APPF: The Australian Plant Phenomics Facility

Bob Furbank, Australian Plant Phenomics Facility, Canberra, Australia

EPSO: The European Plant Science Organisation
EPSO Workshop on Plant Phenotyping
November 02-03, 2009
Forschungszentrum Jülich, Germany

Forschungszentrum Jülich, Germany
ICG-3: Phytosphere
Jülich Plant Phenotyping Centre (JPPC)
Website: http://www.jppc.de

http://www.plantphenomics.com/phenotyping2009

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

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CSIRO

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An Australian Government Initiative
National Collaborative Research Infrastructure Strategy

Government of South Australia

ACPFG

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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

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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

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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

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

Key technologies

- ***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 / salt tolerance
- ***Fluorescence imaging***
  - Physiological state of photosynthetic machinery
- ***Hyperspectral imaging***
  - Carbohydrates, pigments and protein
- ***Carbon isotope ratio***
  - Transpiration efficiency, photosynthetic pathway (TDL/MS)
- ***FTIR Imaging Spectroscopy***
  - Cellular localisation of metabolites (sugars, protein, aromatics)

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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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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

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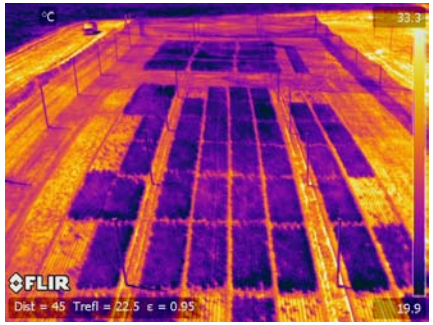
High Resolution Plant Phenomics Centre
From growth cabinet to the field



‘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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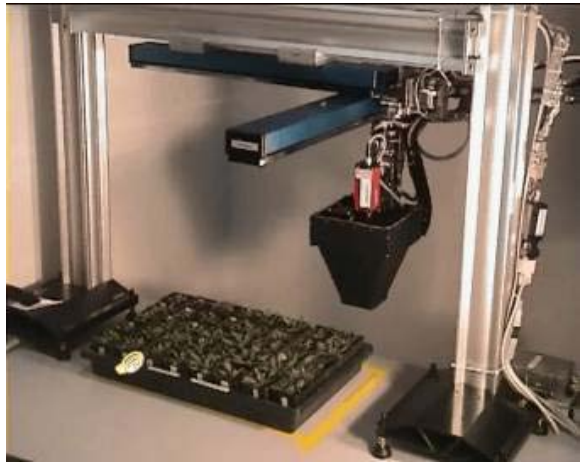
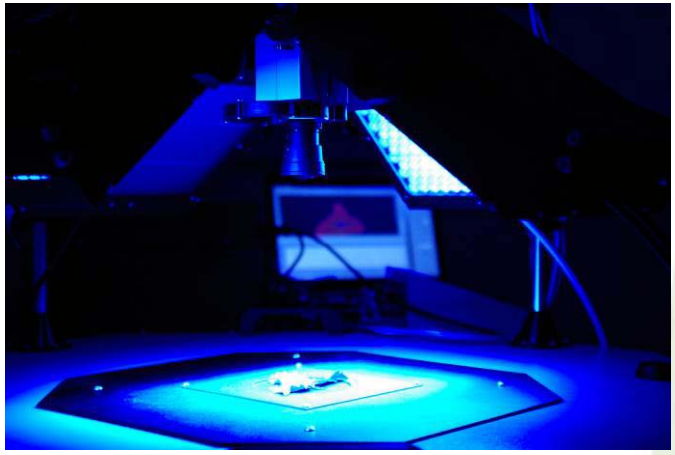
Model plant module

Fluorogro-scan



TrayScan



RGB / FIR in-Cabinet

- **Growth and morphology**
- **Photosynthetic performance (Chl Fluor) under defined environmental conditions**

- **IR screening for leaf temperature**
- **Automated destructive sampling for metabolites, protein, DNA and RNA, delta^{13}C**

Target plants : *Arabidopsis, Tobacco, Brachypodium and seedling screens*

Isolating Photosynthetic and Photorespiratory Mutants

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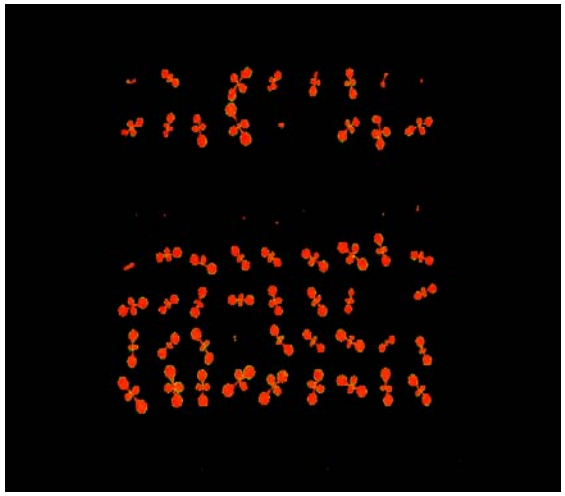
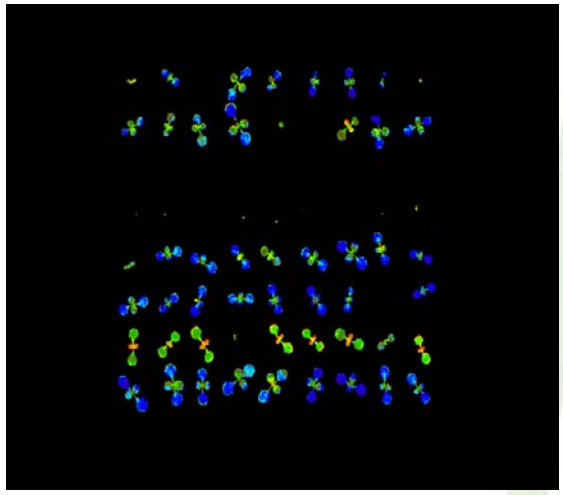
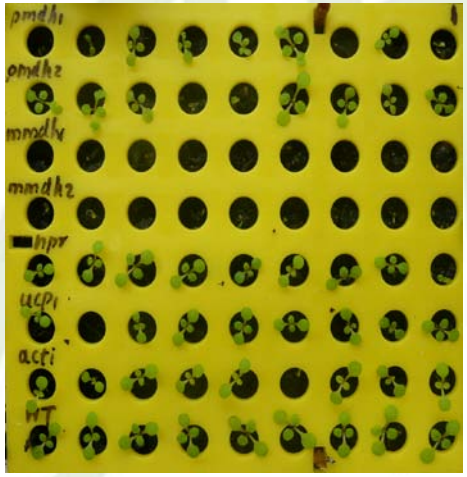
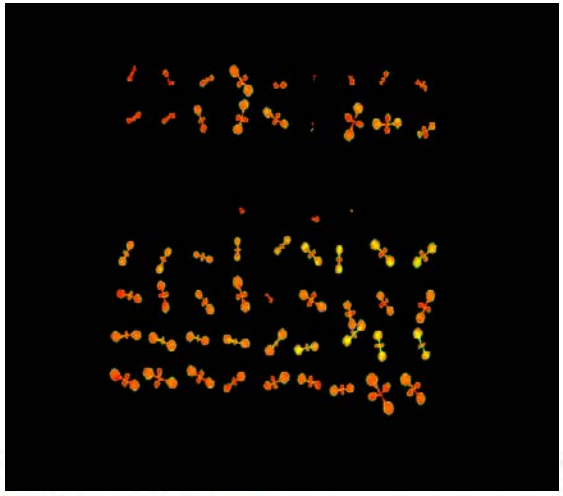
Fv/Fm

