Antifungal Plant Defensins: Mechanisms of Action and Genetic Engineering for Disease Resistance

Fusarium Head Blight Forum
December 2016

Antifungal plant defensins

- Small (45-70 amino acids), cysteine-rich, cationic peptides present throughout plant kingdom
- Rich diversity of defensin genes in every plant
- Antifungal activity at micromolar concentrations
- Apoplast or vacuole targeted and different modes of antifungal action
- Expression in transgenic plants confers resistance to fungal pathogens

Superfamily of cysteine-rich peptides in *Medicago truncatula*

Medicago truncatula defensins

X_{10}-**C**$_1$-X_8-G-X_1-**C**$_2$-X_{5-8}-**C**$_3$-X_3-**C**$_4$-X_{2-3}-E-X_{4-5}-G-X_1-**C**$_5$-X_{6-8}-**C**$_6$-**F**/Y/M/W/I-**C**$_7$-X_3-**C**$_8$-X_n

SIGNAL PEPTIDE — CLEAVAGE SITE — **MATURE DEFENSIN** (50-70 aa)

Nodule-specific **C**ysteine-**R**ich peptides (6 Cys)

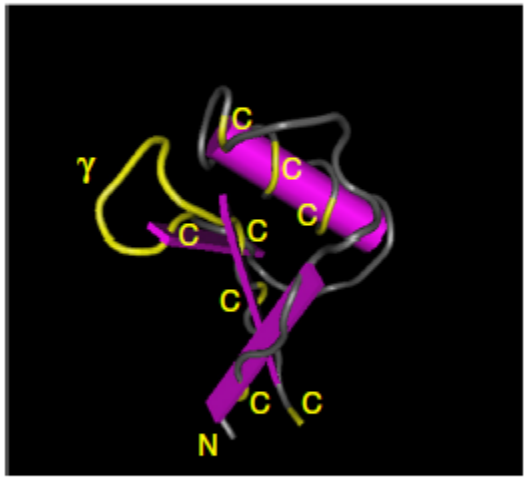
X_{4-12}-**C**$_1$-X_5-**C**$_2$-X_{1-8}-**C**$_3$-X_{5-8}-**C**$_4$-X_{4-16}-**C**$_5$-X_1-**C**$_6$-X_n

SIGNAL PEPTIDE — CLEAVAGE SITE — **MATURE PEPTIDE** (30-55 aa)

Nodule-specific **C**ysteine-**R**ich peptides (4 Cys)

X_{2-7}-**C**$_1$-X_{5-6}-**C**$_2$-X_{9-14}-**C**$_3$-X_4-**C**$_4$-X_n

SIGNAL PEPTIDE — CLEAVAGE SITE — **MATURE PEPTIDE** (30-55 aa)

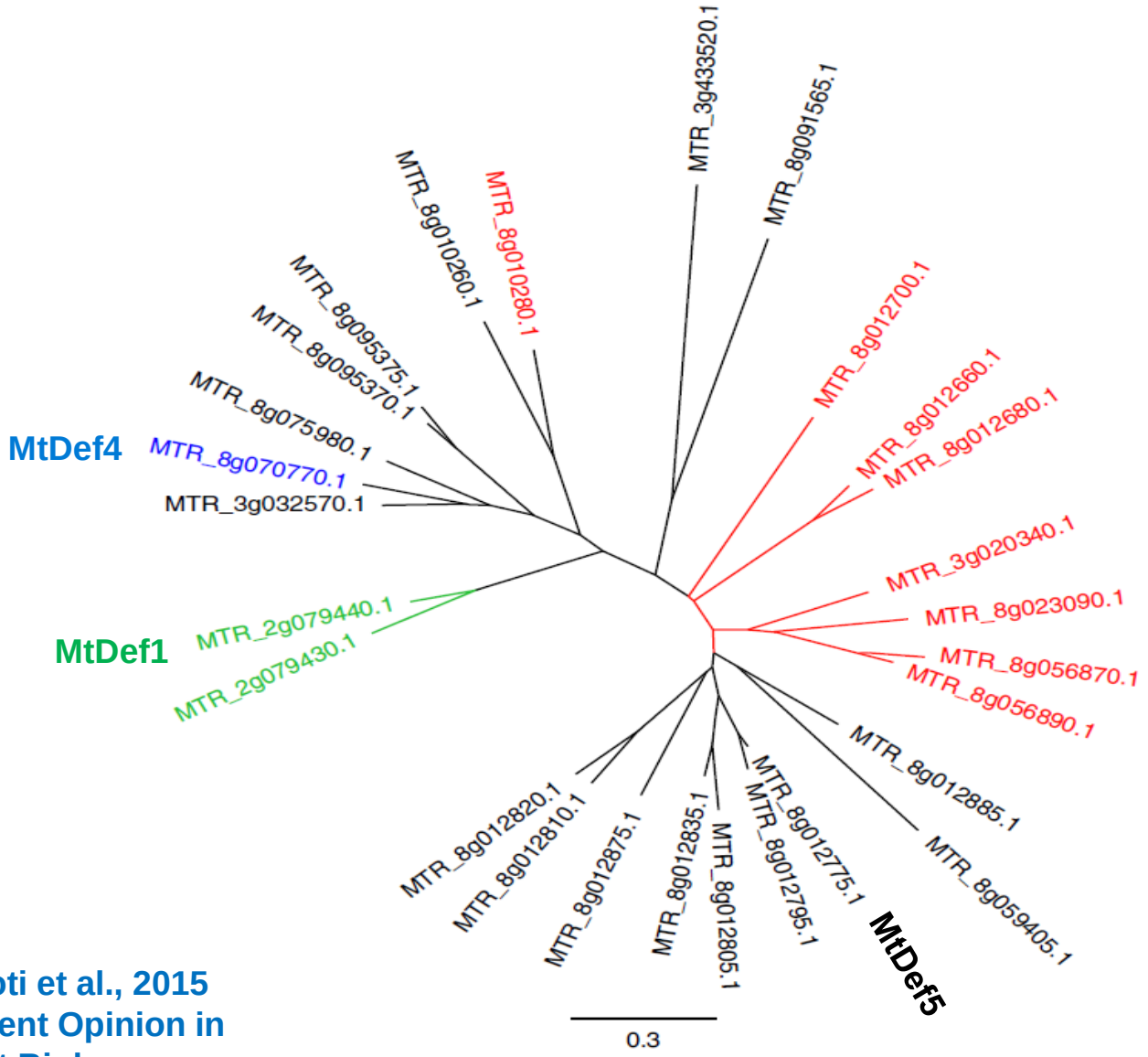


MtDef4

Current Opinion in Plant Biology

Maroti et al., 2015
Current Opinion in Plant Biology

Defensin genes in *M. truncatula*



Maroti et al., 2015
Current Opinion in
Plant Biology

MtDef4

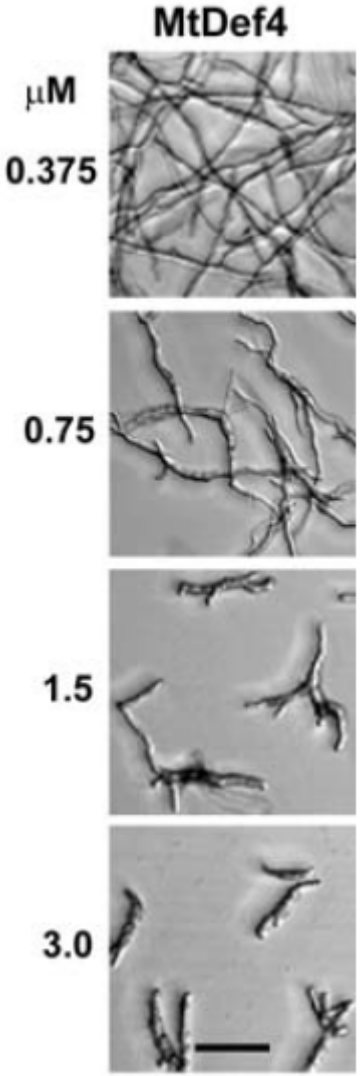
Signal peptide **Mature Def4**

MARSVPLVSTIFVFLLLLVATGPSMVAEARTCESQSHKFKGPCASDHNCASVCQTERFSG**GRCRGFRRRC**FCTTHC

γ-core motif **G X C X_{3-9} C**

- **Apoplast-targeted mature protein of 47-amino acids**
- **Expressed in all organs of *M. truncatula***
- **Homologs present in all plants**
- **Present in food chain**

