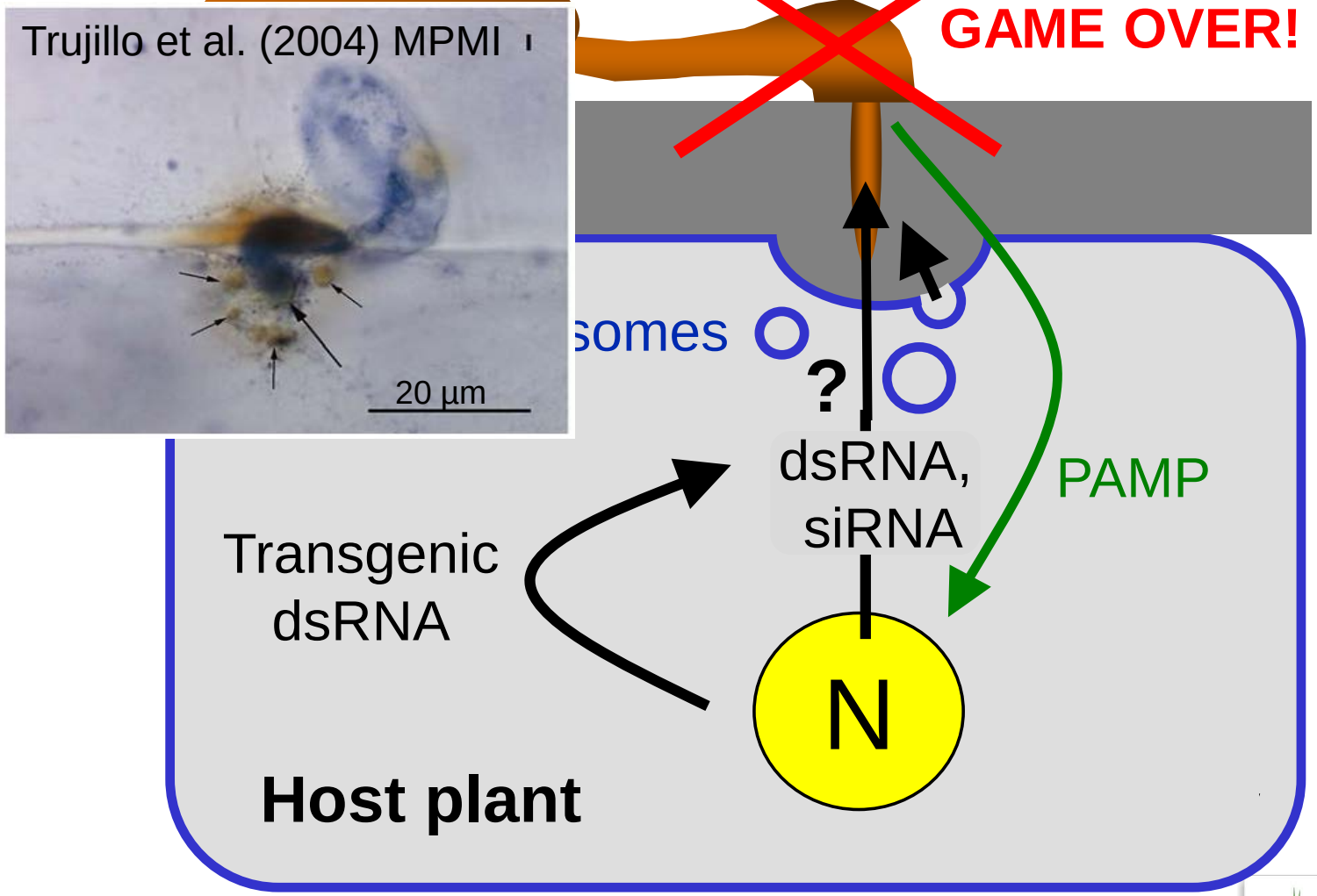
# Host-Induced Gene Silencing in the Fusarium-Wheat interaction

Wanxin Chen and Patrick Schweizer

# What is Host-Induced Gene Silencing (HIGS)?



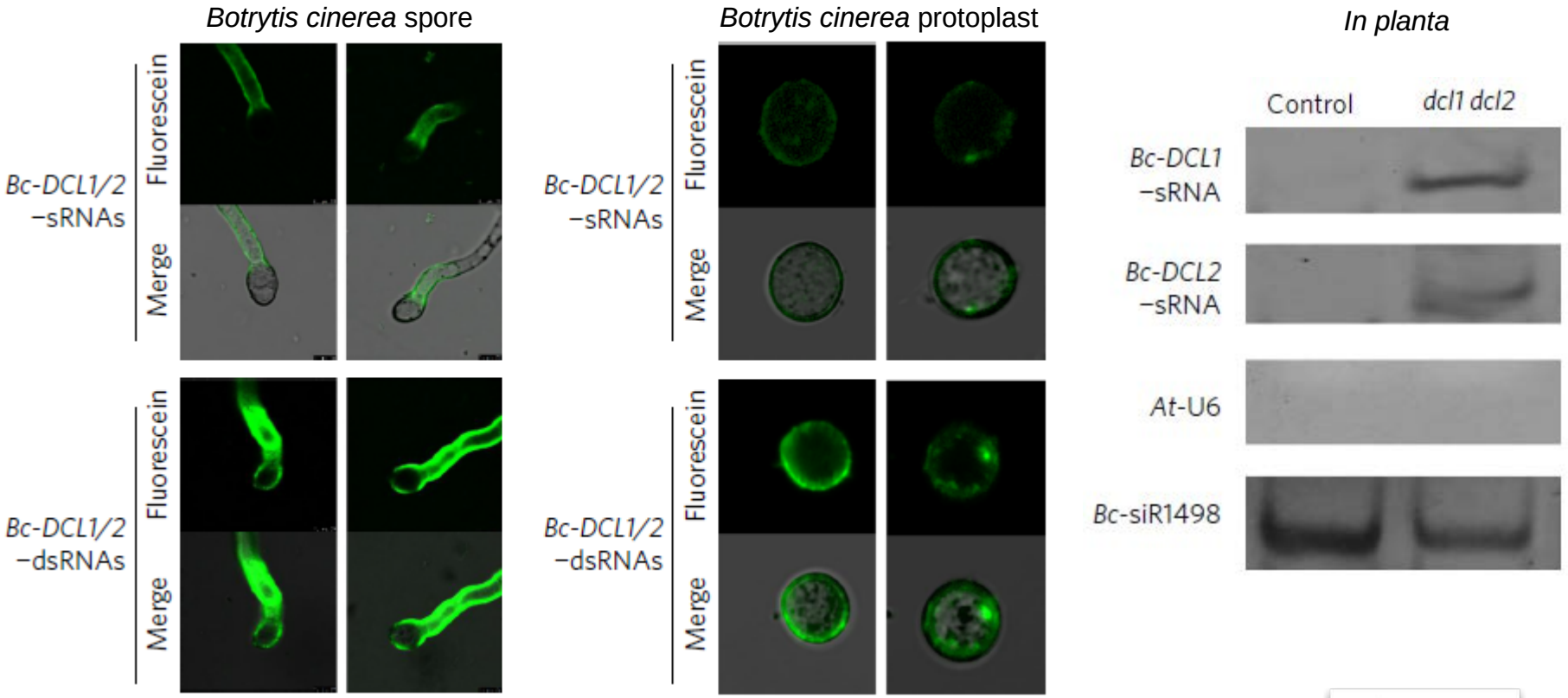
nature plants

ARTICLES

PUBLISHED: 19 SEPTEMBER 2016 | ARTICLE NUMBER: 16151 | DOI: 10.1038/NPLANTS.2016.151

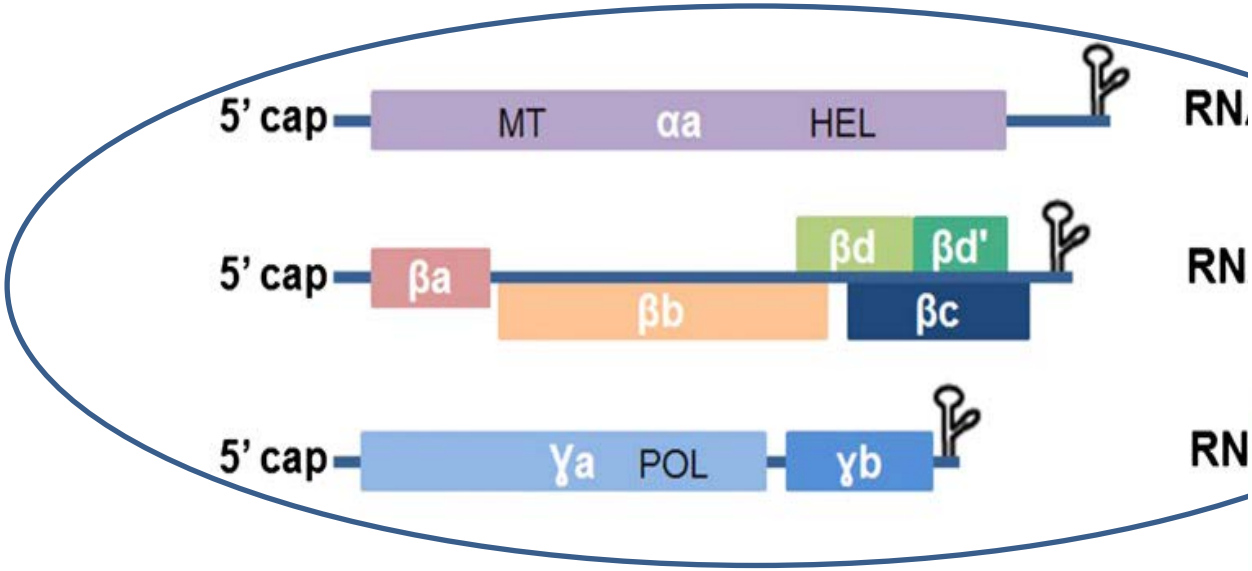
# Bidirectional cross-kingdom RNAi and fungal uptake of external RNAs confer plant protection

