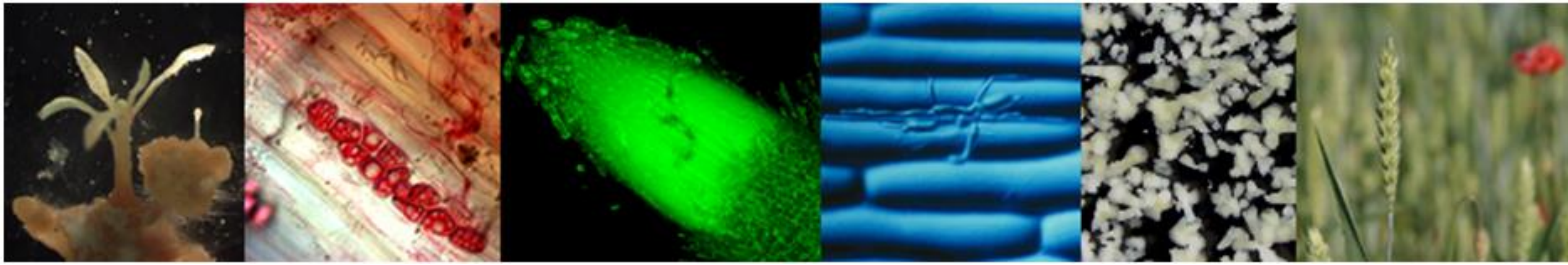


Searching for cross-kingdom communication in plant-microbe interactions and implications for agriculture

Ena Šečić (Prof. Dr. Karl-Heinz Kogel)

Institute for Phytopathology, Justus Liebig University Giessen

Symposium
Modern Agriculture without Chemical Pesticides?
September 03-04, 2018, Neustadt an der Weinstraße

JUSTUS-LIEBIG-UNIVERSITÄT GIESSEN

Gesellschaft für Pflanzenbiotechnologie e.V.

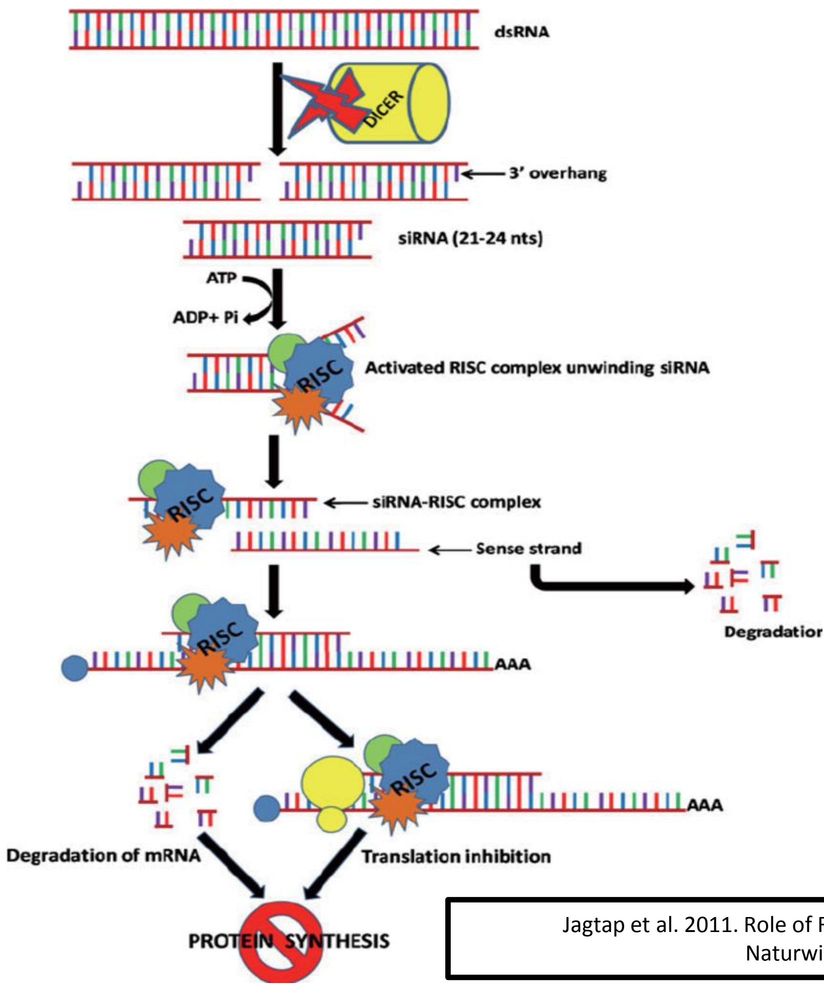
IAPB

PHYTO Phytopathologie JLU Gießen

RNA Interference (RNAi)

DNA → mRNA → Protein

Post transcriptional gene silencing (PTGS); cytoplasm



Mediated by small RNAs (sRNA), involved in endogenous gene silencing and antiviral defense

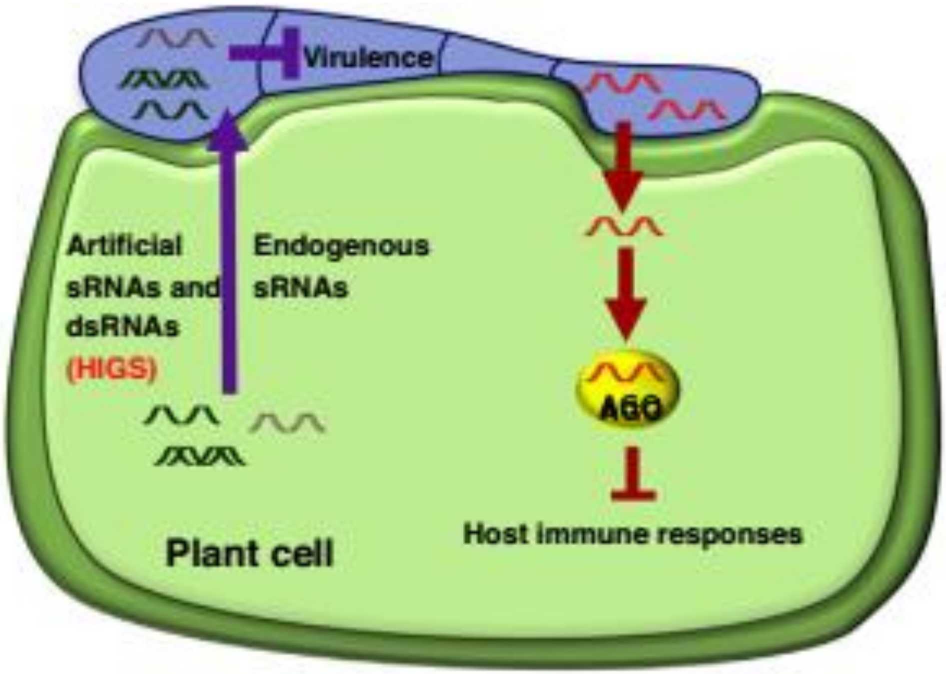
Jagtap et al. 2011. Role of RNA interference in plant improvement. Die Naturwissenschaften 98: 473-92.

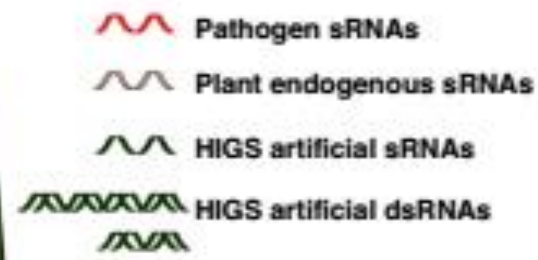
What is CROSS-KINGDOM communication?
(in the context of plant-microbe interactions)?

What is CROSS-KINGDOM communication? (in the context of plant-microbe interactions)?