MtDef4 exhibits broad spectrum antifungal activity *in vitro*



Fungus	IC_{50} (μM) values
Fusarium graminearum	**0.75 – 1.5**
F. pseudograminearum	**0.75 – 1.5**
F. proliferatum	**1.2 – 1.5**
F. verticillioides	**0.75 – 1.0**
Colletotrichum graminicola	**1.0-1.5**

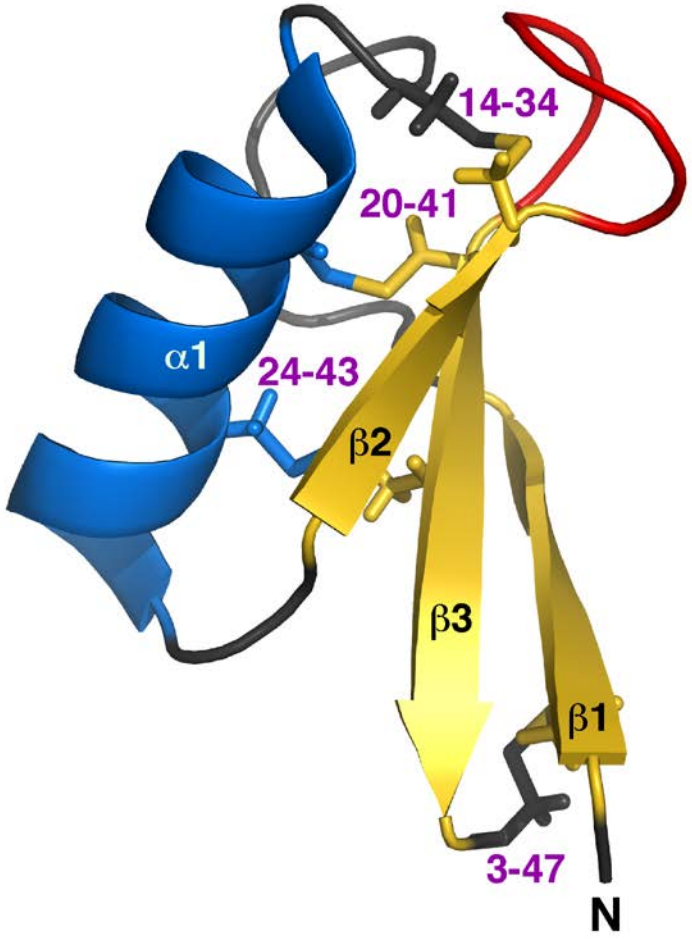
IC_{50} is the concentration at which 50% of the fungal growth is inhibited and is determined by reading the OD of the culture 36-60 h after exposure to the protein. The values are average of three experiments.

Cellular Microbiol 2007
Mol Plant Pathol 2012
Shah lab, unpublished

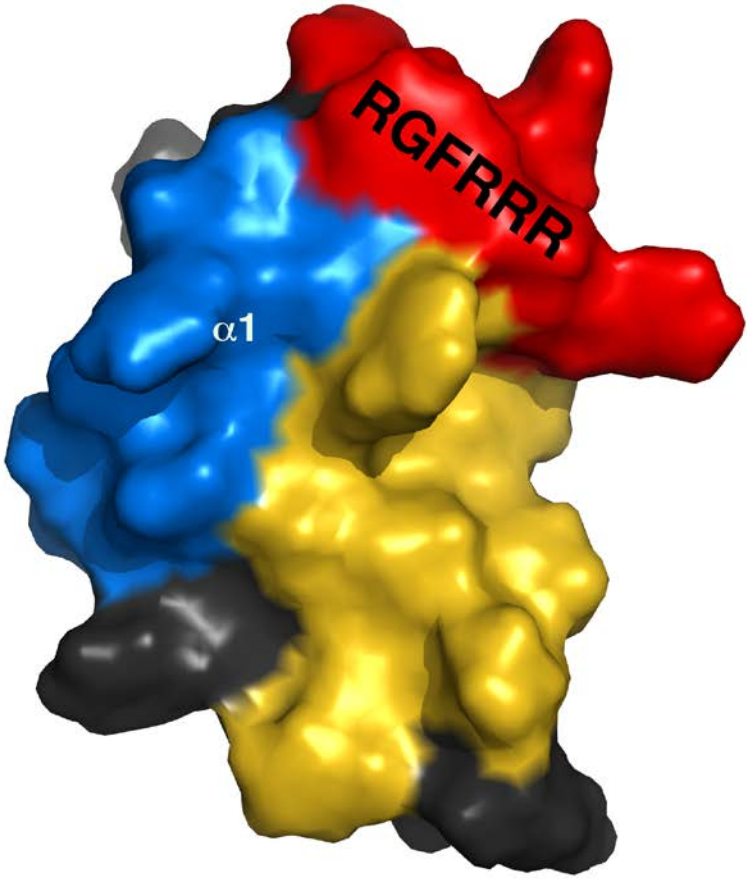
MtDef4 3D structure:Locations of disulfide bonds and γ-core motif

Def4: RTCESQSHKFKGPCASDHNCASVCQTERFSGGRCRGFRRRCFCTTHC

γ-core motif G X C X_{3-9} C



Disulfide bond locations



solvent-accessible surface

PLoS ONE 2013

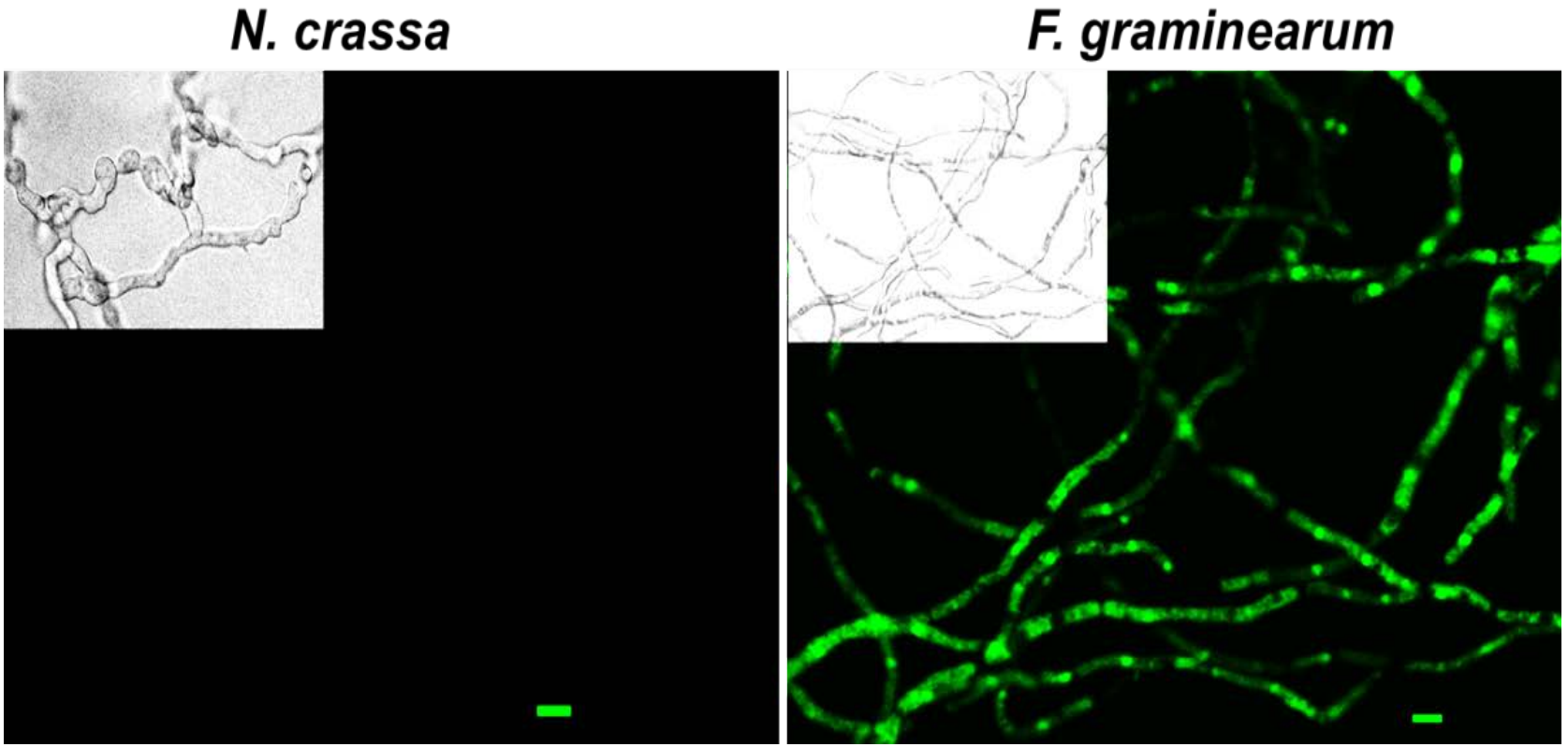
Structure-activity relationships and mode of action of MtDef4

Questions

- What are the structural motifs important for the antifungal activity of MtDef4?
- What is the mode of action of MtDef4?
- Is the mode of action conserved among closely related ascomycete fungi?