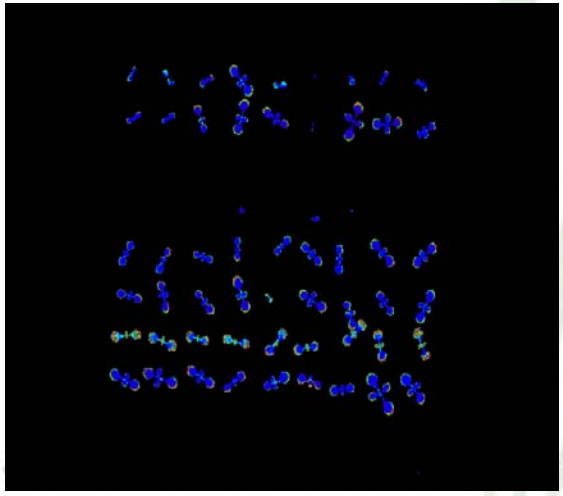
NPQ

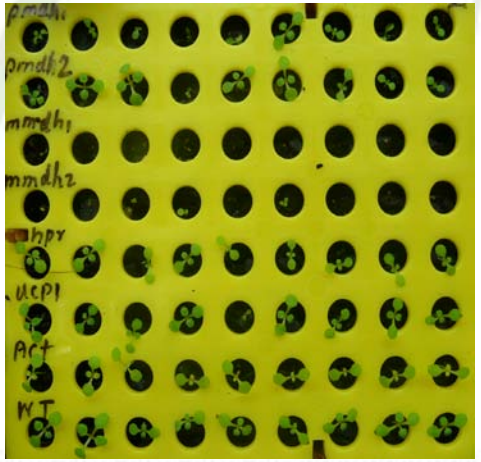












Badger et al., 2009

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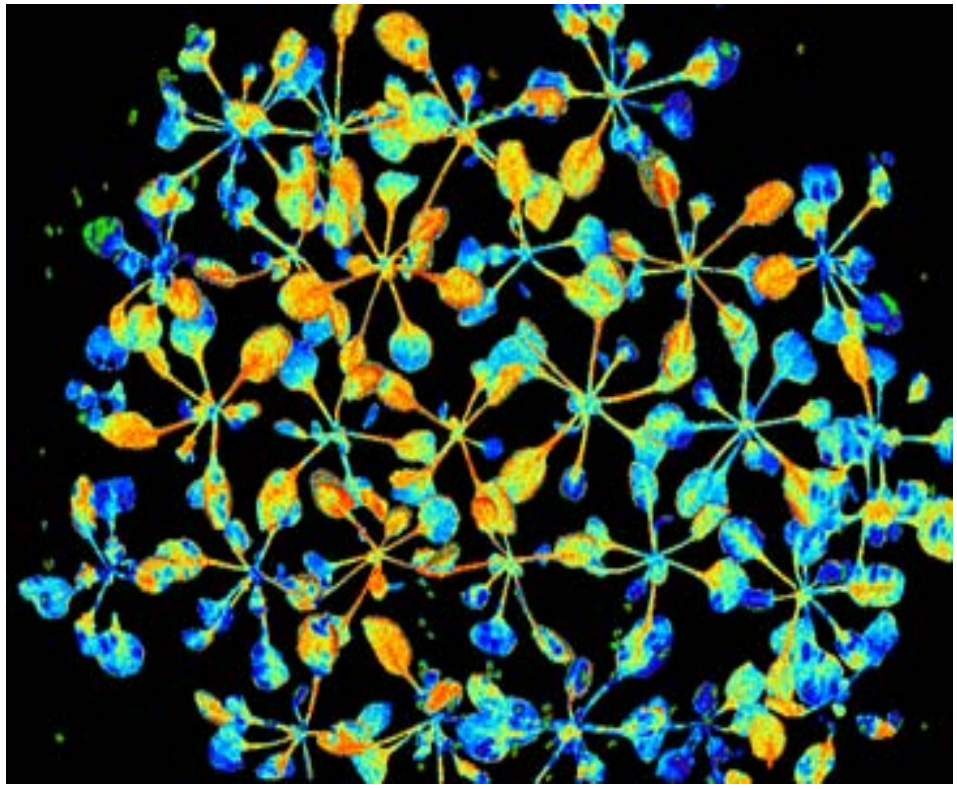
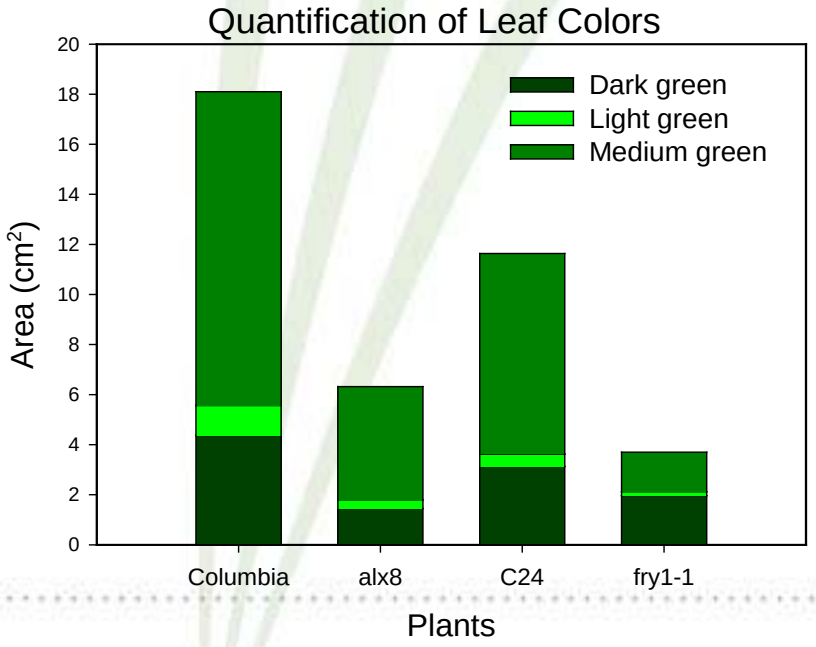
Data Analysis: non-destructive Growth Analysis and morphological clustering



- **Leaf area / growth analysis (eg heterosis and drought stress)**
- **Photosynthetic mutants**
- **Lesions / pathogen attack**
- **Architecture / morphology**
- **Morphological clustering**
- **PODD phenotypic dBase**



