Genetic Manipulation of Susceptibility to Fusarium Head Blight

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Fusarium Head Blight

- **Few sources of effective wheat or barley germplasm conferring resistant to FHB in wheat or barley. This makes crop improvement difficult.**

- **Transgenics are an alternative but gene assays in transgenic wheat or barley is slow and expensive and has only a moderate capacity. OK for testing single genes, but not efficient enough for *de novo* gene discovery.**

- **Approach: Use higher capacity model plants to assay genes for their ability to confer resistance to *Fusarium*.**

A pipeline for gene discovery and deployment

Select potential antifungal genes

Isolate selected genes

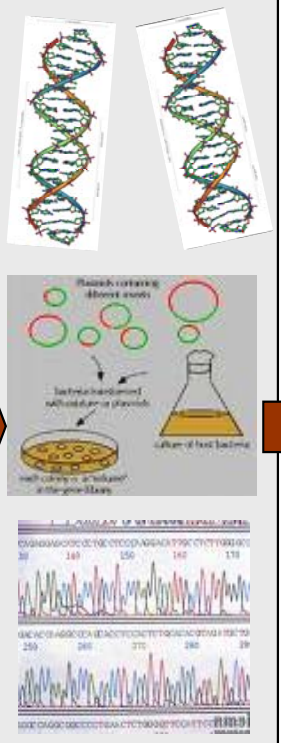
Physcomitrella: Create gene knockouts and overexpressors

Physcomitrella: Assay plants for FHB resistance

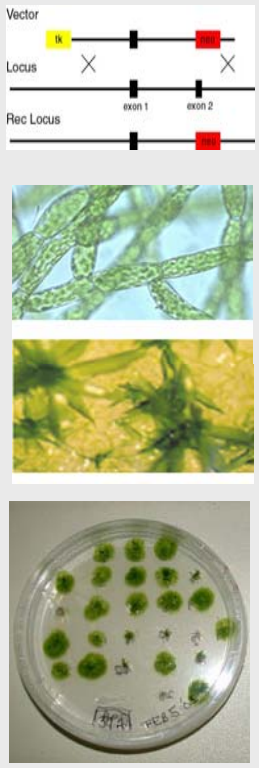
Wheat: assay selected genes for FHB resistance

Wheat: Stable Transgenics

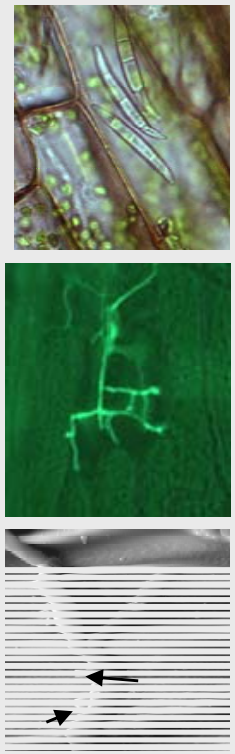
Based on genetic, molecular data from wheat and other species



Clone and sequence genes

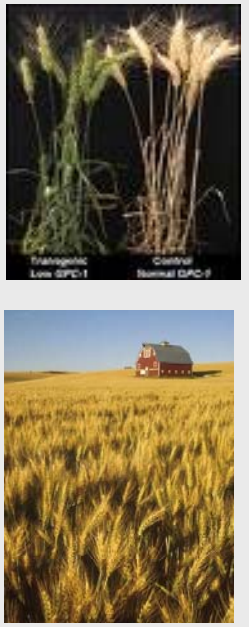


Mutate or express genes in Physcomitrella



Expose mutant plants to FHB, DON

Transient assay in wheat using VIGS (Scofield Lab)



Transformed FHB-Resistant Wheat, Barley (Dahleen Lab)

Physcomitrella patens

Grows like yeast but is a multicellular plant

Haploid gametophyte dominates life cycle

Genome size: 511Mbp; 27 chromosomes

Genome sequence completed in 2007

Functional conservation with higher plants and yeast

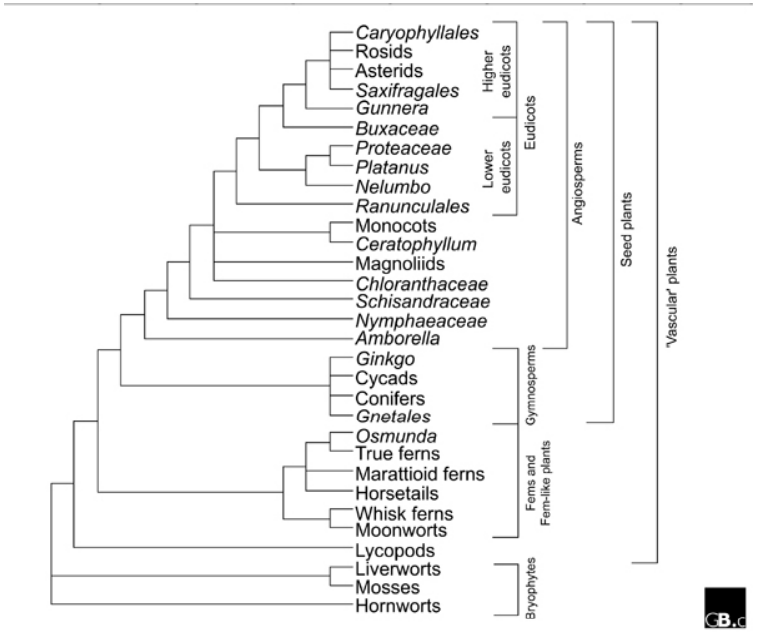
Undergoes high efficiency homologous recombination

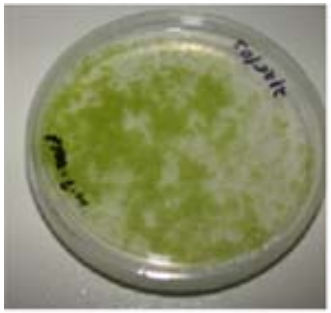
Allows targeted gene replacement for gene knockout or site-specific mutation etc.

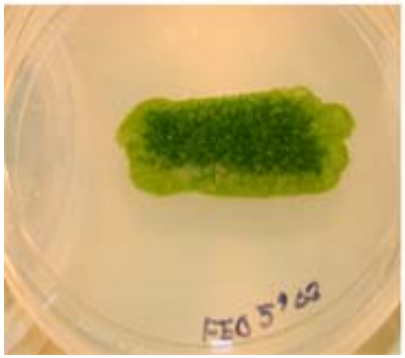
Model system for functional genomics

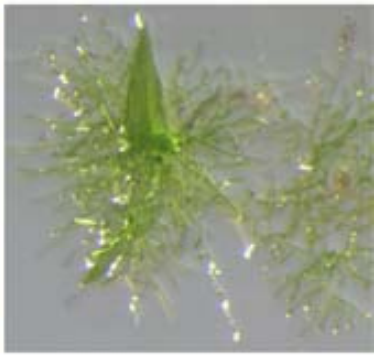
Our Targets:

Disease, Cell Death, Toxin Action, Induced Immunity, Cell Wall

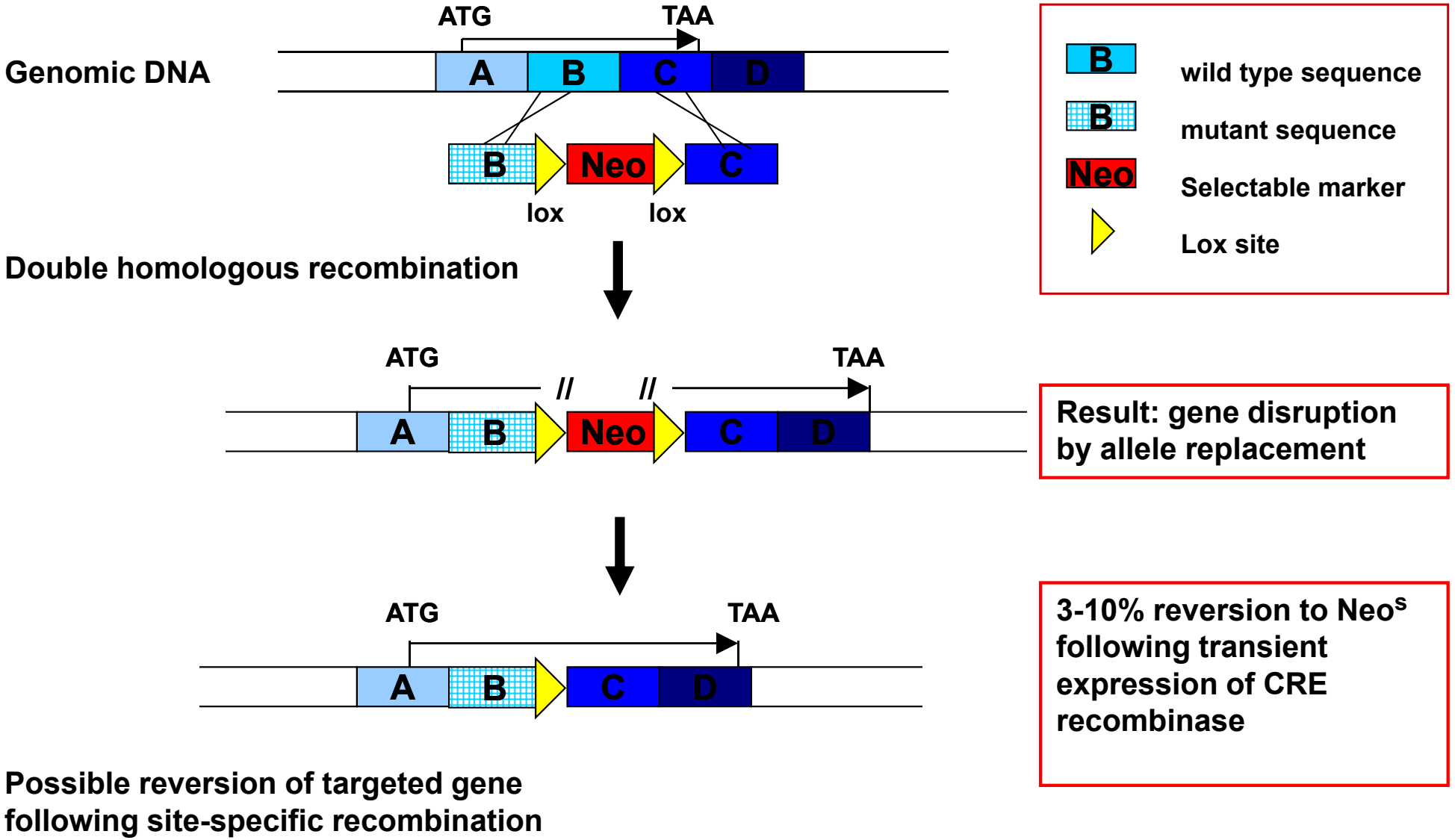








Gene knockout by homologous recombination



Recombination rates in Physcomitrella

Efficiency (GT/GT+IR): **4% (.5kb) up to 100% (2-4 kb) in *Physco***

0.005-0.1% in angiosperms

95% in *S. cerevisae*

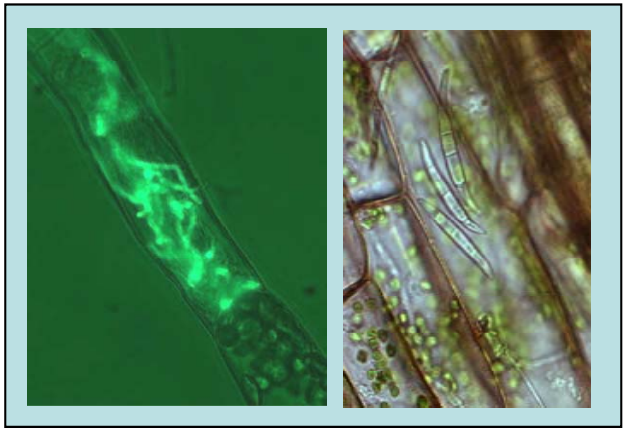
1-30% in *N. crassa*

0.1-1% in mouse ES cells

- **Essentially any gene or genomic sequence in the *Physcomitrella* genome can be deleted quickly and precisely.**
- **Can complement knockout mutants with genes from other plants.**
- **Foreign genes can be introduced into a specific locus and expressed in a predictable manner (no position effects).**

Can *Fusarium* infect *Physcomitrella patens?*

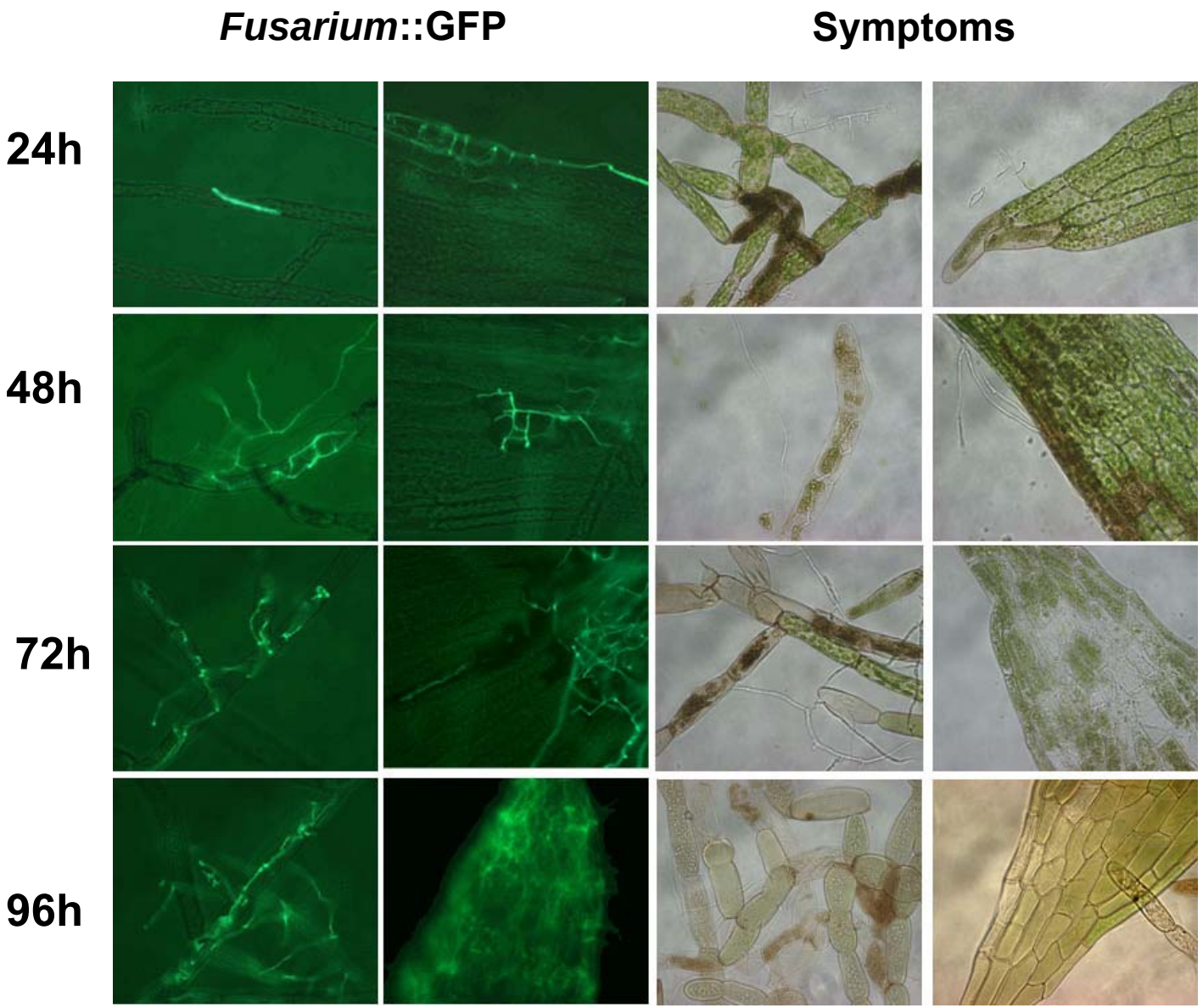
Can it be Infected?



