Inhibiting infections by *Xcm*

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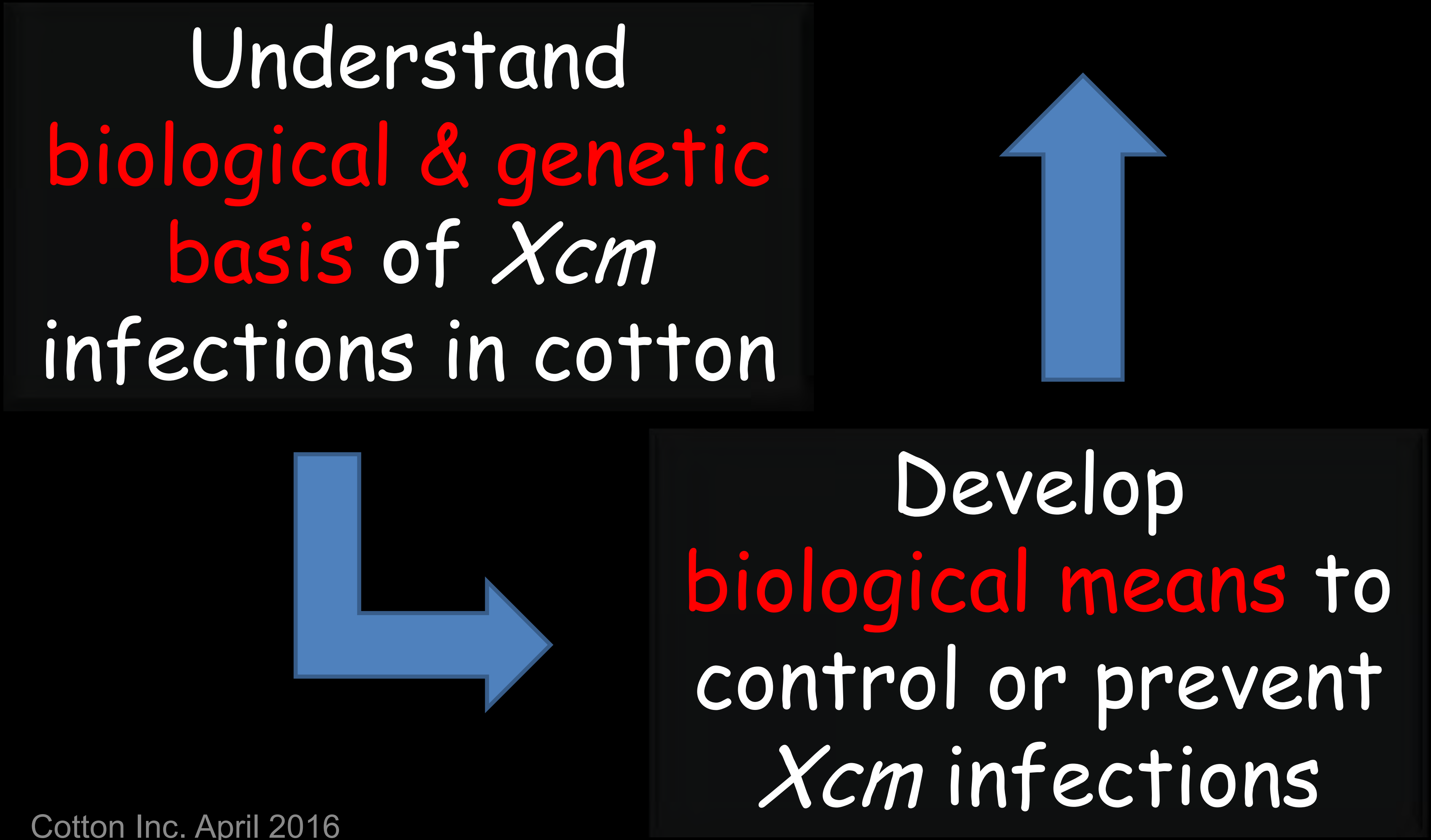
Cotton Inc. April 2016

AgriLIFE RESEARCH
Texas A&M System

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

☹

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Importance & complexity of the cotton genome

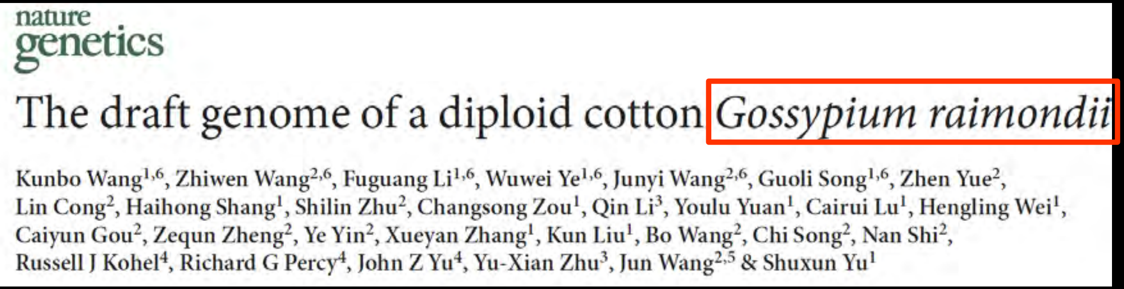
Gossypium spp.

- 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

Li et al., Nat Genet 2014
Wang et al., Nat Genet 2012
Paterson et al., Nature 2012

So many genes in cotton!



nature genetics

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 Yue[2], Lin Cong[2], Haihong Shang[1], Shilin Zhu[2], Changsong Zou[1], Qin Li[3], Youlu Yuan[1], Cairui Lu[1], Hengling Wei[1], Caiyun Gou[2], Zequn Zheng[2], Ye Yin[2], Xueyan Zhang[1], Kun Liu[1], Bo Wang[2], Chi Song[2], Nan Shi[2], Russell J Kohel[4], Richard G Percy[4], John Z Yu[4], Yu-Xian Zhu[3], Jun Wang[2,5] & Shuxun Yu[1]

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

Andrew H. Paterson[1], Jonathan F. Wendel[2], Heidrun Gundlach[3], Hui Guo[1], Jerry Jenkins[4,5], Dianchuan Jin[6], Danny Llewellyn[7], Kurtis C. Showmaker[8], Shengqiang Shu[4], Joshua Udall[9], Mi-jeong Yoo[2], Robert Byers[9], Wei Chen[6], Adi Doron-Faigenboim[10], Mary V. Duke[11], Lei Gong[2], Jane Grimwood[4,5], Corrinne Grover[2], Kara Grupp[2], Guanjing Hu[2], Tae-ho Lee[1], Jingping Li[1], Lifeng Lin[1], Tao Liu[6], Barry S. Marler[1], Justin T. Page[9], Alison W. Roberts[12], Elisson Romanel[13], William S. Sanders[8], Emmanuel Szadkowski[2], Xu Tan[1], Haibao Tang[1,14], Chunming Xu[2,15], Jinpeng Wang[6], Zining Wang[1], Dong Zhang[1], Lan Zhang[6], Hamid Ashrafi[16], Frank Bedon[7], John E. Bowers[1], Curt L. Brubaker[7,17], Peng W. Chee[18], Sayan Das[1], Alan R. Gingle[1], Candace H. Haigler[19], David Harker[9], Lucia V. Hoffmann[20], Ran Hovav[10], Donald C. Jones[21], Cornelia Lemke[1], Shahid Mansoor[1,22], Mehboob ur Rahman[22], Lisa N. Rainville[1], Aditi Rambani[9], Umesh K. Reddy[23], Jun-kang Rong[1], Yehoshua Saranga[24], Brian E. Scheffler[11], Jodi A. Scheffler[11], David M. Stelly[25], Barbara A. Triplett[26], Allen Van Deynze[16], Maite F. S. Vaslin[27],