Seedling screens with IR thermography: osmotic stress tolerance

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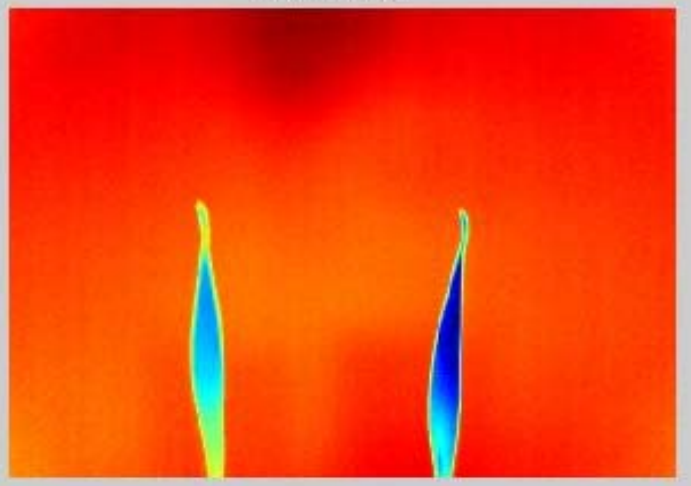
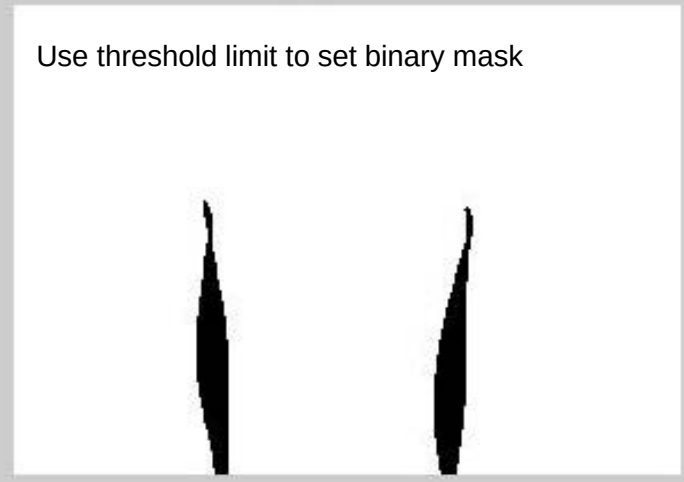
Input Image



Segmented

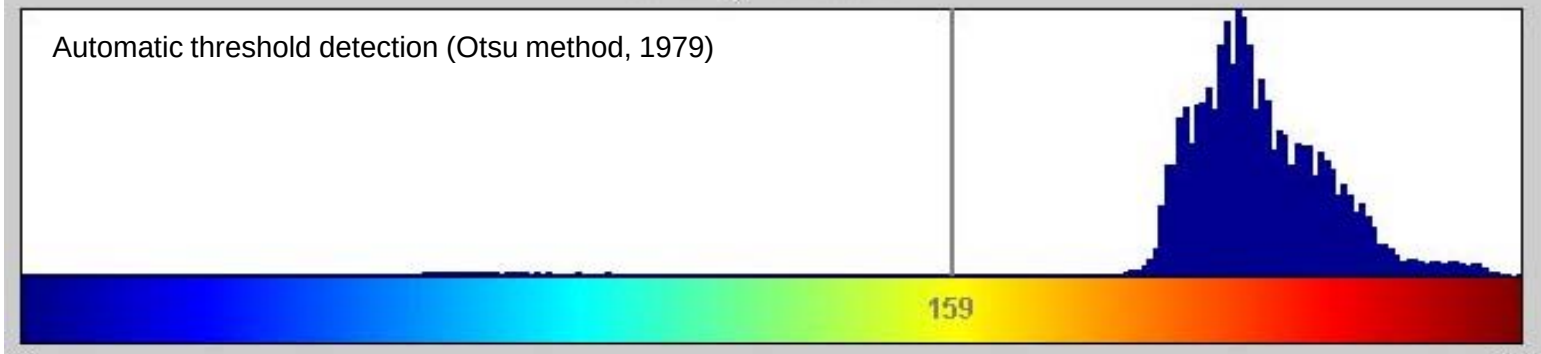
Use threshold limit to set binary mask



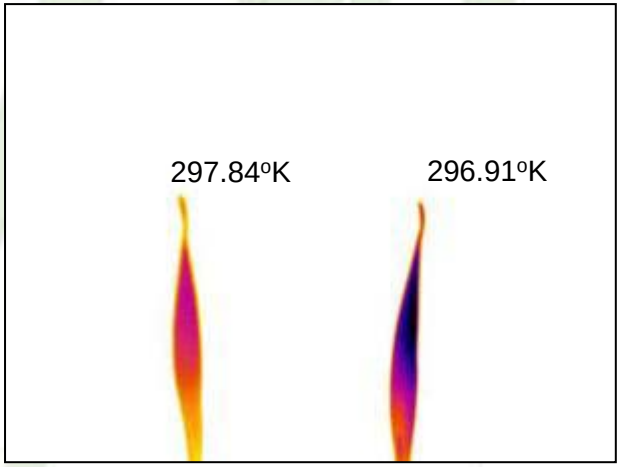
Thermograph: matrix of temperature [640x480]
(8-bit false colour image for visualisation)

Intensity Distribution

Automatic threshold detection (Otsu method, 1979)