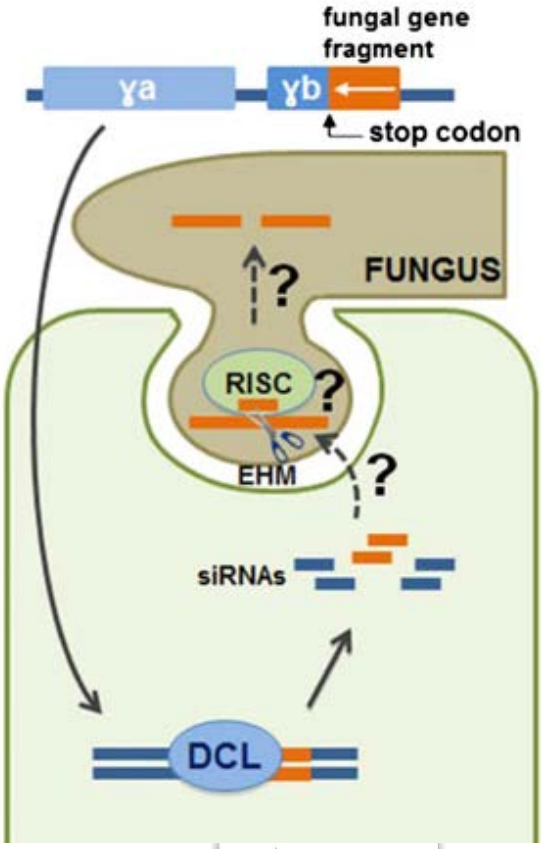
Ming Wang[1], Arne Weiberg[1†], Feng-Mao Lin[2], Bart P. H. J. Thomma[3], Hsien-Da Huang[2] and Hailing Jin[1*]



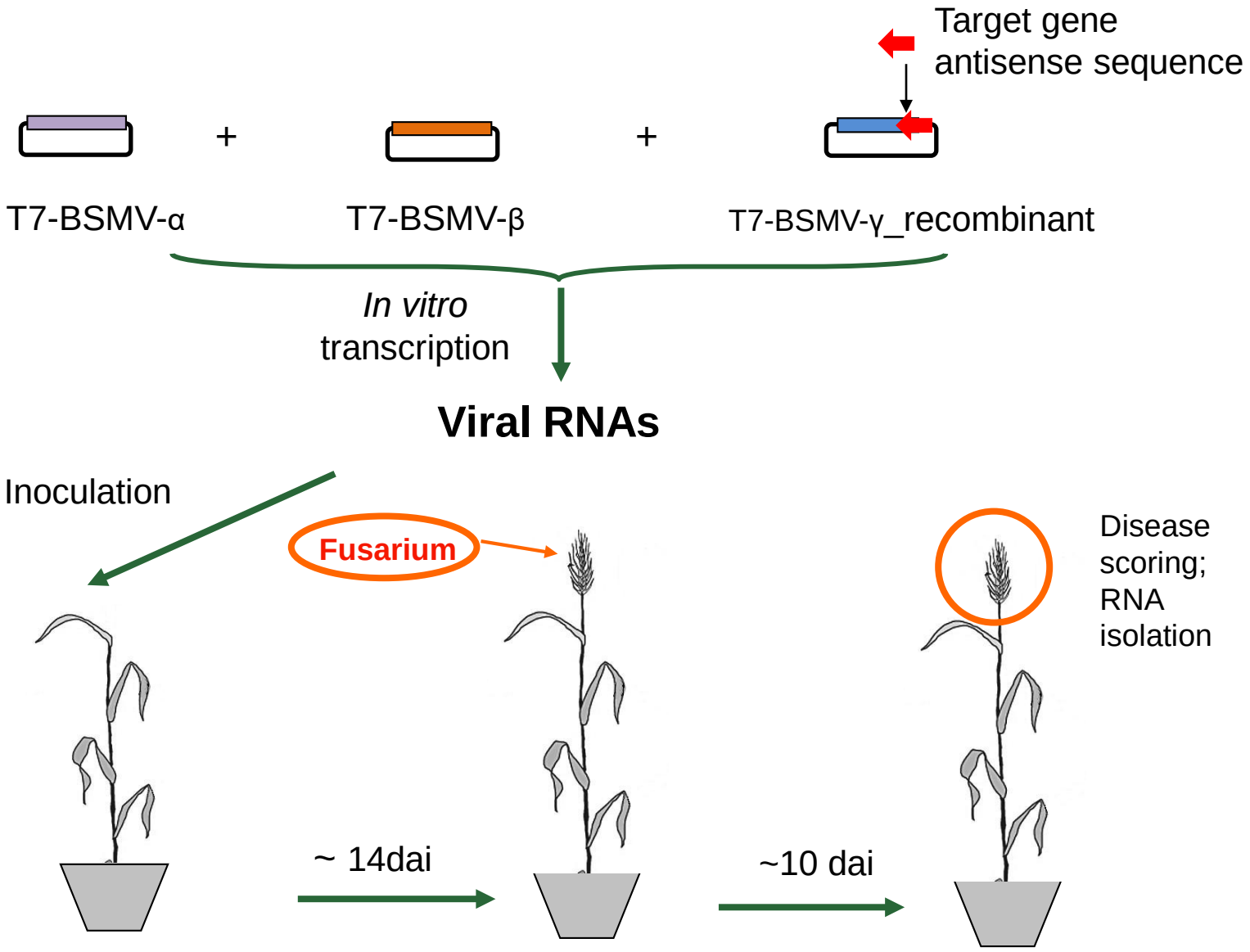
# Virus Induced Gene Silencing (VIGS) - a tool for functional genomic studies

## *Barley Stripe Mosaic Virus* (BSMV)

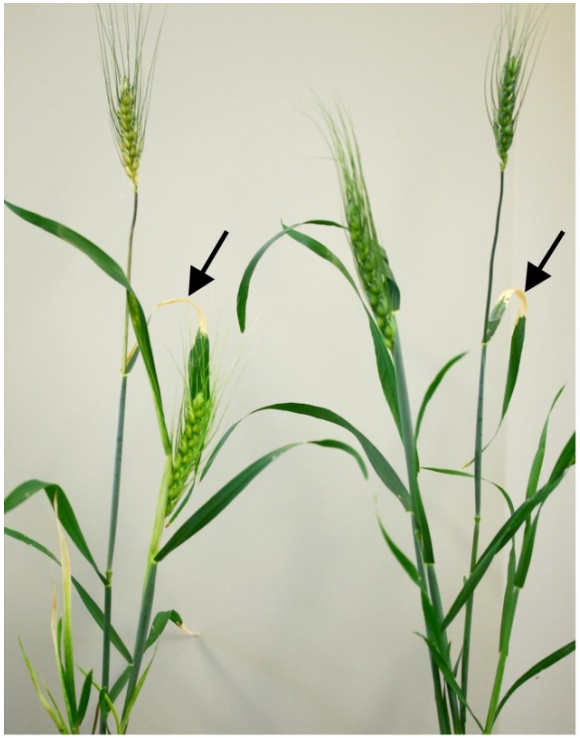



Lee WS, *et al*. *Plant Physiol.* , Oct . 2012, Vol. 160


Target gene
antisense sequence
+
+
T7-BSMV-α
T7-BSMV-β
T7-BSMV-γ_recombinant
In vitro
transcription
Viral RNAs
Inoculation
Fusarium
Disease
scoring;
RNA
isolation
~ 14dai
~10 dai
Adult wheat plant
Point inoculation of Fusarium

# Using fast-maturing dwarf cultivar Apogee as VIGS model plant



BSMV:00 Mock

6-week-old plants

Mock BSMV:00 BSMV:PDS

20 d.a.i. with BSMV

Mock BSMV:00

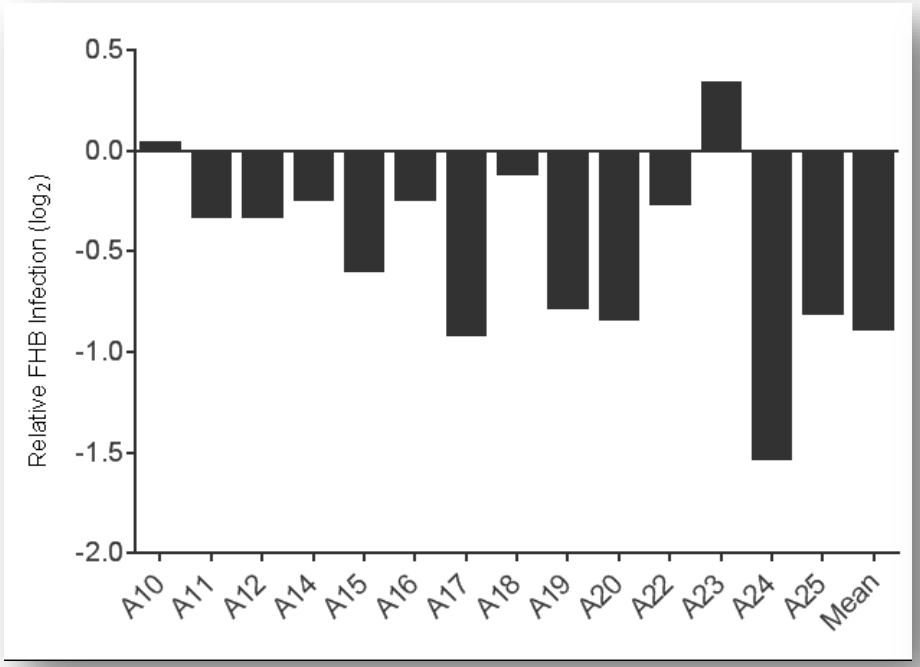
11 d.a.i. with *F.culmorum*

PDS: wheat *Phytoene desaturase* gene

Chen et al. (2016), J. Exp. Botany.