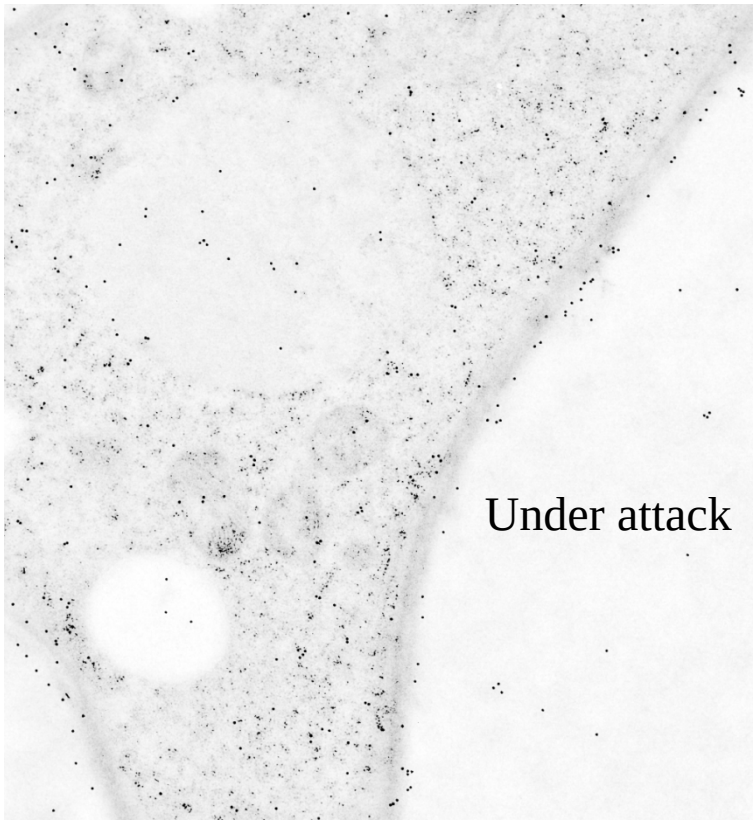
MtDef4 differs in its ability to permeabilize the fungal plasma membrane of *N. crassa* and *F. graminearum*

MtDef4



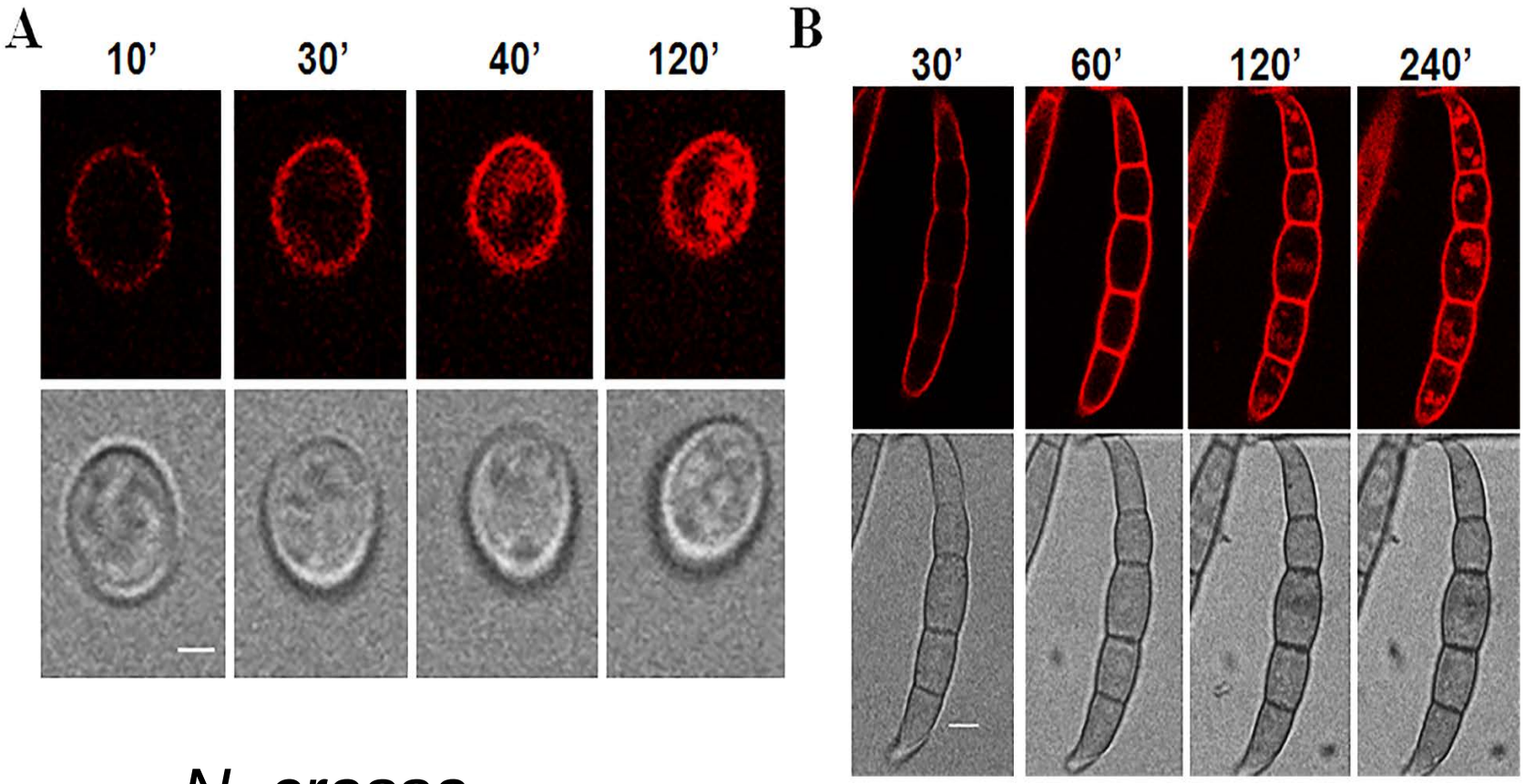
SYTOX Green uptake assay to study membrane permeabilization

MtDef4 is internalized by *F. graminearum* cells



PLoS ONE 2013

Internalization kinetics of MtDef4 in *N. crassa* and *F. graminearum*



N. crassa

F. graminearum

Molecular Microbiol 2016

Molecular Microbiology (2016) **100**(3), 542–559 ■

doi:10.1111/mmi.13333
First published online 1 April 2016

Antifungal mechanisms of a plant defensin MtDef4 are not conserved between the ascomycete fungi *Neurospora crassa* and *Fusarium graminearum*

