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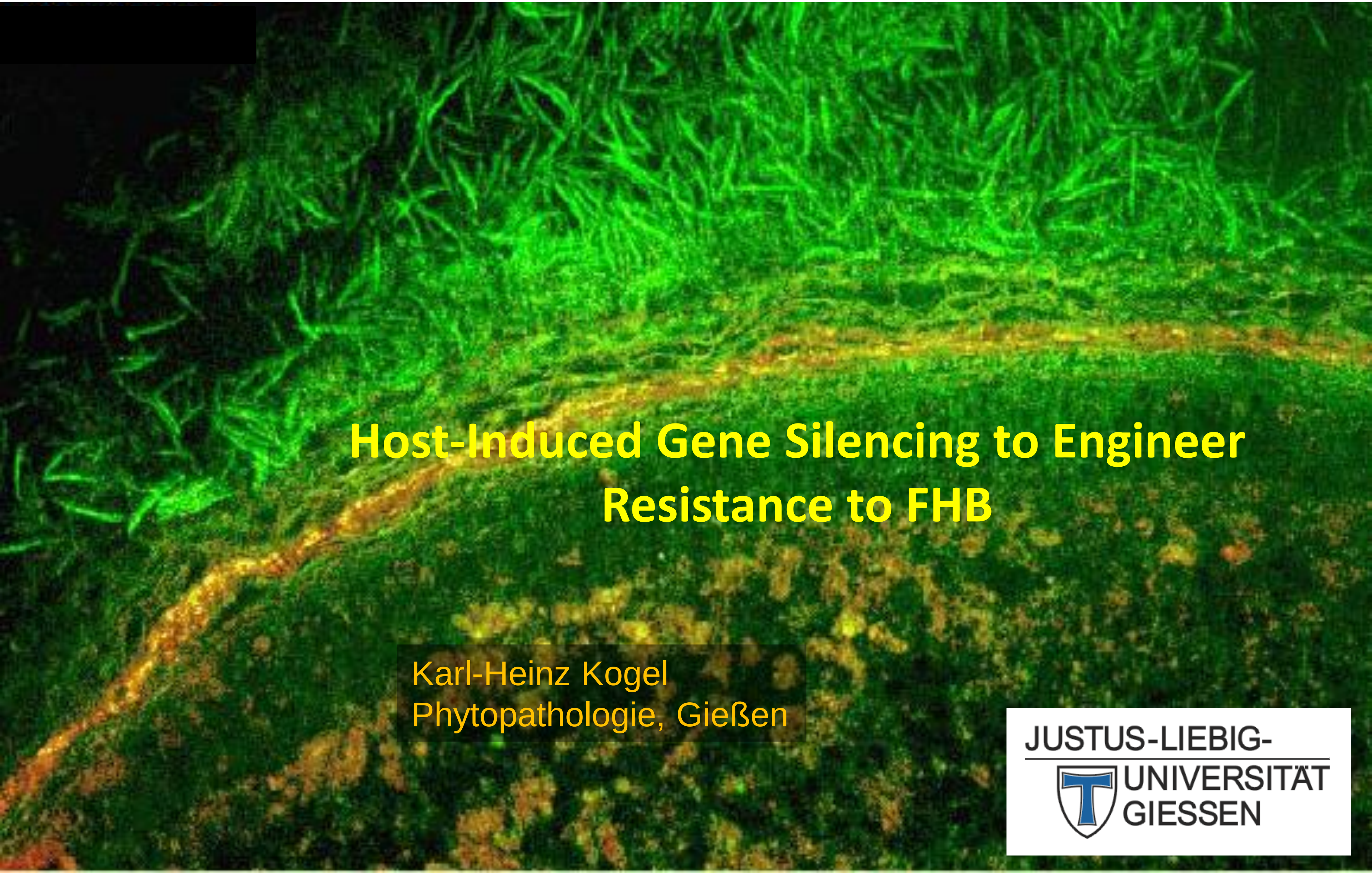
RNA-Based Plant Protection



Host-Induced Gene Silencing to Engineer Resistance to FHB

Karl-Heinz Kogel
Phytopathologie, Gießen

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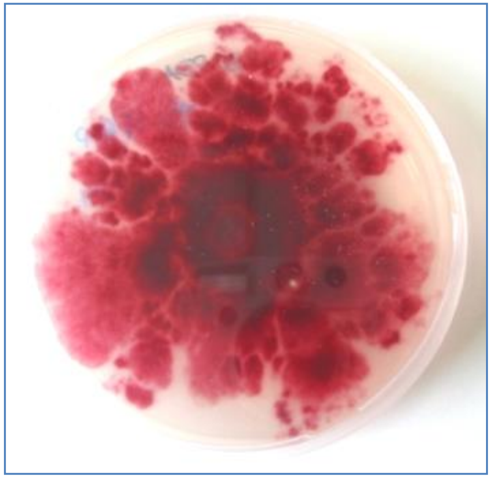
RNA-Based Plant Protection

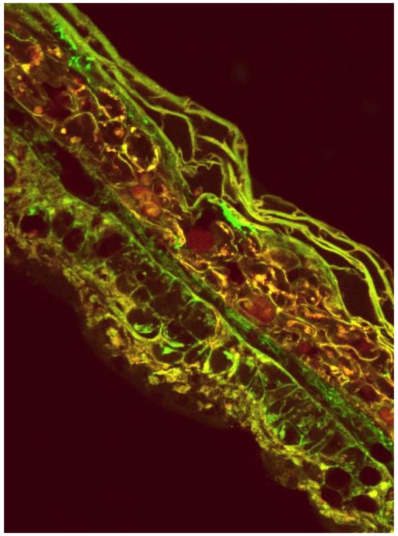
Is there an agronomic potential for application ?

The Plant Pathogen *Fusarium graminearum*

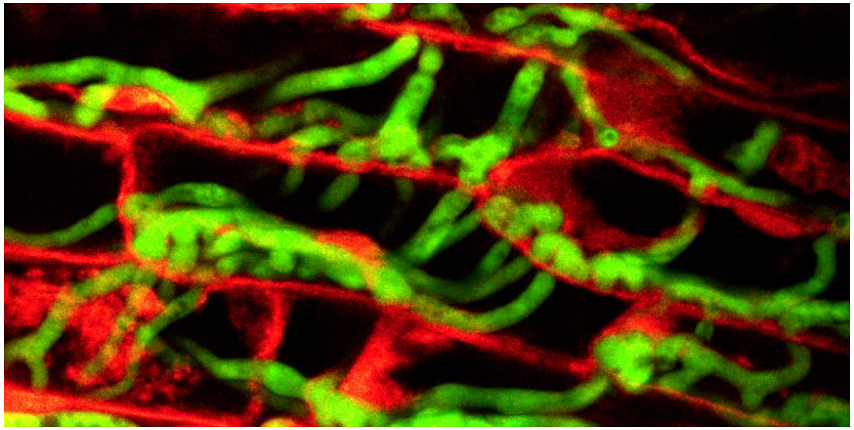
Macroconidia



Head blight disease



Toxin syndrome



Necrotrophic growth

Jansen et al. 2005 PNAS 102

Fusarium species threaten harvest and food safety

Fusarium Head Blight

Toxicity

Zearalenon LD_{50}
7 mg kg^{-1} (body weight, mouse oral)

Copper treatments LD_{50}

500 - 2000	mg kg^{-1}	(mouse oral)
660	mg kg^{-1}	(birds)
0.052	mg l^{-1}	(fish)

Modern pesticide LD_{50}
>5000 mg kg^{-1} (mouse oral)

