

Expression/suppression of haustorial transcripts

Questions to address:

- Can we silence rust genes by RNAi in wheat plants?
- Can we use this for functional analysis of rust genes, or for rust control?
- How can we best express rust effector proteins in wheat cells?

Stripe rust, *P. striiformis*



Resistant variety

Susceptible variety

Precedence for *in planta* gene silencing?

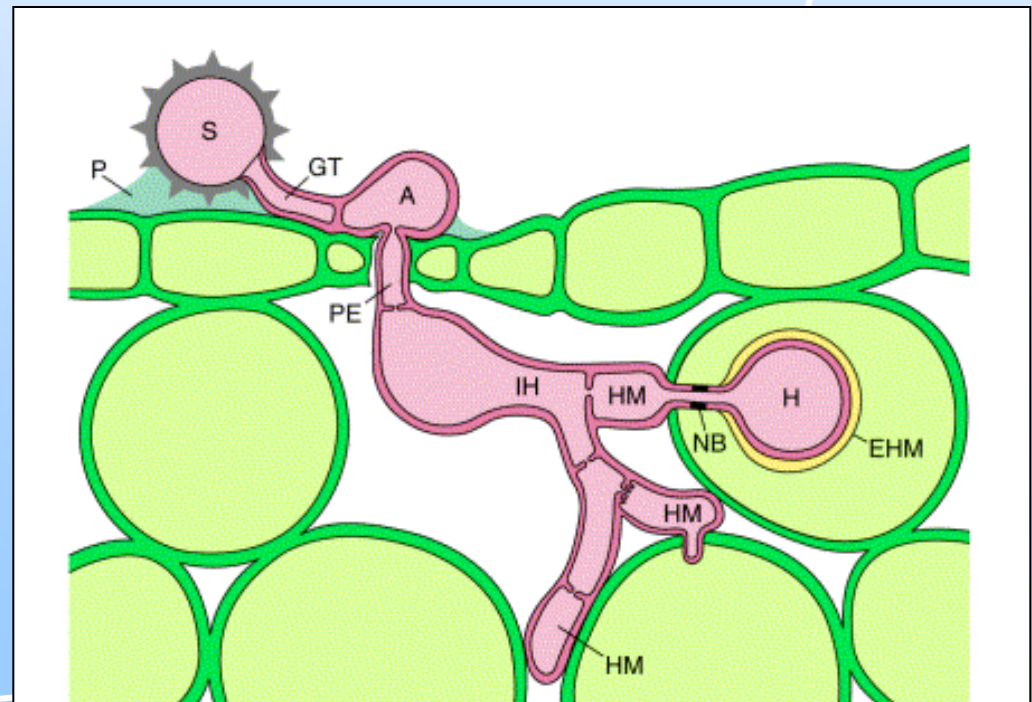
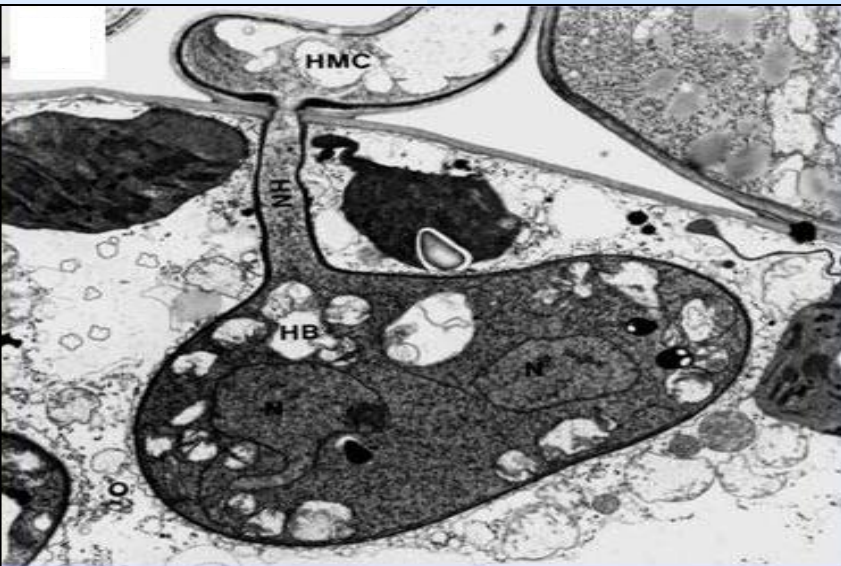
- Silencing by pathogen gene expression in plants has been demonstrated for some viruses, sedentary nematodes (e.g. root knot) and even parasitic weeds.
- Rusts colonize living plant cells like the other organisms where the approach works.