- **Weiberg et al**. 2013. Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. *Science* 342:118-123.
- **Wang M et al.** 2017a. Botrytis small RNA Bc-siR37 suppresses plant defense genes by cross-kingdom RNAi. *RNA Biol* 14:421-428.
- **Wang B et al.** 2017b. Puccinia striiformis f. sp. tritici microRNA-like RNA 1 (Pst-milR1), an important pathogenicity factor of Pst, impairs wheat resistance to Pst by suppressing the wheat pathogenesis-related 2 gene. *New Phytol* 215:338-350.
- **Zhang et al. 2016**. Cotton plants export microRNAs to inhibit virulence gene expression in a fungal pathogen. *Nat Plants 2*, doi:10.1038/nplants.2016.153.
- **Cai et al. 2018.** Cross-Kingdom RNAi - nature's blueprint for modern crop protection technology. *Current Opinion in Microbiology* 46:58-64, doi 10.1016/j.mib.2018.02.003.

Cross-kingdom RNAi in plant-microbe interactions





Main properties of this communication:

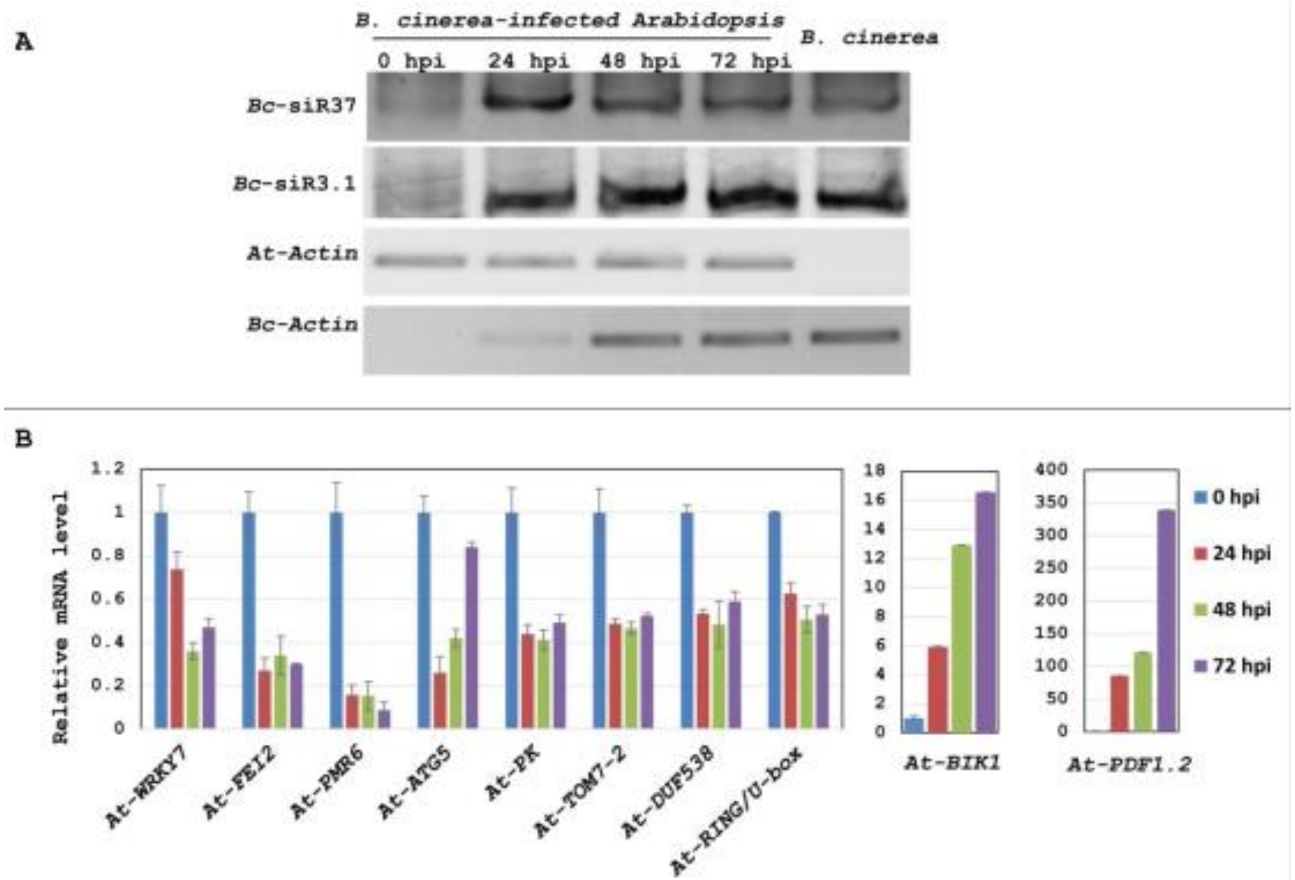
- Bidirectional
- Sequence-specific
- Organism-specific

Detected in plant-microbe and animal-microbe systems (even without a functional RNAi machinery)

Adjusted from: Cai et al. 2018. Cross-Kingdom RNAi - nature's blueprint for modern crop protection technology. DOI 10.1016/j.mib.2018.02.003.

Pathogen / Microbe ⊣ Plant

Botrytis cinerea – Arabidopsis / tomato



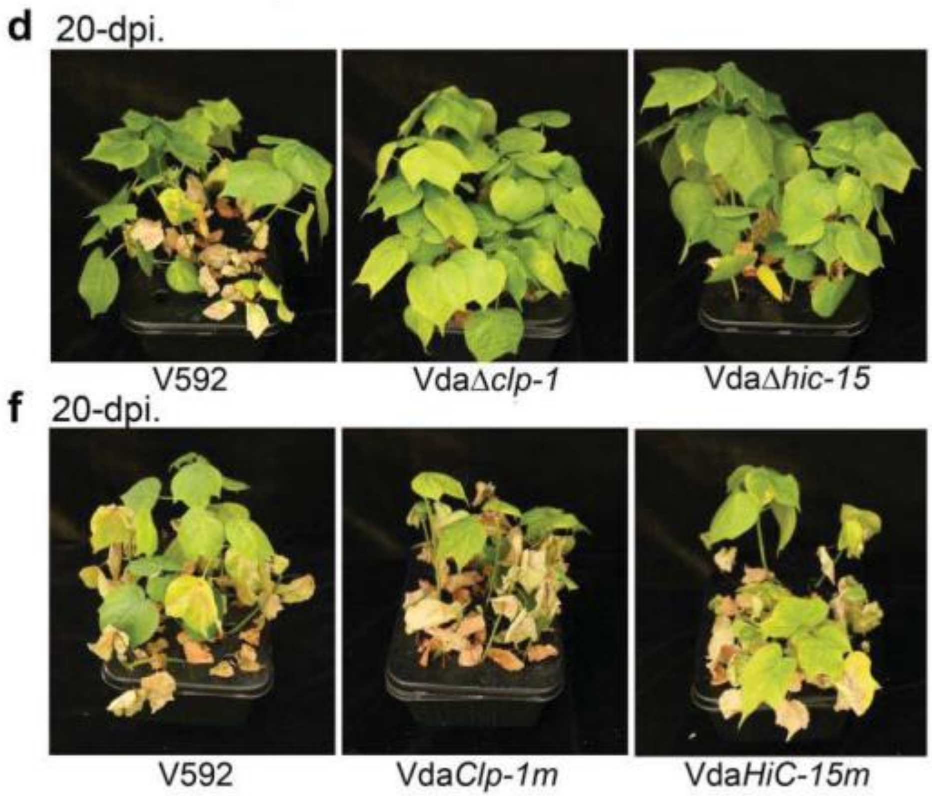
Bc sRNA expressed in the Bc-Arabidopsis system and the corresponding target gene downregulation

From: Wang M et al. 2017a. Botrytis small RNA Bc-siR37 suppresses plant defense genes by cross-kingdom RNAi. RNA Biol 14:421-428.