RNA-based crop protection exploits RNA interference

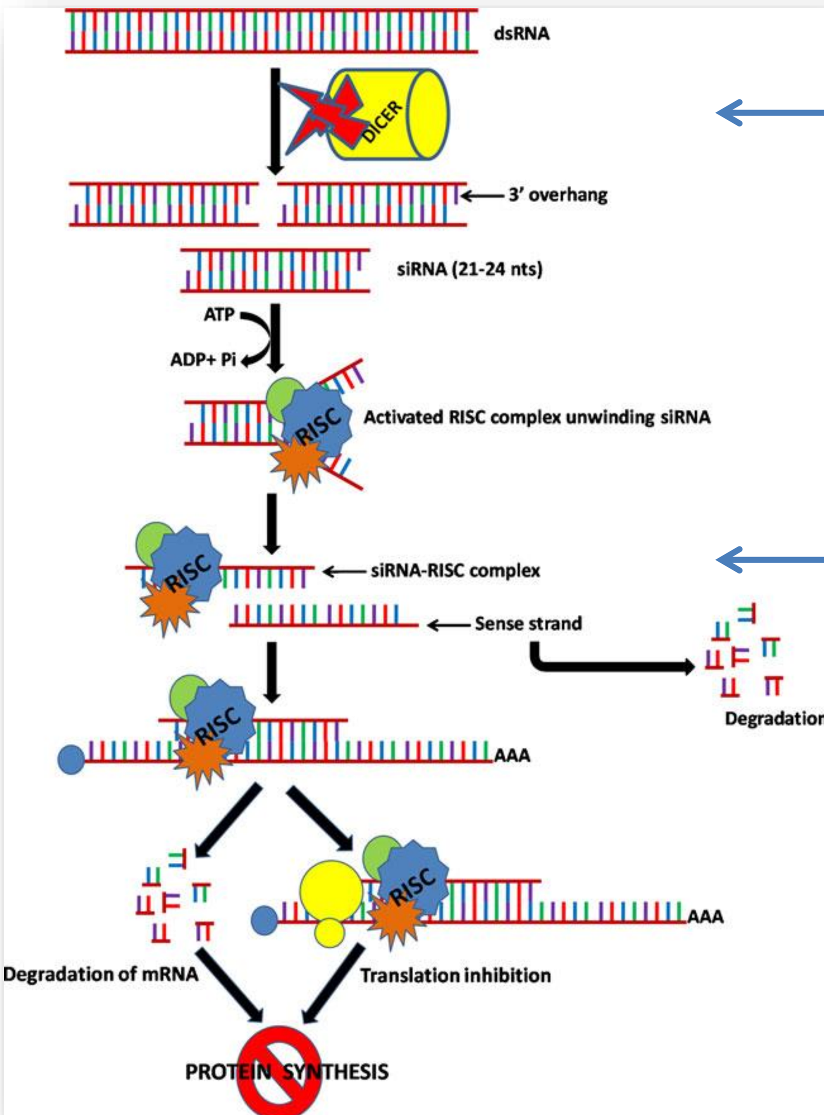
Different mechanisms of RNA interference (gene silencing)

transcriptional gene silencing (TGS)

post-transcriptional gene silencing (PTGS)

Post-transcriptional gene silencing

Andrew Z. *Fire* and Craig C. Mello (Noble Prize 2006)



Key enzymes:

DICER

RNase III –type endonuclease

4 DICER-like proteins in Arabidopsis

Argonaute

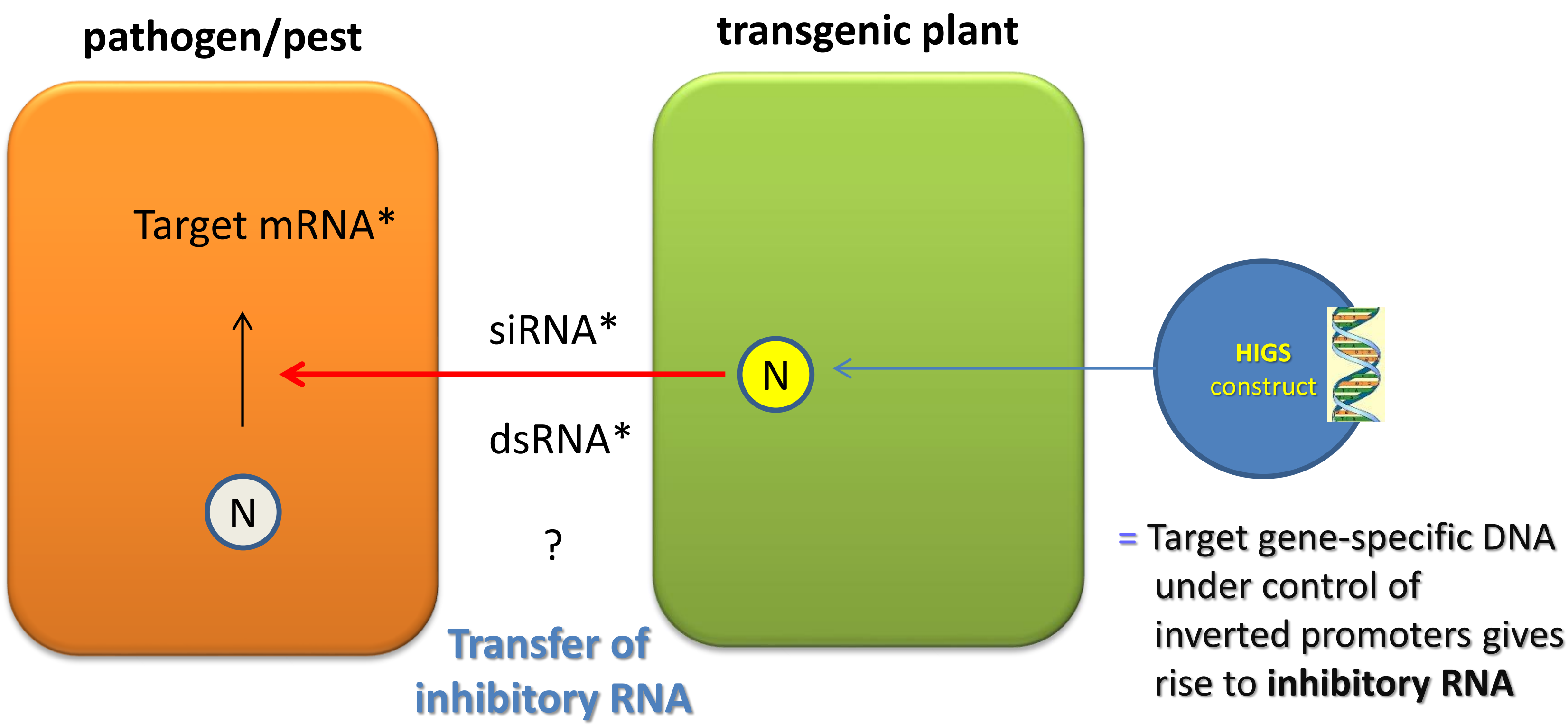
RNase III –type endonuclease

10 Argonauts in Arabidopsis

Image from Jagtap et al. 2011

Host-Induced Gene Silencing

oversimplified model



*Lethal target gene
siRNA: DICER-released small interfering RNA; ds: double stranded RNA; N: nucleus

