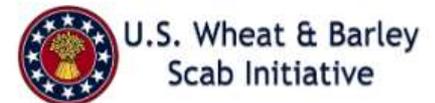


# The functional characterisation of candidate small secreted *Fusarium graminearum* effectors

**Kim Hammond-Kosack**

**Wheat Pathogenomics Team**



**7<sup>th</sup> Dec 2020**

*Fusarium graminearum*  
*Fusarium culmorum*

17 species of Fusaria  
are capable of causing  
Fusarium Head Blight  
disease on wheat



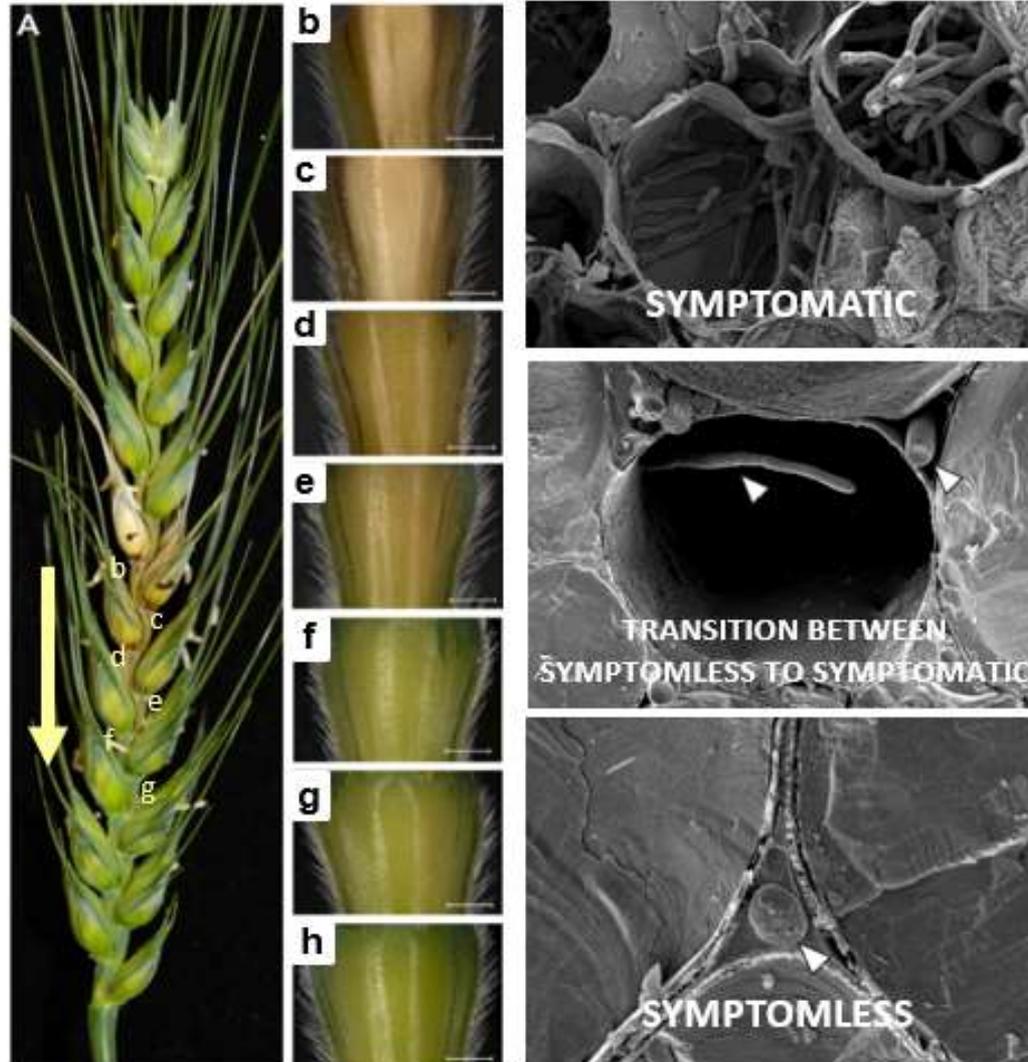
normal grain

diseased grain

# *F. graminearum* exhibits a biphasic lifestyle

A transition from symptomless to symptomatic infection over ~10 mm

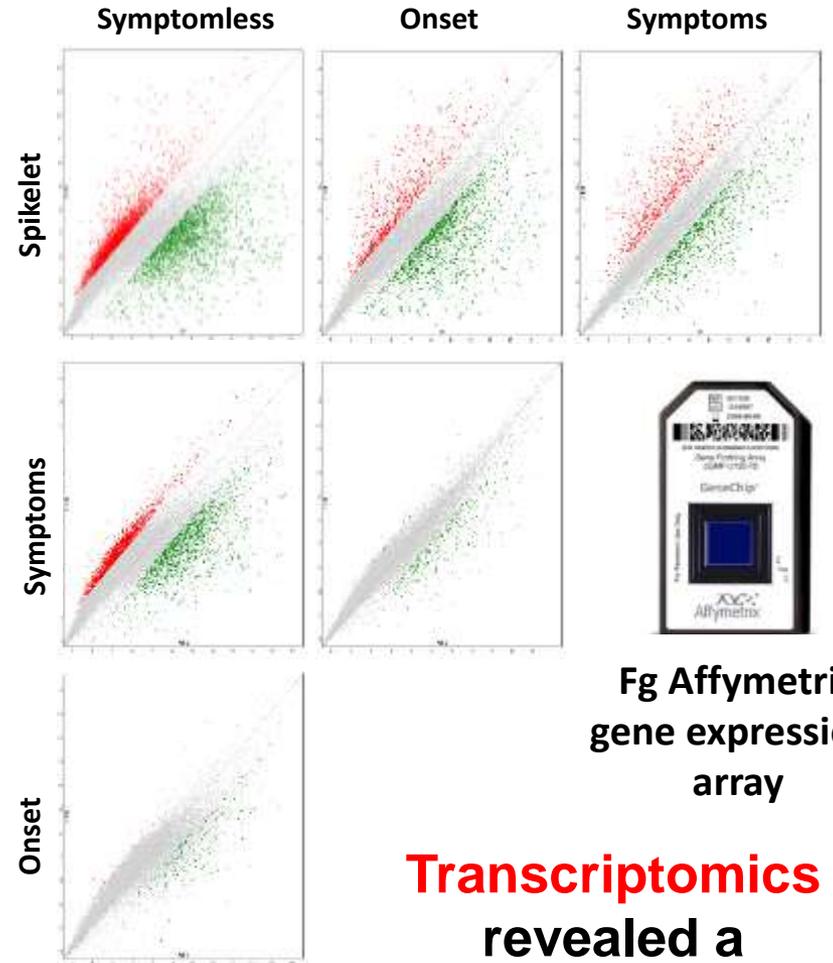
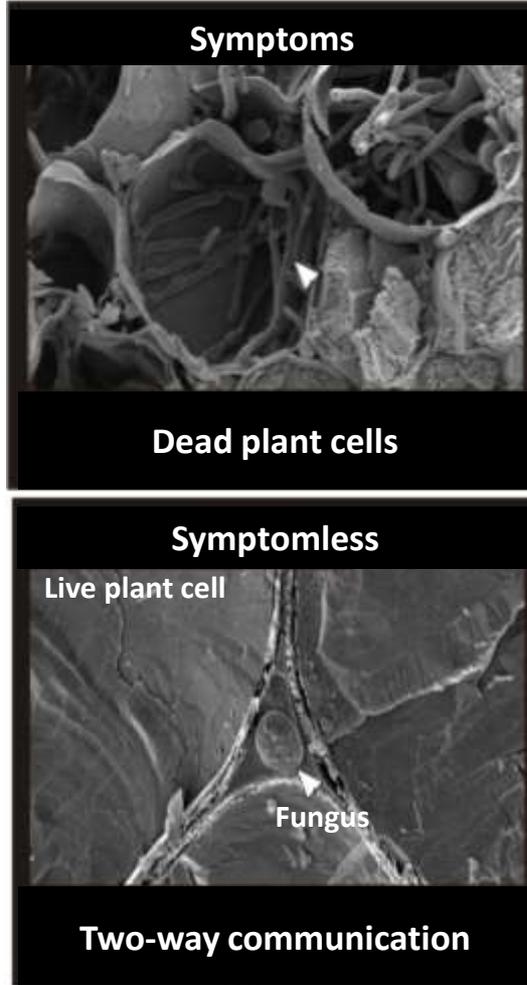
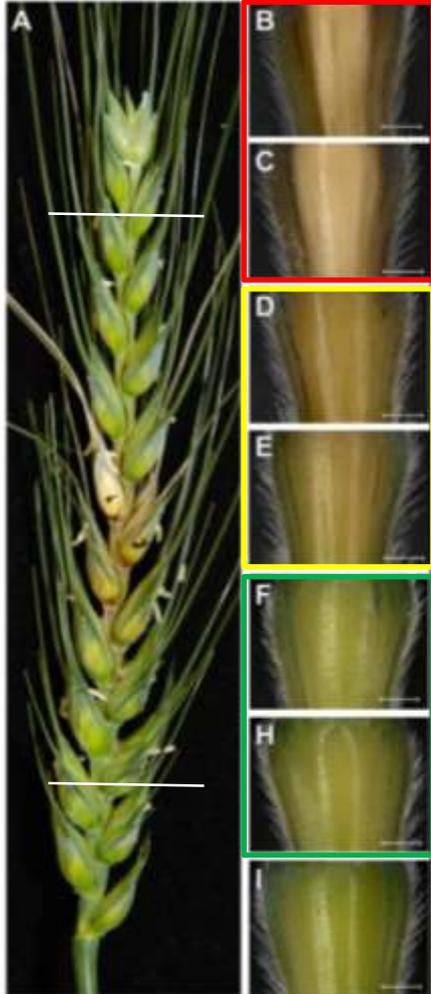
7 days post  
point  
inoculation



# Transcriptomics defined a novel spatial temporal pattern of *Fusarium* expression

Day 7

rachis



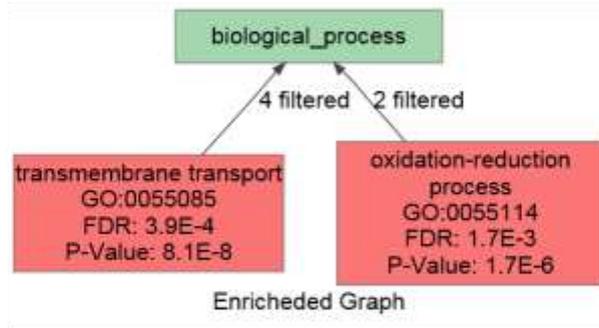
**Transcriptomics**  
revealed a  
**biphasic infection**  
**process**

- Full symptoms
- Onset of symptoms
- Symptomless

Brown et al. (2017) Mol. Plant Pathol.  
18, 1295-1312 (e-12564)

