Designing Disease Resistant Plants

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Soybean production in the USA

- 72.7 million acres (equal to corn)
- 2.8 billion bushels
- $30 billion industry in USA
- $1-2 billion is lost annually to soybean cyst nematode

Plant parasitic nematodes reduce crop yield

- Cause $100 billion in crop losses each year worldwide
- All plant crops are susceptible to nematodes

Belonolaimus Meloidogyne Heterodera Pratylenchus
Hoploaimus Globodera Ditylenchus Rotylenchulus

Genetically engineer plants resistant to nematodes

- Approaches
  - Over-express genes to provide resistance
  - Turn off soybean genes to provide resistance
  - Turn off critical nematode genes important to nematode survival

Timeline of SCN at 4, 6, 8 days after infection in a resistant (Peking) and susceptible (Kent) soybean cultivar

Peking 4D Peking 6D Peking 8D
Kent 4D Kent 6D Kent 8D

Laser Capture Microdissection

- Collect a homogeneous population of syncytial cells – 3, 6 and 9 dai
- Determine gene expression patterns in those cells

Leica LCM System
Klink et al. 2007b Planta
**Pipeline Soybean Assay**

- Grow seedlings 7 days
- Transform by agro inoculation
- Grow in greenhouse
- Trim non-transformed roots
- Green fluorescent roots are inoculated with SCN. Assay at 35 days

**Transformation Pipeline**

1. Identify soybean genes
2. Identify SCN genes
3. Over expression
4. Silencing
5. pRAP shuttle vectors
6. Transform soybean roots
7. Challenge with SCN
8. Identify new resistance

**Promoters of seven of nine genes greatly enhancing susceptibility contain an auxin TF binding site**

<table>
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<tr>
<th>Predicted Function</th>
<th>Location (nt)</th>
<th>FI OX</th>
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<tr>
<td>Oligopeptide transport</td>
<td>yes</td>
<td>254</td>
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<tr>
<td>UDP glucuronate 4-epimerase</td>
<td>yes</td>
<td>232</td>
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<tr>
<td>Unknown</td>
<td>yes</td>
<td>229</td>
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<td>Cupin domain</td>
<td>yes</td>
<td>214</td>
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<tr>
<td>Ca²⁺ kinase</td>
<td>yes</td>
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<tr>
<td>Pectate lyase</td>
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<td>197</td>
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<tr>
<td>Lipase</td>
<td>yes</td>
<td>195</td>
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<tr>
<td>Peroxidase</td>
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<tr>
<td>Auxin permease</td>
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Silencing several of these genes provided some resistance.

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RKN and SCN – two different genera - can be controlled by over-expression of genes.

PAD4 overexpression to control RKN and SCN

Silencing host genes using RNAi

Silencing host genes using RNAi
Silence nematode genes

- RNAi produced in the root
- Nematode feeds and ingests RNAi
- Nematode target transcript is degraded

Gene silencing RNAi constructs against root-knot nematode

- Non-transformed Control
- LDH gene
- ATP gene
- Empty vector control
- MS-70 gene
- TP gene

Broad nematode control is coming

- We can achieve broad control of two genera of nematodes
  - Meloidogyne (RKN)
  - Heterodera (SCN)
- Over-express genes to decrease nematode development
- Silence plant and nematode genes to decrease nematode development

More possibilities???

- Are plants with these constructs resistant to other biotrophic pests and pathogens?
- Can we take a similar approach manipulating jasmonic acid to control necrotrophs?
- Can we combine approaches to engineer in plants broad resistance to pathogens???