



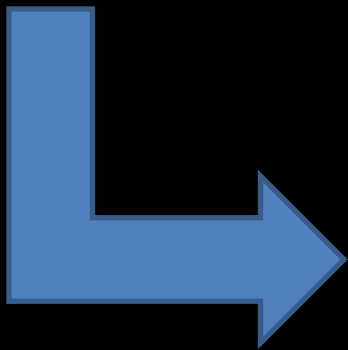
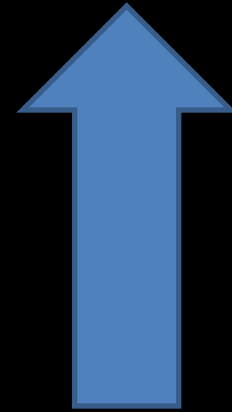
Inhibiting infections by *Xcm*

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Inhibiting infections by *Xcm*

Understand
biological & genetic
basis of *Xcm*
infections in cotton



Develop
biological means to
control or prevent
Xcm infections

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



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)

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

The draft genome of a diploid cotton *Gossypium raimondii*

Kunbo Wang^{1,6}, Zhiwen Wang^{2,6}, Fuguang Li^{1,6}, Wuwei Ye^{1,6}, Junyi Wang^{2,6}, Guoli Song^{1,6}, Zhen Yue², Lin Cong², Haihong Shang¹, Shilin Zhu², Changsong Zou¹, Qin Li³, Youlu Yuan¹, Cairui Lu¹, Hengling Wei¹, Caiyun Gou², Zequn Zheng², Ye Yin², Xueyan Zhang¹, Kun Liu¹, Bo Wang², Chi Song², Nan Shi², Russell J Kohel⁴, Richard G Percy⁴, John Z Yu⁴, Yu-Xian Zhu³, Jun Wang^{2,5} & Shuxun Yu¹

Repeated polyploidization of *Gossypium* genomes and the evolution of spinnable cotton fibres

Andrew H. Paterson¹, Jonathan F. Wendel², Heidrun Gundlach³, Hui Guo¹, Jerry Jenkins^{4,5}, Dianchuan Jin⁶, Danny Llewellyn⁷, Kurtis C. Showmaker⁸, Shengqiang Shu⁴, Joshua Udall⁹, Mi-jeong Yoo², Robert Byers⁹, Wei Chen⁶, Adi Doron-Faigenboim¹⁰, Mary V. Duke¹¹, Lei Gong², Jane Grimwood^{4,5}, Corrinne Grover², Kara Grupp², Guanjing Hu², Tae-ho Lee¹, Jingping Li¹, Lifeng Lin¹, Tao Liu⁶, Barry S. Marler¹, Justin T. Page⁹, Alison W. Roberts¹², Elisson Romanel¹³, William S. Sanders⁸, Emmanuel Szadkowsk¹⁴, Xu Tan¹, Haibao Tang^{1,14}, Chunming Xu^{2,15}, Jinpeng Wang⁶, Zining Wang¹, Dong Zhang¹, Lan Zhang⁶, Hamid Ashrafi¹⁶, Frank Bedon⁷, John E. Bowers¹, Curt L. Brubaker^{7,17}, Peng W. Chee¹⁸, Sayan Das¹, Alan R. Gingle¹, Candace H. Haigler¹⁹, David Harker⁹, Lucia V. Hoffmann²⁰, Ran Hovav¹⁰, Donald C. Jones²¹, Cornelia Lemke¹, Shahid Mansoor^{1,22}, Mehboob ur Rahman²², Lisa N. Rainville¹, Aditi Rambani⁹, Umesh K. Reddy²³, Jun-kang Rong¹, Yehoshua Saranga²⁴, Brian E. Scheffler¹¹, Jodi A. Scheffler¹¹, David M. Stelly²⁵, Barbara A. Triplett²⁶, Allen Van Deynze¹⁶, Maite F. S. Vaslin²⁷, Xueyan Zhang¹, Jun Wang^{2,5} & Shuxun Yu¹

Genome sequence of the cultivated cotton *Gossypium arboreum*

Fuguang Li^{1,11}, Guangyi Fan^{2,11}, Kunbo Wang^{1,11}, Fengming Sun^{2,11}, Youlu Yuan^{1,11}, Guoli Song^{1,11}, Qin Li^{3,11}, Zhiying Ma^{4,11}, Cairui Lu¹, Changsong Zou¹, Wenbin Chen², Xinming Liang², Haihong Shang¹, Weiqing Liu², Chengcheng Shi², Guanghui Xiao³, Caiyun Gou², Wuwei Ye¹, Xun Xu², Xueyan Zhang¹, Hengling Wei¹, Zhifang Li¹, Guiyin Zhang¹, Junyi Wang², Kun Liu¹, Russell J Kohel⁵, Richard G Percy⁵, John Z Yu⁵, Yu-Xian Zhu³, Jun Wang^{2,6-10} & Shuxun Yu¹

Genome sequence of cultivated Upland cotton (*Gossypium hirsutum* TM-1) provides insights into genome evolution

Fuguang Li^{1,9}, Guangyi Fan^{2,9}, Cairui Lu^{1,9}, Guanghui Xiao^{3,4,9}, Changsong Zou^{1,9}, Russell J Kohel^{5,9}, Zhiying Ma^{6,9}, Haihong Shang^{1,9}, Xiongfeng Ma^{1,9}, Jianyong Wu^{1,9}, Xinming Liang^{2,9}, Gai Huang^{3,4}, Richard G Percy⁵, Kun Liu¹, Weihua Yang¹, Wenbin Chen², Xiongming Du¹, Chengcheng Shi², Youlu Y Wuwei Ye¹, Xin Liu², Xueyan Zhang¹, Weiqing Liu², Hengling Wei¹, Shoujun Wei¹, Guodong Huang², Xianlong Zhang⁷, Shuijin Zhu⁸, He Zhang², Fengming Sun², Xingfen Wang⁶, Jie Liang², Jiahao Wang², Qiang He², Leihuan Huang², Jun Wang², Jinjie Cui¹, Guoli Song¹, Kunbo Wang¹, Xun Xu², John Z Yu⁵, Yu-Xian Zhu^{3,4} & Shuxun Yu¹

So many genes in cotton!

