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
Australian Plant Phenomics Facility

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

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)
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
The Plant Accelerator

- 4,485 m² building, 2,340 m² of greenhouses, 250 m² for growth chambers
- Grow >100,000 plants annually in a range of conditions
- 4 x 140 m² 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
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 m² lab space and ‘research hotel’
- Imaging modules interfaced with 245 m² greenhouse, 260 m² growth cabinets
- Large field site with distributed sensor networks portable ‘phenomobile’ and 15m imaging tower
Model plant module

- 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

Fv/Fm  NPQ

Badger et al., 2009
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

Quantification of Leaf Colors

<table>
<thead>
<tr>
<th>Plants</th>
<th>Area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Columbia</td>
<td>18</td>
</tr>
<tr>
<td>alx8</td>
<td>4</td>
</tr>
<tr>
<td>C24</td>
<td>10</td>
</tr>
<tr>
<td>fry1-1</td>
<td>2</td>
</tr>
</tbody>
</table>

Legend:
- Dark green
- Light green
- Medium green
Seedling screens with IR thermography: osmotic stress tolerance

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

Temperature data averaged for each plant and saved in EXCEL spreadsheet.

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

Automatic threshold detection (Otsu method, 1979)

Use threshold limit to set binary mask

Intensity Distribution

Δ = 0.93°C
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

- 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

Max ETR=0.2
Max NPQ=1.25
Digital estimation of biomass validated for a range of species

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

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

\[ y = 2.5938 \times 10^{-5}x - 3.0280 \times 10^{-2} \]

\( R^2 = 9.0591 \times 10^{-1} \)

Above-ground dry matter (g)

Bio-volume (pixels)

\( y = 31.553x + 32.357 \)

\( R^2 = 0.9865 \)

Leaf Area (cm\(^{-2}\))

Plant Volume

Digital estimation of biomass validated for a range of species

- Wheat
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www.plantphenomics.org.au
System can quantify morphometric parameters e.g. canopy density, wilting

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

Clipper

Vlamingh

Bettina Berger TPA
Application of Colour Classification: Boron Toxicity Screens

Treated with 100 \( \mu \text{M GeO}_2 \), 8 d

<table>
<thead>
<tr>
<th>Line</th>
<th>Green area</th>
<th>Necrosis area</th>
<th>% Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sahara</td>
<td>30739</td>
<td>4232</td>
<td>12%</td>
</tr>
<tr>
<td>Clipper</td>
<td>11640</td>
<td>15321</td>
<td>57%</td>
</tr>
</tbody>
</table>

Julie Hayes, Margie Pallotta and Tim Sutton
QTL for Ge toxicity 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
Colour classification: Tissue tolerance index in monococcum wheat

Leaf colour classification

- Necrosis
- Chlorosis
- Green area

MDR 043 C  ||  MDR 043 T

Accessions

Projected shoot "area"


www.plantphenomics.org.au
Crop Plant Root Module: NIR imaging of soil moisture at TPA and HRPPC

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

Data courtesy of Lemnatec
Rhizotron Shoot and Root Growth Imaging System HRPPC

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

Watt, Nagel, Sirault, Furbank
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
Ground-based: Phenomobile and Imaging tower

- 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

Gives 1m² area coverage at 2M boom height

15m tow behind tower
UAV High Throughput Imaging

10m² plots
600 plots per image
Acquisition time
10 min
FIR plus RGB

Scott Chapman  CSIRO
Brisbane
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
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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