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 Lu[1], Changsong Zou[1], Wenbin Chen[2], Xinming Liang[2], Haihong Shang[1], Weiqing Liu[2], Chengcheng Shi[2], Guanghui Xiao[3], Caiyun Gou[2], Wuwei Ye[1], Xun Xu[2], Xueyan Zhang[1], Hengling Wei[1], Zhifang Li[1], Guiyin Zhang[4], Junyi Wang[2], Kun Liu[1], Russell J Kohel[5], Richard G Percy[5], John Z Yu[5], Yu-Xian Zhu[3], Jun Wang[2,6–10] & Shuxun Yu[1]

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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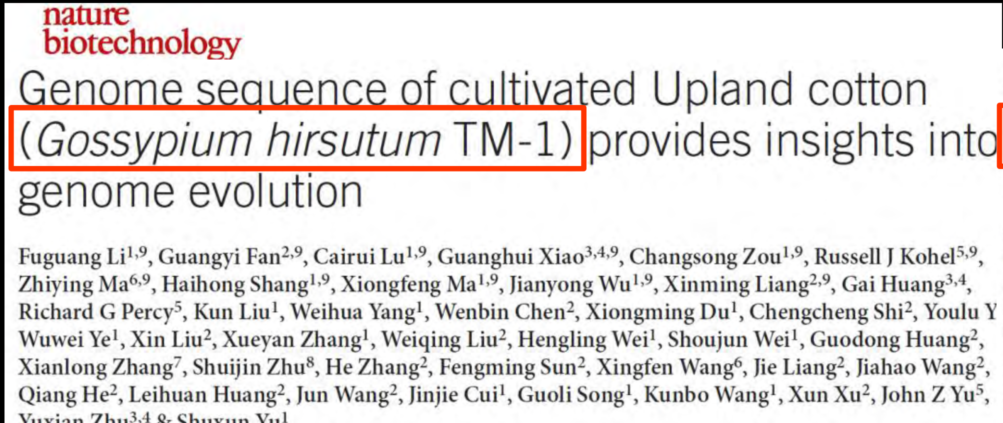


nature biotechnology

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 Percy[5], Kun Liu[1], Weihua Yang[1], Wenbin Chen[2], Xiongming Du[1], Chengcheng Shi[2], Youlu Y Wuwei Ye[1], Xin Liu[2], Xueyan Zhang[1], Weiqing Liu[2], Hengling Wei[1], Shoujun Wei[1], Guodong Huang[2], Xianlong Zhang[7], Shuijin Zhu[8], He Zhang[2], Fengming Sun[2], Xingfen Wang[6], Jie Liang[2], Jiahao Wang[2], Qiang He[2], Leihuan Huang[2], Jun Wang[2], Jinjie Cui[1], Guoli Song[1], Kunbo Wang[1], Xun Xu[2], John Z Yu[5], Yuxian Zhu[3,4] & Shuxun Yu[1]

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 Zhang[2], Christopher A Saski[4], Brian E Scheffler[5], David M Stelly[6], Amanda M Hulse-Kemp[6], Qun Wa Bingliang Liu[1], Chunxiao Liu[1], Sen Wang[1], Mengqiao Pan[1], Yangkun Wang[1], Dawei Wang[2], Wenxue Ye[1], Lijing Chang[1], Wenpan Zhang[1], Qingxin Song[3], Ryan C Kirkbride[3], Xiaoya Chen[7], Elizabeth Dennis[8], Danny J Llewellyn[8], Daniel G Peterson[9], Peggy Thaxton[10], Don C Jones[11], Qiong Wang[1], Xiaoyang Xu[1], Hua Zhang[1], Huaitong Wu[1], Lei Zhou[1], Gaofu Mei[1], Shuqi Chen[1], Yue Tian[1], Dan Xiang[1], Xinghe Li[1], Jian D Qiyang Zuo[2], Linna Tao[2], Yunchao Liu[2], Ji Li[2], Yu Lin[2], Yuanyuan Hui[2], Zhisheng Cao[2], Caiping Cai[1], Xiefei Zhu[1], Zhi Jiang[2], Baoliang Zhou[1], Wangzhen Guo[1], Ruiqiang Li[2] & Z Jeffrey Chen[1,3]

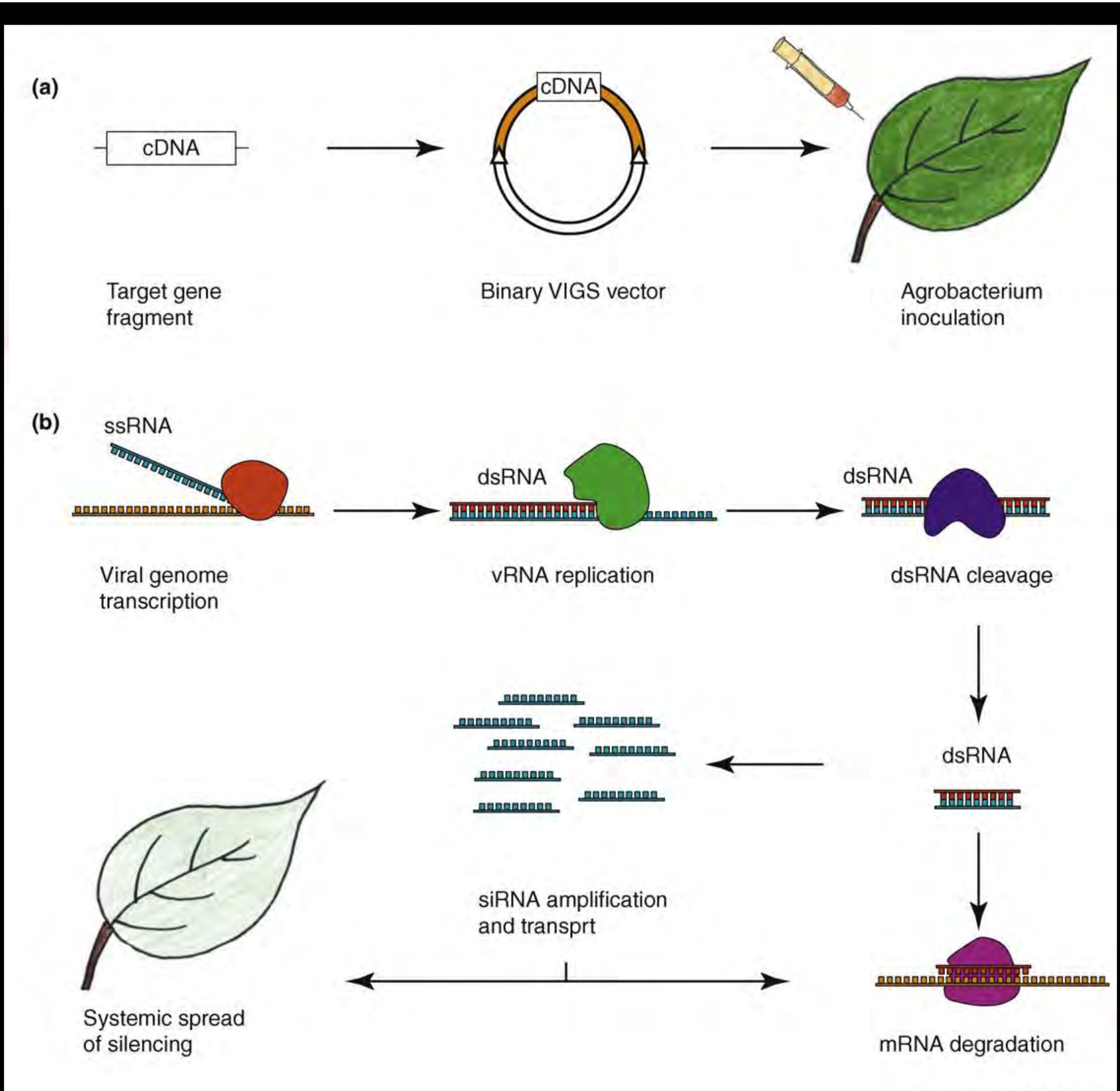
How to study cotton gene functions?
Functional Genomics