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

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Sequencing of allotetraploid cotton (*Gossypium hirsutum* L. acc. TM-1) provides a resource for fiber improvement

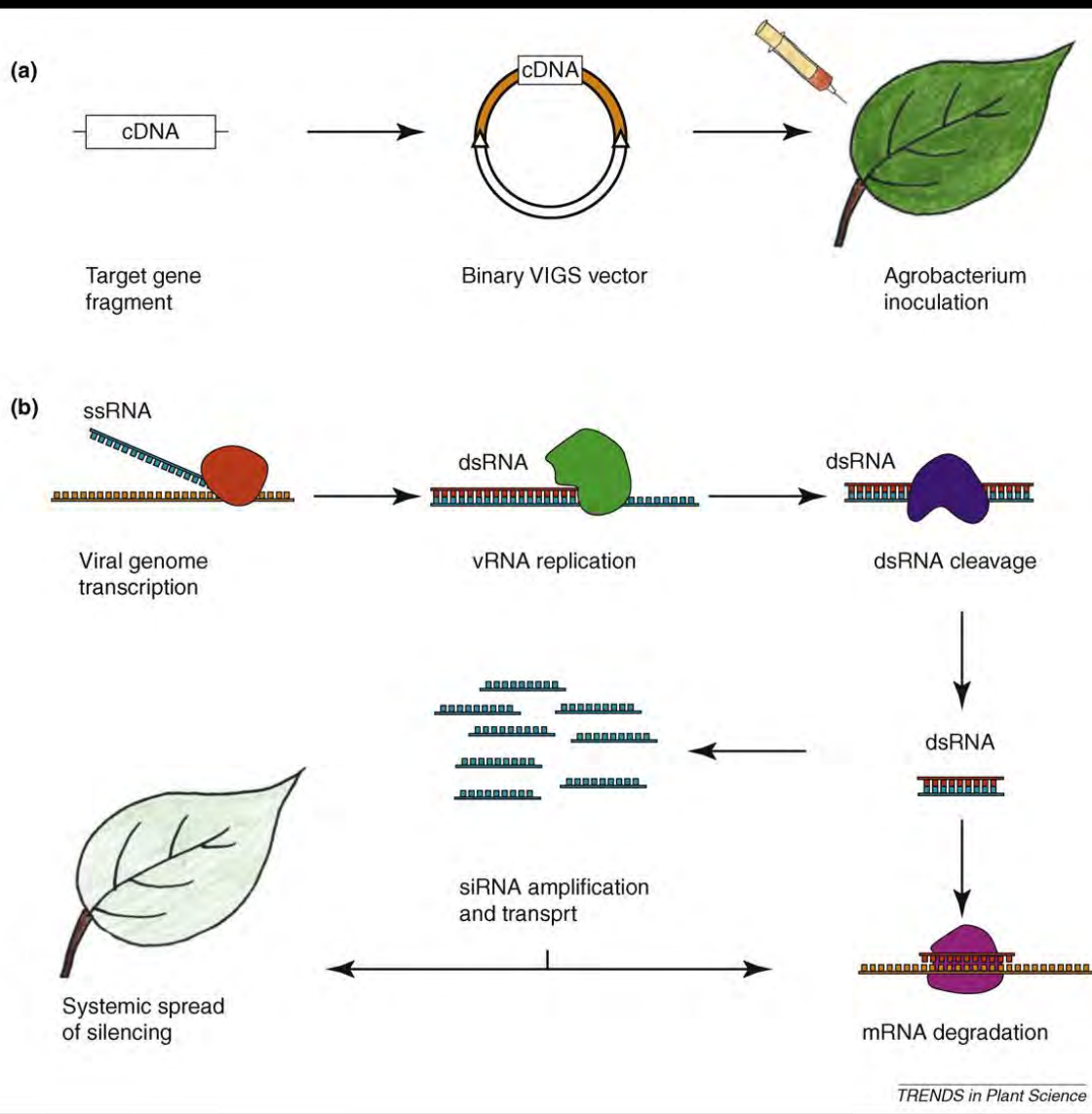
Tianzhen Zhang^{1,12}, Yan Hu^{1,12}, Wenkai Jiang^{2,12}, Lei Fang^{1,3,12}, Xueying Guan^{1,3,12}, Jiedan Chen^{1,12}, Jinbo Zhang², Christopher A Saski⁴, Brian E Scheffler³, David M Stelly⁶, Amanda M Hulse-Kemp⁶, Qun Wang¹, Bingliang Liu¹, Chunxiao Liu¹, Sen Wang¹, Mengqiao Pan¹, Yangkun Wang¹, Dawei Wang², Wenxue Ye¹, Lijing Chang¹, Wenpan Zhang¹, Qingxin Song³, Ryan C Kirkbride³, Xiaoya Chen⁷, Elizabeth Dennis⁸, Danny J Llewellyn⁸, Daniel G Peterson⁹, Peggy Thaxton¹⁰, Don C Jones¹¹, Qiong Wang¹, Xiaoyang Xu¹, Hua Zhang¹, Huaitong Wu¹, Lei Zhou¹, Gaofu Mei¹, Shuqi Chen¹, Yue Tian¹, Dan Xiang¹, Xinghe Li¹, Jian I Qiyang Zuo², Linna Tao², Yunchao Liu², Ji Li², Yu Lin², Yuanyuan Hui², Zhisheng Cao², Caiping Cai¹, Xiefei Zhu¹, Zhi Jiang², Baoliang Zhou¹, Wangzhen Guo¹, Ruiqiang Li² & Z Jeffrey Chen^{1,3}

