Rationale

- Challenge to increase yield in the face of climate change and diminishing water resources
- Genetic improvement via modern plant breeding is the most sustainable and economic approach to meet this challenge
- Development of superior heat and drought tolerant cultivars has been slow and difficult
- Breeding progress could be improved by development of new ways to connect phenotype to genotype

Research Objectives

- To develop a genotyping-by-sequencing (GBS) approach for multiplex genotyping of a cotton RIL population
- To develop a field-based, proximal remote sensing approach for high-throughput phenotyping (HTP) of adaptive traits in a cotton RIL population

Genotyping-By-Sequencing

“...massively parallel sequencing of multiplex reduced-representation genomic libraries.”

“massively parallel sequencing” = sequencing on Illumina HiSeq platform

“multiplex” = using DNA barcode (unique 5-10bp)
- DNA sequence synthesized on the adapter
- pool 48-384 samples together

“reduced-representation” = use restriction enzyme to capture only the low-copy portion of the genome flanking restriction sites
- methylation-sensitive restriction enzymes

Elshire et al. 2011 PLoS ONE 6(5): e19379

GBS: Library Construction

Applying GBS in upland cotton
- TM1 x NM24016 RIL population
  - 94 RILs + 2 parents
  - 96-plex GBS library (PstI-MspI)
  - Sequence 2x on Illumina HiSeq

J. Poland

TM-1 NM24016 RIL population

TM-1: genetic standard for *G. hirsutum*

NM24016: an elite *G. hirsutum* line with considerable (1/3) but stable introgression from several *G. barbadense* lines
GBS: Bioinformatics Pipeline

- Parse reads by barcode
- Collapse identical reads
- Identify tags that differ by 1 or 2 bases
- Fisher Exact Test: Reject $H_0$, $P < 0.001$
- Assign genotype scores to lines for biallelic SNPs

GBS: SNP Calls for RILs

- Constructed genetic map has 500 SNPs with 500 SSRs – low polymorphism rate and segregation distortion

HTP: Sensors, Platform, and Vehicle

- Canopy Tm
- Infrared thermometer
- Plant height
- Ultrasonic Transducer
- Vegetation Indices
- High-clearance tractor
- Average speed of 2.82 km/h
- 1 data point/meter (1 Hz)

HTP: Canopy Temperature

Central Arizona: clear skies, very limited rain, high temperatures
Population: TM-1 x NM24016 of 94 RILs ($Gossypium$ hirsutum L.)
Treatment: 100 and 50% ET (2 reps) drip irrigation

Spatial analysis to control for soil variation when calculating BLUPs

- Dry Rep2: 33–40°C
- Wet Rep1: 29–30°C
- Dry Rep1: 31–40°C

1 pm on day 224 (12-Aug)

Heritability estimates are 0.52 0.11 for wet at 1 pm and 0.58 0.09 for dry at 1 pm

Significant Time-by-Treatment Interaction for Canopy Temperature

- Treatment $P < 0.05$
- Time $P < 0.0001$
- Treatment*Time $P < 0.0001$
Repeatability of $\Delta$ in Canopy Tm

$R^2 = 0.54$

Consistent phenotypic response to stress

QTL Analysis: Canopy Temperature

<table>
<thead>
<tr>
<th>TRT</th>
<th>DOY</th>
<th>Time</th>
<th>Chr</th>
<th>cM</th>
<th>Left Marker</th>
<th>Right Marker</th>
<th>LOD</th>
<th>PVE(%)</th>
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<td>19.15</td>
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Inclusive CIM with a 0.05 Type I Error Rate

HTP: Normalized Difference Vegetation Index (NDVI)

$NDVI = \frac{(NIR-\text{red})}{(NIR+\text{red})}$

NIR 820 nm
Red 670 nm

Wilting Index (WI)

$WI = \frac{(NDVI_{pm} - NDVI_{pm})}{NDVI_{pm}}$

 Significant Time-by-Treatment Interaction for NDVI

Heritability estimates are 0.79 0.04 for wet 1 pm and 0.59 0.09 for dry 1 pm

Repeatability of Wilting Index

$R^2 = 0.76$

Consistent phenotypic response to stress

QTL Analysis: Wilting Index

<table>
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<tr>
<th>DOY</th>
<th>Time</th>
<th>Chr</th>
<th>cM</th>
<th>Left Marker</th>
<th>Right Marker</th>
<th>LOD</th>
<th>PVE(%)</th>
<th>Add</th>
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<tr>
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Inclusive CIM with a 0.05 Type I Error Rate
Conclusions

• Constructed a GBS genetic map for tetraploid cotton RIL population without a need for a reference genome or downstream SNP assays

• Developed a field-based HTP approach to rapidly phenotype 100s to 1000s of plots for several canopy traits

• Canopy temperature and wilting index are moderately to highly heritable as well as repeatable QTL on a temporal scale

Next Steps for HTP

• HTP for screening 1,000 cotton cultivars and day-neutral landrace lines for stress tolerance

• HTP for evaluating very large cotton RIL populations (e.g., cotton NAM populations)

• Investigate G × E in multiple crops with HTP for many different phenotypes (disease, yield…)

• Testing new imaging and non-contact sensor technologies for phenotyping

Optical Remote Sensing with Light Detection And Ranging (LIDAR)

Bob Strand and Andy French

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Richard Percy
David Fang

GBS: Sample Genotyping Costs

J. Poland
**GBS: Bioinformatics Pipeline**

Parse sequences to samples using barcode

Collapse identical tags (matrix of counts – presence/absence)

**SNPs:**
- Identifying tags: 1) differing by 1 or 2 bp
- Segregating in population
- Not in same line

Map reads to genetic map using reference markers

**SNPs**
- Fisher exact test for significance, BIN mapping
- ML to position in interval between markers

**Tags**
- (Dominant markers) binomial probably for significance testing, ML to position within interval (matrix of tags/SNPs with genotype calls by line)

**de novo genetic map**

Poland et al. 2012 *PLoS ONE* 7(2): e32253
Phenotypic Variability of Canopy Temperature

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Frequency</th>
<th>Mean</th>
<th>Std Dev</th>
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<tr>
<td>32.28</td>
<td>32.96</td>
<td>Mean</td>
<td>1.94</td>
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<tr>
<td>37.98</td>
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<td>Mean</td>
<td>1.72</td>
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Wet and Dry Plots at 1 pm on Day 224

Relationship between ∆ Canopy Tm and Wilting Index

R² = 0.49

How do we connect phenotype to genotype for complex adaptive traits?

HTP: Plant Height

R² = 0.81

“Worst drought in years...”

Source: U.S. Drought Monitor

Source: U.S. Drought Monitor