Plant

Pathogen / Microbe

Cotton - *Verticillium dahliae*

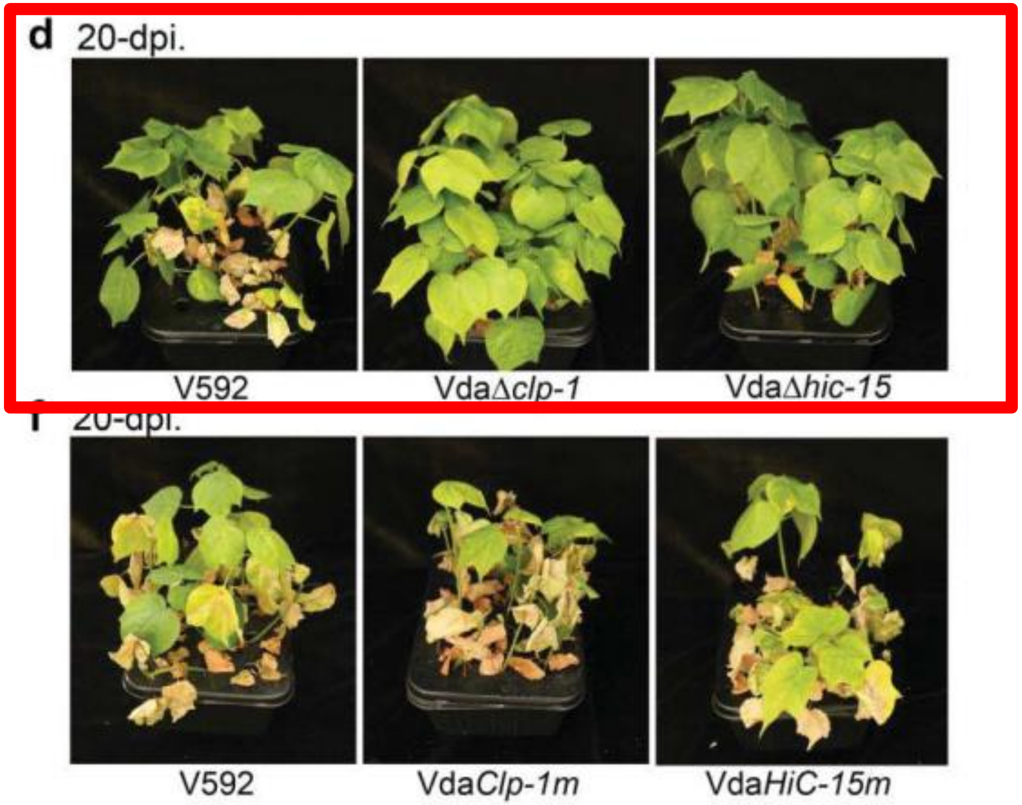


Mutant Vd strains with knock-out of mRNA targets (d) and with targets modified to be ck-RNA resistant (f) have resistant (d) and susceptible (f) reponses on cotton plants

From: Zhang et al. 2016. Nat Plants 2, doi:10.1038/nplants.2016.153.

Plant —| Pathog Microbe

Cotton - *Verticillum dahliae*



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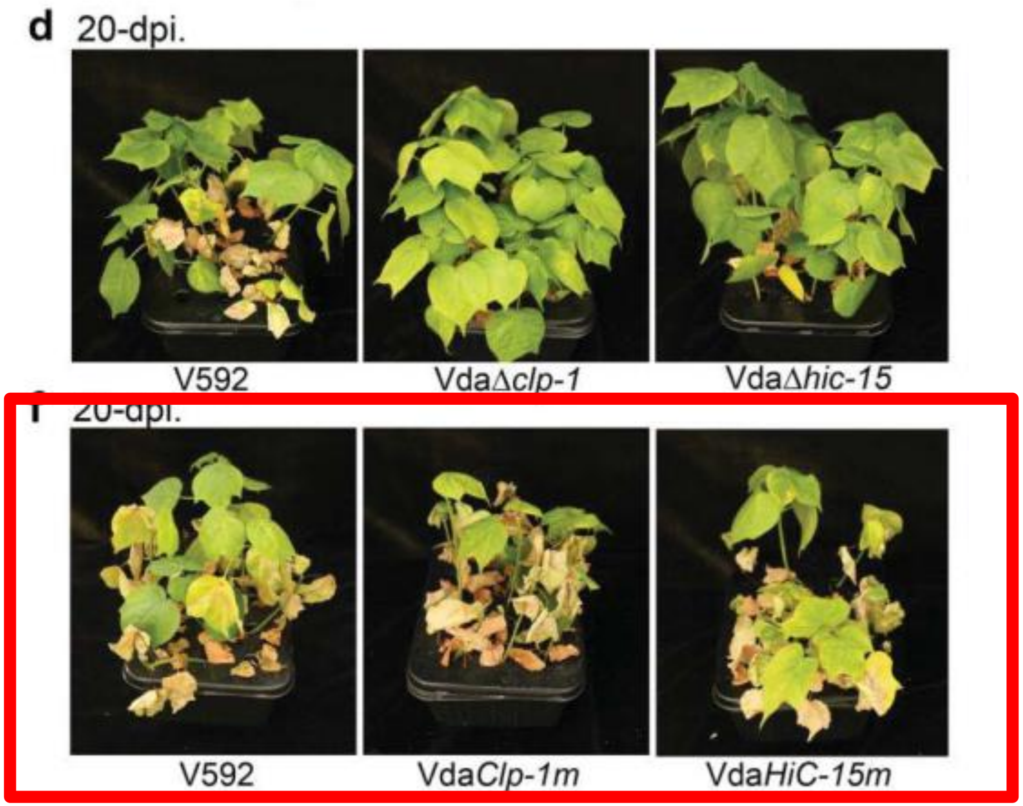
Plant | Pathogen / Microbe

Cotton - *Verticillium dahliae*



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From: Zhang et al. 2016. Nat Plants 2, doi:10.1038/nplants.2016.153.

Approaches to detect CROSS-KINGDOM communication

Cross-kingdom communication - identification pipeline

Obtaining of an sRNA library from the pathogen-microbe system

↓

Identification of sRNA candidates from interacting organisms

↓

Prediction of mRNA targets of the sRNA candidates

↓

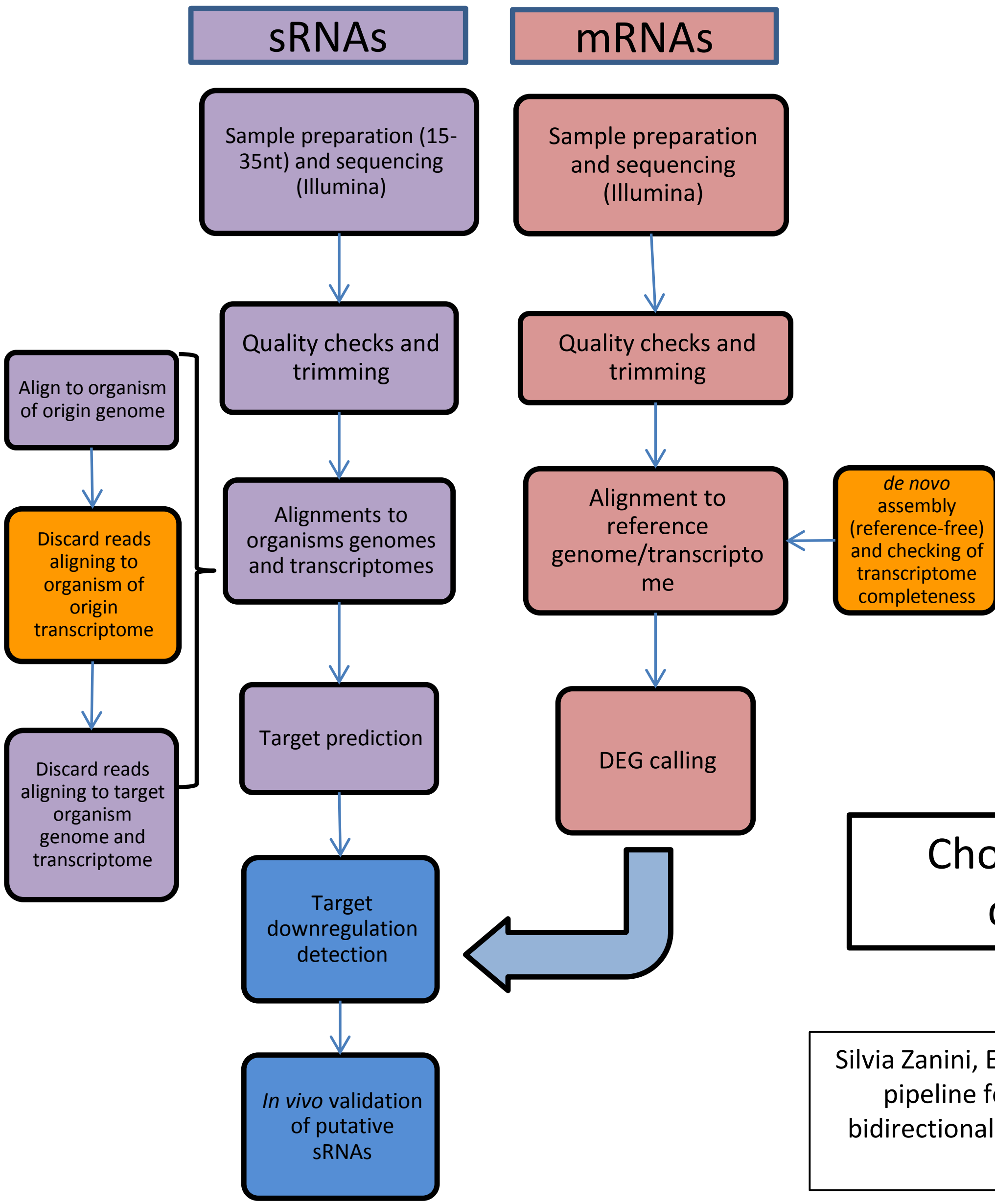
Examination of transcript levels of the predicted target genes