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





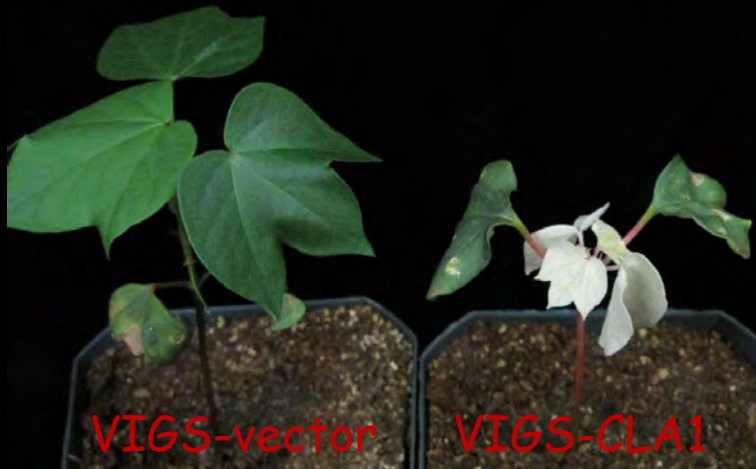
Agrobacterium-mediated VIGS



10-day-old seedling



Agro-infiltration



VIGS-vector

VIGS-CLA1

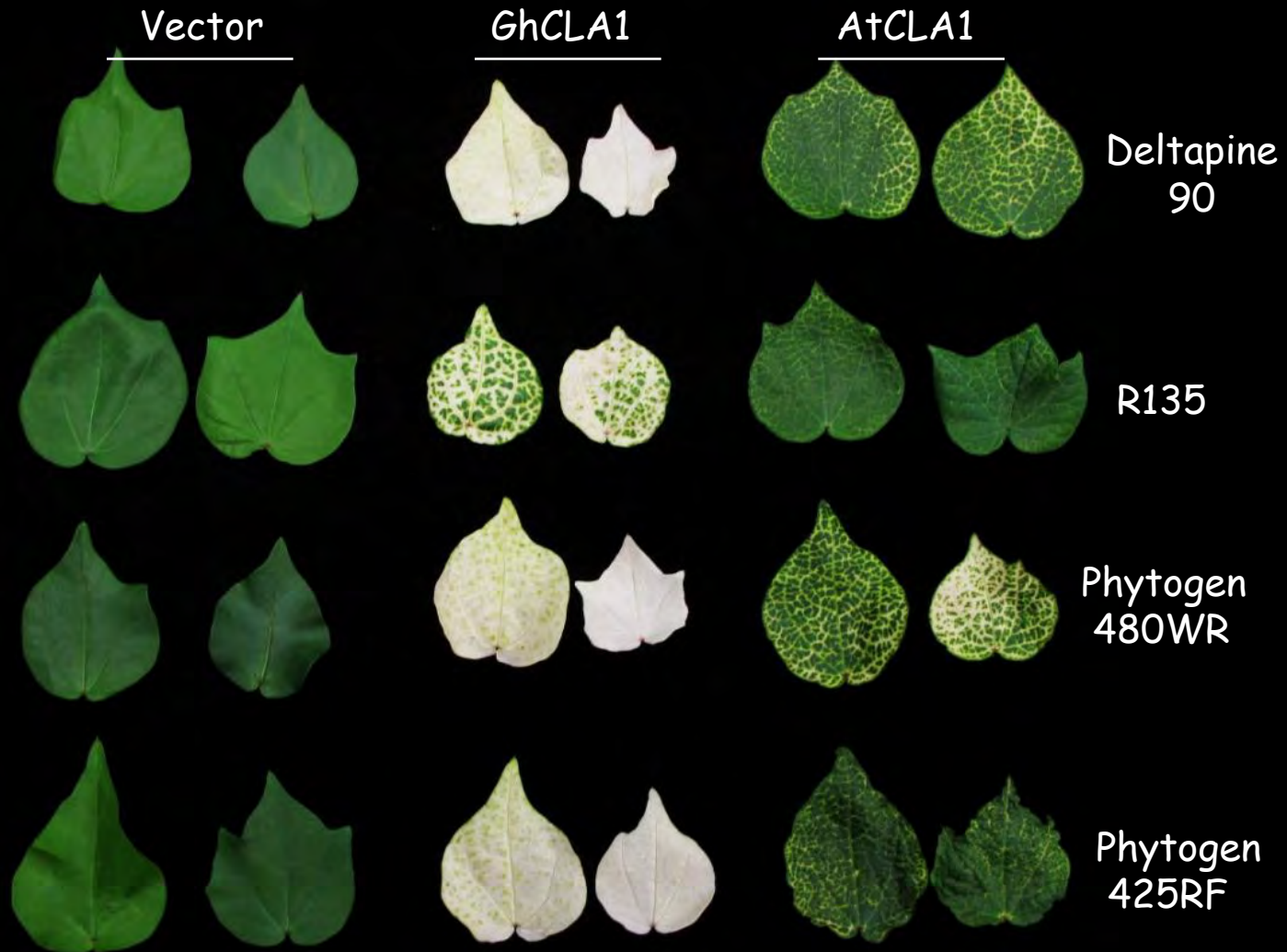
Phenotypic analysis
2-week-post-infiltration

