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DON INDUCES GENES THAT INCREASE WHEAT SUSCEPTIBILITY TO FHB.

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Genomics program on cereal/*Fusarium* interaction – Goals

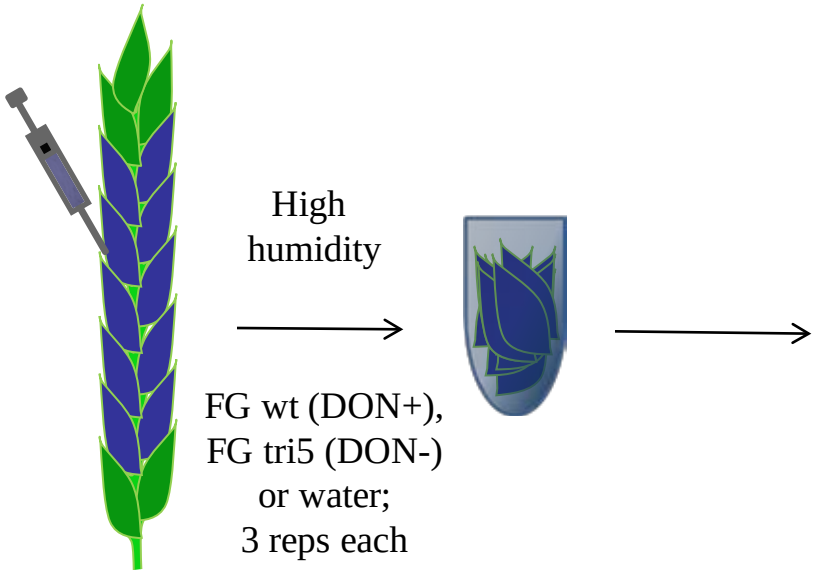
- To identify molecular mechanisms that enhance the defense response of wheat to *Fusarium graminearum* infection
- To define molecular mechanisms used by *Fusarium* to invade cereals

Comparing susceptible vs resistant varieties by microarray

- Genes which expression is associated with resistance to FHB

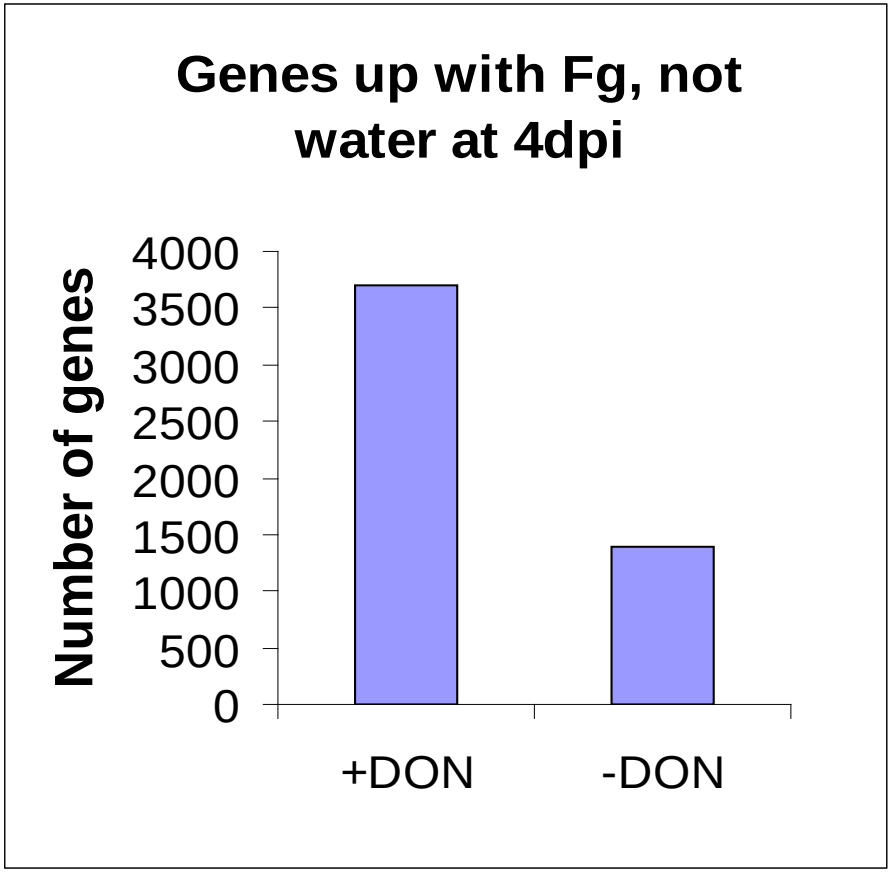
- Genes which expression is associated with susceptibility to FHB

Global expression profiling of wheat heads infected with FG producing or not DON



- Affymetrix GeneChip Wheat Genome Array
- RMA normalization
- Analysis of profiles within experiment
- Candidate list finalised by observation over whole database

Wheat sequences up-regulated by Fg infection.