Stripe rust
P. striiformis



Silencing considerations

- Will the silencing signal enter haustoria? Through the extrahaustorial membrane & matrix, haustorial cell wall and membrane
- How far does the silencing signal extend? Haustorial mother cells? Intercellular hyphae?
- Will VIGS work as a transient delivery system?



Kurt Mendgen and Matthias Hahn

Initial isolation of stripe rust haustorial sequences

- ~ 5000 haustorium EST were assembled into 1253 unigenes
- ~15 predicted to code for secreted proteins using SignalP 3.0 algorithm, iPSORT
- Variable in whether they are haustoria specific in their expression.



Chuntao Yin

Purified haustoria



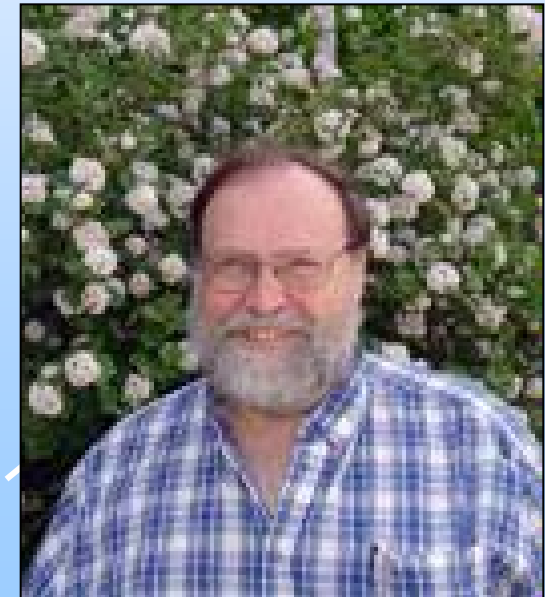
Cooperators: Zhensheng Kang, Xianming Chen

Silencing Assay Methods

- Infect wheat seedlings (2-3 leaves) with BSMV virus carrying rust gene fragment
- Inoculate with rust ~10-20 days later
- Isolate RNA from doubly infected plants ~7 days later
- Compare expression in seedlings infected with recombinant VIGS construct to seedling infected with VIGS vector; use EF1 (or β -tubulin) gene as internal control



Double
infection



Jim Jurgenson, UNI

PST5a23 results:

- Haustorial EST; predicted 108 amino acid secreted protein
- Expression: infected leaf/urediniospores 3617:1
haustoria/infected leaf 29:1
- Weak homology to a predicted 106 aa *P. graminis* secreted protein (E~10E-4)

Expression of the Pst5a23 gene in nine seedlings infected with the Pst5a23 construct

<u>Seedling #</u>	<u>VIGS/ virus control</u>
1	0.63
2	0.14
3	0.38
4	1.38
5	0.42
6	0.37
7	0.28
8	0.12
9	0.43

Silencing ranged from 0 to 8-fold in different seedlings

PST12h2 results

- Haustorial EST; putative secreted protein
- Expression: infected leaf/urediniospores 23:1
haustoria/infected leaf 91:1
- No homology in fungal databases

Expression of the Pst12h2 gene in nine seedlings infected with the Pst12h2 construct