CLA1: Cloroplastos alterados 1 involved in chloroplast development

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

Gao *et al.*, *JoVE*. 2011

VIGS in diverse cotton genetic backgrounds



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

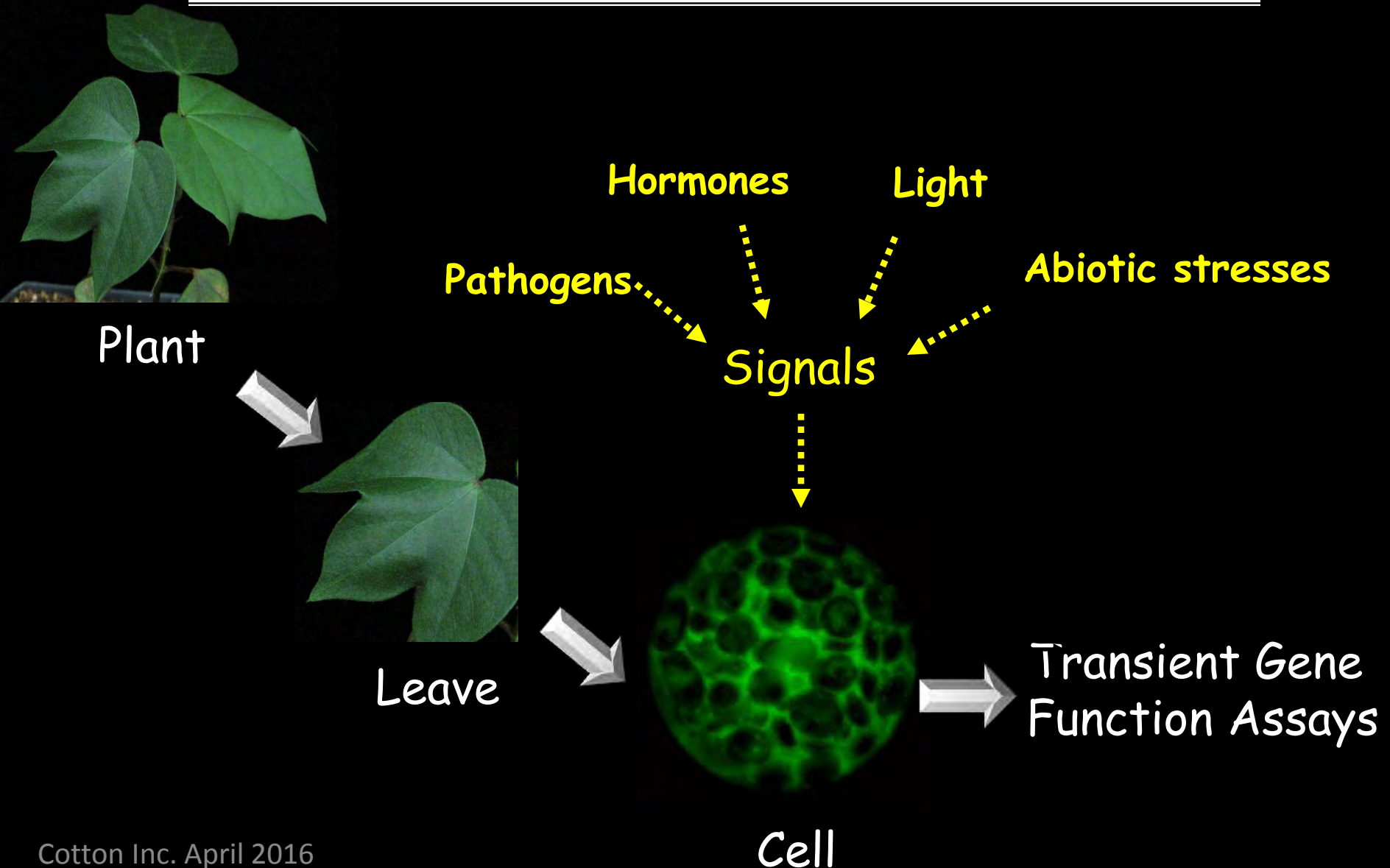
Targeted breeding program or transgenic approach



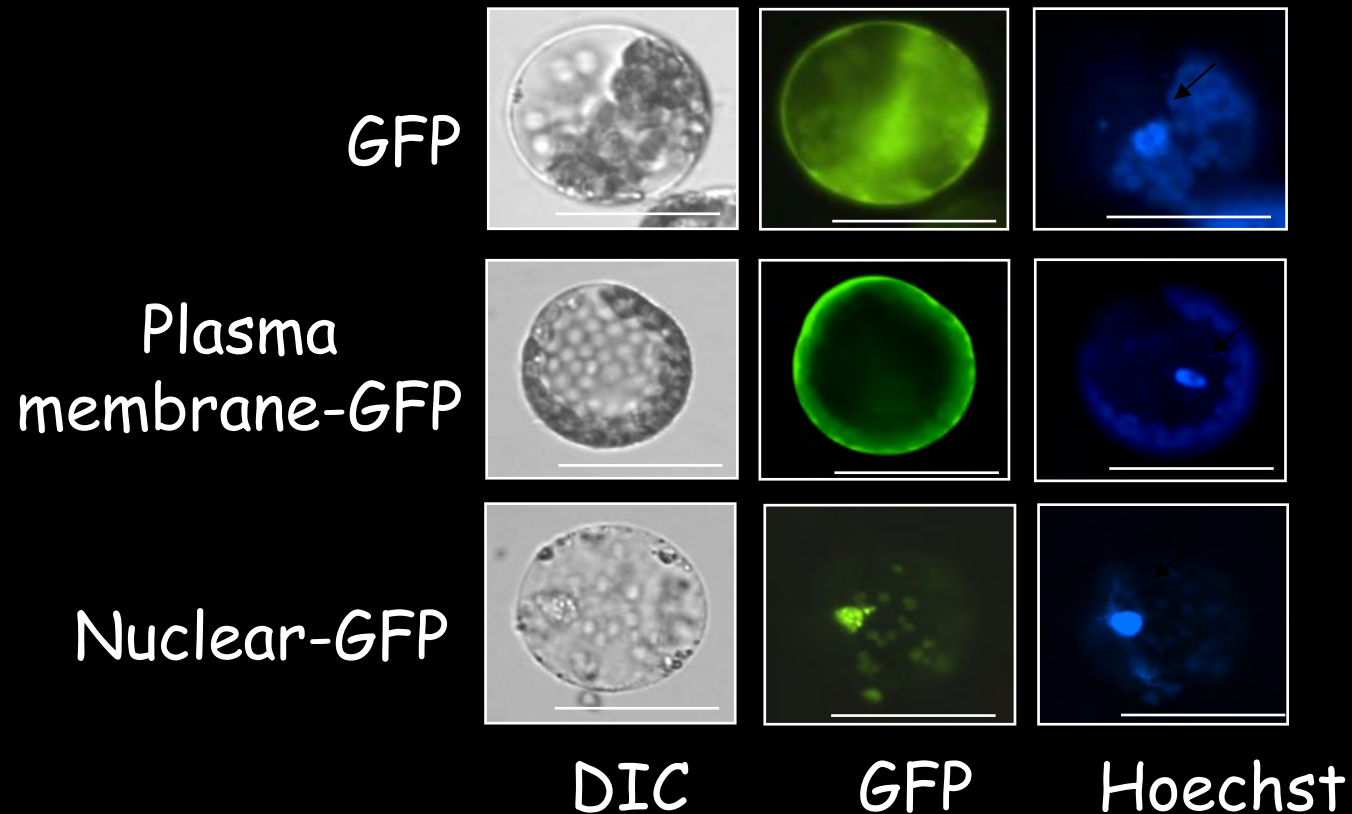
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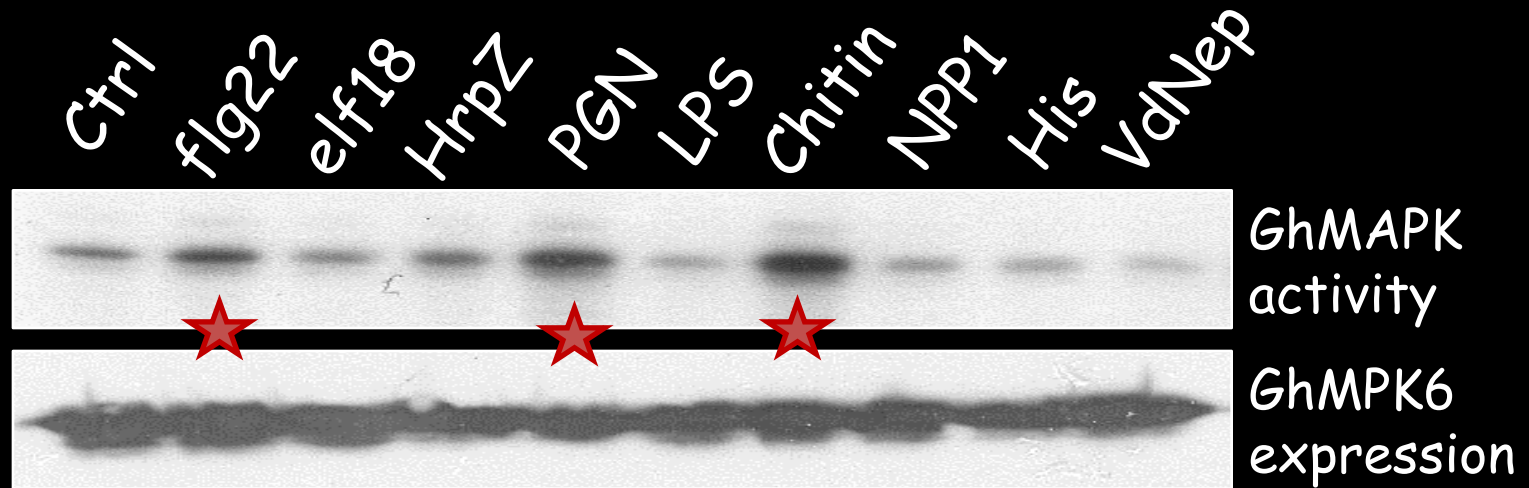
Transient gain-of-function assay in cotton