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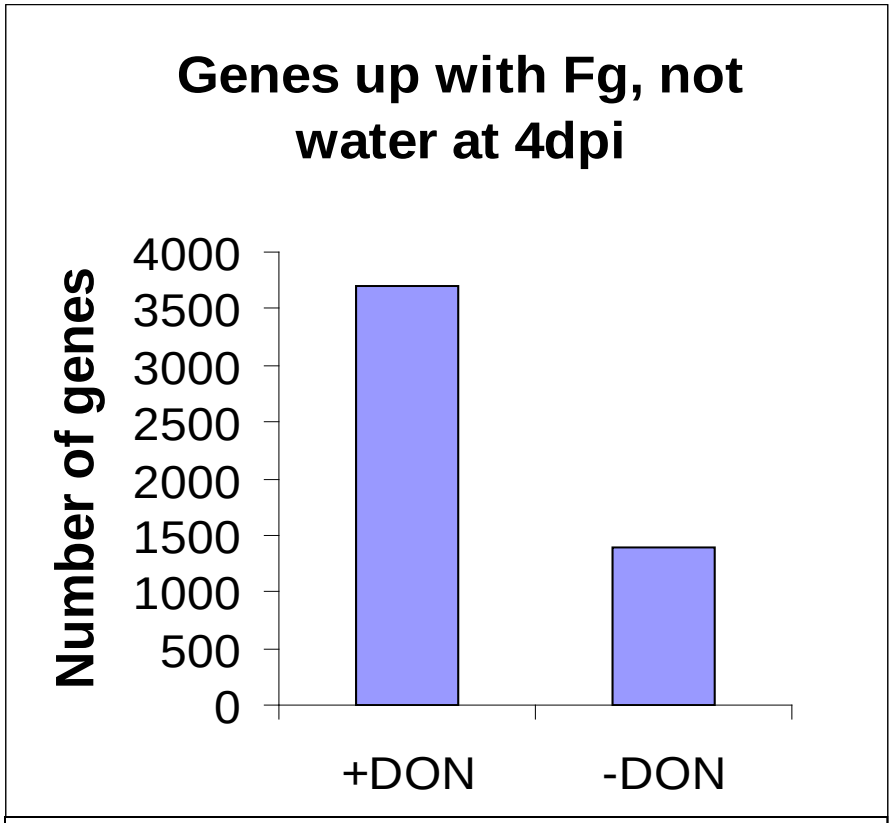
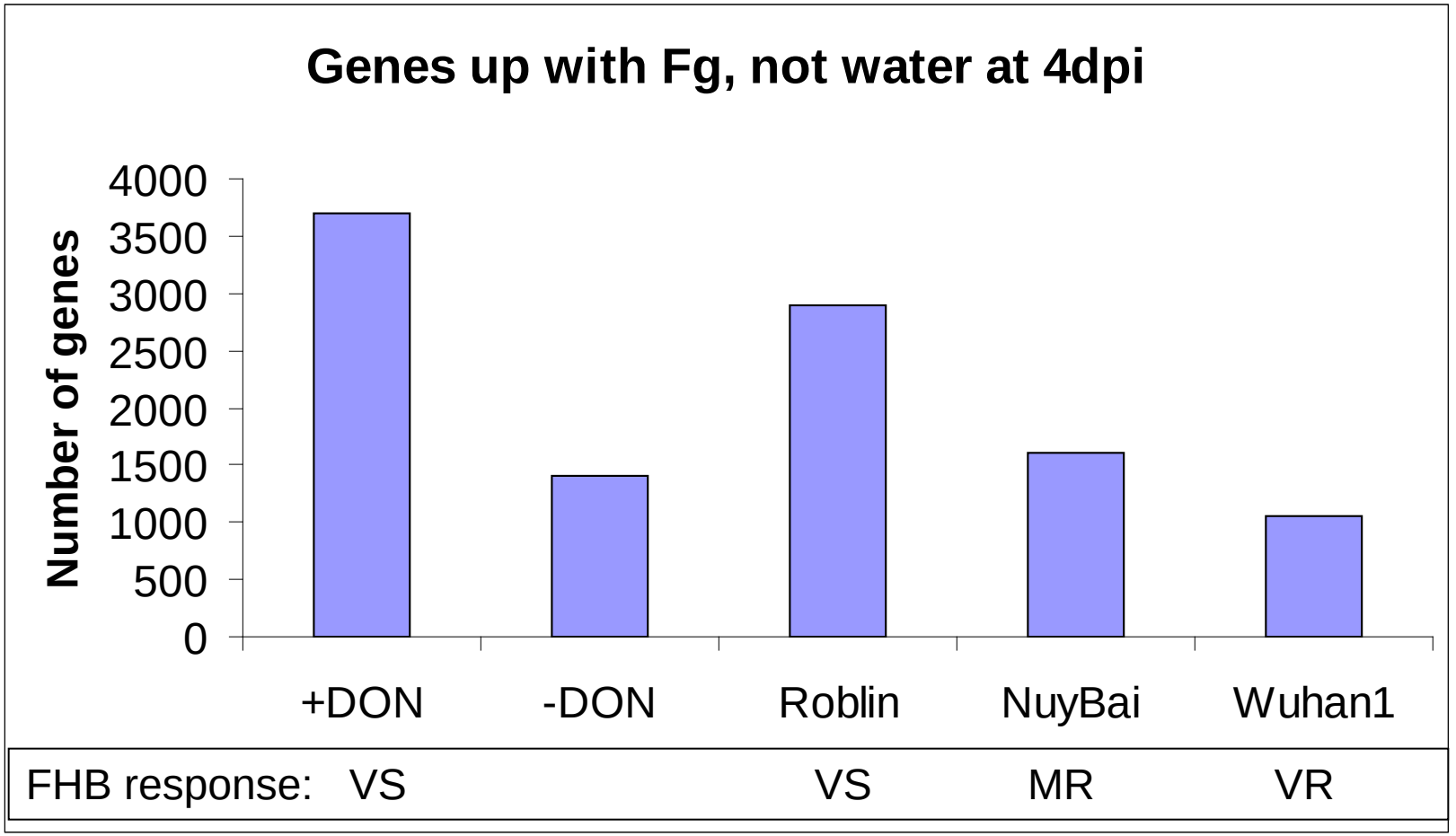