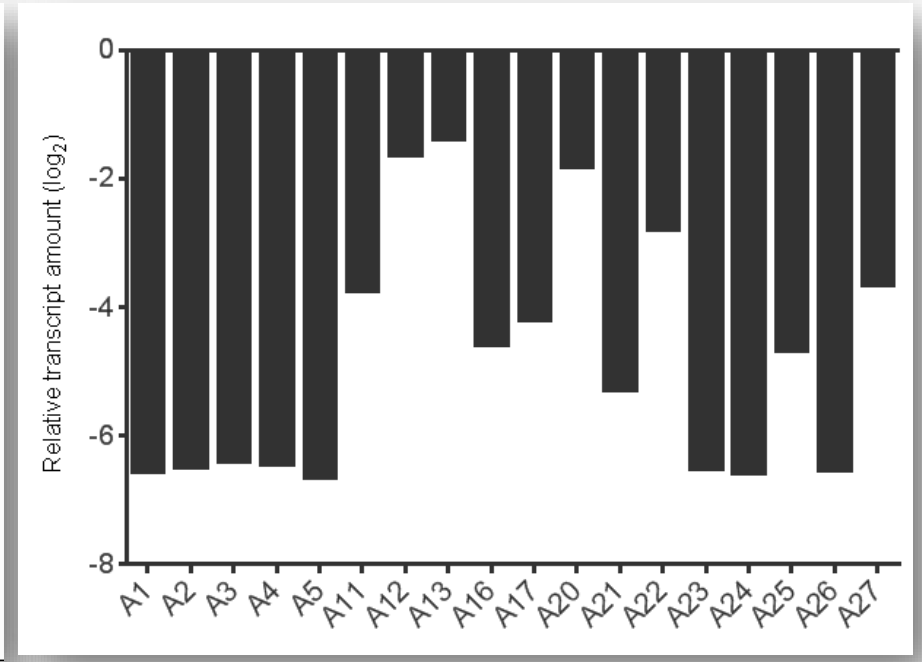
# Silencing of wheat *ARF2* gene by BSMV:*Arf2* protected plants from FHB infection

Relative FHB infection ($log_2$)




EXP 2 | EXP 3 | EXP 4

Relative transcript amount($log_2$)




EXP 1 | EXP 2 | EXP 3 | EXP 4

P = 0.0026 (one sample t test)

P < 0.0001 (Wilcoxon signed rank test)

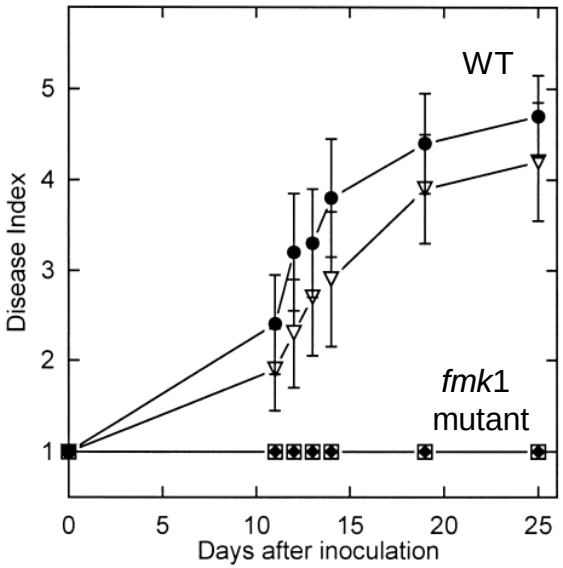
# Fungal targets for the wheat VIGS-FHB interaction

1. *Fgl*1 (a *Fusarium* secreted lipase)
2. *Fmk*1 (a *Fusarium* pathogenicity MAP Kinase)
3. *Gls*1 (*Fusarium* Glucan synthase I)
4. *Chs*V ( *Fusarium* Chitin synthase V )

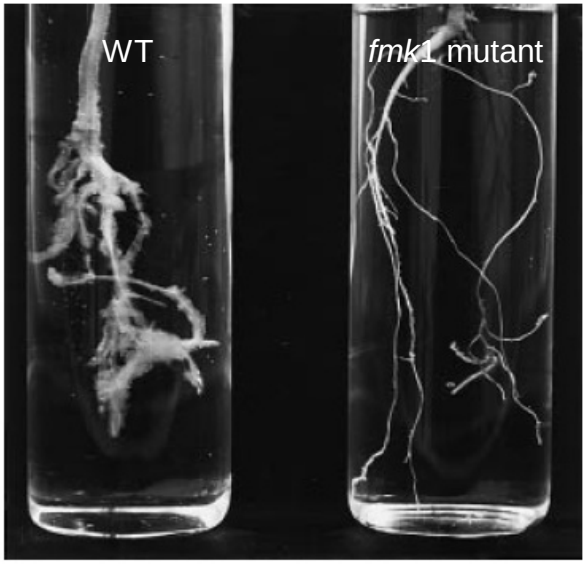
# *Fusarium graminearum Fgl*1 knock out mutant failed to spread within wheat spikes

