Phenotyping of common bean and cowpea

The need to exploit opportunities afforded by genomics.

S. Beebe
I. Rao
Current Applications in SNP Genotyping:

- Cowpea
  - 1052 SNP converted to Kaspar
  - Use in Back Crossing and MARS
- Bean
  - BeanCAP: Diversity panels of Mesoamericans and Andeans
  - GCP: 300 African parental lines
  - 1500 SNP converted to Kaspar
  - MARS population for drought
Costs of Genotyping

- Reducing by one-tenth every 3 years
- Current wave: SNP
- Next wave: whole genome sequencing

Costs of Phenotyping

- Some automation but still expensive
- Much still field based at same cost
  - (or higher?)
General Questions

• Do we have the right genetic materials to justify the investment?
  – Invest in what we have?
  – Or wait for better materials?

• When to invest in what?
  – Genotype now?
  – Phenotype now, genotype later?

• How to improve the quality of the phenotypic data? (better heritability)
3 Challenges of Phenotyping

1. Working with complex traits
   I am assuming that phenotyping is not a problem for simple traits (?)
   Many disease traits are already into MAS

2. On large populations (> 200 ?)

3. Reliability of data
Complex Traits

- Drought tolerance
- Tolerance to Edaphic problems
- Physiologically and genetically complex
- Much interaction (GxE) and local adaptation
- Likely interaction among stresses
  - Drought x Soil fertility
  - Drought x Heat
Large populations

• > 200 individuals or lines
• Difficult to manage large field trials…
  – large CVs
• How can we optimize statistical design?
  – Lattice designs…still too limited?
  – Multiple lattice linked by common checks?
  – “Latin square” …sudoku style
Population Size vs Population Structure

- Biparental crosses
  - Narrow base, effects may be specific
  - Two alleles

- Association mapping
  - Broad base, many genetic backgrounds
  - Different allele frequencies

- MAGIC populations
  - Several parents (8?)
  - Multiple gene interactions
Reliability of Data

Yield based evaluations...

• Field sites for stress
  – For drought and soil problems, variability is great!
  – Fields that are uniform with non-stress management express variability under stress

• *Is this less of a problem with cowpea?*
  – Are lowland soils of West Africa more uniform?
Consultancy of Charles Wortmann, Ph.D., U. of Nebraska

Variables

- Water quality
- Soil water holding capacity
- Irrigation facilities
- Weather patterns
- Planting dates

Site descriptions and recommendations

- Melkassa, Ethiopia*
- Thika, Kenya
- Kabete, Kenya
- Katumani, Kenya
- Kiboko, Kenya
- Kandiyani, Malawi
- Kasinthula, Malawi*
- Chiredzi, Zimbabwe
- Selian, Tanzania*
- Madiira, Tanzania
Reliability of Data (Yield)

- Need excellent Trial Management!
  - Biotechnology does not fix this!
  - Soil preparation !!!!
  - Water management
(Reliability of Data)

- Trait evaluation
  - Simpler inheritance, fewer genes…
  - More reliable and better heritability?
Trait-based Selection

- How important is the trait in determining yield?
- Does the same QTL always control the trait?

Yield

Trait

QTL
• Trait based selection

  – Only part of a broader phenotype…miss interaction among traits?

  – Requires great confidence in the value of the trait for the desired phenotype
The case of Drought

- A common commitment in CRSP, TL-1, and TL-2
- Significant genetic variability
- Some traits identified
**Correlation (r) between grain yield and plant traits**

* *, **, *** Significant at 0.05, 0.01 and 0.001 probability levels, respectively

<table>
<thead>
<tr>
<th>Plant traits</th>
<th>Irrigated</th>
<th>Rainfed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf area index (m²/m²)</td>
<td>-0.48***</td>
<td>-0.14*</td>
</tr>
<tr>
<td>Canopy biomass (kg ha⁻¹)</td>
<td>0.38***</td>
<td>0.53***</td>
</tr>
<tr>
<td>Canopy temperature depression (°C)</td>
<td>0.12</td>
<td>0.19**</td>
</tr>
<tr>
<td>Pod partitioning index (%)</td>
<td>0.87***</td>
<td>0.83***</td>
</tr>
<tr>
<td>Stem biomass reduction (%)</td>
<td>0.10</td>
<td>0.40***</td>
</tr>
<tr>
<td>Pod harvest index (%)</td>
<td>0.66***</td>
<td>0.61***</td>
</tr>
<tr>
<td>Harvest index (%)</td>
<td>0.89***</td>
<td>0.86***</td>
</tr>
<tr>
<td>Seed N content (%)</td>
<td>-0.73***</td>
<td>-0.62***</td>
</tr>
<tr>
<td>Seed P content (%)</td>
<td>-0.59***</td>
<td>-0.60***</td>
</tr>
<tr>
<td>Seed TNC content (mg g⁻¹)</td>
<td>0.29***</td>
<td>0.13</td>
</tr>
</tbody>
</table>

Andean reference collection (81)-terminal drought

CIAT, 2009
Methodology for root phenotyping in field
Field training:
Katumani, Kenya, Jan. 2008
TL-2 Project
## Equipment on site

