Using Maker-Assisted Selection to Improve Hard Winter Wheat FHB Resistance

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FHB in KS:
- 10% acreage annually, eastern quarter
- 40-50% acreage sporadically, central KS
- Yield loss: 1.9% (6 mi bushels) in 2008

FHB in OK:
- Acreage: 2008 1-2%; 2009 >20%
- Yield loss: 3%, up to 25% in eastern districts

Source: US. Wheat Association

Major US. Wheat Growing Regions
HWW FHB in greenhouse experiments

Percentage of HWW accessions

0-10
10.01-20
20.01-30
30.01-40
40.01-50
50.01-60
60.01-70
70.01-80
80.01-90
90.01-100

PSS (%)
Status of FHB resistance in US HWW

- HWW cultivars are mainly MS to S

- *Fhb1* has been used in breeding for more than 10 years, to date *Fhb1* presents only in a few elite breeding lines, not in any cultivar

- Several local HWW were identified with FHB resistance, but QTL have not been determined
FHB related research in my lab

- Identify ‘diagnostic’ markers for *Fhb1*
- Develop markers for other QTL from Chinese and US resistant sources
- Develop high-throughput markers using next-generation-sequencing (GBS, GBMAS, Genotyping by multiple amplicon sequencing)
- Transfer *Fhb1* into US HWW backgrounds using marker-assisted backcross
Develop ‘diagnostic’ markers for *Fhb1* through fine mapping
Develop 12 markers in the *Fhb1* region based on reference sequence

Screen a diversity panel

Potential ‘diagnostic’ markers

Fine mapping define *Fhb1* region using SSR, 92K SNP and GBS

NIL population

Marker screen 5000 Bc7F2 for recombination in *Fhb1* region

Ning7840/Clark*7

Differentially expressed genes

RNA-Seq
**Fhb1 region marker haplotypes assayed in an diversity panel (166 acc.)**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>cfb6067</th>
<th>SNP319</th>
<th>umn10</th>
<th>cfb6059</th>
<th>FHB1-STS1</th>
<th>FHB1-STS2</th>
<th>FHB1-STS4</th>
<th>cfb6057</th>
<th>cfb6056</th>
<th>cfb6055</th>
<th>STS256</th>
<th>cfb6078</th>
<th>Represent Var.</th>
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<tbody>
<tr>
<td>Hap1</td>
<td>293</td>
<td>G</td>
<td>247</td>
<td>240</td>
<td>other</td>
<td>237</td>
<td>332</td>
<td>273</td>
<td>317</td>
<td>225</td>
<td>243</td>
<td>208</td>
<td>Chinese Spring</td>
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<tr>
<td>Hap2</td>
<td>293</td>
<td>G</td>
<td>255</td>
<td>238</td>
<td>other</td>
<td>237</td>
<td>332</td>
<td>273</td>
<td>317</td>
<td>224</td>
<td>244</td>
<td>null</td>
<td>Dahuangpl</td>
</tr>
<tr>
<td>Hap3</td>
<td>290</td>
<td>G</td>
<td>255</td>
<td>244</td>
<td>other</td>
<td>237</td>
<td>332</td>
<td>279</td>
<td>317</td>
<td>225</td>
<td>243</td>
<td>208</td>
<td>Clark</td>
</tr>
<tr>
<td>Hap4</td>
<td>293</td>
<td>A</td>
<td>258</td>
<td>null</td>
<td>other</td>
<td>246</td>
<td>302</td>
<td>null</td>
<td>224</td>
<td>250</td>
<td>205</td>
<td>ND2419</td>
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<td>Hap5</td>
<td>null</td>
<td>A</td>
<td>258</td>
<td>null</td>
<td>null</td>
<td>220</td>
<td>302</td>
<td>null</td>
<td>225</td>
<td>250</td>
<td>208</td>
<td>Sumai 3</td>
<td></td>
</tr>
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</table>

**PSS(%)**

- **Fhb1 marker haplotypes**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>2</th>
<th>5</th>
<th>71</th>
<th>16</th>
<th>72</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hap1</td>
<td>79.0</td>
<td>80.0</td>
<td>55.0</td>
<td>70.0</td>
<td>27.0</td>
</tr>
<tr>
<td>Hap2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hap3</td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>Hap4</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Hap5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FHB1-STS1 for *Fhb1*

**FHB1-R**

**FHB1-S**
Mean PSS for two groups in the diversity panel contrasting in FHB1-STS1 alleles

![Bar chart showing mean PSS for two FHB1-STS1 alleles: FHB1-STS1-other with PSS of 94% and FHB1-STS1-220 with PSS of 27%]
Meta-analysis of FHB resistance QTL in Chinese wheat landraces
## FHB resistant Chinese landraces