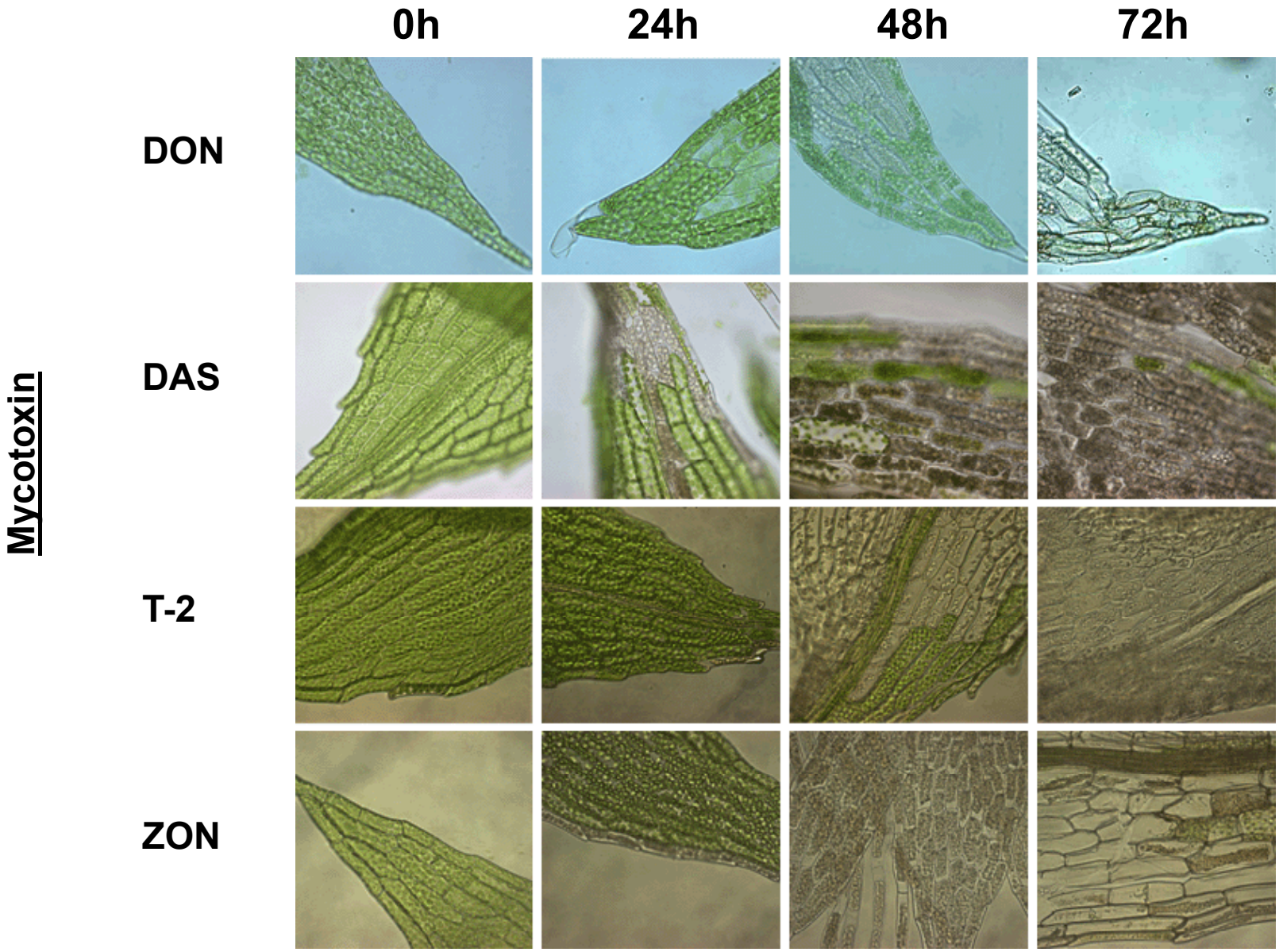
www.wheatlifemagazine.com

Is it a relevant model for diseases of crop plants?

Infection of Physcomitrella by *Fusarium graminearum*

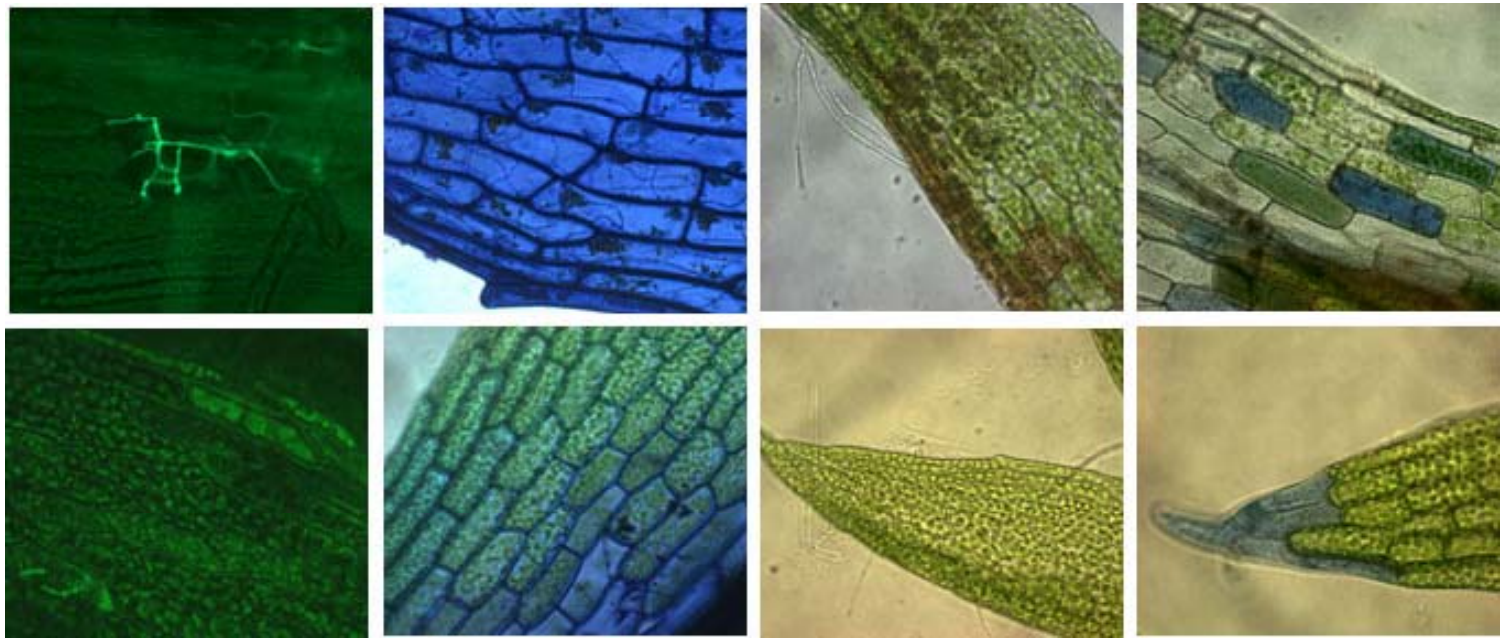


Physcomitrella is sensitive to multiple FHB mycotoxins

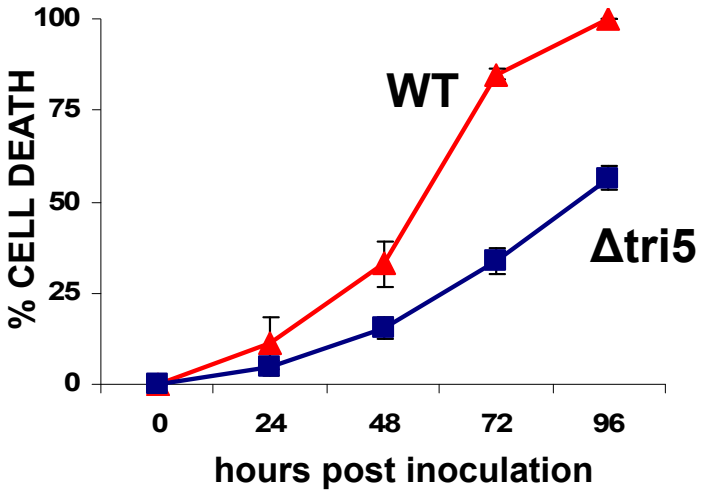


Tricothecenes contribute to *F. graminearum* virulence

(A)



(B)



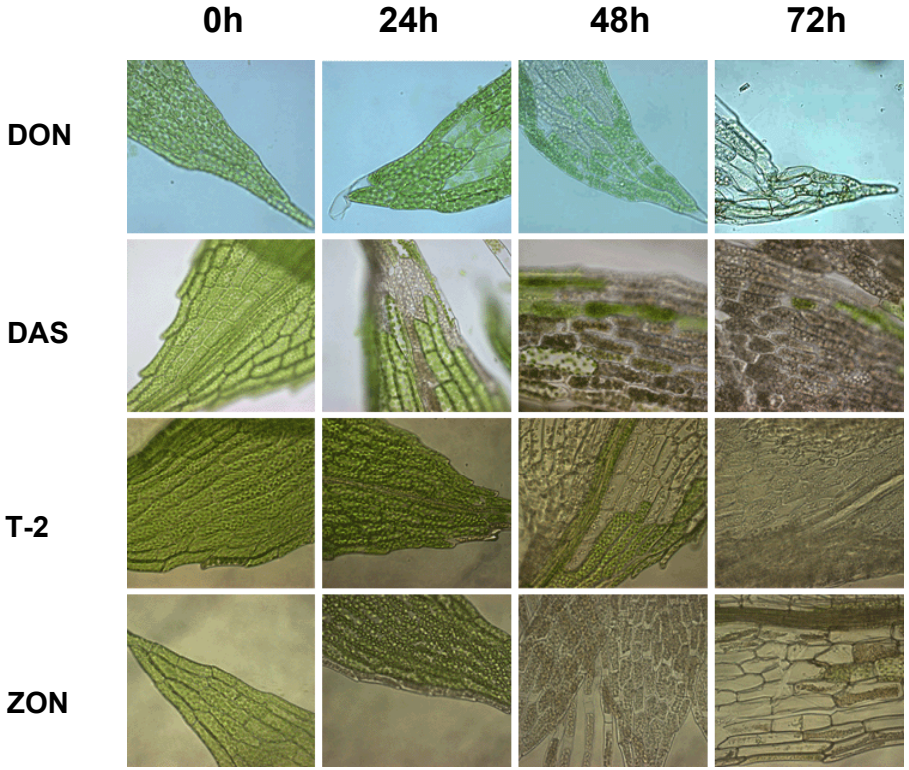
The Δ*tri5 strain of F. graminearum* does not produce DON or DAS

Programmed Cell Death (PCD)

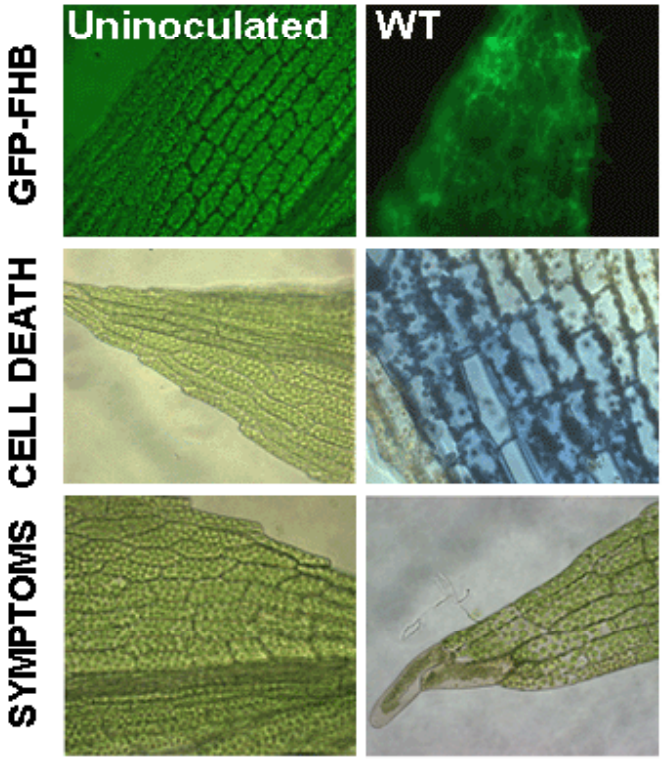
Is Programmed Cell Death a target for Fusarium toxins?