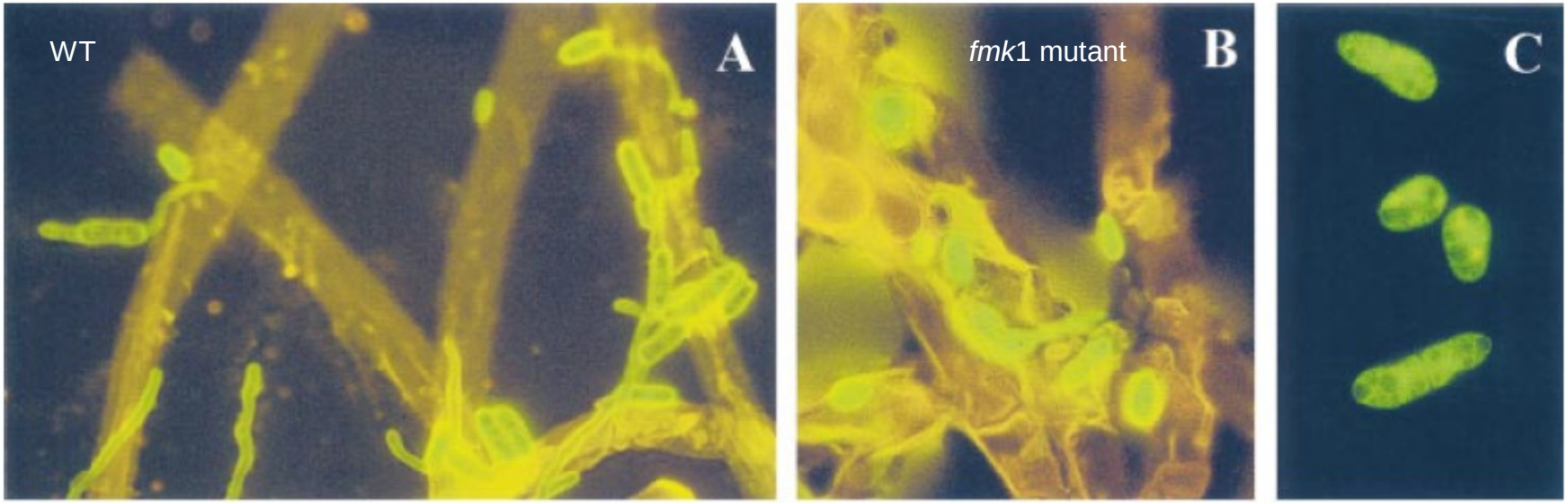

Christian A. Voigt, et al. The Plant Journal (2005) 42

# *Fmk*1 is essential for *Fusarium* pathogenicity on plants







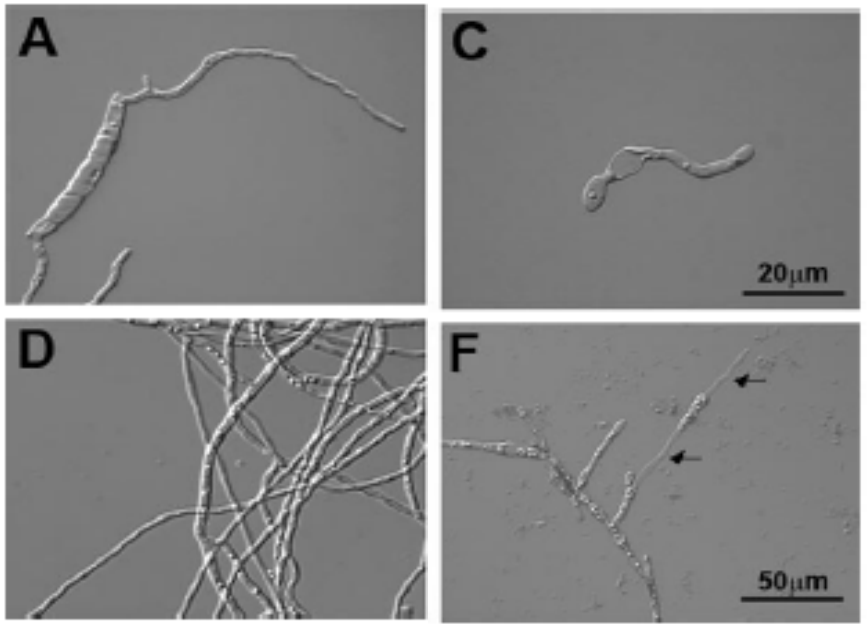




Antonio Di Pietro, et al. Molecular Microbiology (2001) 39(5), 1140±1152

# *β-1,3-Glucan Synthase* I is essential for fungal cell-wall biosynthesis

*Fusarium solani*

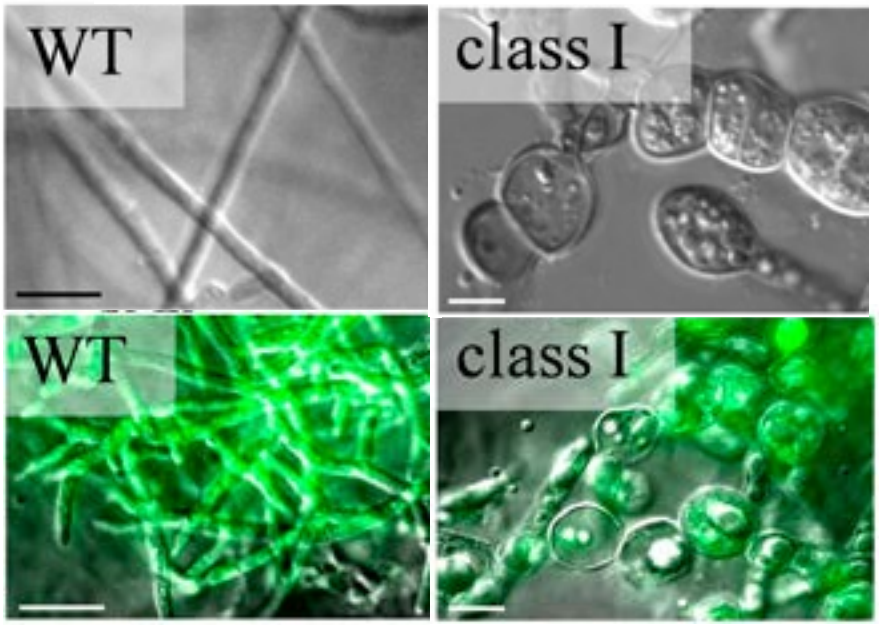
WT | *Fks*1 RNAi mutant




Young-sil Ha, et al. EUKARYOTIC CELL, July 2006, p. 1036–1042

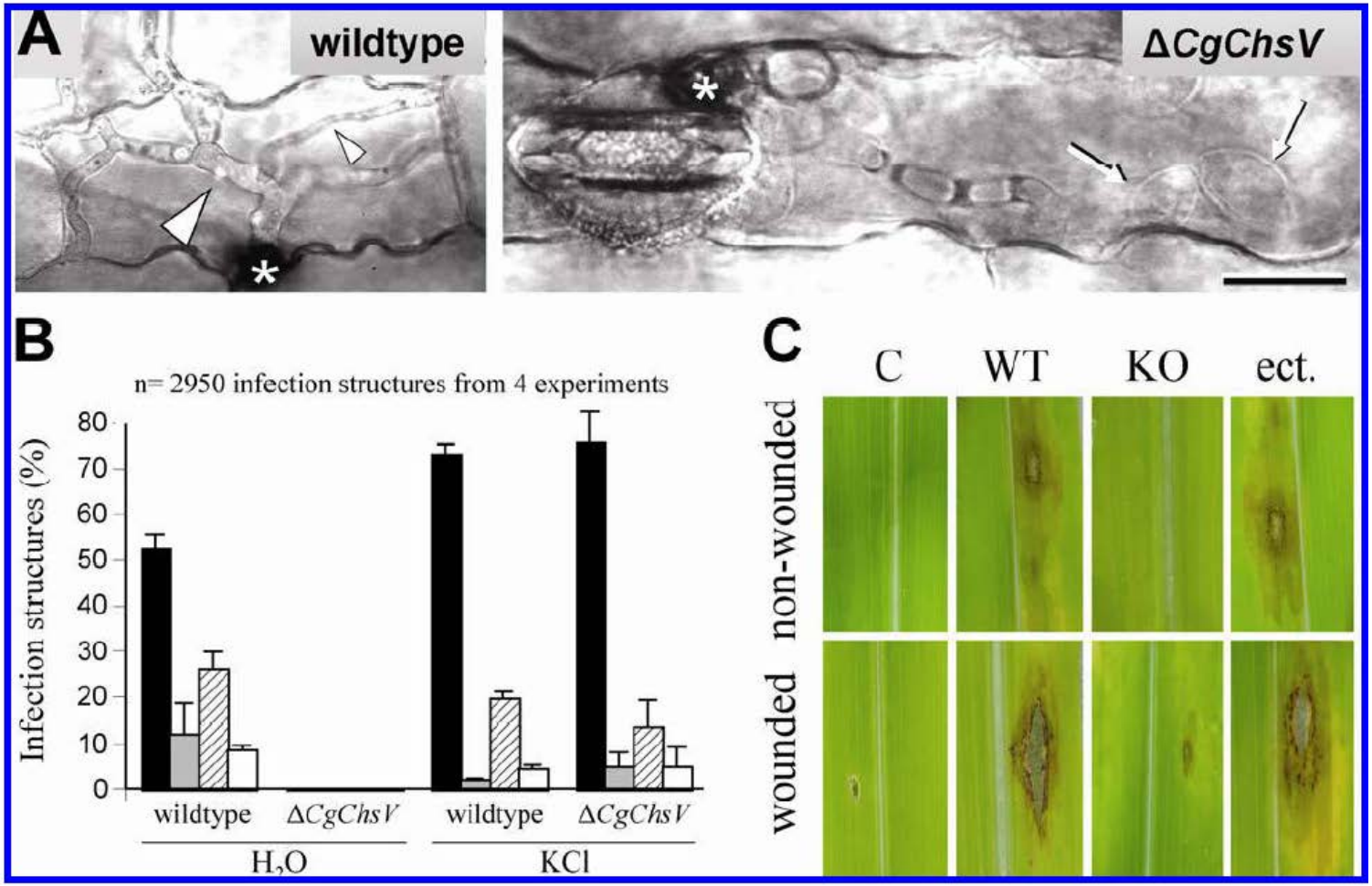
*Colletotrichum graminicola*

WT | *Gls*1 RNAi mutant



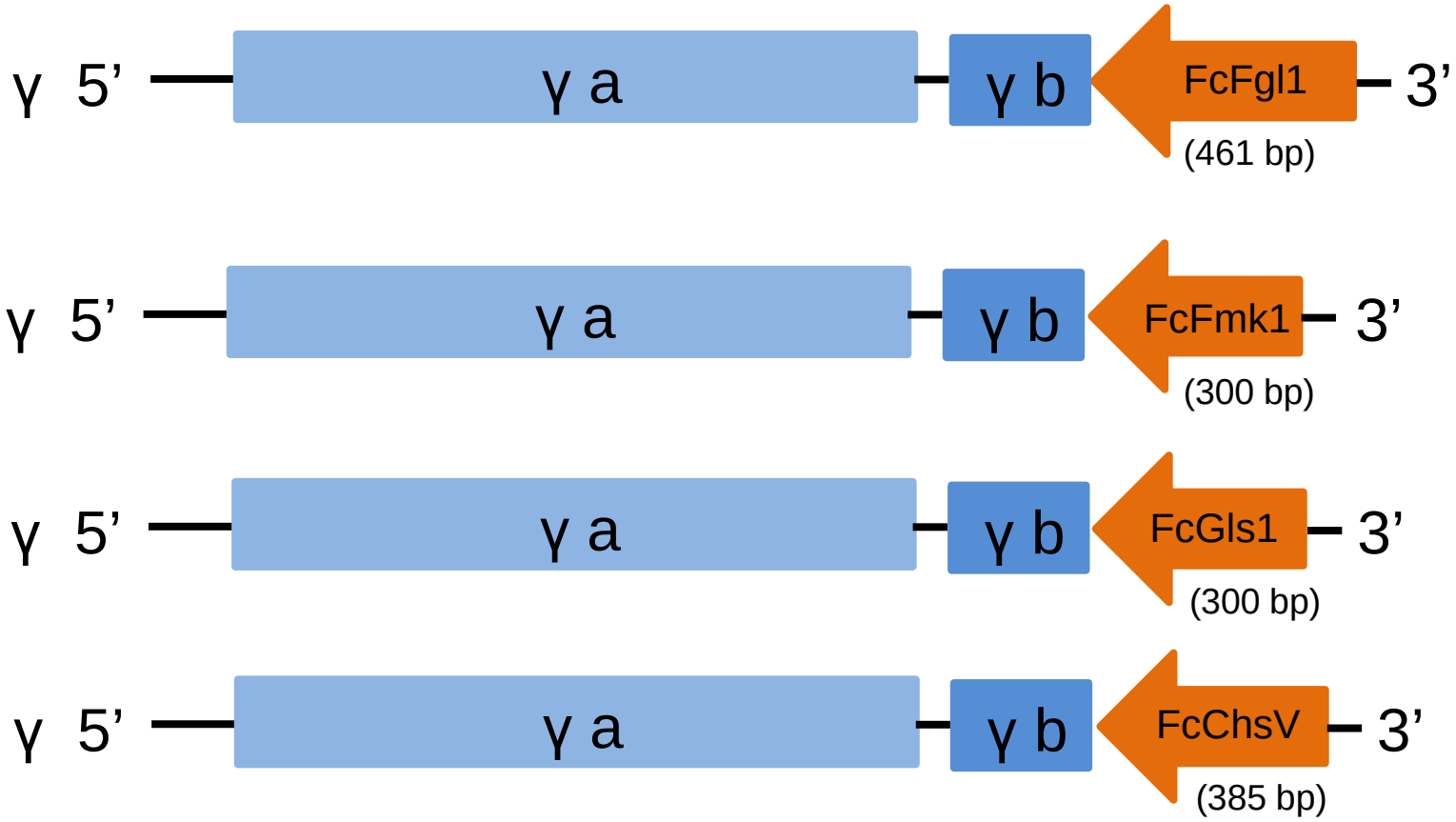

Ely Oliveira-Garciaa and Holger B. Deising. The Plant Cell, Jun 2013, 25(6):2356-78

# *Chitin synthase* V is essential for hyphal growth, appressorium differentiation and pathogenicity of *Collectotrichum graminicola*