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



100 mM control

$\Delta = 0.93^{o}C$

Temperature data averaged for each plant and saved in EXCEL spreadsheet

Physiological consequences of osmotic stress

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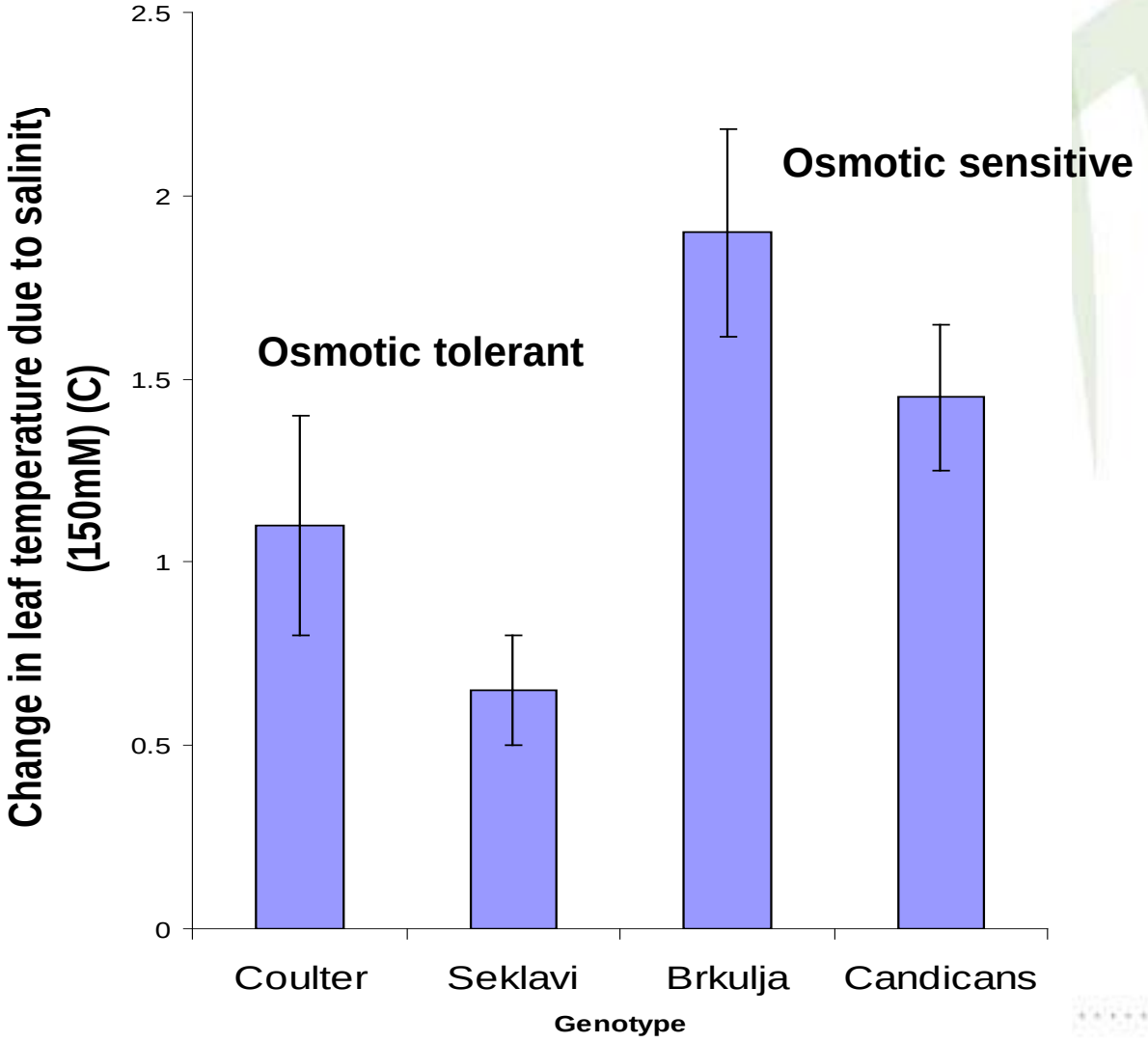
Barley and wheat genotypes of known osmotic tolerance screened by growth analysis and at 2 leaf stage by IR imaging. Rankings were identical

Sirault, James and Furbank Functional Plant Biology (2009)



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

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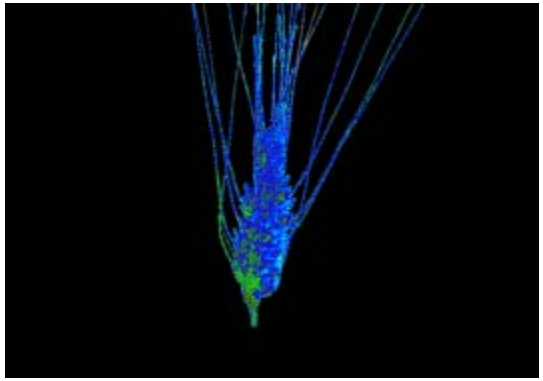
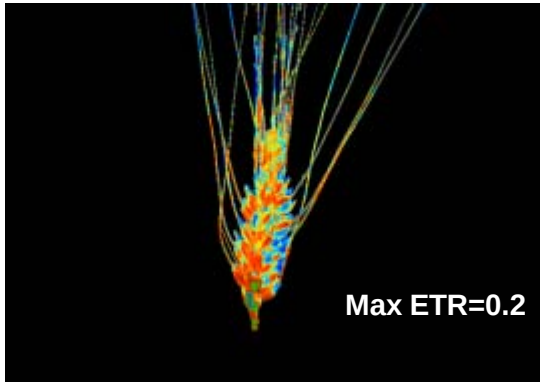
- Whole of lifecycle photosynthesis and growth
- Dynamic growth and carbon allocation to plants organs
- Transpiration and water use
- Hyperspectral detection of leaf protein and CHO





Digital estimation of biomass validated for a range of species

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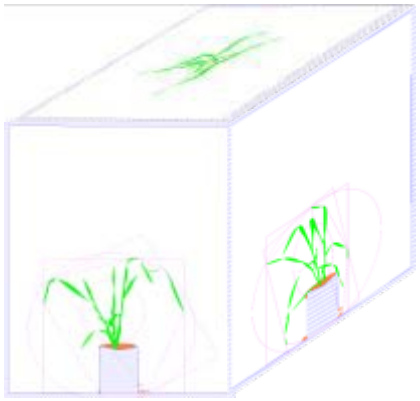
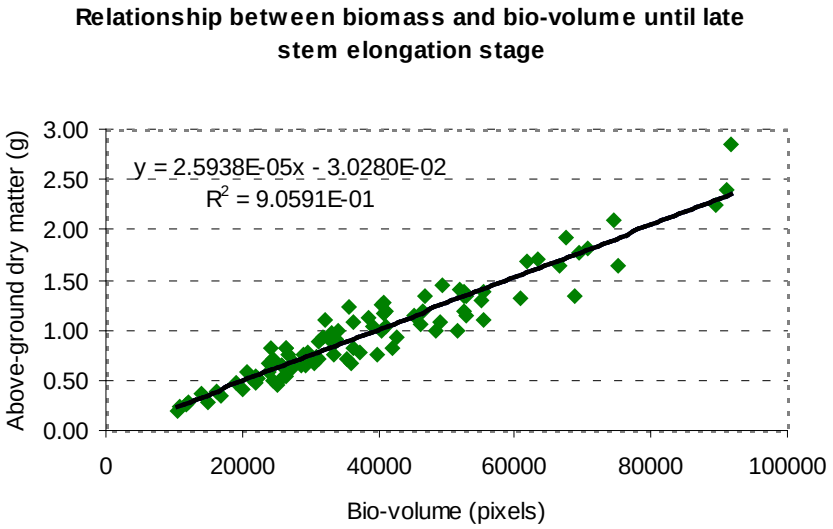
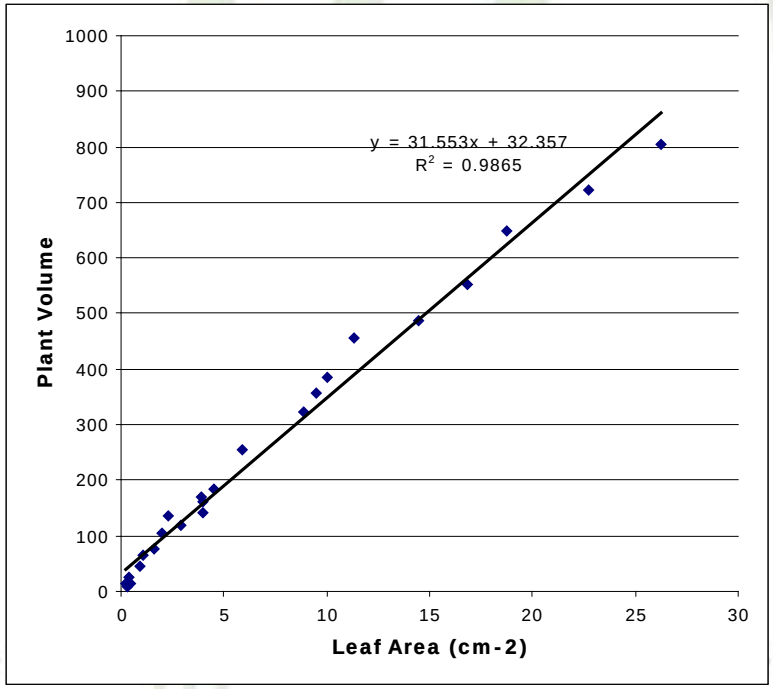
- Wheat
- Rice
- Barley
- Cotton
- Chickpea
- Cowpea
- Flaveria
- Arabidopsis