- *Fusarium* mycotoxins contribute to virulence on Physcomitrella and wheat
- Both *Fusarium* and mycotoxins cause cell death on host plants
- **Programmed Cell Death (PCD)** is a genetically controlled process
- Mutating genes that control host cell death may suppress symptoms and attenuate virulence

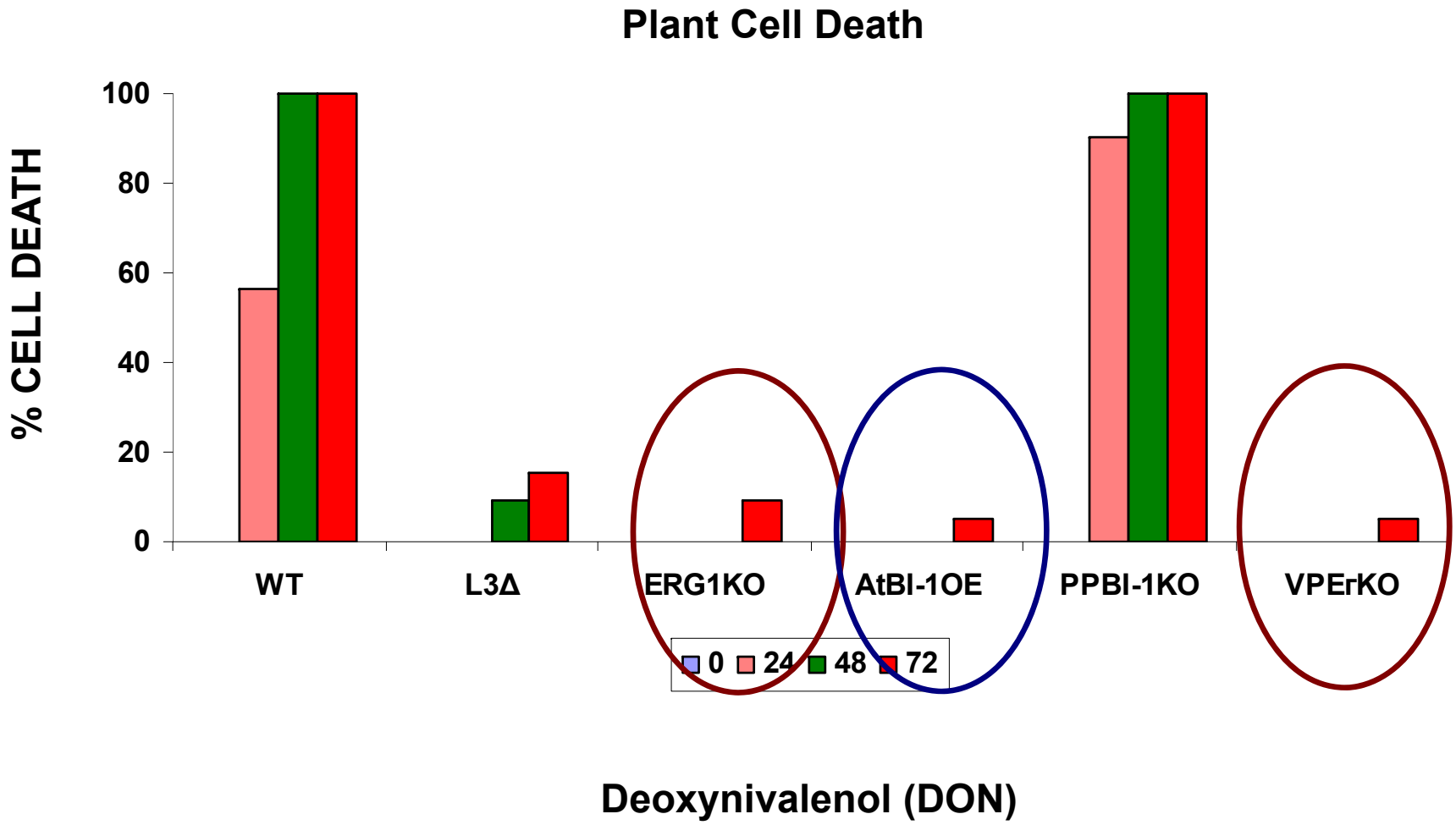
Mycotoxins



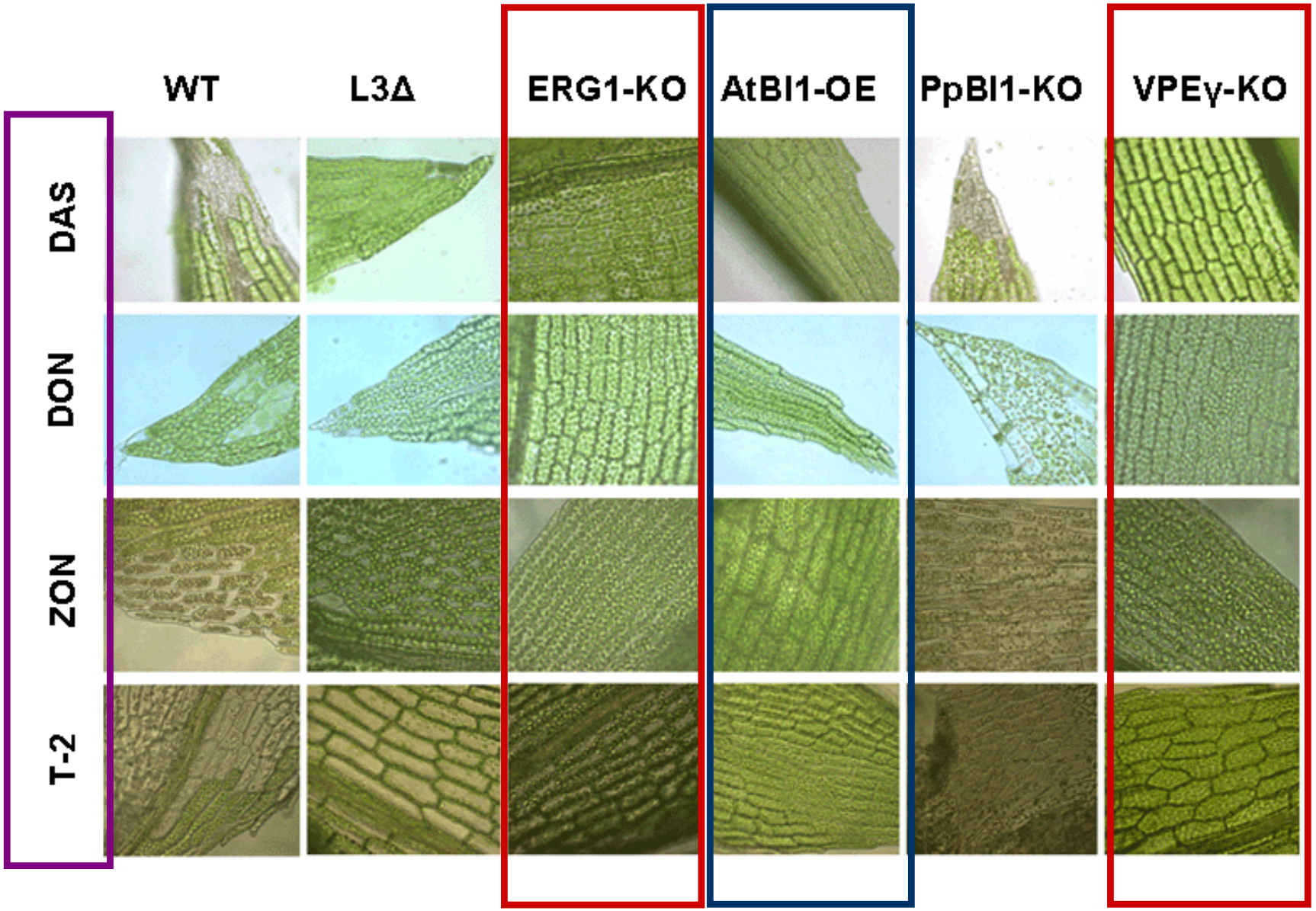
Fusarium graminearum



PCD is required for host cell death and sensitivity to DON

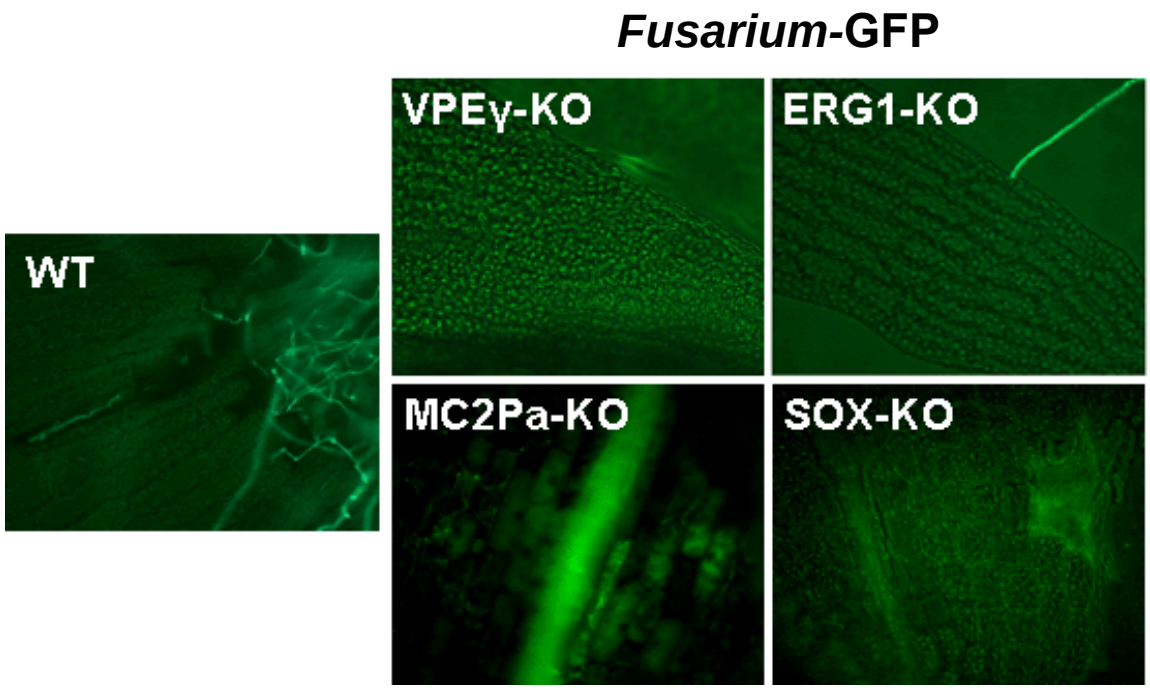


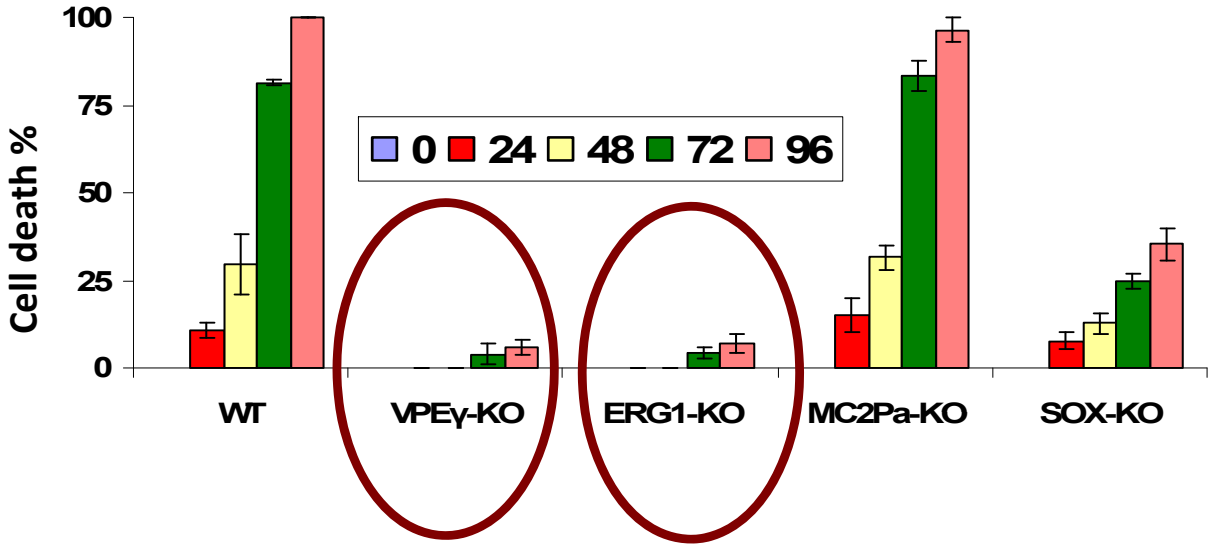
PCD mutant plants are resistant to multiple mycotoxins



Plant Cell Symptoms

Some PCD mutant plants are resistant to FHB



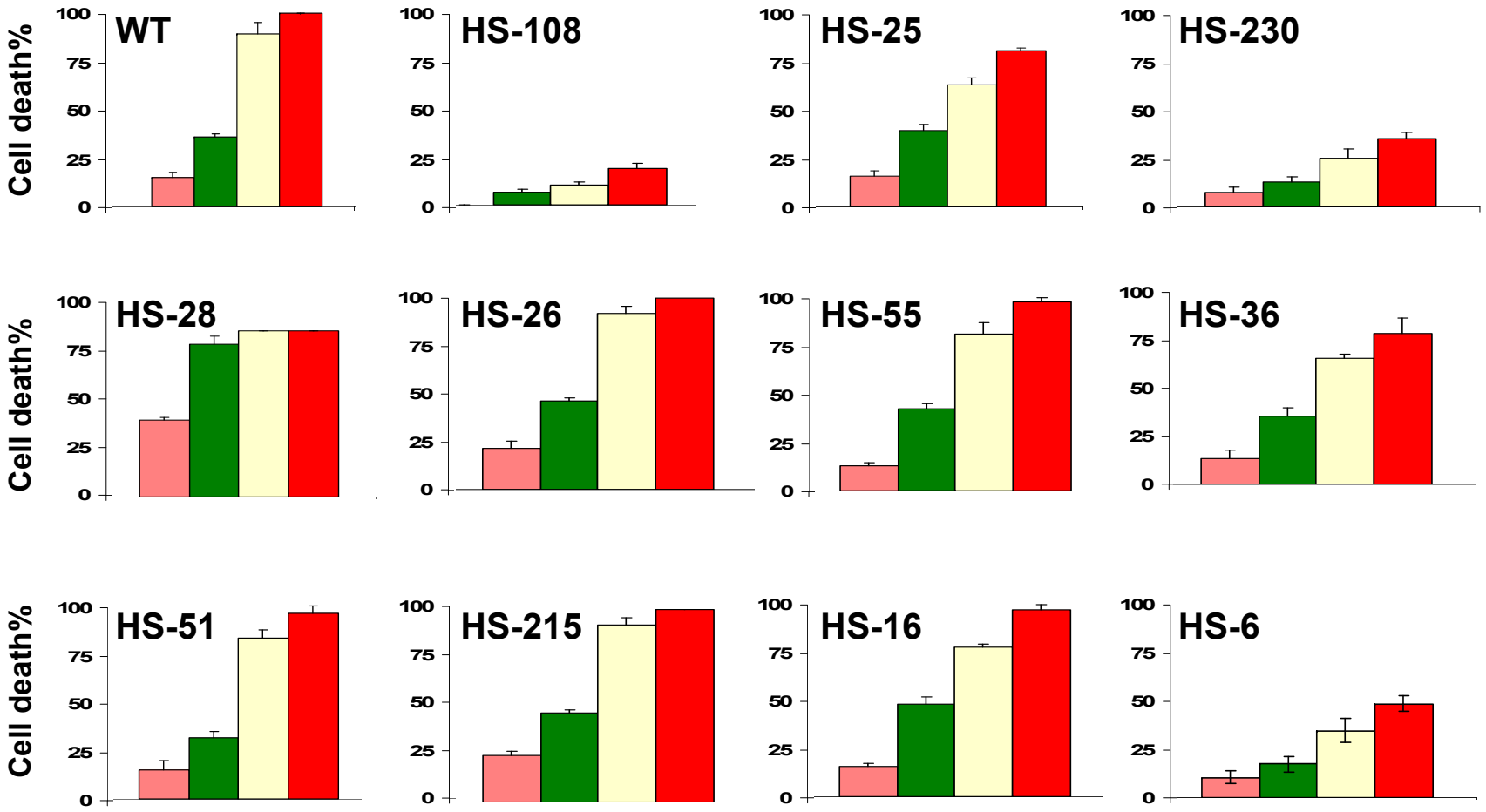


KO = Gene Knockout Plant

OE = Overexpressing Transgenic Plant

Medium Throughput Assay for Gene Function

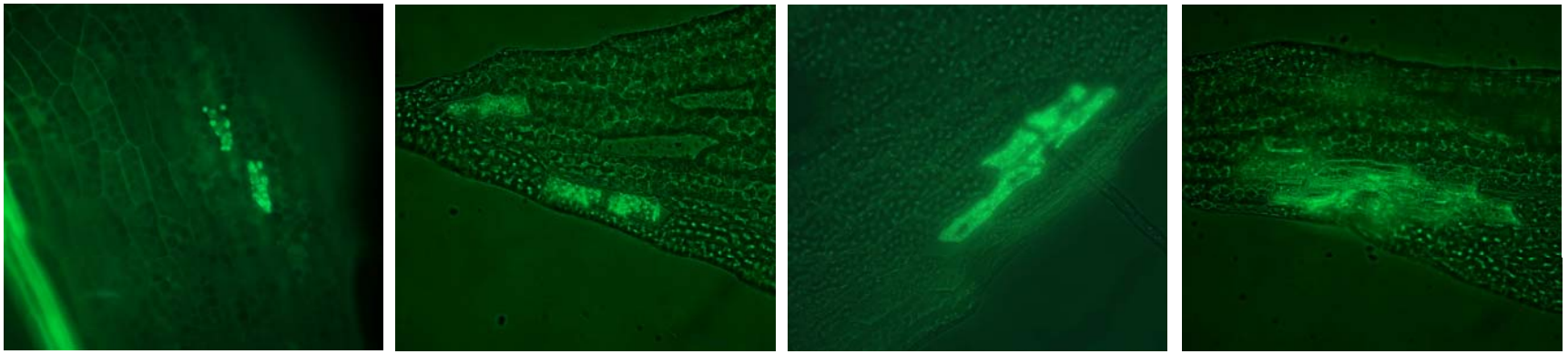
Assay Gene Knockout Plants for Reduction of Symptoms