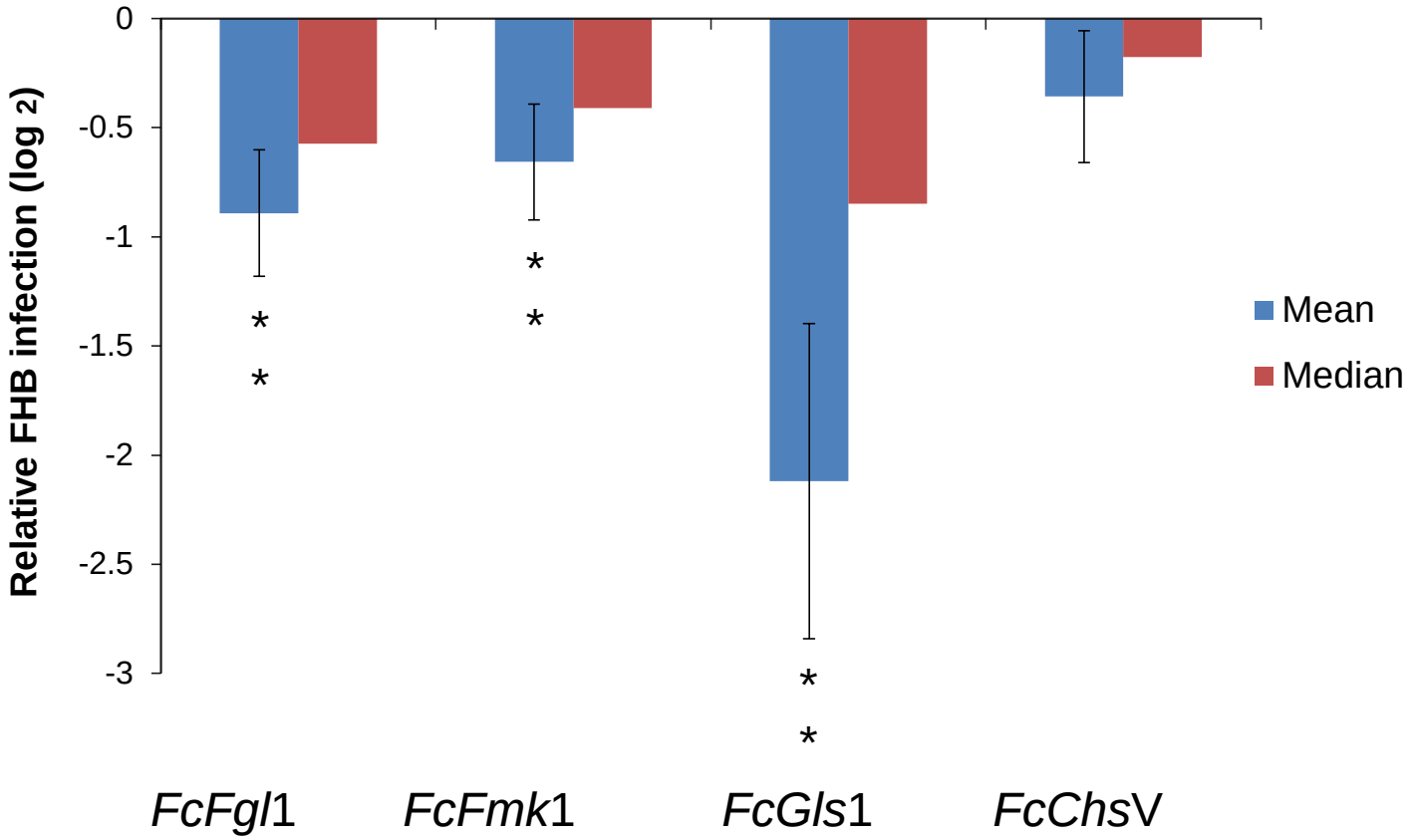



Stefan Werner et al. MPMI Vol. 20, No. 12, 2007

# HIGS target sequences were cloned into T7-BSMV: γ vectors



# Relative FHB infection (log2) of BSMV:HIGS –FHB plants




Wilcoxon signed rank test; mean from 3-5 independent experiments; *, p<0.05; **, p<0.005; ***, p<0.0005

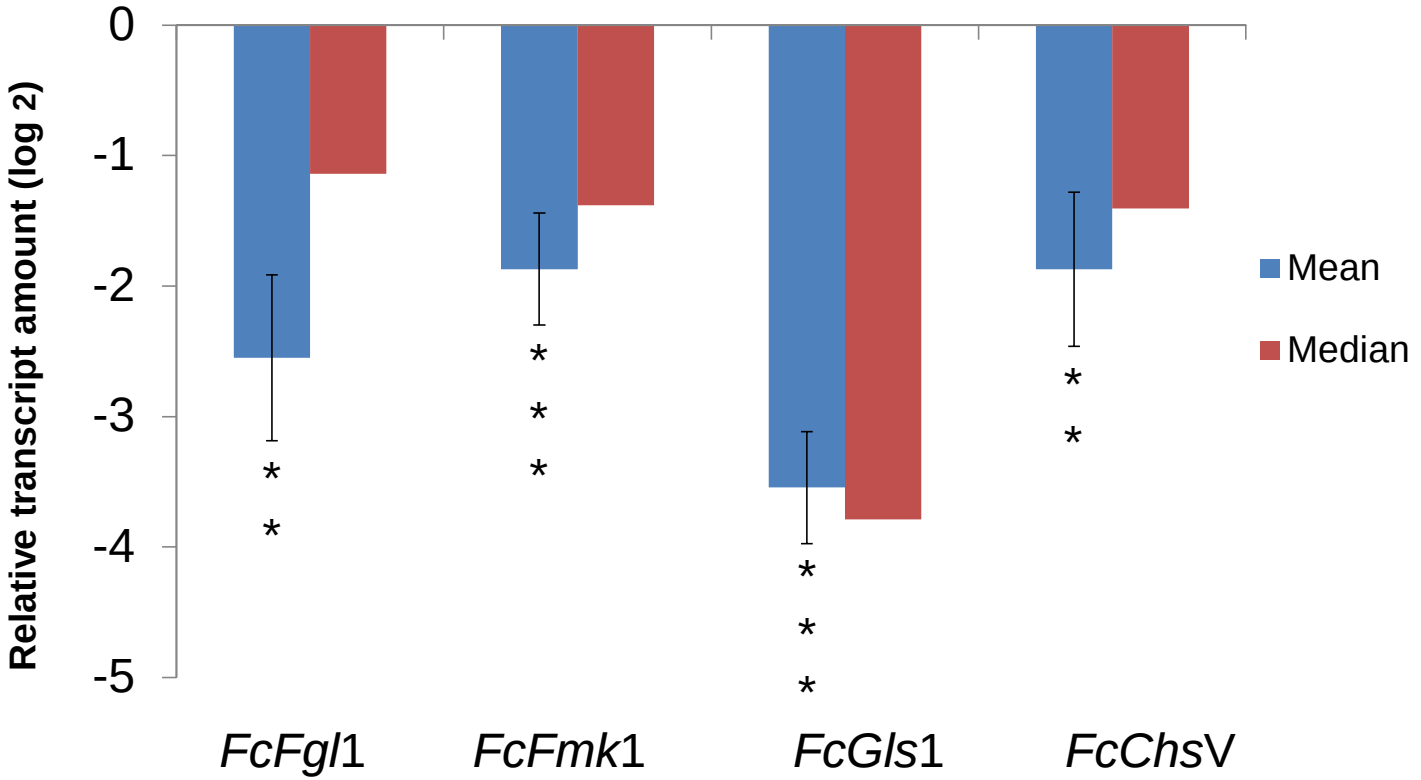
# Point inoculation of VIGS plant with *Fusarium culmorum*

**BSMV:00 + FHB**

**BSMV:*Fgl*1+FHB**

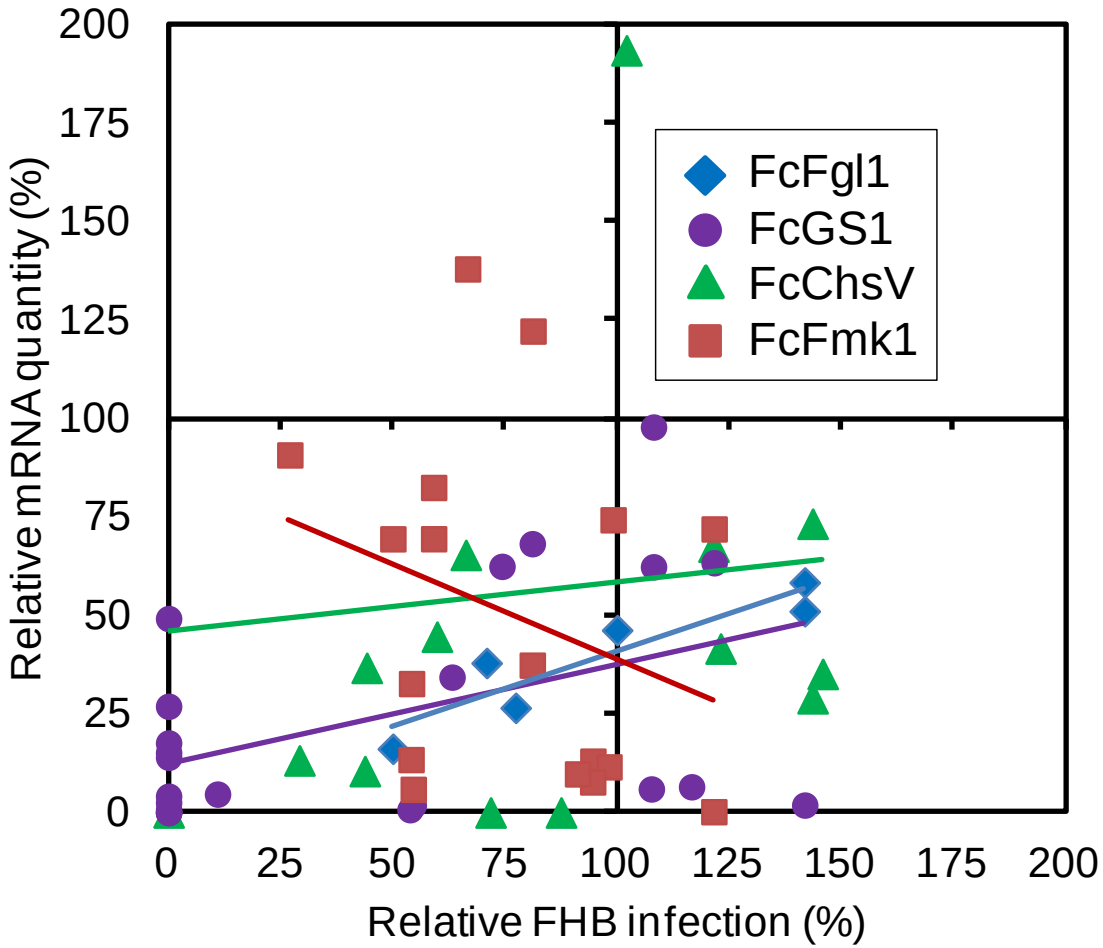
Disease severity (DS)= (Infected spikelets-2)/(Total spikelets-2)