# DON mycotoxin promotes symptomless Fusarium infection

Blast2GO analysis of **up-regulated** genes during symptomless infection



**DON inhibits** plant defences

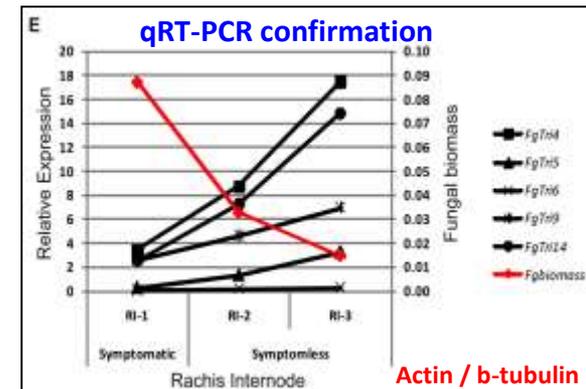
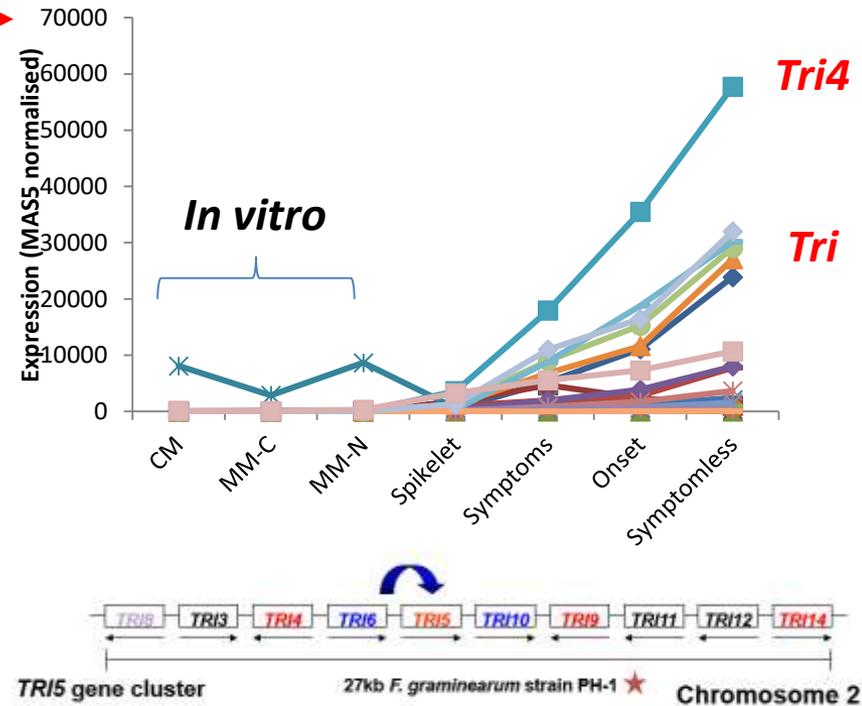


- Symptoms
- Onset
- Symptomless

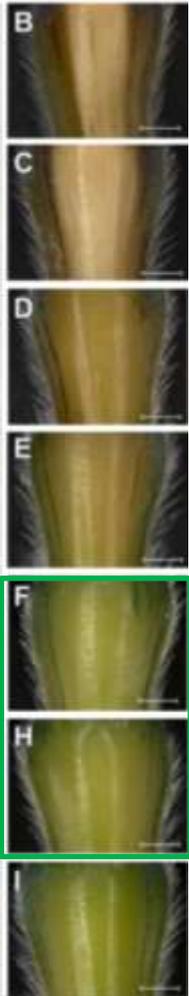


Cuzick (2008) *New Phytologist* 177(4); 990-1000

Induction of **DON** biosynthesis

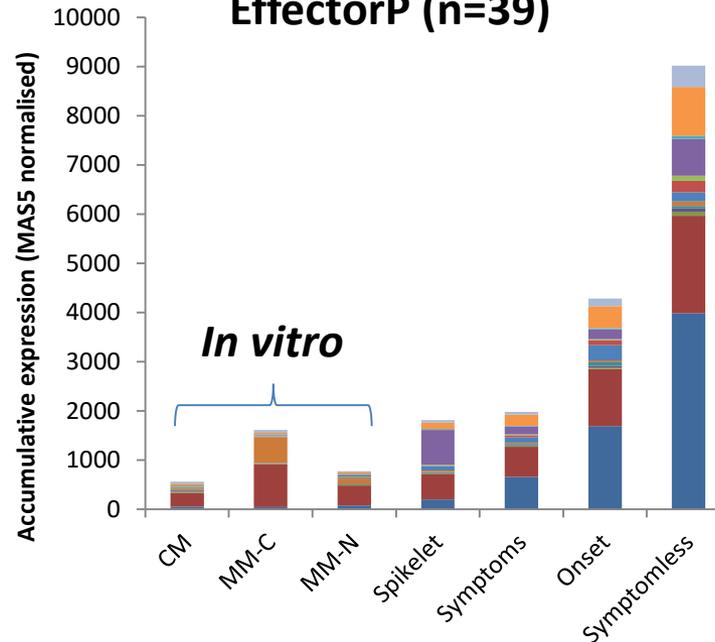


# Identification of numerous candidate effectors for characterisation



Accumulation of gene transcripts coding for **small**, cysteine-rich, repeat containing, **secreted** proteins

EffectorP (n=39)



Most effector genes highly expressed in symptomless phase (n=21) are **highly conserved** in multiple *Fg* isolates (n=16 Illumina seq)

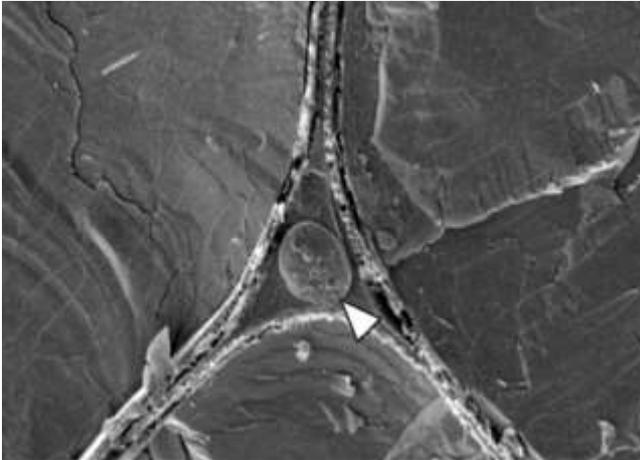
■ Symptoms

■ Onset

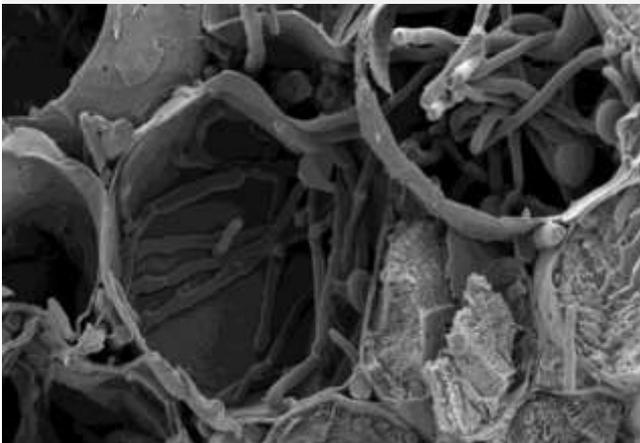
■ Symptomless

# Hypothesis: Secreted effector proteins contribute to virulence

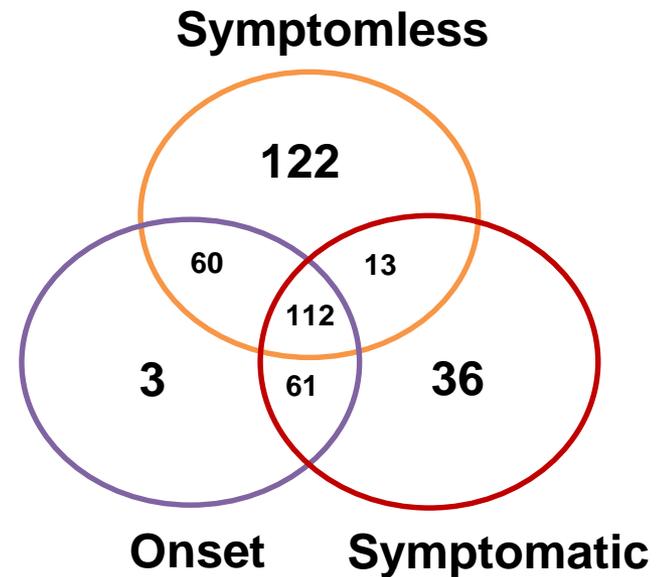
Effectors **SUPPRESSING**  
host defences



Effectors **ACTIVATING** host  
cell death

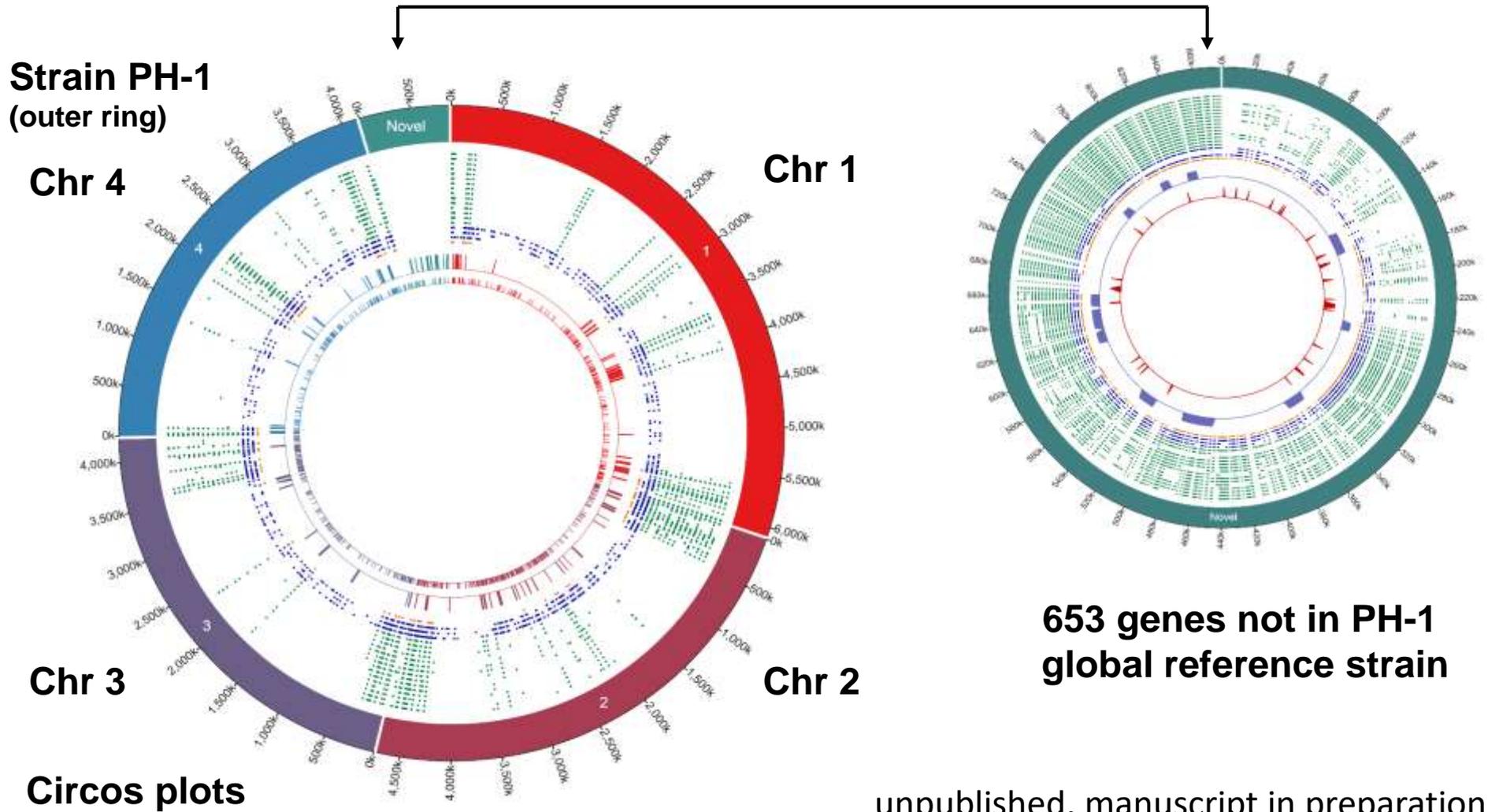


FgSSP effectors  
produced have **phase-  
specific expression**



# Identifying the *Fg* effectors in core and variable parts of the **PANGENOME** for 21 strains- **Brazil**, **USA** and **Australia**