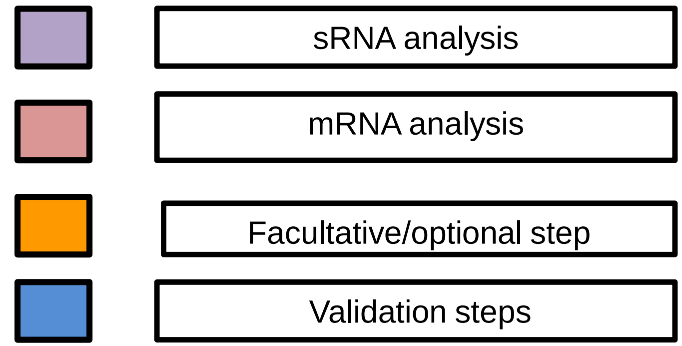
↓

Further validation of sRNA-mRNA interaction (knock-out of RNAi genes, mRNA targets, sRNA precursors)

Purple: *In silico* steps

Green: *In vitro/in vivo* steps





Choice of software for this analysis depends on project specifics

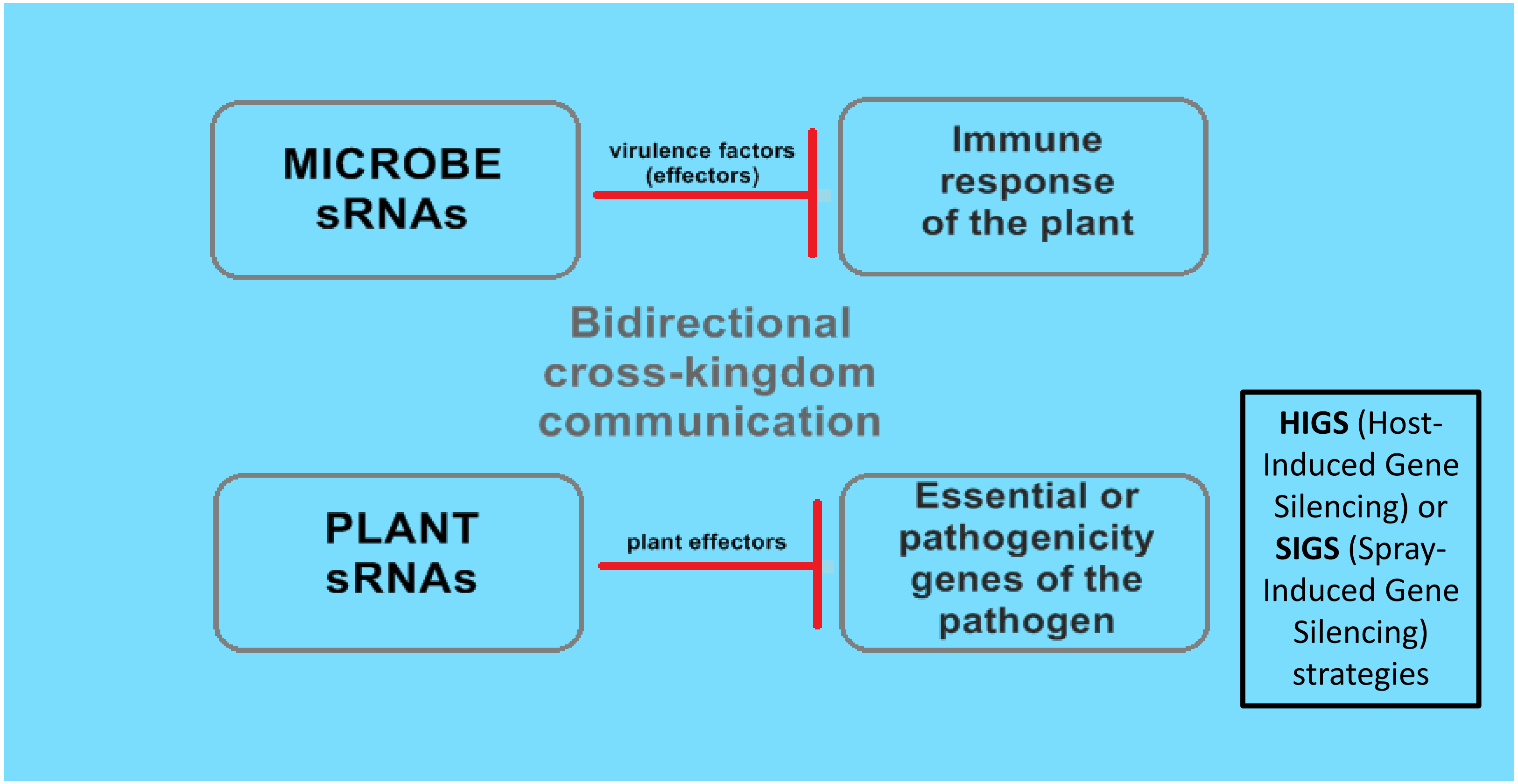
Silvia Zanini, Ena Šečić, Lukas Jelonek, Karl-Heinz Kogel, „A bioinformatics pipeline for the analysis and target prediction of RNA effectors in bidirectional communication during plant-microbe interactions“, Front. Plant Sci., 20 August 2018

Fundamental research questions:

- Mechanistic background of cross-kingdom communication?
- How are sRNAs transported between organisms?
- Target conservation in microbes with different lifestyles?
- Target detection for design of plant protection strategies

Agronomic application strategies:

- HIGS and RNAs as fungicides / SIGS strategies


MICROBE sRNAs
virulence factors (effectors)
Immune response of the plant
Bidirectional cross-kingdom communication
PLANT sRNAs
plant effectors
Essential or pathogenicity genes of the pathogen
HIGS (Host-Induced Gene Silencing) or **SIGS** (Spray-Induced Gene Silencing) strategies

Application potential (ck-communication and RNAi protection strategies)

Chemical-based protection strategies to be avoided due to:

- **Development of pathogen resistance**
- **Soil/Water pollution**
- **Pathogens difficult to control**

NOVEL PROTECTION STRATEGIES ARE CONSTANTLY REQUIRED

AGRICULTURAL APPLICATION OF dsRNA AS A SOLUTION

Direct delivery via the crop plant (GMO)