Proof-of-Concept

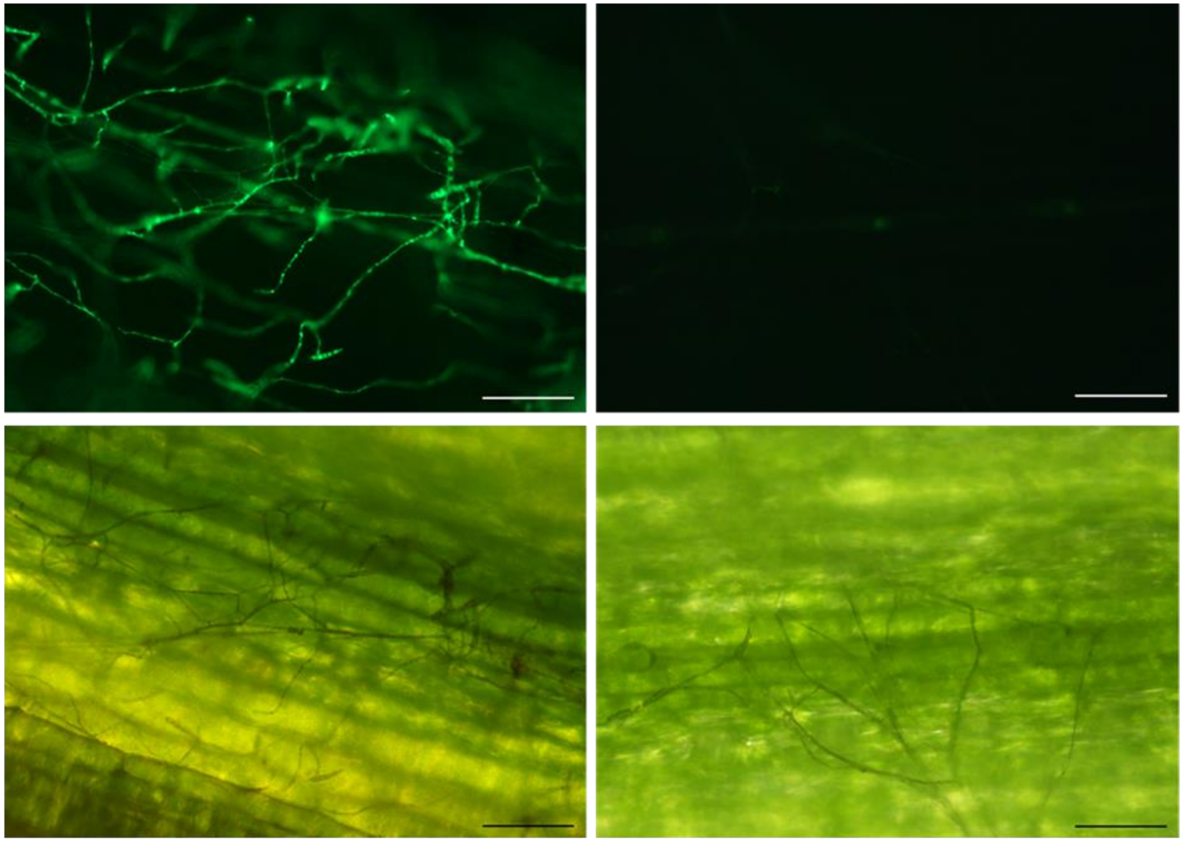
dsRNA-GFP plants inoculated with [§]GFP-tagged *Fusarium graminearum*

Control*

dsGFP-RNA plants

excitation 395 nm

visible light



*Golden Promise inoculated with Fg-GFP

[§]GFP = Green Fluorescence Protein

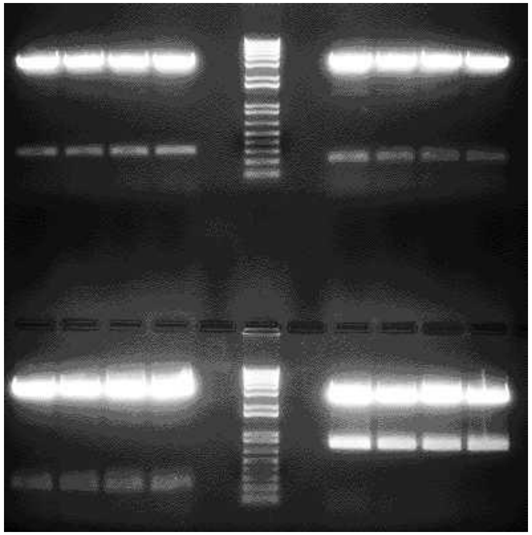
Host-Induced Gene Silencing

Selection of the right target gene
is a critical success factor
of HIGS applications

The target is a critical success factor for HIGS applications

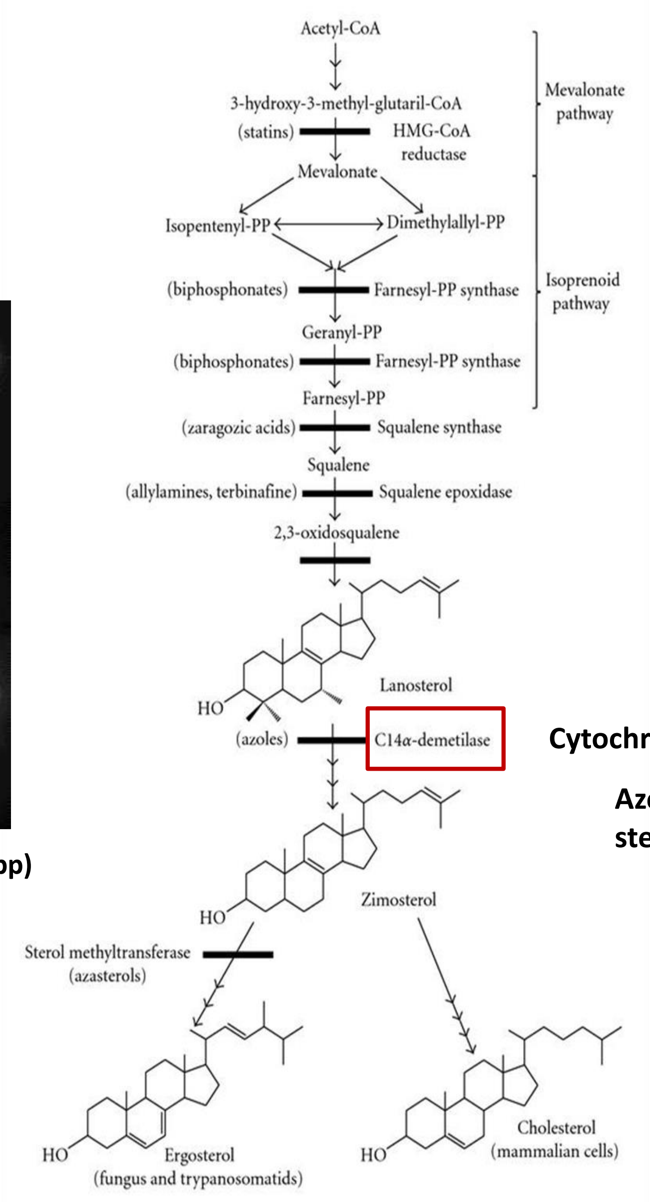
Fusarium graminearum has three *CYP51* genes

cyp51A (294 bp) **cyp51B (232 bp)**



cyp51C (250 bp) **cyp51ABC (791 bp)**

Gene fragments were amplified from genomic DNA with specific primers and cloned into pGEM-T easy vector



Ergosterol biosynthesis

Cytochrome P450 sterol 14α-demethylase (CYP51)

Azole fungicides =
sterol demethylation Inhibitors (DMI)