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

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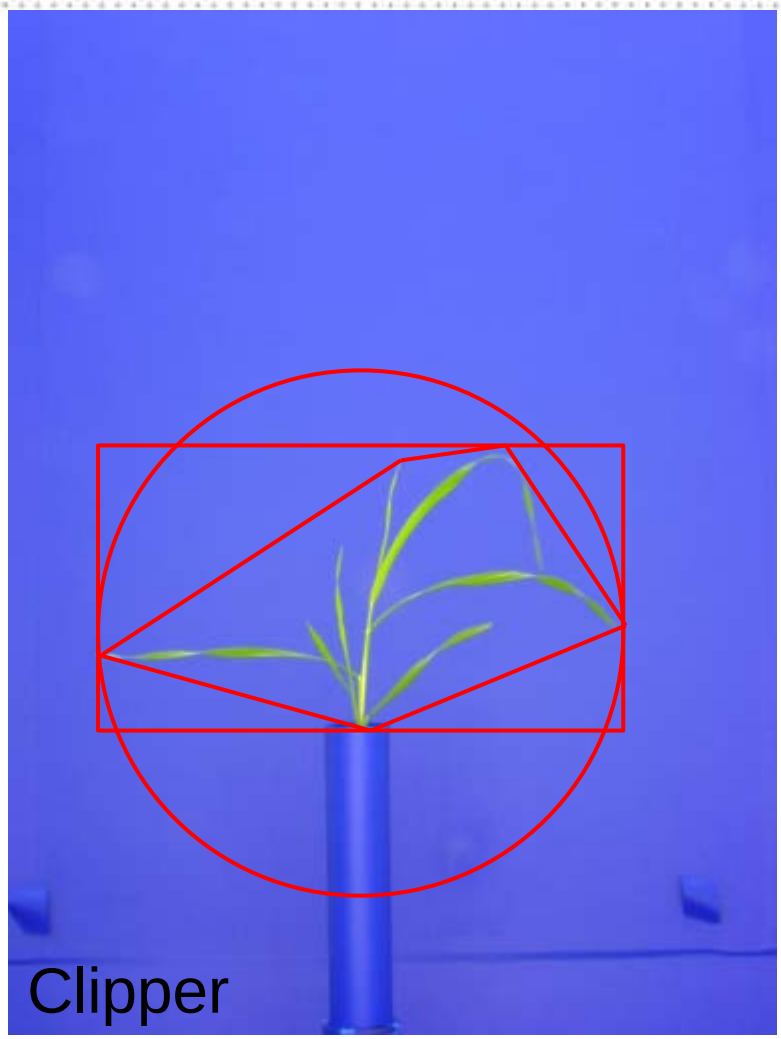
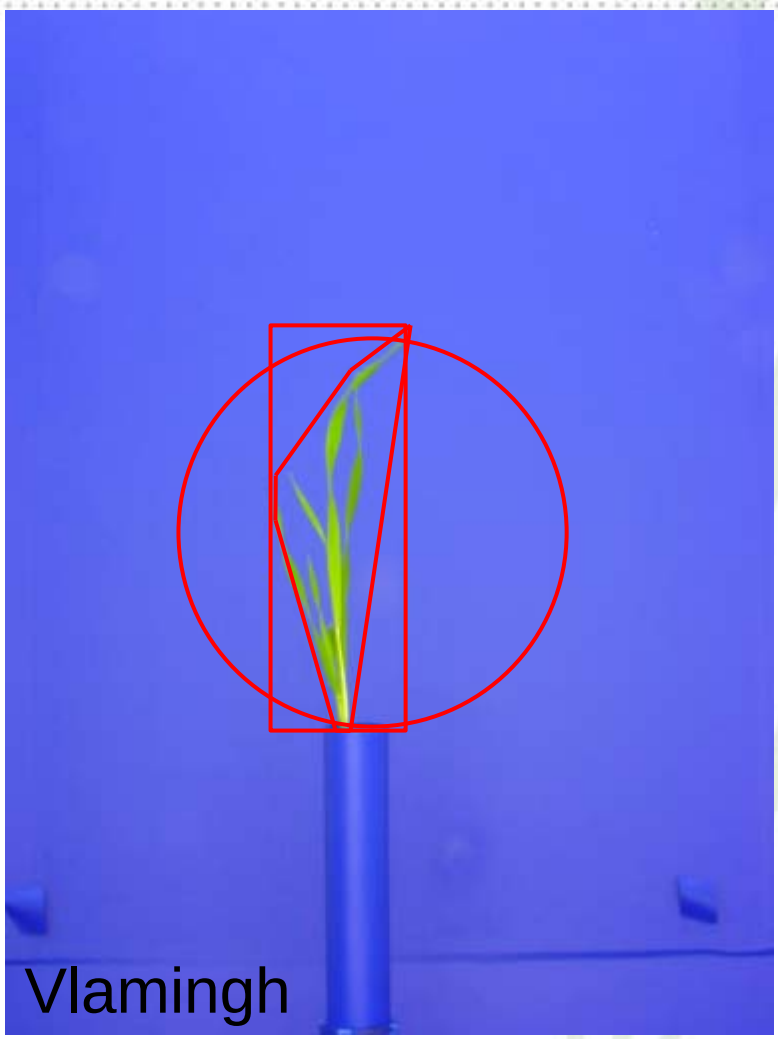