Dimeric MtDef5

Signal peptide **Mature Def5A**

MTSSASKFYTIFIFVCLAFLFISTSEVEAKLCQKRSTTWSGPCLNTGNCKRQCINVEHATFGACHRQGFGFACFCYKKC

APKKVEPKLCERRSKTWSGPCLISGNCKRQCINVEHATSGACHRQGIGFACFCKKKC γ-core motif

Linker **Mature Def5B** γ-core motif

```
Def5A KLCQKRSTTWSGPCLNTGNCKRQCINVEHATFGACHRQGFGFACFCYKKCAPKKVEP
Def5B KLCERRSKTWSGPCLISGNCKRQCINVEHATSGACHRQGIGFACFCKKKC
                                      γ-core motif
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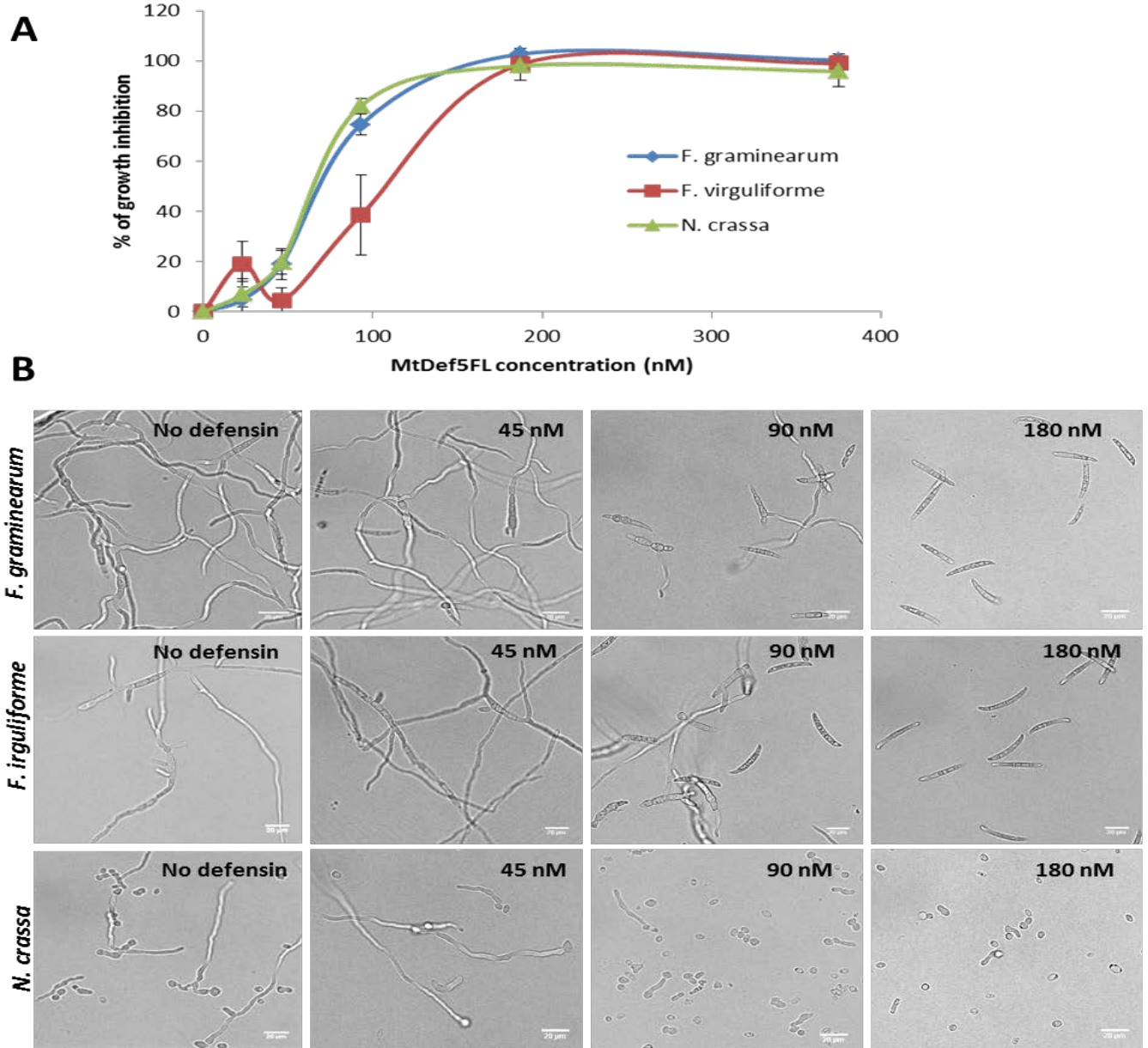
- Apoplast-targeted dimeric protein of 107 amino acids
- Net charge +16
- Expressed in root, stem and nitrogen-fixing nodules
- Present in all dicots

Dimeric MtDef5 exhibits potent antifungal activity at nanomolar concentrations



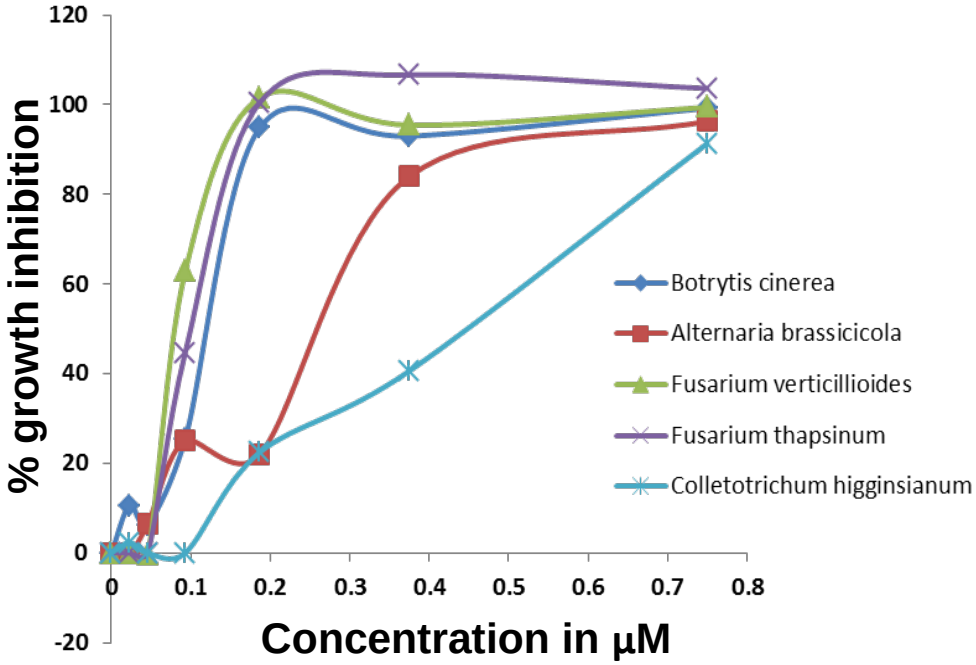
In vitro antifungal activity of various defensins against *F. graminearum*

	IC_{50} (nM)	IC_{100} (nM)
MsDef1	1500-3000	24000
MtDef4	750-1500	6000
Dimeric MtDef5	90-120	180-250

MtDef5 >16 fold more potent than MsDef1

MtDef5 >8 fold more potent than MtDef4

MtDef5 exhibits broad spectrum antifungal activity