De Souza et al. 2009

The inhibitory dsRNA *CYP3RNA*

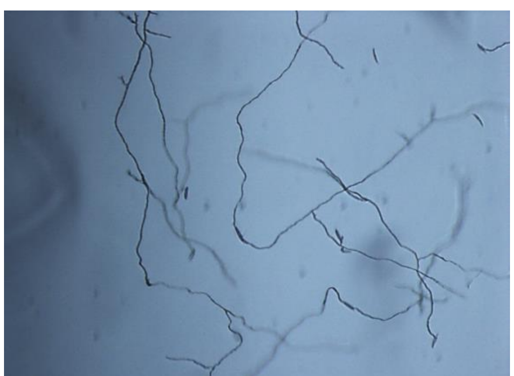
Clone sequences of CYP51A (**294nt**)
CGGTCCATTGACAATCCCCGTCTTTGGTAGCGATGTCGTATACGATTGTCCCAACTCGAAGCTCATGGAACAAAAGAAGTTTGTCAAGTTTGGCCTTACGCAAAA
AGCACTCGAGTCACACGTCCAGTTAATCGAGCGAGAGGTTCTTGACTACGTCGAAACTGATCCATCCTTTTCTGGCAGAACTAGCACCATCGATGTCCCCAAGGC
AATGGCTGAGATAACAATCTTTACTGCCTCACGTTCTTTGCAGGGTGAGGAAGTTCGGAGAAAACTCACTGCCGAGTTTGCTGC

Clone sequences of CYP51B (**220nt**)
CAGCAAGTTTGACGAGTCCCTGGCCGCTCTCTACCACGACCTCGATATGGGCTTCACCCCCATCAACTTCATGCTTCACTGGGCCCCTCTCCCCTGGAACCGTA
AGCGCGACCACGCCCAGCGCACTGTTGCCAAGATCTACATGGACACTATCAAGGAGCGCCGCGCCAAGGGCAACAACGAATCCGAGCATGACATGATGAAGCA
CCTTATGAACTCT

Clone sequences of CYP51C (**238nt**)
ATTGGAAGCACCGTACAATATGGCATCGACCCGTACGCTTTTTTCTTCGACTGCAGAGATAAATACGGCGACTGCTTTACCTTTATTCTCCTTGGCAAATCAACGA
CTGTCTTTCTTGGTCCCAAGGGCAATGACTTTATCCTCAACGGCAAACACGCCGATCTCAACGCCGAGGACGTTTATGGGAAACTTACCACGCCCGTGTTTGGTG
AGGAGGTTGTTTATGACTGCTCCAATG

***CYP3RNA**

dsRNA: *CYP3RNA*

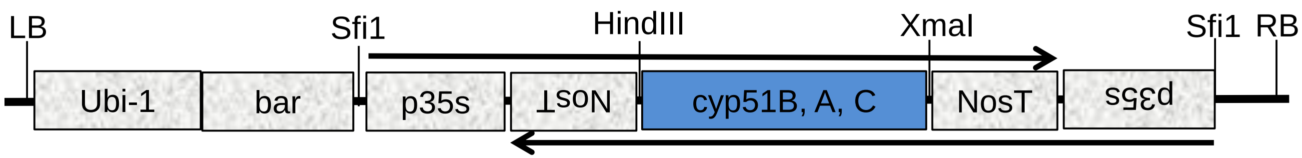


buffer

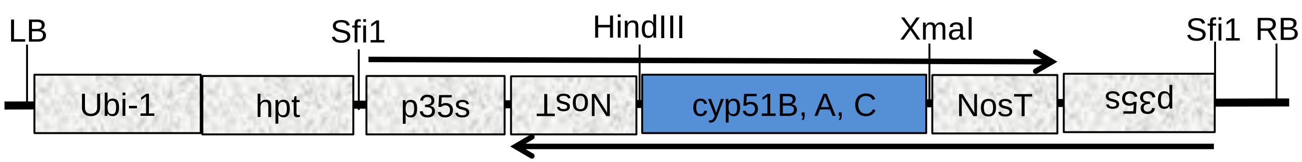
*Synthesis of dsRNA for *in vitro* studies was performed using BLOCK-iT RNAi TOPO Transcription Kit (Invitrogen)