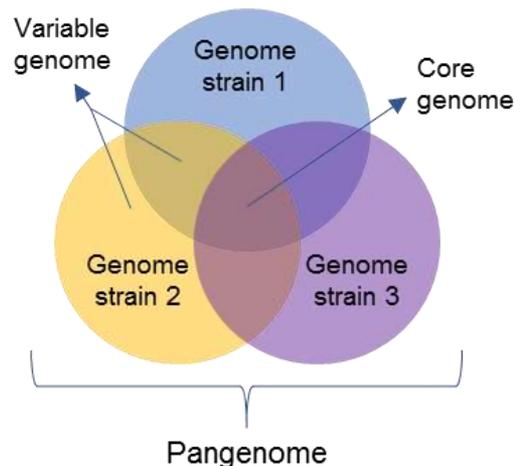
## 37Mb haploid genome



# *F. graminearum* pangenome – 21 strains



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	Core	Variable
Secretome	784	118 (15%)
<b>Effectors</b>	<b>161</b>	<b>35 (21.7%)</b>

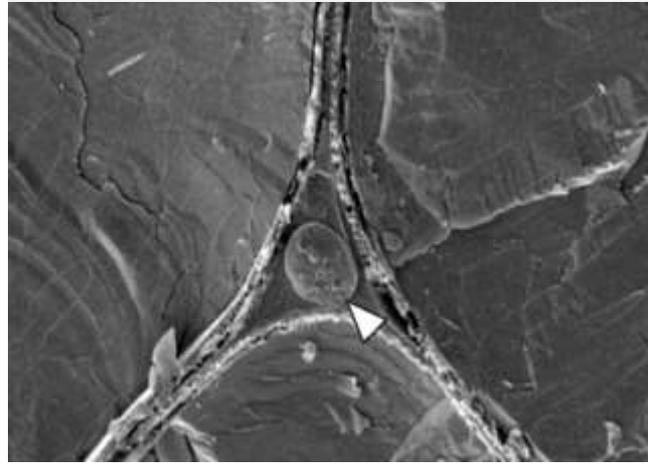
Description	Number of genes
Pangenome	14,798
Variable	1,798 (12%)
<b>Core</b>	<b>13,000</b>
<b>PH-1 genome (global ref)</b>	<b>14,160</b>
Genes present in one strain	195
New annotations (not present in PH-1)	653

- Presence of new 9 secondary metabolites cluster in the variable genome
- Pangenome was created using PanSeq software
- Reads were mapped using bwa and the cut-off of 80% reads map coverage was set using bedtools

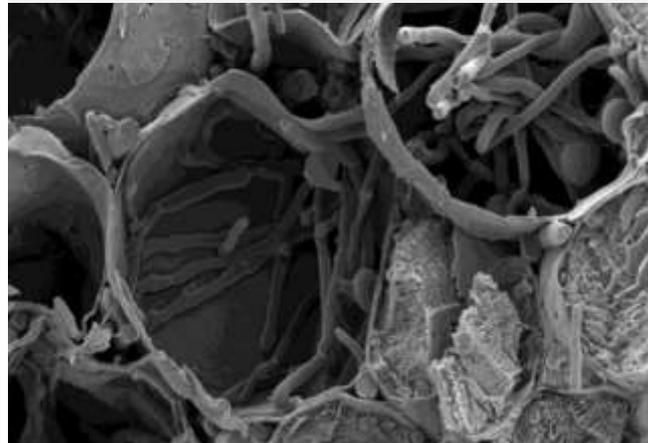
unpublished, manuscript in preparation

# Hypothesis: Secreted effector proteins contribute to virulence

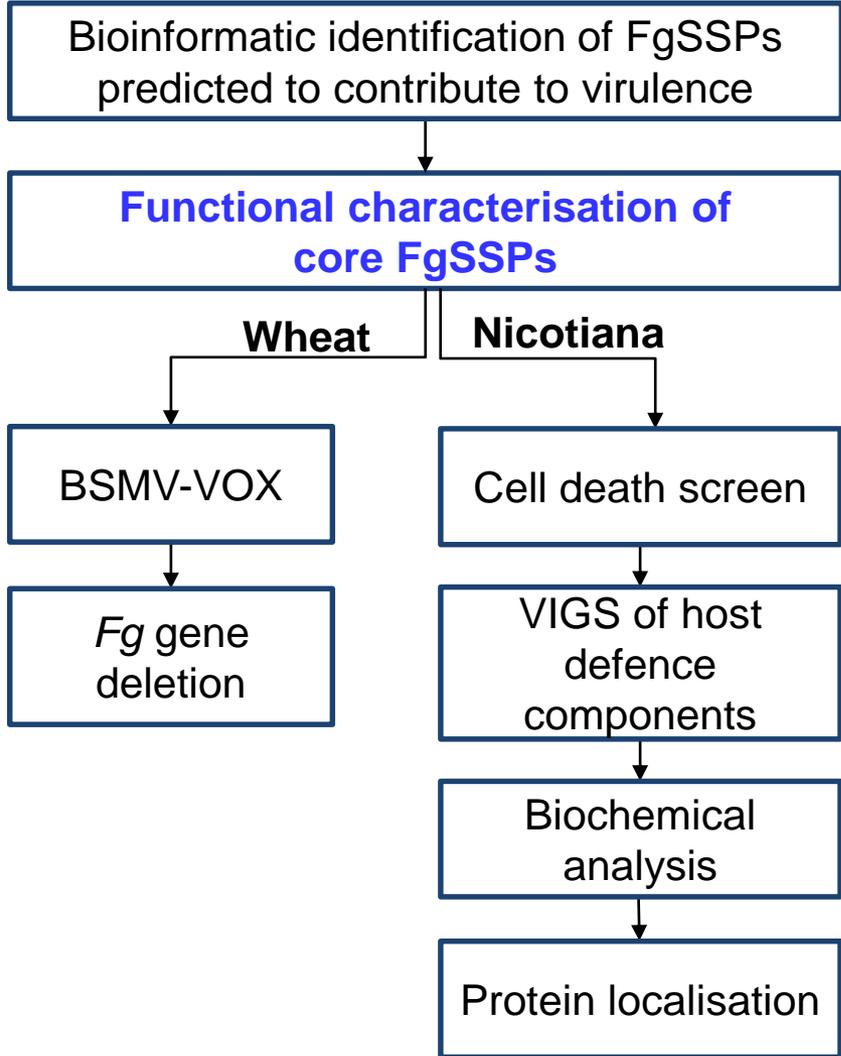
Effectors **SUPPRESSING** host defences



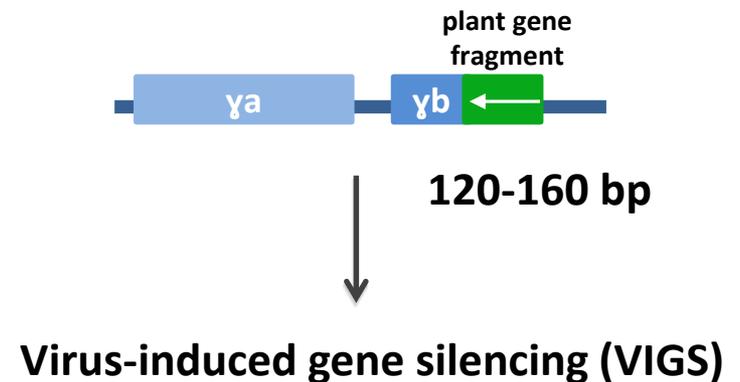
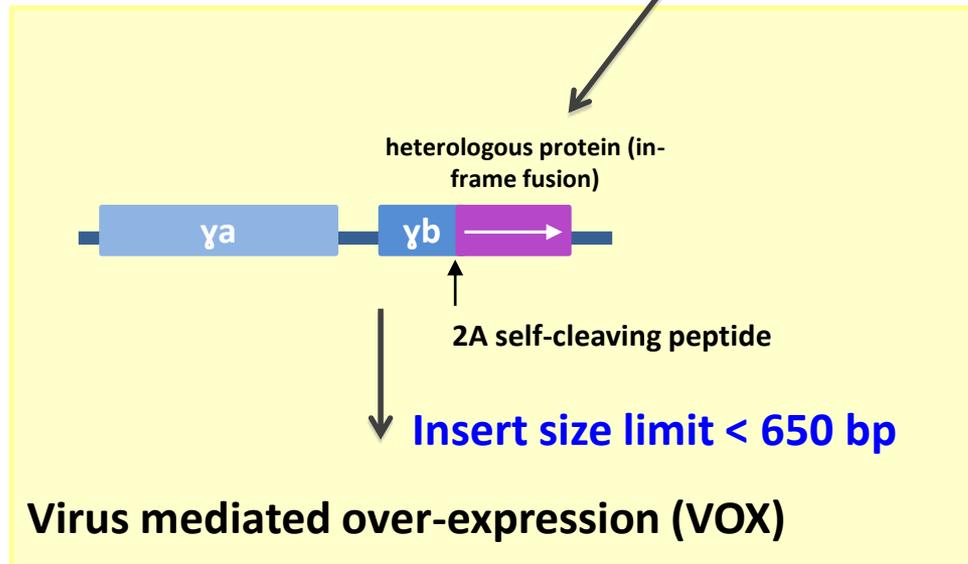
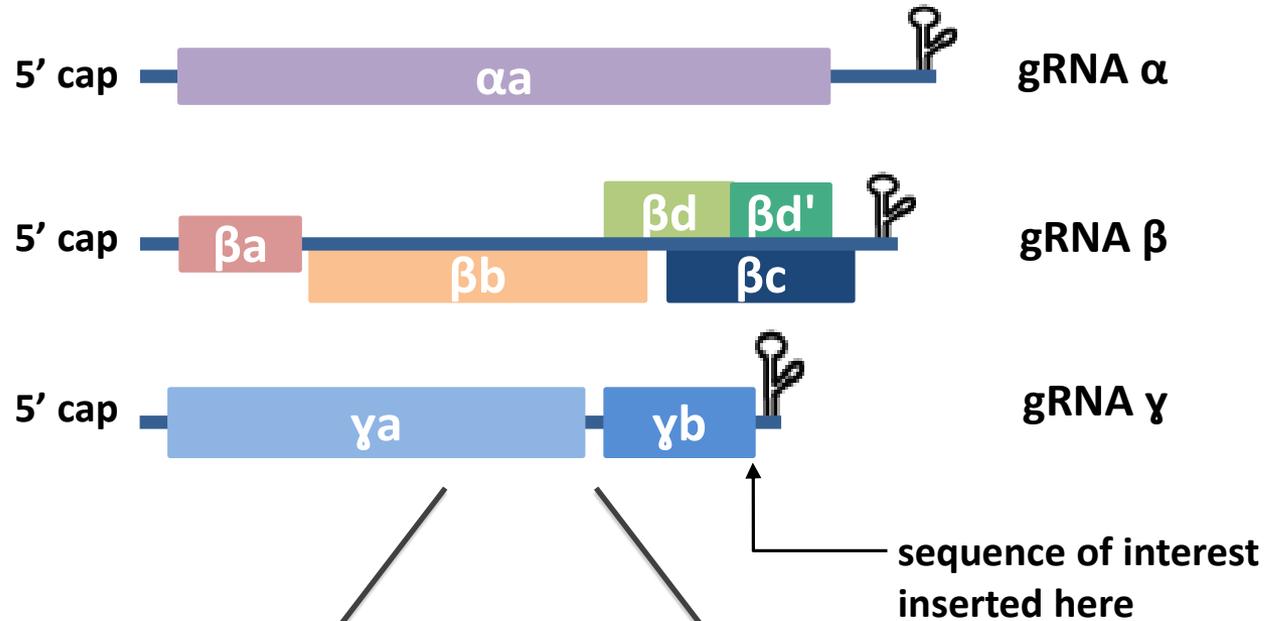
Effectors **ACTIVATING** host cell death