<table>
<thead>
<tr>
<th>EQUIPMENT</th>
<th>COUNTRIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Davis Vantage Pro2 Weather Station.</td>
<td>ETH, KYA, MWI, TNZ, ZIM</td>
</tr>
<tr>
<td>Laptop computer</td>
<td>&quot;</td>
</tr>
<tr>
<td>Watermark soil moisture system with Meters.</td>
<td>&quot;</td>
</tr>
<tr>
<td>Sensor for Soil moisture system.</td>
<td>&quot;</td>
</tr>
<tr>
<td>Ohaus Explorer Pro Toploading Balance.</td>
<td>&quot;</td>
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<tr>
<td>Digital camera SONY DSC-H50/B</td>
<td>&quot;</td>
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<tr>
<td>ET Gauge</td>
<td>&quot;</td>
</tr>
<tr>
<td>SPAD 502DL Chlorophyll meter</td>
<td>&quot;</td>
</tr>
<tr>
<td>Soil Corers</td>
<td>&quot;</td>
</tr>
<tr>
<td>SC-1 Porometer</td>
<td>ETH, KYA, MWI</td>
</tr>
<tr>
<td>Turf-Tec Infrared Turf Thermometer with probe</td>
<td>&quot;</td>
</tr>
<tr>
<td>Hand-held FluorPen with firmware upgrade</td>
<td>&quot;</td>
</tr>
<tr>
<td>WHINRIZO Prosoftware on CDROM</td>
<td>&quot;</td>
</tr>
<tr>
<td>Calibrate Color Optical Scanner</td>
<td>&quot;</td>
</tr>
<tr>
<td>Root positioning system for STD scanners</td>
<td>&quot;</td>
</tr>
</tbody>
</table>
QTL for Multiple Traits

• MD 23-24 x SEA 5
• Phenotypic data for QTL on…
  – Intermittent drought yield (3 years)
  – Pod Partitioning Index
  – Pod harvest Index
  – Rooting depth
  – etc
QTL with drought yield

Effect MD 23-24

Effect SEA 5

GL 1 2 3 4 5 6 7 8 9 10 11
Physiological parameters

Pod Partitioning Index

(Pod biomass / Shoot biomass @ mid pod fill) x 100

Pod Harvest Index

(Grain / Total pod biomass) x 100
Evaluation of rooting depth of RILs
Trait-based QTL

Yield

Trait

QTL

Trait

QTL

Trait

QTL

Trait

QTL
Options for (semi-) Automated Phenotyping

- Canopy temperature
- Chlorophyll
- Ground cover
- Root development
- ...
Rainout shelter at CIAT-HQ
Canopy temperature depression using infrared thermography (left) and infrared thermometer (right).

CIAT, 2010
Phenotyping for Soil Constraints

• Field evaluations…

• Example of Aluminum –
  – The worst case scenario
Phenotyping for Al resistance

Germination

4 days

2 days

0 µM Al

20 µM Al

Germination 2 days

2 days

Image analysis (WinRhizo)

Primary root elongation
Total root length per plant
Mean root diameter

CIAT, 2008
Identification of Al resistant genotypes

![Graph showing Al-resistant genotypes]

- **ICA QUIMBAYA**
- **BRB 198**
- **G 24601**
- **G 5273**
- **ICA PIJA**
- **O G 19833**
- **RAB 655**
- **INB 109**
- **MAM 38**
- **ALB 218**
- **INB 603**
- **ALB 604**
- **G 40001**
- **INB 605**
- **INB 602**
- **BAT 477**
- **ALB 220**
- **VAX 1**

Al-inhibited primary root elongation (%)
$P. \text{coccineus}$  
(resistant) 

$P. \text{vulgaris}$  
(susceptible)
Correlation of Root and Shoot traits with Yield under High Aluminum*

<table>
<thead>
<tr>
<th>ROOT TRAITS - NUTRIENT SOLUTION</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>% Root inhibition, (high vs low Al)</td>
<td>- 0.15</td>
</tr>
<tr>
<td>Root Biomass</td>
<td>0.07</td>
</tr>
<tr>
<td>Total root length</td>
<td>0.19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SHOOT TRAITS - SOIL TUBES</th>
<th></th>
</tr>
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<tbody>
<tr>
<td>Shoot biomass</td>
<td>0.29**</td>
</tr>
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<tr>
<td>Root biomass</td>
<td>0.10</td>
</tr>
<tr>
<td>Total root length (m / plant)</td>
<td>0.27**</td>
</tr>
<tr>
<td>Root diameter</td>
<td>- 0.23*</td>
</tr>
</tbody>
</table>

*Louis Butare, Ph.D. thesis
Application to Low P

• Significant genetic variability

• Several traits identified
  – (Jonathan discussed)
Summary

• Genotyping is upon us! Phenotyping is lagging

• Yield testing is a “necessary headache” as the primary trait and as a point of reference for other options

• Traits may be used either as selection criteria or as the basis of QTL analysis

• Traits need careful assessment for stability of their relationship with yield

• Trait QTL also need careful assessment for stability
• Thank you