In planta experiment

Vector for plant transformation

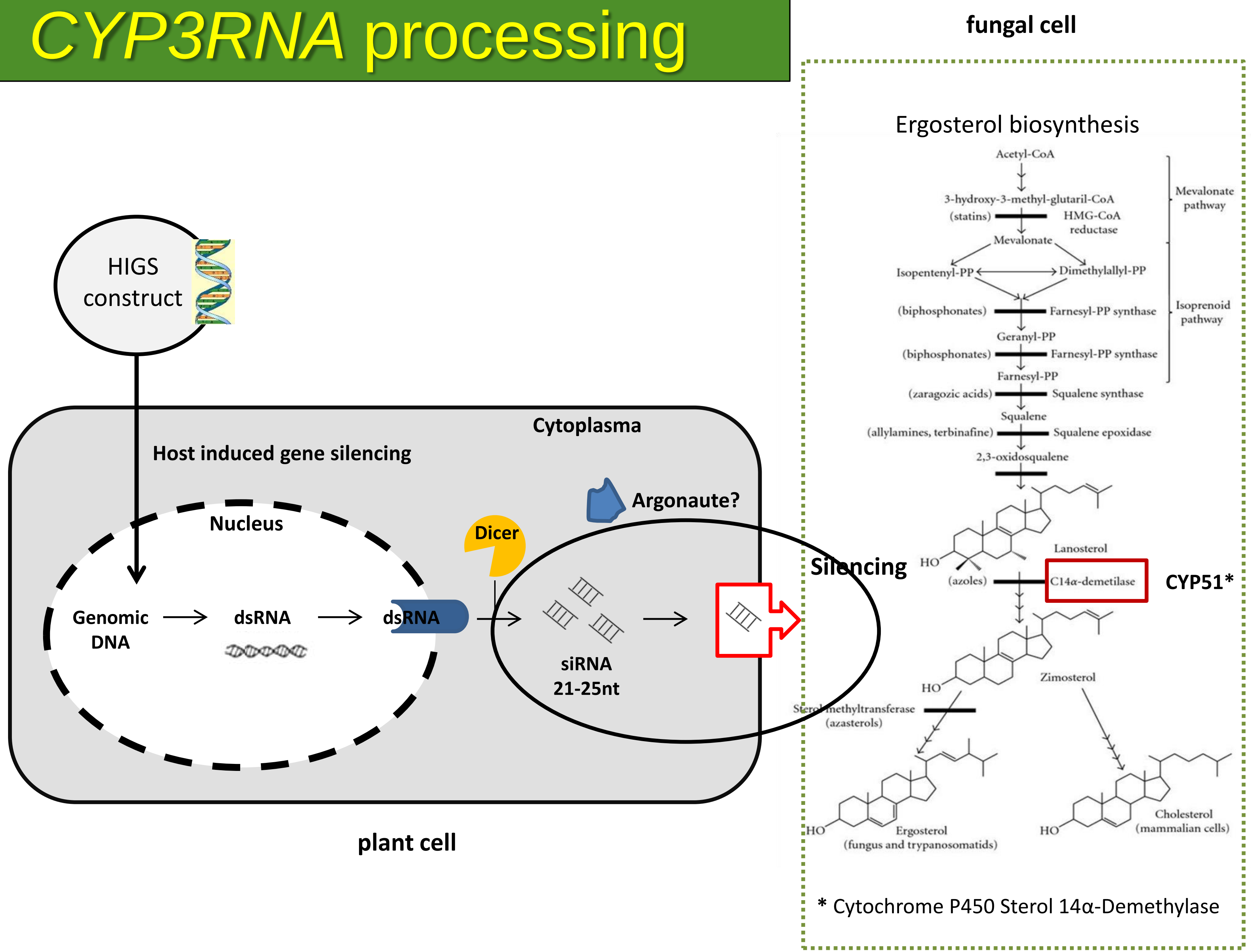


Arabidopsis



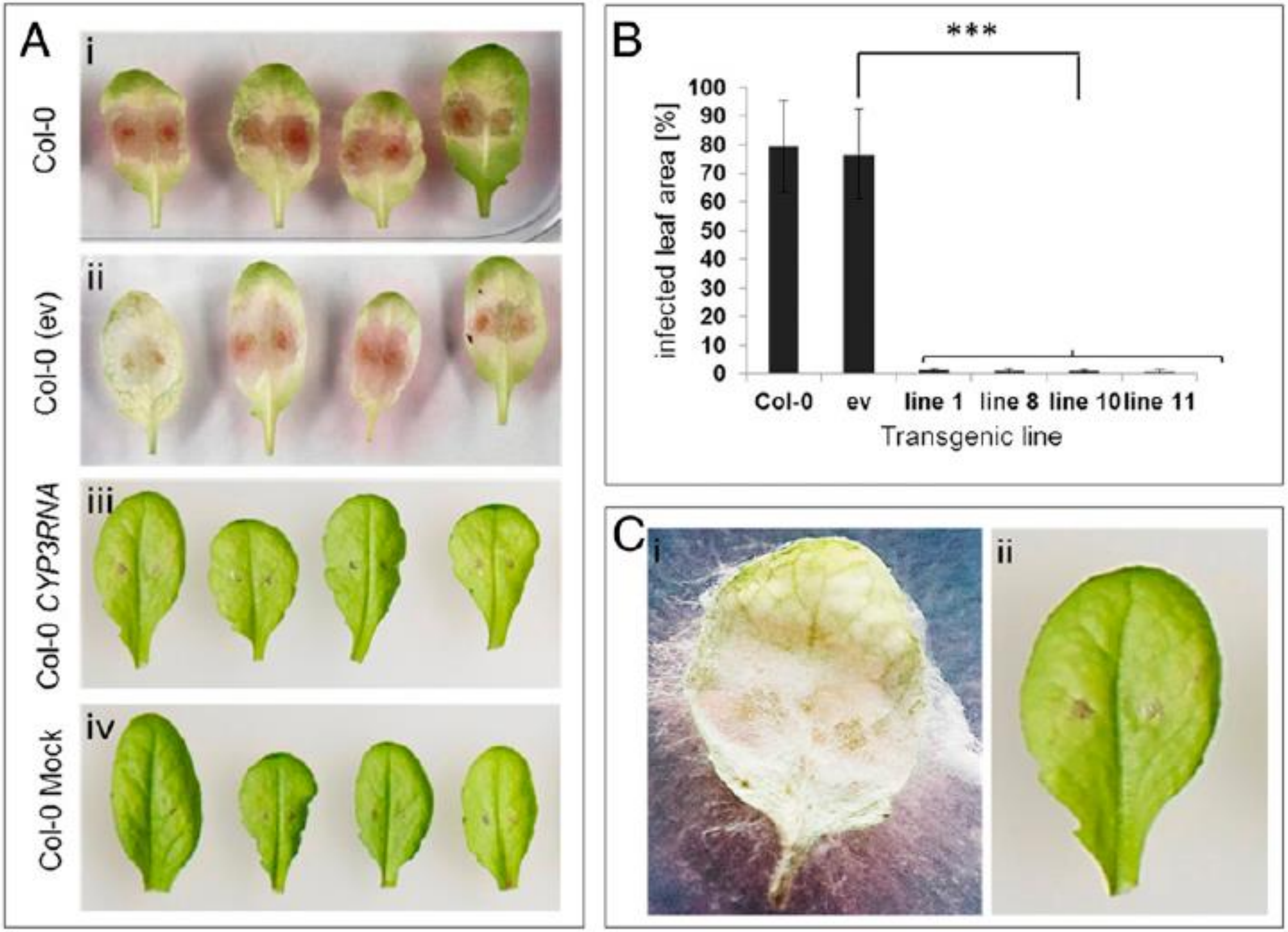
Barley

CYP3RNA processing



C*YP3RNA* expression inhibits infection

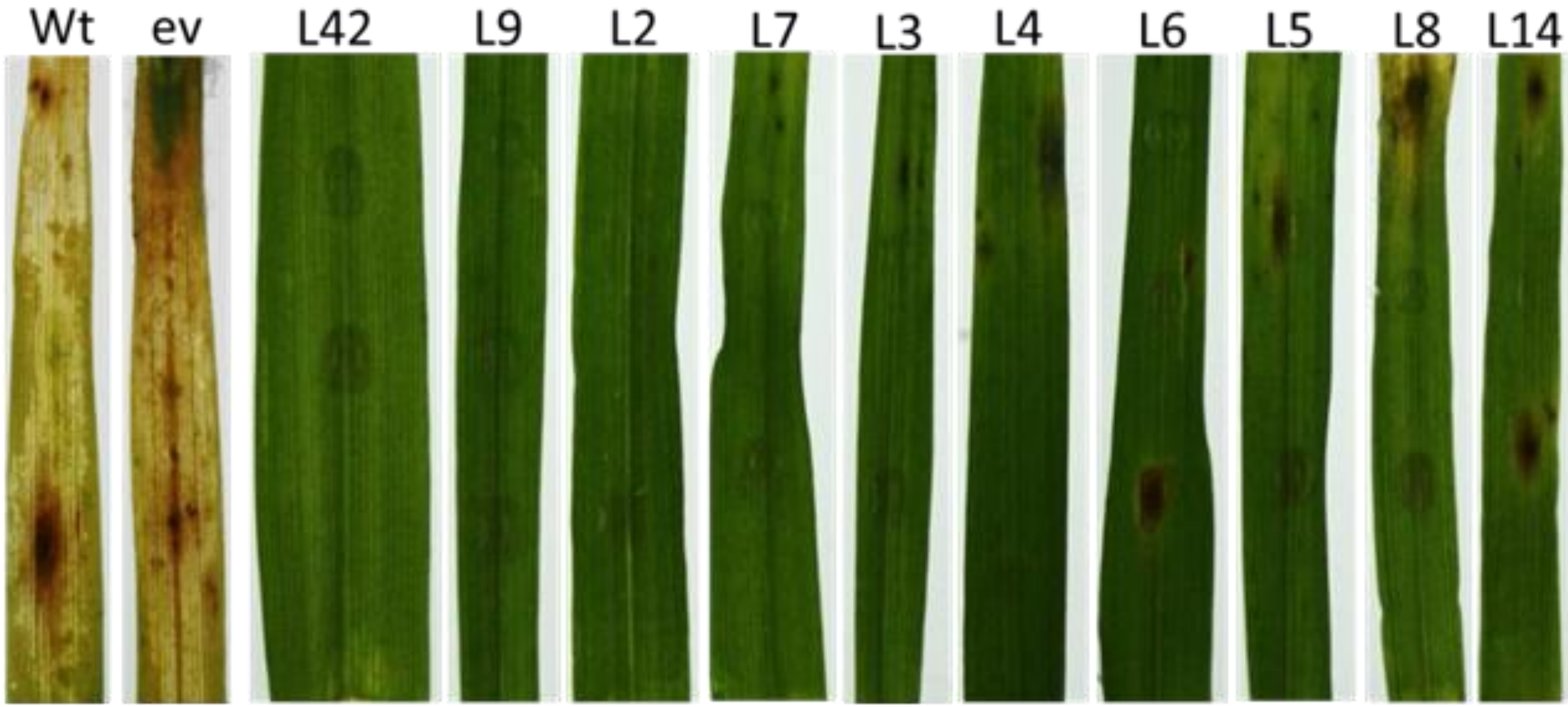
Arabidopsis



Koch et al. 2013, PNAS 110

CYP3RNA expression inhibits infection

Barley



Wild type

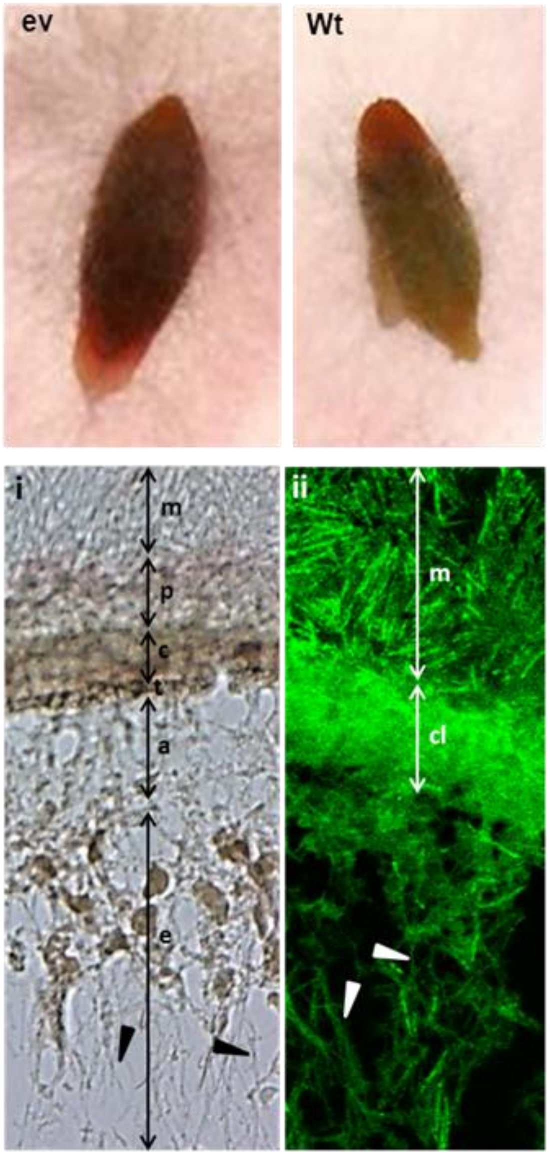
transgenic lines expressing *CYP3RNA*

transgenic line expressing empty vector

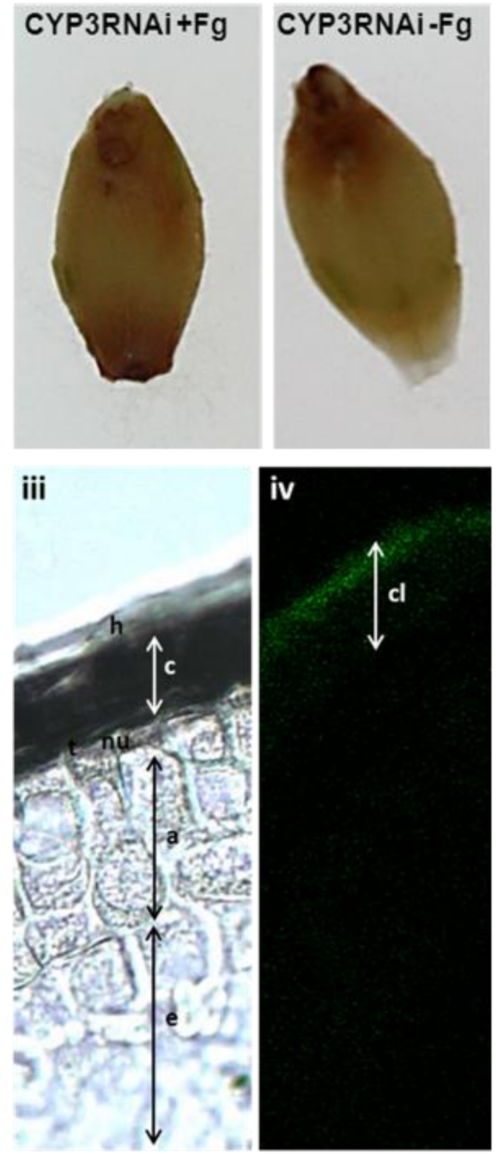
wt = cv. Golden Promise

Strong inhibition of Fusarium Head Blight

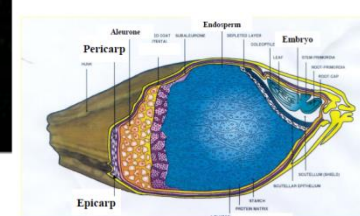
Barley



Control



CYP3RNA



Strong silencing of fungal *CYP51* expression *in planta*

Barley

F usarium infected

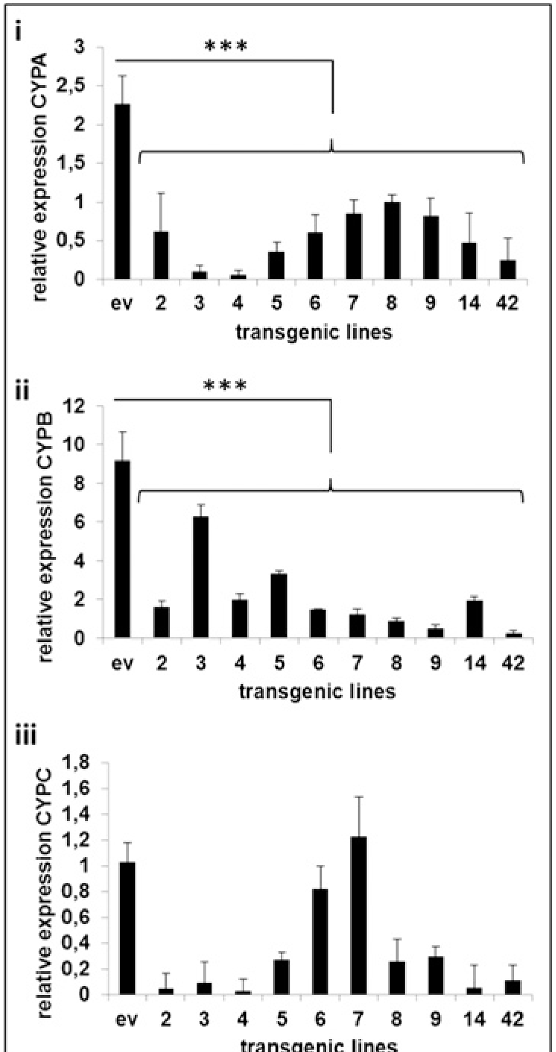