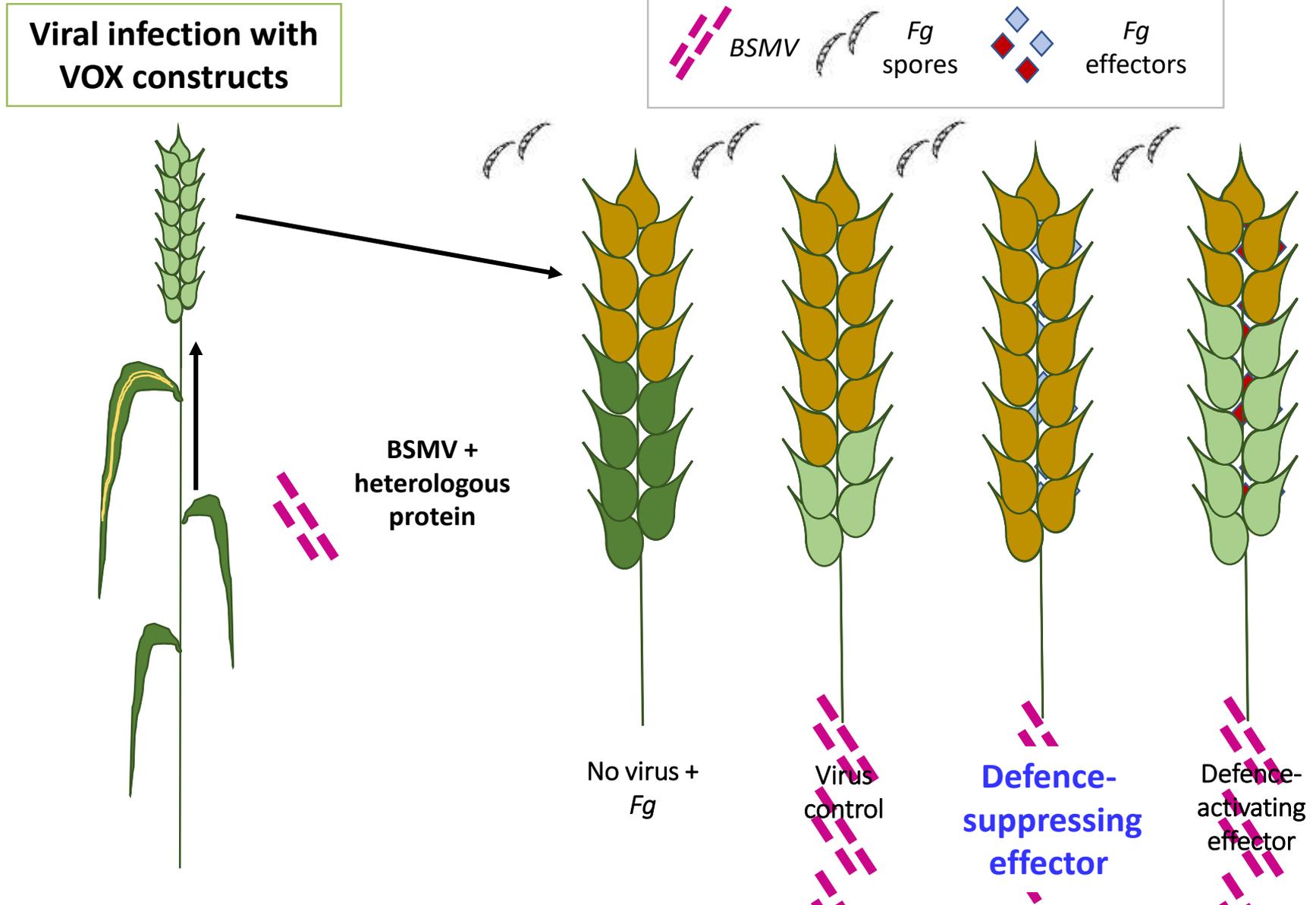
## Discovery pipeline



# Barley stripe mosaic virus (BSMV) as a tool for functional genomics

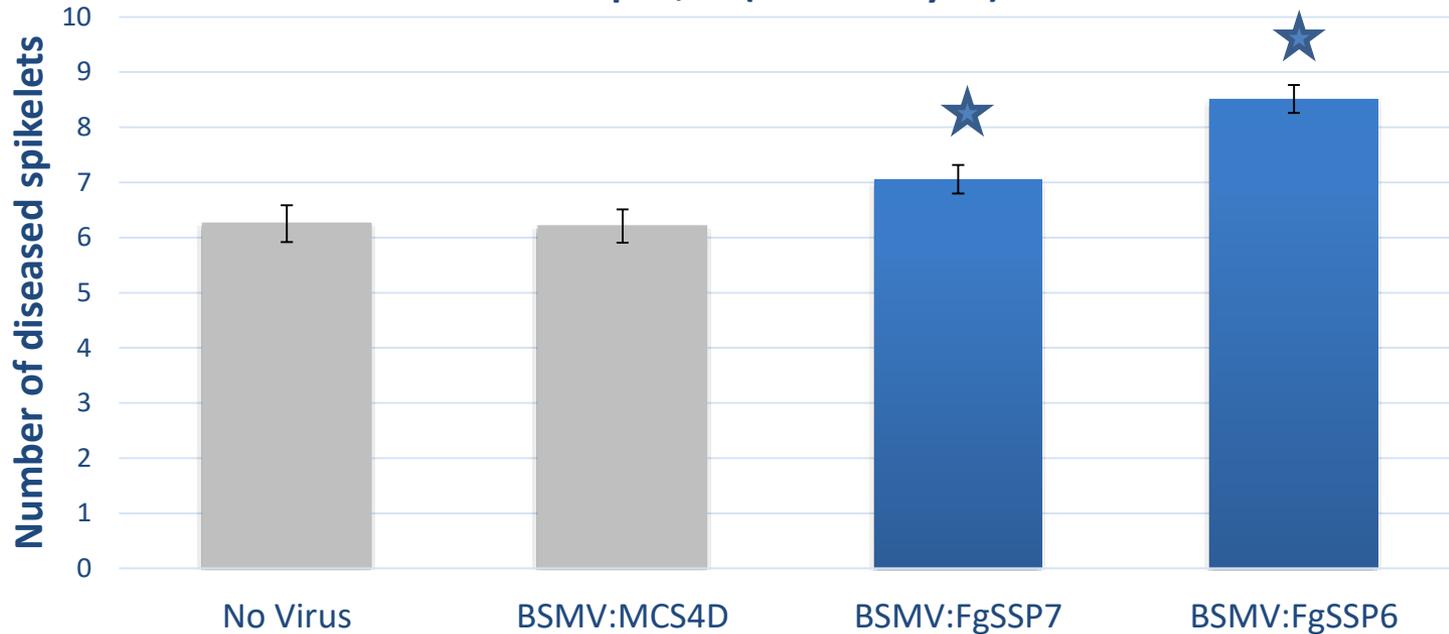


# Barley Stripe Mosaic Virus - VOX

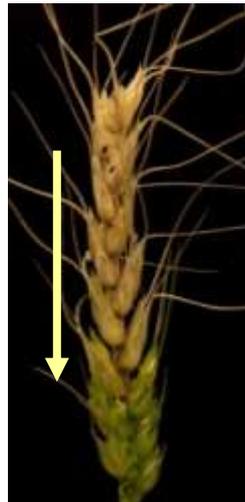


# VOX results for two annotated *Fg* effectors

Data 12 days after inoculation  
n=32 p<0,05 (GLM analysis)



**FHB**  
**diseased**  
**ears**  
Photos 12dai

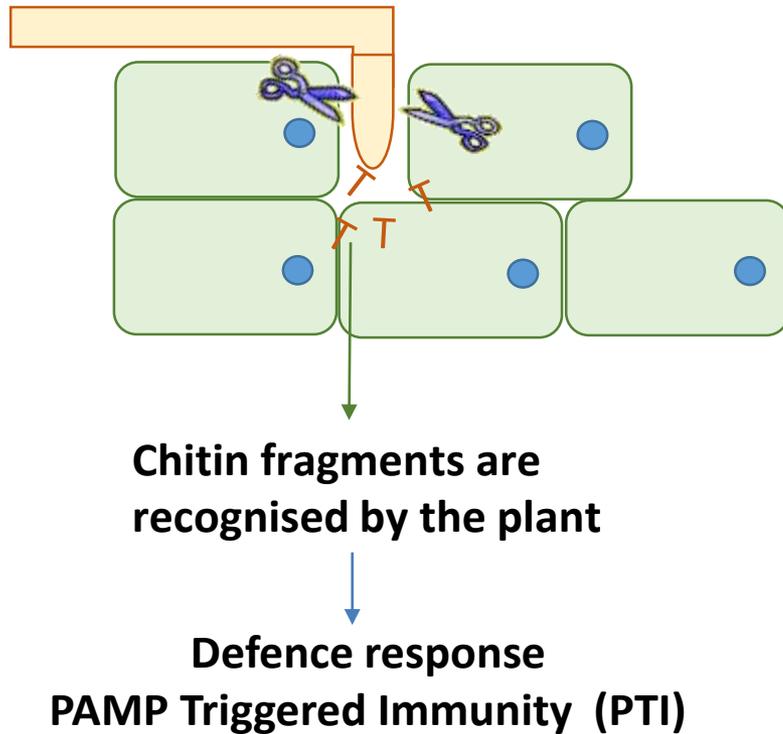


Ana  
Machado Wood  
(RRes)

# Fg SSP6 and Fg SSP7 are annotated as cerato-platanin proteins (CPPs)

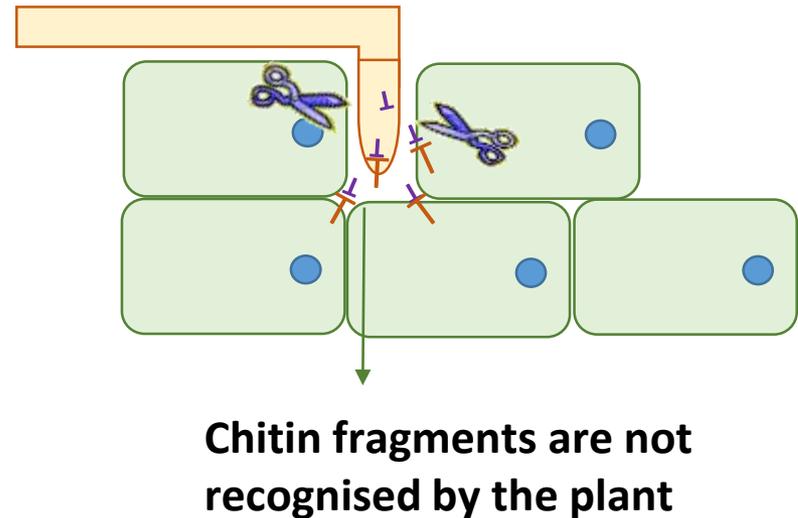
## Possible mode of action?