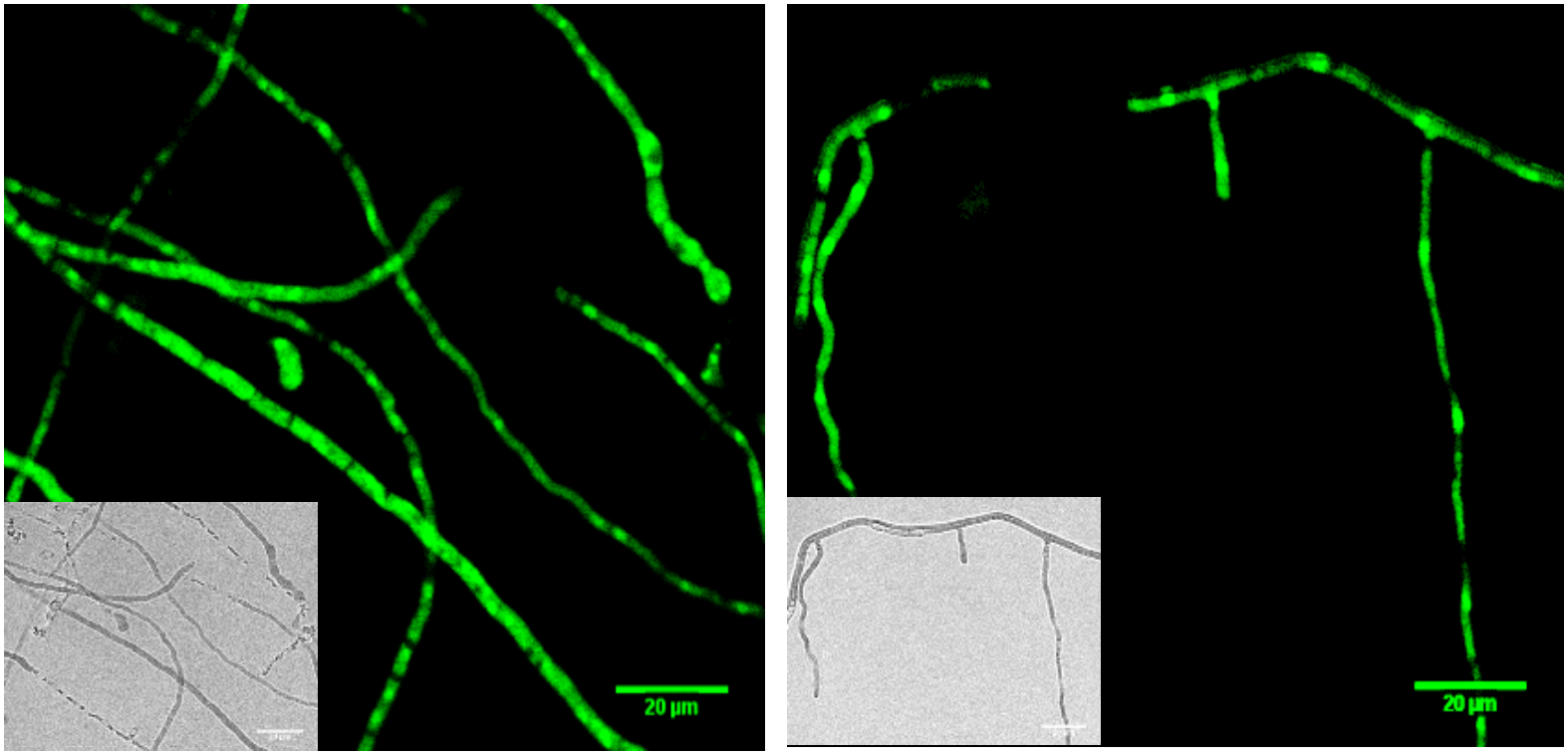
Dimeric MtDef5 exhibits similar *in vitro* antifungal activity against *Alternaria brassicicola*, *Botrytis cinerea, F. verticillioides, F. thapsinum* and *Colletotrichum higginsianum*

MtDef5 permeabilizes the plasma membrane of *N. crassa* and *F. graminearum*

MtDef5

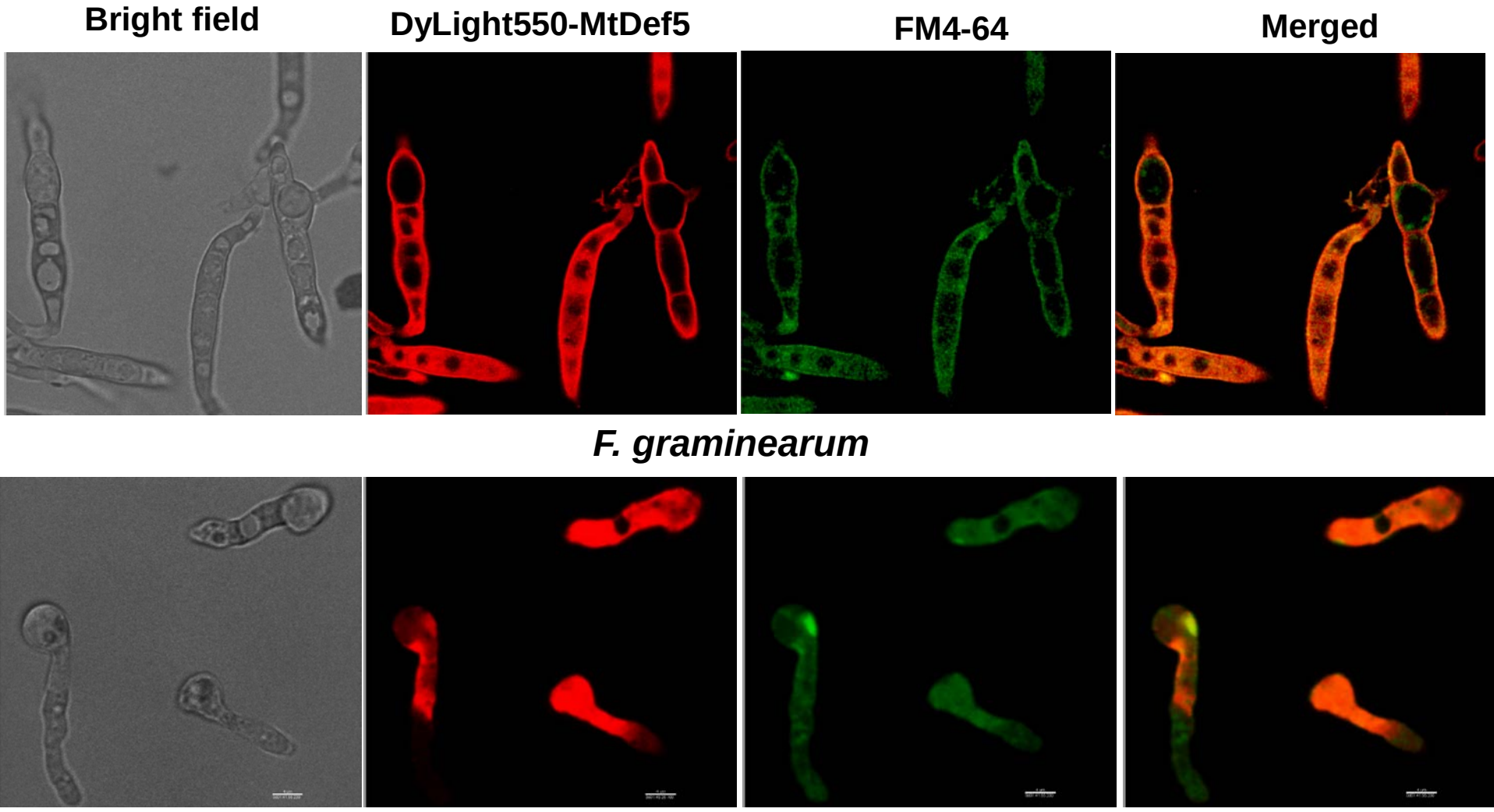
N. crassa

F. graminearum

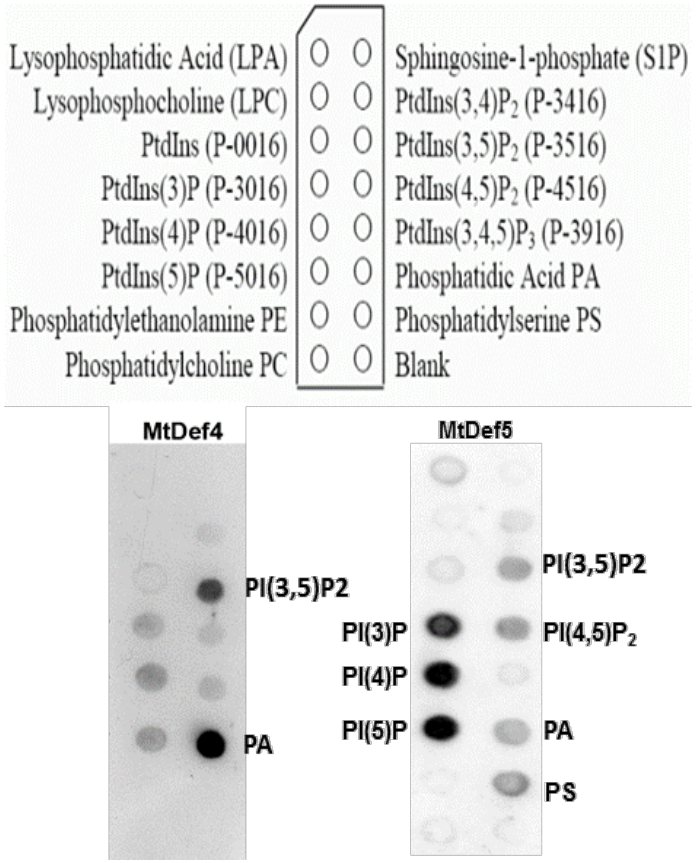


SYTOX Green uptake assay to study membrane permeabilization

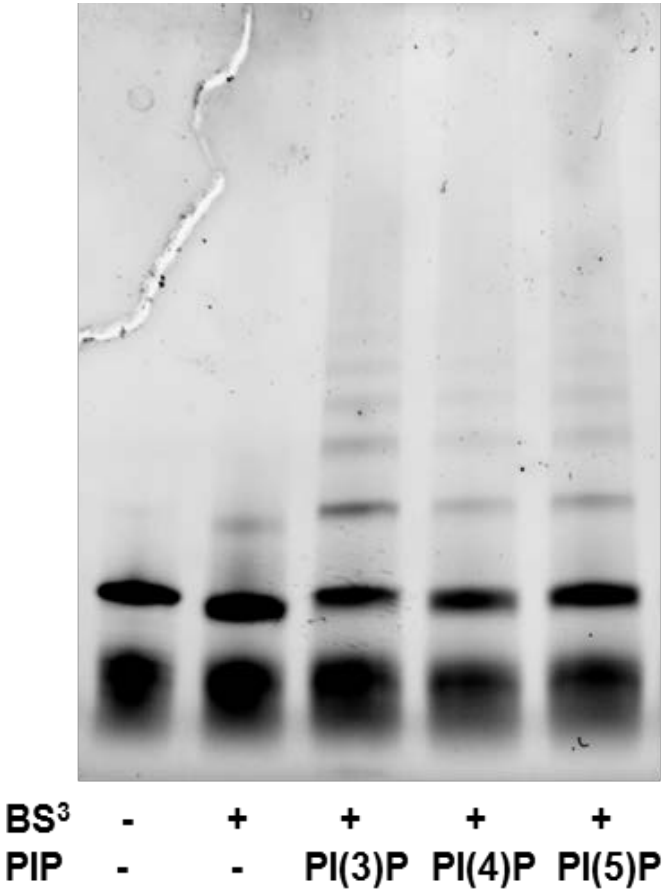
MtDef5 is internalized by *F. graminearum* and *N. crassa*



MtDef4 binds to phosphatidic acid, whereas MtDef5 binds to phosphatidylinositol monophosphates



PIP induced oligomerization of MtDef5

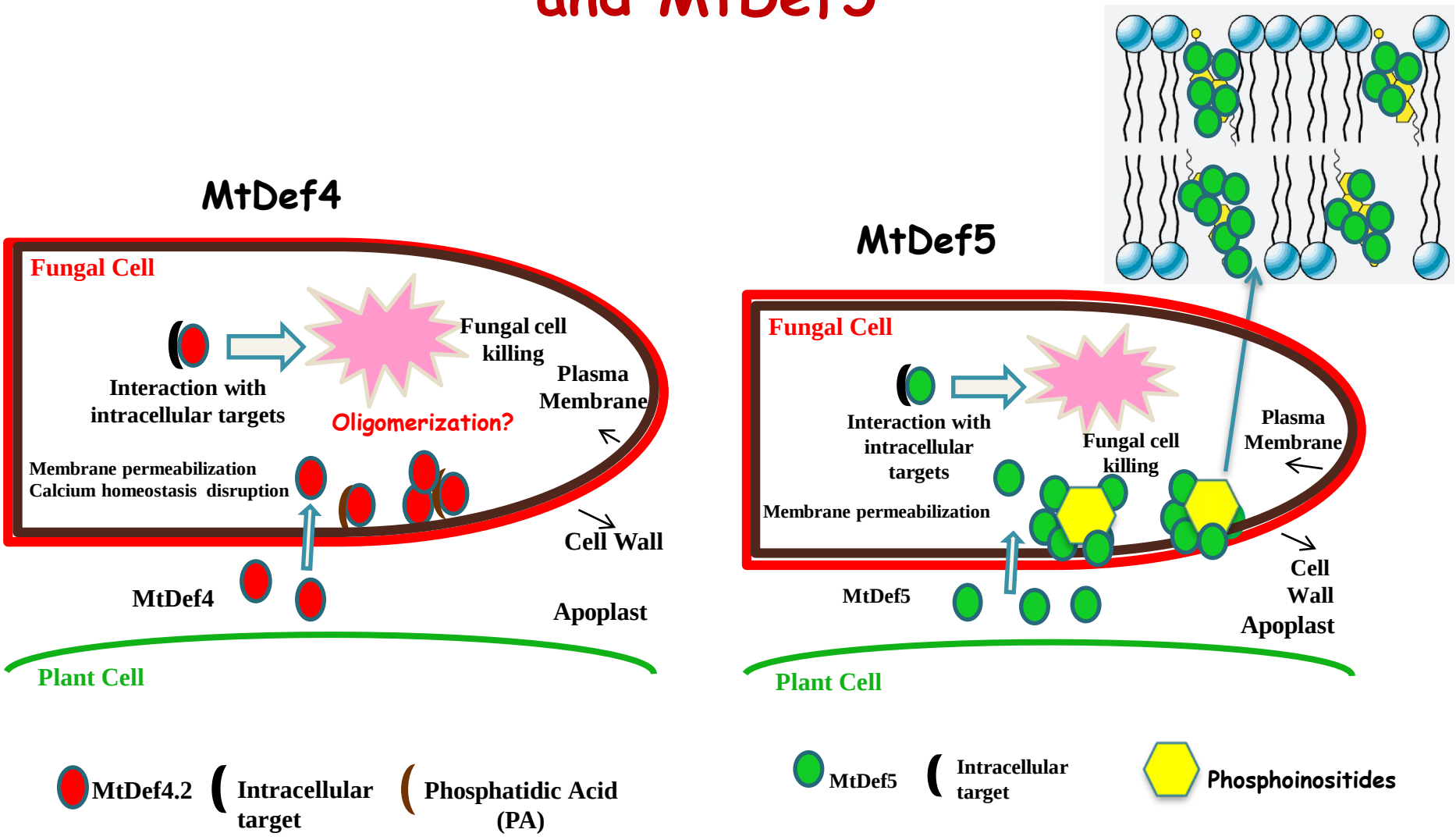


PLoS ONE 2013

PA and PIPs are pivotal lipid second messengers in fungi

- Essential phospholipids in membrane formation and signal transduction in fungi
- Important for cell growth, development and stress responses
- Regulate cell function though direct interaction with effector proteins involved in vesicle trafficking and cytoskeletal rearrangements