<u>Seedling #</u>	<u>VIGS/ virus control</u>
Exp.1-1	0.63 ± .03
2.	0.71 ± .03
3	0.58 ± .02
4	0.50 ± .01
5	1.01 ± .06
6	1.85 ± .04
Exp.2-1	1.45 ± .03
2	0.58 ± .13
3	0.62 ± .02
4	0.68 ± .01

PST12j12 results

- Putative secreted protein, haustorial EST, 133 amino acids
- Expression: infected leaf/urediniospores 104:1
haustoria/infected leaf 748:1
- No homology in fungal databases

Expression of the Pst12j12 gene in nine seedlings infected with the Pst12j12 construct

<u>Seedling #</u>	<u>VIGS/ virus control</u>
Exp1-1	0.50 ± .03
2	1.51 ± .13
3	0.36 ± .02
4	0.30 ± .03
5	0.43 ± .01
6	0.27 ± .13
Exp2-1	0.34 ± .01
2	0.40 ± .09
3	0.60 ± .03
4	1.12 ± .04
5	0.47 ± .01

PST12o3 results

- Haustorial EST; homology to *Uromyces fabae* hexose transporters
- Expression: infected leaf/urediniospores 0.16:1
haustoria/infected leaf 13:1

Expression of the Pst12o3 gene in nine seedlings infected with the Pst12o3 construct

<u>Seedling #</u>	<u>VIGS/ virus control</u>
Exp1-1	0.18 ± .04
2	0.08 ± .01
3	0.46 ± .13
4	0.20 ± .05
5	0.27 ± .08
Exp2-1	0.84 ± .15
2	0.15 ± .00
3	0.38 ± .01
4	0.13 ± .01
5	0.20 ± .01

PST5a1 results

- Putative chitinase, haustoria EST
- Expression: infected leaf/urediniospores 5981:1
haustoria/infected leaf 151:1
- Homologous to gene family in *P. graminis*, most homologous gene was 82% ID for 179 amino acids

Expression of the Pst5a1 gene in nine seedlings infected with the Pst5a1 construct

<u>Seedling #</u>	<u>VIGS/ virus control</u>
Exp1-1	0.59 ± .06
2	0.70 ± .04
3	0.30 ± .01
4	0.51 ± .00
5	1.62 ± .09
6	0.20 ± .01
Exp2-1	0.50 ± .02
2	0.69 ± .01
3	1.23 ± .12
4	0.82 ± .04
5	0.66 ± .04
6	0.60 ± .03

PST2a5 results

- Haustorial EST; putative secreted protein
- Expression:
infected leaf/urediniospores 120:1
haustoria/infected leaf 92:1
- No homology in fungal databases

Seedling #	VIGS/ virus control
Exp 1-1	2.13 ± .69
2	3.10 ± .09
3	1.01 ± .02
4	0.48 ± .04
5	1.68 ± .92
6	2.71 ± .17
Exp 2-1	0.35 ± .12
2	0.92 ± .08
3	0.54 ± .00
4	1.62 ± .30
5	0.85 ± .04
Exp 3-1	1.67 ± .11
2	1.86 ± .04
3	27.43 ± 3.47
4	0.45 ± .04
5	0.69 ± 0.13
6	4.71 ± 0.16

PST9F18 results

- Haustorial EST; putative secreted protein
- Expression:
infected leaf/urediniospores 0.001:1
haustoria/infected leaf 419:1
- No homology in fungal databases

Seedling #	VIGS/ virus control
Exp 1-1	1.15 ± .05
2	0.74 ± .03
3	9.37 ± .34
4	3.66 ± .17
5	1.06 ± .14
6	3.03 ± .08
Exp 2-1	1.63 ± .02
2	6.81 ± .54
3	0.78 ± .06
4	1.54 ± .11
5	0.66 ± .06
6	3.58 ± .21

Expression in control seedlings for PST2a5
PST9F18 varied 15 fold and 9 fold!

