Rust Resistance Gene Cloning
perfect markers and cassette development

Peter Dodds

BGRI technical workshop March 2014
Outlook: R gene pyramids via GM gene cassettes

- Stacking of multiple R genes in a single location

  eg.

  Segregates as one locus

Need: Multiple cloned R genes  (=> perfect markers)

  Ability to stack into a transgene cassette
### Outlook: R gene pyramids via GM gene cassettes

#### Available genes:

<table>
<thead>
<tr>
<th>APR</th>
<th>Lr34/Yr18</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Krattinger et al 2009 Science 323:1360</td>
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<tr>
<td></td>
<td>= ABC transporter</td>
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<tr>
<td></td>
<td>Yr36</td>
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<tr>
<td></td>
<td>Fu et al 2009 Science 323:1357</td>
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<tr>
<td></td>
<td>= START Kinase</td>
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<tr>
<td>R</td>
<td>Sr33</td>
</tr>
<tr>
<td></td>
<td>Periyannan et al 2013 Science 341:786</td>
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<td></td>
<td>Sr35</td>
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<td></td>
<td>Saintenac et al 2013 Science 341:783</td>
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<tr>
<td></td>
<td>Lr1, Lr10, Lr21</td>
</tr>
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<td></td>
<td>= CC-NB-LRR immune receptors</td>
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</tbody>
</table>
**APR Cloning Targets: Lr67**

**Lr67**  
$[=Yr46=Sr55=Pm46=Ltn3]$  
(leaf rust, stripe rust, stem rust, powdery mildew, leaf tip necrosis)

*chromosome 4DL*

- cosegregation of all five phenotypes at *Lr67* locus
- mutation experiments indicates a single gene affects all five phenotypes
APR Cloning Targets: Sr2 and Lr46

Sr2
- physical contig across locus fully sequenced, 1.2Mbp
- 3 candidates testing in transgenic wheat
- new mutants and recombinants in locus

Lr46/Yr29
- multi-pathogen resistance with Ltn
- mapped on 1BL
- sequencing a physical contig across locus
- several mutants
R Cloning Targets: SrR = Sr50

1DL.1RS (Imperial)
- Gli-1, Glu-3
- SrR
- Sec-1
- NOR

1BL.1RS (Petkus)
- Gli-1, Glu-3
- Pm8
- Lr26, Sr31, Yr9
- Sec-1
- NOR

(Shepherd 1973)
Physical contig at Sr50 locus : 1RS chromosome sorted library (Dolezel)

Smallest deletion mutant includes 6 NB-LRR genes

2 EMS mutants carry small deletions in one NB-LRR gene

Rohit Mago
R Cloning Targets: SrR = Sr50

Sr50 transgenics express stem rust resistance

Stem Rust

Fielder #19 #13

Leaf Rust
• Derived from *Aegilops tauschii* AUS18913

• Confers all stage partial resistance to stem rust
  (Australian, Ug99 race group, Ethiopian and Yemeni isolates)

• Located on chromosome 2DS

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**Sr46**  **susc**
R Cloning Targets: Sr46

Genetic map, chr. 2DS

Sr46

Ae. tauschii BAC contig

Brachypodium 5g region

Sr46 candidate gene altered in three mutants (CC-NB-LRR)
Effective against worldwide stem rust races (Australia, Canada, India, US and Ug99 etc.)

Two sources:

- **T. boeoticum**
  - Middle East part of Israel
  - Gerechter- Amitai et al., 1971

- **T. monococcum**
  - Canada
  - Kerber and Dyck, 1973

**Schomburgk**
(Australian wheat cultivar)

**W3534**
(Standard differential for Sr22 -Australia)
R Cloning Targets: Sr22

Schomburgk x Westonia (1200 F2)

Schomburgk x Westonia (1200 F2)

T. monococcum

4044 (Sr22) x 4069 (1200 F2)

BE498985

4445409

4554964

4556232

AT7D7094

EMS mutants

➔ 1300 M2 heads – screened at PBI, Cobbitty

➔ 6 Mutants

With Matt Rouse
U. Minnesota

With Matt Rouse
U. Minnesota

Sam Periyannan
R gene cis-stacks

Resistance gene cis-stacks

Genes available

Lr34/Yr18, Lr21, Lr67/Yr46
Sr22, Sr33, Sr35, Sr46, Sr50
Yr36

Durability ??
R gene cis-stacks: wheat transformation

Transformation efficiency

Fielder = 41%
Gladius = 32%
Westonia = 45%
Mace = 25%

Mick Ayliffe
R gene cis-stacks: a 2-gene stack

Transformed into durum wheat cultivar Stewart

2 confirmed transgenics with both genes
- show leaf rust resistance
- to test for stripe rust
R gene cis-stacks: a 3-gene stack

Co-transformation

150 Fielder transgenics produced - screening now for co-transformation
Current activities

• Complete cloning of Sr2, Lr46

• Confirm Sr22 and Sr46 by transformation

• Develop gene stacking approaches
  insertion site targeting

• Understanding resistance gene function
  can we tweak them to improve function?

• R genes from wider sources: Barley, Brachypodium
  can’t be deployed conventionally