

Subcellular targeting of plant defensin
MtDef4 determines the outcome of
plant-pathogen interactions in transgenic
Arabidopsis

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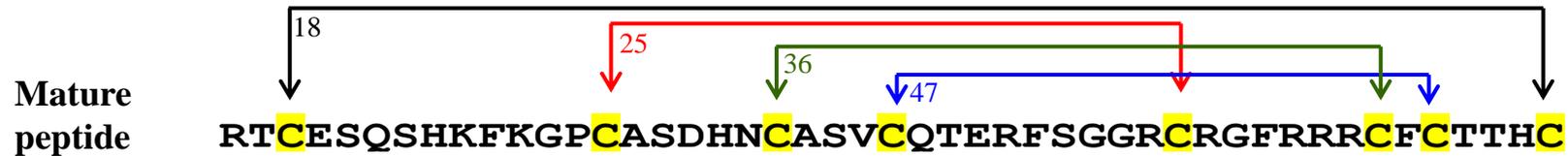


What are plant defensins?

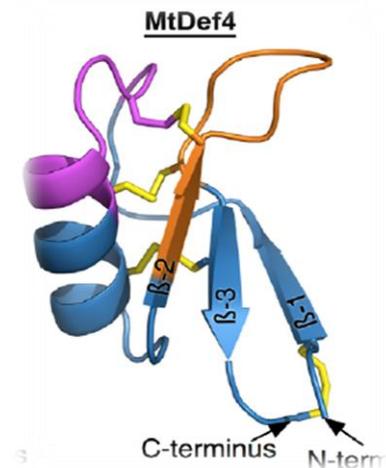
- Plant defensins are 45-54 amino acids long cysteine-rich antifungal peptides
- Comprise of one α - helix and three antiparallel β - sheets, stabilized by 4 disulfide bonds
- Majority of plant defensins are extracellular, a few are vacuolar
- Constitutively expressed and induced by abiotic and biotic stresses
- Greenhouse and field efficacy of plant defensins for control of fungal and oomycete pathogens in transgenic plants already demonstrated



Antifungal plant defensin MtDef4 derived from *Medicago truncatula*

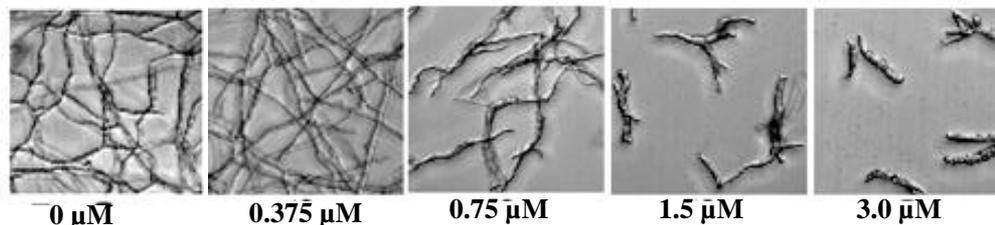


- Naturally secreted in extracellular space
- Present in alfalfa sprouts, hence already in food chain
- Homologs of MtDef4 are present in many other plants - both legume and non-legume
- *In silico* analysis showed that MtDef4 is expressed in a variety of tissue types and during various stages of seed filling in *M. truncatula*



MtDef4 exhibits broad spectrum activity against *Fusarium* spp.

IVAF assay of MtDef4 against *Fusarium graminearum* PH-1



Ramamoorthy *et al.*, 2007

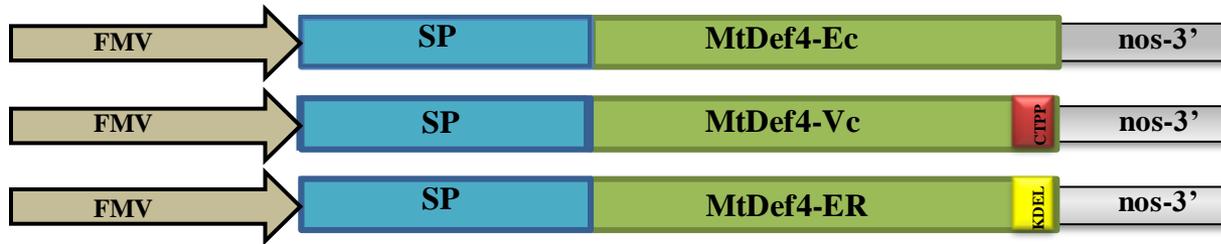
Causes devastating Fusarium Head Blight disease of wheat and barley and produces harmful mycotoxins

