility

- A new opportunity to take a forward genetic approach to elucidate more components that contribute to particular plant function in a wide range of species

- A new opportunity to document germplasm collections in a standardised way – rapidly and reliably

- Contacts

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