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

Jagdeep Kaur
12/05/11
What are plant defensins?

- Plant defensins are 45-54 amino acids long cysteine-rich antifungal peptides
- Comprise of one $\alpha$ - helix and three antiparallel $\beta$ - 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

Shah Lab, unpublished data
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

<table>
<thead>
<tr>
<th>Disease</th>
<th>Fungus</th>
<th>Isolate</th>
<th>IC₅₀ (µM)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHB of cereals</td>
<td><em>F. graminearum</em></td>
<td>PH-1</td>
<td>0.75 - 1.5</td>
</tr>
<tr>
<td>Crown rot of wheat</td>
<td><em>F. pseudograminearum</em></td>
<td>9095</td>
<td>0.5 - 1.0</td>
</tr>
<tr>
<td>Ear rot of maize</td>
<td><em>F. proliferatum</em></td>
<td>19</td>
<td>1.2 - 1.6</td>
</tr>
<tr>
<td>Ear rot of maize</td>
<td></td>
<td>37-2</td>
<td>0.8 - 0.9</td>
</tr>
<tr>
<td>Ear rot of maize</td>
<td></td>
<td>310</td>
<td>1.2 - 1.5</td>
</tr>
<tr>
<td>Ear rot of maize</td>
<td><em>F. verticillioides</em></td>
<td>ISU94445</td>
<td>1.3 - 1.8</td>
</tr>
<tr>
<td>Ear rot of maize</td>
<td></td>
<td>ISU94482</td>
<td>1.2 - 1.5</td>
</tr>
</tbody>
</table>

ᵃConcentration required to inhibit 50% hyphal growth.

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

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

Shah Lab, unpublished data
Gene constructs used for transforming *Arabidopsis thaliana*

Three homozygous lines for each construct were selected for further characterization.

- **Extracellular targeting** = native signal peptide
- **Vacuolar targeting** = 15 aa barley lectin sequence
- **ER retention** = Lys-Asp-Glu-Leu (KDEL)

Shah Lab, unpublished data
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*

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


Jansen et al., 2005

Shah Lab, unpublished data
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 (5x10⁵ spores/ml) and spores were counted at 7 dpi

<table>
<thead>
<tr>
<th>Line</th>
<th>Spore number/ml/2 plants*10⁴</th>
<th>Mean ± SEM&lt;sup&gt;a&lt;/sup&gt;</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>13.42 ± 1.761</td>
<td>n.a.</td>
<td></td>
</tr>
<tr>
<td>Ec4-3</td>
<td>3.30 ± 0.505</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>Ec5-1</td>
<td>10.44 ± 3.695</td>
<td>0.4143</td>
<td></td>
</tr>
<tr>
<td>Ec15-5</td>
<td>5.94 ± 1.308</td>
<td>0.0012</td>
<td></td>
</tr>
<tr>
<td>Ve9-1</td>
<td>9.55 ± 1.138</td>
<td>0.0705</td>
<td></td>
</tr>
<tr>
<td>Ve25-8</td>
<td>14.32 ± 2.083</td>
<td>0.7435</td>
<td></td>
</tr>
<tr>
<td>Ve28-3</td>
<td>13.27 ± 2.630</td>
<td>0.9635</td>
<td></td>
</tr>
<tr>
<td>ER5-2</td>
<td>14.35 ± 2.672</td>
<td>0.7731</td>
<td></td>
</tr>
<tr>
<td>ER12-2</td>
<td>11.60 ± 2.585</td>
<td>0.5745</td>
<td></td>
</tr>
<tr>
<td>ER16-4</td>
<td>16.28 ± 1.661</td>
<td>0.2428</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Values show the mean of six biological replicates ± SEM. Analyzed using Student’s *t*-test.

Shah Lab, unpublished data
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

Shah Lab, unpublished data
Transgenic Arabidopsis lines are resistant to *F. graminearum* and DON accumulation

- Silique inoculation was done using PH-1 (1x10^6 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

<table>
<thead>
<tr>
<th>Line</th>
<th>Disease Severity</th>
<th>DON (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SEM</td>
<td>P value</td>
</tr>
<tr>
<td>WT</td>
<td>2.99 ± 0.062</td>
<td>/</td>
</tr>
<tr>
<td>Ec4-3</td>
<td>2.48 ± 0.063</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vc28-3</td>
<td>2.37 ± 0.063</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ER16-4</td>
<td>2.52 ± 0.064</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WT</td>
<td>3.05 ± 0.056</td>
<td>/</td>
</tr>
<tr>
<td>Ec5-1</td>
<td>2.72 ± 0.055</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Vc9-1</td>
<td>2.71 ± 0.068</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ER12-2</td>
<td>2.65 ± 0.053</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>WT</td>
<td>3.09 ± 0.042</td>
<td>/</td>
</tr>
<tr>
<td>Ec15-5</td>
<td>2.93 ± 0.038</td>
<td>0.0091</td>
</tr>
<tr>
<td>Vc25-8</td>
<td>2.73 ± 0.042</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ER5-2</td>
<td>2.69 ± 0.051</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Shah Lab, unpublished data
Transgenic Arabidopsis lines accumulate less fungal biomass compared to WT

Shah Lab, unpublished data
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

Shah Lab, unpublished data
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

Shah Lab, unpublished data
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Shah Lab, unpublished data