Leaves

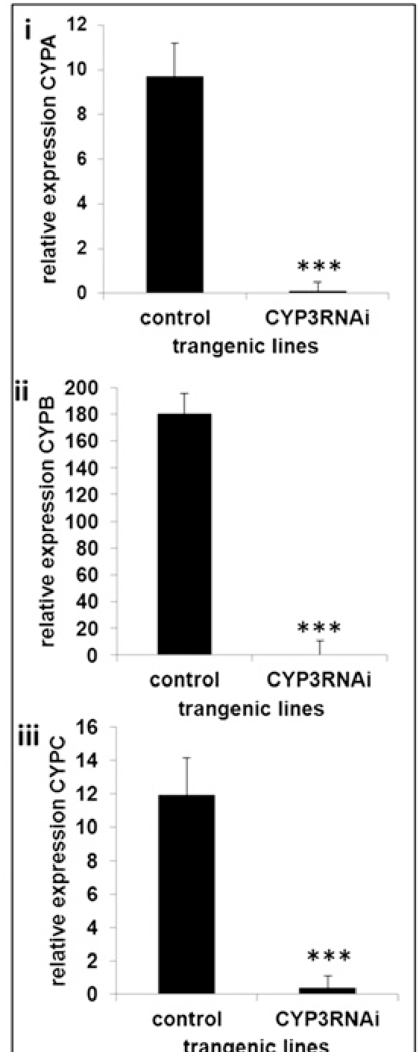
Roots

CY51A expression

CY51B expression

CY51C expression





Normalized with fungal ß-tubulin

Off-target analysis

Table S1. Prediction of *CYP3RNA* off-target transcripts

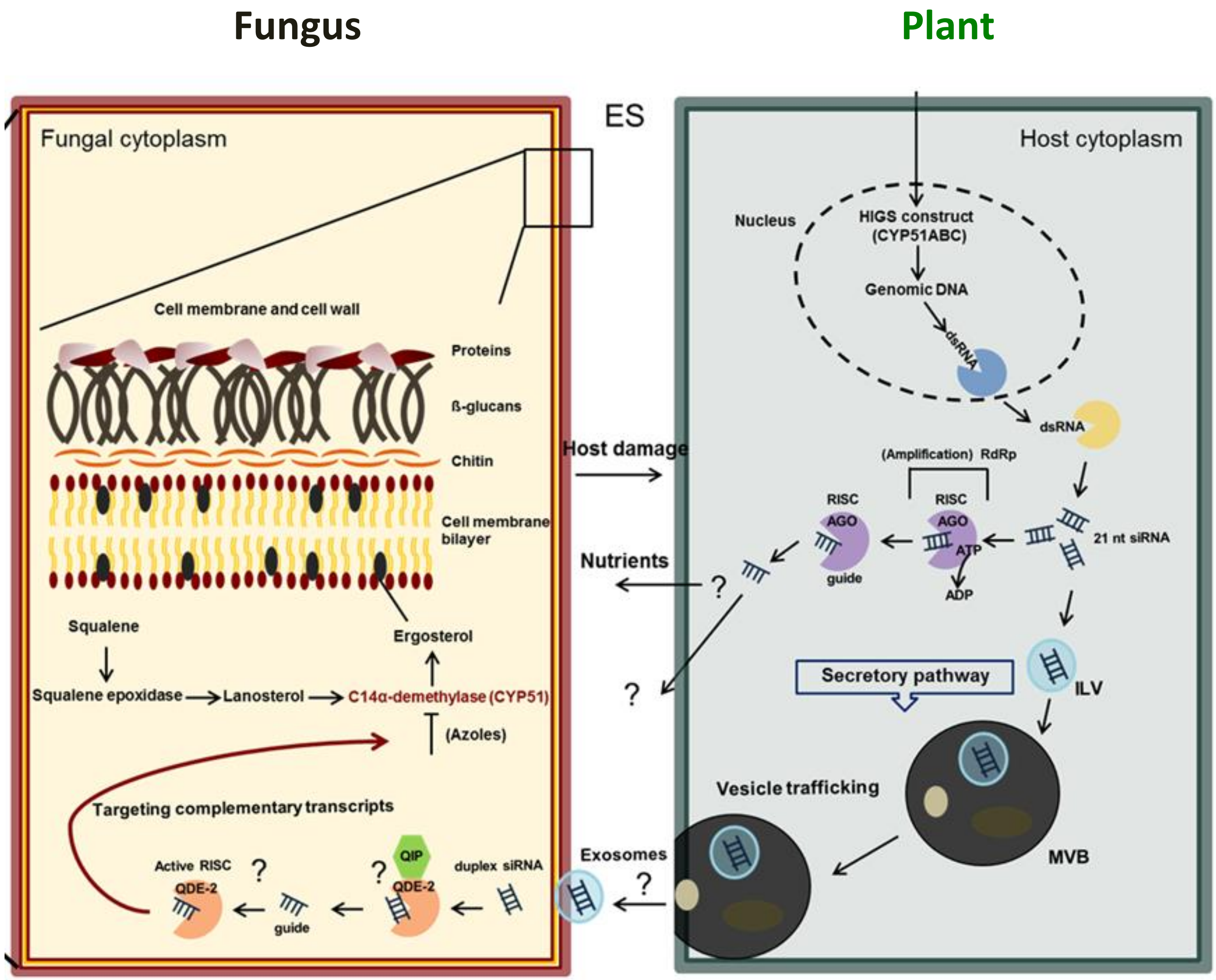
Organism	Query name*		All hits†	Efficient hits‡
	Gene	Description		
Fusarium graminearum	FGSG_01000§	*CYP51B*	200	46
	FGSG_04092§	*CYP51A*	274	126
	FGSG_11024§	*CYP51C*	218	95
Arabidopsis thaliana	AT2G17330	*CYP51A1*	0	0
	AT1G11680	*CYP51A2*	0	0
Hordeum vulgare	Published database (1)		0	0
Hyaloperonospora arabidopsidis	Published database (2)		0	0
Rhizophagus irregularis	Published database (3)		0	0
Piriformospora indica	Published database (4)		0	0
Homo sapiens	Published database (5)		0	0