	+DON	-DON
Fungal DNA (pg):	1938	672
DON (ppm):	731	0

Wheat sequences up-regulated by Fg infection.



Types of differential expression patterns observed in Roblin heads infected with FG producing or not DON, at 4dpi, relative to water treatment

Predicted gene function	FG wt (DON+)	FG tri5 (DON-)
Antranilate N-benzoil transferase	↑	no
Cytochrome b561	↓	no

Types of differential expression patterns observed in Roblin heads infected with FG producing or not DON, at 4dpi, relative to other experiments

Predicted gene function	FG wt (DON+)	FG tri5 (DON-)	NuyBai	Wuhan 1	CS	7EL
FHB response	VS		R	VR	S	VR
Antranilate N-benzoil transferase	↑	no	↑	no	↑	no
Cytochrome b561	↓	no	no	no	↓	no

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FHB response	VS		R	VR	S	VR	S	R	S	R
inoculation	point	point	point	point	point	point	point	point	spray	spray
Antranilate N-benzoil tfase	↑	no	↑	no	↑	no	↑	↑	no	no
Cytochrome b561	↓	no	no	no	↓	no	↓	↓	no	no

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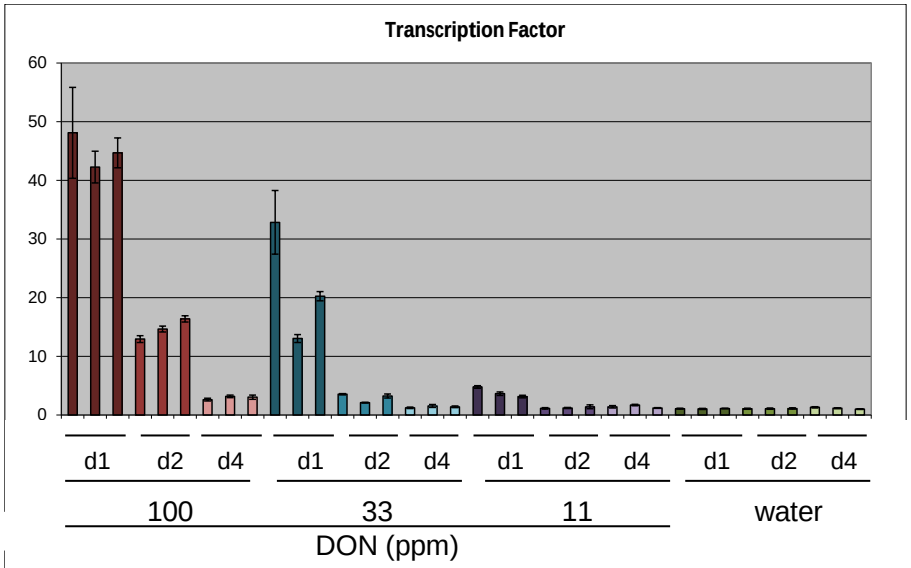
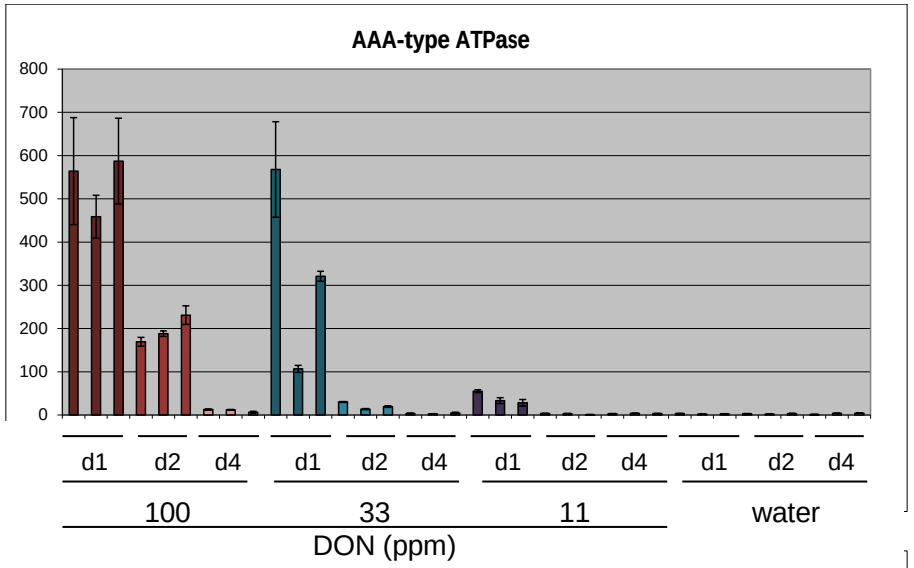
inoculation	point	point	point	point	point	point	point	point	spray	spray
Antranilate N-benzoil tfase	↑	no	↑	no	↑	no	↑	↑	no	no
Cytochrome b561	↓	no	no	no	↓	no	↓	↓	no	no
Flavonol 3-sulfotransferase	↑↑	no	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑
Protein kinase	↑	no	↑	no	↑↑	↑	↑↑	↑↑	↑	↑