Subcellular localization in cotton protoplasts



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

Cotton comparative, functional and biochemical genomics

Drought tolerance
PARP & MAPKs



Root Rot



Cotton receptor-like protein GhVe1 and receptor-like kinase GhBAK1 mediate *Verticillium wilt* resistance.



VIGS-control



VIGS-GhVe1



VIGS-GhBAK1



VIGS-Ctrl GhVe1
GhVe1



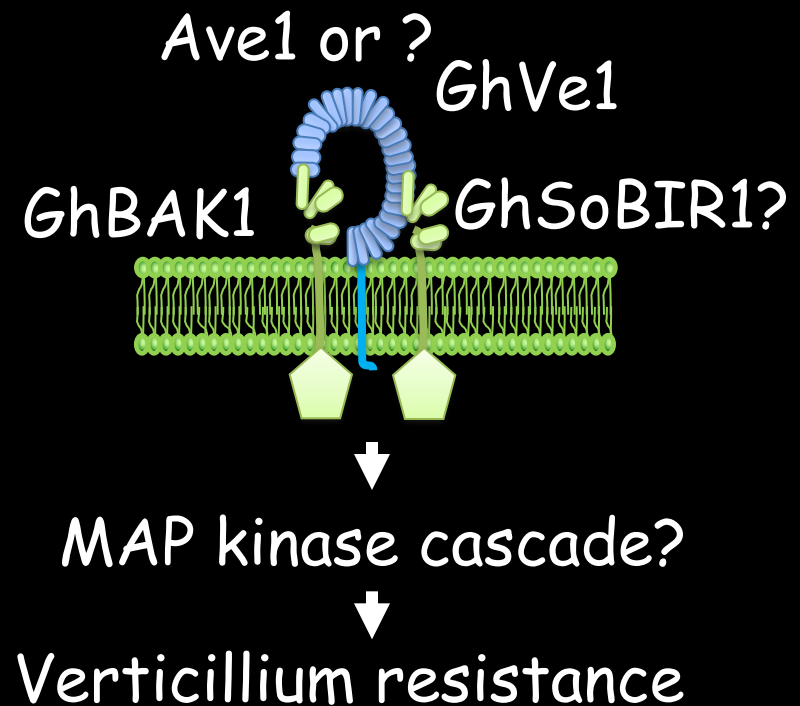
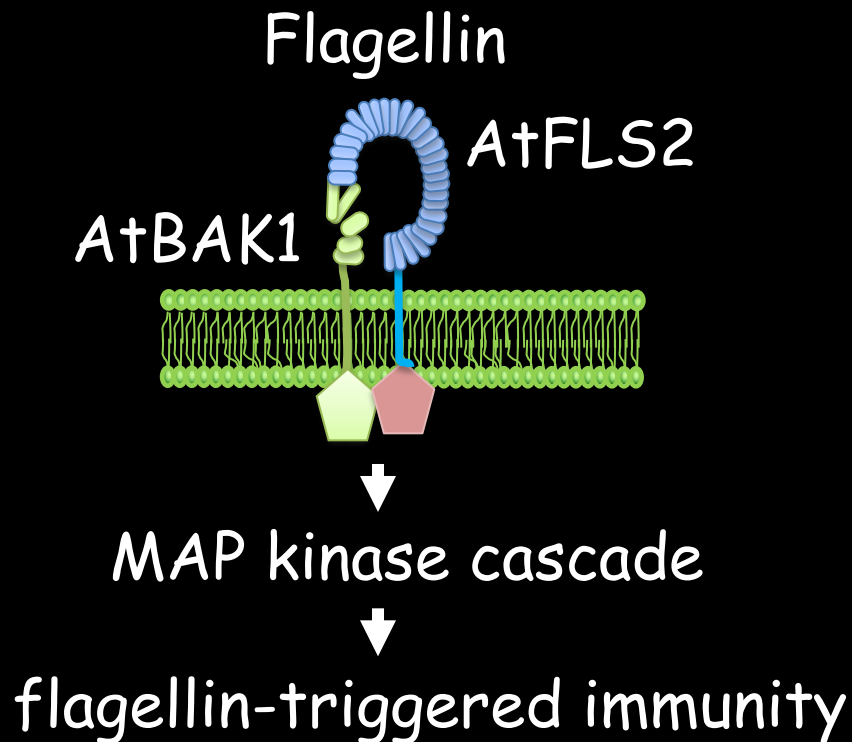
VIGS-Ctrl GhBAK1
GhBAK1