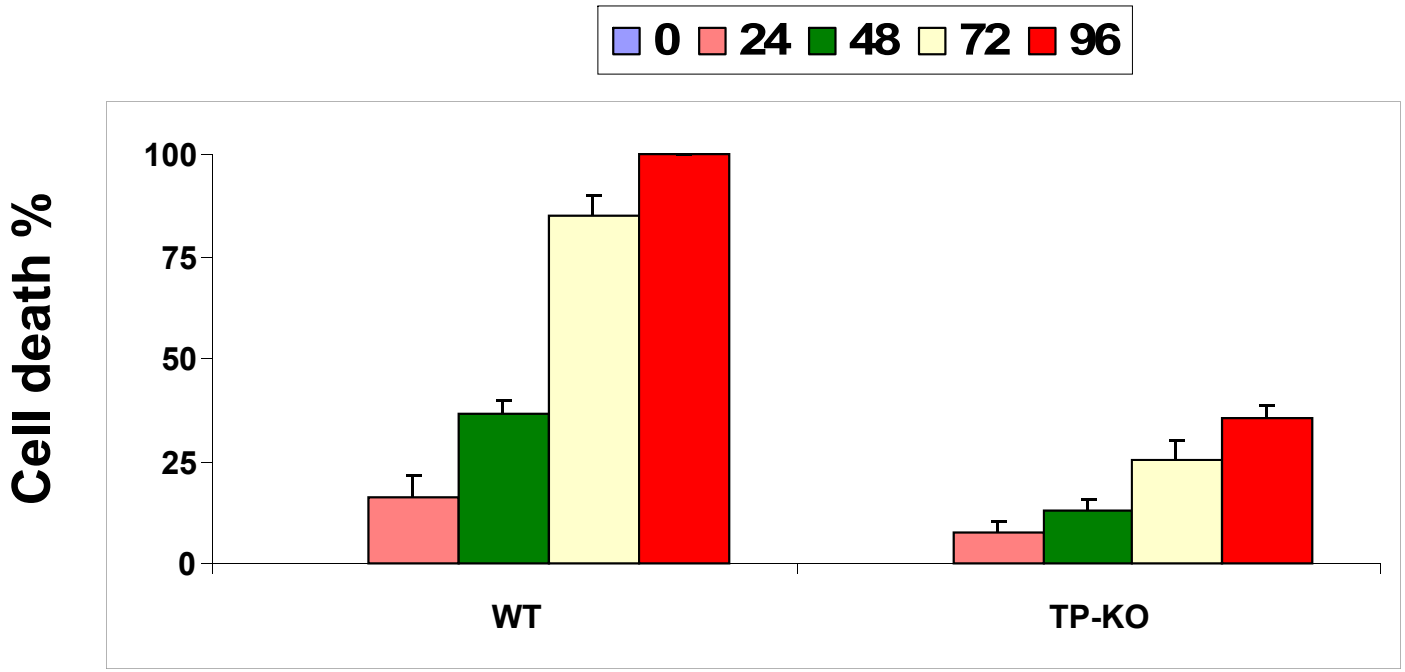
Lots of 'failures' –you need a high capacity system in order to select those rare genes that do confer resistance Fusarium.

Fusarium graminearum infection in TP-KO plants

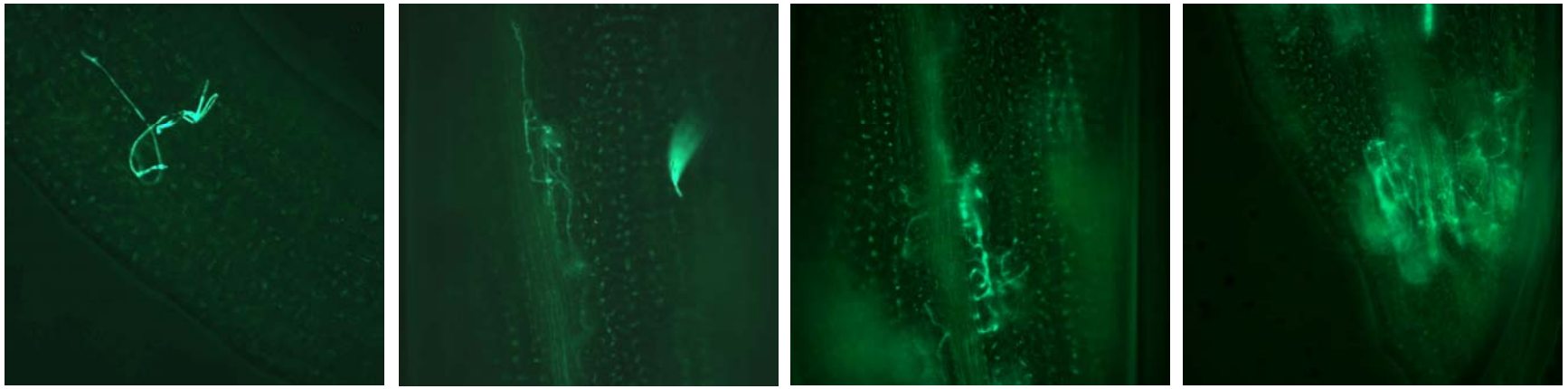
TP- KO

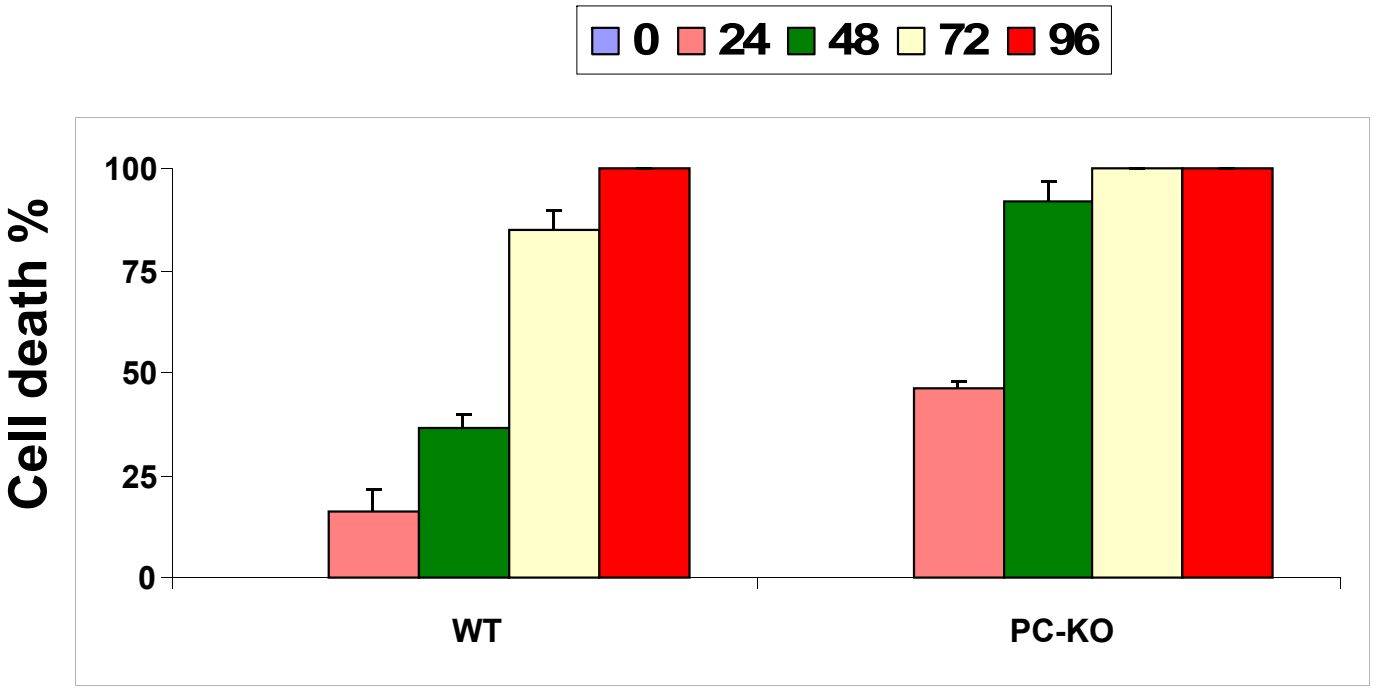


24h 48h 72h 96h

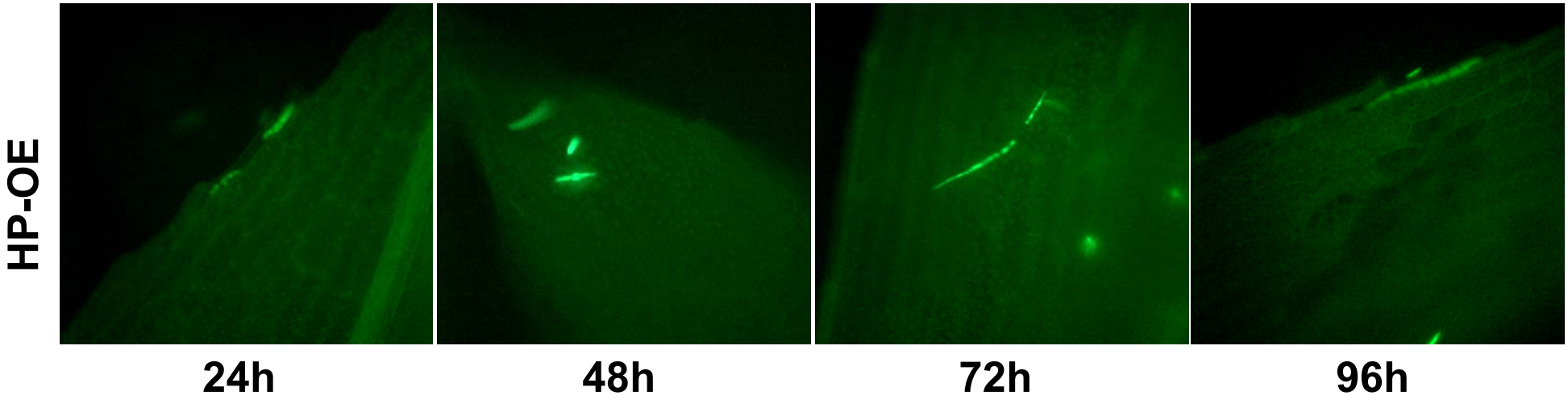


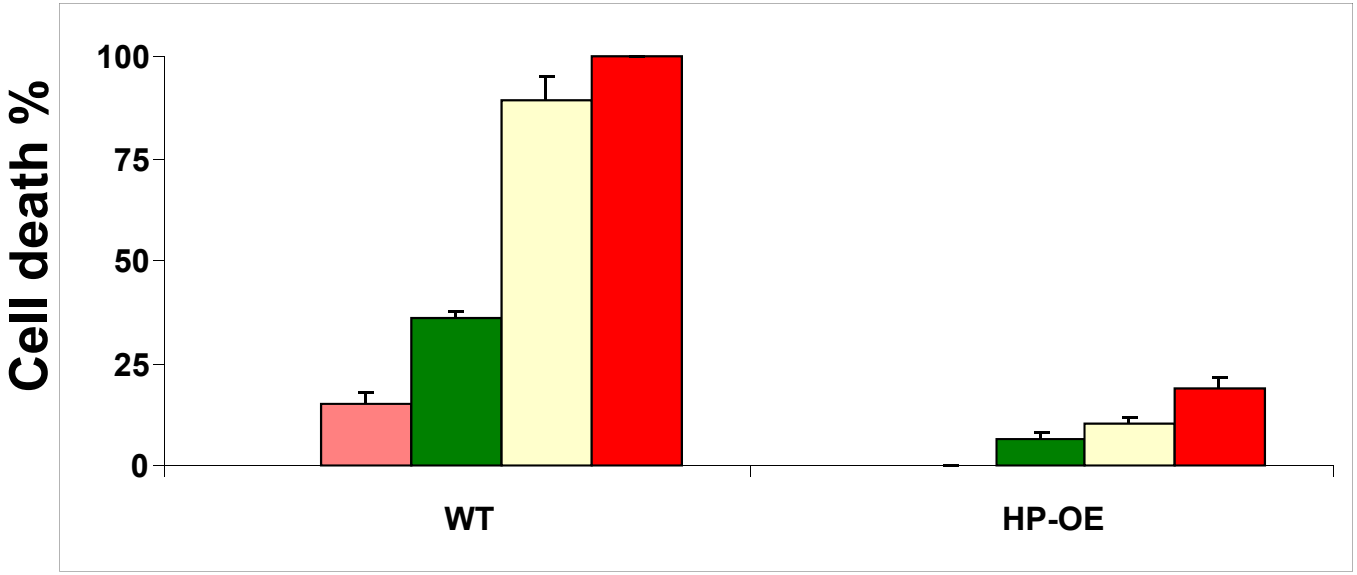
Fusarium graminearum infection in PC-KO plants



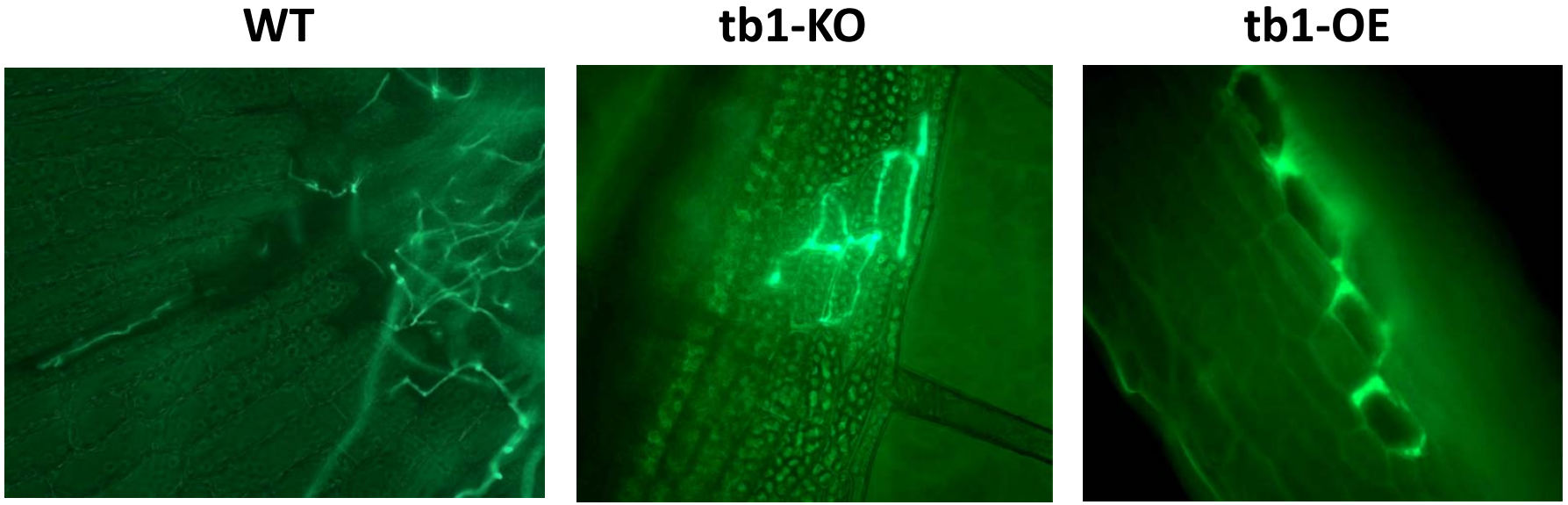


Fusarium graminearum infection in HP-OE plants





Fusarium graminearum infection of WT, tb1-KO and tb1-OE plants



Physcomitrella patens **infected with** ***Fusarium graminearum*****:GFP**

Mutation of tb1 affects the ability of Fusarium to enter into cells.