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>PSS (%, greenhouse)</th>
<th>PSS (%, field)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ning7840</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td>Wangshuibai</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>Huangcandou (HCD)</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Haiyanzhong (HYZ)</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td>Huangfangzhu (HFZ)</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Baisanyuehuang (BSYH)</td>
<td>7</td>
<td>11</td>
</tr>
</tbody>
</table>
5 Chinese landraces/Wheaton or Jagger

$F_{6-8}$ RILs

FHB phenotyping

GBS/SSR

QTL mapping for each pop

Consensus map

Meta QTL analysis

Convert GBS to KASP-SNP and GBMAS for MAS
**Genotyping-by-sequencing**

- **gDNA**
  - RE1
  - RE2

  \[\text{restriction with 2 enzymes (RE)}\]

  - wanted
  - wanted

  \[\text{adapter ligation} \]

  - 5'
    - barcode adapter
    - barcode
    - RE1 site
    - RE2 site
  - common adapter
  - 3'

  \[\text{amplification} \]

  - Ion A primer
  - ion P1 primer binds to Ion Sphere™

  \[\text{emulsion PCR (pooled)} \]

  - PGM™ Sequencing

  \[\text{alignment of reads, SNP calling} \]
6232 SNPs were called with 20% missing data

~5000 SNPs were mapped to 36 linkage groups

Average marker density was 0.755cM per marker
## QTL in HYZ using a GBS map

<table>
<thead>
<tr>
<th>Locus</th>
<th>Flanking markers</th>
<th>Combined mean</th>
<th>LOD</th>
<th>R² %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5A</td>
<td>GBS3127~Xbarc316</td>
<td>6.0</td>
<td></td>
<td>9.4</td>
</tr>
<tr>
<td>2B</td>
<td>GBS1711~GBS5561</td>
<td>5.0</td>
<td></td>
<td>7.6</td>
</tr>
<tr>
<td>6B</td>
<td>GBS0576~GBS0208</td>
<td>5.3</td>
<td></td>
<td>8.5</td>
</tr>
<tr>
<td>7D</td>
<td>GBS0983~Xwmc121</td>
<td>2.9</td>
<td></td>
<td>4.8</td>
</tr>
<tr>
<td>2D</td>
<td>GBS5276~Xcfd51</td>
<td>3.9</td>
<td></td>
<td>5.8</td>
</tr>
<tr>
<td>4D</td>
<td>Xwmc52~GBS4813</td>
<td>4.1</td>
<td></td>
<td>6.2</td>
</tr>
<tr>
<td>6D</td>
<td>GBS0984~GBS5458</td>
<td>3.4</td>
<td></td>
<td>5.0</td>
</tr>
</tbody>
</table>
Consensus map for chromosome 3BS
Meta-QTLs on 3BS
Meta-QTL on 3AS
Meta-QTLs on 2D
Meta-QTLs on 3D and 4D
Summary

- Many important QTL reported to date can be identified in Chinese landraces

- *Fhb1* is the QTL with the largest effect in most Chinese resistance landraces, but not in HYZ

- HYZ lacks *Fhb1* and its resistance is conditioned by seven minor QTL, suggesting accumulation of multiple minor QTL also can achieve a high level of resistance

- QTL on 3AS, 3BS(2), 3D, 2D and 4D detected in multiple populations are more stable QTL for breeding

- GBS-SNP were found for most of these QTL and conversion of them to KASP and high-throughput GBMAS markers is in progress
Marker-assisted backcross to transfer \textit{Fhb1} into HWW
Fhb1 X HWW

BC1F1

MAS-BC

BC2F1

MAS

BC2F2

MAS

BC2F3 GH FHB test

BC2F4 field FHB test

Germplasm release
Transfer *Fhb1* to Wesley, Trego and Harding

- *Fhb1* donors: Sumai 3 and its soft wheat derivatives
- Recurrent parents: Wesley, Trego, Harding
- ~100 Bc2F4-5 lines per cross tested in Tri-state breeding nurseries
WesleyFhb1 lines and Wesley in field (2012)
WesleyFhb1 lines and Wesley in field (2013)
WesleyFhb1 and Wesley in greenhouse
# PSS for *Fhb1* in three HWW