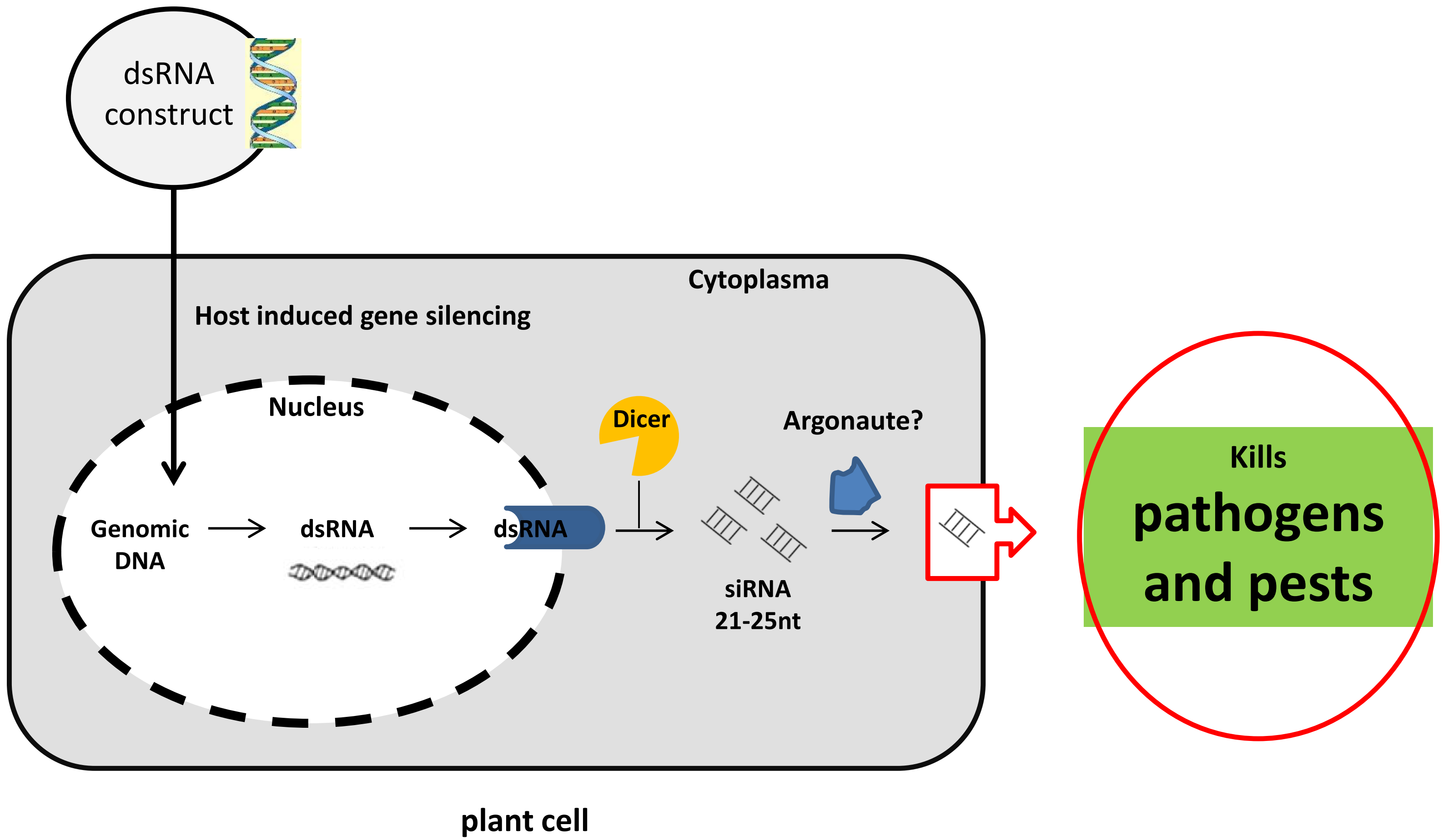
(Host-Induced Gene Silencing, HIGS*)

Delivery via spray-application (RNA as fungicide)

(Spray-Induced Gene Silencing, SIGS)

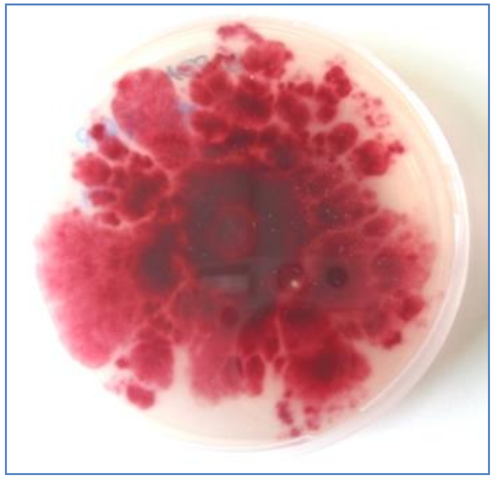
*Nowara et al. (2010) Plant Cell 22:3130-3141