Disease	Fungus	Isolate	IC ₅₀ (μM) ^a
FHB of cereals	<i>F. graminearum</i>	PH-1	0.75 - 1.5
Crown rot of wheat	<i>F. pseudograminearum</i>	9095	0.5 - 1.0
Ear rot of maize	<i>F. proliferatum</i>	19	1.2 - 1.6
		37-2	0.8 - 0.9
		310	1.2 - 1.5
	<i>F. verticillioides</i>	ISU94445	1.3 - 1.8
		ISU94482	1.2 - 1.5

^aConcentration required to inhibit 50% hyphal growth.

- Use *Arabidopsis thaliana* as model system to test the effect of antifungal defensin MtDef4 *in planta*

Gene constructs used for transforming *Arabidopsis thaliana*



Extracellular targeting

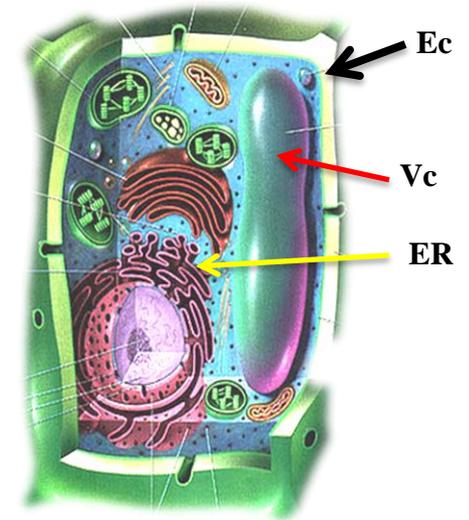
Vacuolar targeting

ER retention

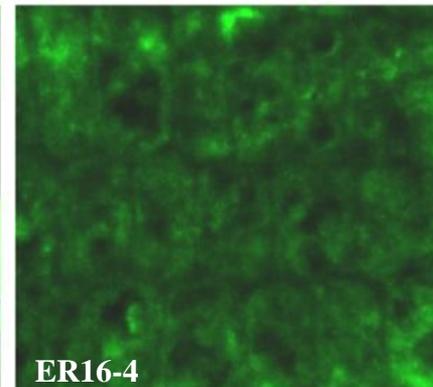
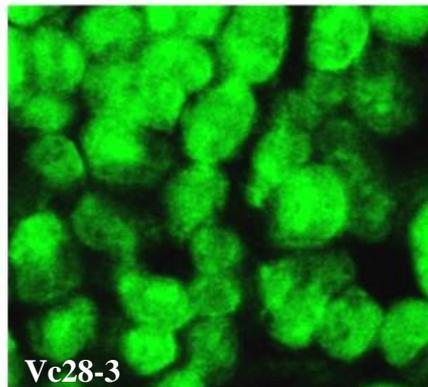
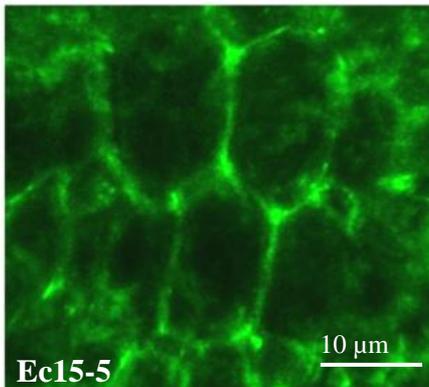
= native signal peptide

= 15 aa barley lectin sequence

= Lys-Asp-Glu-Leu (KDEL)



Three homozygous lines for each construct were selected for further characterization



Testing transgenic Arabidopsis lines against pathogens with different lifestyles

Test transgenic Arabidopsis lines targeted to different subcellular compartments for resistance to