Fusarium cerealis isolate NRRL13721	JN416614¶	*CYP51A*	190	83
Fusarium austroamericanum isolate NRRL28718	JN416607¶	*CYP51A*	117	50
Fusarium vorosii isolate 67C1	JN416608¶	*CYP51A*	116	50
Fusarium acaciae-mearnsii isolate NRRL26752	JN416603¶	*CYP51A*	94	43

*Simulations were run using Si-Fi software (v3.1) for predicting off-targets prediction (http://labtools.ipk-gatersleben.de).

†Number of 21-mer siRNA sequences with perfect match to the query sequence.

‡Number of 21-mer siRNA sequences with perfect match to the query sequence that fulfill additional criteria for efficient RNAi (See Si-Fi software).

What type of inhibitory RNA is transferred ?

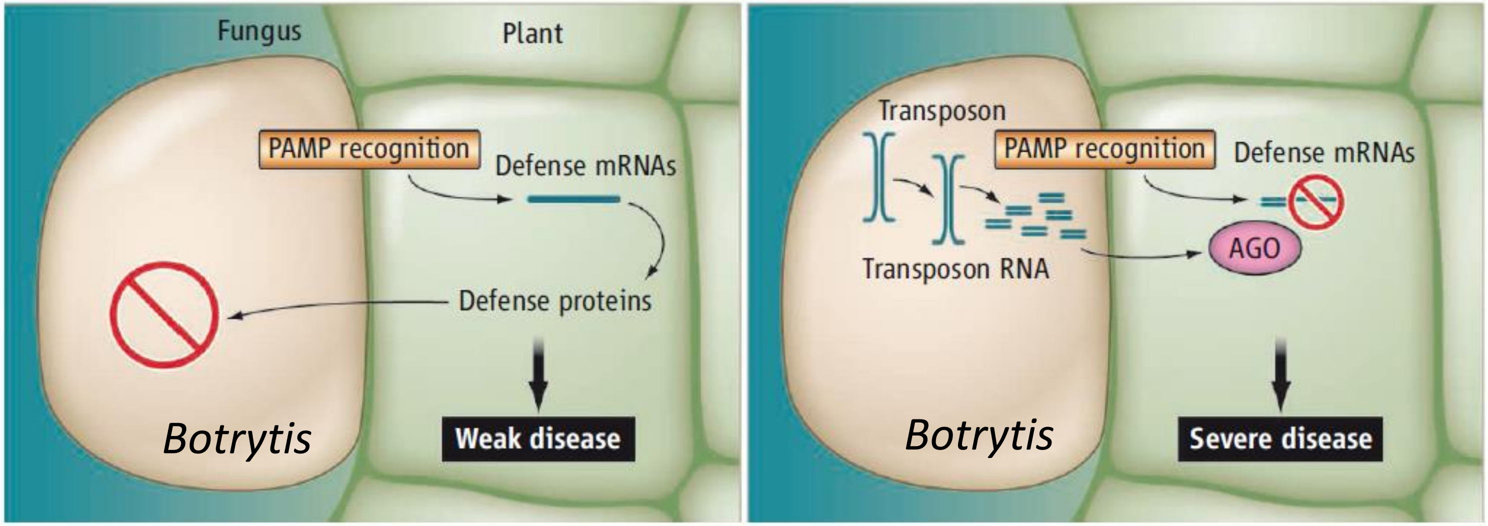


ILV, intraluminal vesicles, MVB, multivesicular bodies

Outlook – HIGS amenable to plant breeding ?