- 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

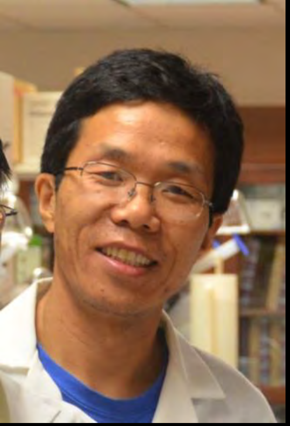
Virus-induced gene silencing



TRENDS in Plant Science

Modified from Becker and Lange, *Trends in Plant Science*. 2010

Agrobacterium-mediated VIGS



10-day-old seedling

Agro-infiltration

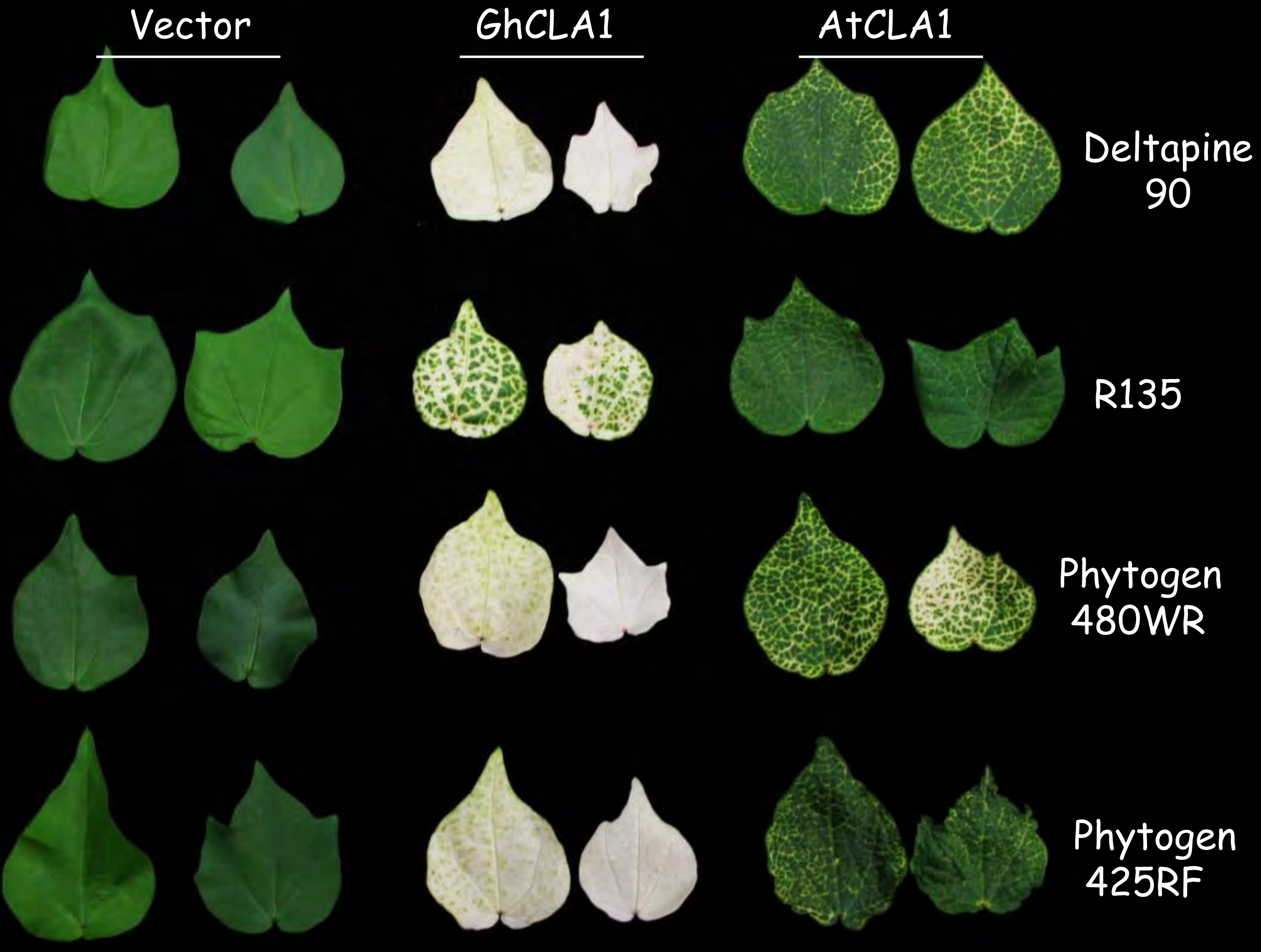
Phenotypic analysis

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



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*

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Transient gain-of-function assay in cotton