### Control WT plants



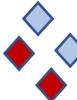
- Chitin binding ability **Yes**  

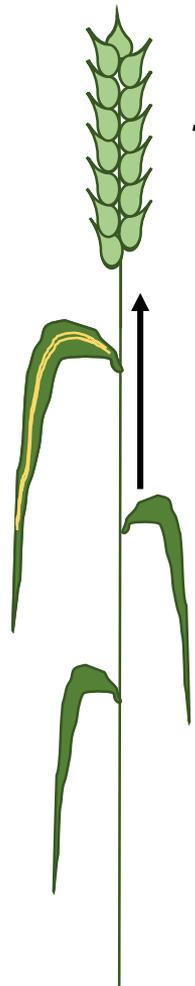
### VOX plants



# Barley Stripe Mosaic Virus - VOX

Viral infection with  
VOX constructs

 BSMV  *Fg* spores  *Fg* effectors



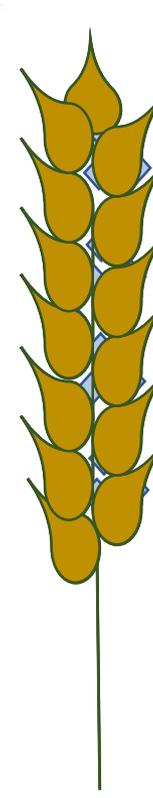
BSMV +  
heterologous  
protein



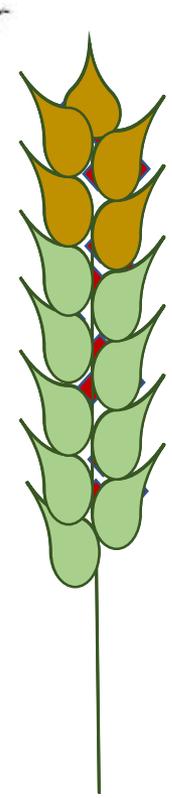
No virus +  
*Fg*



Virus  
control

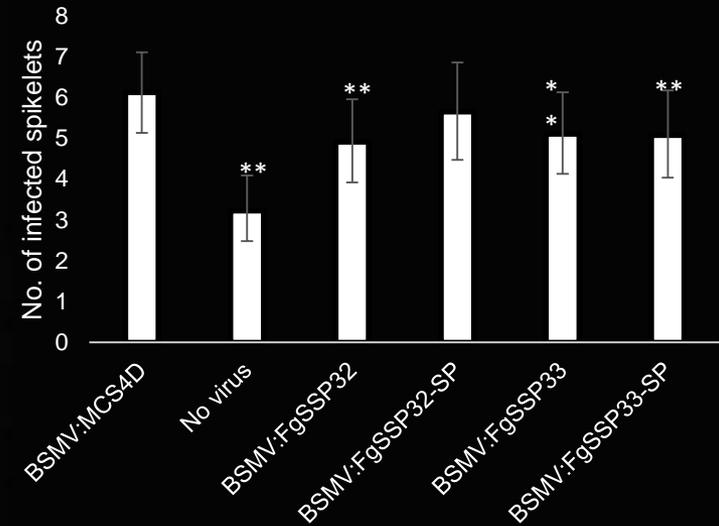


Defence-  
suppressing  
effector



Defence-  
activating  
effector

# Overexpression of FgSSP32 & FgSSP33 leads to reduction in fungal pathogenicity



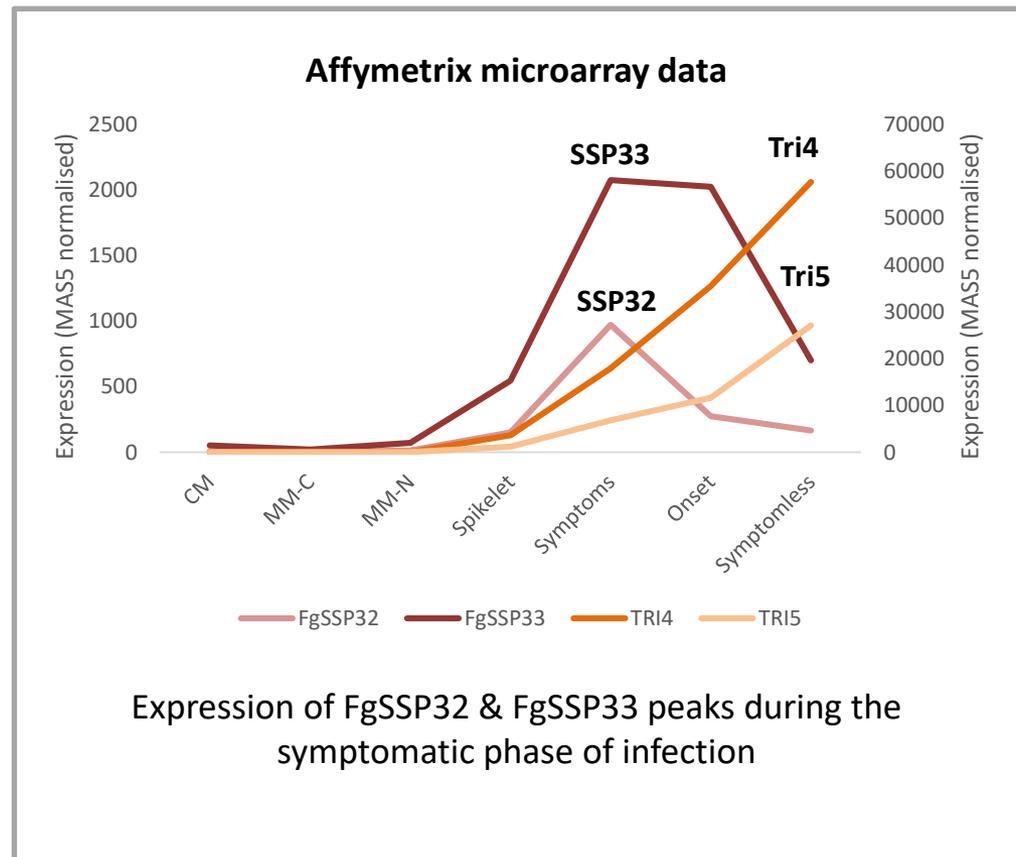
- Pooled BSMV-VOX results across 3 experimental reps and statistically analysed using GLMM analysis (+/- S.E.M,  $p < 0.05$ ).
- Overexpression leads to decrease in fungal disease symptoms
- FgSSP32 phenotype depends on the presence of the signal peptide

# Characteristics of FgSSP32 and FgSSP33

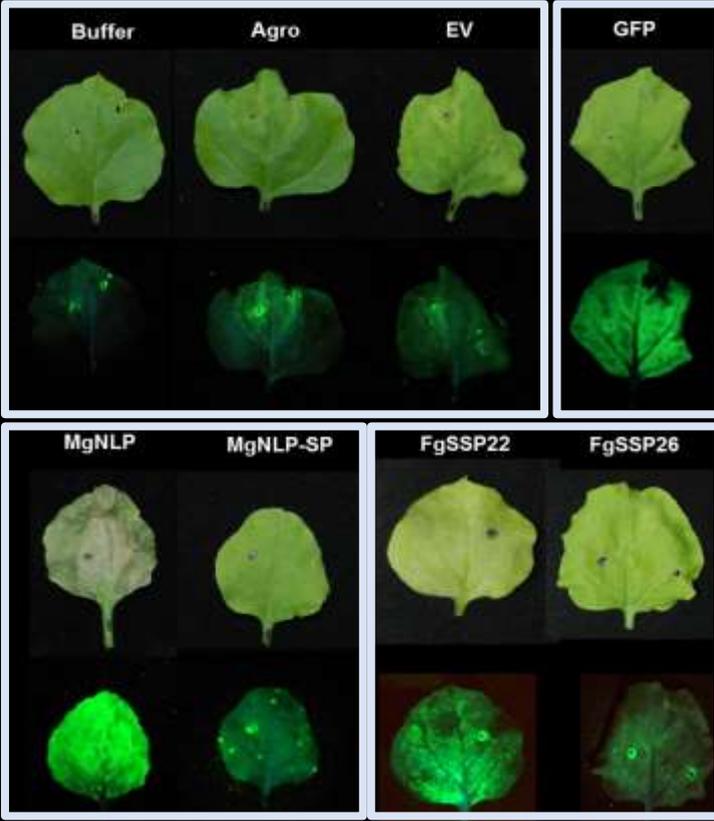
**FgSSP32** – < 60 aa mature protein – *F. graminearum* specific

**FgSSP33** – < 60 aa mature protein

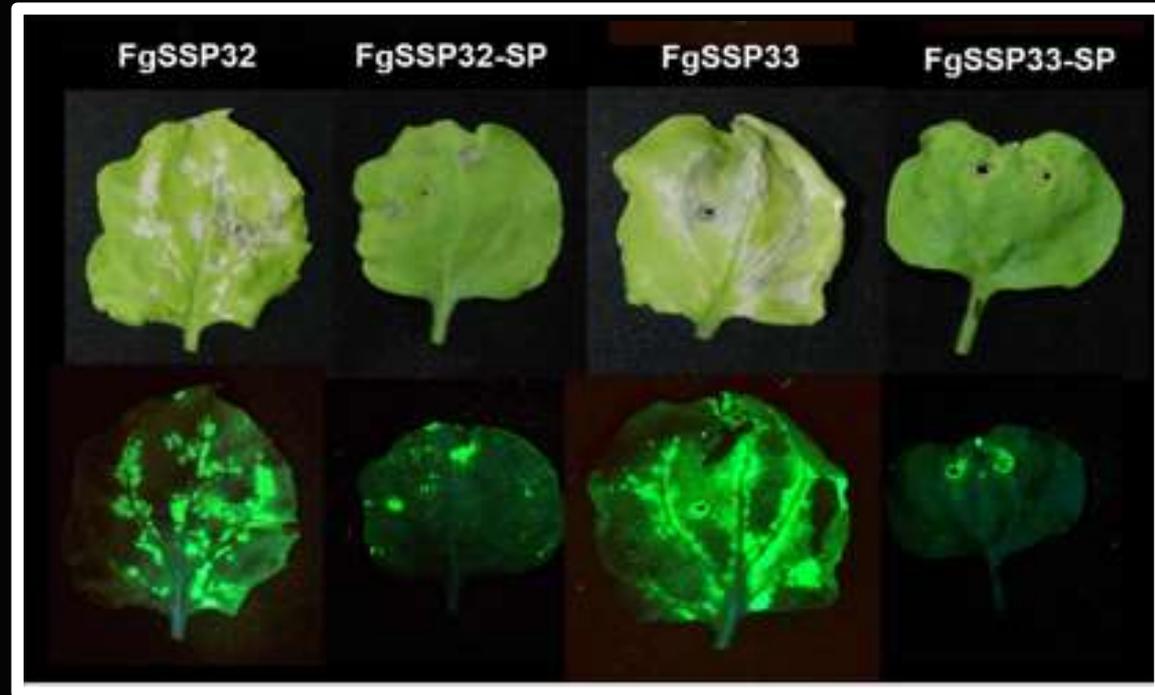
FgSSP33 homologues **are restricted to the Fusarium genus** (n = 14)  
including several vascular wilt species