Effect are manifested at or near the plant cell surface and vacuole.

Mechanism is not understood, but may be revealing something important about how Fusarium colonizes its host. Interactions at the cell surface may be important.

Pipeline for discovery and deployment of genes effective against FHB

Select potential antifungal genes

Isolate selected genes

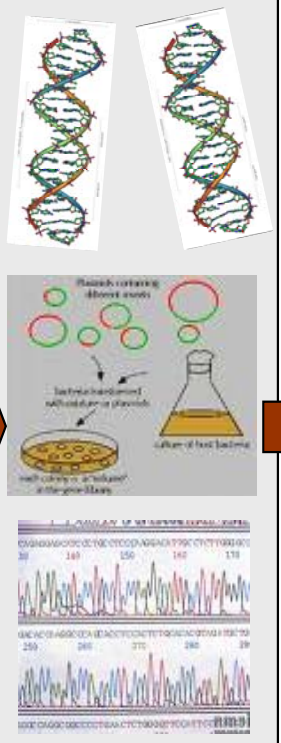
Physcomitrella: Create gene knockouts and overexpressors

Physcomitrella: Assay plants for FHB resistance

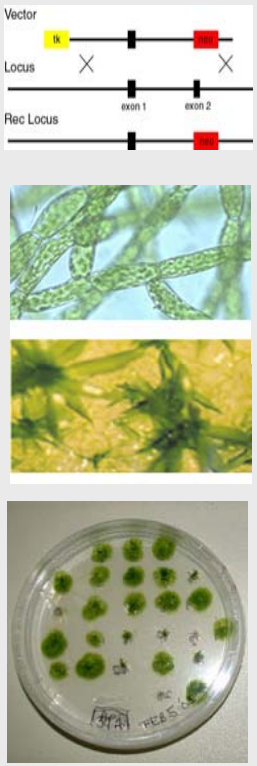
Wheat: assay selected genes for FHB resistance

Wheat: Stable Transgenics

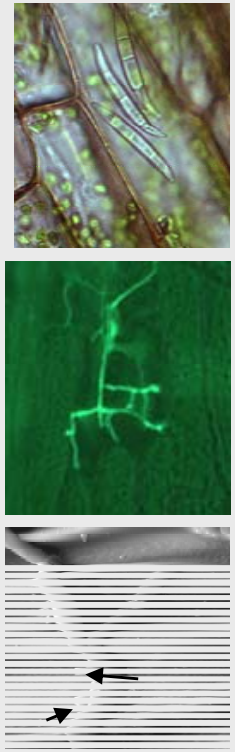
Based on genetic, molecular data from wheat and other species



Clone and sequence genes

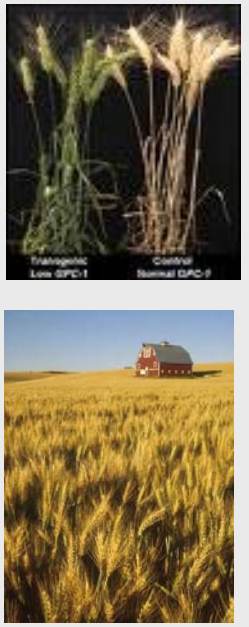


Mutate or express genes in Physcomitrella



Expose mutant plants to FHB, DON

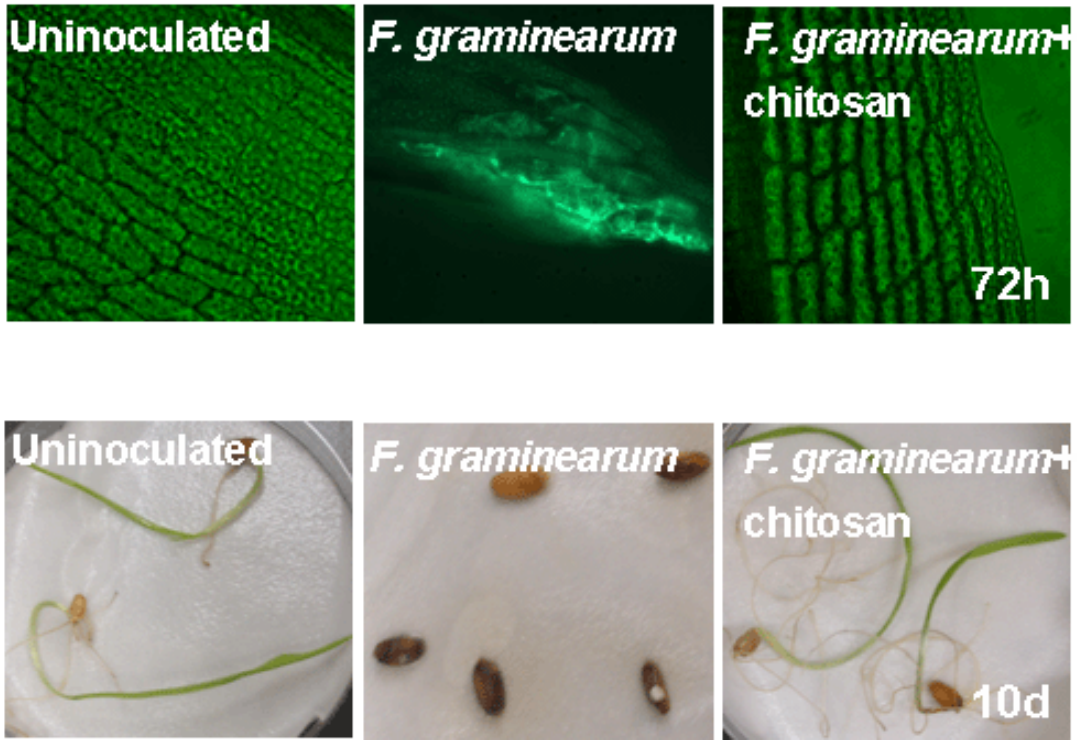
Transient assay in wheat using VIGS (Scofield Lab)

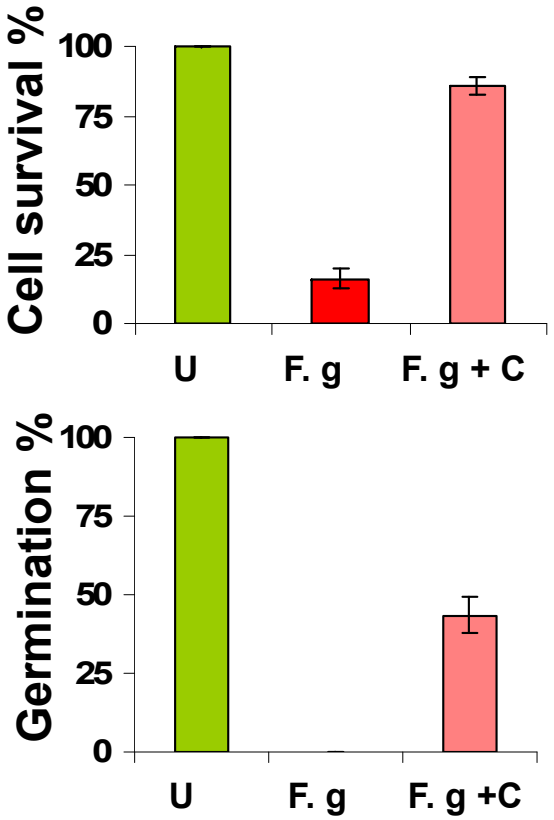


Transformed FHB-Resistant Wheat, Barley (Dahleen Lab)

Induced Immunity

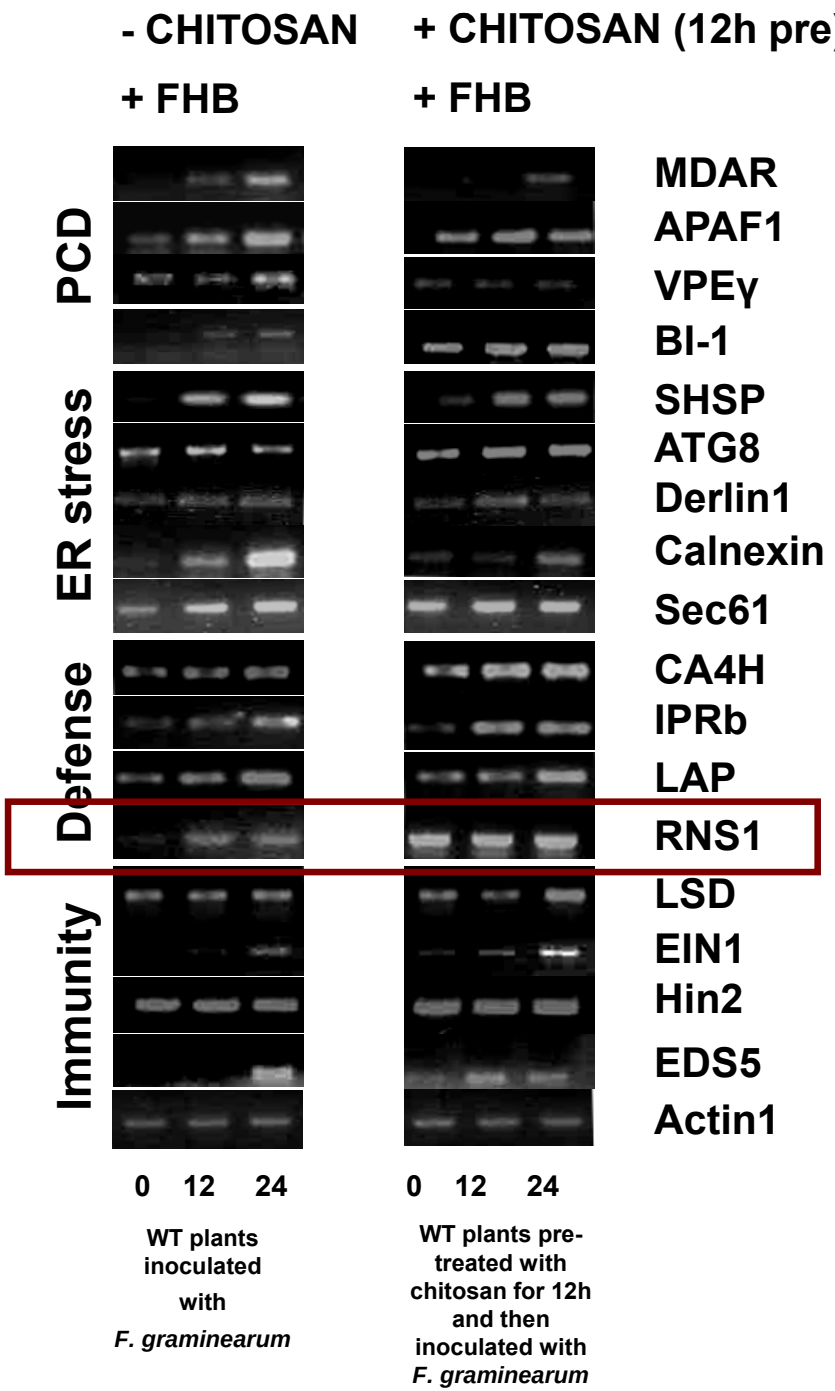
Chitosan induces immunity in *Physcomitrella* and wheat





Chitosan pre-treatment induces immunity against *Fusarium graminearum* in both Physcomitrella and wheat. However, the response is more pronounced in Physcomitrella.