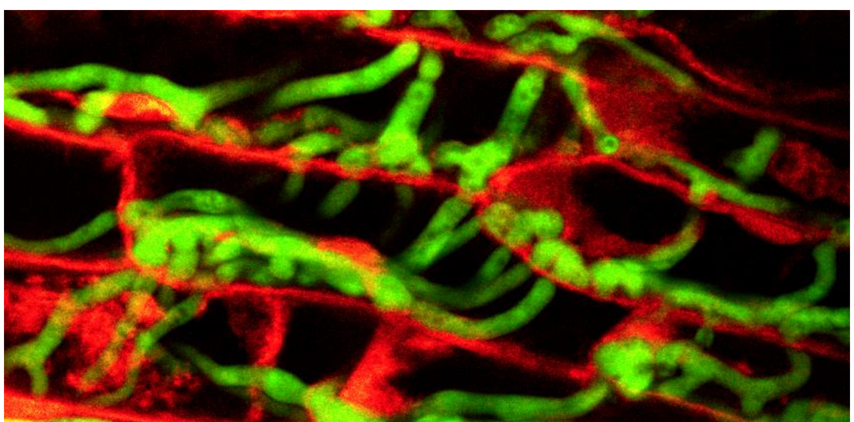
HIGS – A GMO plant protection strategy



Fusarium graminearum

Macroconidia

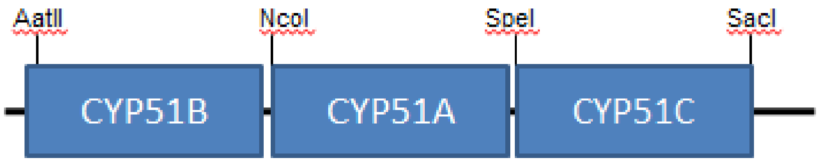




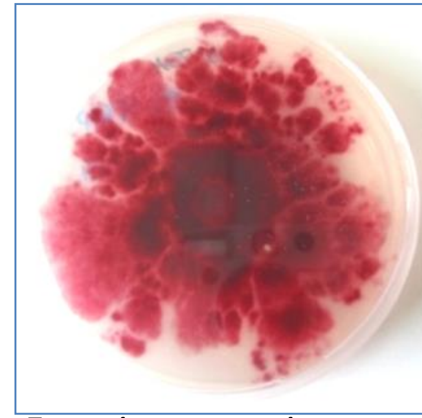
Jansen et al. 2005 PNAS 102

Head blight disease

CYP3-dsRNA inhibits Fusarium *in vitro*

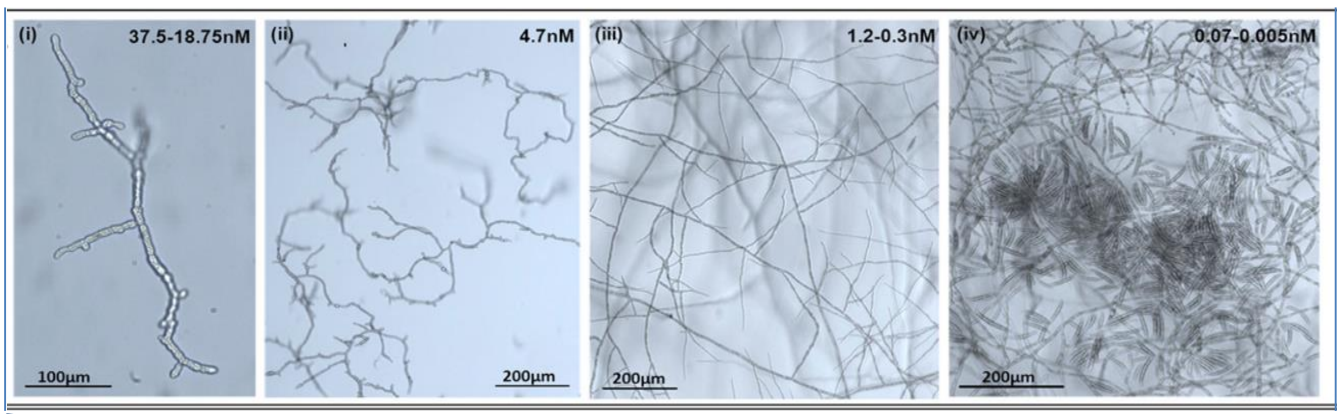


CYP3-dsRNA (791 nt long)



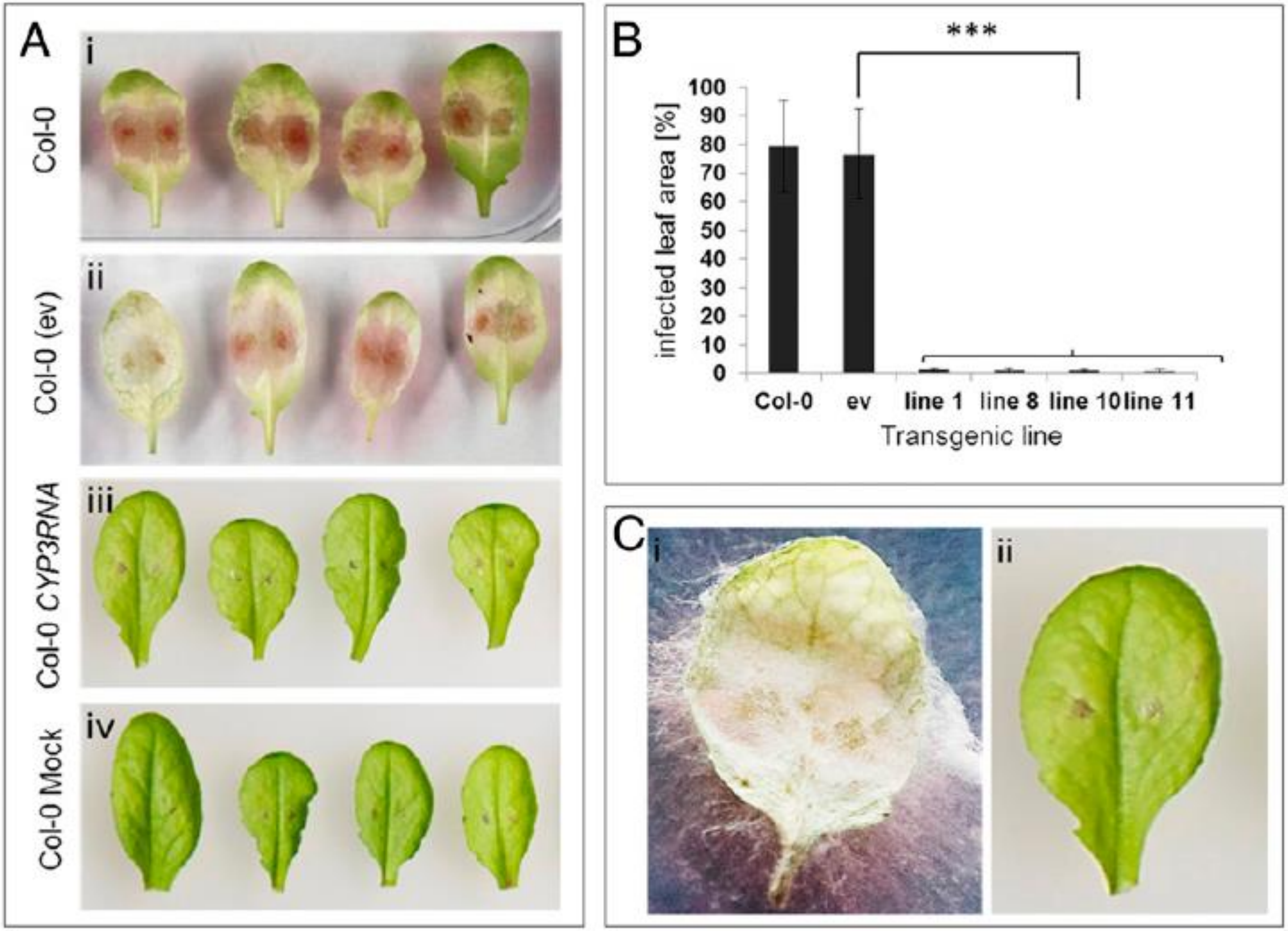
Fusarium graminearum

In vitro growth inhibition by *CYP3*-dsRNA



Koch et al. 2013, PNAS 110:19324
Koch et al. 2016, Plos Pathog. 10.1371/journal.ppat.1005901

CYP3-dsRNA inhibits Fusarium infection on transgenic Arabidopsis plants

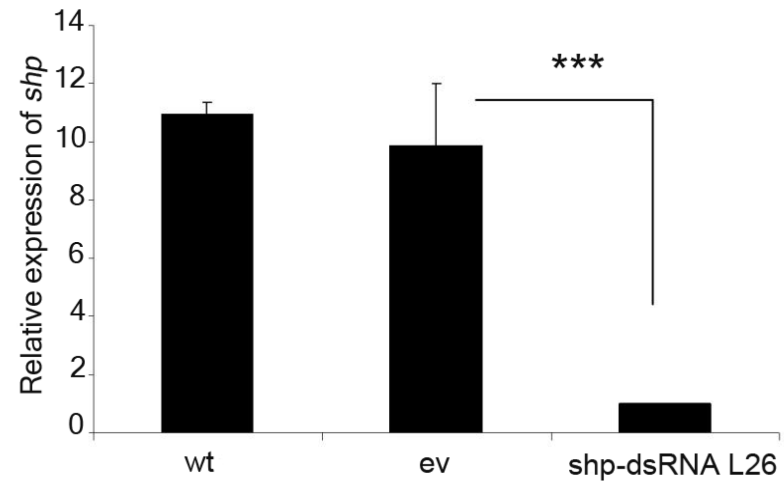


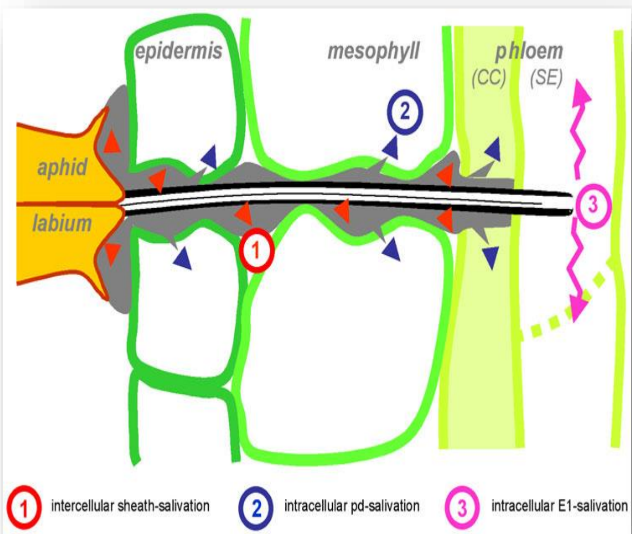
Fusarium graminearum strain Fg-IFA65

Koch et al. 2013, PNAS 110

Aphid target „SHEATH PROTEIN“ (shp)