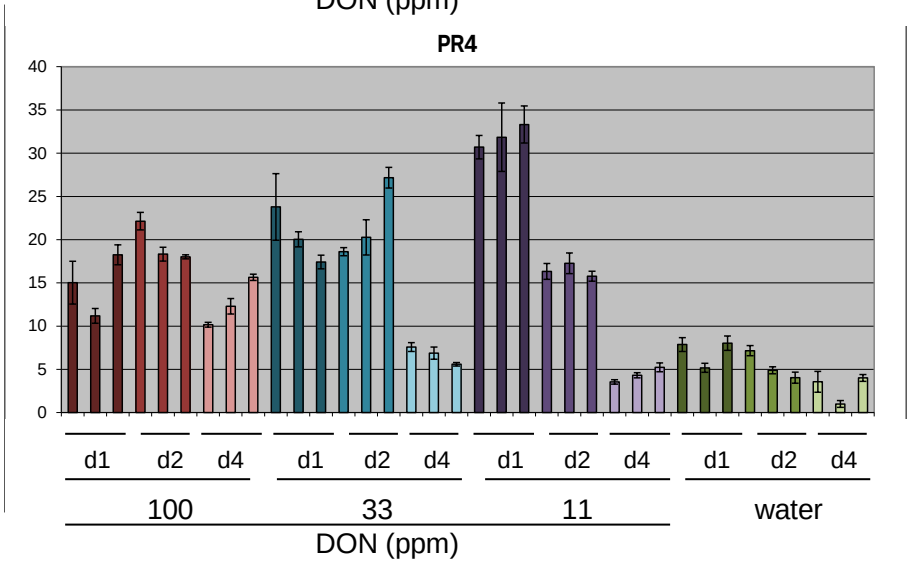
Genes which expression is associated with DON+