# Relative transcript amount ($log_2$) of target genes in BSMV:HIGS – FHB plants




One sample t test; mean from 2-4 independent experiments; *, p<0.05; **, p<0.005; ***, p<0.0005

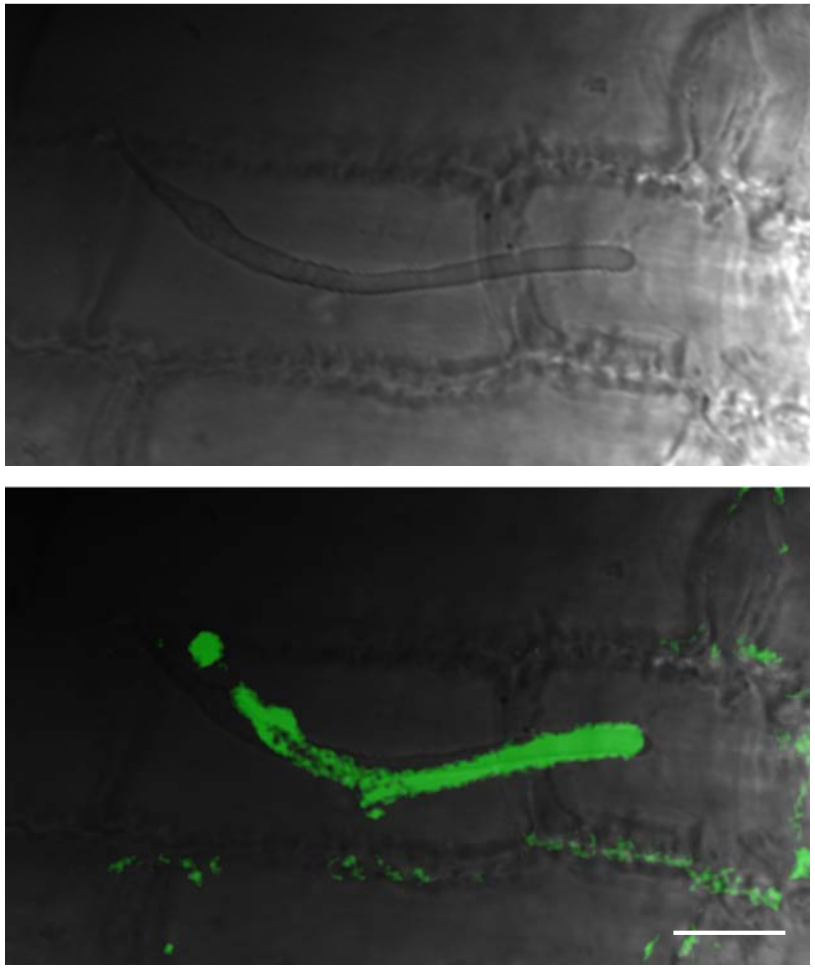
# Linear regression of relative HIGS target-gene mRNA levels and relative FHB infection




Correlation for plants of BSMV:*Fgl*1 and BSMV:*Gls*1 are significant.
(P< 0.05; R= 0.87 and 0.38 respectively)

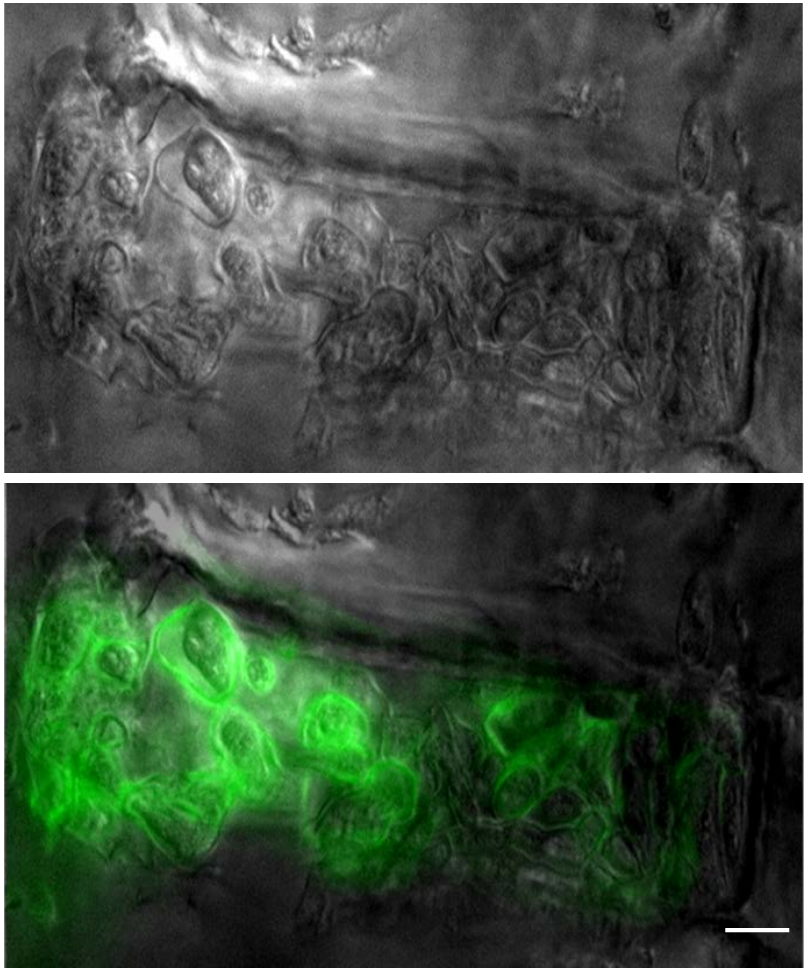
# Microscopy of *F. culmorum* hyphae growth in BSMV pre-infected plants 3 dai with WGA staining

