Fine-mapping of a QTL for nonhost resistance to *Blumeria graminis* f. sp. *tritici* in barley

Cynara Romero, Reza Aghnoum, Rients Niks
Outline of presentation

- Introduction
- Material and Methods
  - Development of fine-mapping population
  - Recombinants screening
- Results
  - Fine-mapping to a region of 0.2cM
  - Synteny with *Brachypodium* and rice
  - Candidate genes
- Future prospects
Acknowledgements

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Introduction

What are we interested in?
Genes responsible for the nonhost status of a plant species to a non-adapted pathogen

How to study this?
Barley-rust model system – “near nonhost”

- **SusPtrit**: experimental barley line with increased susceptibility to several heterologous rusts
  - Also increased susceptibility to non-adapted powdery mildews (*Blumeria graminis* f. spp.)
Nonhost resistance of barley to *Bgt*

RIL mapping population:

\[ \text{Vada} \times \text{SusPtrit} \]

(VxS Jafary *et al.*, 2006)

- Screened at seedling stage for susceptibility to the wheat powdery mildew:

*Blumeria graminis* f. sp. *tritici* (*Bgt*)

Barley powdery mildew (*B. graminis* f. sp. *hordei*)
Nonhost resistance of barley to *Bgt*

- **Phenotyping**
  - Macroscopically: micro-colonies (scale 0-5)
  - Microscopically: haustorium formation
- **Quantitative, polygenic inheritance**
- **QTL mapping**
  - Large effect QTL on 5H:
    - *Rbgtq1*

Vada as donor of resistance allele

**LOD 19.9**
Explained phenotypic effect:
> 40%
“*Rbgtq1*”
Development of a fine-mapping population

VxS: 152 RILs

- RILs with the highest scores
- RILs with the lowest scores

Crossings to develop fine-mapping populations
**F₂ recombinants screening**

- **370** F₂ seedlings sampled: genotyping by KASPar assay (outsourcing lab)  
  (September 2014)

- **90** heterozygous recombinants

Set seeds:

**F₃ RECOMBINANTS**

**Chr 5H**

First genotyping screen

- Marker +33
  - 4.2 cM

- Marker +16
  - 4.4 cM

- Marker -6
  - 6.8 cM

- Marker -49

LOD -1: 5.4 cM region

Peak marker
**F₃ recombinants screening**

1st round of fine-mapping (180 F₃ plants)  
(January 2015)

Genotyped by LightScanner® technology  
(high-resolution melting analysis)

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Chr 5H

First genotyping screen

**1st round of fine-mapping**  
(180 F₃ plants)  
(January 2015)

*LOD -1: 5.4 cM region*

**Marker -49**

**Marker -6**

**Marker +16**

**Marker +33**

**Peak marker**

**Peak marker**

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**Rbgtq1 is located between markers -6 and -49**
F₃ recombinants screening

New markers developed

LOD -1: 5.4 cM region
New markers for *Rbgtq1*

- DNA from F₃ plants was tested with new markers in between M-6 and M-49;

- One “special” plant (*1_1.35*):

  Score: 2.5  
  Resistant phenotype
Attention focused in the region between markers “A” and “F”

More markers developed

2nd and 3rd rounds of fine-mapping on a total of 358 F₃ plants
**F$_3$ recombinants screening**

<table>
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<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
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**line 1_1**

Average of scores for the progeny of line 1_1

- **Rbg$tq1** is to the “right” of marker A

**line 2_13**

Average of scores for the progeny of line 2_13

- **Rbg$tq1** is not between markers E and F

**line 3_15**

Average of scores for the progeny of line 3_15

- **Rbg$tq1** is not in the region between markers D and F

Resistance allele: dominant
**F₃ recombinants screening**

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<td>VV</td>
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**Average of scores for the progeny of line 1_1**

- SS: 4.6
- HH: 2.8
- VV: 2.7

**Average of scores for the progeny of line 2_13**

- SS: 4.3
- HH: 2.5
- VV: 2.8

**Average of scores for the progeny of line 3_15**

- SS: 0.18
- HH: 0.24
- VV: 0.22

*Rbg tq1* between markers A and D!
F₃ recombinants screening

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<th>PHENOTYPE</th>
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*Rbgtdq1* is to the “right” of marker B!
*Rbg* is in a region of 0.2 cM

<table>
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<th>Marker code #</th>
<th>Marker code</th>
<th>Linkage Group</th>
<th>Position Consensus Map (cM)</th>
<th>Position on Morex map (cM)</th>
<th>HOST vs NONHOST</th>
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<td>Marker +33</td>
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</table>
Synteny with *Brachypodium* and rice genomes

“genomes of grass species resemble one another when gene order is considered”

(Poursarebani et al., 2013)
Rice

10 genes between markers B and D

≈ 37.4 kb

Marker B
Protease inhibitor
Protease inhibitor
Protease inhibitor
Acetyltransferase
Acetyltransferase

Marker C
Glutamate dehydrogenase
Ribosomal protein
5'-3'-exonuclease

Marker D

≈ 121.8 kb

Barley

Marker B
Protease inhibitor
Protease inhibitor
Protease inhibitor
Acetyltransferase
Acetyltransferase

Marker C
Acetyltransferase
Acetyltransferase

Marker D

0.2 cM

Rice

28 genes between markers B and D

Brachypodium

extra set of genes: not in barley or rice
To wrap up...

- *Rbgtq1* – resistance in barley to *Bgt*
  - Dominant
  - LOD -1: 5.4 cM region
  - Fine-mapping: 0.2 cM region
  - Physical distance: in rice about 38 kb
Future prospects

- Screening BAC libraries of Vada and SusPtrit to pick out candidate genes
- Candidate gene validation
  - Transient (over)expression and silencing
- Histology studies – inoculation with other forms of *B. graminis*
Thanks for your attention!

Questions?