No evidence of silencing was found for any *constitutively* transcribed genes

Gene	Range of gene expression	# seedlings
β -tubulin	0.73 – 1.5	12
EF1	0.80 – 1.53	6
GAPDH	0.81 – 1.77	6
Actin	0.85 – 1.70	6
EPSPS (<i>P. graminis</i>)	0.87 – 1.51	6

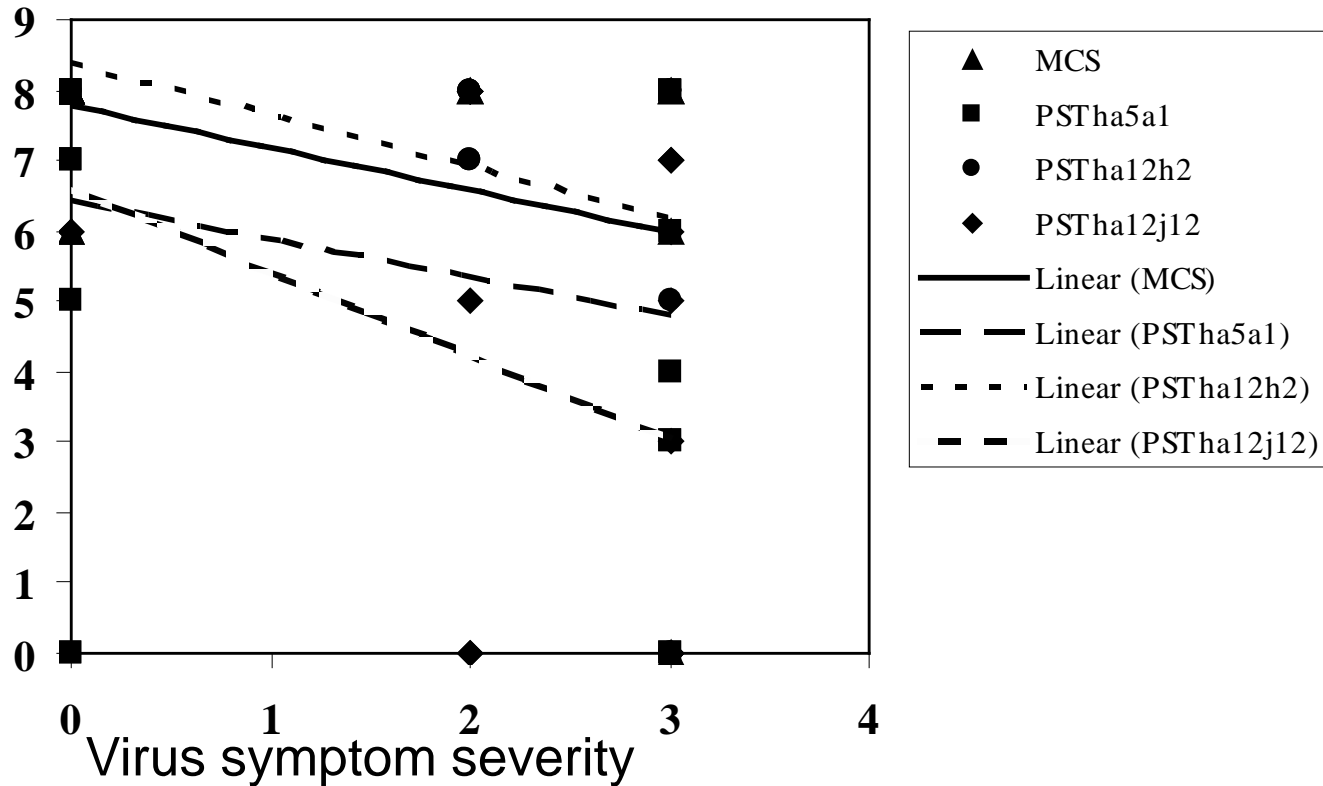
Ability to detect silencing depends on expression patterns of rust genes