Plant

Leave

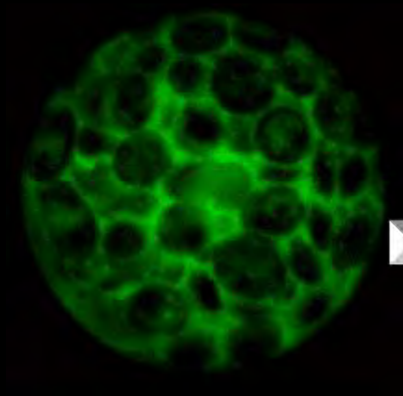
Hormones

Light

Pathogens

Abiotic stresses

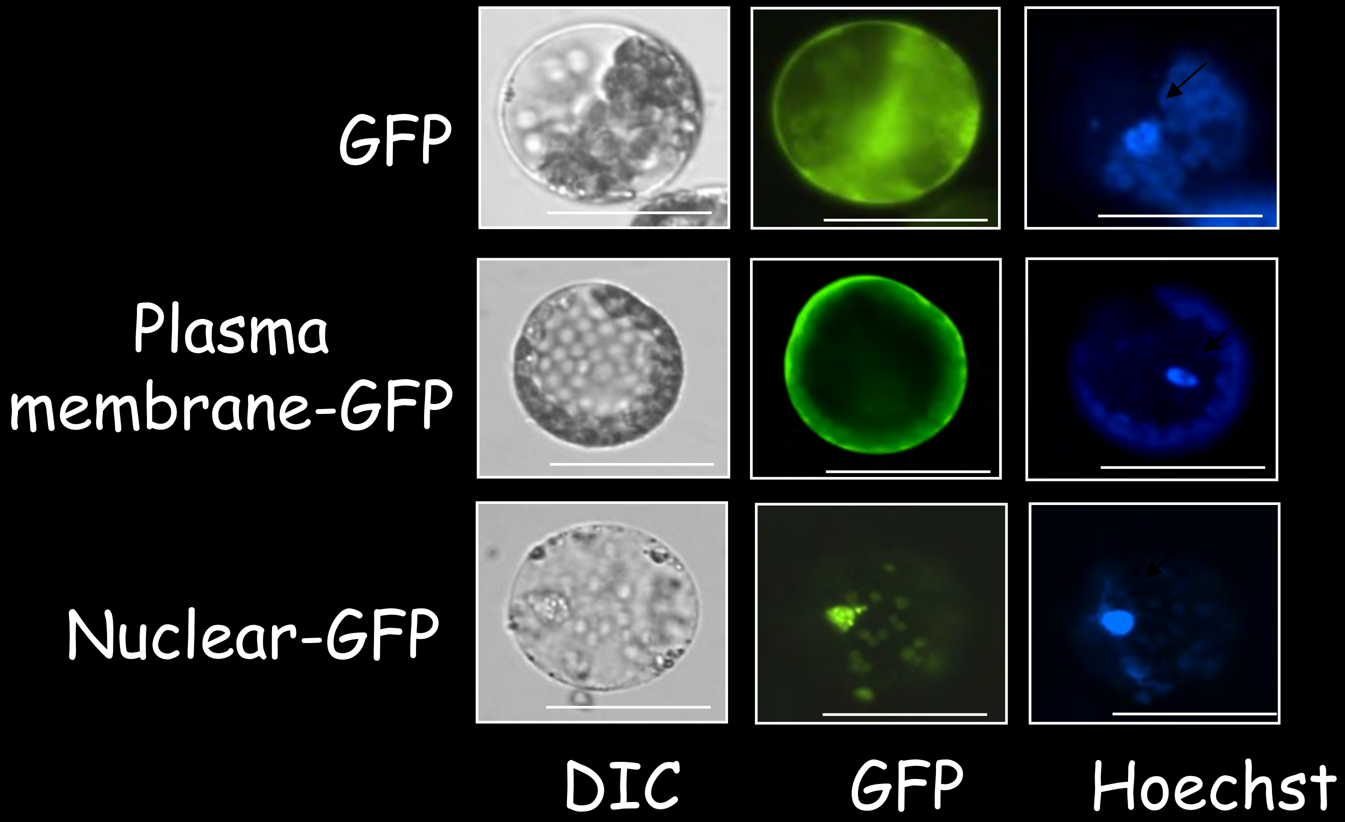
Signals



Cell

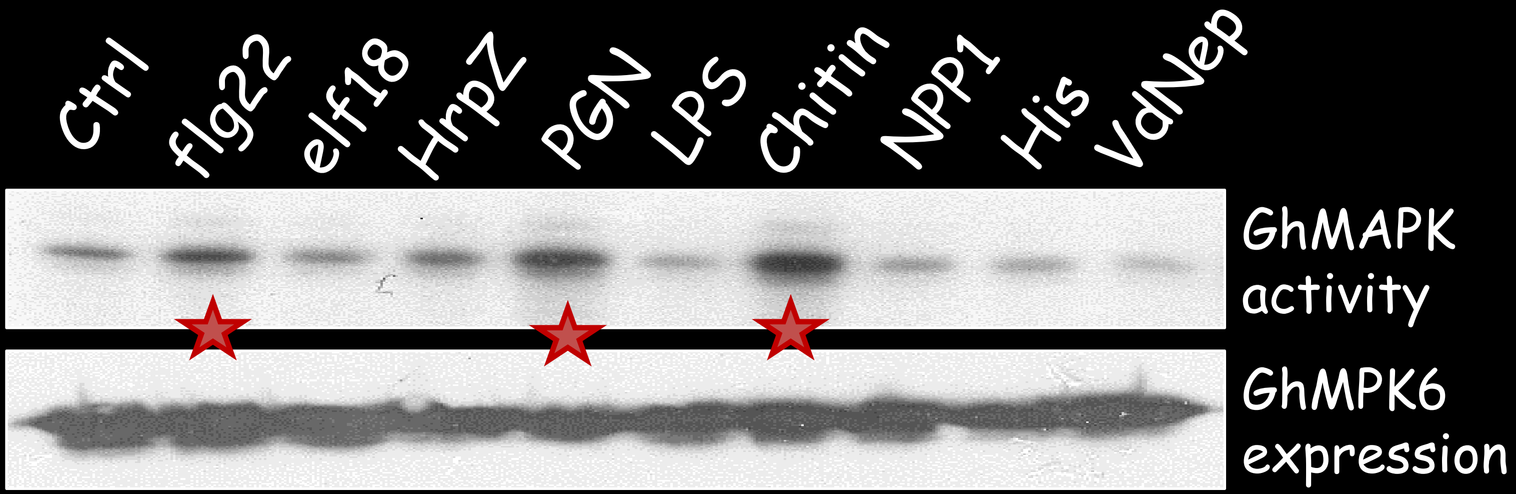
Transient Gene Function Assays

Subcellular localization in cotton protoplasts



Gao et al., The Plant Journal. 2011

Activation of convergent MAP kinase signaling by pathogen elicitors



Immuno-complex kinase assay to detect cotton MAPK6 activity

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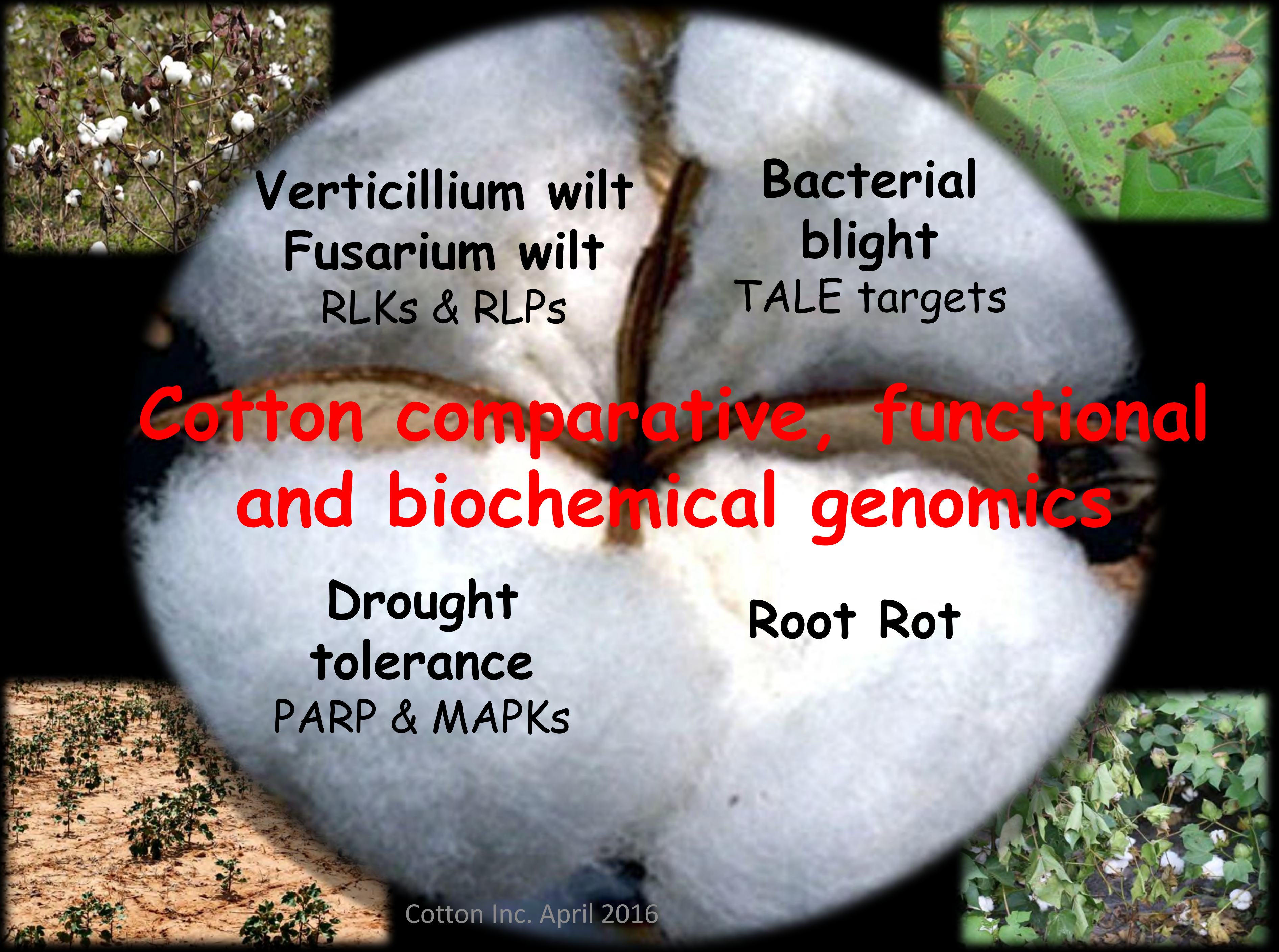
Gao *et al.*, *The Plant Journal.* 2011