BSMV:00-FHB



Bar = 10 µm

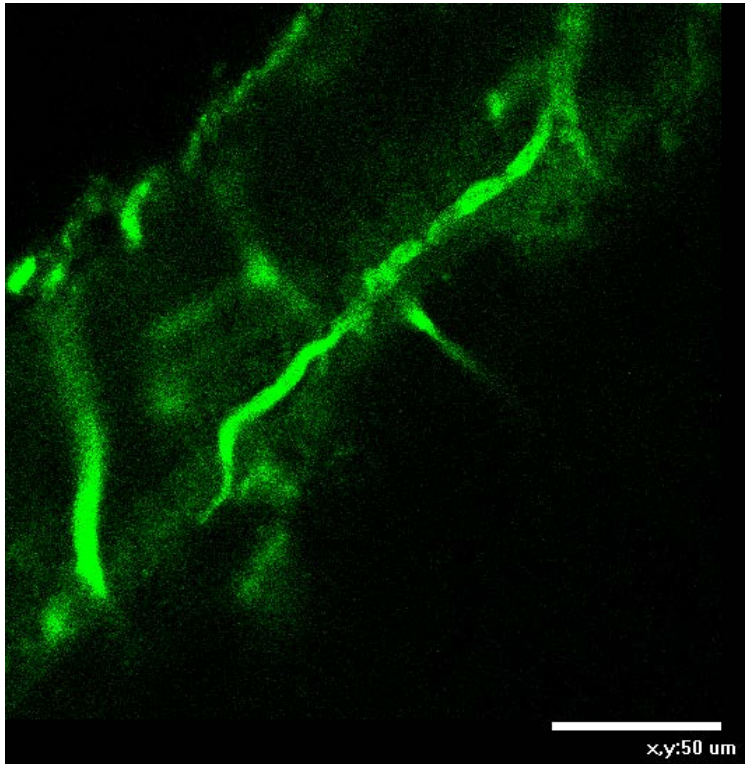
BSMV:*Gls*1-FHB



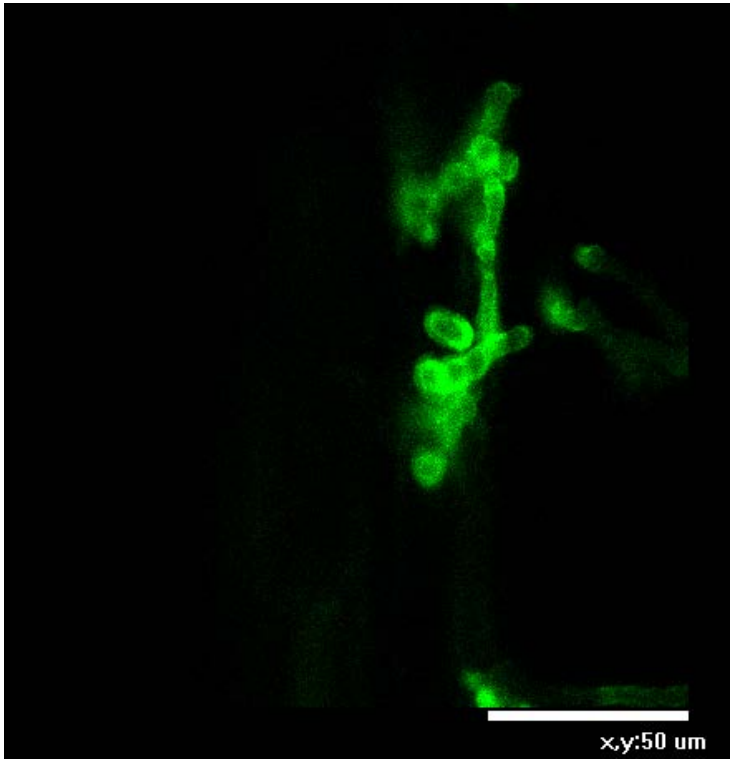
Bar = 10 µm

# Microscopy of *F. graminearum* hyphae growth in BSMV pre-infected plants 3 dai with WGA staining

BSMV:00-FHB

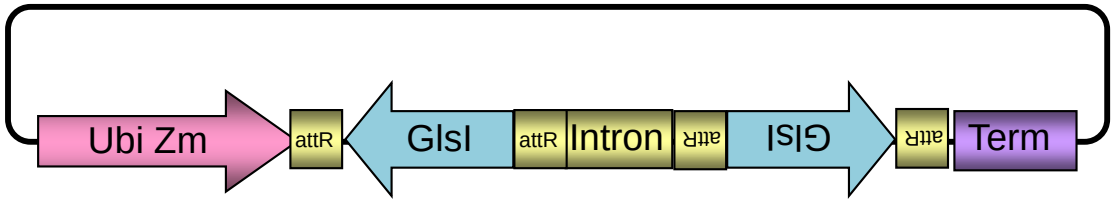



BSMV:*Gls*1-FHB

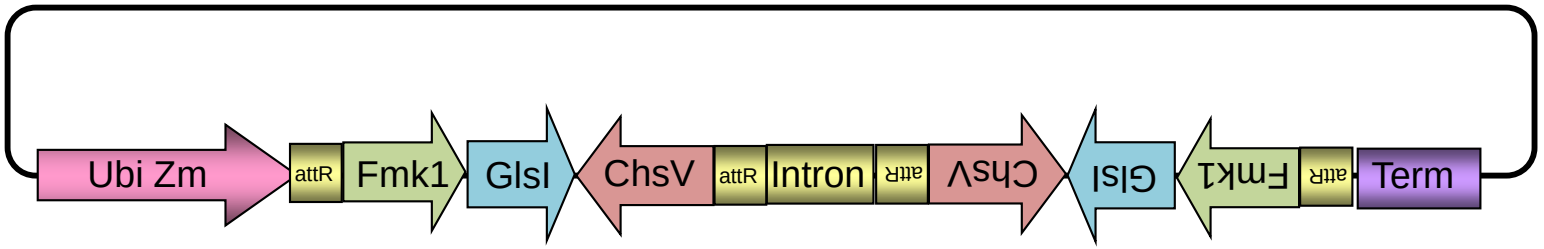


# Two HIGS RNAi constructs for wheat transformation

Single construct: pIPKb027-GlsI

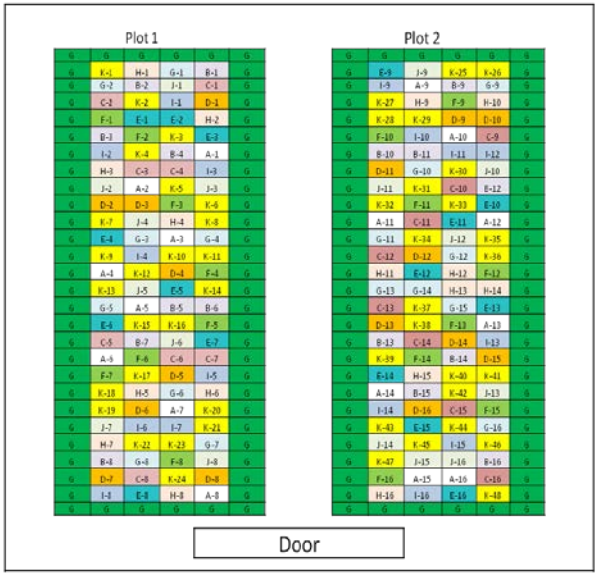



Triple construct: pIPKb027-GlsI-ChsV-Fmk1



# Greenhouse experiment with field-like conditions.

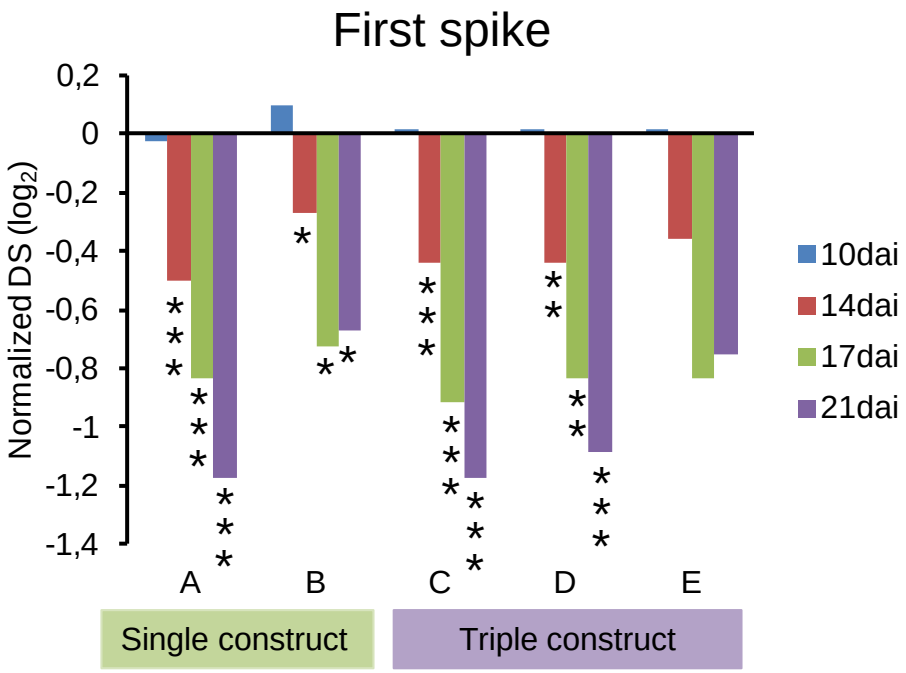
**336** HIGS transgenic
**144** azygous control
**144** wildtype Bobwhite