<table>
<thead>
<tr>
<th>Line</th>
<th>GH PSS</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>262(ND2928/Wesley*2)F3/WesleyF4</td>
<td>0.079</td>
<td></td>
</tr>
<tr>
<td>267(ND2928/Wesley*2)F3/WesleyF4</td>
<td>0.090</td>
<td></td>
</tr>
<tr>
<td>568(ND2928/Wesley*2)F3/WesleyF4</td>
<td>0.192</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Wesley</strong></td>
<td><strong>0.584</strong></td>
<td><strong>79.5%</strong></td>
</tr>
<tr>
<td>277(ND2710/Trego*2F3//TregoF4</td>
<td>0.324</td>
<td></td>
</tr>
<tr>
<td>219(ND2710/Trego*2F3//TregoF4</td>
<td>0.253</td>
<td></td>
</tr>
<tr>
<td>27(ND2710/Trego*2F3//TregoF4</td>
<td>0.203</td>
<td>0.26</td>
</tr>
<tr>
<td><strong>Trego</strong></td>
<td><strong>0.722</strong></td>
<td><strong>63.7%</strong></td>
</tr>
<tr>
<td>167(Harding*2/Sumai3)F3Harding/F4</td>
<td>0.534</td>
<td></td>
</tr>
<tr>
<td>61(Harding*2/Sumai3)F3Harding/F4</td>
<td>0.580</td>
<td></td>
</tr>
<tr>
<td>31(Harding*2/Sumai3)F3Harding/F4</td>
<td>0.350</td>
<td>0.49</td>
</tr>
<tr>
<td><strong>Harding</strong></td>
<td><strong>0.507</strong></td>
<td><strong>0.02</strong></td>
</tr>
</tbody>
</table>
Transfer *Fhb1* from Ning7840 to Overland, Overlay and Jagger
FHB severity and DON content between parents and their Fhb1 lines in Overland, Overley and Jagger backgrounds

<table>
<thead>
<tr>
<th></th>
<th>GH PSS (%) (3 seasons)</th>
<th>Field PSS (%) (2 years)</th>
<th>FDK (%) (2 years)</th>
<th>DON (ppm) (2014)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overland-Fhb1 lines (49)</td>
<td>21.26</td>
<td>33.08</td>
<td>20.61</td>
<td>14.44</td>
</tr>
<tr>
<td>Overland</td>
<td>42.00</td>
<td>46.00</td>
<td>41.00</td>
<td>32.20</td>
</tr>
<tr>
<td>FHB reduction due to Fhb1 (%)</td>
<td>49.37</td>
<td>28.09</td>
<td>49.74</td>
<td>55.16</td>
</tr>
<tr>
<td>Overley-Fhb1 lines (20)</td>
<td>46.31</td>
<td>28.94</td>
<td>17.20</td>
<td>10.94</td>
</tr>
<tr>
<td>Overley</td>
<td>95.00</td>
<td>77.00</td>
<td>68.00</td>
<td>27.60</td>
</tr>
<tr>
<td>FHB reduction due to Fhb1 (%)</td>
<td>51.25</td>
<td>62.42</td>
<td>74.70</td>
<td>60.36</td>
</tr>
<tr>
<td>Jagger-Fhb1 lines (59)</td>
<td>40.39</td>
<td>19.44</td>
<td>11.93</td>
<td>10.48</td>
</tr>
<tr>
<td>Jagger</td>
<td>83.00</td>
<td>58.00</td>
<td>31.00</td>
<td>15.10</td>
</tr>
<tr>
<td>FHB reduction due to Fhb1 (%)</td>
<td>51.33</td>
<td>66.49</td>
<td>61.50</td>
<td>30.57</td>
</tr>
</tbody>
</table>
Transfer *Fhb1* from Wesley-Fhb1 to 11 HWW from 5 States

- 11 locally adapted recurrent parents:
  - ND: Decade, Jerry,
  - NE: NE01481, NE6607,
  - KS: Everest, KS061406LN-47,
  - SD: SD08080, Lyman,
  - OK: Garrison, Ruby Lee, OK06617-Rht

- Markers: Xumn10 and Xsnp8
Progress

Completed projects:

- \textit{Fhb1} carrying NILs are available for Wesley, Harding, Trego, Overland, Overley and Jagger

5 state MAB project in progress:

- DH: 1st set sent to AZ for increase and 2nd set DH production in progressing
- 11 Bc_2F_4 pop. were planted for field FHB evaluation this fall and greenhouse FHB evaluation is in progress
**Summary**

*Fhb1* significantly improves resistance in most HWW backgrounds but FHB resistance levels varied with resistance levels of recurrent parents, thus avoiding use of highly susceptible cultivars as recurrent parents have a higher chance to select highly resistant lines.

Although many backcross progenies show slightly lower yield than recurrent parents, some *Fhb1* lines have similar yield as recurrent parents.

Marker selected *Fhb1* lines still show different levels of resistance in each population, phenotypic selection will be useful to improve the level of resistance in selected lines.

*WesleyFhb1* can be useful *Fhb1* donor for HWW.
GBS is a cost effective system for QTL mapping and SNP discovery

Single SNP marker may not be diagnostic for a QTL when a large set of germplasm are screened. Thus several SNP (haplotype) may be needed to tag a QTL

GBMAS using Ion Proton can be a good system for high throughput screening of multiplex SNP for MAS in breeding
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