Cotton comparative, functional and biochemical genomics

Verticillium wilt
Fusarium wilt
RLKs & RLPs

Bacterial blight
TALE targets

Drought tolerance
PARP & MAPKs

Root Rot

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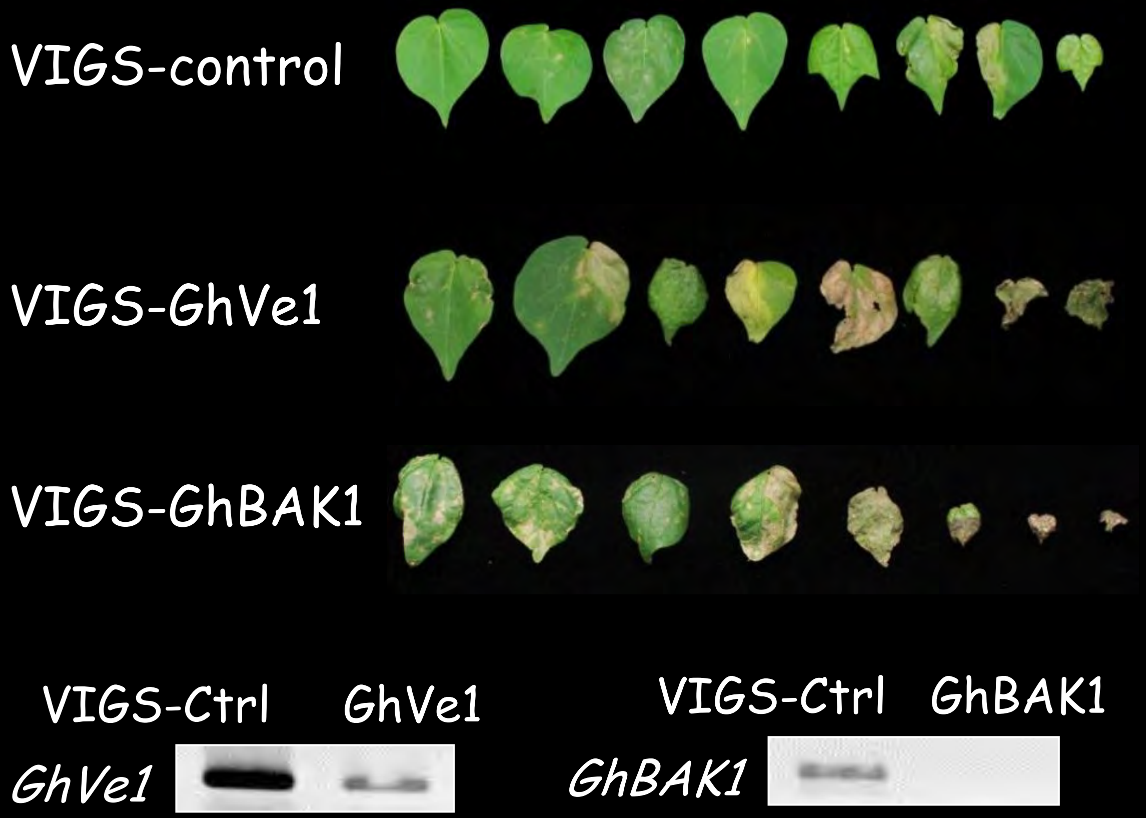
Cotton receptor-like protein GhVe1 and receptor-like kinase GhBAK1 mediate *Verticillium wilt* resistance.



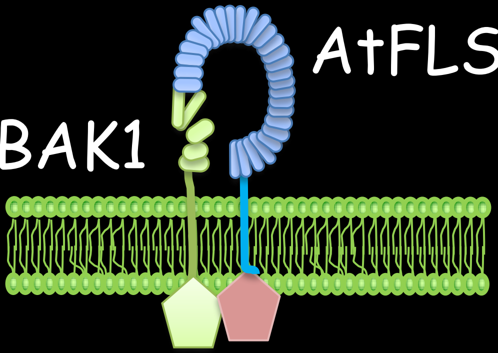
Gao *et al.*, *The Plant Journal*. 2011

Gao *et al.*, *Journal of Integrative Biology*. 2013

RLK/RLP complex in cotton *Verticillium wilt* resistance

Arabidopsis-Pseudomonas

Flagellin



AtFLS2

AtBAK1

↓

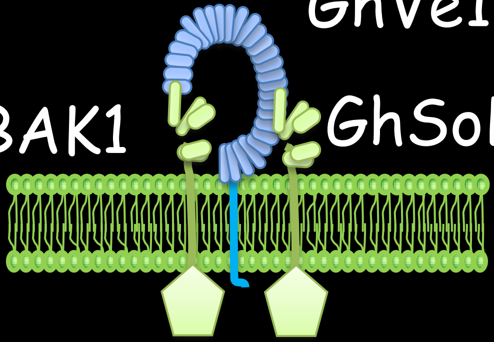
MAP kinase cascade

↓

flagellin-triggered immunity

Cotton-Verticillium

Ave1 or ?



GhVe1

GhBAK1

GhSoBIR1?

↓

MAP kinase cascade?

↓

Verticillium resistance

Felix et al., Plant J. 1999; Gomez-Gomez & Boller, Mol Cell 2000; Chinchilla et al, Plant Cell 2006; Chinchilla et al, Nature 2007; Heese et al., PNAS 2007; Sun et al., Science 2013

Cotton resistance to *Fusarium wilt* The role of receptor kinases

VIGS-Control.