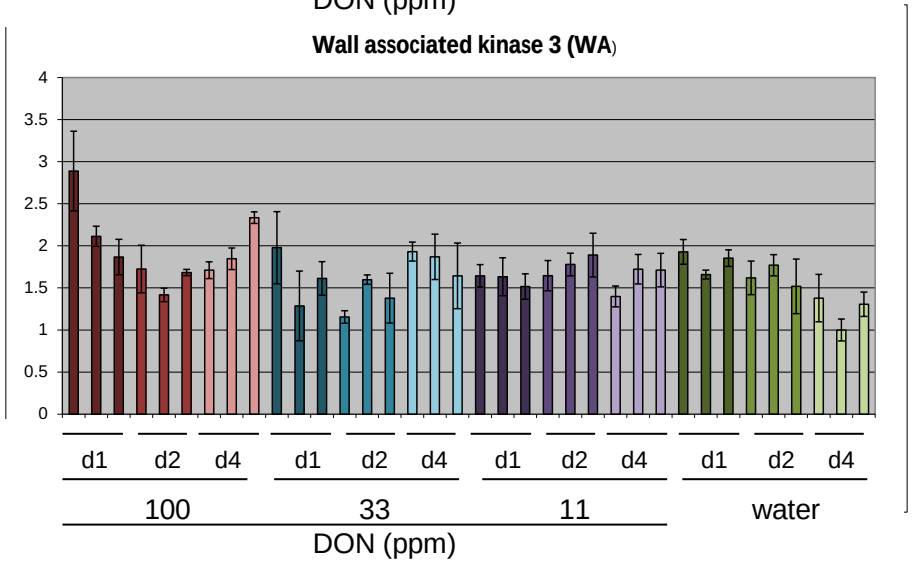
Category	Predicted gene function	Q-PCR
Secondary metabolism	Anthranilate N-benzoyltransferase	√
	Flavonol 3-sulfotransferase	√
	Tyrosine decarboxylase	√
	Gibberellin-2-oxidase	√
Regulation of gene expression	Wall-associated kinase 3	√
	Receptor protein kinase	√
	Protein kinase	√
	WRKY2 transcription factor	√
	TF-like protein 1	√
	TF-like protein 2	√
	Cys2/His2 zinc-finger protein	√
	SOS2-like protein	√
Protein synthesis+degradation	Asparagine-tRNA synthetase	
	Aspartyl-tRNA synthetase	
	Lysyl-tRNA synthetase	
	Valyl tRNA synthetase	
	ubiquitin / ribosomal protein CEP52	
Other	AAA-type ATPase	√
	MRP-like ABC transporter	√
	integral membrane protein	
	unknown 1	√
	unknown 2	
	unknown 3	
	unknown 4	
	unknown 5	

