Regulation of mycotoxin production and kinome analysis in *Fusarium graminearum*

Chenfang Wang

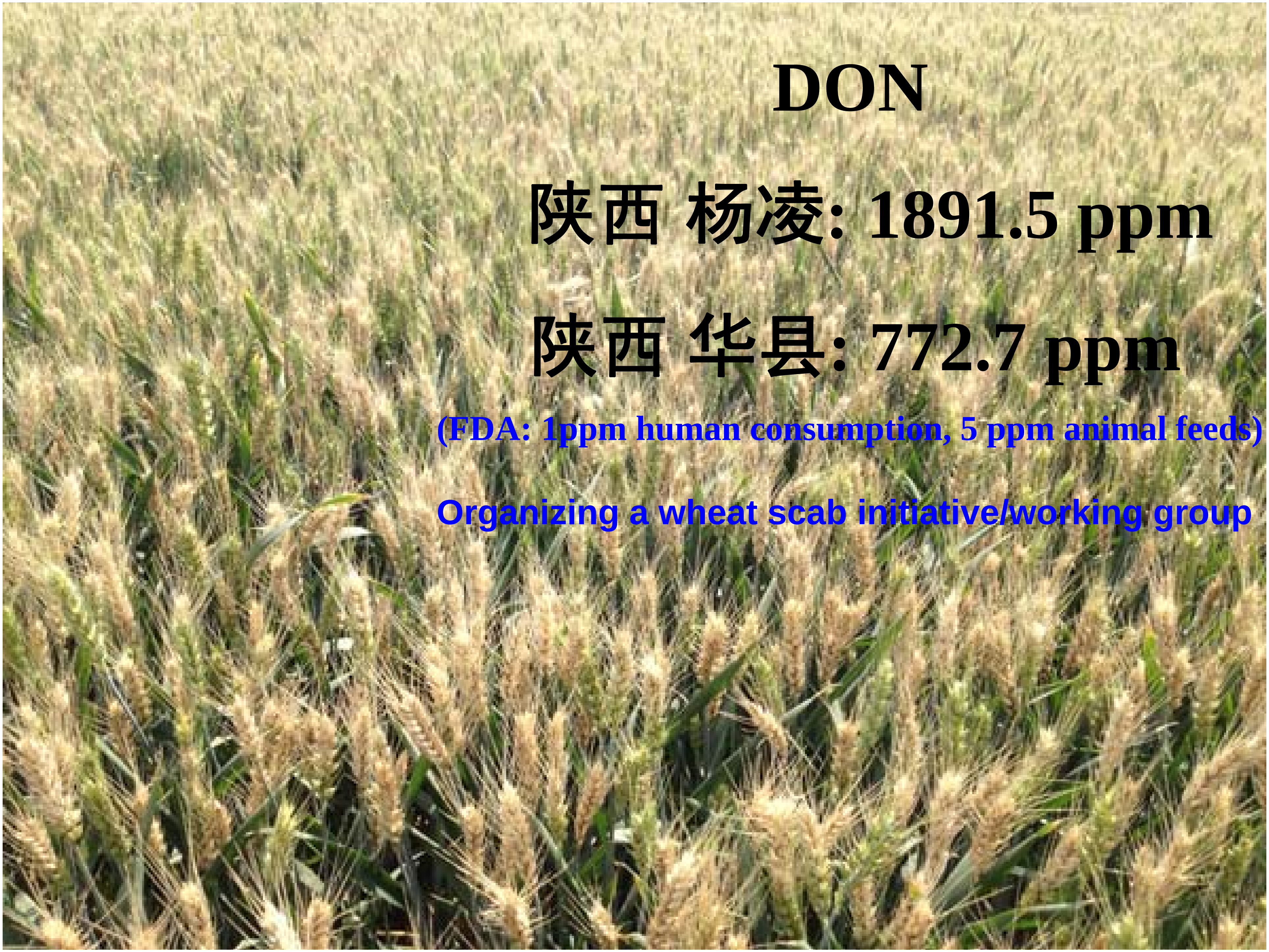
Northwest Agricultural & Forestry University

Regulation of mycotoxin production and kinome analysis in *Fusarium graminearum*

Chenfang Wang

Jin-Rong Xu, Purdue University
Purdue – NWAFU Joint Research Center

Nationwide Outbreak of Wheat Scab in China - 2012



DON

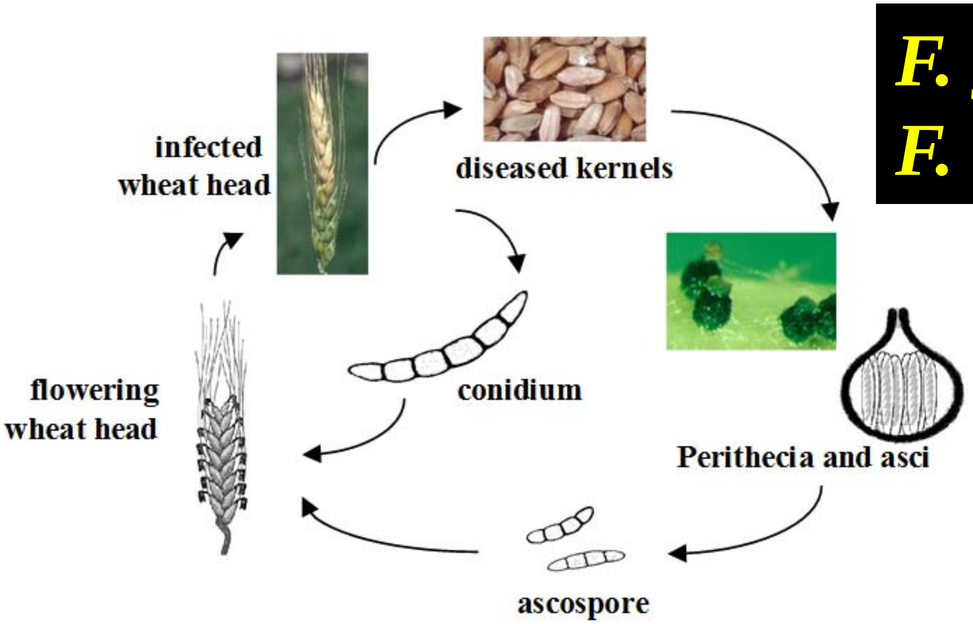
陕西 杨凌: 1891.5 ppm

陕西 华县: 772.7 ppm

(FDA: 1ppm human consumption, 5 ppm animal feeds)