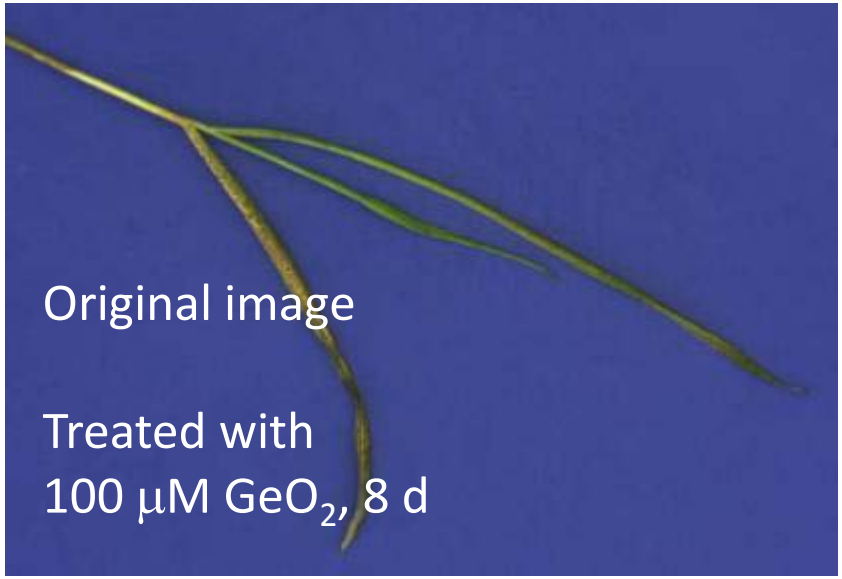
Object properties

- **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**

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

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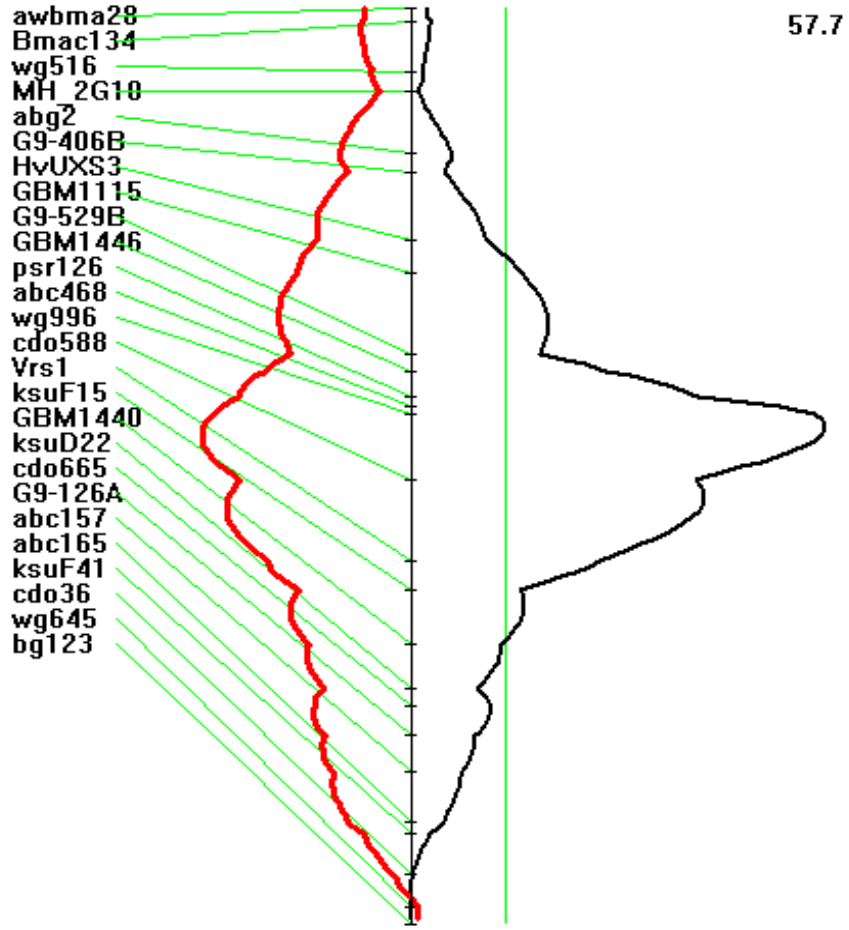
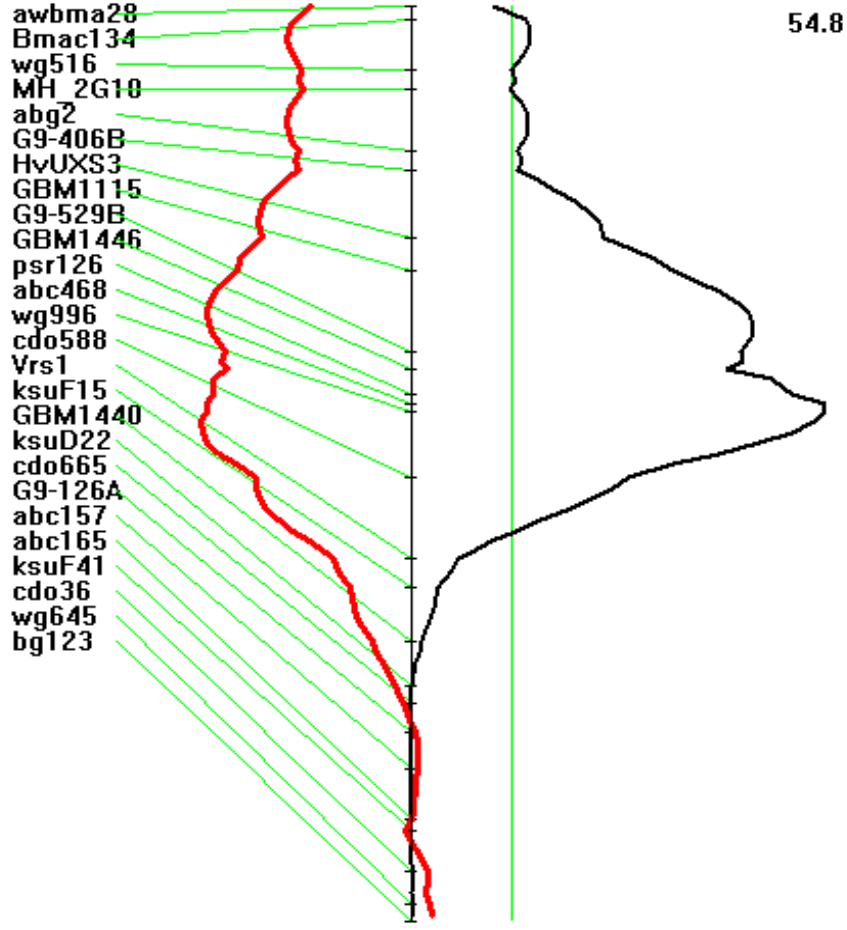
QTL for Ge tolerance identified using LemnaTec similar to QTL for B tolerance (1999)