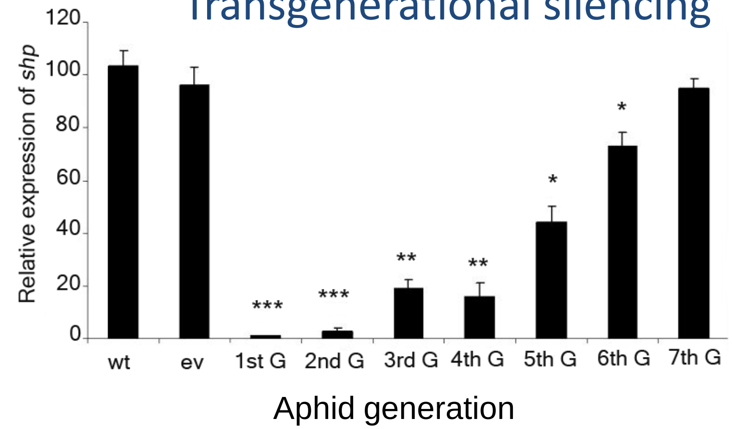
www.lastrefuge.co.uk





Abdellatef et al. 2014, Plant Biotech. J. 13:849

Transgenerational silencing

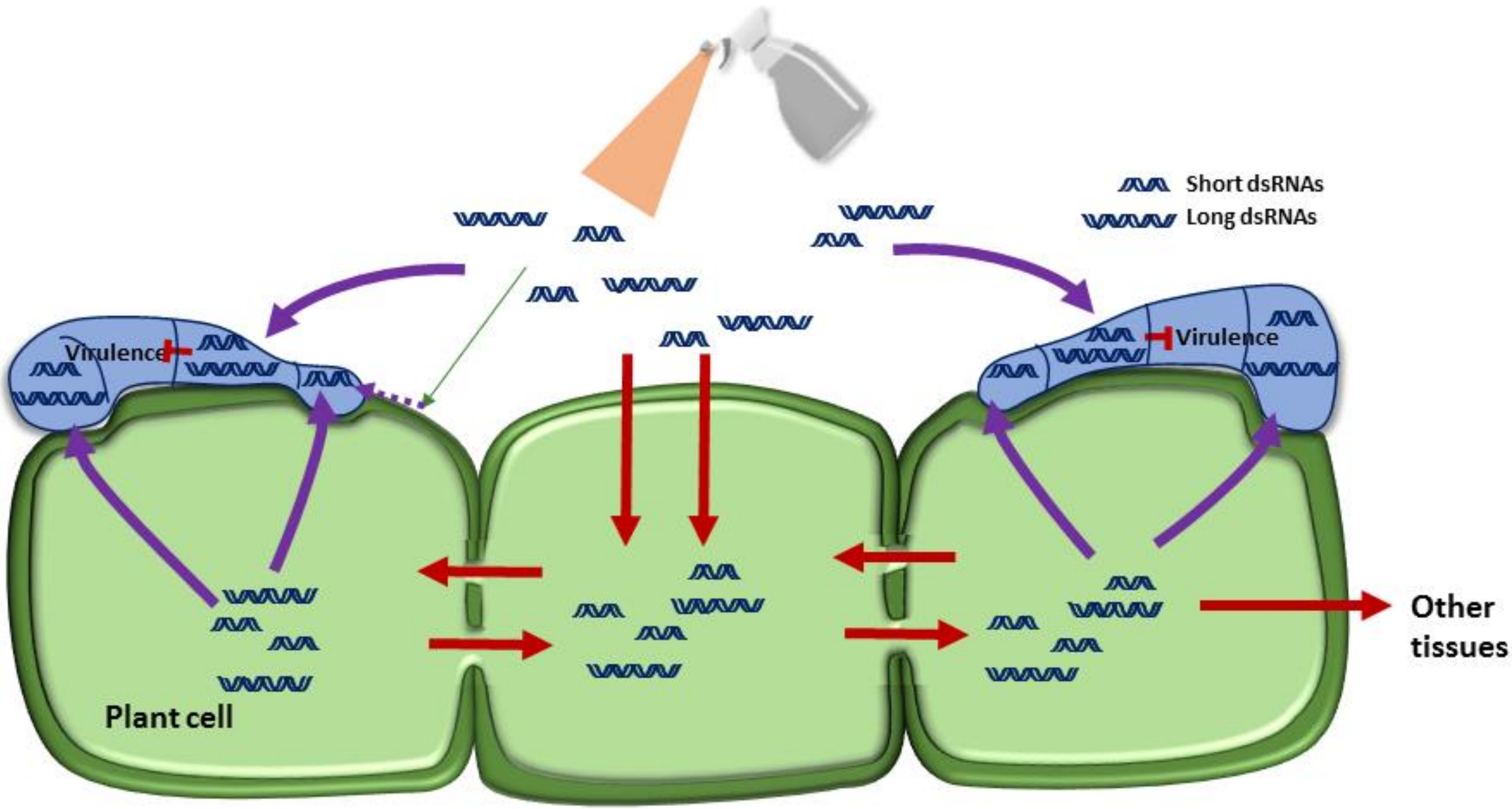


One week on *shp*-dsRNA plants

Seven generations on wt plants

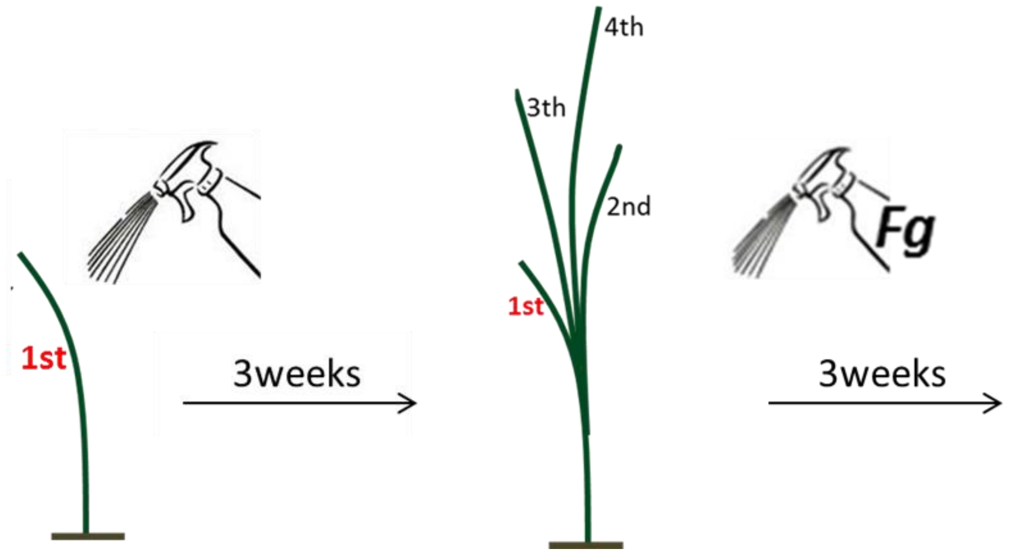
SIGS – dsRNA as a fungicide

in vitro	*in planta*	*on planta*
		
✓	✓	?