Biotrophs

- Keep the host cells alive
- Intercellular growth in the host
- Obligate pathogens
- *Hyaloperonospora arabidopsidis*



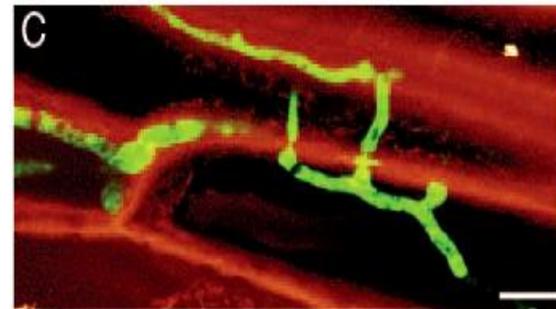
<http://en.wikipedia.org/wiki/File:Hyaloperonospora-parasitica-hyphae-haustoria.jpg>

Shah Lab, unpublished data



Hemibiotrophs

- Kill the host plant cells
- Growth is inter- and intracellular
- Non-obligate pathogens
- *F. graminearum*



Jansen *et al.*, 2005



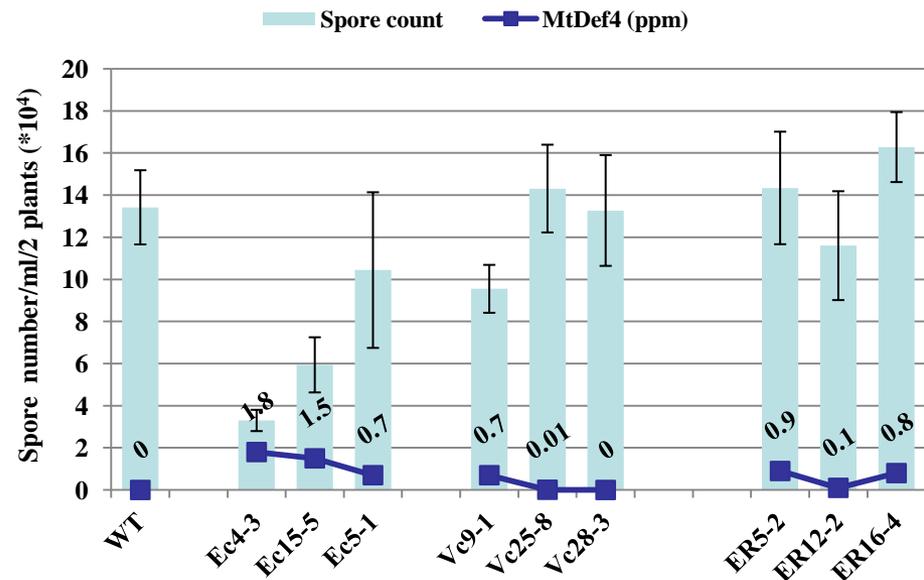
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MtDef4 targeted to extracellular space confers strong resistance to the biotroph *H. arabidopsidis*