B toxicity - leaf symptoms

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



Ge toxicity - leaf symptoms

Hayes et al., unpubl., using LemnaTec

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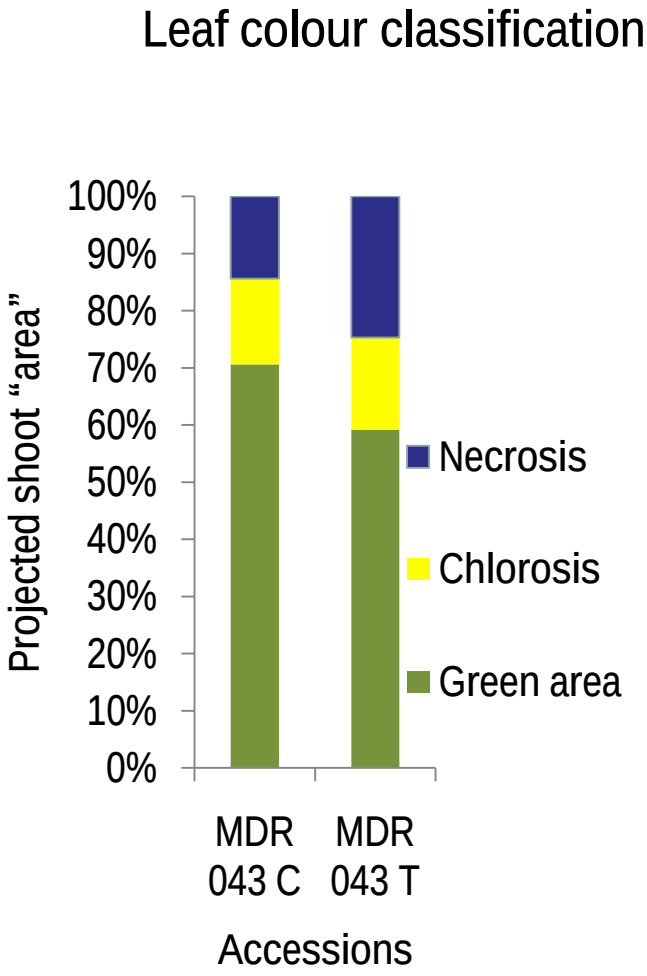
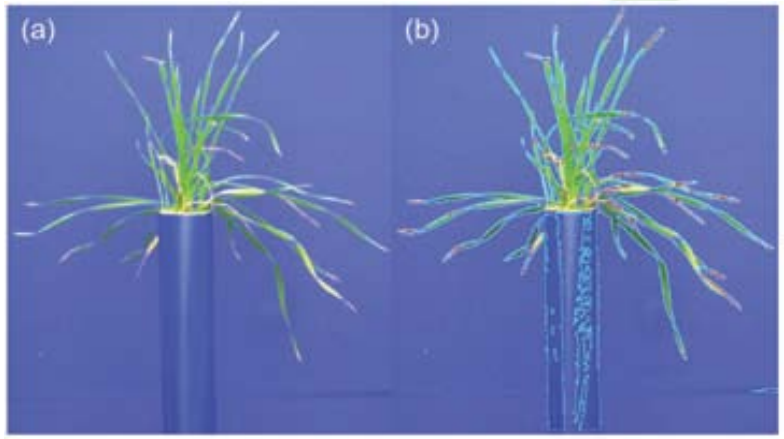
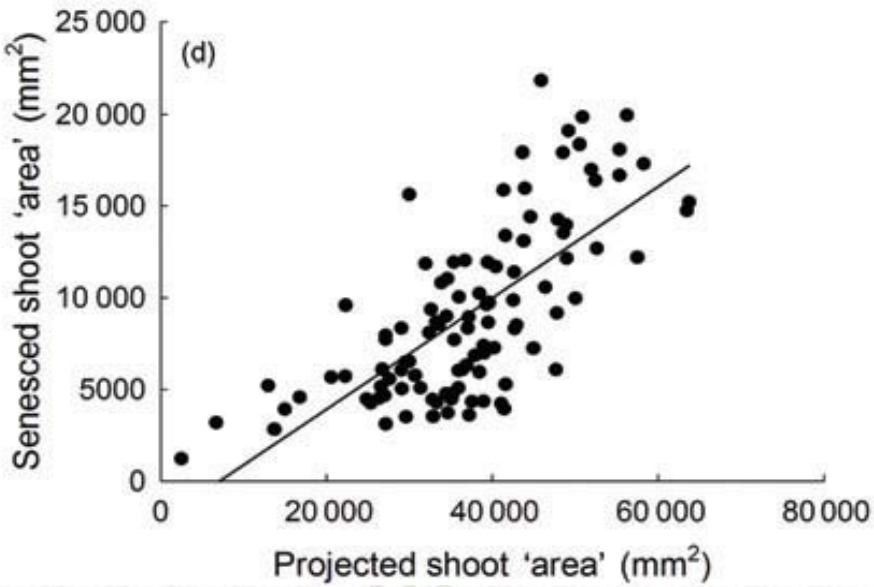
Colour classification: Tissue tolerance index in monococcum wheat







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

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

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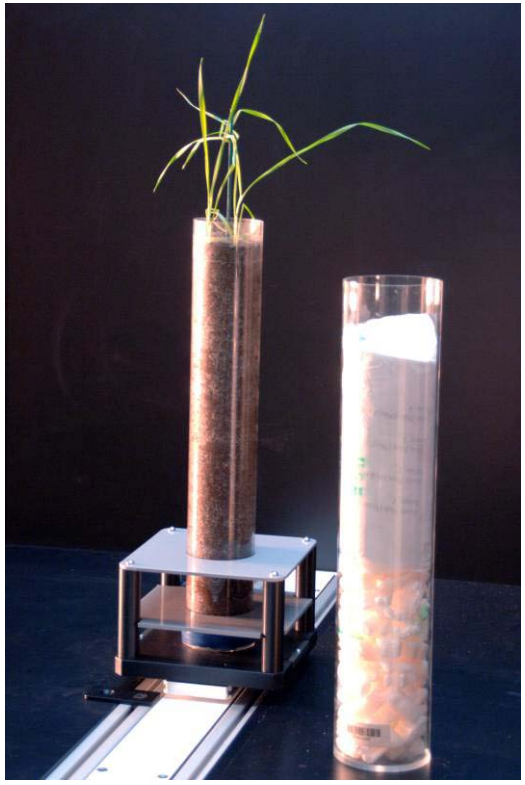
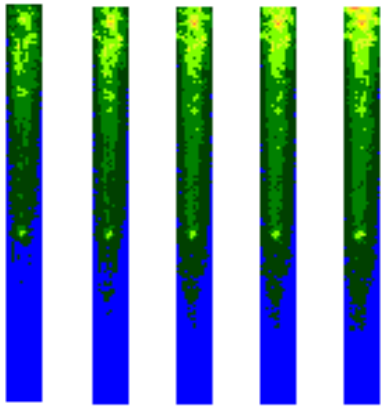
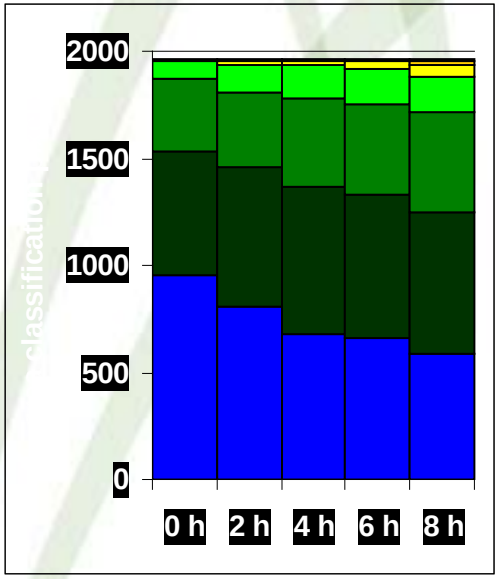