Testing the timing of rust inoculation after virus inoculation

Fold change in expression level of PST12j12		
10 day	15 day	20 day
0.41 ± 0.02	0.67 ± 0.07	0.28 ± 0.06
0.28 ± 0.01	0.29 ± 0.01	1.28 ± 0.11
1.24 ± 0.04	1.37 ± 0.09	1.24 ± 0.25
0.19 ± 0.02	0.66 ± 0.05	0.65 ± 0.02
0.125 ± 0.003	0.68 ± 0.10	0.71 ± 0.03
0.36 ± 0.03	0.45 ± 0.07	0.96 ± 0.11

Virus infection/symptoms can affect rust ratings

Rust symptom severity



- Virus symptoms affect rust development on some leaves
- Virus symptom levels are not well correlated with amount of silencing.
- An ideal cultivar would not show severe virus symptoms

Some wheat lines silence well with fewer virus symptoms



- 1: healthy plant
- 2: inoculated with BSMV::MCS
- 3: inoculated with BSMV::PDS

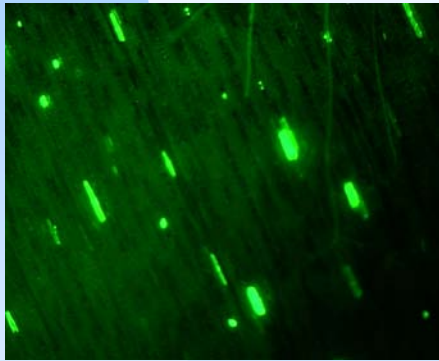
Potential assays for transient expression of effectors

Biolistic delivery

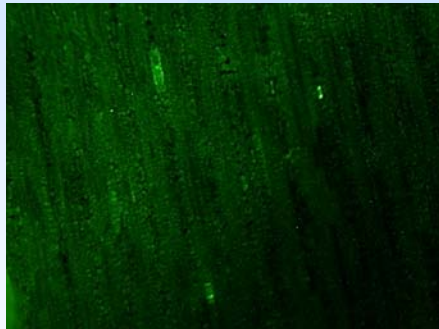
Rxo1 Maize

Plasmids

GFP only



GFP and
AvrRxo1



Bacterial delivery of AvrRxo1 by T3SS



Rxo1 maize, *rxo1* maize

Other possibilities:

Agrobacterium delivery?

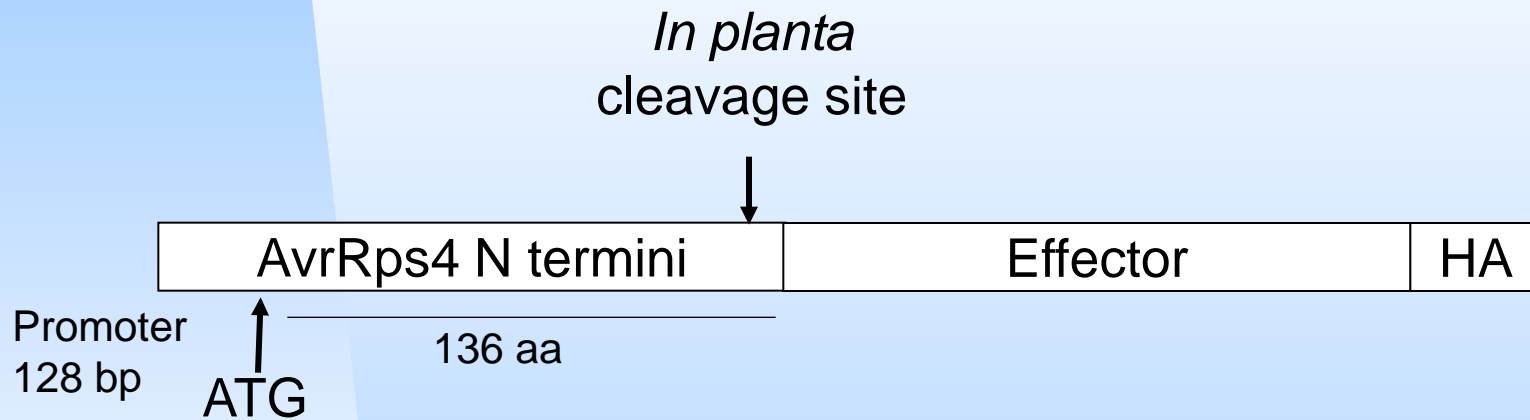
Protoplast transformation?