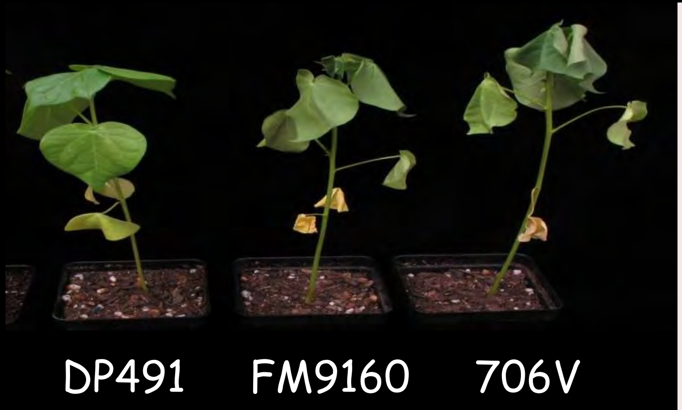
VIGS-Gene 1

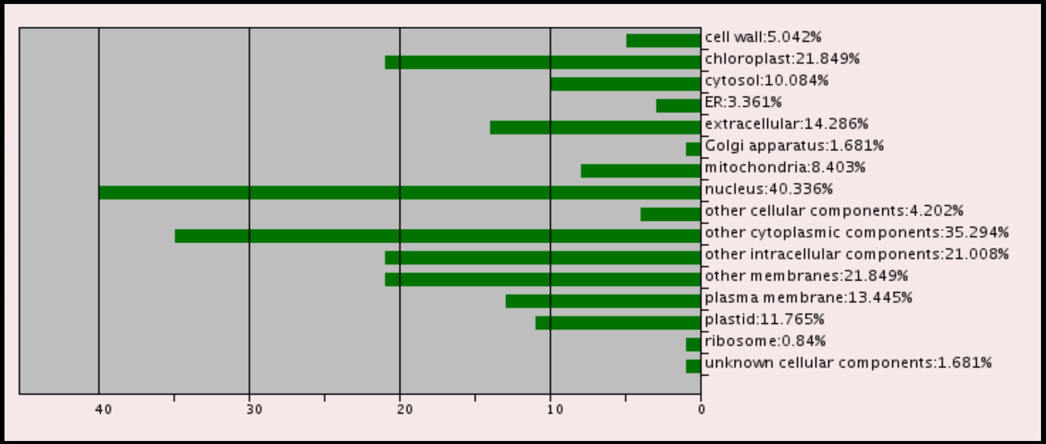
H_2O

FOV10 RACE1

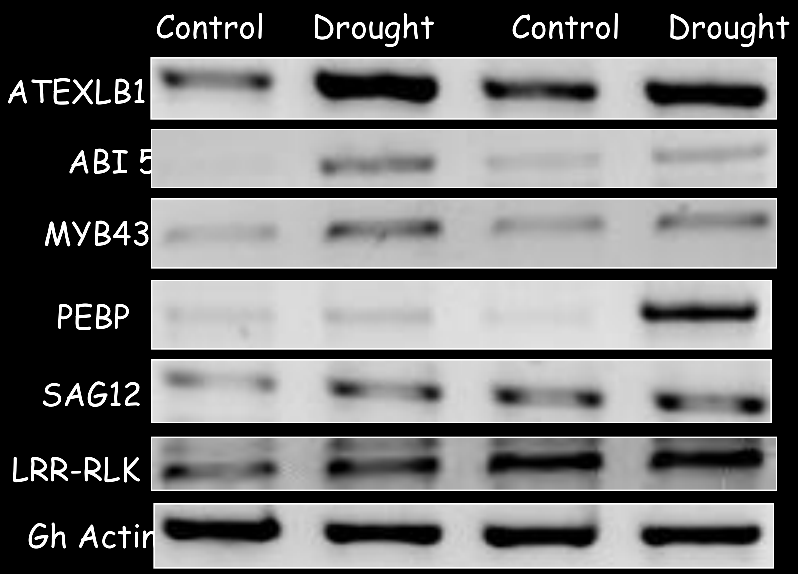
unpublished

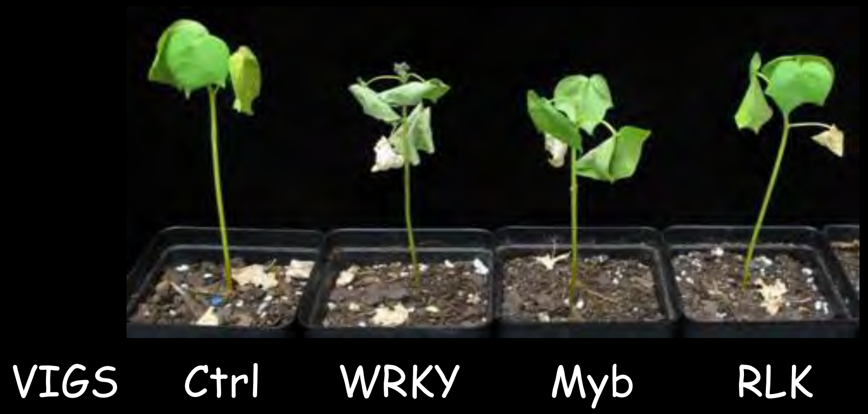
RNA-seq to mine novel genes in cotton drought stress





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





unpublished

Determinatives of cotton drought stress

Two weeks after VIGS; Drought treatment: 12 days

unpublished

Bacterial blight of cotton (BBC)

Pathogenicity of *Xcm*

The Type III Secretion System

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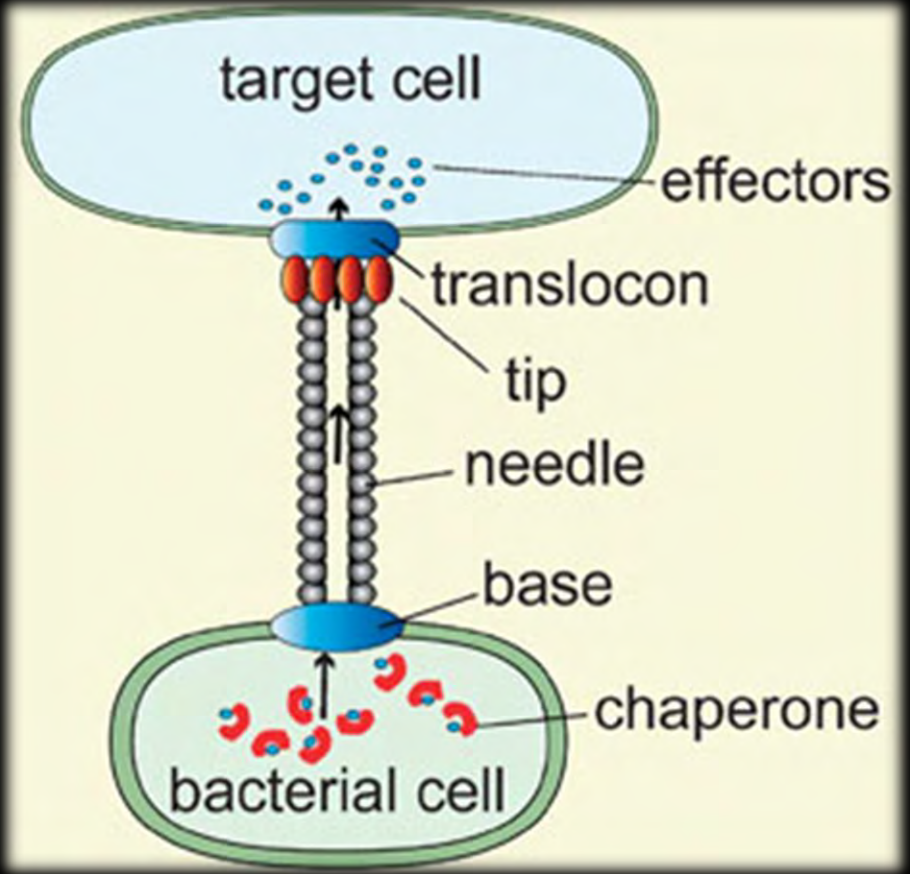
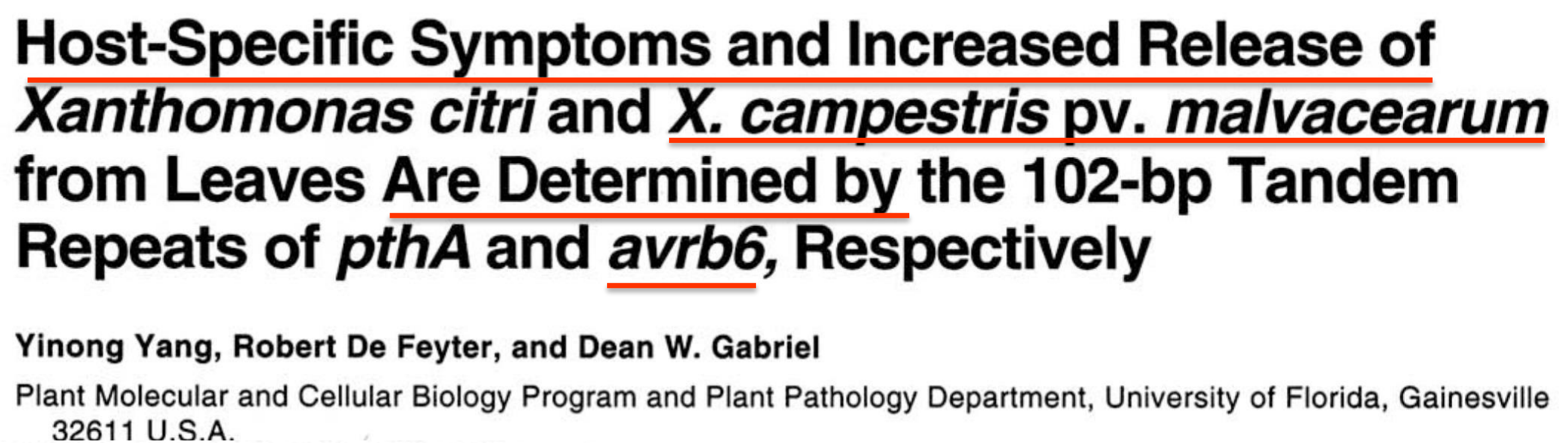