Genes which expression is directly induced by DON







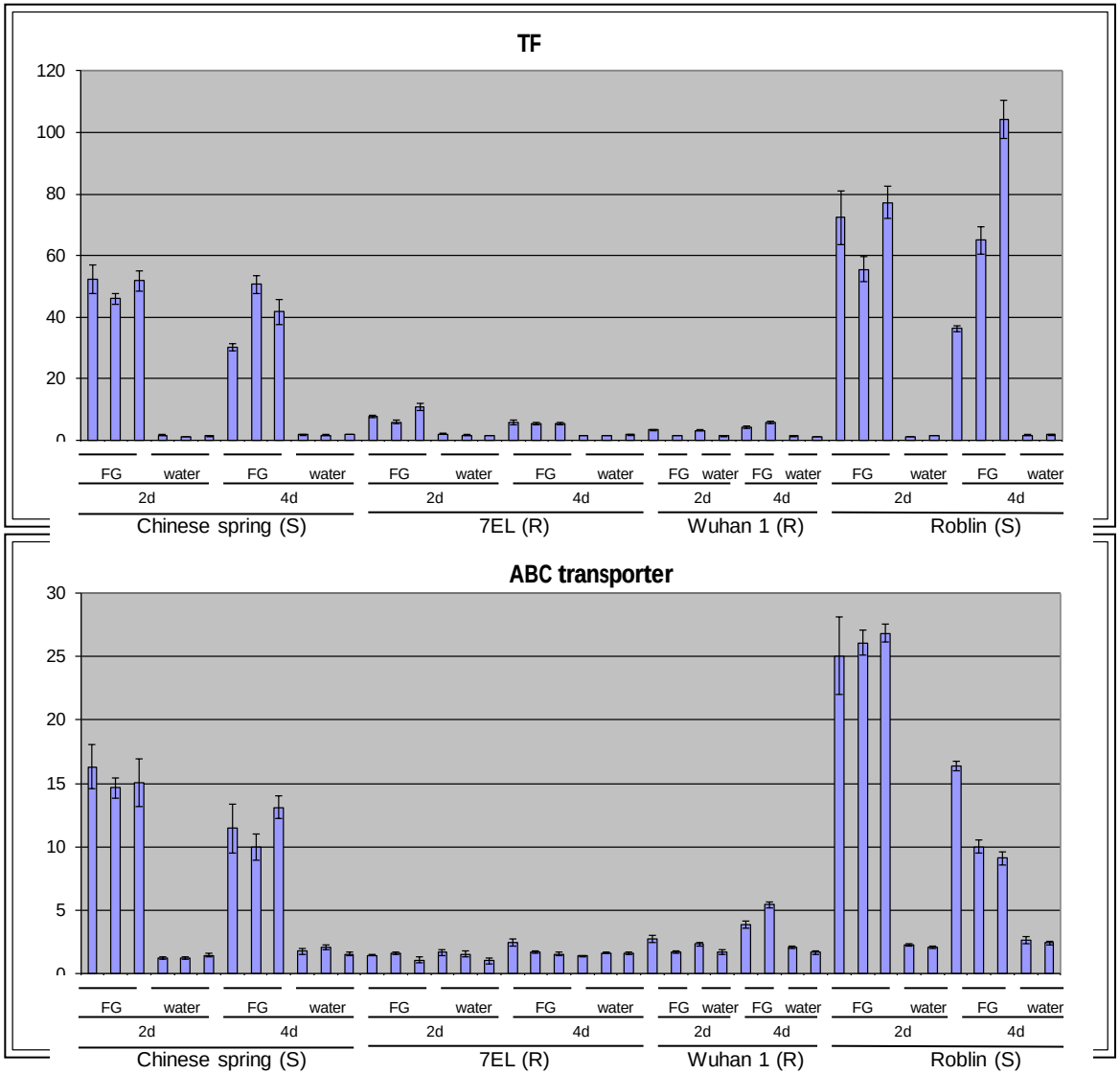


Induction by FG+DON vs DON alone

Predicted gene function	Fg+DON[8]	DON[†]	
Transcription factor –like protein 1	70*	45*	←
WRKY2-like transcription factor	1600	1000	
Cis2/His2 zinc-finger protein	700	180	
Wall associated kinase	75	0	
AAA-type ATPase	400	550	
MRP-like ABC transporter	8	7	←
Unknown	550	70	

[‡]FG wt (DON+), 4days after inoculation
[†]100 ppm/floret, 2 florets of a central spikelet; 24 hrs after treatment
*Fold induction relative to water control

Expression profile in FHB-susceptible and resistant wheat



Two candidate genes for transient silencing using BSMV system

Predicted gene function	Low expression in resistant	Comment
TF-like protein 1	7ES, Wuhan 1	AtNFXL1, a negative regulator of defense, induced by Trichothecenes in Arabidopsis
MRP-like ABC transporter	7ES, Wuhan 1	Homolog to MRP 9 and MRP15 of Arabidopsis

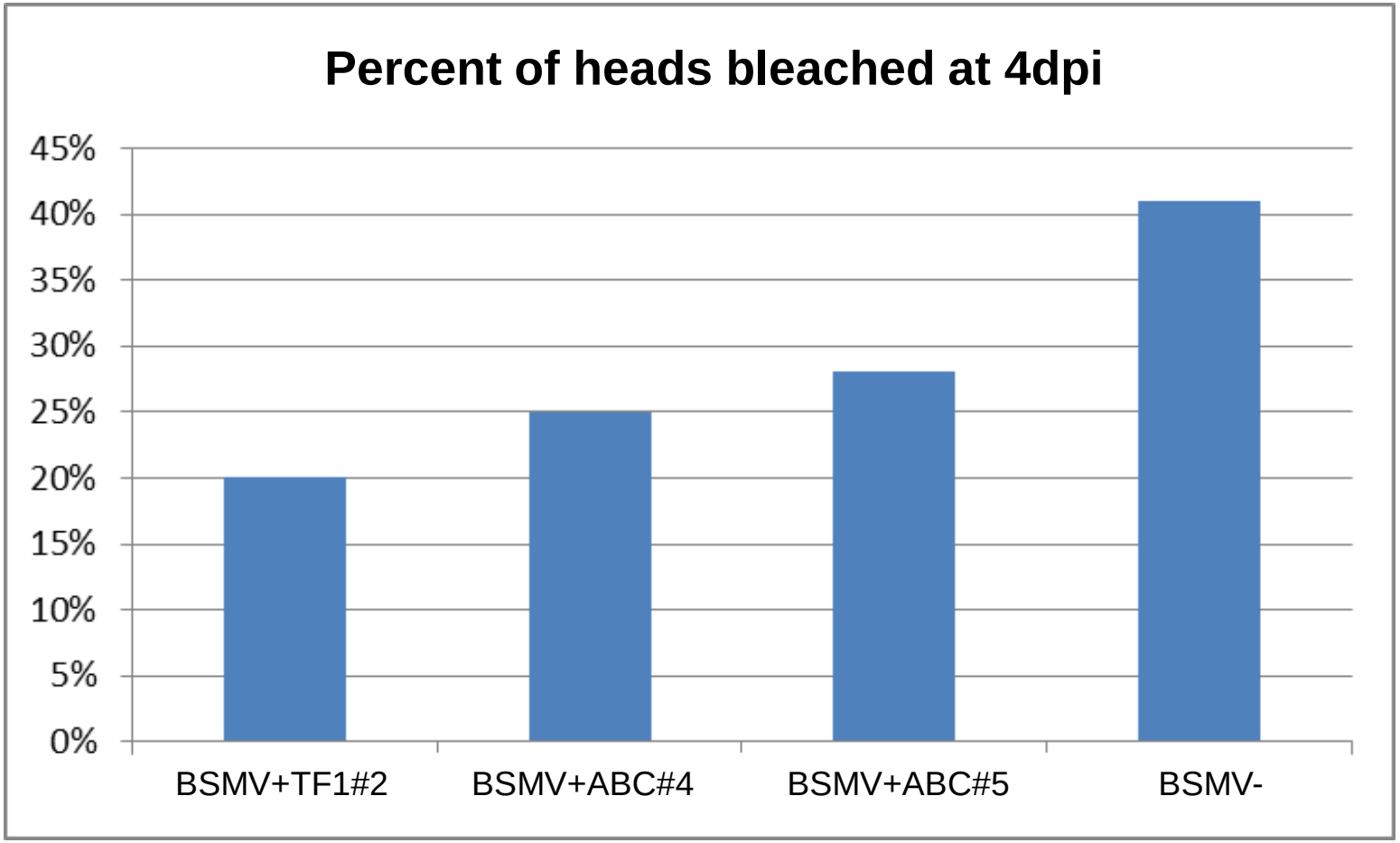
Transient silencing in susceptible Roblin: Tracking different FHB symptoms