# FgSSP32 & FgSSP33 induce vascular-localised cell death in *Nicotiana*



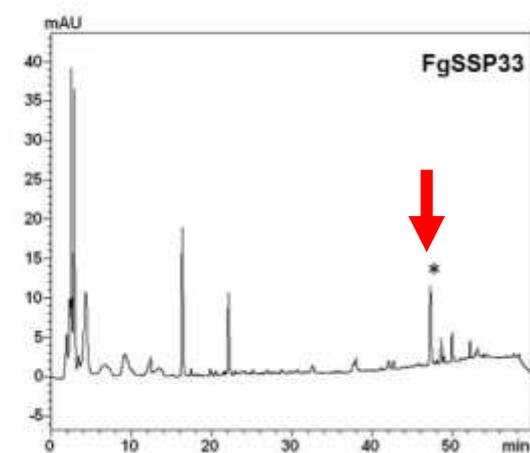
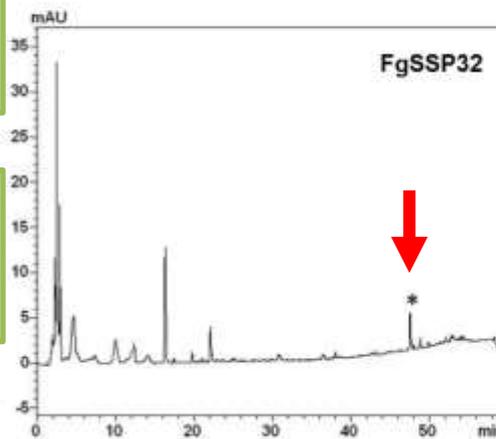
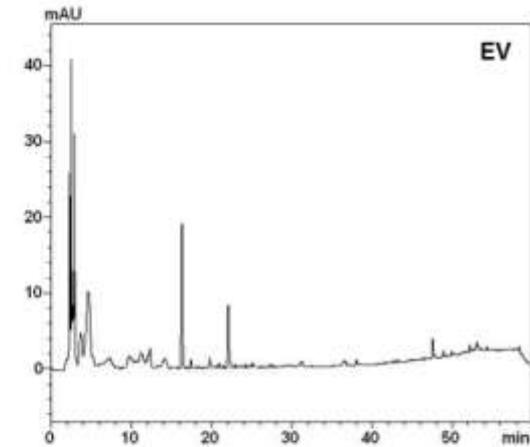
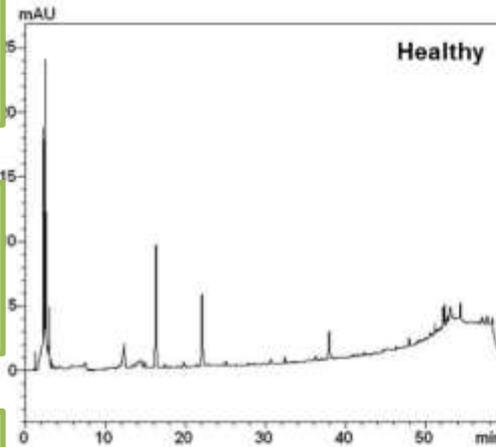
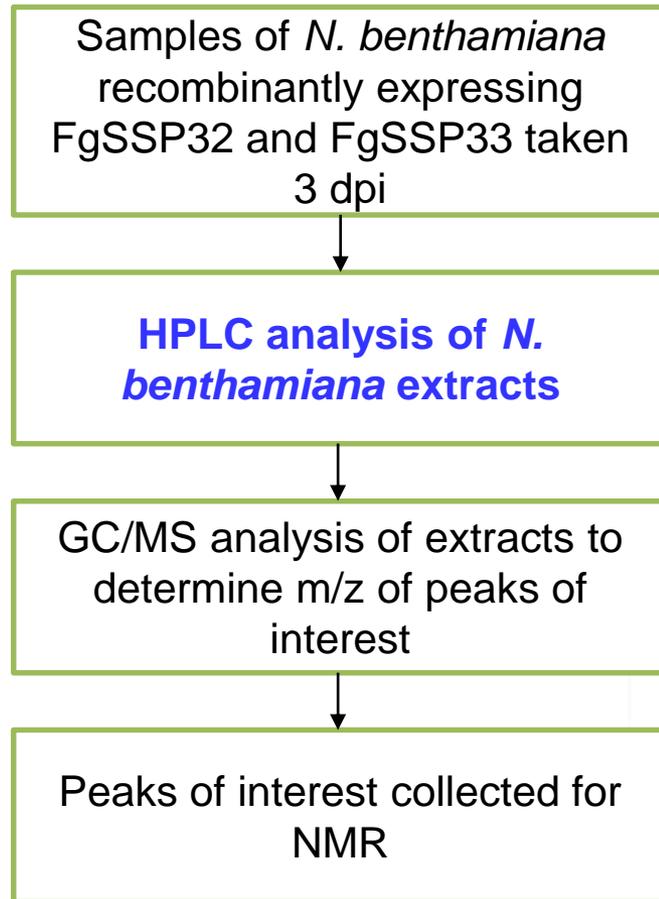
## Agroinfiltration overexpression bioassay



- Necrosis is dependent on the presence of the signal peptide → apoplastic localisation integral to phenotype

# Biochemical analysis of *N. benthamiana* recombinantly expressing FgSSP32 and FgSSP33

## High-performance liquid chromatography analysis:



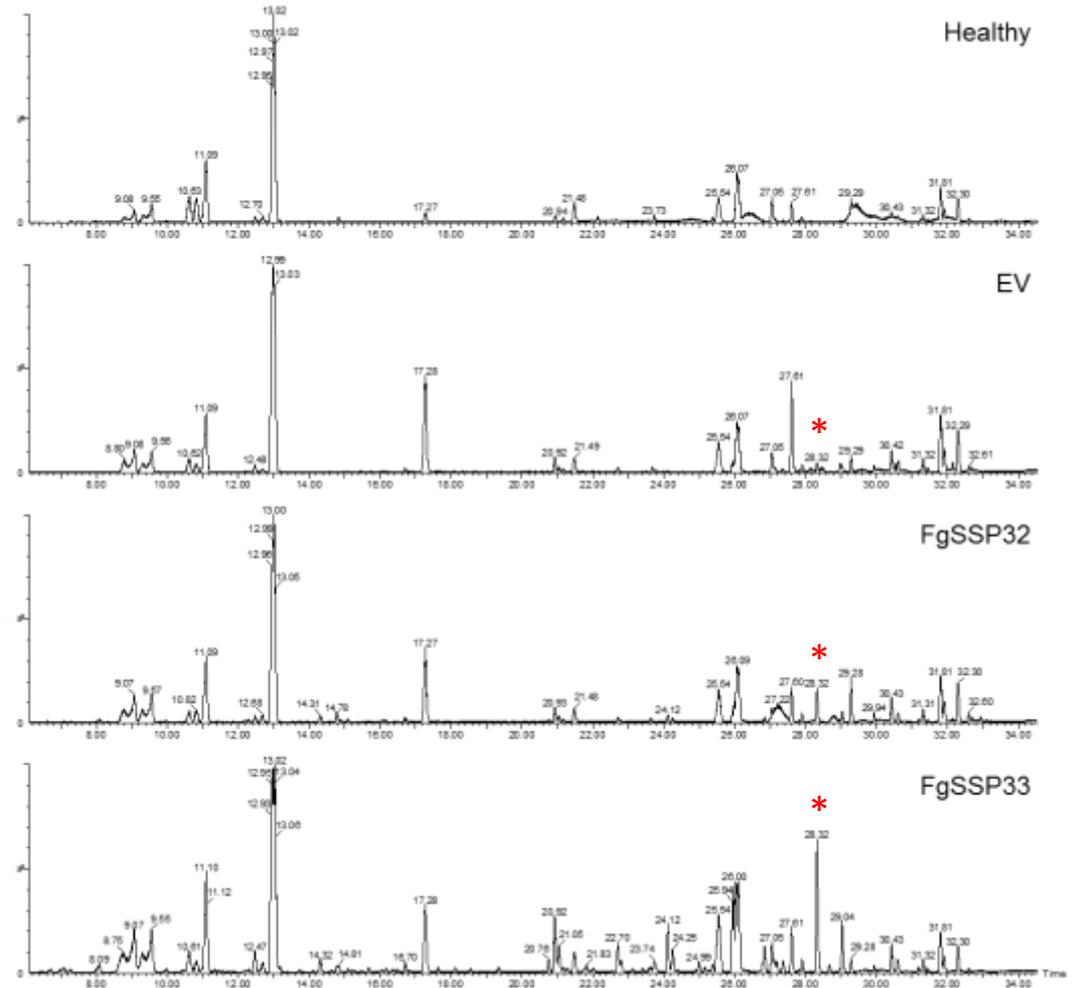
# Biochemical analysis of *N. benthamiana* recombinantly expressing FgSSP32 and FgSSP33

Samples of *N. benthamiana* recombinantly expressing FgSSP32 and FgSSP33 taken 3 dpi