Delannoy et al., Annu. Rev. Phytopathol. 2005

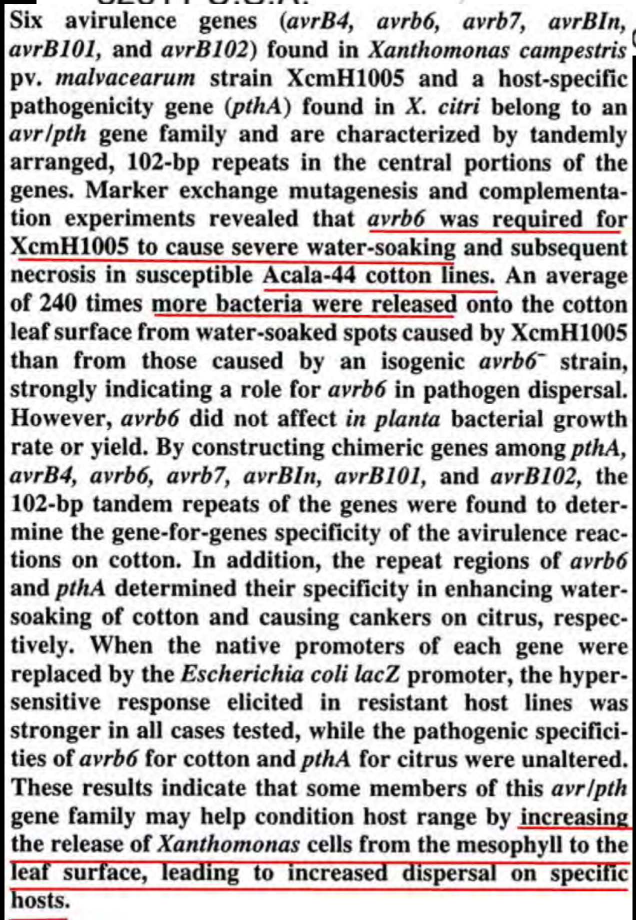


Host-Specific Symptoms and Increased Release of *Xanthomonas citri* and *X. campestris* pv. *malvacearum* from Leaves Are Determined by the 102-bp Tandem Repeats of *pthA* and *avrb6*, Respectively

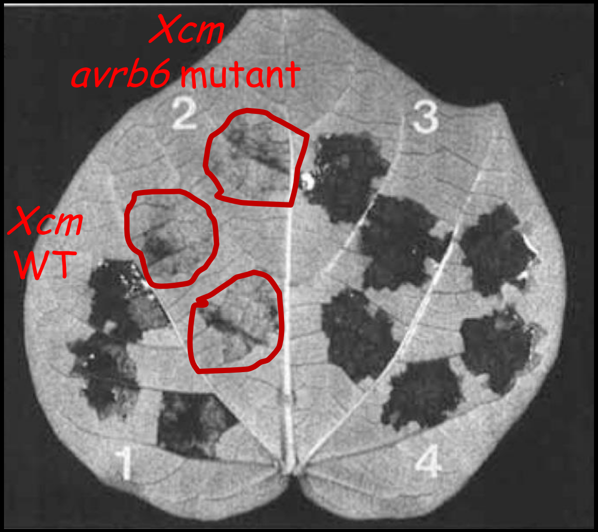
Yinong Yang, Robert De Feyter, and Dean W. Gabriel

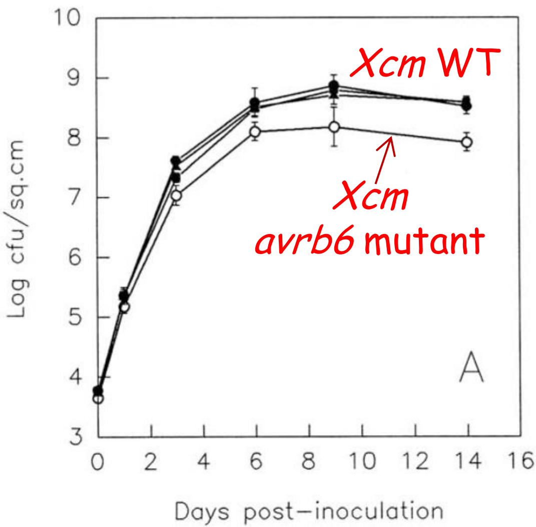
Plant Molecular and Cellular Biology Program and Plant Pathology Department, University of Florida, Gainesville 32611 U.S.A.

d 18 January 1994.



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





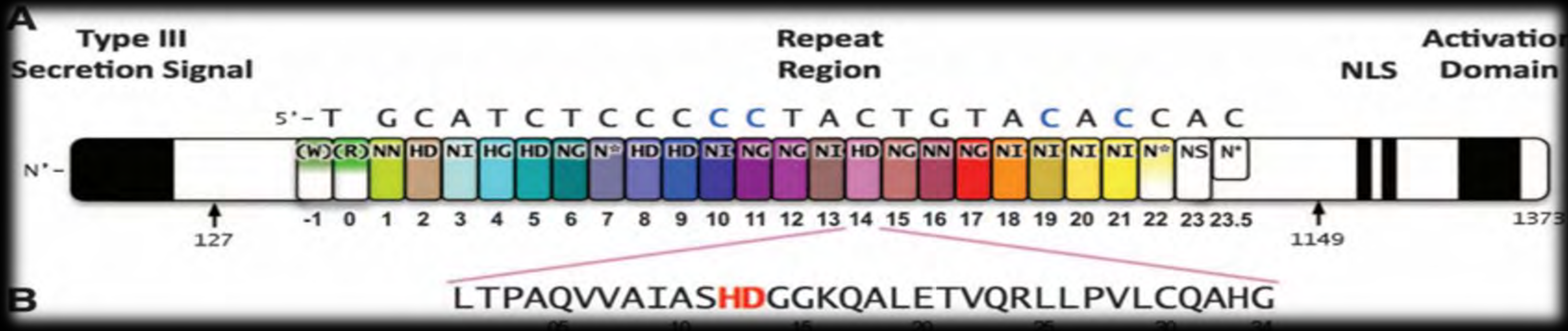
Water-soaking symptom

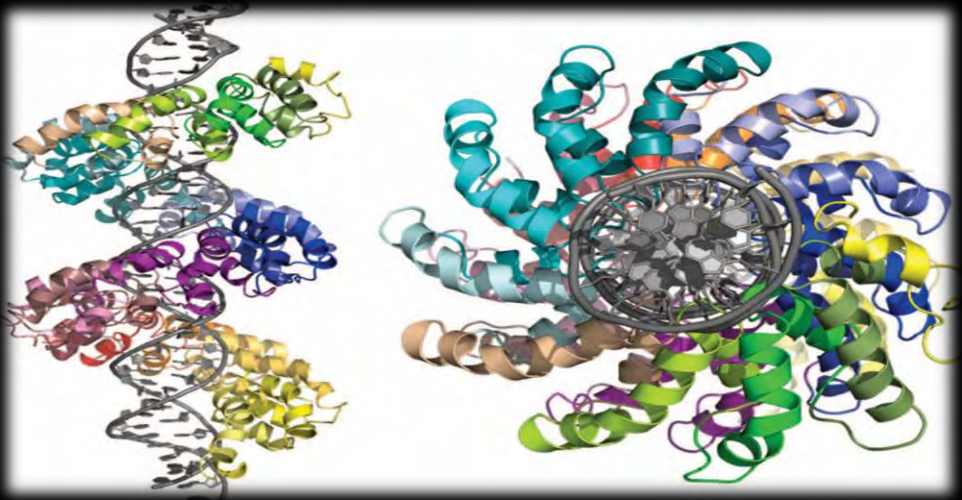
Bacterial release from mesophyll to leaf surface