Organizing a wheat scab initiative/working group

Unique features of *Fusarium graminearum*



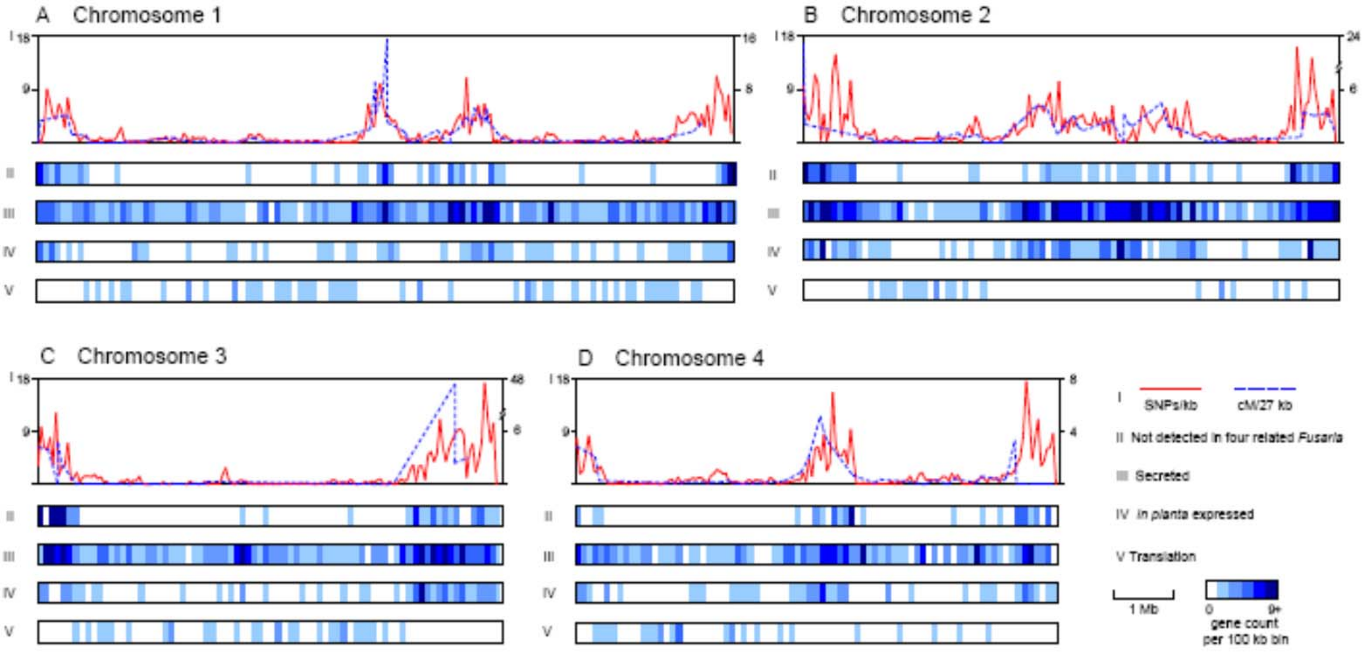
F. graminearum
F. asiaticum

- **Ascospores – primary inoculum**
- **Sexual reproduction critical in its infection cycle**
- **No microconidia**
- **‘Tissue-specific’ for flowering heads**

wheat, barley, Brachypodium, and others

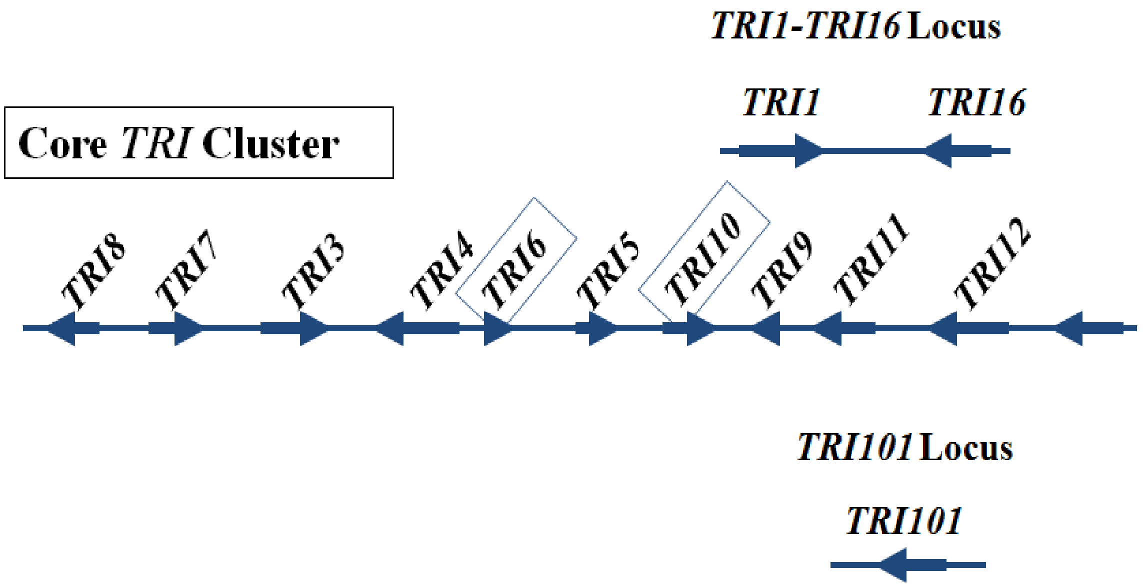
Unique genomic features of *F. graminearum*

- Very few repetitive sequences (0.03%, 15x less than Sc)
- No active transposable elements
- Rare recent duplications
- Localized polymorphism and pathogen specialization



Cuomo et al., 2008. Science. 317: 1400-1402.

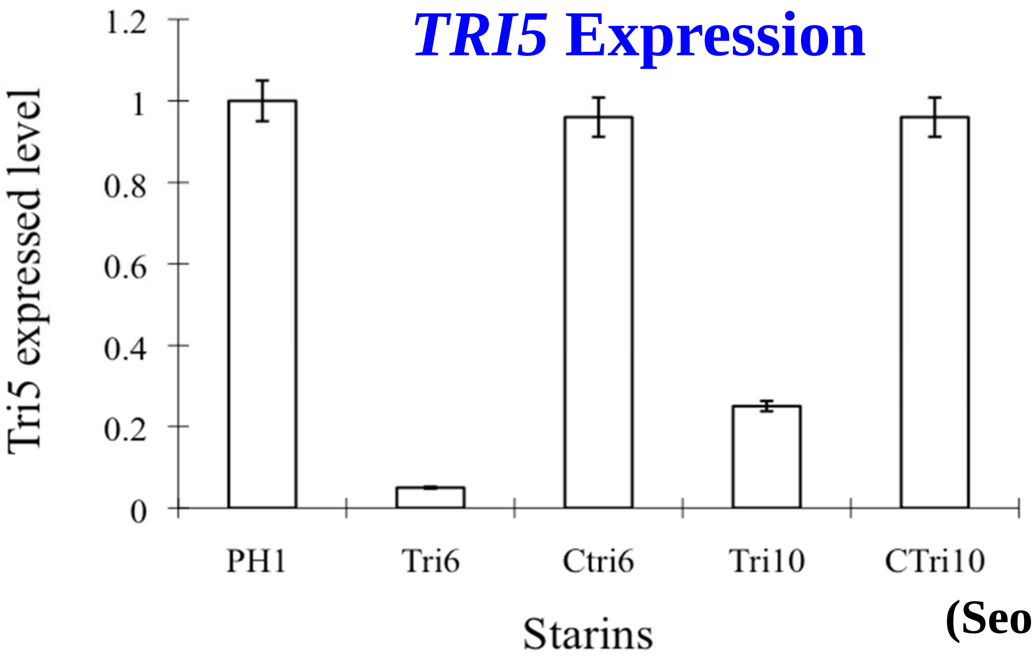
Many *TRI* genes were up-regulated during plant infection



TRI Gene Functions

TRI3	C-3 Acetylation	*TRI9*	‘Unknown’
TRI4	Trichodiene Oxygenation	***TRI10***	**Transcription factor**
TRI5	Trichodiene Synthesis	*TRI11*	C-15 Hydroxylation
TRI6	**Transcription factor**	***TRI12***	Transporter
TRI7	C-4 Acetylation	*TRI13*	C-4 Hydroxylation
TRI8	C-3 Deacetylation	*TRI14*	
TRI101	Trichothecene 3-0-acetyltransferase	*TRI6*	

Tri6: Cys2His2 zinc-finger domain
Tri10: Fungal specific Zn(2)-Cys(6) binuclear cluster domain