No example has been found so far showing that a crop produces small RNAs to target its pathogen/pest

However: *Botrytis cinerea* targets plant defense genes by small RNAs



From: Baulcombe 2013
Comments on Weiberg et al. 2013

It is too early to speculate whether breeding approaches on these plant targets could be a realistic strategy.

Acknowledgments

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Dr. Aline Koch

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transgenic barley

M.Sc. Eltayb Abdellatef

RNAi mutants

Dagmar Biedenkopf

Technical assistance

Excellent effects of HIGS against the grain aphid *Sitobion avenae**

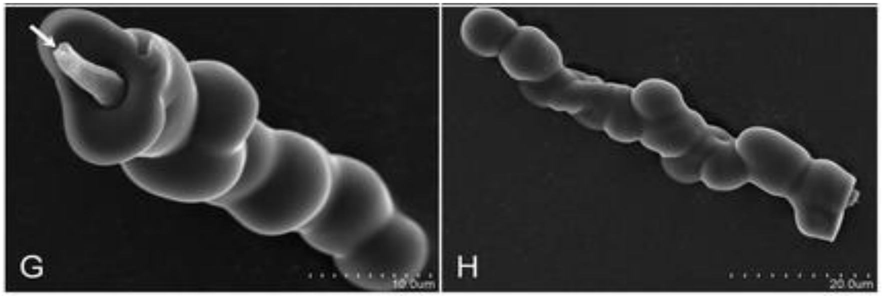
Eltayb Abdellatef

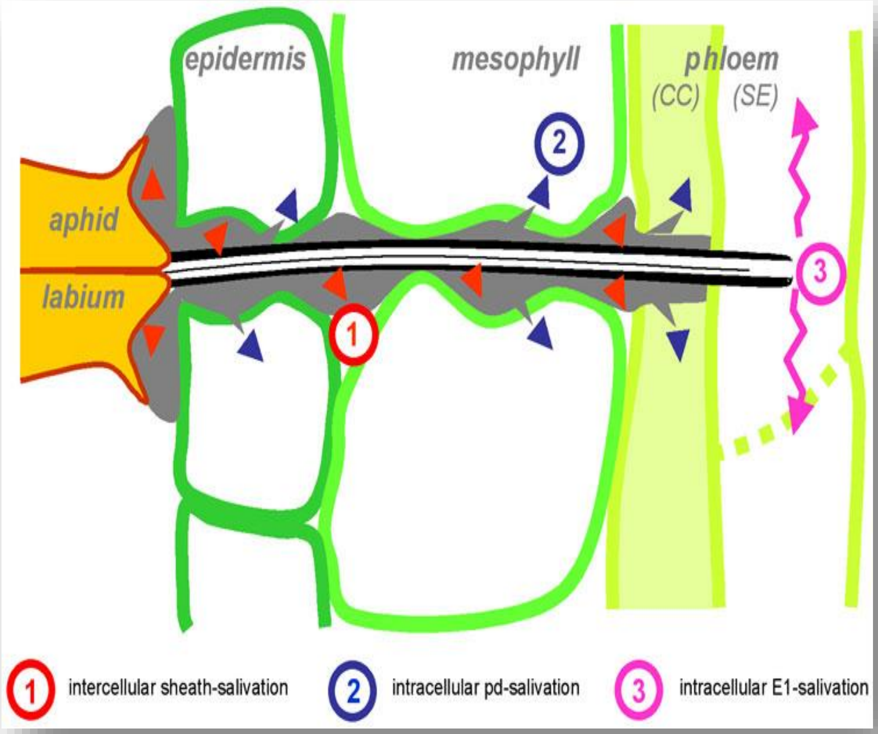
*Collaboration with A. Vilcinskas and T. Will, Inst. f. Entomology, JLU Gießen

Silencing of Salivary Sheath Protein SHP in *S. avenae*

- Aphids feed on sap suck from sieve tube of vascular plants. During this process aphids secrete gel saliva that forms a sheath to enclose the stylet.

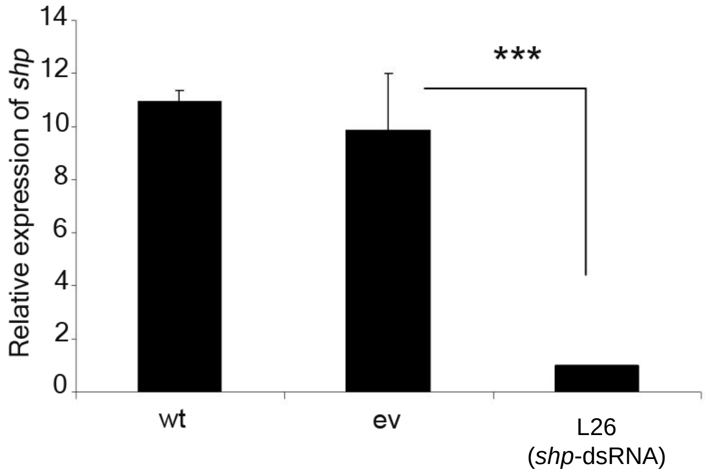
- The stylet sheath is built up by different proteins, though SHP seem essential because it forms the structural backbone of the sheath.



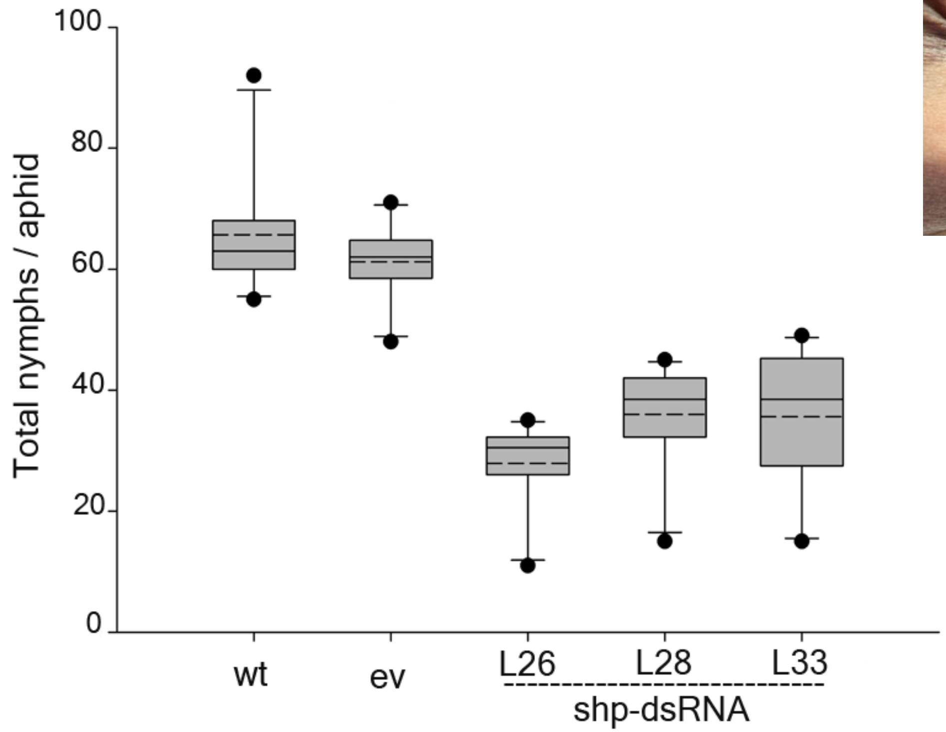


Tjallingii W F J. Exp. Bot. 2006;57:739-745

Reduced expression of *shp* in aphids fed on transgenic barley



Reproduction rate, growth development, and survival rate was negatively affected



Silencing of *shp* is transmitted transgenerationally