Host-Induced Gene Silencing to Engineer Resistance to FHB

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RNA-Based Plant Protection

Is there an agronomic potential for application?
The Plant Pathogen
*Fusarium graminearum*

Jansen et al. 2005 PNAS 102

Macroconidia

Toxin syndrome

Head blight disease

Necrotrophic growth
Fusarium species threaten harvest and food safety

**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)

Fusarium Head Blight
RNA-based crop protection exploits RNA interference

Different mechanisms of RNA interference (gene silencing)

- Transcriptional gene silencing (TGS)
- Post-transcriptional gene silencing (PTGS)

DNA → mRNA → Protein

Small inhibitory RNAs
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 Argonautes in Arabidopsis

Image from Jagtap et al. 2011
Host-Induced Gene Silencing

oversimplified model

pathogen/pest

Target mRNA*

N

Transgenic plant

siRNA*

dsRNA*

? Transfer of inhibitory RNA

N

HIGS construct

= Target gene-specific DNA under control of inverted promoters gives rise to inhibitory RNA

*Lethal target gene

siRNA: DICER-released small interfering RNA; ds: double stranded RNA; N: nucleus
dsRNA-GFP plants inoculated with $\text{GFP}$-tagged *Fusarium graminearum*

Control*  
\[ \text{excitation 395 nm} \]

\[ \text{visible light} \]

*Golden Promise inoculated with Fg-GFP

$\text{GFP} = \text{Green Fluorescence Protein}$
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

Azole fungicides = sterol demethylation Inhibitors (DMI)

De Souza et al. 2009
Synthesis of dsRNA for *in vitro* studies was performed using BLOCK-iT RNAi TOPO Transcription Kit (Invitrogen).

Clone sequences of **CYP51A** (294nt)

CGGTCCATTGACAATCCCCGTCTTTGGTAGCGATGTCGTATACGATTGTCTCACAACGTGAAGCTCATGGAACAAAAGAAGTTTGTCAAGTTTGGCCTTACGCAAAA
AGCACTCGAGTCACACGTCAGTTAATCGAGCGAGAGTTCTTTGACTACGTGCAAATGACTCATCCTTTTTGTCGAGAAGCTCACTGACCATCGATGTCCCCAAGGC
AATGGGCTGAGATAAACACTTTTACTGCTACCCATTGCTCTTTCAGGGGTTCACCCCATCAACTCTCATGCTCTCGACCTCGATATGGGCTTCCACCCCCATCAGAACTAGCACCATCGATGTCCCCAAGGC
AATGGCTGAGATAAACACTTTTACTGCTACCCATTGCTCTTTCAGGGGTTCACCCCATCAACTCTCATGCTCTCGACCTCGATATGGGCTTCCACCCCCATCAGAACTAGCACCATCGATGTCCCCAAGGC

Clone sequences of **CYP51B** (220nt)
CAGCAAGTTTGACGAGTCCCTGGCCGCTCTCTCTACCCAGCTGATTAGGCGCTACGCTGCAGAGATATTATACGGCGACTGCCTTAAAATTCTCCTTGGCAAATCGA
AGGGAGCACCAGCCAGGGGACTGTGGTCCAGTCCAGGAGACTATCGATGACACTATCAAGAGCGCCGGCGCAAGGGAACACGATCCGAAGCATGATGAACTGACATTGAAGCA
CTCTGTGAACCTCT

Clone sequences of **CYP51C** (238nt)
ATTGGGAACCGTAACTATTTGGCGTGACCCGTACTCTTTTTCTTGACTGCAGAGATAAATACGGCGACTGTCTTTACCTTTTATTCTCTCTTTGCCAATACGGG
AGTTGGCAAGTTTGACGAGTCCCTGGCCGCTCTCTCTACCCAGCTGATTAGGCGCTACGCTGCAGAGATATTATACGGCGACTGCCTTAAAATTCTCCTTGGCAAATCGA
AGGGAGCACCAGCCAGGGGACTGTGGTCCAGTCCAGGAGACTATCGATGACACTATCAAGAGCGCCGGCGCAAGGGAACACGATCCGAAGCATGATGAACTGACATTGAAGCA
CTCTGTGAACCTCT

The inhibitory dsRNA *CYP3RNA*

*dsRNA: CYP3RNA*  
*buffer*

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

**pGEM-T Easy cyp51 part B, A, C**
In planta experiment

Vector for plant transformation

Arabidopsis

Barley
**CYP3RNA processing**

**Plant cell**

- **Genomic DNA** → dsRNA → dsRNA → siRNA 21-25nt

**Cytoplasm**

- **Dicer**

**Nucleus**

- **Host induced gene silencing**

**Fungal cell**

- **Ergosterol biosynthesis**
  - Acetyl-CoA → 3-hydroxy-3-methyl-glutaril-CoA → Mevalonate → Dimethylallyl-PP → Farnesyl-PP
  - Geranyl-PP → Squalene synthase → Squalene
  - (allylamine, terbinafine) → Squalene epoxidase → 2,3-oxidosqualene

- **CYP51***

*Cytochrome P450 Sterol 14α-Demethylase*
CYP3RNA expression inhibits infection

Arabidopsis

Koch et al. 2013, PNAS 110
CYP3RNA expression inhibits infection

Barley

Wt  ev  L42  L9  L2  L7  L3  L4  L6  L5  L8  L14

Wild type  transgenic lines expressing CYP3RNA  transgenic line expressing empty vector

wt = cv. Golden Promise
Strong inhibition of Fusarium Head Blight

Barley

Control vs. CYP3RNA

CYP3RNAi+Fg vs. CYP3RNAi-Fg
Strong silencing of fungal CYP51 expression \textit{in planta}

Barley

Fusarium infected Leaves

CY51A expression

CY51B expression

CY51C expression

Normalized with fungal β-tubulin
# Off-target analysis

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

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

*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?

Fungus

Plant

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.

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

Dr. Aline Koch

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transgenic barley

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RNAi mutants

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

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