Proposed models for modes of action of MtDef4 and MtDef5



Does MtDef4 provide resistance to fungal pathogens in transgenic crops?

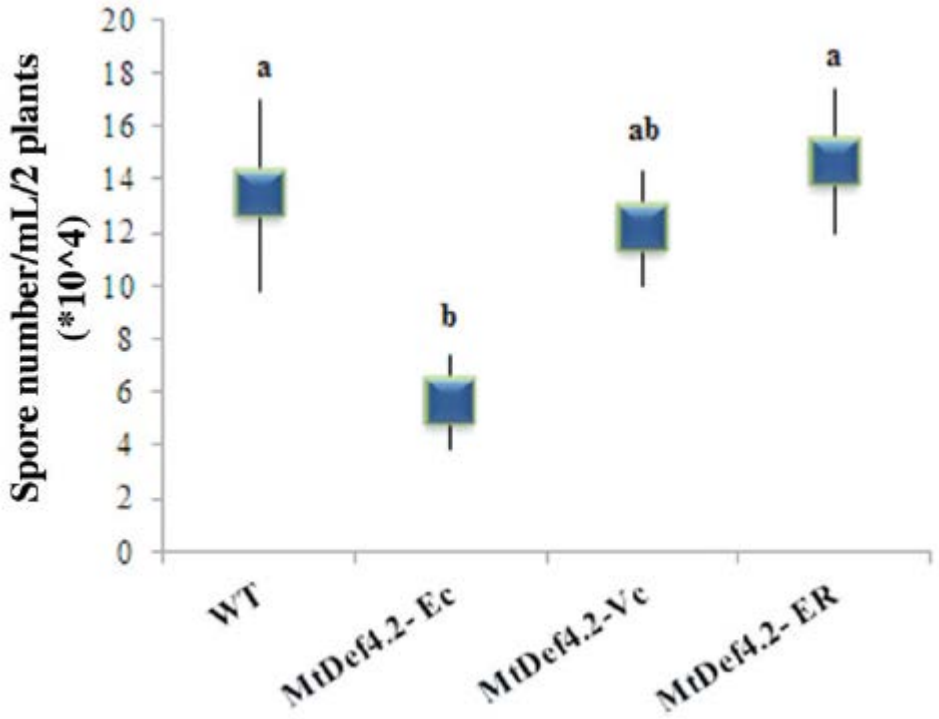
Results

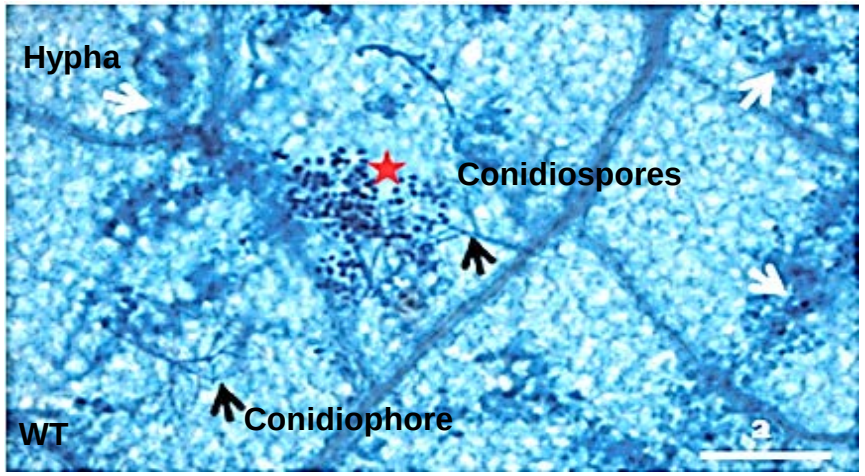
- In transgenic Arabidopsis, apoplast-targeted MtDef4 confers strong resistance to the oomycete biotroph *Hyaloperonospora arabidopsidis*, but intracellularly targeted MtDef4 does not.

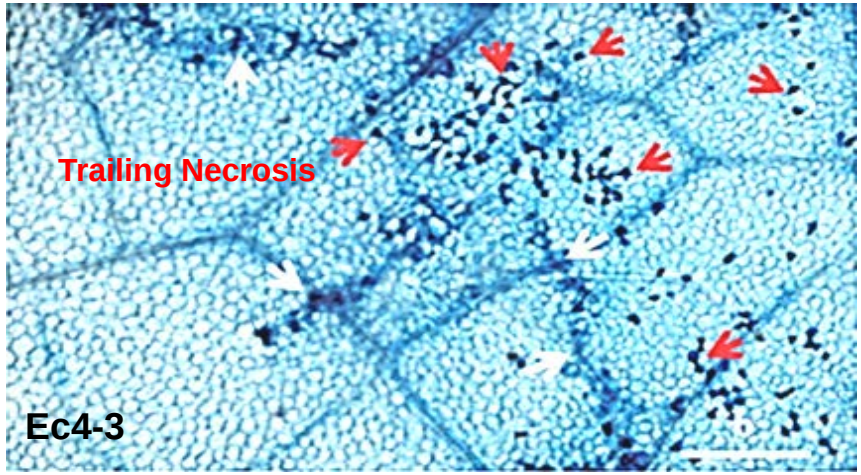
- Against hemibiotroph fungal pathogen *F. graminearum*, low level of resistance was observed. However, there was significant reduction in mycotoxin deoxynivalenol.

Molecular Plant Pathology 2012

Extracellularly targeted MtDef4 confers robust resistance to biotrophic oomycete *Hpa* in transgenic Arabidopsis







Mol Plant Pathology, 2012

Wheat leaf rust disease

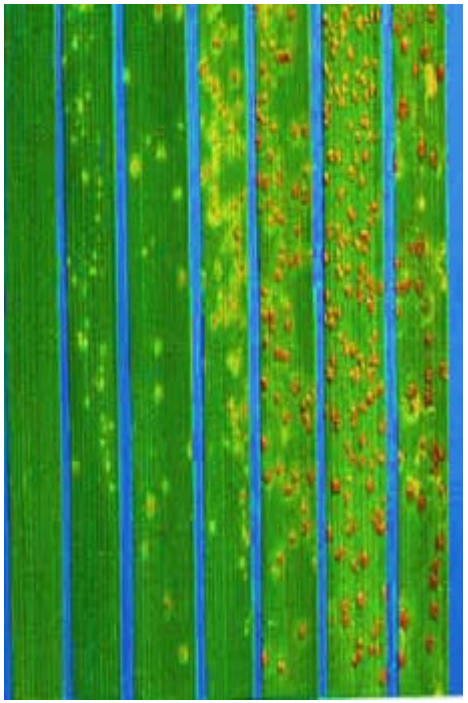
- Wheat rusts are obligate biotrophic fungal pathogens
- Leaf rust is caused by an obligate basidiomycete pathogen *Puccinia triticina*