RLK/RLP complex in cotton *Verticillium* wilt resistance

Arabidopsis-Pseudomonas

Cotton-Verticillium



Cotton resistance to *Fusarium wilt*

The role of receptor kinases

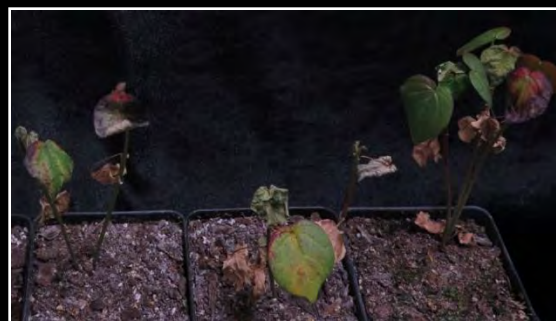
VIGS-Control.

VIGS-Gene 1

H₂O



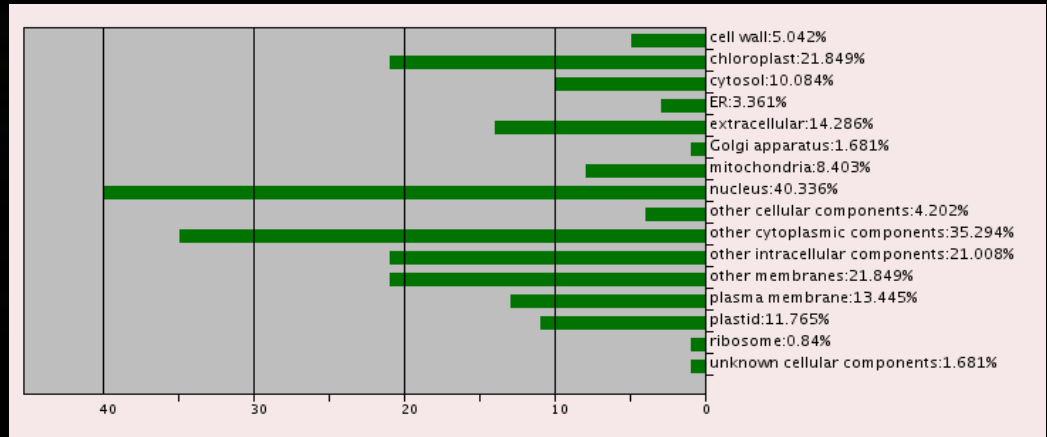
FOV10 RACE1



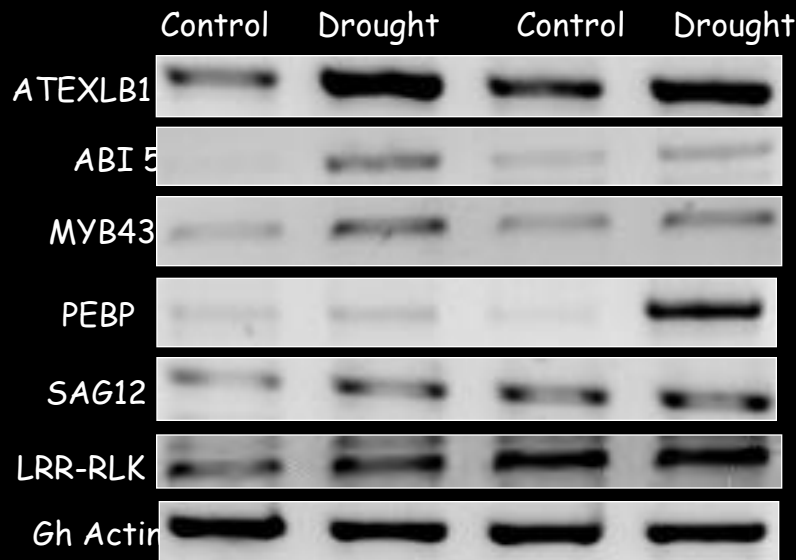
RNA-seq to mine novel genes in cotton drought stress



DP491 FM9160 706V



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



VIGS Ctrl WRKY Myb RLK



Determinatives of cotton drought stress



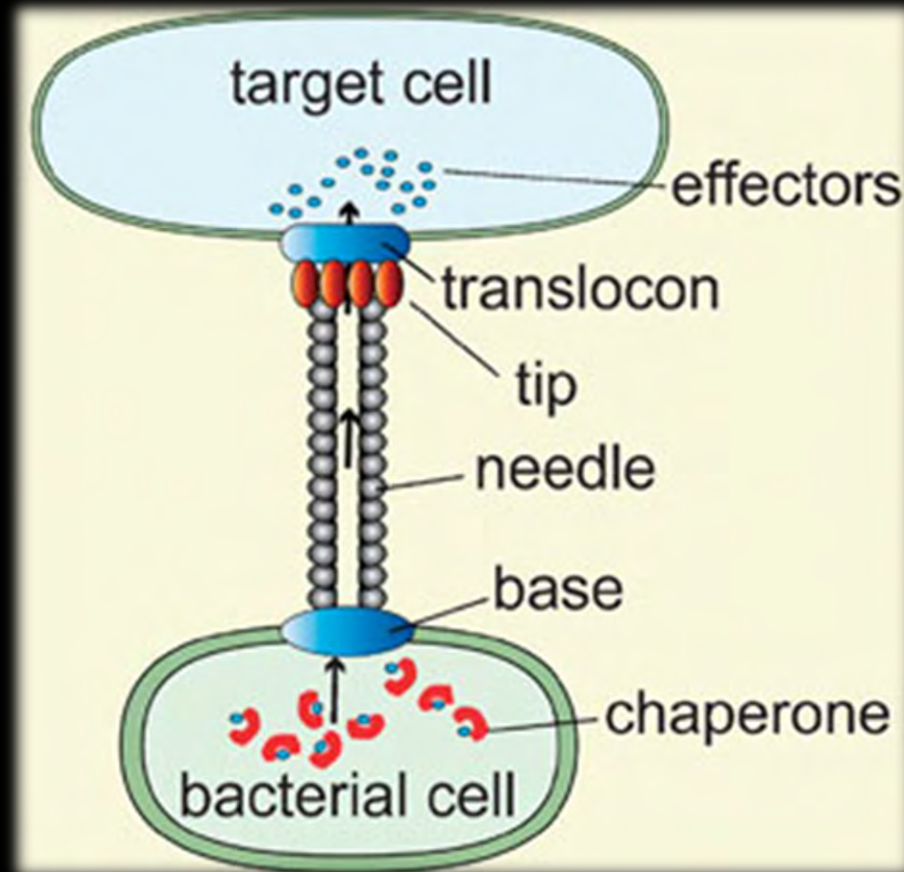
Two weeks after VIGS; Drought treatment: 12 days