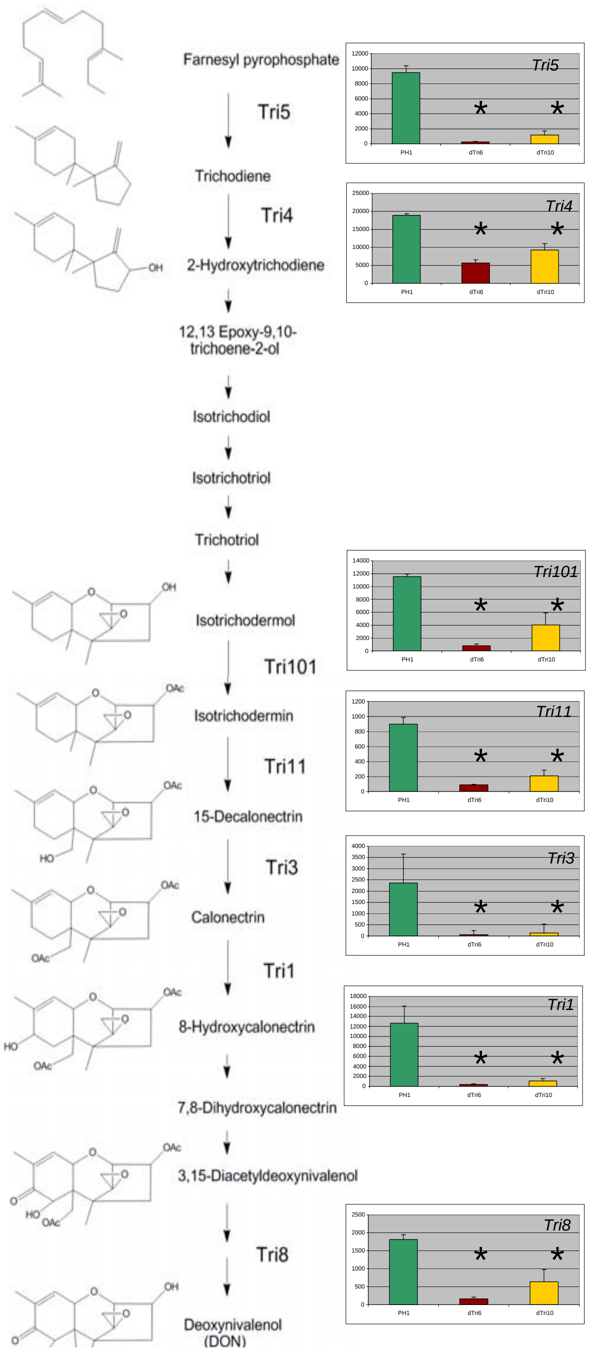

***TRI5* Expression**

(Seong et al., 2010)

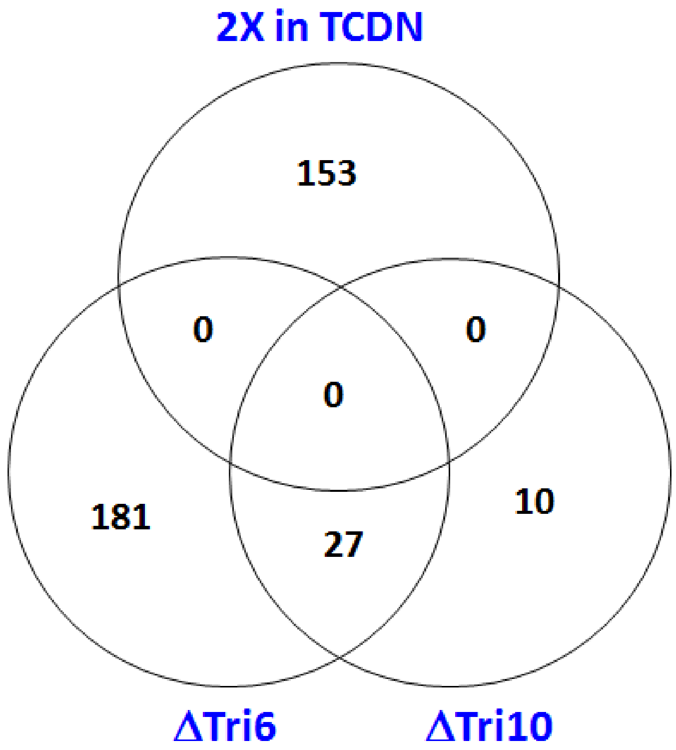
tri6 tri10 double mutants

-Both Tri6 and Tri10 are important for *TRI5* expression
-Tri6 appears to be more important than Tri10

WT *tri6* *tri10*



TRI genes down-regulated in the *tri6* and *tri10* mutants

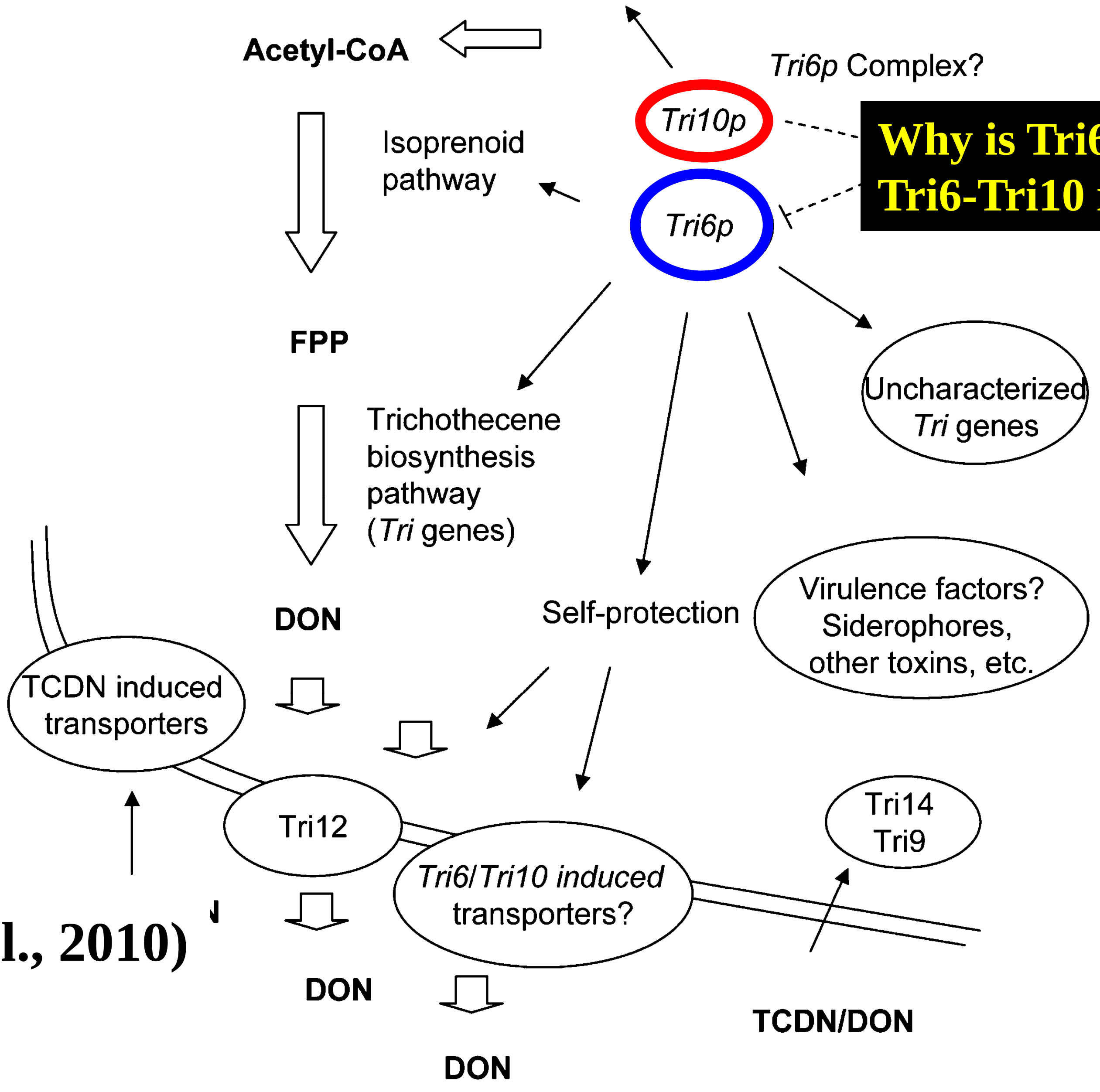


Microarray analysis
(Seong et al., 2010)

