Inhibiting infections by Xcm

Libo Shan

Department of Plant Pathology & Microbiology
Institute for Plant Genomics & Biotechnology
Texas A&M University
Cotton

Soft Cool Stylish Versatile
Environmentally friendly Natural
Fashionable Comfortable Quality fabric
Youthful Traditional Breathes Sporty

Cotton Research

Long growth cycle Difficult Complex
recalcitrant for transformation

Cotton Inc. April 2016
**Importance & complexity of the cotton genome**

**Gossypium spp.**

- natural textile fiber & major oilseed crop
  1st value-added crop in USA

- Unique model for cell wall biosynthesis & polyploidization

- Diversity of germplasm
  45 diploid species (A or D genome)
  5 tetraploid species (AD genome)

- Complexity of genome
  *G. hirsutum*: ~2.5 Gb (95% cotton production)

*References*

Li et al., Nat Biotechnol 2015
Zhang et al., Nat Biotechnol 2015
Cotton Inc. April 2016
Li et al., Nat Genet 2014
Wang et al., Nat Genet 2012
Paterson et al., Nature 2012
2012: *G. raimondii* - ~41,000 protein-coding genes

2014: *G. arboreum* - ~41,000 protein-coding genes

2015: *G. hirsutum* - ~32,000 A-subgenome, ~34,000 D-subgenome

\[
\text{So many genes in cotton!}
\]
How to study cotton gene functions?

Functional Genomics

- Explore cotton gene functions in cotton NOT other plants.

- Transient loss-of-function assays (two weeks)
  
  *Agrobacterium*-mediated VIGS

- Transient gain-of-function assays (two days)
  
  *Agrobacterium*-mediated transient assay
  Cotton protoplast transfection assay
Virus-induced gene silencing

(a) cDNA → Binary VIGS vector → Agrobacterium inoculation

(b) ssRNA → dsRNA → dsRNA cleavage → dsRNA

Viral genome transcription → vRNA replication → dsRNA

Systemic spread of silencing → siRNA amplification and transport → mRNA degradation

Modified from Becker and Lange, Trends in Plant Science. 2010
Agrobacterium-mediated VIGS

10-day-old seedling → Agro-infiltration → Phenotypic analysis

VIGS-vector VIGS-CLA1

CLA1: Cloroplastos alterados 1 involved in chloroplast development

Gao et al., The Plant Journal. 2011
Gao et al., JoVE. 2011

Cotton Inc. April 2016
VIGS in diverse cotton genetic backgrounds

Vector        GhCLA1        AtCLA1

Deltapine 90
R135
Phytogen 480WR
Phytogen 425RF

Cotton Inc. April 2016

Gao et al., The Plant Journal. 2011
Cotton Functional Genomics

Transient loss-of-function assay

Mining essential cotton genes for the traits

Genetic engineering of the master regulator

Increase sustainable yield, resistance and quality
Cotton functional genomics via VIGS library

10-day-old seedlings

Inoculation with Agro-VIGS cDNA library

Target genes are silenced two weeks after inoculation

Screening for disease resistance or other phenotypes

Identifying interested candidates

Sequencing plasmid DNA from Agrobacteria

Targeted breeding program or transgenic approach

~1.9 × 10^6 clones G. hirsutum
Transient gain-of-function assay in cotton
Subcellular localization in cotton protoplasts

GFP

Plasma membrane-GFP

Nuclear-GFP

DIC  GFP  Hoechst
Activation of convergent MAP kinase signaling by pathogen elicitors

Immuno-complex kinase assay to detect cotton MAPK6 activity
Verticillium wilt
Fusarium wilt
RLKs & RLPs
Bacterial blight
TALE targets
Drought tolerance
PARP & MAPKs
Root Rot
Cotton comparative, functional and biochemical genomics
Cotton Inc. April 2016
Cotton receptor-like protein GhVe1 and receptor-like kinase GhBAK1 mediate Verticillium wilt resistance.

Gao et al., The Plant Journal. 2011
Gao et al., Journal of Integrative Biology. 2013
RLK/RLP complex in cotton

Verticillium wilt resistance

Arabidopsis-Pseudomonas

Flagellin

AtFLS2

AtBAK1

MAP kinase cascade

flagellin-triggered immunity

Cotton-Verticillium

Ave1 or ?

GhVe1

GhBAK1

GhSoBIR1?

MAP kinase cascade?