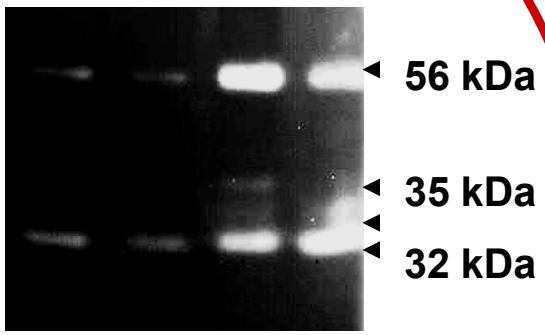
Induced Immunity in Physcomitrella



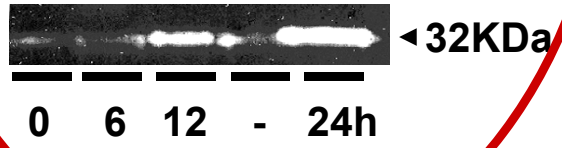
RT-PCR

Role of nucleases in infection of *F. graminearum*

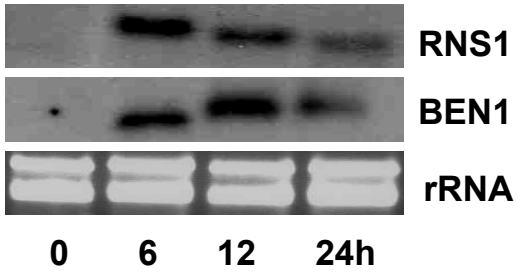
RNase activity gel assay



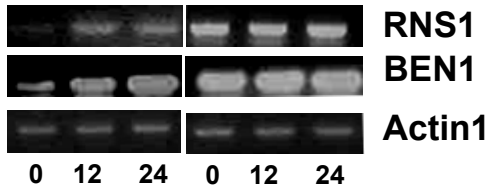
DNase activity gel assay



Gene Expression -Northern blot

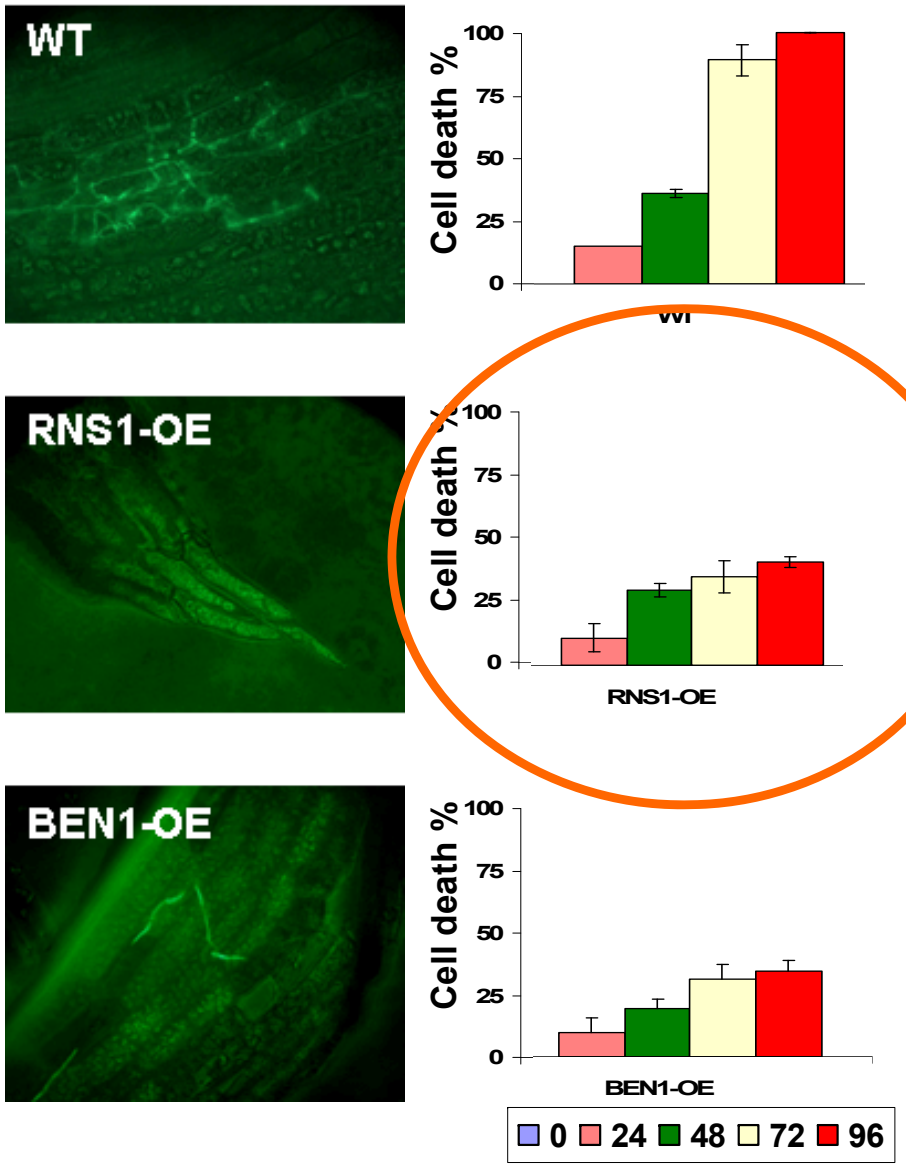


Gene Expression -RT-PCR



Role of nucleases in infection of *F. graminearum*

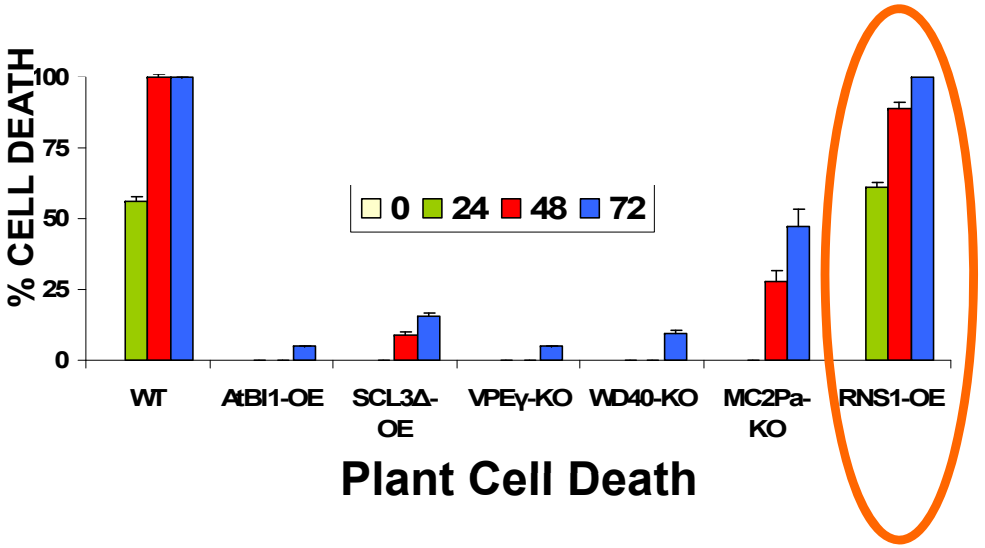
Sensitivity to FHB



Fusarium-GFP

Plant Cell Death

Sensitivity to DON



Plant Cell Death

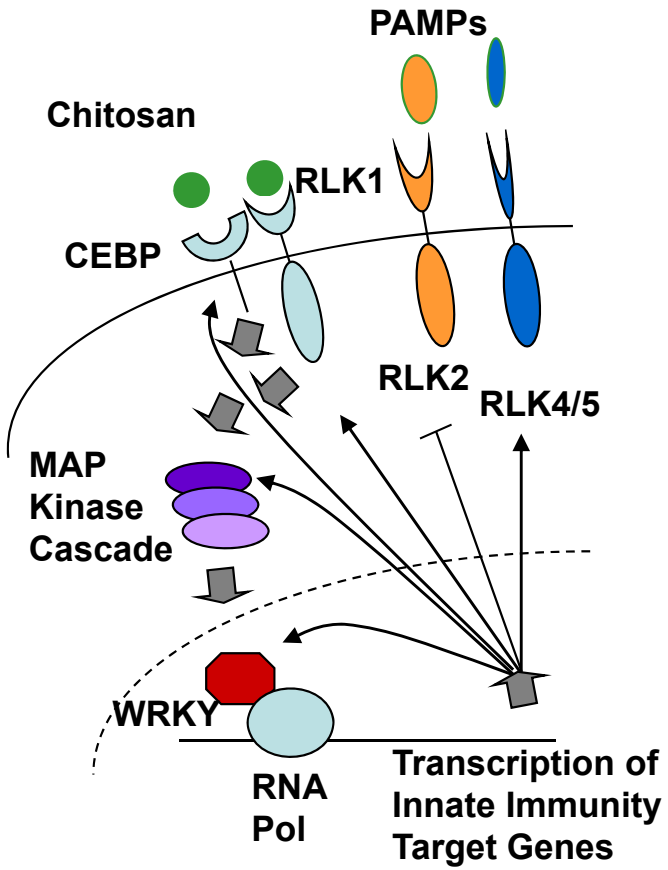
Note that plants overexpressing RNS1 are significantly more resistant to FHB than WT but are still sensitive to DON (as is the WT).

This is consistent with RNS1 having a direct antifungal effect on *F. graminearum.*

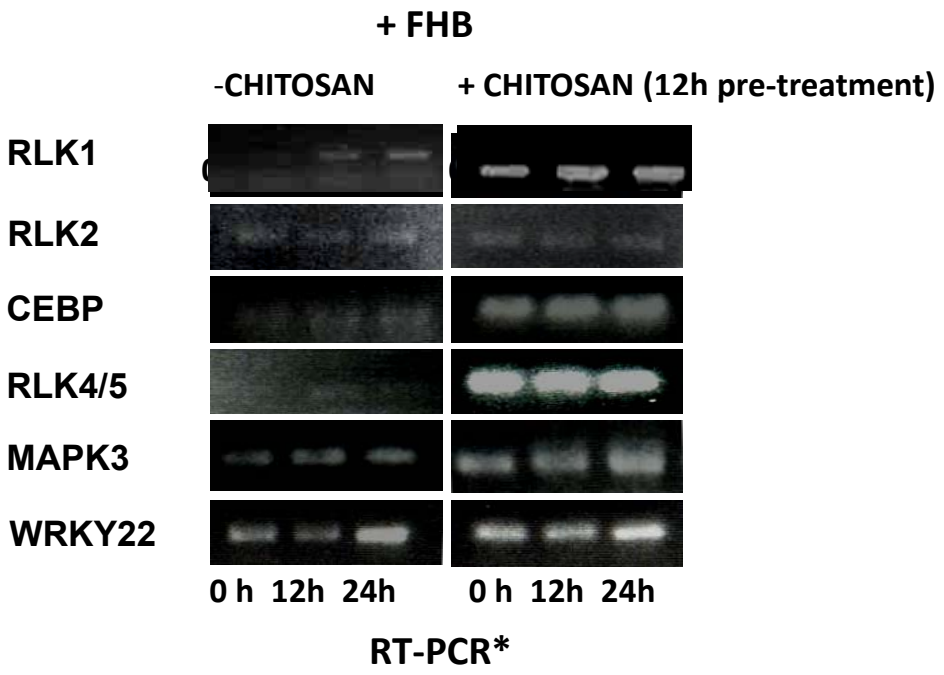
N.B. Exogenous RNase is lethal to *F. graminearum*

Induced Immunity

Chitosan Induced Immunity



PAMP Signaling Genes



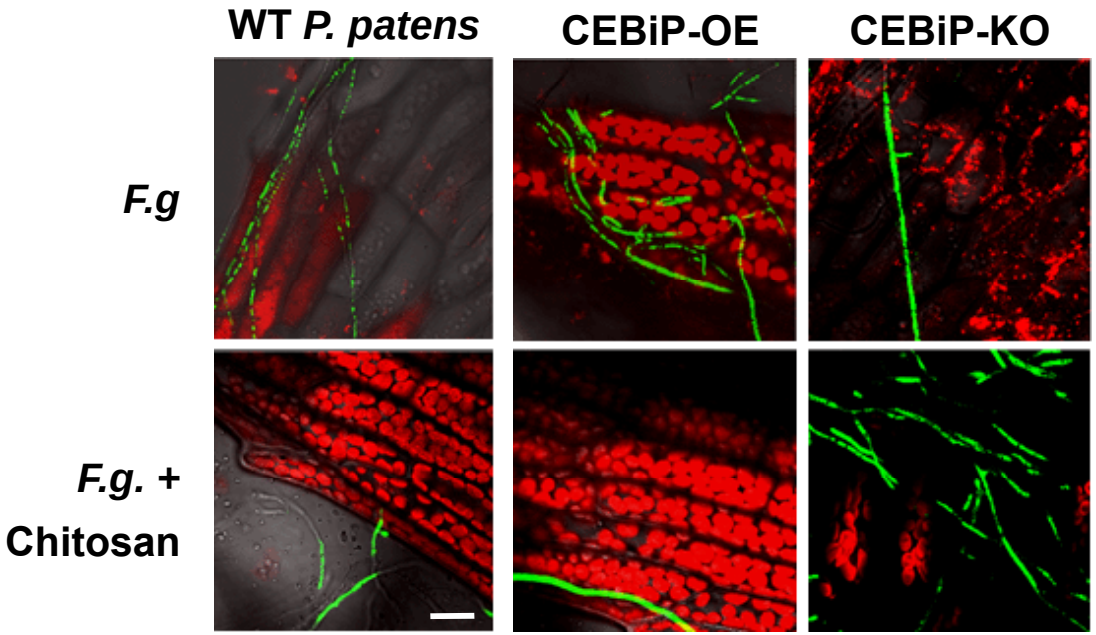
Chitosan pre-treatment induces many of the components invoved in chitosan and other PAMP (elicitor) signaling.

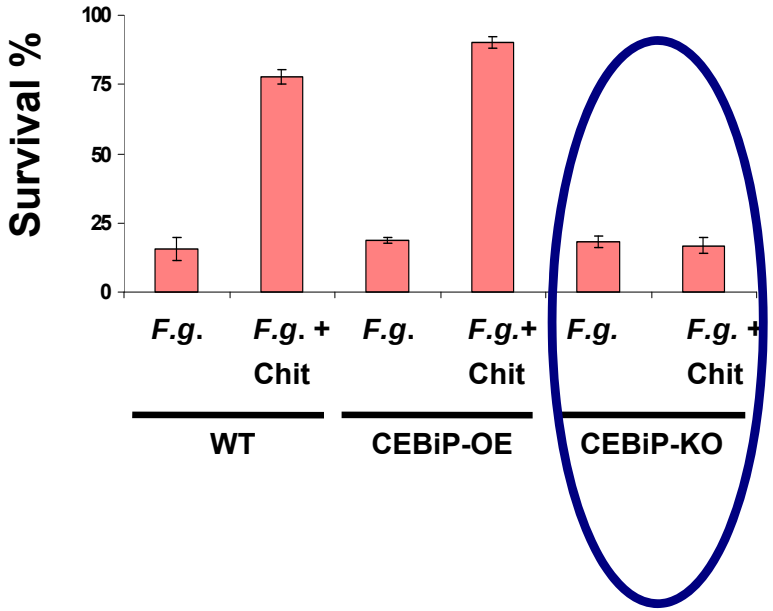
PAMP = Pathogen Associated Molecular Pattern