1/37 genes positively regulated by Tri10
87/208 genes positively regulated by Tri6

Tri10: quite specific for trichothecene biosynthesis
Tri6: other genes related to plant infection

Evolution: co-regulation of isoprenoid and trichothecene biosynthesis genes in *F. graminearum*



Acetyl-CoA

Tri6p Complex?

Tri10p

Tri6p

Isoprenoid pathway

FPP

Trichothecene biosynthesis pathway (*Tri* genes)

Uncharacterized *Tri* genes

DON

Self-protection

Virulence factors? Siderophores, other toxins, etc.

TCDN induced transporters

Tri12

Tri6/Tri10 induced transporters?

Tri14
Tri9

DON

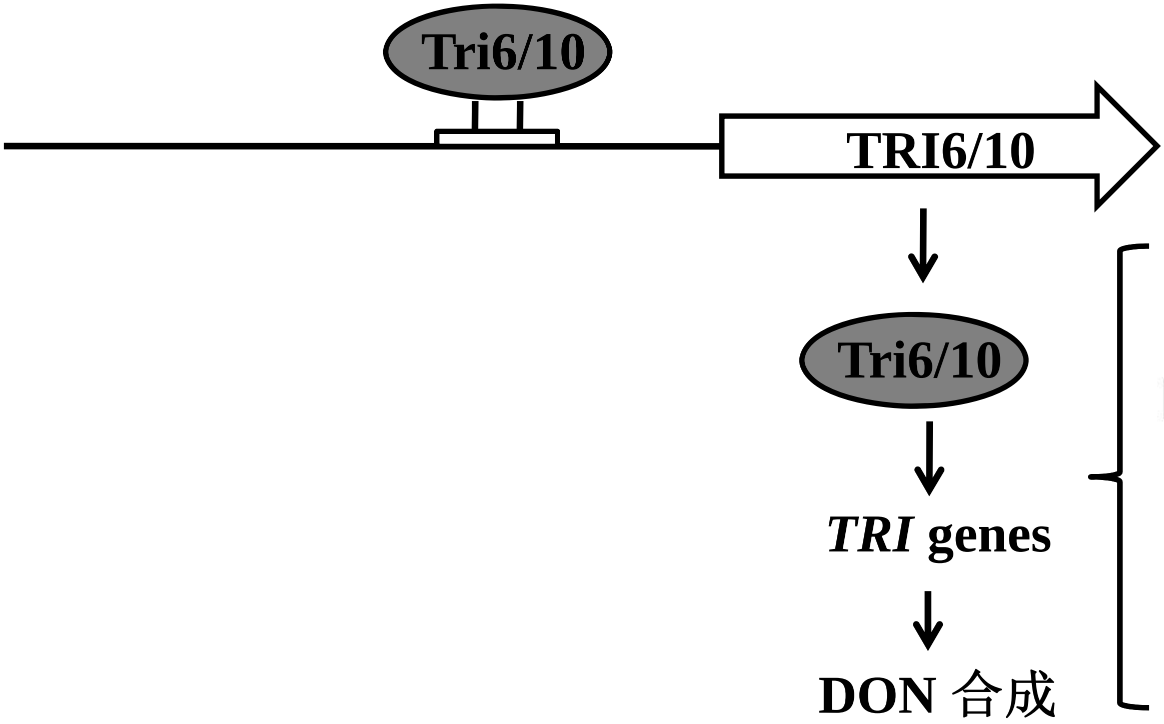
DON

TCDN/DON

Why is Tri6 global?
Tri6-Tri10 relationship?

(Seong et al., 2010)

Tri6-Tri10 interaction may be conditional -



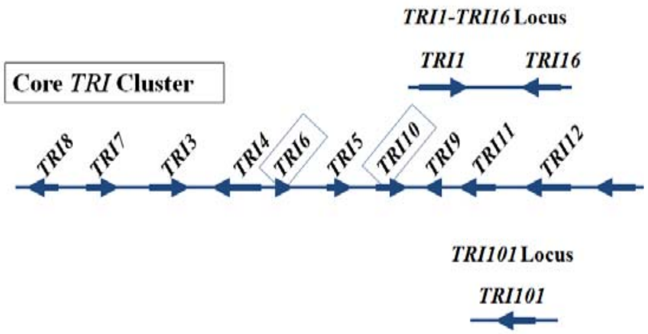
Tri6/10

TRI6/10

Tri6/10

TRI genes

DON 合成



Environmental or host factors affect DON production may converge on Tri6/Tri10

pH: acidic pH induced TRI genes

Nitrogen sources

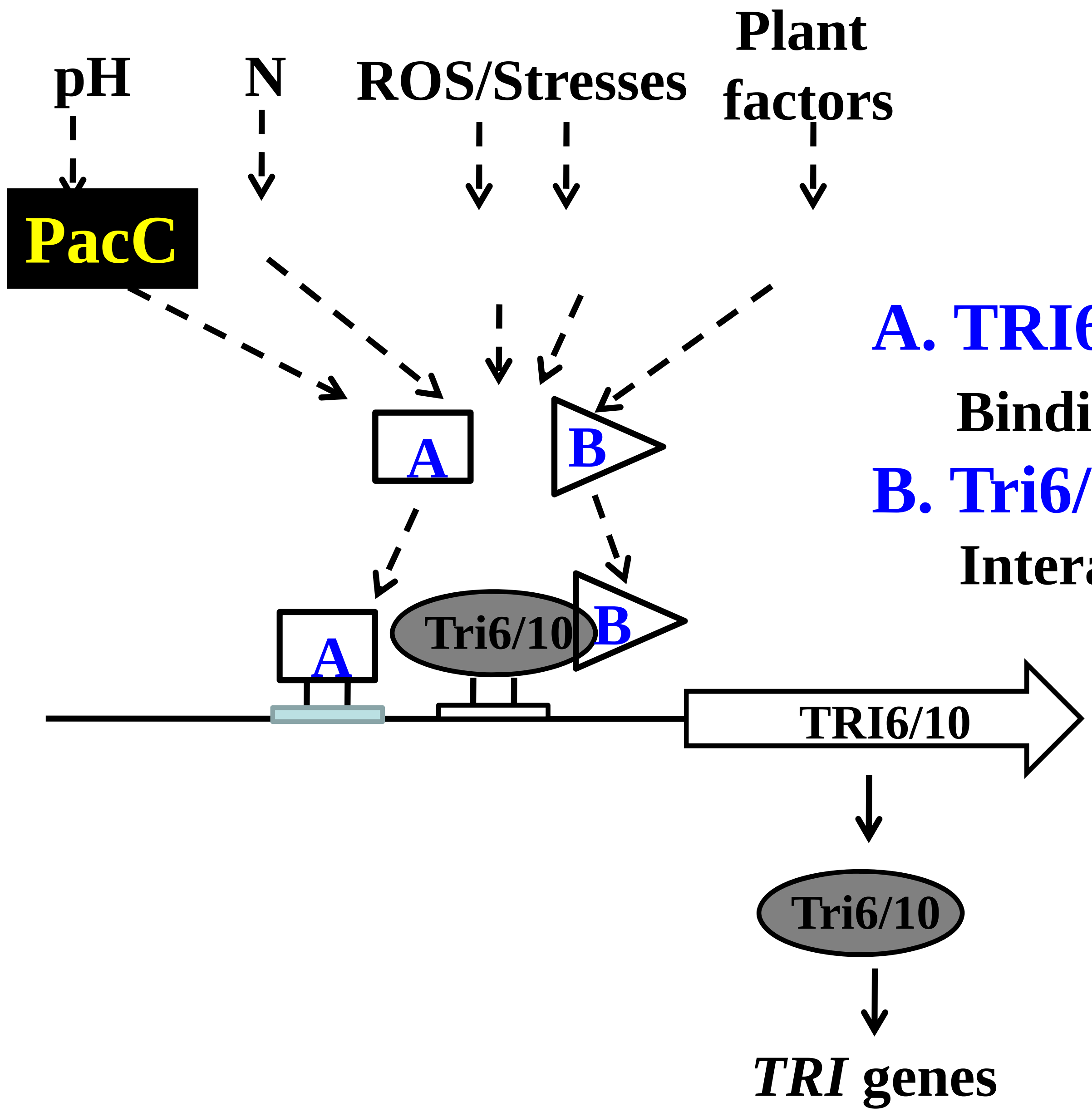
NH_4 inhibits DON synthesis
Polyamines stimulates (Gardiner et al., 2010)