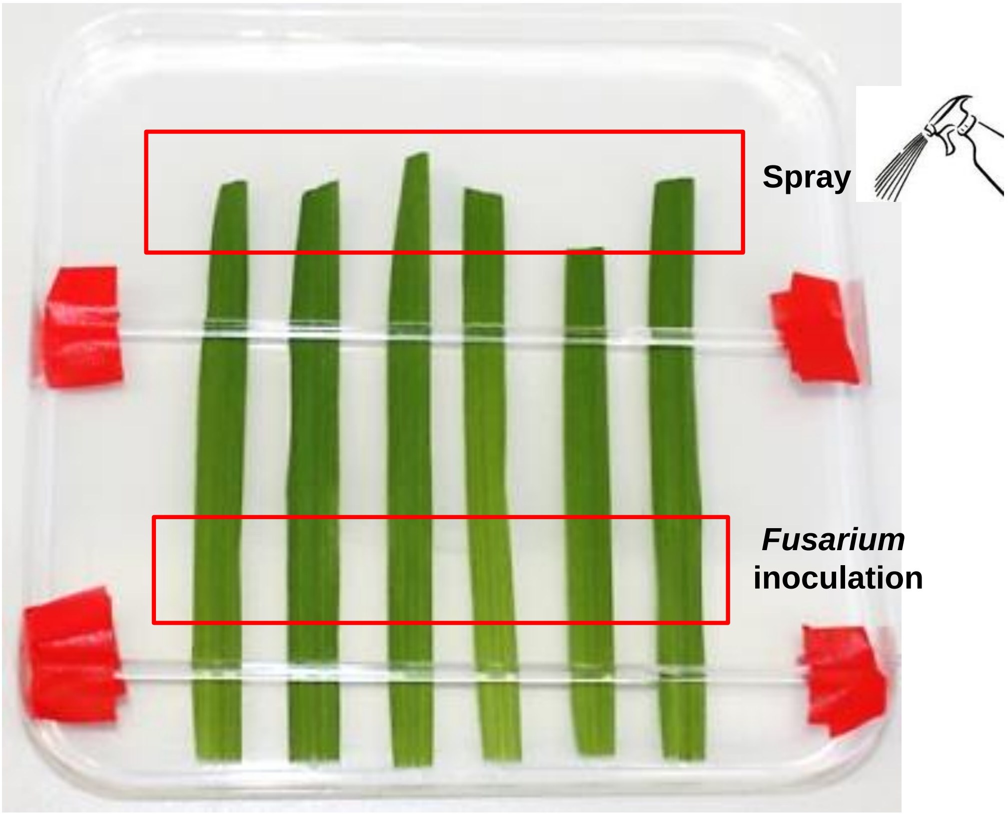


Adjusted from: Cai et al. 2018. Cross-Kingdom RNAi - nature's blueprint for modern crop protection technology. DOI 10.1016/j.mib.2018.02.003.

SIGS-mediated control of *F. graminearum* on whole barley plants



Systemic Setup: spray-induced gene silencing (SIGS)



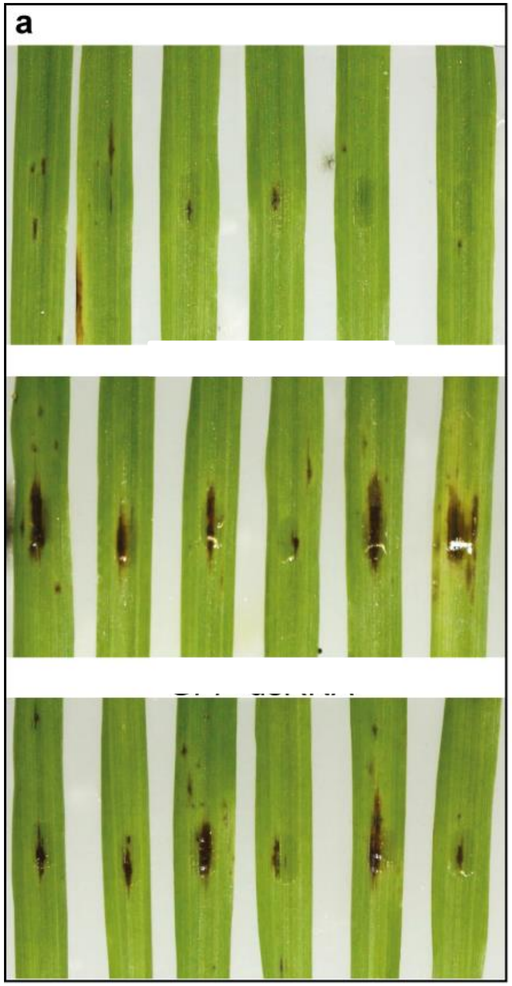
SIGS-mediated systemic control of *Fusarium graminearum*

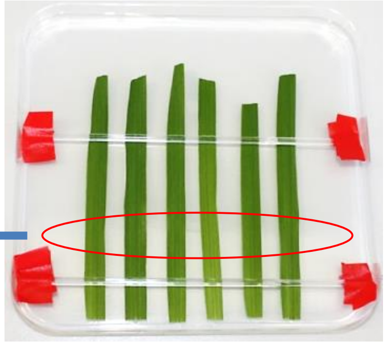
systemic effect

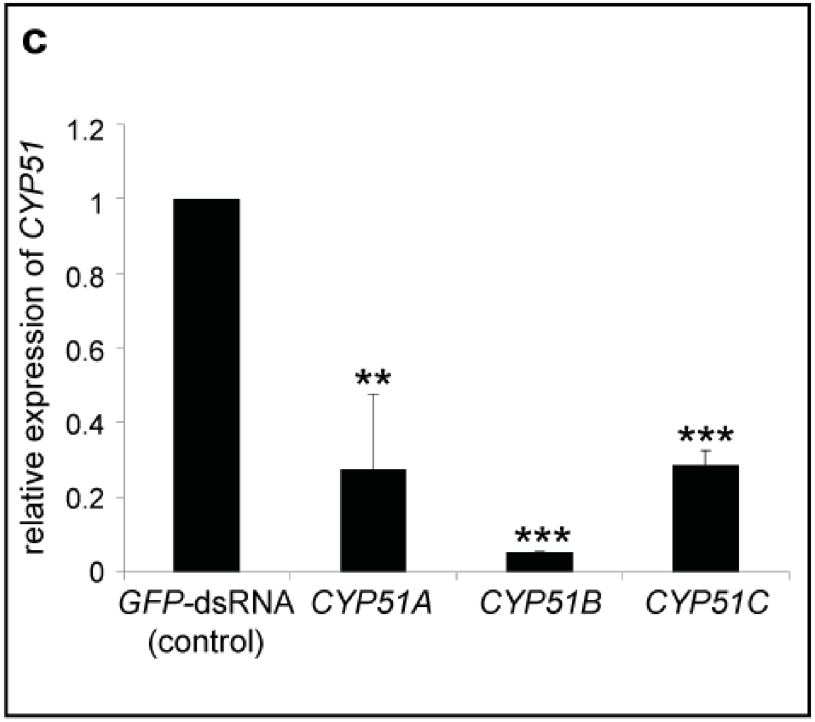
CYP3-dsRNA

Buffer control

GFP-dsRNA control







Koch et al. 2016, Plos Pathog. 10.1371/journal.ppat.1005901

RNAi-based crop protection: Application and potential drawbacks

- **dsRNA design strategies**
- **Stability of dsRNA (formulation potential)**
- **Off-target prediction and precautions**
- **Emergence of resistant/tolerant microbes/pests?**
- **Accesibility for application and cost considerations**

Off-target search

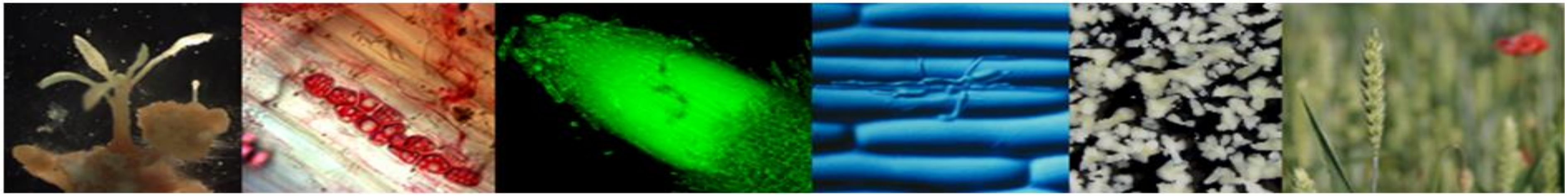
Table S1. Prediction of *CYP3RNA* off-target transcripts

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).



THANK YOU FOR ATTENTION!

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