Infected Spikelets

Bleached Spikelets

Infected Rachis

Transient silencing in susceptible Roblin: FHB disease symptom reduction

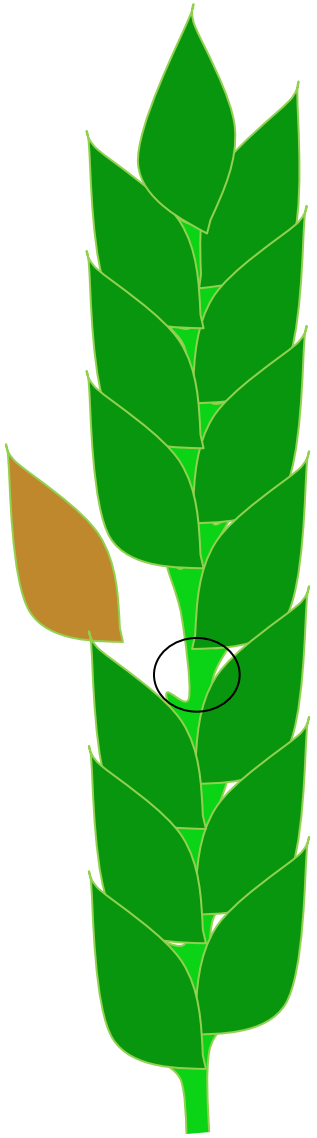


Ø Reduce blocking of vascular tissues at inoculation site by FG

Transient silencing in susceptible Roblin: FHB disease symptom reduction

Reduction at 4 dpi:

- Percent of heads bleached: 10 to 20 %
- Spread of infection from inoculated spikelet to rachis: ca 15%



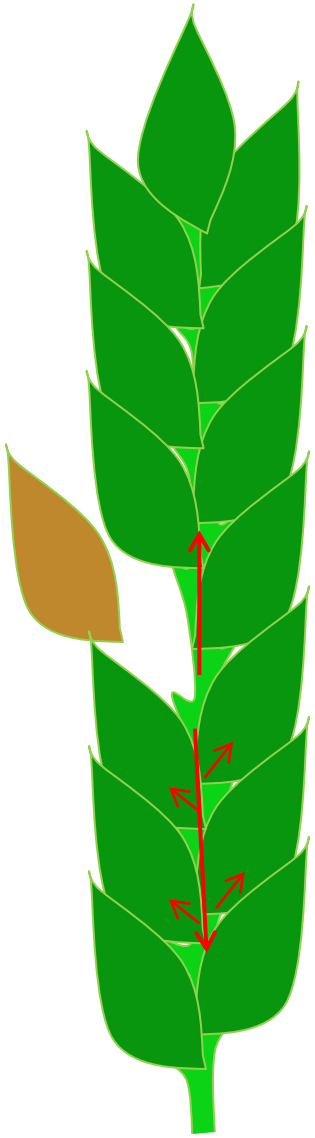
Transient silencing in susceptible Roblin: FHB disease symptom reduction

Reduction at 4 dpi:

- Spread of infection from inoculated spikelet to rachis: ca 15%
- Percent of heads bleached: 10 to 20 %

Reduction at 8 dpi:

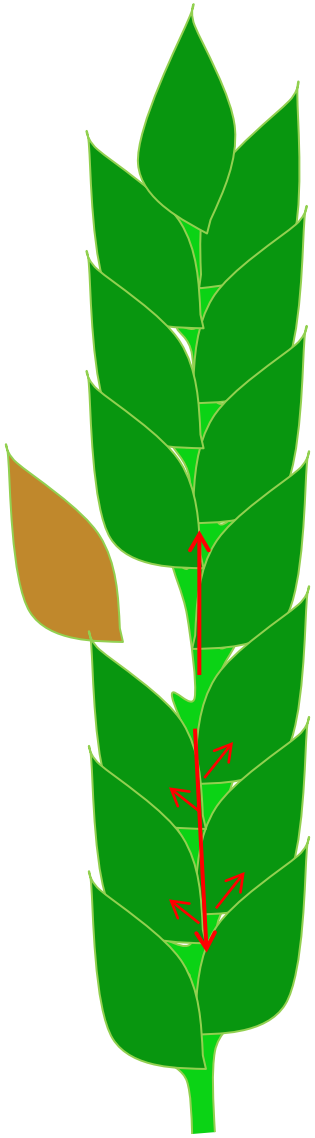
- Number of infected rachis segments: 10 to 20%
- Number of infected spikelets: ca 30%