Verticillium resistance

Cotton resistance to *Fusarium wilt*

The role of receptor kinases

---

VIGS-Control.  
VIGS-Gene 1

**H₂O**  
**FOV10 RACE1**

Cotton Inc. April 2016
RNA-seq to mine novel genes in cotton drought stress

RNA-seq: upregulated genes upon 2-day and 4-day drought treatment

<table>
<thead>
<tr>
<th>Gene</th>
<th>Control</th>
<th>Drought</th>
<th>Control</th>
<th>Drought</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATEXLB1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABI 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MYB43</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEBP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SAG12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LRR-RLK</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gh Actin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Cotton Inc. April 2016
Determinatives of cotton drought stress

Two weeks after VIGS; Drought treatment: 12 days

Control  SnRK  MPK  CBF3  NFYA5  MYB2  MYC2  ABA8  DREB

Cotton Inc. April 2016
Bacterial blight of cotton (BBC)

Pathogenicity of Xcm
The Type III Secretion System

Cotton Inc. April 2016

Delannoy et al., Annu. Rev. Phytopathol. 2005
Host-Specific Symptoms and Increased Release of *Xanthomonas citri* and *X. campestris pv. malvacearum* from Leaves Are Determined by the 102-bp Tandem Repeats of *pthA* and *avrb6*, Respectively

Yinong Yang, Robert De Feyter, and Dean W. Gabriel

Plant Molecular and Cellular Biology Program and Plant Pathology Department, University of Florida, Gainesville 32611 USA

Six avirulence genes (*avrB*, *avr6*, *avr7*, *avrB1n*, *avrB101*, and *avrB102*) found in *Xanthomonas campestris pv. malvacearum* strain XcmH1005 and a host-specific pathogenicity gene (*pthA*) found in *X. citri* belong to an *avr/pth* gene family and are characterized by tandemly arranged, 102-bp repeats in the central portions of the genes. Marker exchange mutagenesis and complementation experiments revealed that *avrb6* was required for XcmH1005 to cause severe water-soaking and subsequent necrosis in susceptible Acala-44 cotton lines. An average of 240 times more bacteria were released onto the cotton leaf surface from water-soaked spots caused by XcmH1005 than from those caused by an isogenic *avrb6* strain, strongly indicating a role for *avrb6* in pathogen dispersal. However, *avrb6* did not affect in planta bacterial growth rate or yield. By constructing chimeric genes among *pthA*, *avrB4*, *avr6*, *avr7*, *avrB1n*, *avrB101*, and *avrB102*, the 102-bp tandem repeats of the genes were found to determine the gene-for-genes specificity of the avirulence reactions on cotton. In addition, the repeat regions of *avrb6* and *pthA* determined their specificity in enhancing water-soaking of cotton and causing cankers on citrus, respectively. When the native promoters of each gene were replaced by the *Escherichia coli lacZ* promoter, the hypersensitive response elicited in resistant host lines was stronger in all cases tested, while the pathogenic specificities of *avrb6* for cotton and *pthA* for citrus were unaltered. These results indicate that some members of this *avr/pth* gene family may help condition host range by increasing the release of *Xanthomonas* cells from the mesophyll to the leaf surface, leading to increased dispersal on specific hosts.
Type III Effectors

Transcription Activator-Like (TAL) Effectors

Moscou and Bogdanove, Science 2009
Mak et al., Science 2012

Cotton Inc. April 2016
One of the major virulence factors from \textit{XcmH1005} (Race 4).

- Induces strong water-soaking on specific lines of cotton

\[ \sim 10 \text{ TALEs cloned from } Xcm, \text{ but no any } R \text{ or } S \text{ genes have been found from cotton.} \]
Determinatives of TAL effectors in cotton bacterial blight

In collaboration with Dr. Adam Bogdanove
Cotton Inc. April 2016
Biological inhibiting infection by *Xcm*

Biological inhibiting *Xcm* infection specifically blocking the effector binding site without interfering its endogenous functions

**CRISPR-Cas9 System**

- Revolutionary genome editing tool
- Mutate TALe recognition site in cotton
- Used in rice to create resistant rice to BBR
- Create Cotton that has broad spectrum resistance to BBC

Cotton Inc. April 2016
Acknowledgements

Texas A&M Univ.
Kevin Cox
Dr. Fanhong Meng
Dr. Fangjun Li
Tri Tran
Dr. Xiquan Gao
Dr. Maoying Li
Dr. Ping He
Dr. Tom Isakeit

Texas Agricultural Experiment Station
Lubbock
Dr. Terry Wheeler

Texas A&M AgriLife Research
Dr. Jane Dever

Boyce Thompson Institute
Dr. Zhangjun Fei
Dr. Yi Zheng

Cornell Univ.
Dr. Adam Bogdanove
Katie Wilkins

Univ. of Florida
Dr. Dean Gabriel

Donald Danforth Plant Science Center
Dr. Rebecca Bart
Mark Wilson

USDA-ARS, Texas A&M Univ.
Dr. John Yu

Cotton Inc. April 2016