Genomics assisted breeding for disease resistance in oats

Gnanesh Nanjappa
Diseases of oats

- **Crown rust** (*Puccinia coronata f. sp. avenae*)
- **Stem rust** (*Puccinia graminis f. sp. avenae*)
Resistant Breeding

- More than 100 oat crown and 17 stem rust resistance genes are known
- Major single gene seedling resistance
- Partial resistance (APR) is believed to be more effective
- An estimated $400 million in lost production was prevented
Limitations

- A number of race-specific resistance genes have been mapped.
- Limited use of these markers in oat breeding.
- Since no rust resistance genes have yet been located to a specific chromosome position.
- Many genes could be alleles or the same gene.
Objectives

- Develop and deploy markers linked to effective crown and stem rust resistance genes
- Chromosomal location
- High throughput marker-assisted selection
- Gene pyramiding
Pc91

Chromosome location and allele-specific PCR markers for marker-assisted selection of the oat crown rust resistance gene Pc91.


Pc91 is a seedling crown rust resistance gene

*A. Longiglumus* × *A. Magna*

2x 4x
Plant materials

- **F₂ populations**
  - AC Morgan × Stainless
  - SW Betania × Stainless
  - AC Morgan × CDC Morrison

- **RIL**
  - CDC Sol-Fi × HiFi

Race LRGB (CR254) at the seedling stage.
## Segregation of oat crown rust resistance

<table>
<thead>
<tr>
<th>Populations</th>
<th>Gen</th>
<th>Total</th>
<th>Res</th>
<th>Sus</th>
<th>ER</th>
<th>$\chi^2$</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC Morgan × Stainless</td>
<td>$P_1$</td>
<td>20</td>
<td>-</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$P_2$</td>
<td>19</td>
<td>19</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$F_1$</td>
<td>10</td>
<td>10</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$F_2$</td>
<td>342</td>
<td>211</td>
<td>131</td>
<td>3:1</td>
<td>32.2</td>
<td>0.0001</td>
</tr>
<tr>
<td>SW Betania × Stainless</td>
<td>$P_1$</td>
<td>9</td>
<td>-</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$P_2$</td>
<td>17</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$F_2$</td>
<td>345</td>
<td>233</td>
<td>112</td>
<td>3:1</td>
<td>10.2</td>
<td>0.0014</td>
</tr>
<tr>
<td>AC Morgan × CDC Morrison</td>
<td>$P_1$</td>
<td>30</td>
<td>-</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$P_2$</td>
<td>30</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$F_2$</td>
<td>294</td>
<td>193</td>
<td>101</td>
<td>3:1</td>
<td>13.7</td>
<td>0.0002</td>
</tr>
<tr>
<td>CDC Sol-Fi × HiFi</td>
<td>$P_1$</td>
<td>10</td>
<td>-</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$P_2$</td>
<td>10</td>
<td>10</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>$F_7$</td>
<td>90</td>
<td>40</td>
<td>50</td>
<td>1:1</td>
<td>1.1</td>
<td>0.2918</td>
</tr>
</tbody>
</table>
How KASPgenotyping works

competitive allele specific forward tailed primer followed by hybridisation of fluorescent labelled primer to tail region
Genotyping data from the 3 KASPar SNP assay of 16 North American oat lines

*opt-0350-KOM4c2*, *opt-0350-KOM5c1*, *opt-0350-KOM6c2*

- **Pc91**
  - non carriers
  - carriers
  - no template controls
Genotyping data from the oPt-0350-KOM4c2 KASPar SNP assay

<table>
<thead>
<tr>
<th>Populations</th>
<th>Gen</th>
<th>Total</th>
<th>Res</th>
<th>Seg</th>
<th>Sus</th>
<th>Missing</th>
<th>ER</th>
<th>$\chi^2$</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AC Morgan × Stainless</td>
<td>F$_2$</td>
<td>146</td>
<td>20</td>
<td>35</td>
<td>81</td>
<td>10</td>
<td>1:2:1</td>
<td>86.75</td>
<td>0.0001</td>
</tr>
<tr>
<td>SW Betania × Stainless</td>
<td>F$_2$</td>
<td>142</td>
<td>25</td>
<td>75</td>
<td>40</td>
<td>2</td>
<td>1:2:1</td>
<td>3.93</td>
<td>0.1403</td>
</tr>
<tr>
<td>AC Morgan × CDC Morrison</td>
<td>F$_2$</td>
<td>170</td>
<td>54</td>
<td>79</td>
<td>36</td>
<td>1</td>
<td>1:2:1</td>
<td>4.55</td>
<td>0.1028</td>
</tr>
<tr>
<td>CDC Sol-Fi × HiFi</td>
<td>F$_7$</td>
<td>90</td>
<td>40</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td>1:1</td>
<td>1.11</td>
<td>0.2918</td>
</tr>
</tbody>
</table>

Legend:
- **Pc91**
  - Red: non carriers
  - Green: segregating
  - Blue: carriers
  - Black: no template controls
Comparative maps of the oat crown rust *Pc91* region with oat consensus map on oat chromosome 7C-17A.