*** "Non-quantitative" RT-PCR**

Chitosan-Induced Immunity Against FHB

Chitosan-induction immunity in WT, CEBiP OE and CEBiP KO *Physcomitrella* plants

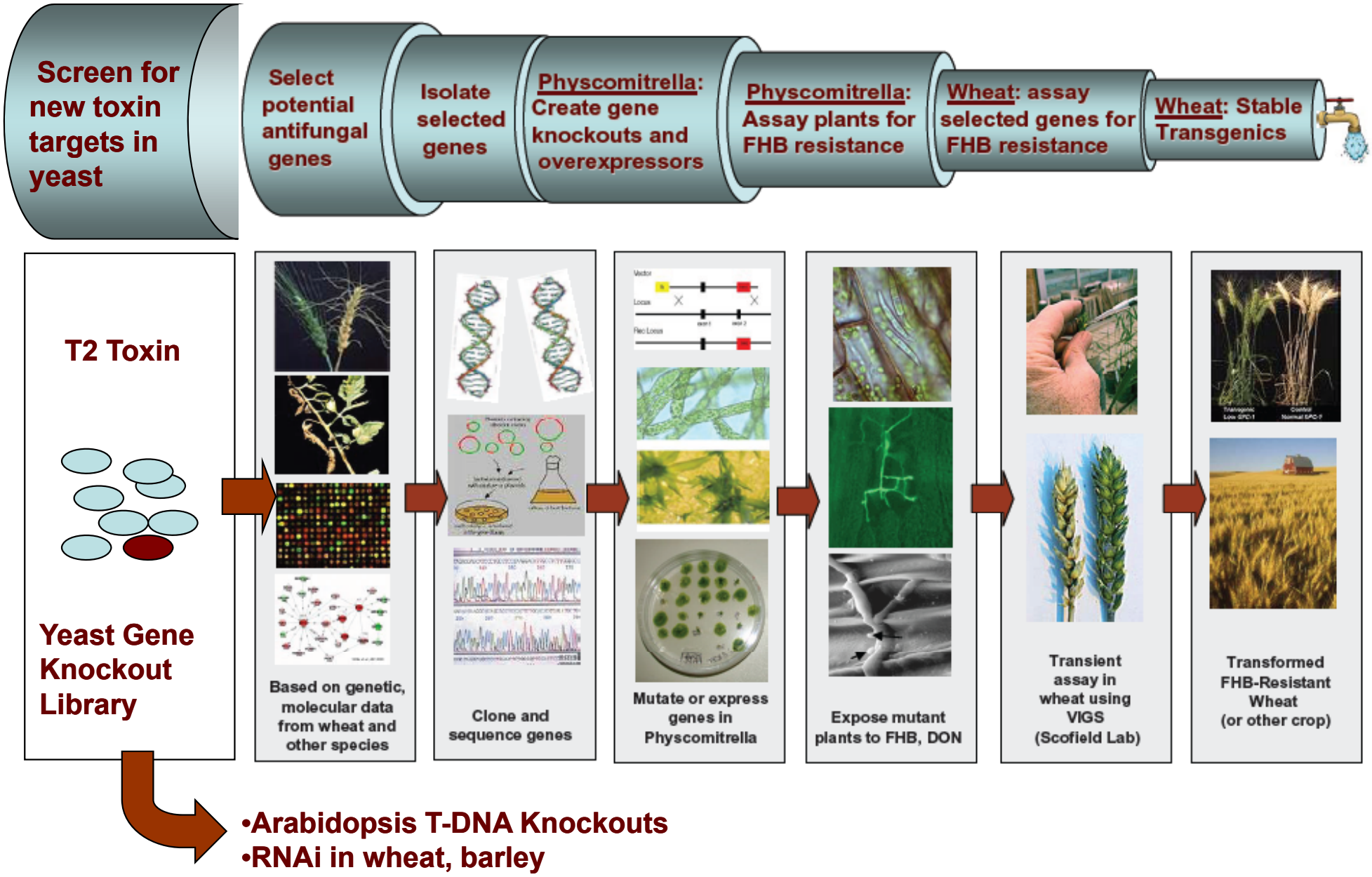




Yeast Gene Knockout Library Screen

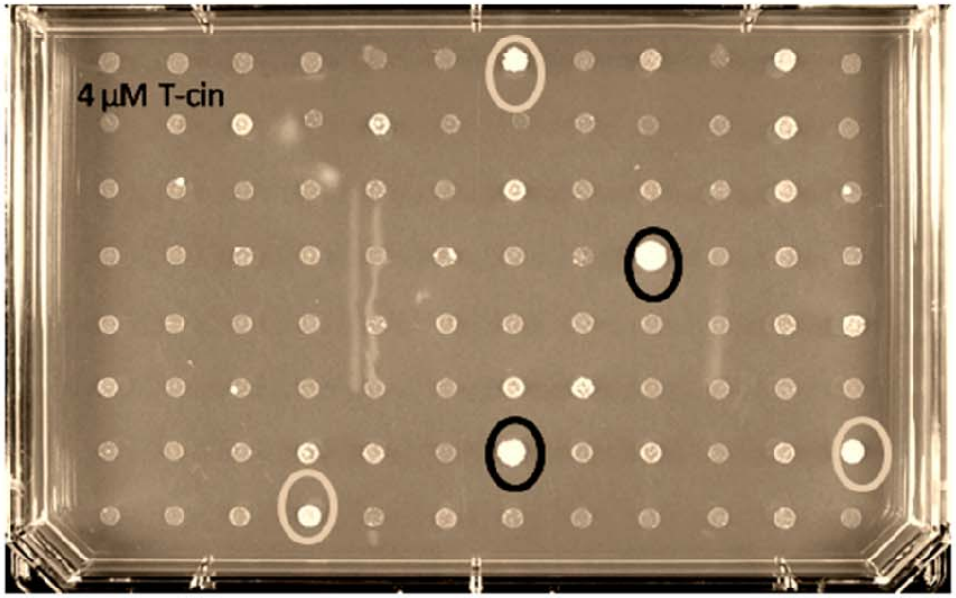
Nilgun Tumer's Lab
Rutgers University

Screens for new cellular targets of trichothecene toxins

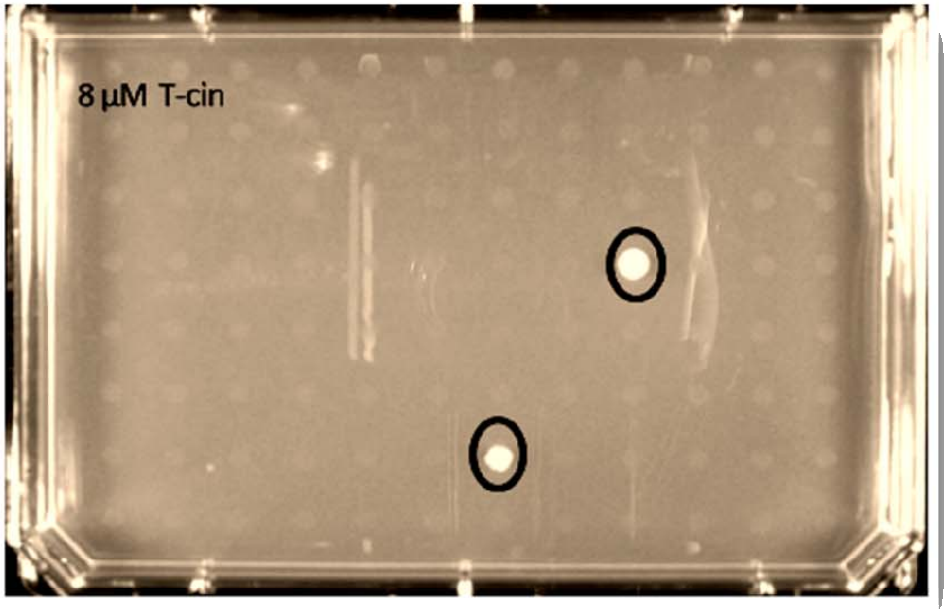


Selection of resistant strains on plates using different concentrations of T-cin

4 μM T-cin



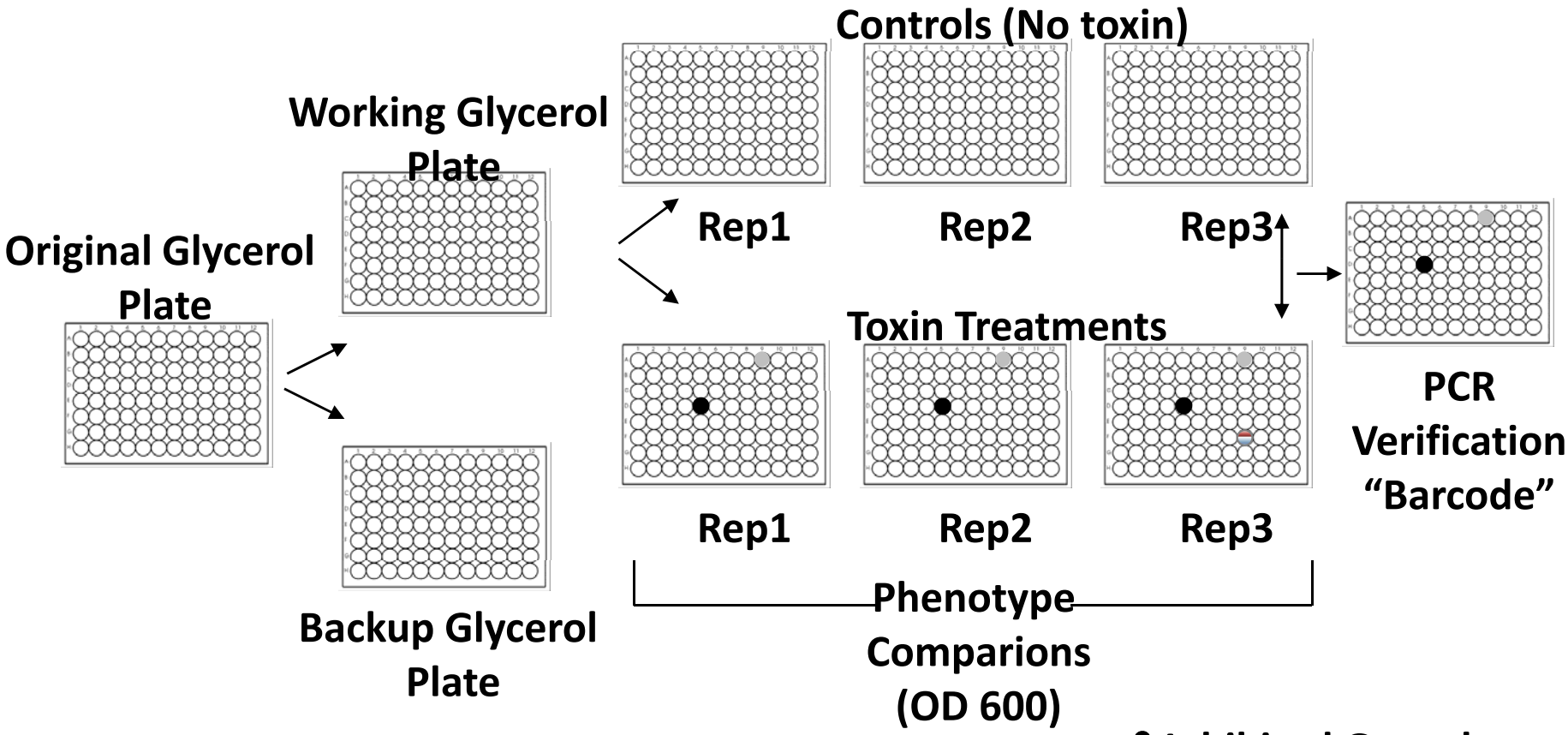
8 μM T-cin



Double printed omniplates identified mutants that were resistant only at 4μM (circled white) or at 8 μM (circled black) T-cin

McLaughlin, Tumer et al., 2009 PNAS in press.

A genome-wide high throughput screen of yeast gene deletion library to identify novel tricothecene targets

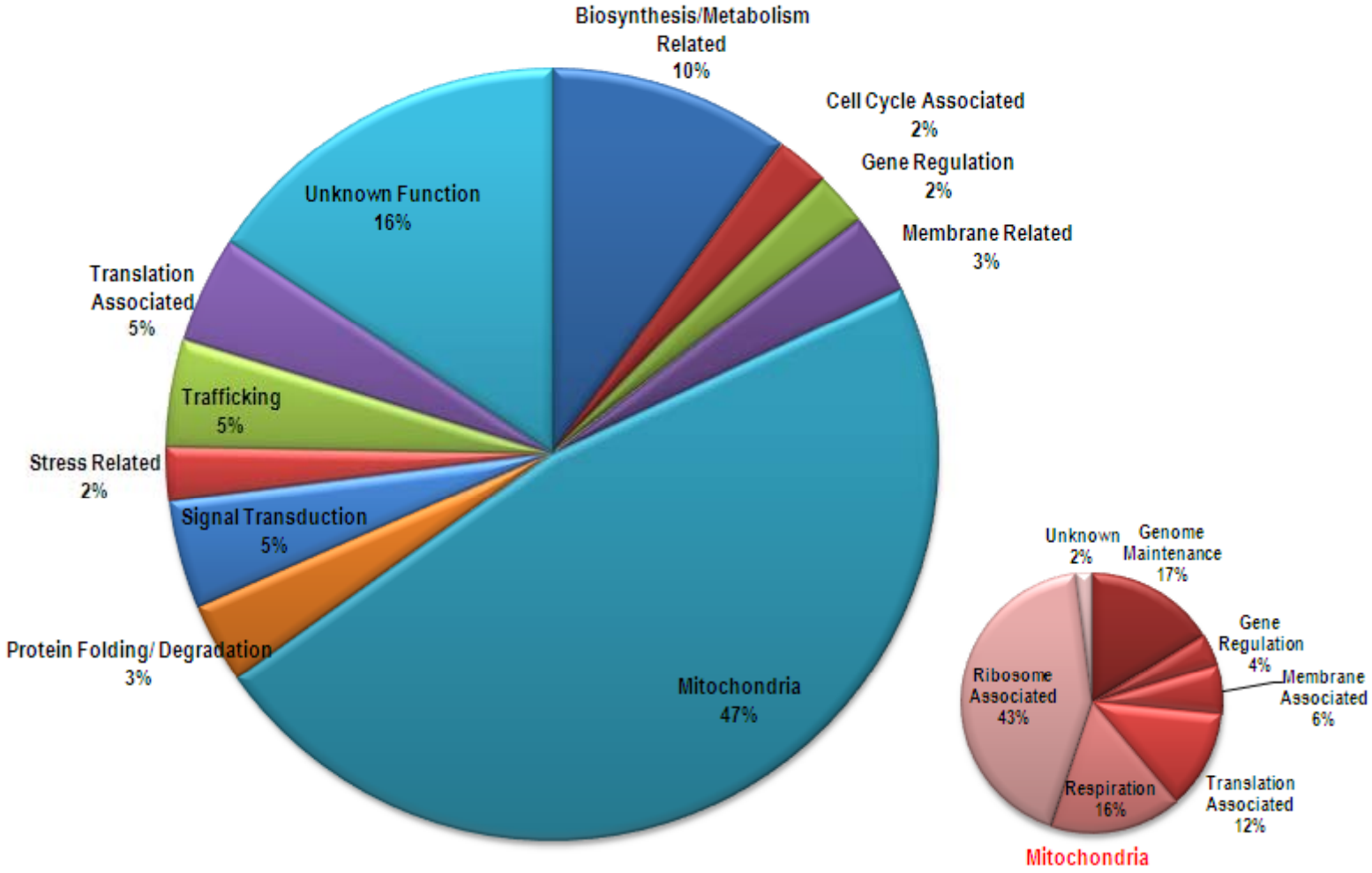


4720 strains on 72 plates
350 slow growing strains on 4 plates
Screened twice at 4, 8, 12 and 24 µM

- Inhibited Growth (Susceptibility)
- Increased Growth (Resistance)
- False Positive

McLaughlin, Tumer et al., 2009 PNAS in press.

Mitochondria play a critical role in Tricothecin toxicity



These <u>NUCLEAR</u>-encoded genes, targeted to the mitochondrion, represent novel tricothecene targets. Their contribution to the susceptibility of plants to *Fusarium* can now be tested in *Physcomitrella,* Arabidopsis and wheat.

Gene Knockouts Help Define Cellular Mechanisms

PCD

Reactive Oxygen Species

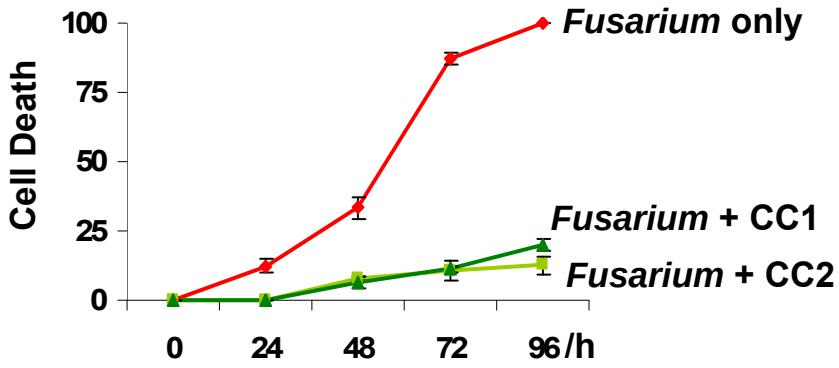
Induced Immunity

Cell Wall

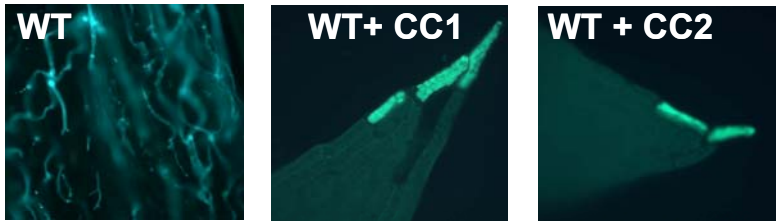
Can we simulate the effects of these mutants by chemical treatment?