- Leaf rust incurred a 14% yield loss in winter wheat in Kansas in 2007

Leaf rust – *Puccinia triticina*

Rust Evaluation of Transgenic Wheat Lines

TABLE 1-1 Major infection type classes for stem rust and leaf rust

Infection type[a]	Host Response	Symptoms
0	Immune	No visible uredia
;	Very resistant	Hypersensitive flecks
1	Resistant	Small uredia with necrosis
2	Resistant to moderately resistant	Small to medium sized uredia with green islands and surrounded by necrosis or chlorosis
3	Moderately resistant/ moderately susceptible	Medium sized uredia with or without chlorosis
4	Susceptible	Large uredia without chlorosis
X	Resistant	Heterogeneous, similarly distributed over the leaves
Y	?	Variable size with larger uredia towards the tip
Z	?	Variable size with larger uredia towards the leaf base

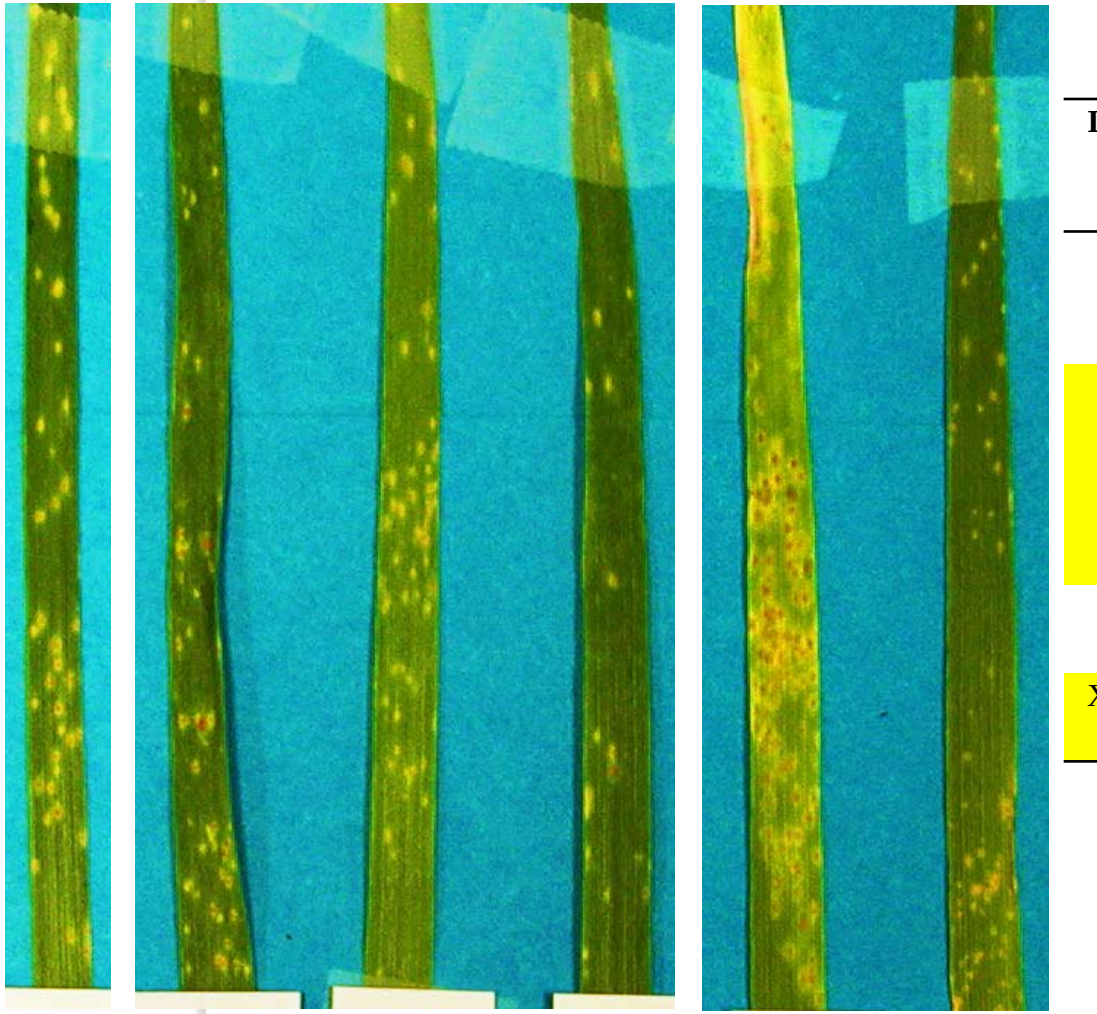


0 ; 1 2 3 3+ 4

Taken from “Wheat Rusts-An Atlas of Resistance Genes” Eds. RA McIntosh, CR Wellings and RF Park

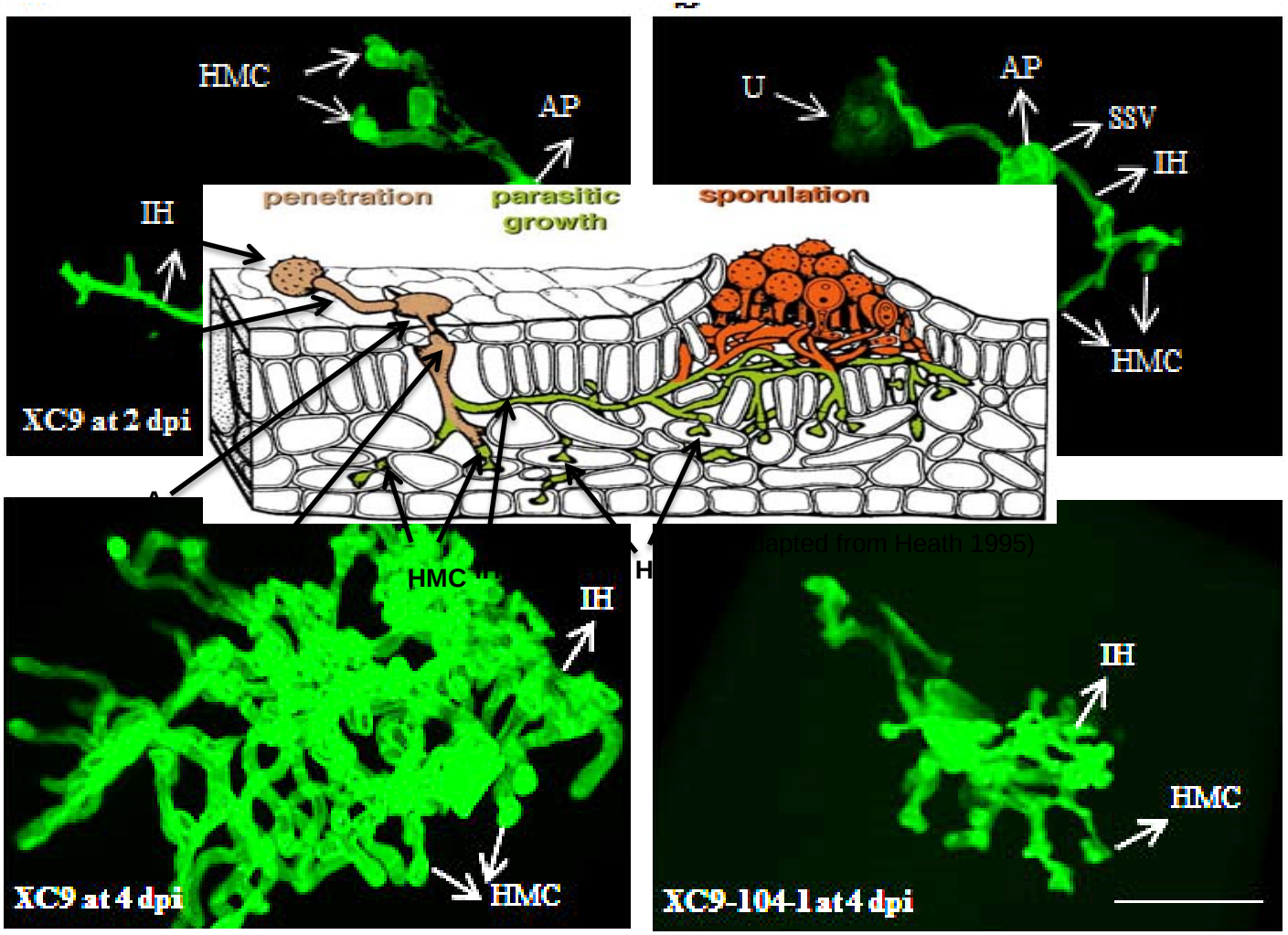
Transgenic wheat lines are resistant to leaf rust

Puccinia triticina pathotype MCPSS was used in seedling infection type assay