Gnanesh et al. (*In prep.*)
Conclusions

- The KASPar SNP assay accurately postulated the $Pc91$ status of 16 North American oat breeding lines.
- KASPar SNP genotyping assay was validated on four populations segregating for $Pc91$
- Co-dominant KASP marker oPt-0350-KOM4c2-MAB
- This is the first report of localization of $Pc91$ to the oat chro 7C-17A.
Other genes


- Crown rust resistance gene \textit{Pc94} and \textit{Pc68}

- \textit{SR=Pg13}, and for gene(s) res to SR race NA67.
## Other genes

<table>
<thead>
<tr>
<th>Population</th>
<th>Gen</th>
<th>Total</th>
<th>R</th>
<th>S</th>
<th>Seg</th>
<th>ER</th>
<th>$\chi^2$</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pc94</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OT3033 × OT3024</td>
<td>F₆</td>
<td>90</td>
<td>41</td>
<td>42</td>
<td>7</td>
<td>1:1</td>
<td>0.01</td>
<td>0.9126</td>
</tr>
<tr>
<td>(2010)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OT3033 × OT3024</td>
<td>F₇</td>
<td>185</td>
<td>77</td>
<td>95</td>
<td>13</td>
<td>1:1</td>
<td>1.88</td>
<td>0.1699</td>
</tr>
<tr>
<td>(2012)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leggett × D921-643</td>
<td>F₂</td>
<td>175</td>
<td>128</td>
<td>47</td>
<td>-</td>
<td>3:1</td>
<td>0.32</td>
<td>0.5705</td>
</tr>
<tr>
<td>NA67</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OT7030 × Leggett</td>
<td>F₆</td>
<td>225</td>
<td>101</td>
<td>118</td>
<td>6</td>
<td>1:1</td>
<td>1.32</td>
<td>0.2507</td>
</tr>
<tr>
<td>(2011)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OT7030 × Leggett</td>
<td>F₆</td>
<td>224</td>
<td>105</td>
<td>116</td>
<td>3</td>
<td>1:1</td>
<td>0.54</td>
<td>0.4593</td>
</tr>
</tbody>
</table>
Identification of a major QTL for adult plant crown rust resistance in oat line MN841801 using Illumina GoldenGate assay.


- **8 QTLs controlling APR** have been detected in oat during the last two decades
- **QTLs detected were not consistent across environment**
Material and methods

- AC Assiniboia × MN841801 (AM)
- 167 RIL (F_{7:10})
- *P. coronata* isolate CR251 (race BRCB)
- Four environments (SK11, SK12, MB01 and MB02)
- DS, IT, CI (DS × IT)
- 240 DArT + Illumina GoldenGate assay (6KSNP Chip).
- MapDisto v. 1.7
- CIM MLE, QGene v. 4.0
Preliminary results

Mapping
- In total 1445 loci (SNPs/DArTs)
- 40 LG
- Spanning 1600 cM

QTL detection
- 1 major QTL across 4 env
- LOD = 8.8 - 47.0
- $R^2$ (%) = 22.0 - 73.5
- AE = 3.3 - 19.2
Major QTL

‘AC Assiniboia × MN841801’
Lin et al. (In prep)
Comparative mapping of one of the chromosomes

A total of nine markers on LG 24 exhibited a good agreement with marker order.

In general, a good congruence was observed.

Oat consensus map
Oliver et al. (2013)

'AC Assiniboia × MN841801'
Lin et al. (In Prep)
Summary

- The SNP and DArT data integrated very well and the generated map is about 1,600 cM.

- In all the 4 envi a major APR QTL explaining up to 74% of the PV identified.

- This is the 1st reported APR QTL in oat with a major and consistent effect in multiple envi.

- A single QTL seg in this pop makes it a suitable candidate for use in MAB and map-based cloning.
Acknowledgements

Jennifer Fetch
Curt McCartney
Jim Menzies
Tom Fetch
James Chong
Taye Zegeye

Nick Tinker
Rebekah Oliver
Eric Jackson
Oat CORE Group

Yang Lin
Aaron Beattie
Randy Kutcher
Peter Eckstein
Isobel Parkin
THANK YOU