Bacterial blight of cotton (BBC)

Pathogenicity of *Xcm*

The Type III Secretion System



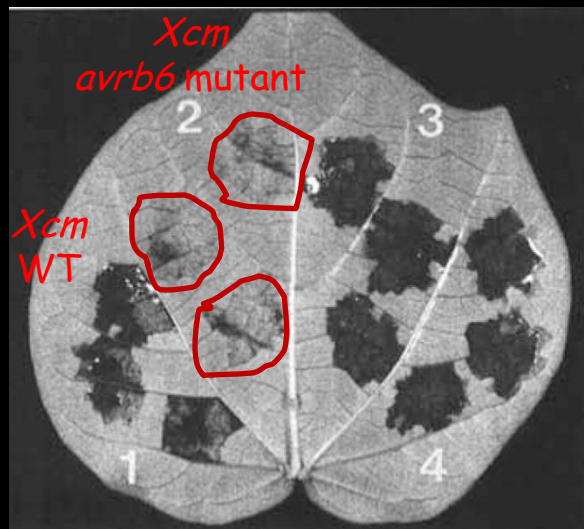
Host-Specific Symptoms and Increased Release of *Xanthomonas citri* and *X. campestris* pv. *malvacearum* from Leaves Are Determined by the 102-bp Tandem Repeats of *pthA* and *avrb6*, Respectively

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

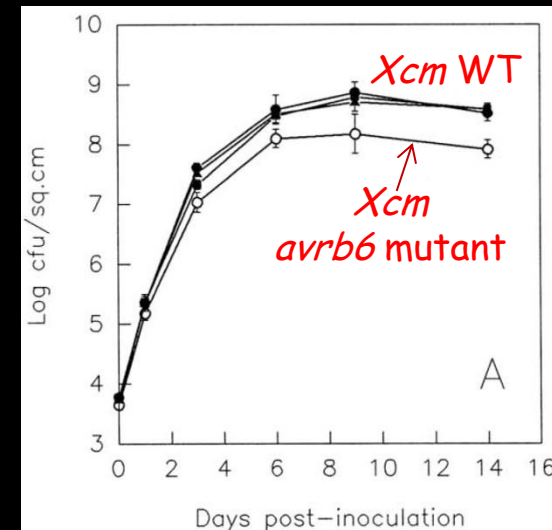
Plant Molecular and Cellular Biology Program and Plant Pathology Department, University of Florida, Gainesville 32611 U.S.A.

Six avirulence genes (*avrB4*, *avrb6*, *avrb7*, *avrBln*, *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*, *avrb6*, *avrb7*, *avrBln*, *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.

18 January 1994.



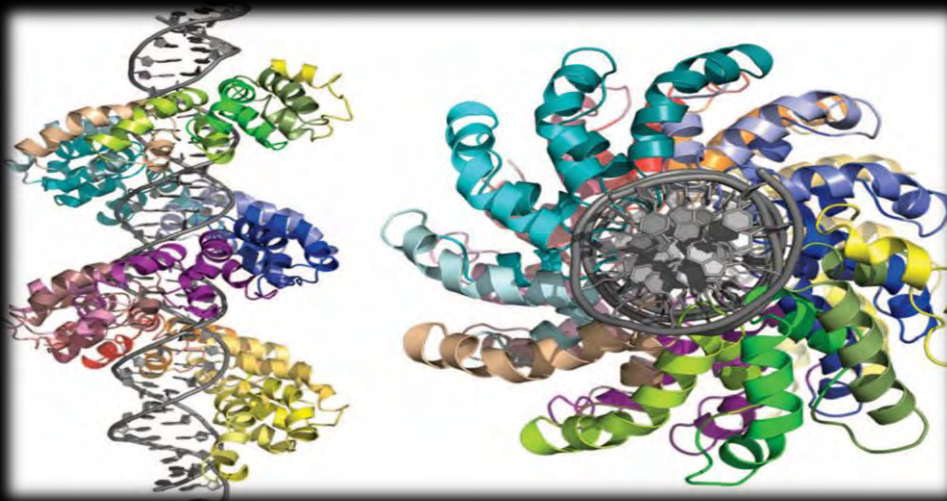
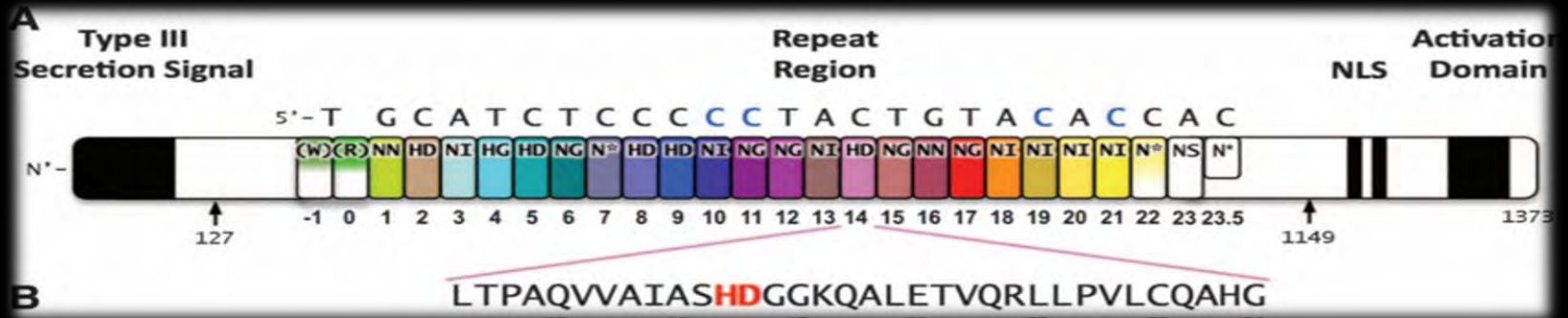
Water-soaking symptom