ROS + other stresses

Plant factors (flowering heads)

(DON not produced in rice scab)

‘Tri6/Tri10 model’



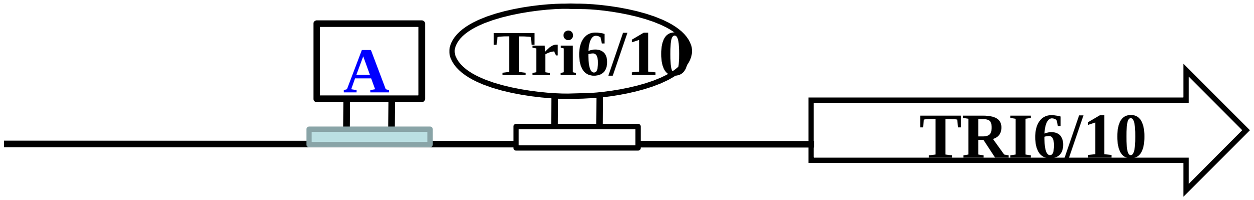
A. TRI6/10 expression

Binding to TRI6/10 promoter

B. Tri6/10 activities

Interacting with Tri6/10

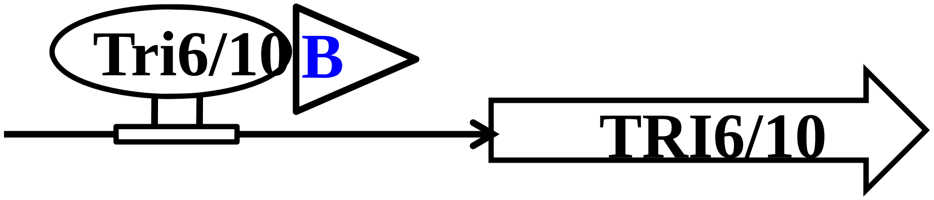
A type factors: binding to *TRI6* promoter



Conserved PacC-binding sites:

GCCAAG (-512) *TRI6* promoter

- yeast one-hybrid assays
- searching for conserved promoter elements



B type factors: Tri6–interacting proteins

- Screening 2 yeast two-hybrid libraries

- Affinity purification -MS analysis
  - TRI6-GFP
  - TRI6-3xFLAG
  - TRI6-S-tag
  - (may need to be over-expressed)

WT

Mutants

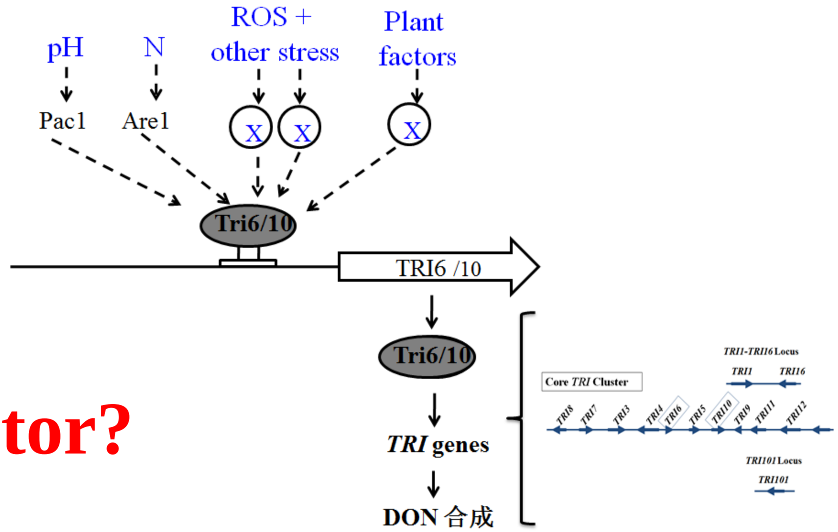
Three MAPK pathways

(Gpmk1, Mgv1, FgHog1)