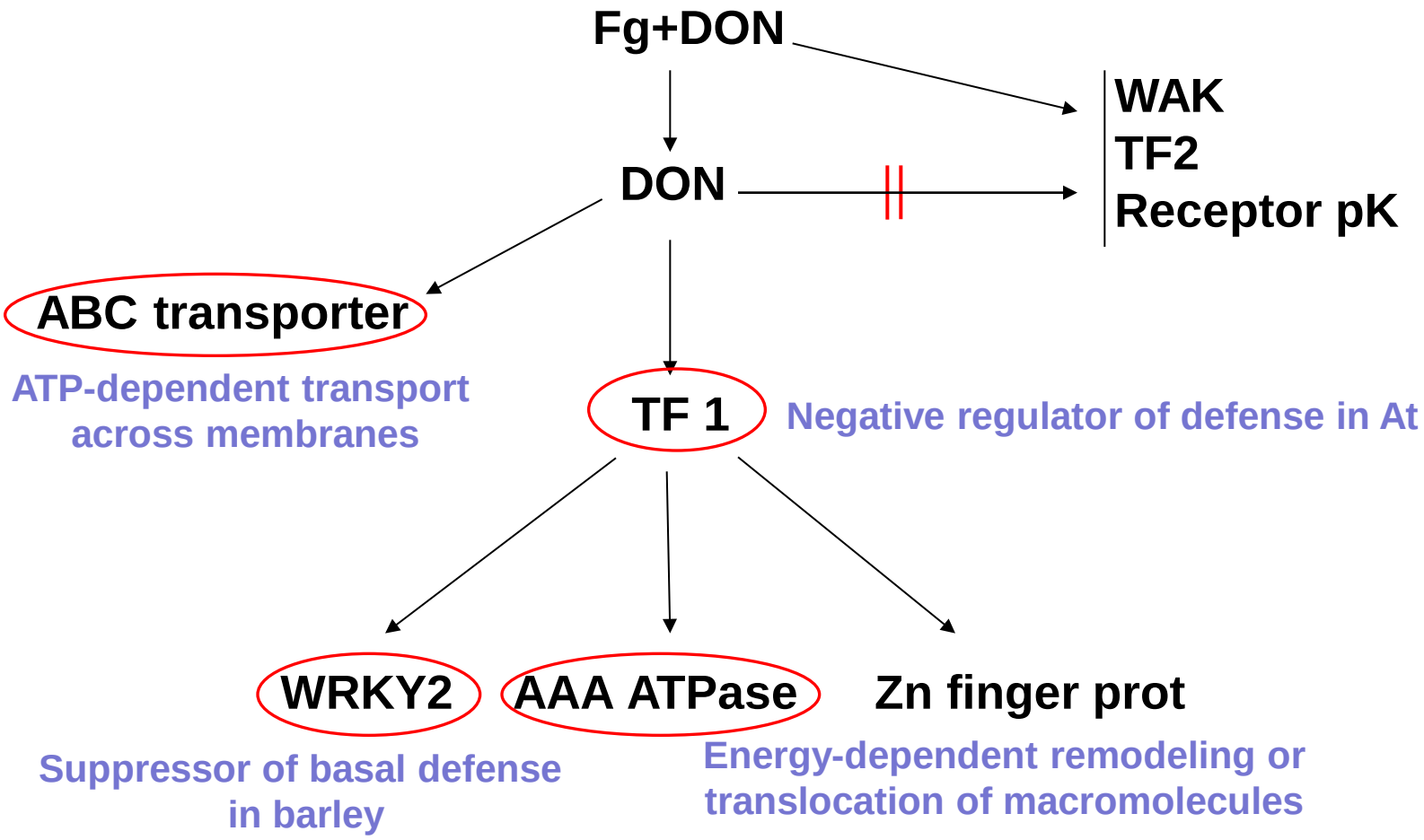
Transient silencing in susceptible Roblin: FHB disease symptom reduction

- Expression of both the transcription factor-like gene 1 and the MRP-like ABC transporter contributes to susceptibility to *F. graminearum*

- The transcription factor 1 contributes to regulation of other DON-associated genes



A potential expression network




Questions?
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