HPLC analysis of *N. benthamiana* extracts

GC/MS analysis of extracts to determine m/z of peaks of interest

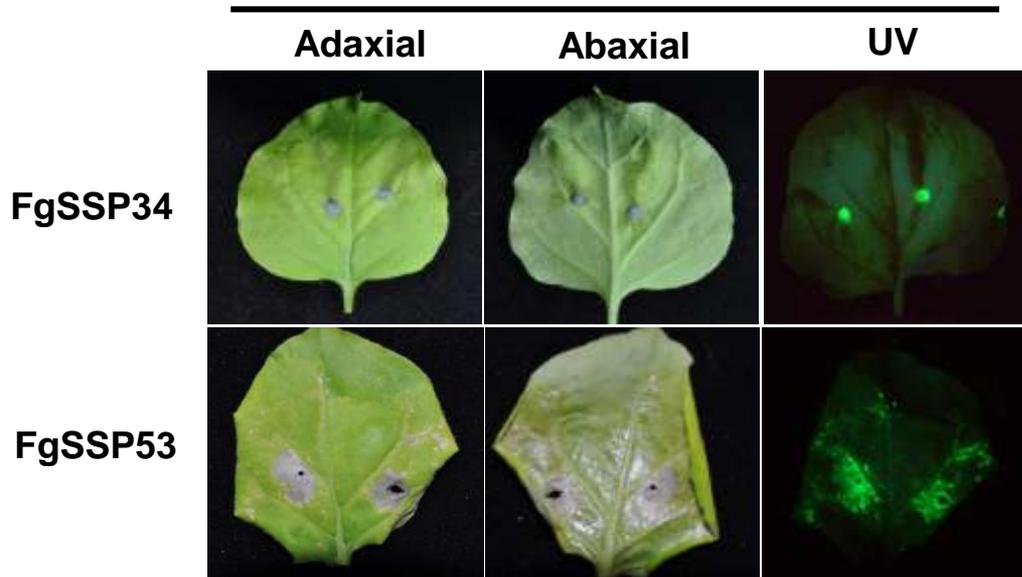
Peaks of interest collected for NMR



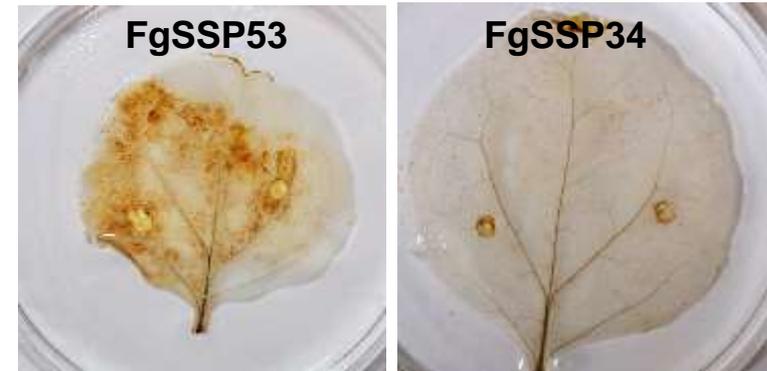
m/z value – has been determined

# Characterisation of FgSSP34 and FgSSP53 in *N. benthamiana* (> 55 aa)

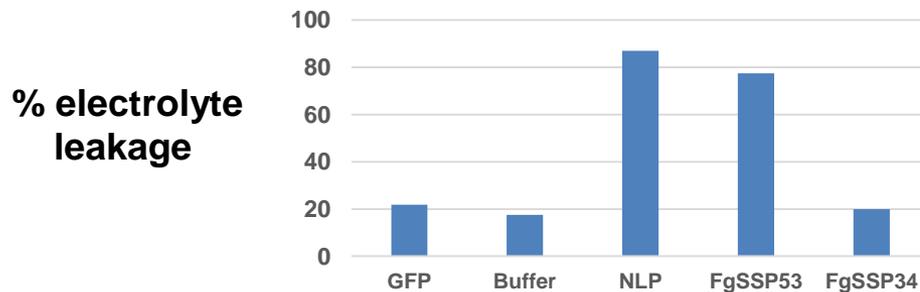
## Necrosis Assay



## DAB staining for Reactive Oxygen Species



Electrolyte leakage of Agro-infiltrated *N. benthamiana* 4dpi



**BSMV-VOX in wheat: FGSSP53 results in significantly less FHB disease**

# Removal of the signal peptide (SP) sequence from FgSSP53 results in no necrosis in *N. benthamiana*

Working hypothesis : Host recognition occurs in the apoplast

## Necrosis Assay

Adaxial

Abaxial

FgSSP53 minus SP



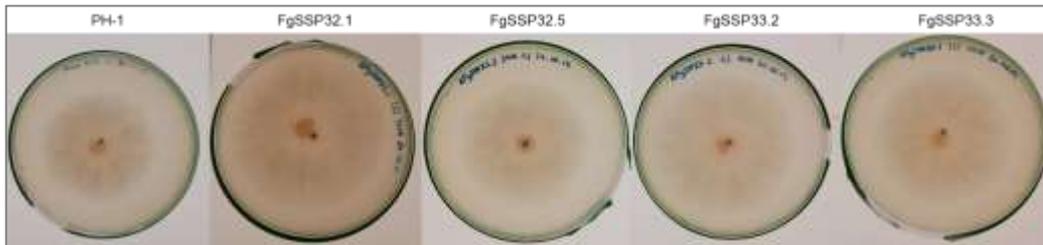
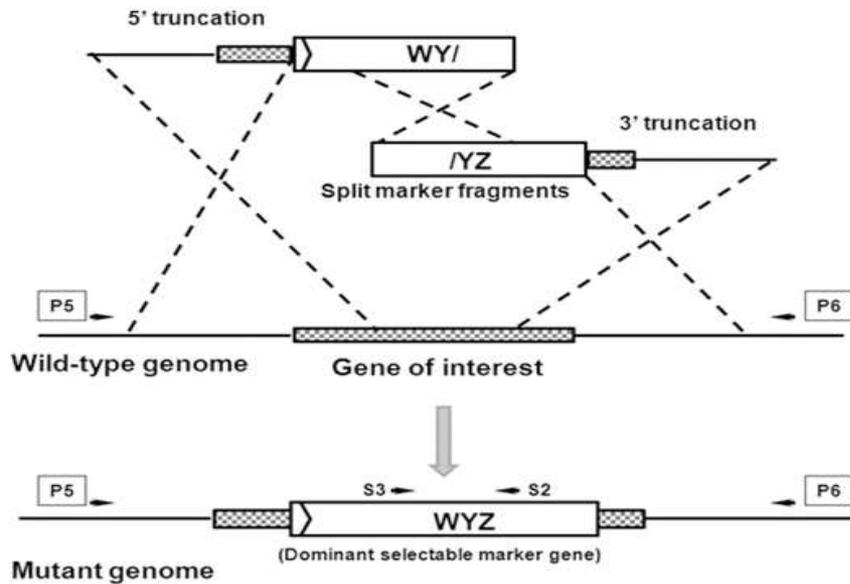
FgSSP53



Agro OD<sub>600</sub> = 1  
Images taken 4dpi

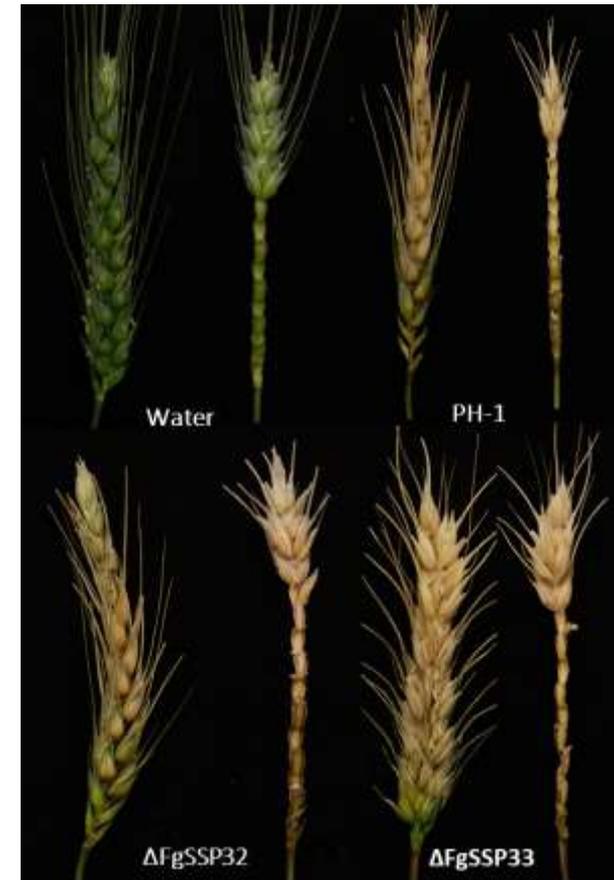
# *FgSSP6*, *FgSSP7*, *FgSSP32* & *FgSSP33* single gene deletion mutants exhibit wildtype pathogenicity

## Split-marker transformation:



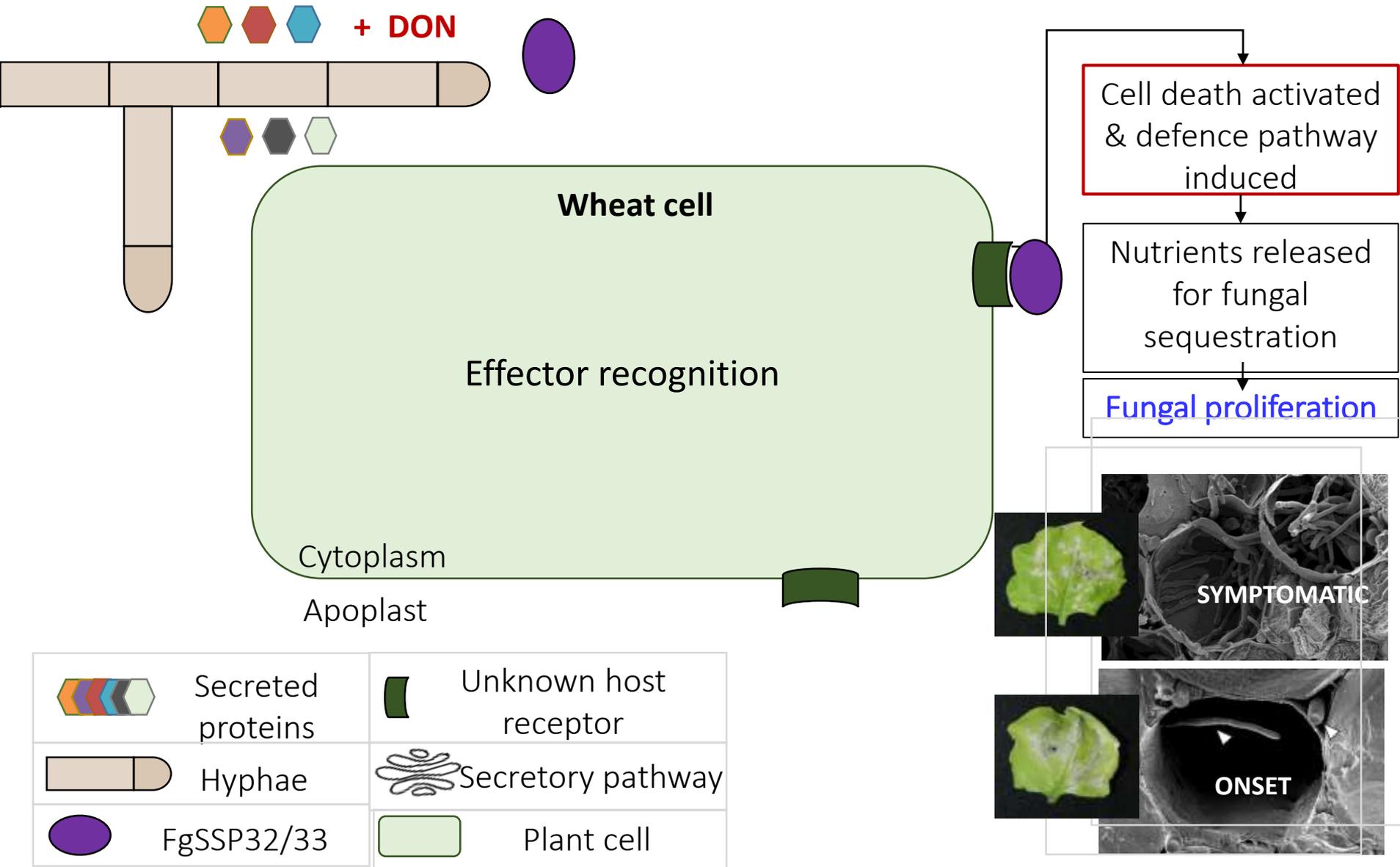
Generation of several independent gene deletion strains

## Floral pathogenicity assay:

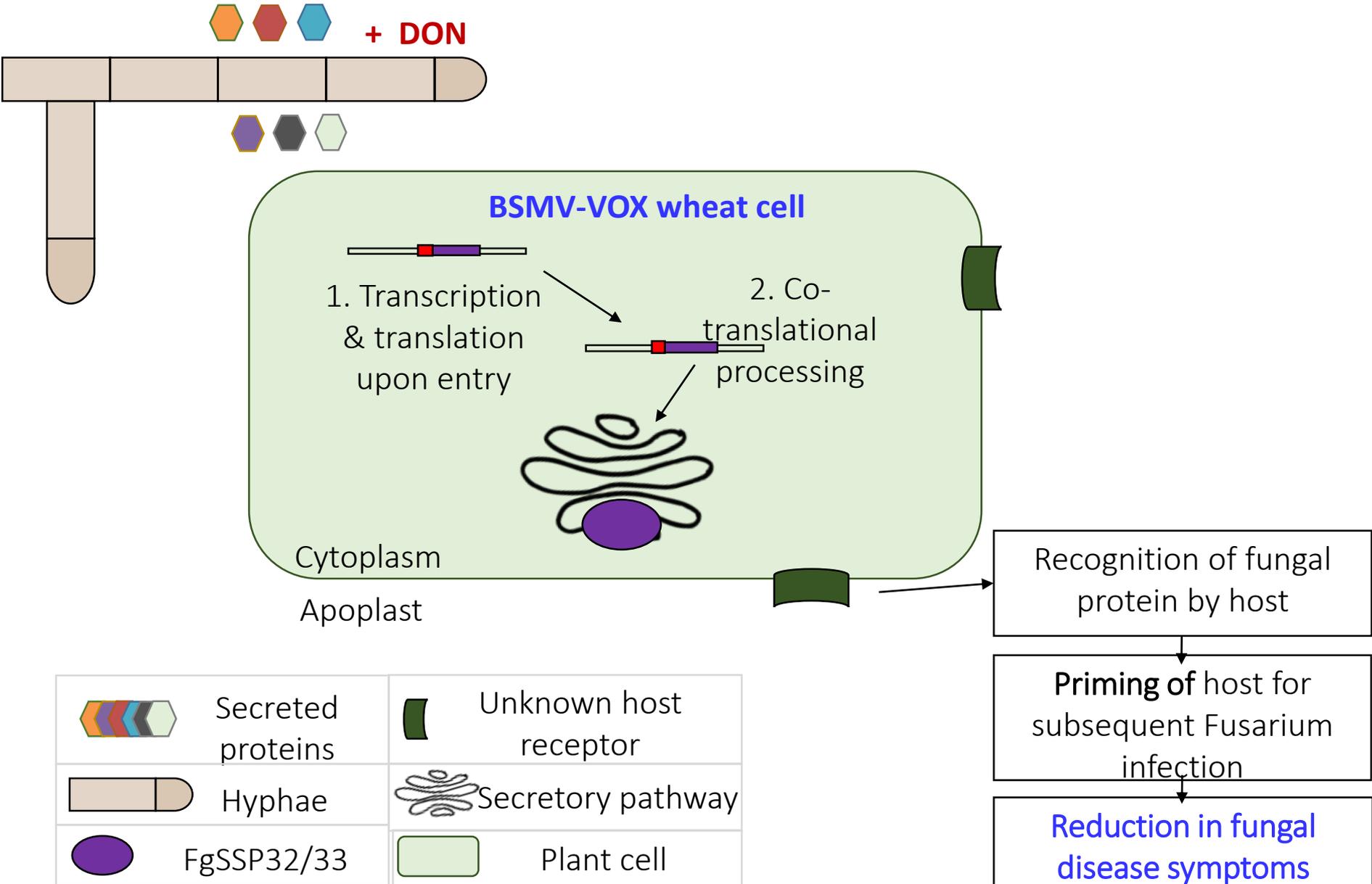


Perhaps a case of genetic redundancy?