The cAMP-PKA pathway

Putative PKA & <u>MAPK</u> phosphorylation sites in Tri6

MAPK docking site



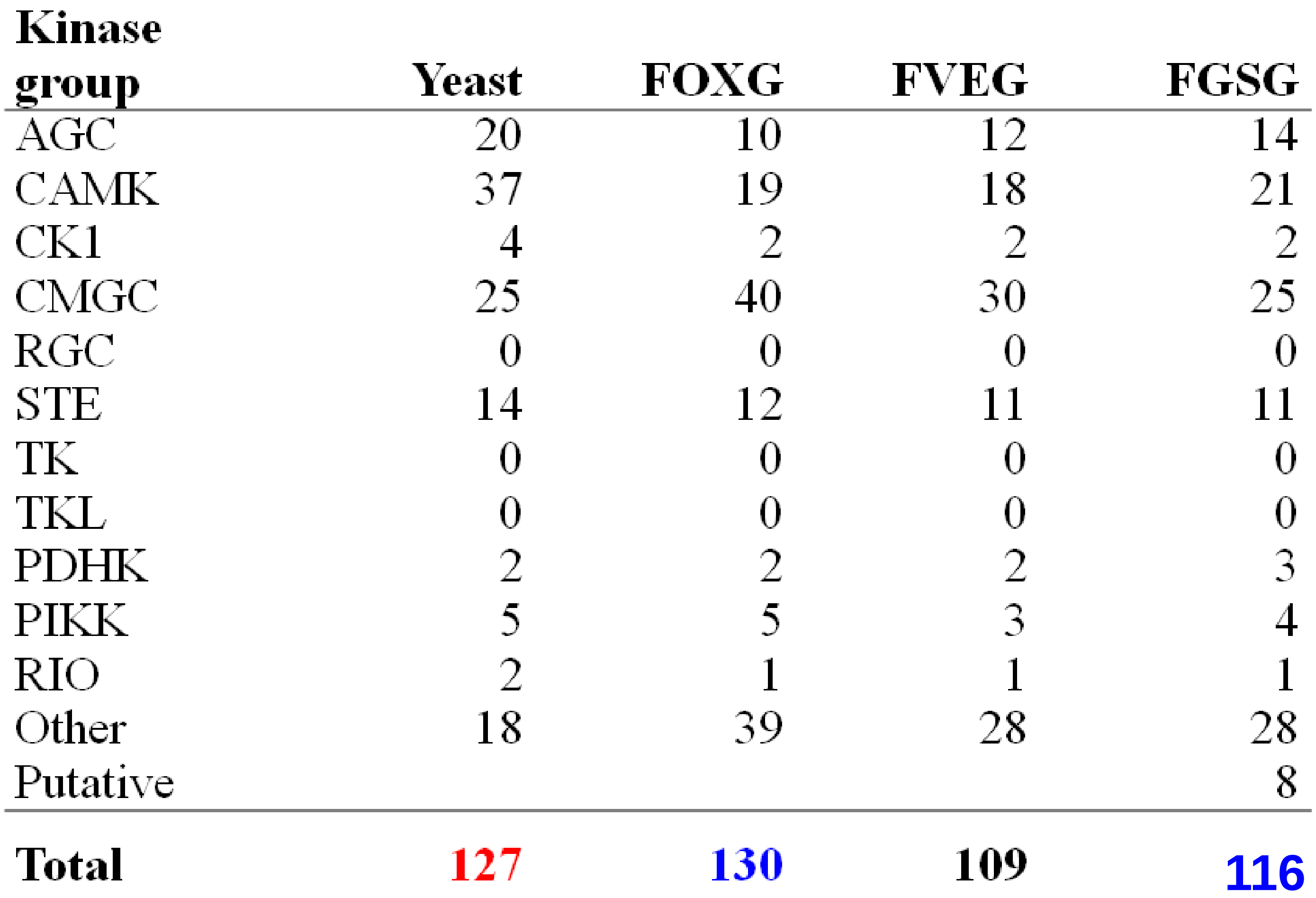
Why Tri6 is ‘global’ regulator?

Project 1. Functional analyses of predicted protein kinase genes (Kinome)

Project 2. Genes unique to *F. graminearum* (‘Uniquitome’)

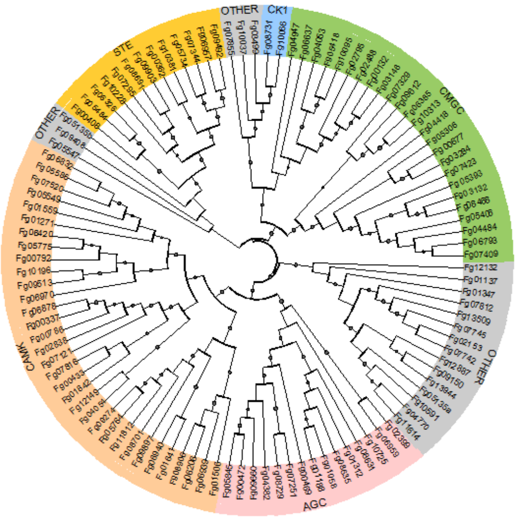
Targeted gene deletion – highly efficient (80%) in *F. graminearum*

Comparative Analysis -Protein Kinase Genes



Kinase group	Yeast	FOXG	FVEG	FGSG
AGC	20	10	12	14
CAMK	37	19	18	21
CK1	4	2	2	2
CMGC	25	40	30	25
RGC	0	0	0	0
STE	14	12	11	11
TK	0	0	0	0
TKL	0	0	0	0
PDHK	2	2	2	3
PIKK	5	5	3	4
RIO	2	1	1	1
Other	18	39	28	28
Putative				8
Total	**127**	**130**	**109**	**116**

F. oxysporum & *F. verticillioides*- Ma et al., 2010. Nature 464: 367-373



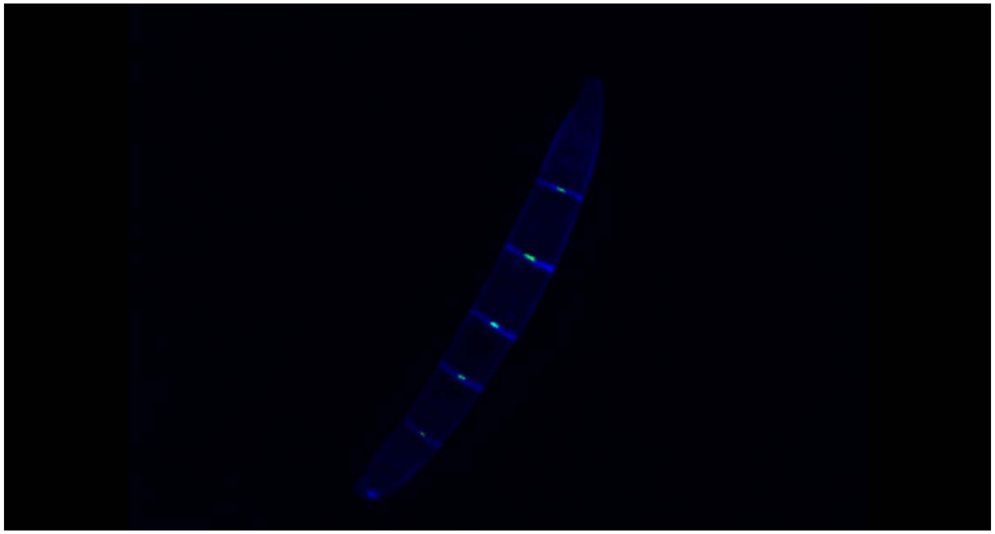
20 genes - essential

Mutants identified for 96 PK gene

At least two mutants for each gene

>30 of them- complementation assays

(subcellular localization)



Specifically localized to the septal pore

17 phenotypes scored - mutants identified

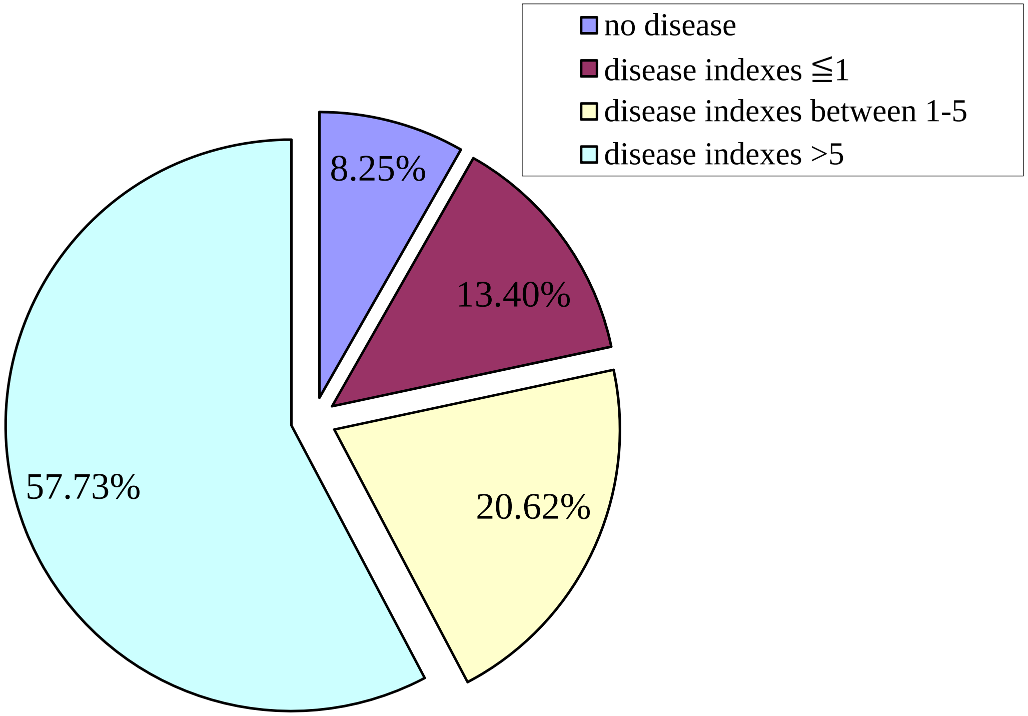
-growth rate
-colony morphology (pigmentation)
-hyphal growth and branching
-conidiation
-conidium morphology & germination
-sexual reproduction
-infection assays with flowering wheat heads
-DON production
-stress responses: NaCl, H_2O_2, SDS, CR
…………………