Advantage of transforming a patch of cells:

Can monitor gene expression or biochemical changes

Can monitor cell death easily

One Version of an Effector Detector Vector; pEDV3



Protein delivered by *P. syringae* DC3000 (or variants)

Many bacterial nonpathogens of wheat cause HR when infiltrated



mock inoculated

Pst DC3000

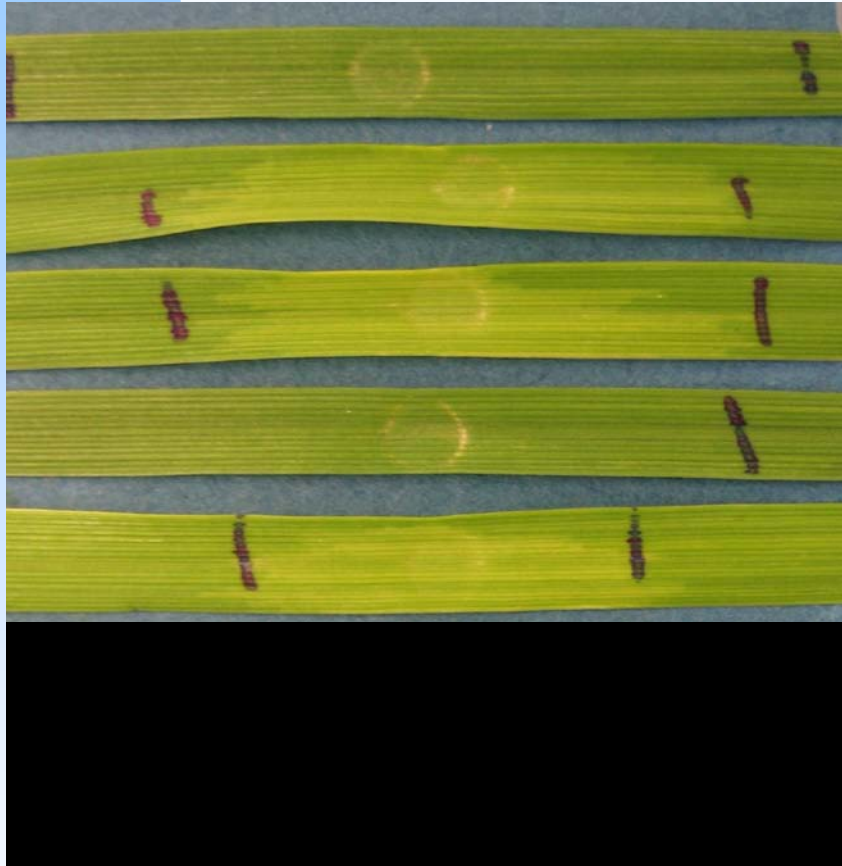
CUCPB5500*

CUCPB5500 w/ pEDV6

* *CUCPB5500* is a DC3000 with multiple effectors deleted and was obtained from Allan Collmer's group

* Some barley cultivars don't react to these isolates

P. fluorescens strain EtHAN delivers bacterial effectors into wheat:



EtHAN strain

EtHAN w/ genomic copy of *avrRpt2*

EtHAN w/ genomic copy of *avrRpm1*

pEDV3 empty expression vector

pEDV6:: *avrRpt2*

pEDV6:: *avrRpm1*

Mock (water)