Yang et al., MPMI 1994

Type III Effectors

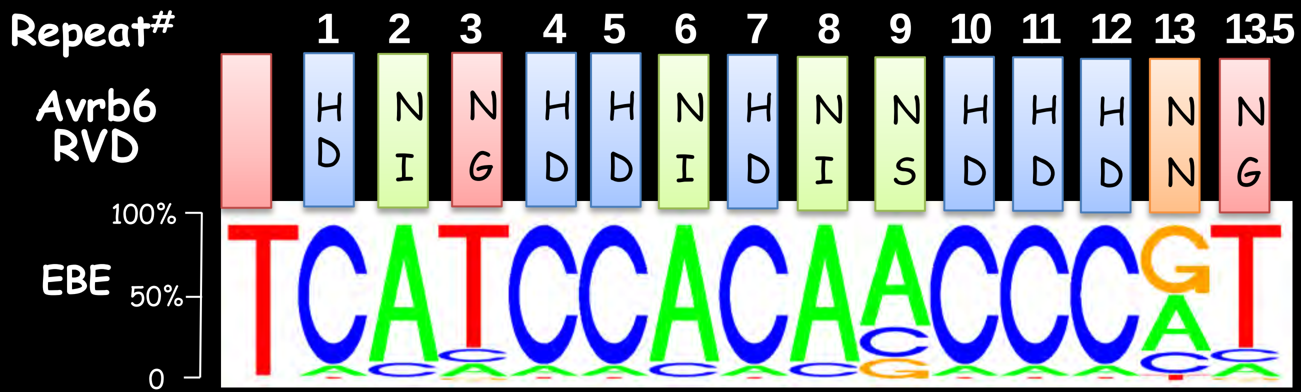
Transcription Activator-Like (TAL) Effectors





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

TAL effector-host gene recognition



Effector Binding Element

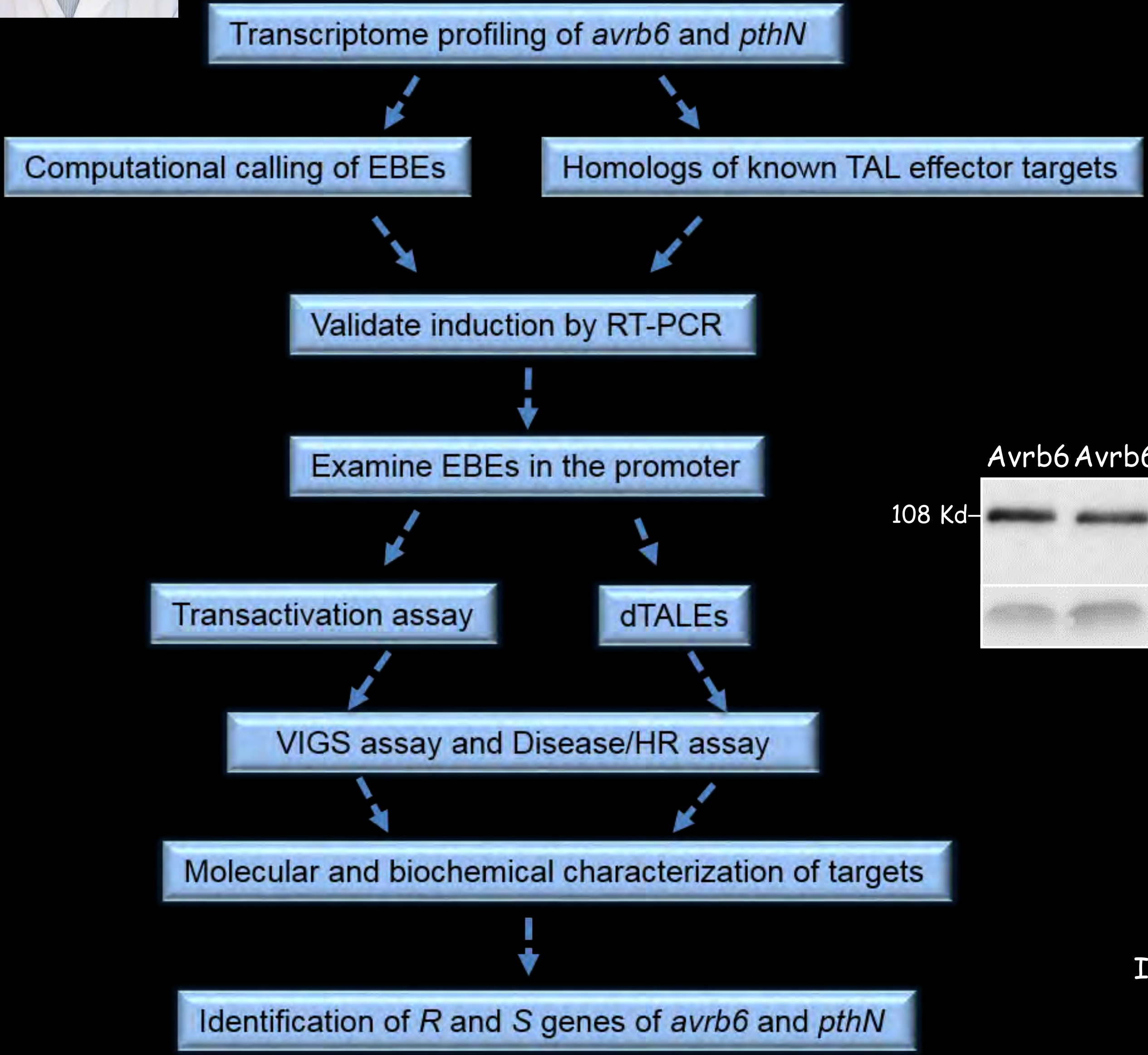
- One of the major virulence factors from *Xcm*H1005 (Race 4).

- 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.

Yang et al., MPMI 1994

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

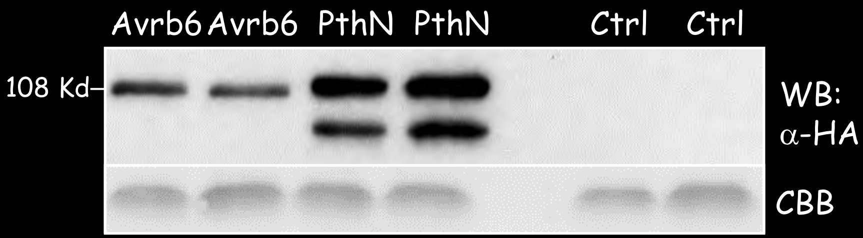
Transactivation assay

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

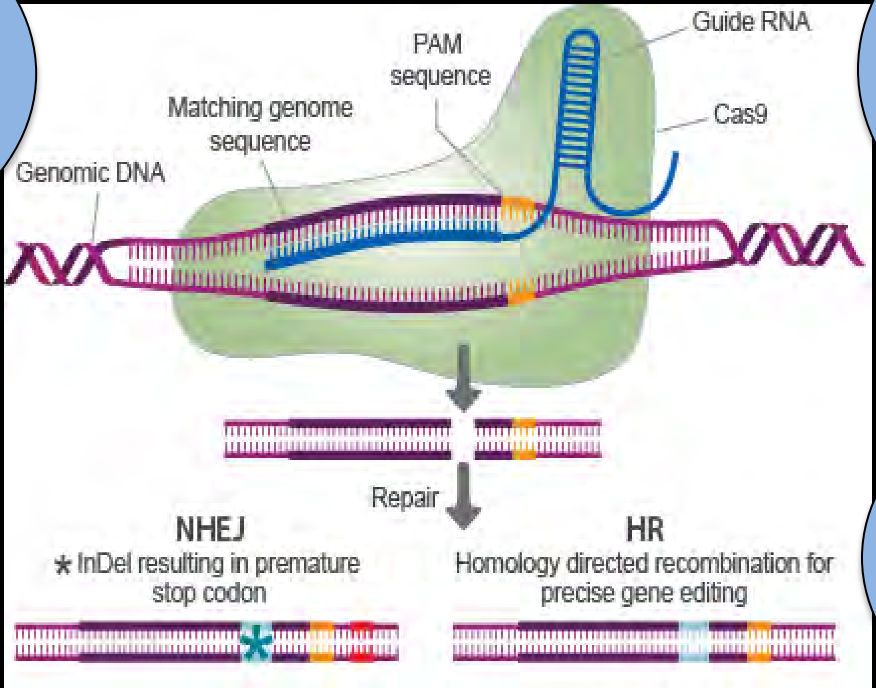
Biological inhibiting infection by *Xcm*

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

CRISPR-Cas9 System

Revolutionary genome editing tool

Used in rice to create resistant rice to BBR



Mutate TALe recognition site in cotton

Create Cotton that has broad spectrum resistance to BBC

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Boyce Thompson Institute

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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

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