Searchable database

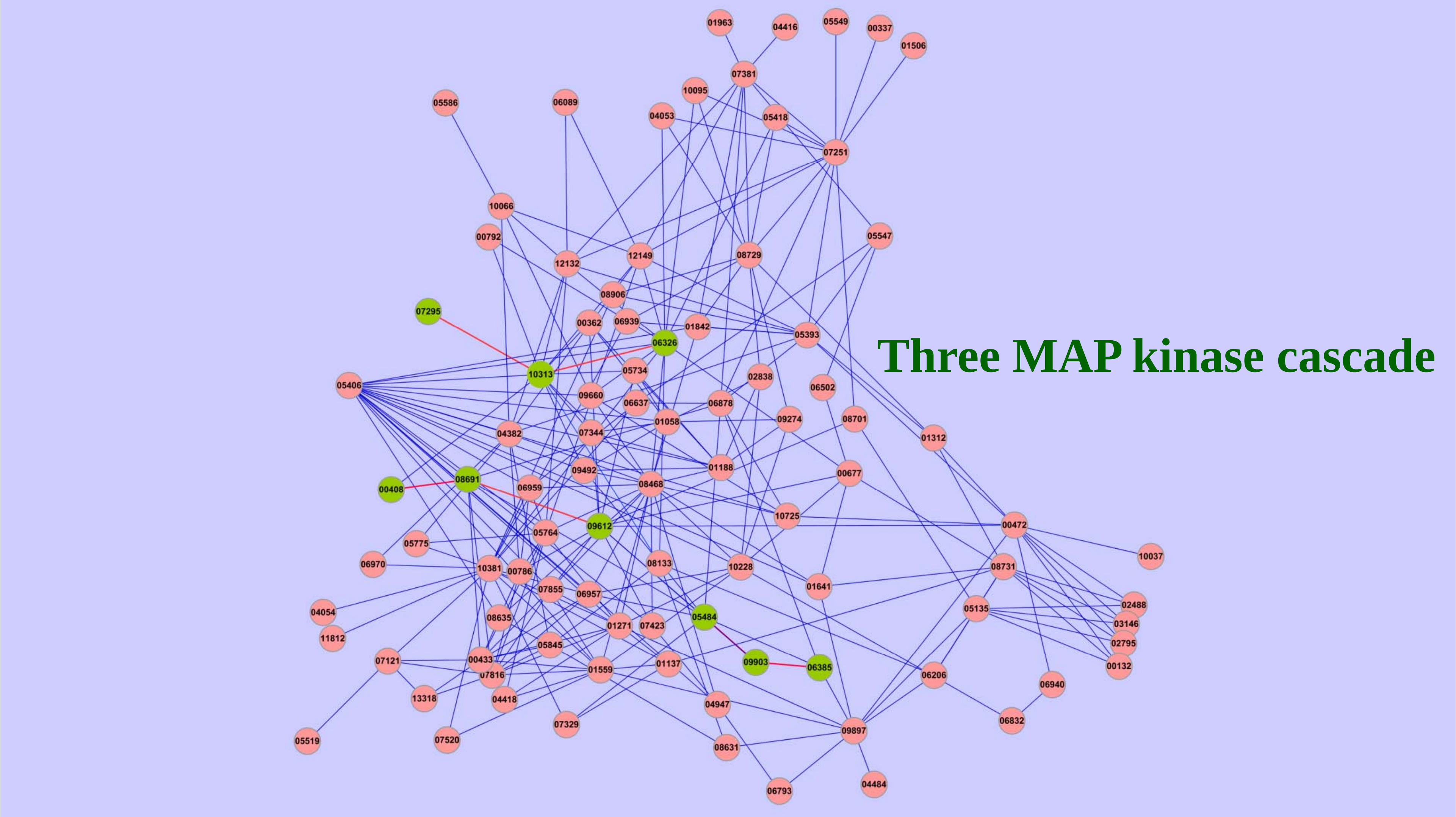
http://www.fgkinome.nwsuaf.edu.cn

Wang et al., PLoS Pathogens. 7(12): e1002460

42 PK genes are important for plant infection



Networks of PK-PK interactions



Three MAP kinase cascade

PK genes essential in *S. cerevisiae* or *S. pombe* but not in *F. graminearum*

F. graminearum genes	*S. cerevisiae* orthologs	*S. pombe* orthologs	Number of mutants
FGSG_02399 [a]	*IPL1*	*ark1*	14
FGSG_00433	*RAD53*	*cds1*	11
FGSG_04947	*CAK1*	-[c]*csk1*	6
FGSG_13318	*MEC1*	SPBC216.05	18
FGSG_10381	*CDC15*	*cdc7*	3
FGSG_08468 [b]	*CDC28*	*cdc2*	12
FGSG_03132 [b]	*CDC28*	*cdc2*	8
FGSG_01188	*CBK1*	*orb6* [d]	7
FGSG_05734	*KIC1*	*nak1* [d]	5
FGSG_07344	- [c]	*sid1*	15
FGSG_08906	- [c]	SPCC70.05C	19
FGSG_04053	- [c]	*prp4*	7

Cdk kinase

Cdk

Key regulator of cell cycle

Two Cdc28/Cdc2 genes

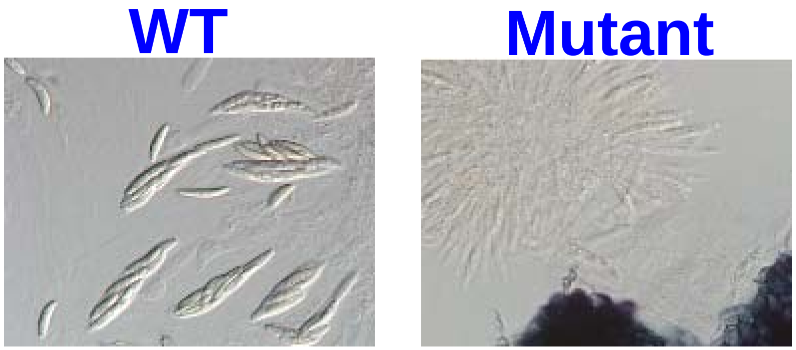
FGSG_03132 mutant :