EtHAN strains were provided by Jeff Chang

P. fluorescens strain EtHAn delivers bacterial effectors into wheat:
DAB staining showing H₂O₂ accumulation



EtHAn strain

EtHAn w/ genomic copy of *avrRpt2*

EtHAn w/ genomic copy of *avrRpm1*

pEDV3 empty expression vector

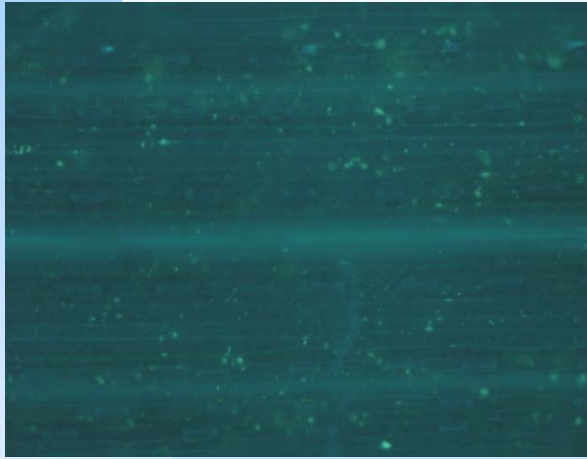
pEDV6:: *avrRpt2*

pEDV6:: *avrRpm1*

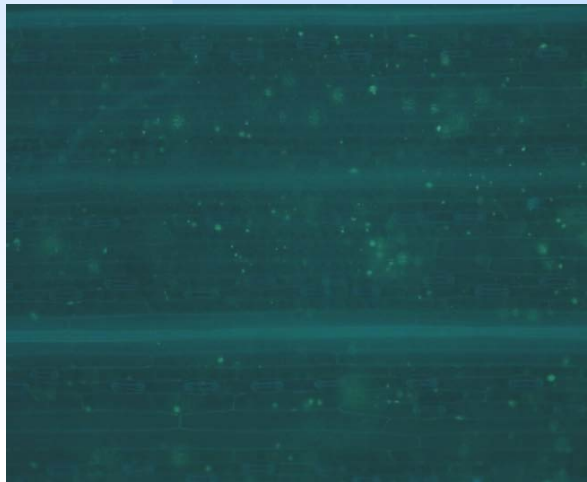
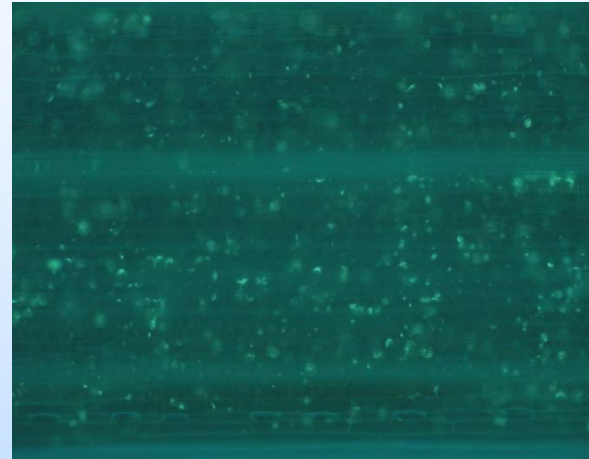
Mock (water)

P. fluorescens strain EtHAn delivering *Stagonospora ToxA* constructs; effects detected by callose staining

pEDV3 vector control



pEDV6::ToxA₄₈₋₅₃₇



pEDV6::ToxA₁₋₅₃₇



Mock (water)



Conclusions from gene delivery experiments

- Pseudomonas species can deliver proteins to wheat cells
- Not clear if they will deliver enough protein of effectors to monitor HR
- We need some rust Avr genes to test bacterial delivery systems

Conclusions from silencing experiments

- Some rust genes can be silenced by in planta RNAi expression
- VIGS system appears sufficient for delivery of silencing signal
- Ability to detect silencing probably depends on gene expression patterns

Stripe rust project cooperators:

Xianming Chen
Christina Cuomo
Zhensheng Kang
Jim Jurgenson
Chuntao Yin