# Current working model for FgSSP32 / FgSSP33 function



# Current working model for FgSSP32 / FgSSP33 function



# Summary & Conclusions

- *Fusarium graminearum* has a biphasic infection lifestyle

Both the symptomless and symptomatic phases are important

- By prematurely expressed individual FgSSPs in wheat the disease level is either increased or decreased

Focus FgSSPs belong to the core proteome and which function apoplastically

- FgSSP32, FgSSP33 and FgSSP53 **trigger cell necrosis** in *Nicotiana benthaminana* but with different spatial patterns

- *Fusarium* mutants with individual *FgSSP* genes deleted have wildtype pathogenicity (genetic redundancy)

BSMV-VOX system is an excellent discovery tool

# Unanswered questions

- **Why do FgSSP32 and FgSSP33 induce vascular - associated necrosis in Nicotiana whereas FgSSP53 does not ?**



Are some of the host receptors that recognise specific FgSSP proteins localised to vascular tissue ?

- **During the symptomless phase what is the interplay between DON and the expressed FgSSP repertoire?**
- **Why do Fusarium species in the FHB complex only cause disease on floral and stem base tissues ?**



# Many thanks to .....



ROTHAMSTED  
RESEARCH

## Rothamsted Research

### *Biointeractions and Crop Protection*

Martin Urban

Neil Brown (WP 2007-2011, 2015-2018)

**Ana Machado Wood \***

**Catherine Walker\*\***

**Claire Kanja\*\***

**Kostya Kanyuka**

Wing-Sham Lee (WP 2011-2017)

**David Withall**

### *Computational and Systems Biology*

**Rob King**

**Dan Smith**

Keywan Hassani-Pak

John Antoniw (WP 2003 – 2013)

## Collaborators

### University of Liverpool

FungalDB team

### Earlham Institute, Norwich

Chris Watkins + team

### EBI PhytoPath, Cambridge

Paul Kersey, Helder Pedro, Nishadi De Silva, ENSEMBL non-vertebrates team

### BROAD and MIPS for *Fg* files

### EMBRAPA – Brazil



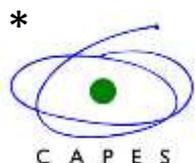
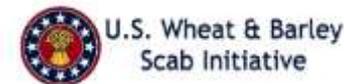
Mauricio Fernandez, Elene Lau

### University of Federal de Viçosa,

Emerson del Ponte, Camila Nicolli  
for Brazilian Fusarium isolates

### USDA – Purdue / Univ Indiana

**Matt Helm**, Steve Scofield, Roger Innes  
**Joint *Fg* effector  
poster No 44**



Biotechnology and  
Biological Sciences  
Research Council

\*\*



University of  
Nottingham  
UK | CHINA | MALAYSIA

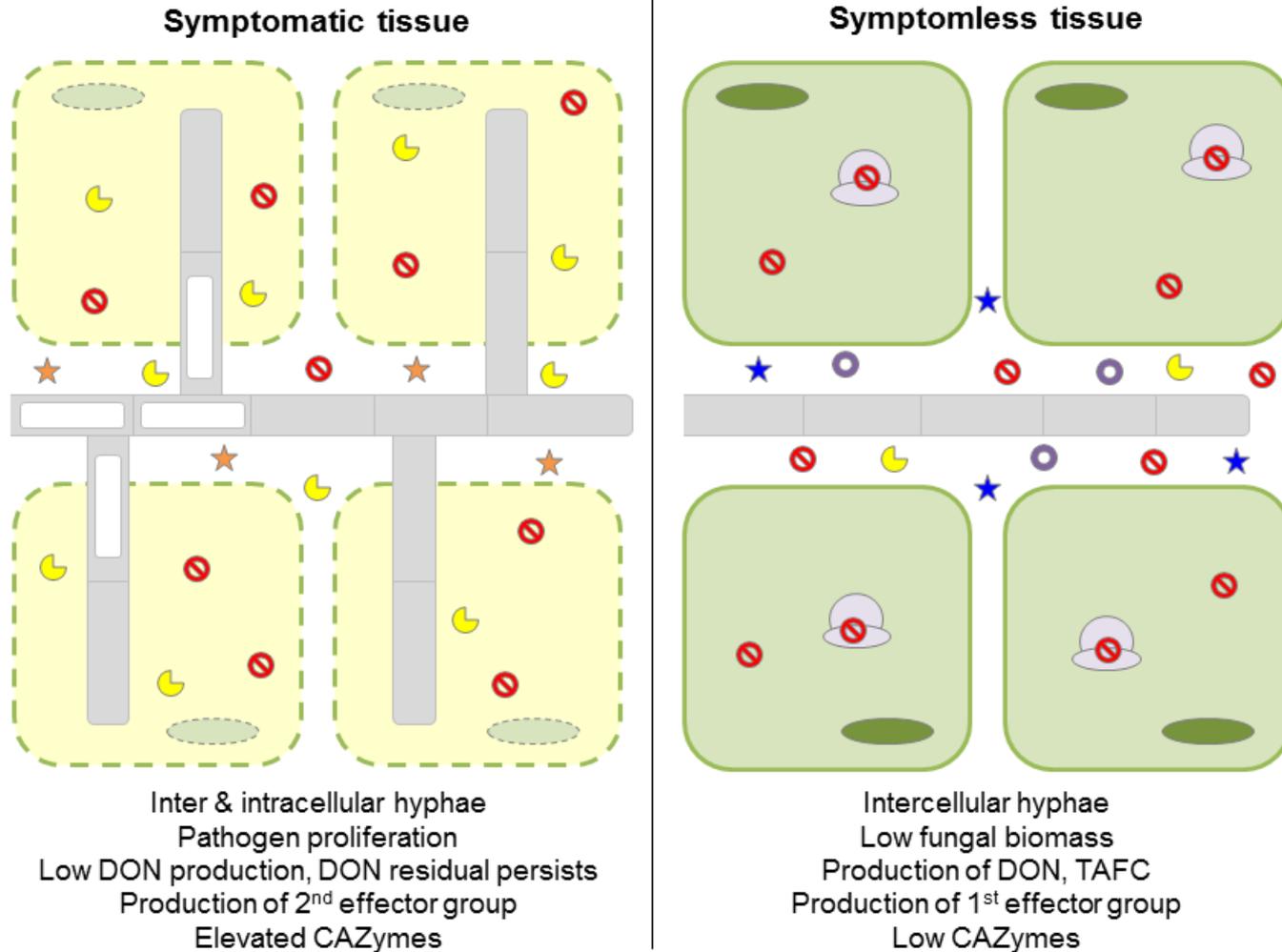


**Thank you**

**Rothamsted Research is situated 25 miles north of London in SE England**

# A spatial temporal model for Fusarium infection

Wheat  
floral  
rachis



Inter & intracellular hyphae  
Pathogen proliferation  
Low DON production, DON residual persists  
Production of 2<sup>nd</sup> effector group  
Elevated CAZymes

Intercellular hyphae  
Low fungal biomass  
Production of DON, TAFC  
Production of 1<sup>st</sup> effector group  
Low CAZymes

- Plant cell
  Plant nucleus
  Hyphae
  TAFC
  DON
  CAZyme
  2<sup>nd</sup> effector group
- Dead plant cell
  Dead plant nucleus
  Vacuolated hyphae
  Plant ribosome
  1<sup>st</sup> effector group

# Relevant publications from the Rothamsted Fusarium team - 1

## Infection biology

Brown, N. A., Urban, M., van de Meene, A. M. L. and Hammond-Kosack, K. E. (2010) The infection biology of *Fusarium graminearum*: Defining the pathways of spikelet to spikelet colonisation in wheat ears. [Fungal Biology](#) 114, 555-571.

Brown, N., Bass, C., Baldwin, T.K., Chen, H., Massot, F., Carion, P.W.C., Urban, M., van de Meene, A. M. L., and Hammond-Kosack, K. E. (2011) Characterisation of the *Fusarium graminearum* – wheat floral interaction. [Journal of Pathogens](#) (2011) Article ID 626345

Dean, R., van Kan, J. , Pretorius; Z., Hammond-Kosack, K. E., Di Pietro, A., Spanu, P., Rudd, J. J., Dickman, M., Kahmann, R., Ellis , J., and Foster, G.D. (2012) The Top 10 Fungal Pathogens in Molecular Plant Pathology. [Molecular Plant Pathology](#) 13: 414–430

Brown, N.A. and Hammond-Kosack, K. E. (2015) Secreted bio-molecules in fungal plant pathogenesis, Chapter 19, p263-310 in the book entitled 'Fungal Bio-molecules: Sources, Applications and Recent Developments (Eds: V. K. Gupta, S. Sreenivasaprasad, and Robert L. Mach) Pp408 Wiley – Blackwell ISBN: 978-1-118-95829-2.

Reviews

## Fusarium genomes and transcriptomics

Brown, N. A., Antoniw, J., and Hammond-Kosack, K. E. (2012) The predicted secretome of the plant pathogenic fungus *Fusarium graminearum*: A refined comparative analysis. [PLoS ONE](#) e0033731.

King, R., Urban, M., Hammond-Kosack, M.C.U. Hassani-Pak, K. and Hammond-Kosack, K.E. (2015) The completed genome sequence of the pathogenic ascomycete fungus *Fusarium graminearum*. [BMC Genomics](#) 16, 544.

King, R., Urban, M., and Hammond-Kosack, K. E. (2017) The annotation of *Fusarium graminearum* (PH-1) version 5.0 [Genome Announcements](#) 5 (2) e01479-16.

Brown, NA, Evans, J., Mead, A and. Hammond-Kosack, K.E. (2017) A spatial temporal analysis of the *Fusarium graminearum* transcriptome during symptomless and symptomatic wheat infection. [Molecular Plant Pathology](#) 18, 1295-1312 (e-12564)

King, R., Brown, N.A., Urban M. and Hammond-Kosack, K. E. (2018) Inter-genome comparison of the Quorn fungus *Fusarium venenatum* and the closely related plant infecting pathogen *Fusarium graminearum*. [BMC Genomics](#) 19, 269

Wood, AM, King, R., Urban, M., Nicolli, C., Del Ponte, E. and Hammond-Kosack, K.E. (2020) Genome Sequence of *Fusarium graminearum* strain CML3066 isolated from a wheat spike in Southern Brazil. [Microbiology Resource Announcements](#) 9(999): e00157-00120

# Relevant publications from the Rothamsted Fusarium team - 2

## Functional genomics

Lee, W-S, Hammond-Kosack, K. E. and Kanyuka, K. (2012) *Barley stripe mosaic virus*-mediated tools for investigating gene function in cereal plants and their pathogens: VIGS, HIGS and VOX. [Plant Physiology](#) 160: 582-590.

Wood, AKM, Walker, C., Lee, W-S, Urban, M and Hammond-Kosack, KE (2020) Functional evaluation of a homologue of plant rapid alkanisation factor (RALF) peptides in *Fusarium graminearum*. [Fungal Biology](#) 124:753-765.

Kanja, C and Hammond-Kosack, KE (2020) Proteinaceous effector discovery and characterisation in filamentous plant pathogens. [Molecular Plant Pathology](#). <https://doi.org/10.1111/mpp.12980>