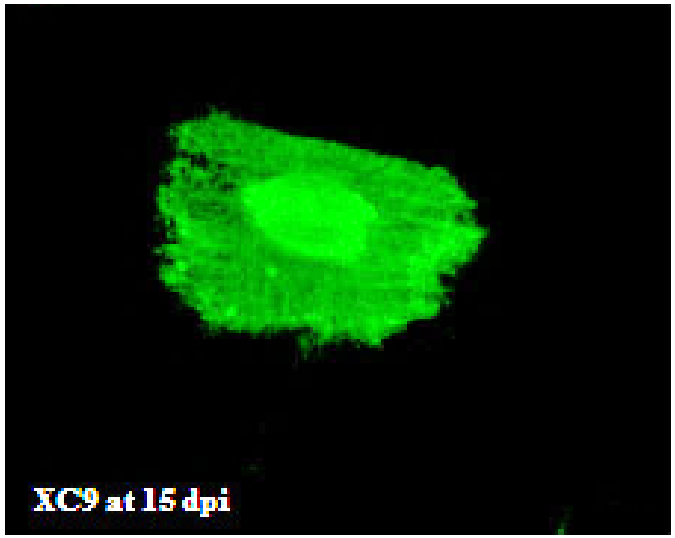
Line name	*Pt* pathotype MCPSS		Rust response
	1st experiment	2nd experiment	
BW	0, 1, 2	1,2	Moderately resistant
A-11-11	;	1,2	Highly resistant
B-4-11	Chlorosis	;1-	Highly resistant
F-10-13	1-2	;	Highly-resistant
XC9	1-2	33+	Moderately susceptible
XC9-104-1	;, fewer pustules	;1-	Highly resistant

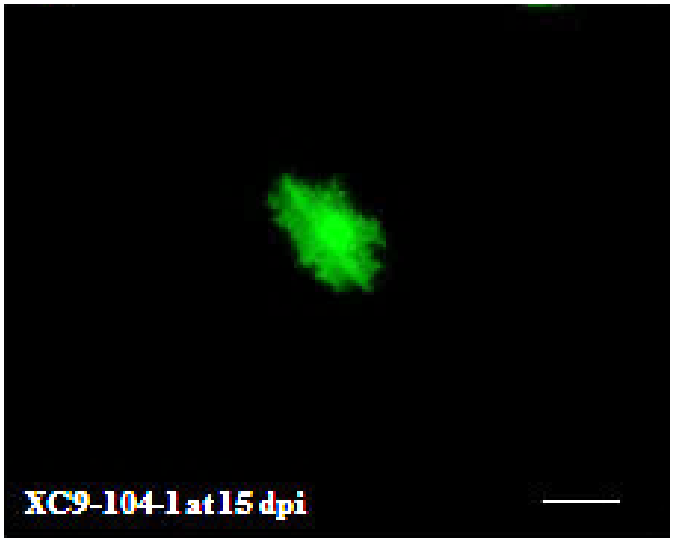
Transgenic wheat line XC9-104 shows pre- and posthaustorial resistance to *Pt*



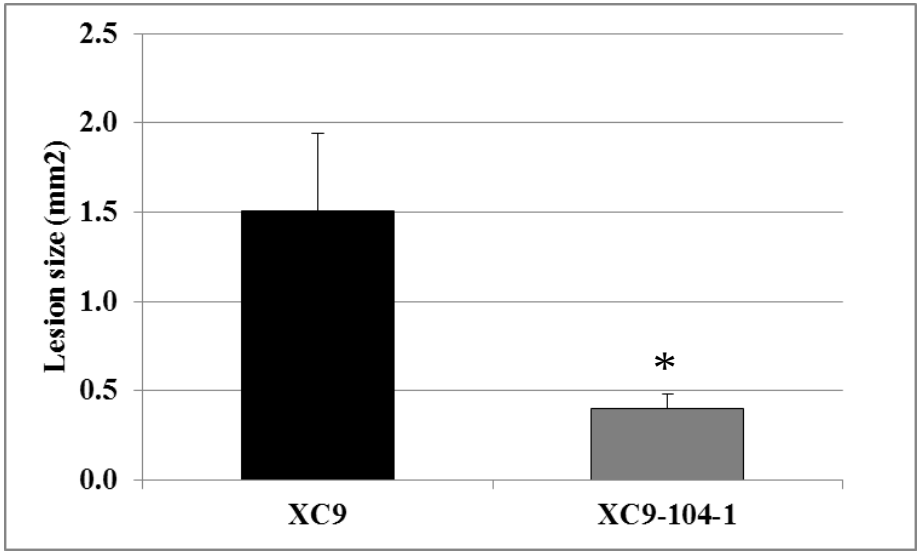
Transgenic Research, 2016

Transgenic wheat line XC9-104 has smaller infection colonies compared to XC9





Transgenic Research, 2016



Future directions

- Identify the amino acid residues involved in the PIP binding, oligomerization, internalization and antifungal activity of MtDef5.
- Elucidate the intracellular targets of MtDef4 and MtDef5.
- Test in planta antifungal activity of MtDef5 in transgenic crops.

Acknowledgements

Modes of Action of Defensins
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MtDef5 subcellular localization in *F. graminearum*