Results of NIR monitoring allow measurement of spatial distribution water content in soil



total development of drying over time

Data courtesy of Lemnatec

Rhizotron Shoot and Root Growth Imaging System HRPPC

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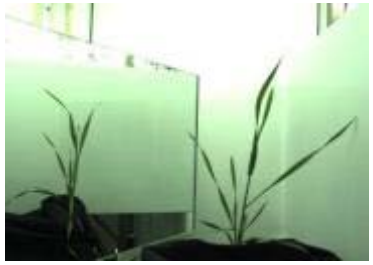
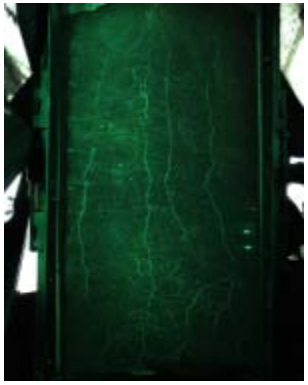
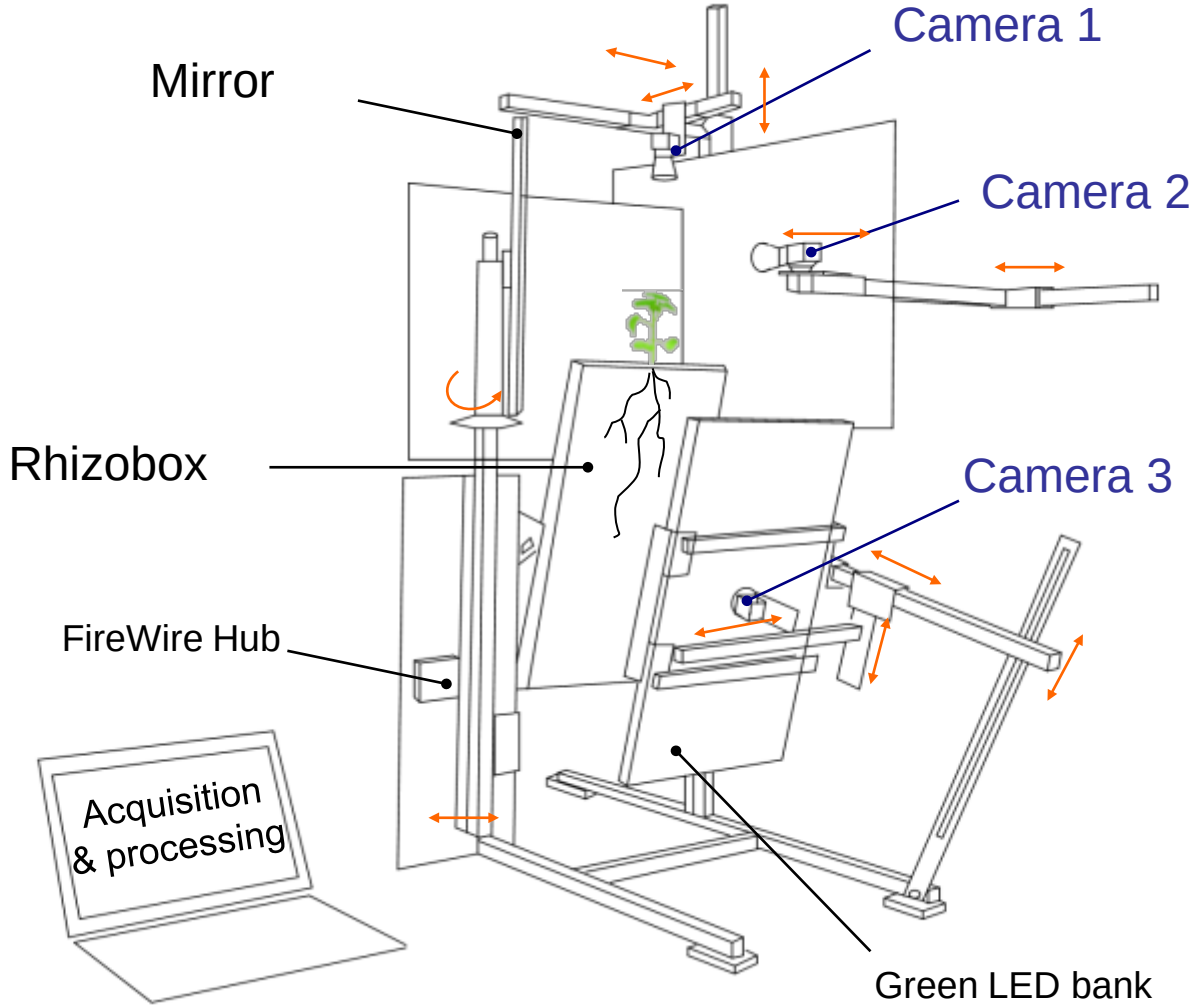
Top view



2 side views



Roots



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

Watt, Nagel, Sirault, Furbank

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Field Module: High Throughput Phenotyping in the field

- 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

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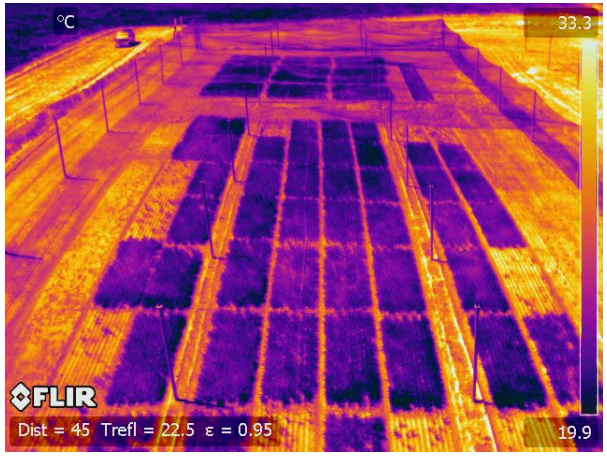
Ground-based : Phenomobile and Imaging tower



15m tow behind tower

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

- **Variable span buggy 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**

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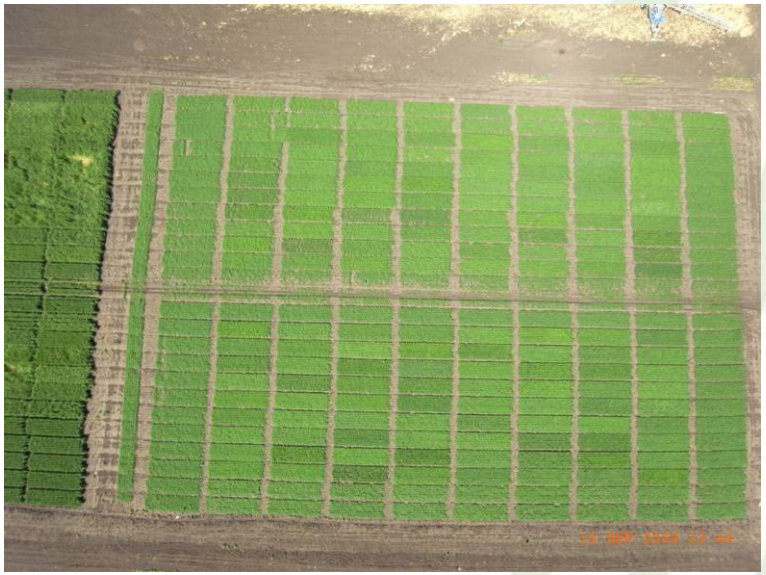
UAV High Throughput Imaging

55mm cube 640X480
Microbolometer weighs 300g



$10m^2$ plots
600 plots per image

Acquisition time
10 min

FIR plus RGB

Scott Chapman CSIRO Brisbane

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Phenonet Distributed Sensor Network

Datacall® Fleck™

- "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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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

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