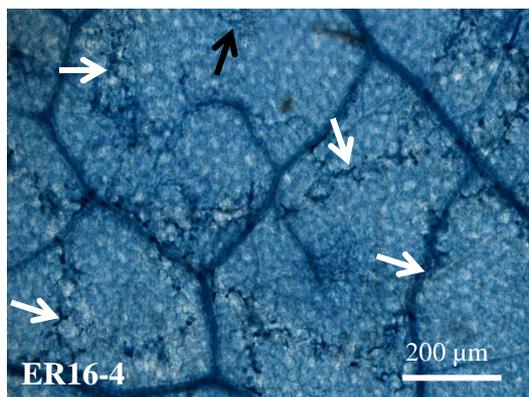
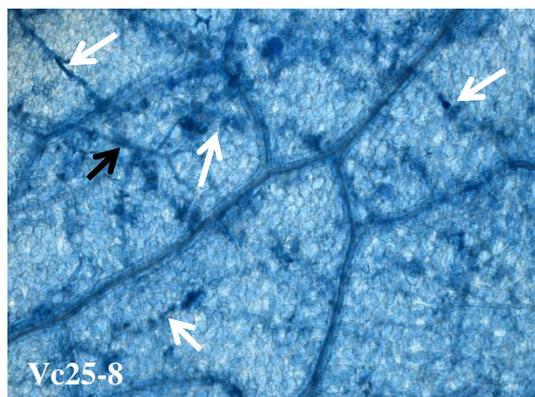
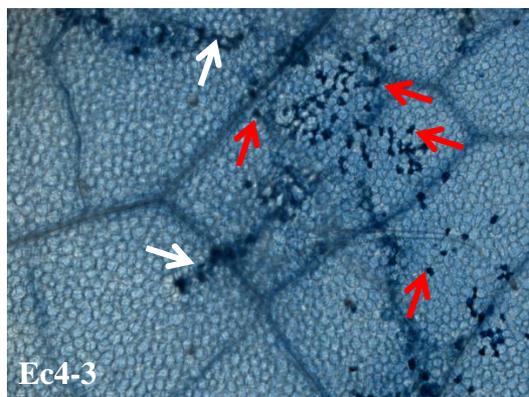
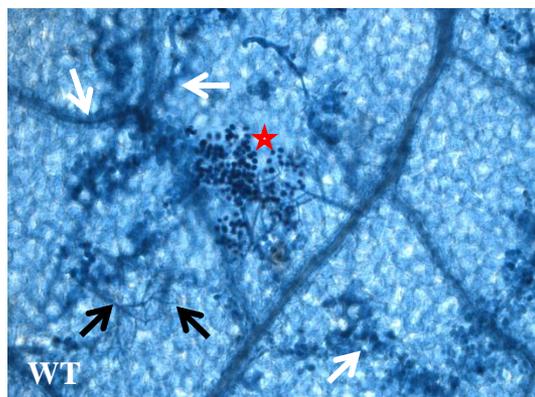
- *H. arabidopsidis* is natural pathogen of *Arabidopsis thaliana*
- *HpaNoco2* was found in Norwich and is virulent on Columbia
- Spray inoculation was done (5×10^5 spores/ml) and spores were counted at 7 dpi

Line	Spore number/ml/2 plants* 10^4	
	Mean \pm SEM ^a	P-value
WT	13.42 \pm 1.761	n.a.
Ec4-3	3.30 \pm 0.505	<0.0001
Ec5-1	10.44 \pm 3.695	0.4143
Ec15-5	5.94 \pm 1.308	0.0012
Vc9-1	9.55 \pm 1.138	0.0705
Vc25-8	14.32 \pm 2.083	0.7435
Vc28-3	13.27 \pm 2.630	0.9635
ER5-2	14.35 \pm 2.672	0.7731
ER12-2	11.60 \pm 2.585	0.5745
ER16-4	16.28 \pm 1.661	0.2428

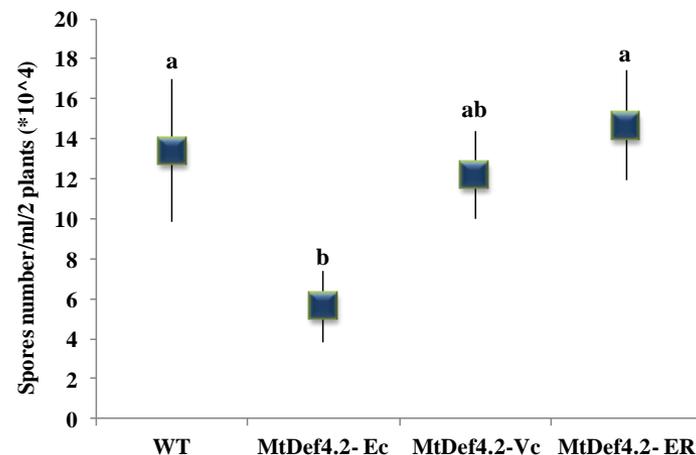
^aValues show the mean of six biological replicates \pm SEM. Analyzed using Student's *t*-test.