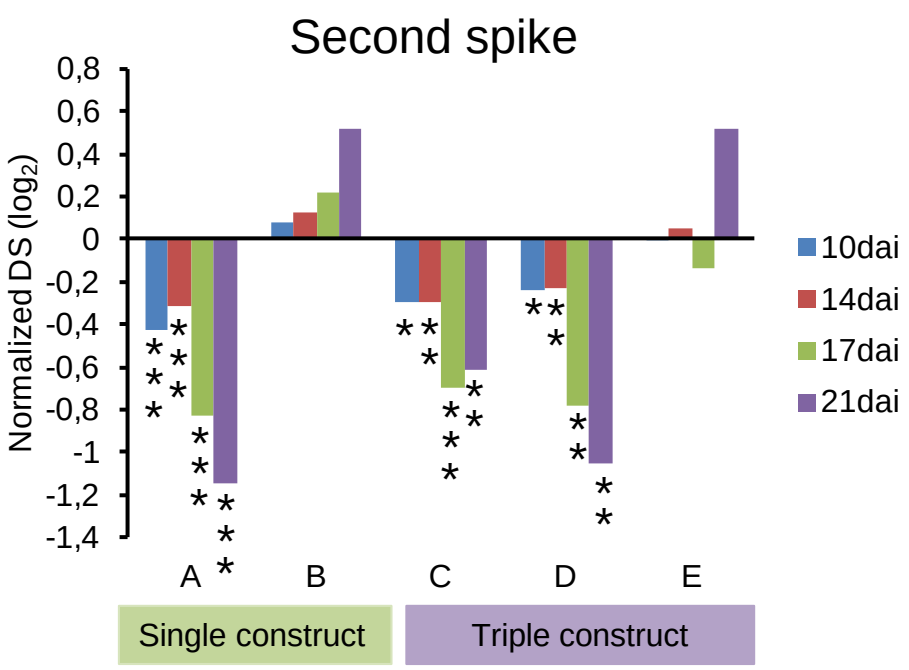



Point inoculated and covered by transparent bag for 48 h

Dates of inoculation were marked by labels in different colors

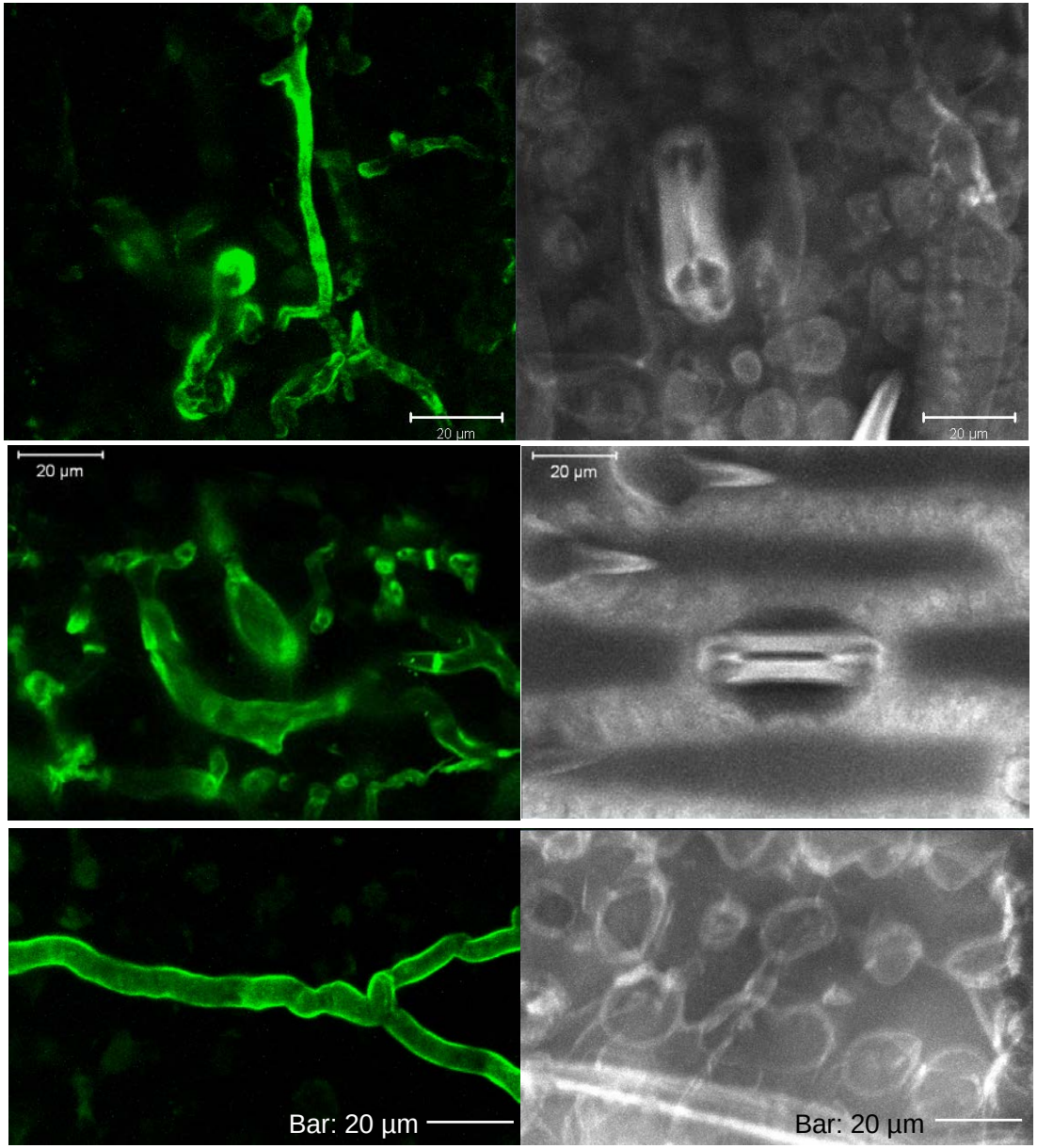
# Relative FHB infection (log2) of HIGS transgenic wheat (T2/T3)



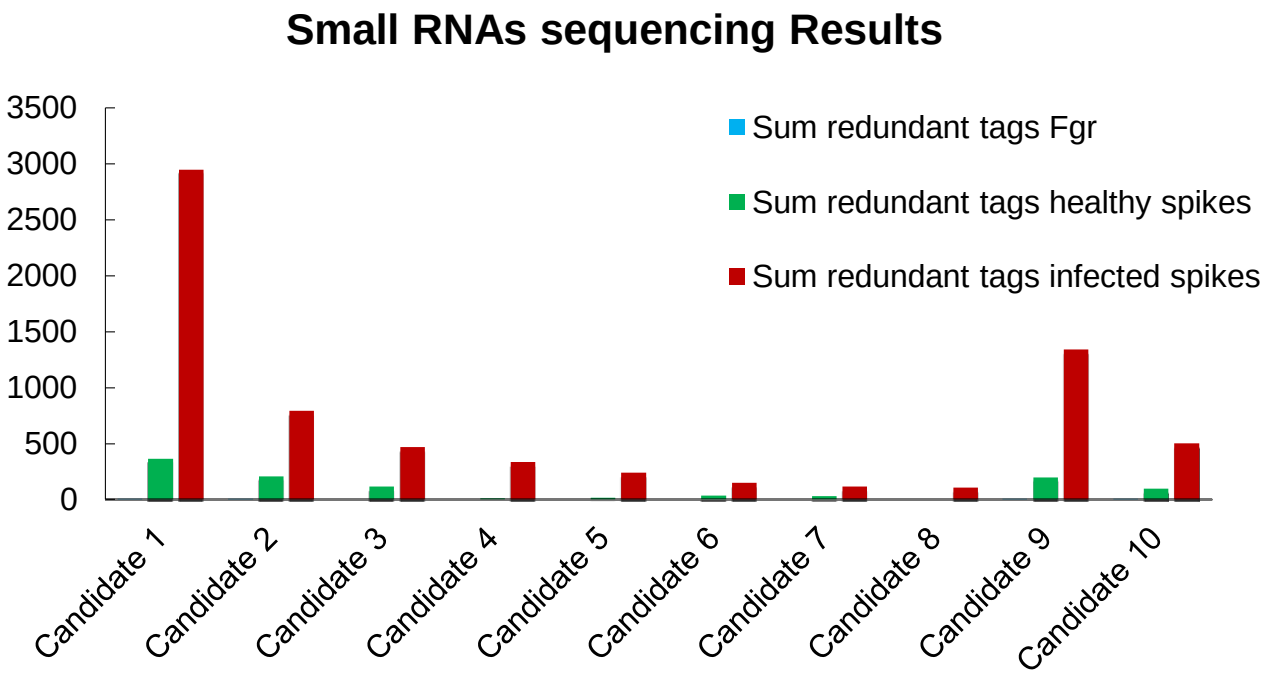




Events A, C & D were significantly more resistant than controls on both first and second spike ( *P < 0.05; * * P< 0.005; * * * P < 0.0005)

# Microscopy of *F.c.* in HIGS-transgenic wheat at 9-10 dai



# Sequencing of Small-RNA libraries from barley- *F.g.* interaction




**VIGS-FHB**

**Target genes encode**:

- Biotin biosynthetic enzyme
- Enzyme required for pre-mRNA 3' formation
- Enzyme involved in the regulation of nuclear positioning
- Methyltransferase
- ATP synthesis related enzyme
- Succinate related enzyme
- tRNA synthetase
- Transcription factor
- Enzyme required for glucose transport
- Lipase metabolism related enzyme

# Summary and Outlook

1. BSMV-mediated gene silencing (VIGS) of *F.c.* target genes (*FcFgl*1, *FcFmk*1 and *FcGls*1) reduced corresponding target transcript amounts and infection of wheat. Targeting *FcChs*V did not give a resistant phenotype.
2. Microscopic analysis of *F.c.* hyphae attacking BSMV:Gls1 pre-infected spikes revealed a phenocopy of stable *Gls1* RNAi events in *C. graminicola*.
3. Statistical analysis of transgenic wheat lines carrying an RNAi construct against *FcGls1,* or a triple-target RNAi construct against *FcGls*1, *FcFmk*1 and *FcChs*V, revealed events significantly reduced FHB infection in field-like conditions.
4. Target candidates of natural HIGS molecules were derived from sequencing of sRNA libraries of *F.g.*-inoculated barley spikes and are currently validated by VIGS.

# Acknowledgments


- Dr. Patrick Schweizer
- Dr. Daniela Nowara
- Karolina Slominska-Durdasiak
- All members in PSG

Prof. Holger Deising (MLU)

Dr. Ely Oliveira Garcia (MLU)

- Christine Kastner (IPK)
- Dr. Jochen Kumlein (IPK)
- Dr. Twan Rutten (IPK)
- Dr.Yusheng Zhao (IPK)

- Dr. Frederic Schmitt (BCS)
- Dr. Bernard Pelissier (BCS)
- Prof. Antonio Di Pietro (UCO)

Bundesministerium für Bildung und Forschung

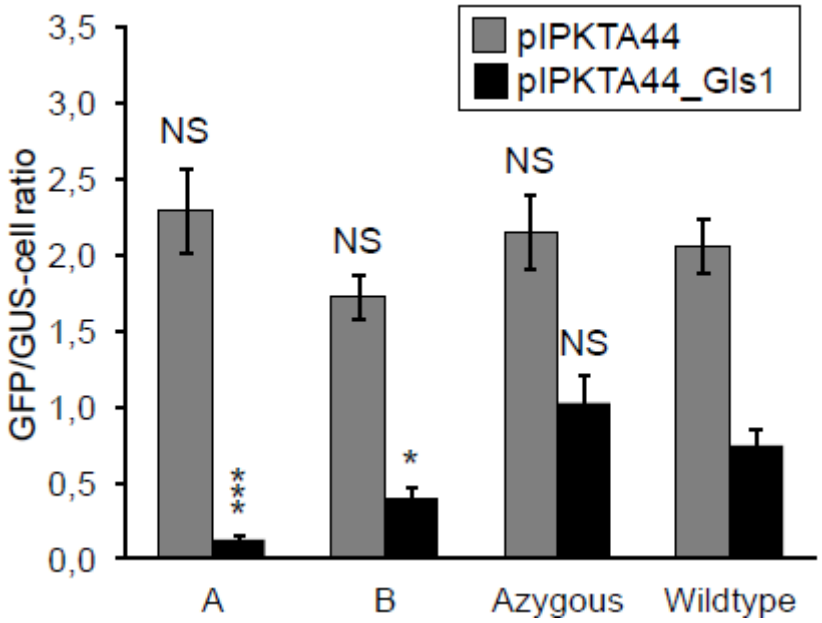
PLANT-KBBE

BAYER

KWS

# Analysis of gene silencing activity of HIGS transgenic wheat (T4/T5)

RNAi reporter assay by instable GFP




Detached leaf assay