Chemical suppression of PCD controls *Fusarium*

(A) Prevention of plant cell death in *Physcomitella*

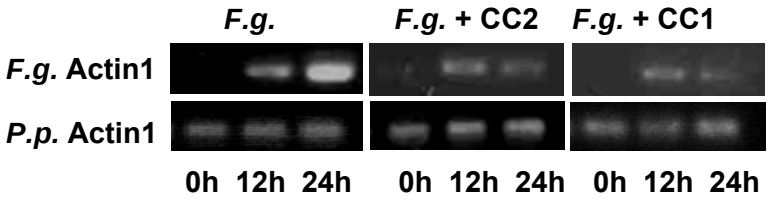


(B) Suppression of *Fusarium* growth *in planta*



Physcomitrella **plants infected with GFP-labeled *Fusarium graminearum***

(C) Reduced *Fusarium* growth *in planta*



RT-PCR

F.g. ***Fusarium graminearum***

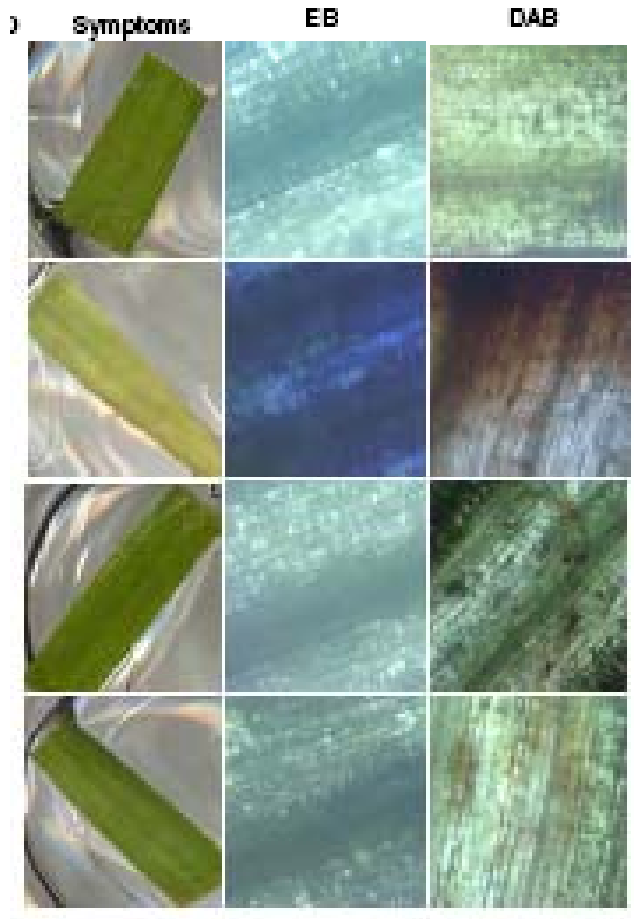
P.p. ***Physcomitrella patens***

CC1 and CC2 PCD suppressors are NOT toxic to *Fusarium*

Chemical suppression of PCD and *Fusarium*

Physcomitrella

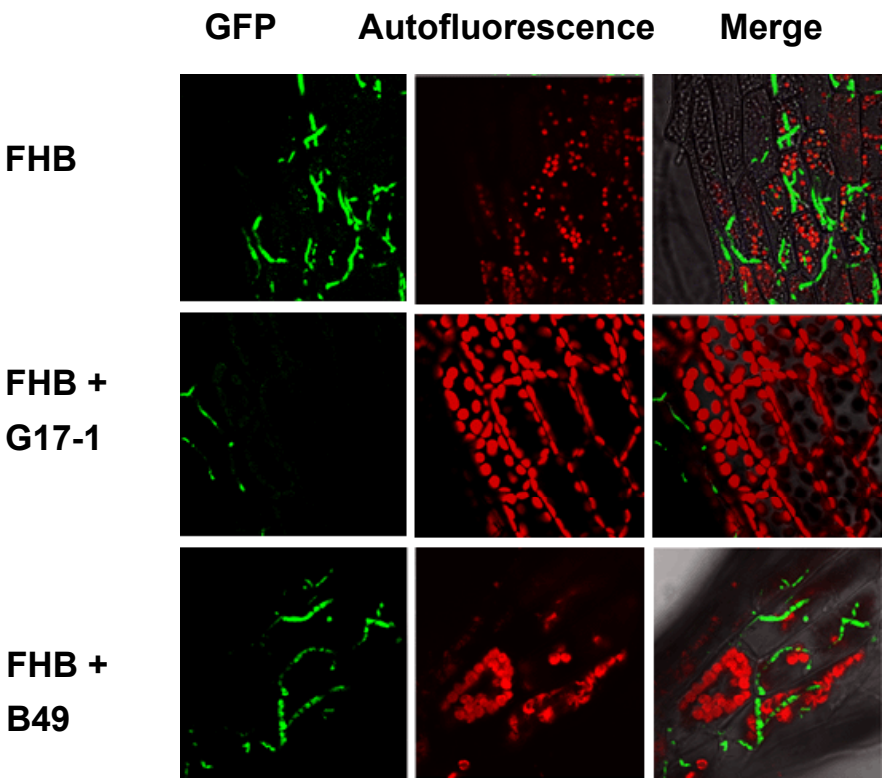
Wheat

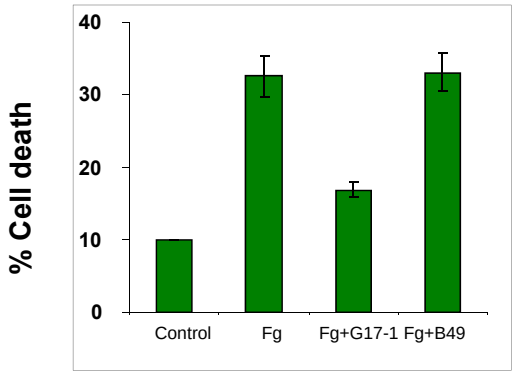


PCD suppressors protect plants from *Fusarium* infection

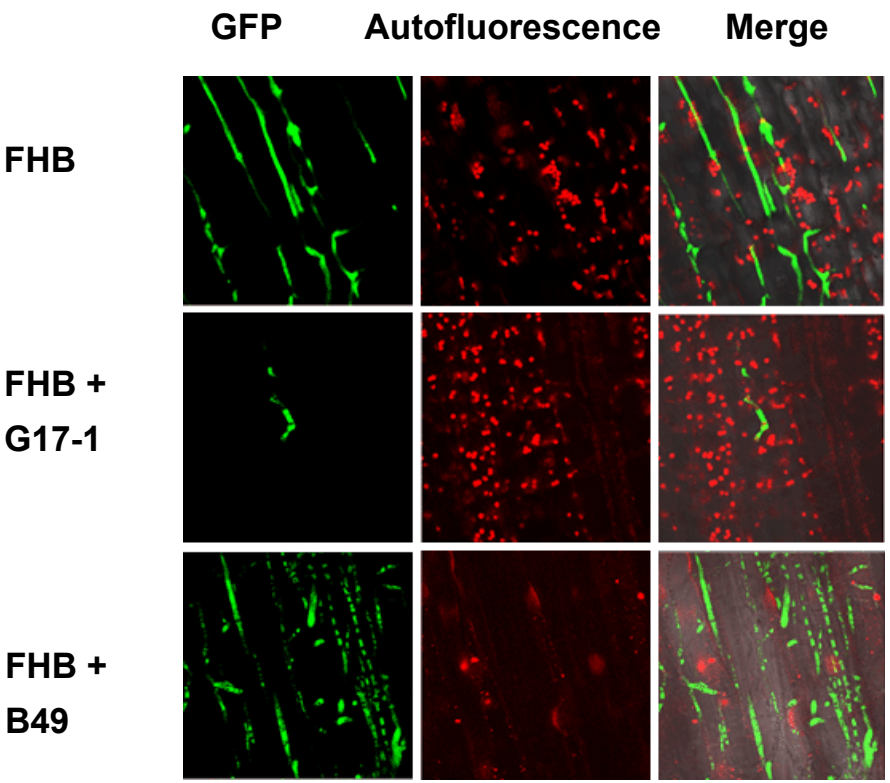
Chemically Inducted Defense Against FHB (non-PCD)

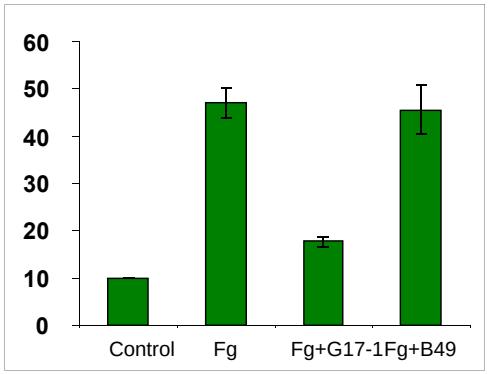
Physcomitrella patens



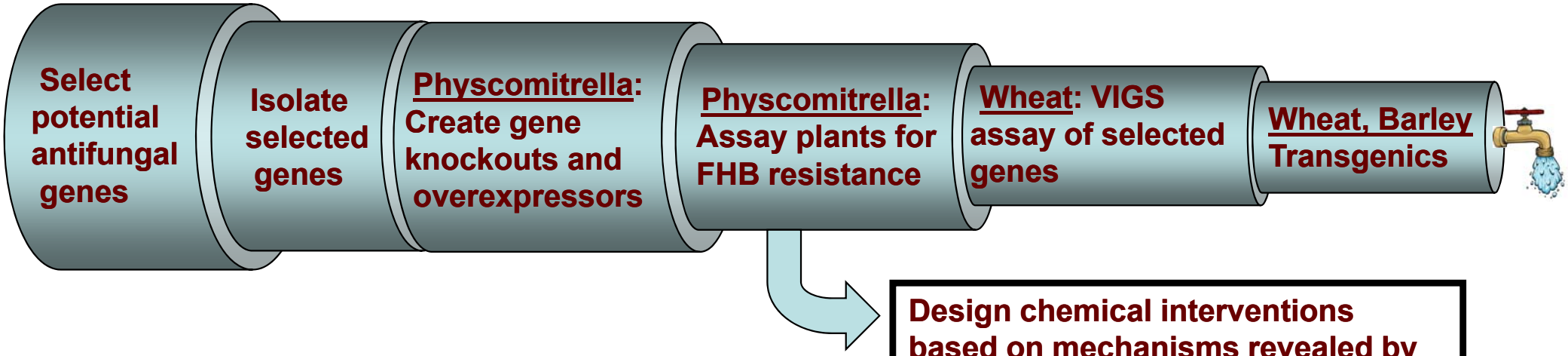


Wheat





An end-run around the gene discovery and deployment pipeline?



Design chemical interventions based on mechanisms revealed by mutant studies

Test efficacy in Physcomitrella, wheat, etc.

Chemical studies can help us understand mechanisms of FHB susceptibility and resistance

May provide novel approaches for chemical control of FHB

Conclusions

- Physcomitrella is a rapid and sensitive assay for genes that control sensitivity to DON and susceptibility to Fusarium.
- We have identified a number of genes whose mutation alters sensitivity to Fusarium.
- There are multiple ways to enhance resistance to Fusarium.
  - Suppress PCD pathway
  - Enhance innate immunity/defense responses
  - Alter the plant cell wall/cell surface
- It is important to test the efficacy of these genes in crop plants
  - Test by VIGS assay in wheat (Scofield, Purdue University)
  - Test by in transgenic crop plants (Dahleen, BioEn-USP)
- Chemical approaches to controlling FHB
  - Physcomitrella gene mutants can suggest novel approaches for the chemical control of FHB (non-toxic, non-fungicidal chemicals)
  - This may have some practical applications

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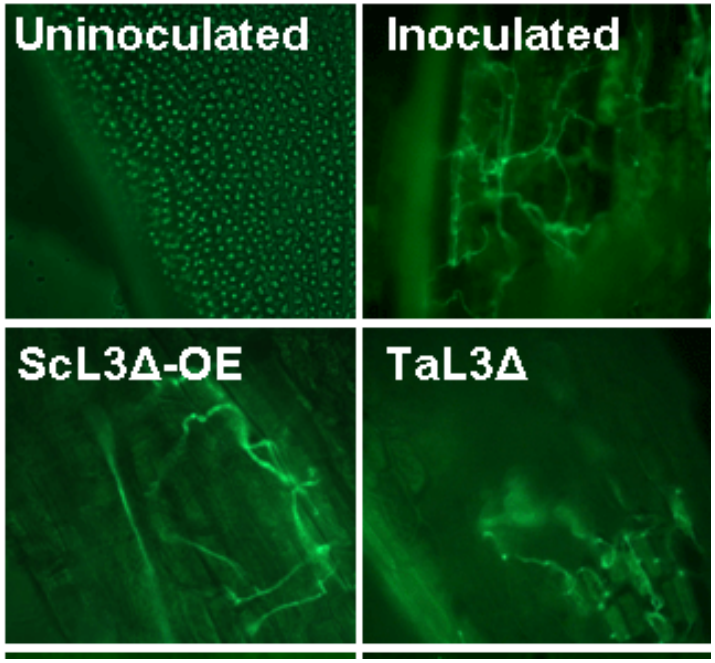
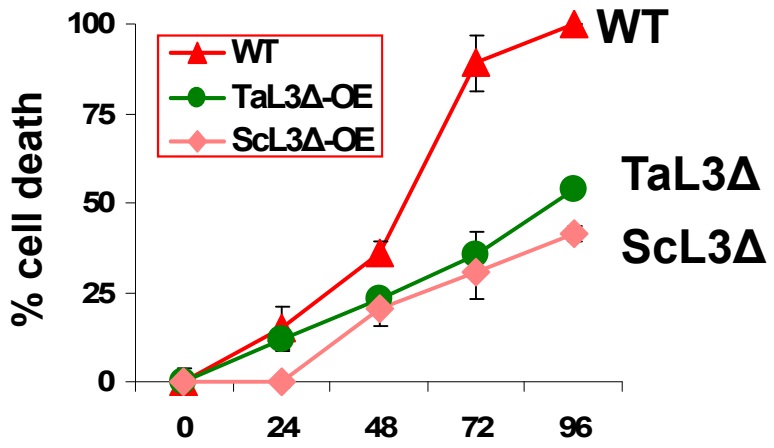
Overexpression of the Ribosomal Protein L3 confers resistance to FHB

***Fusarium*-GFP**



96h

Plant Cell Death



TaL3Δ: Wheat L3Δ gene

ScL3Δ: Yeast L3Δ gene