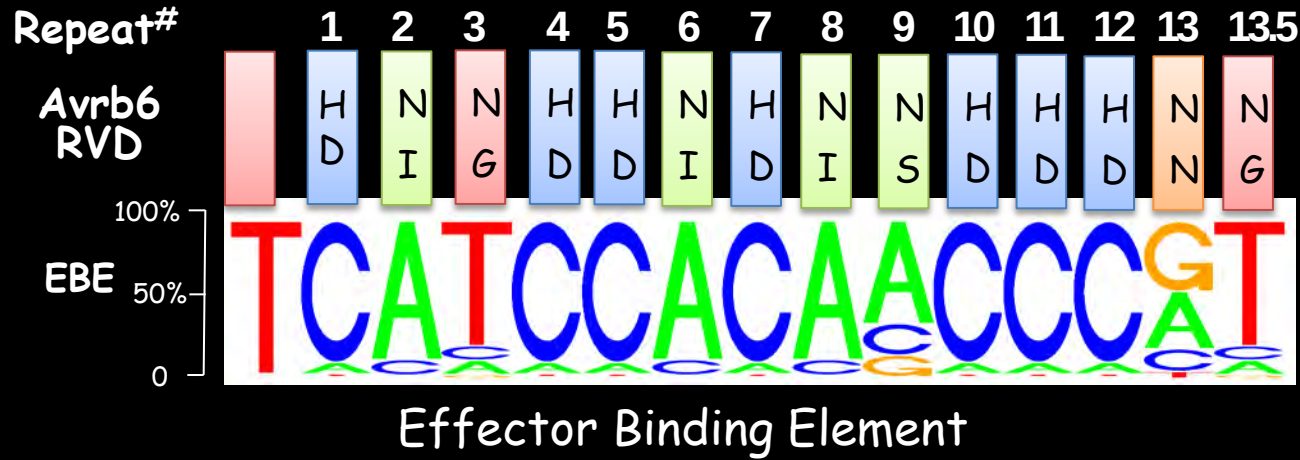
Bacterial release from mesophyll to leaf surface

Type III Effectors

Transcription Activator-Like (TAL) Effectors



TAL effector-host gene recognition



- One of the major virulence factors from *XcmH1005* (Race 4).
- Induces strong water-soaking on specific lines of cotton
- ~10 TALEs cloned from *Xcm*, but **no any R or S genes have been found from cotton.**



Determinatives of TAL effectors in cotton bacterial blight



Transcriptome profiling of *avrb6* and *pthN*

Computational calling of EBEs

Homologs of known TAL effector targets

Validate induction by RT-PCR

Examine EBEs in the promoter

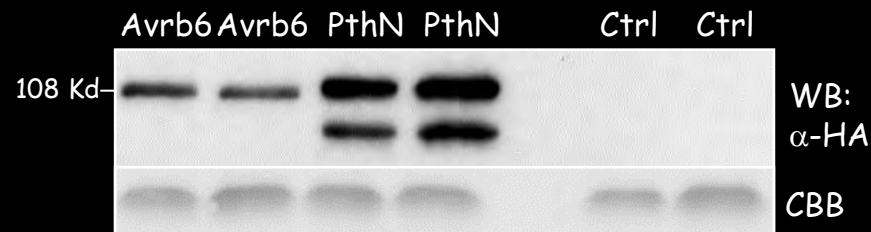
Transactivation assay

dTALEs

VIGS assay and Disease/HR assay

Molecular and biochemical characterization of targets

Identification of *R* and *S* genes of *avrb6* and *pthN*



In collaboration with Dr. Adam Bogdanove

Cotton Inc. April 2016

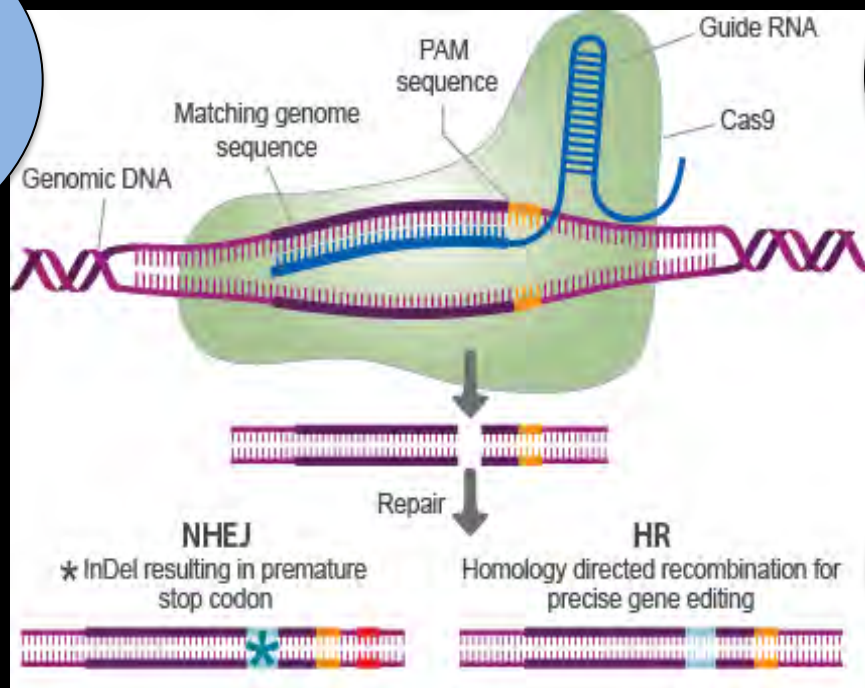
Biological inhibiting infection by *Xcm*

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*



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Texas A&M AgriLife Research

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Boyce Thompson Institute

Dr. Zhangjun Fei

Dr. Yi Zheng

Cornell Univ.

Dr. Adam Bogdanove

Katie Wilkins

USDA-ARS, Texas A&M Univ.

Dr. John Yu

Univ. of Florida

Dr. Dean Gabriel

Donald Danforth Plant Science Center

Dr. Rebecca Bart

Mark Wilson