Extracellular targeting of MtDef4 is sufficient to provide resistance to biotroph

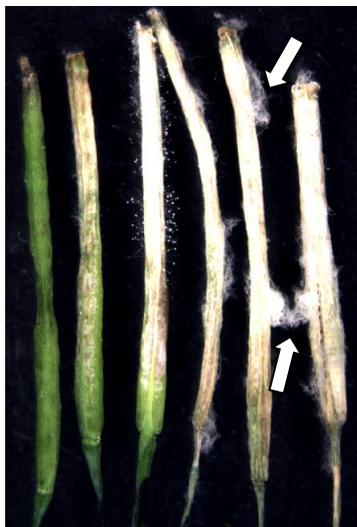


White arrows = Hyphae
Black arrows = Conidiophore
Red arrows = TN, failed infection attempt
Asterisk = Spores



Transgenic Arabidopsis lines are resistant to *F. graminearum* and DON accumulation

- Silique inoculation was done using PH-1 (1×10^6 spores/ml)
- A large number of siliques ranging 150-250 were inoculated for each line and scored on 8 dpi
- Frequency of infected siliques belonging to resistant category was relatively higher in transgenic lines compared to WT



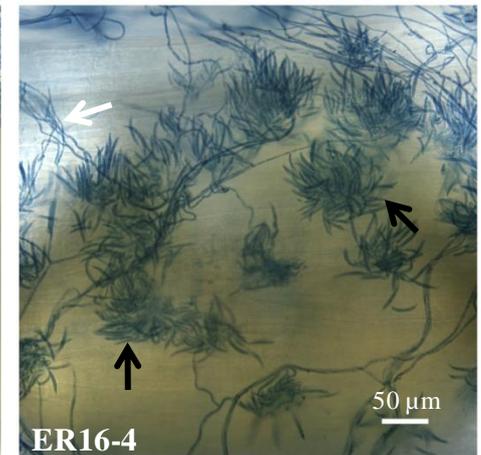
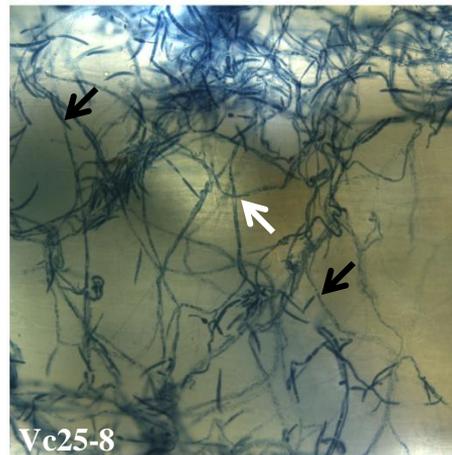
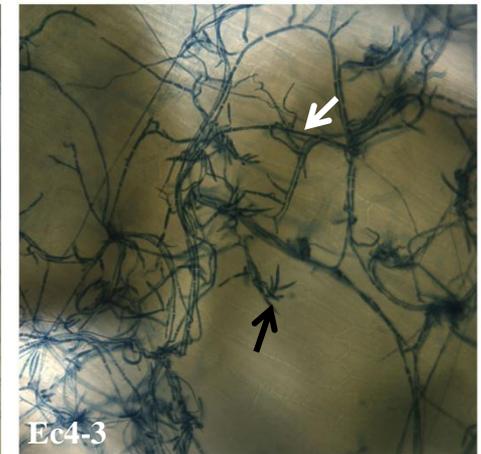
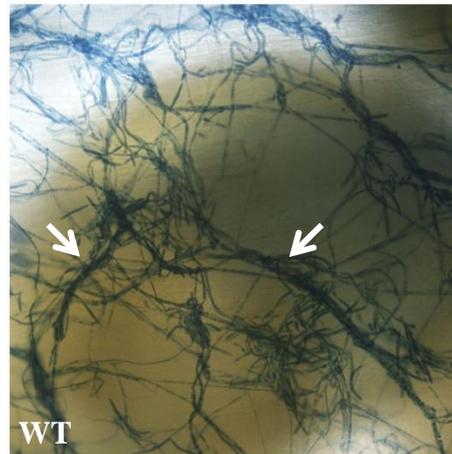
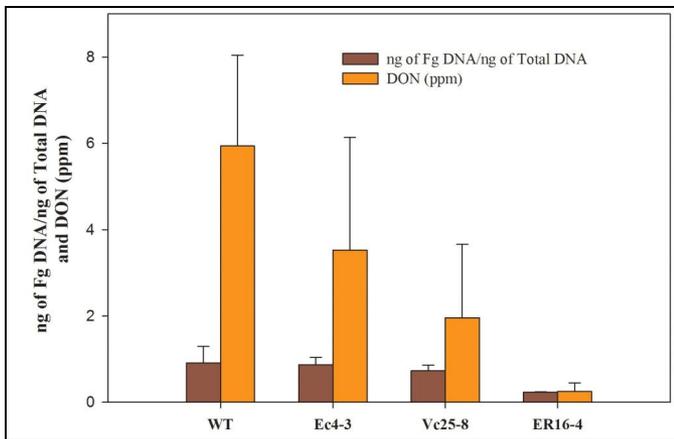
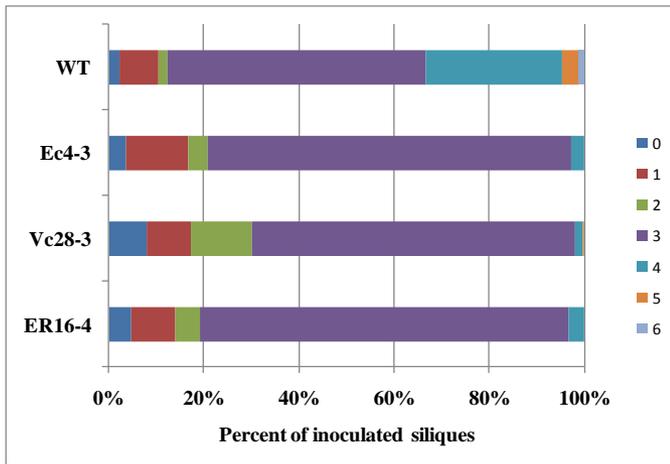
WT (8 dpi)