Reduced 30% in conidiation

FGSG-08468 mutant: **similar to Fg04947 (CDK kinase)**

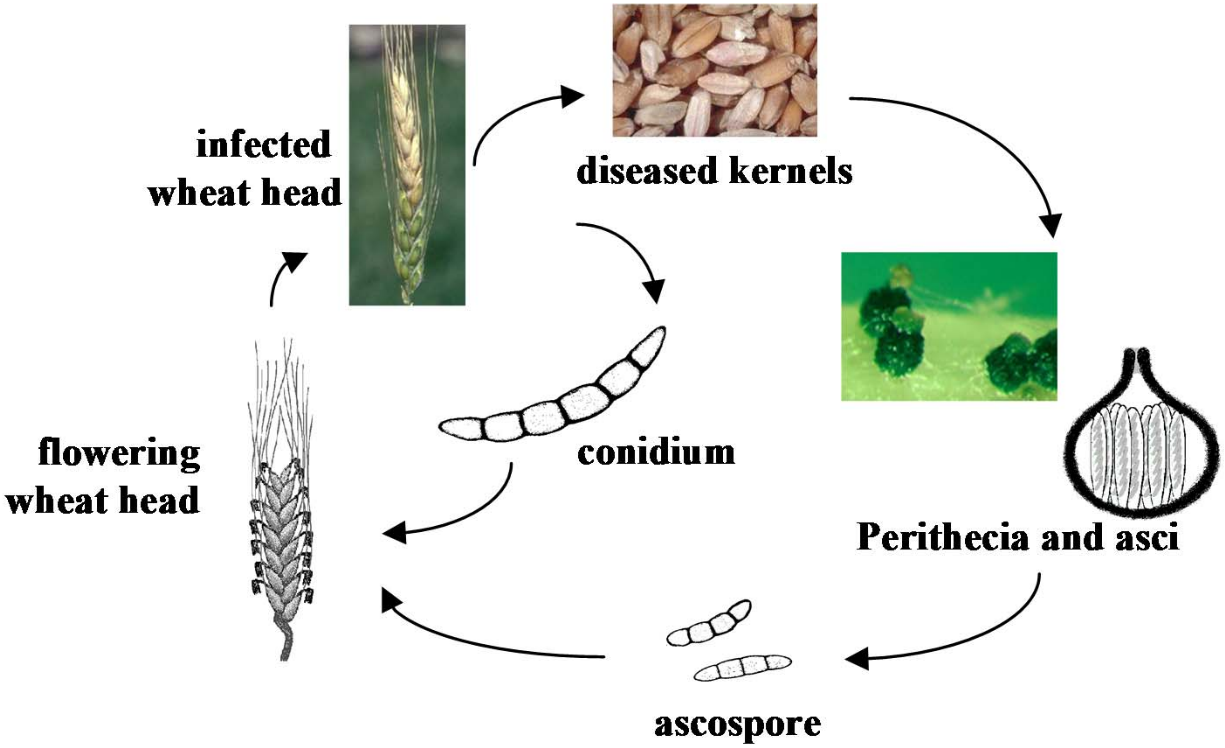
Defective in pathogenesis (DI <1.5)

Blocked in ascosporogenesis



Double mutants – not viable

Cell cycle and fungal pathogenesis



Some CDKs: infection-specific functions

Hyphal growth: *in vitro* vs. *in planta*

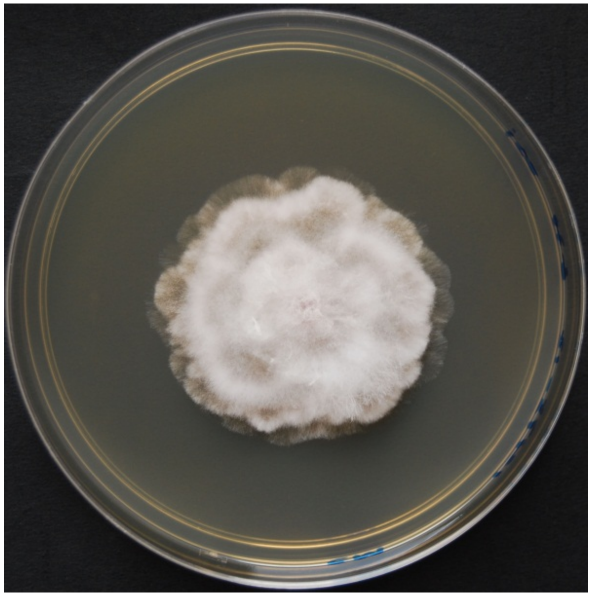
Project 2. Genes unique to *F. graminearum*

-Unique genes with signal peptide (secreted)
-Unique genes with conserved domains

Six of them have distinct functions

***SSP1* mutant**: reduced growth rate

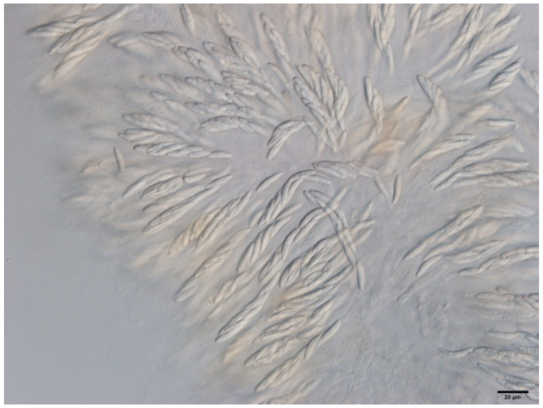
+ colony morphology defects

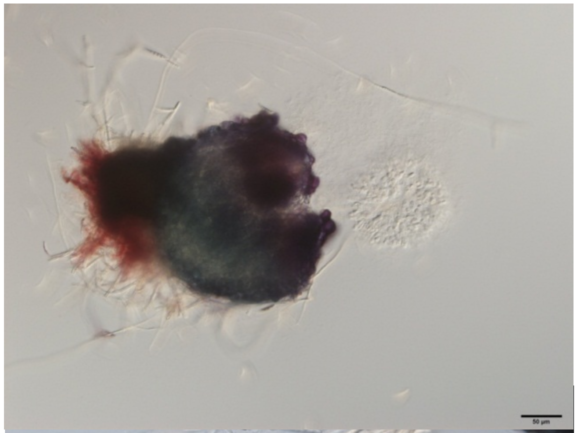


A *F. graminearum* unique gene important for hyphal growth

SSP2 is required for normal sexual reproduction

PH-1

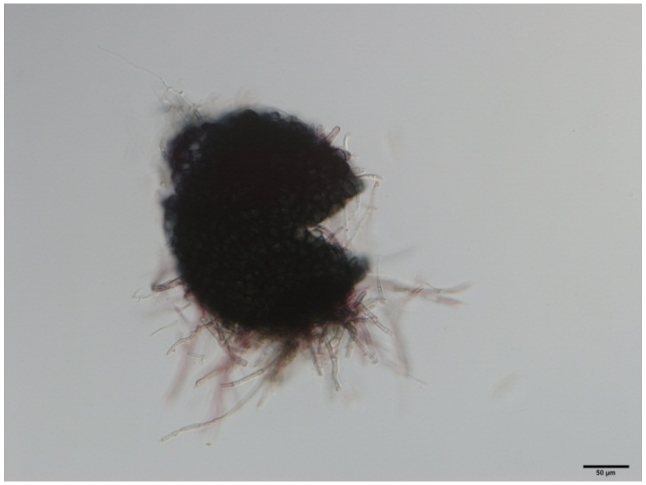




aborted

abnormal

Mutant



Empty perithecia (60%)

Two *F. graminearum* specific genes important for plant infection

PH-1 *ssp3* *sdp1*

***In planta* expression**

Acknowledgements

Northwest Agricultural & Forestry University

Chengfang Wang
Huiquan Liu
Qiaojun Jin
Shijie Zhang
Qian Zheng
Rui Hou
Qijun Xu
+ undergraduate students

Collaborators

Corby Kistler (USDA)
Li-Jun Ma (UMass)

Purdue University

Jin-Rong Xu
Xiaoying Zhou