Genetic and Molecular Characterization of Host-Plant Resistance to Root-knot Nematodes and Fusarium Wilt in Cotton

Phil Roberts & Congli Wang,
Department of Nematology
Univ. of California, Riverside
Project Objectives

1. Determine the genetic basis of resistance to root-knot nematode (RKN) and Fusarium wilt (FW); uniqueness of NemX v. other R genes
2. Develop linked molecular markers to RKN and FW resistance genes;
3. Localize RKN $R$ genes to chromosome genome of cotton;
4. Develop a high resolution linkage map around the $R$ genes.
The Phenotype of Parents for Reaction to RKN and to FOV

<table>
<thead>
<tr>
<th>Genotype</th>
<th>( M. ) \textit{incognita} \textit{(RKN)}*</th>
<th>( F. o. ) \textit{vasinfectum} \textit{(FOV)}**</th>
<th>RKN +FOV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJ-2</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>NemX</td>
<td>R</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td>Pima S-7</td>
<td>S</td>
<td>R</td>
<td>MR</td>
</tr>
</tbody>
</table>

*I. Seedling inoculation method  
**II. Cut-root-dip method
Plant Populations

**NemX**

- **F1**
  - **F2**
  - **F2:3**
  - **F2:7**
  - **137 lines**

**SJ-2**

- **F1**
  - **BC1F1**
  - **Other test crosses**

**Pima S-7**

- **F1**
  - **F2**
  - **170+ plants**
  - **RILs**
  - **200+ plants**

- **BC1F1**
  - **Other test crosses**
F₂ and BCF₁ plants

NemX x Pima S-7
## The Phenotype of Parents and F1 for Reaction to *M. incognita*

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Gall rating</th>
<th>Total eggs (in 1000s)</th>
<th>Eggs/g root</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJ-2</td>
<td>5.58a</td>
<td>259.4a</td>
<td>4,129a</td>
</tr>
<tr>
<td>NemX</td>
<td>1.17b</td>
<td>14.8c</td>
<td>291b</td>
</tr>
<tr>
<td>N901</td>
<td>1.35b</td>
<td>8.0c</td>
<td>175b</td>
</tr>
<tr>
<td>F1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SJ-2 x NemX</td>
<td>5.37a</td>
<td>186.9ab</td>
<td>2,295a</td>
</tr>
<tr>
<td>Sj-2 x N901</td>
<td>5.06a</td>
<td>147.4b</td>
<td>2,487a</td>
</tr>
</tbody>
</table>
### Summary of Segregation Data for *Meloidogyne incognita* Resistance in Cotton Cultivar Acala NemX

<table>
<thead>
<tr>
<th>Parent or generation</th>
<th>Total plants or families</th>
<th>Observed R: I: S ratio</th>
<th>Expected R: I: S ratio</th>
<th>$\chi^2$</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SJ-2</td>
<td>10</td>
<td>0 : 0:10</td>
<td>All S</td>
<td>0</td>
<td>&gt;0.995</td>
</tr>
<tr>
<td>NemX</td>
<td>10</td>
<td>10 : 0: 0</td>
<td>All R</td>
<td>0</td>
<td>&gt;0.995</td>
</tr>
<tr>
<td>F₁</td>
<td>10</td>
<td>0 : 0: 10</td>
<td>All S</td>
<td>0</td>
<td>&gt;0.995</td>
</tr>
<tr>
<td>BC₁</td>
<td>346</td>
<td>91 : 172: 83</td>
<td>86.5 : 173: 86.5</td>
<td>0.382</td>
<td>0.75-0.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(1:2:1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Observed R: Seg: S ratio</th>
<th>Expected R: Seg: S ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>F₂:₃</td>
<td>7 : 45: 32</td>
<td>5.3 : 42: 36.8</td>
</tr>
<tr>
<td>F₂:₃</td>
<td>9 : 71: 52</td>
<td>8.3 : 66: 57.7</td>
</tr>
</tbody>
</table>

R - Resistant; S - Susceptible; I – Intermediate ; Seg – Segregating.
Multiplexed AFLP Protocol

Genomic DNA

Restriction digestion and ligation of adaptors to the fragment ends

Preamplification with adaptor-specific primers and (+1/+1) selective nucleotides

Selective amplification with IRD800 and IRD700 labeled adaptor-specific primers and (+3/+3) selective nucleotides

Gel electrophoresis
**Bulked Segregant Analysis (BSA)**

- BSA is used to make resistant plant DNA pools and susceptible DNA pools to obtain markers that correlate with each resistance trait.
- BSA has proved to be a powerful technique to identify markers linked to resistance genes.

```
P1(S)     P2(R)      RB     SB
```

Individual homozygous F_2 or F_{2:3} resistant plants (7-10)

Individual homozygous F_2 or F_{2:3} susceptible plants (7-10)
AFLP (amplified fragment length polymorphism)
Screening of Parental Polymorphism
AFLP and Bulked Segregant Analysis (BSA)
Potential Markers

200bp

145bp

255bp
Future Work

1. Screen potential markers for linkage to nematode and wilt resistance traits;
2. Use markers to confirm gene interactions that determine resistance phenotype;
3. Convert markers to STS/SCAR forms;
4. Localize resistance genes to the chromosomes;
5. Fine mapping of resistance genes
Reaction to FOV

Cut-root-dip method
4 weeks after inoculation

SJ-2  NemX  PimaS-7
The development of Fusarium wilt Disease in Parents and F₁ plants

Disease index

Days after inoculation

F₁ Pima S-7 x NemX
Pima S-7 x SJ-2
Cut-root-dip method