Line	Disease Severity		DON (ppm)	
	Mean \pm SEM	P value	Mean \pm SEM	P value
WT	2.99 \pm 0.062	/	5.9 \pm 2.10	/
Ec4-3	2.48 \pm 0.063	<0.0001	3.5 \pm 2.60	0.4908
Vc28-3	2.37 \pm 0.063	<0.0001	4.2 \pm 3.2	0.6653
ER16-4	2.52 \pm 0.064	<0.0001	0.2 \pm 0.19	0.0273
WT	3.05 \pm 0.056	/	5.2 \pm 1.18	/
Ec5-1	2.72 \pm 0.055	<0.0001	2.2 \pm 0.52	0.0523
Vc9-1	2.71 \pm 0.068	<0.0001	2.9 \pm 0.49	0.1182
ER12-2	2.65 \pm 0.053	<0.0001	3.5 \pm 1.23	0.3539
WT	3.09 \pm 0.042	/	4.3 \pm 1.92	/
Ec15-5	2.93 \pm 0.038	0.0091	2.9 \pm 1.74	0.6289
Vc25-8	2.73 \pm 0.042	<0.0001	1.9 \pm 1.69	0.3964
ER5-2	2.69 \pm 0.051	<0.0001	1.9 \pm 1.22	0.3495



ER16-4 (8 dpi)

Transgenic Arabidopsis lines accumulate less fungal biomass compared to WT



Summary

- Extracellular, but not intracellular, targeting of MtDef4 in transgenic Arabidopsis is sufficient to provide strong resistance to biotroph *H. arabidopsidis*
- Extracellular or intracellular targeting of MtDef4 confers low level of resistance to *F. graminearum*
- Significant reduction in DON levels are observed in transgenic Arabidopsis line ER16-4



Future directions

- Generate transgenic *Arabidopsis* lines coexpressing extra- and intracellularly targeted MtDef4
- Test these transgenic lines for more robust resistance to *F. graminearum* and DON accumulation



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