Breeding for FHB resistance

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University of Minnesota

Photo: Brian Steffenson
Breeding for FHB Resistance

I  Measuring FHB and DON
II  Screening Germplasm
III  Genetics of Disease Resistance
IV  UM Breeding Scheme
V  Genomics Resources for Breeding
Inoculum Production

Macroconidia - spray

Ascospore – grain spawn

Measuring FHB and DON
Inoculum Application

Macroconidinia application to heads

Ascospores - Grain Spawn applied to ground
Disease Nurseries

**Saint Paul**
Macroconidia Spray

2 Applications timed to Head Emergence

**LATE**

**Crookston**
Ascospore Showers occur over several weeks

**TALL**

Ascopore – Grain Spawn

Measuring FHB and DON
Visual Severity Assessment in the Field
Disease Rating Scale

Steffenson, B. J. et al., 2004 Proceedings of the 2nd International Symposium on FHB

Measuring FHB and DON
Harvest and DON

Hand Sickle and Stationary Thresher

Gas Chromatography / Mass Spectrometry

Measuring FHB and DON
Informative Checks

NABSEN 2010 - 2013

Heading Date
Height
2-row/6-row

Measuring FHB and DON

University of Minnesota
Accounting for Spatial Variation

21 rows x 10 columns

DON randomized

DON Plot Order
Accounting for Spatial Variation

21 rows x 10 columns

FHB Plot Order

DON Plot Order
Type 2 Modified Augmented Design (MAD)

- Each block contains a primary check sub-plot at its center
- An arbitrary number of blocks contain an arbitrary (but >1) number of secondary check sub-plots
  - Numbers depend on field space and experiment size
Method 1 (M1) Correction

- Calculates row and column effects using only the primary checks
- Uses effects to adjust individual plots based on their coordinates

The spatially-adjusted value for a plot in row 2, column 4 is then:

\[ \text{Adj.M1(Plot}_{R2,C4} = \text{Raw(Plot}_{R2,C4} - (Chk1.R_2 - Chk1.GM) - (Chk1.C_4 - Chk1.GM) } \]
Method 3 (M3) Correction

- Uses both primary and secondary checks to calculate weighted block effects
- Un-weighted block effect calculated as: \( \text{Blk.Eff}_{R_i,C_j} = \text{Chk1}_{R_i,C_j} - \text{Chk1.GM} \)

- Identify blocks with secondary checks and calculate the mean of all secondary checks within a block:
  \[
  \frac{\text{Chk2. A} + \text{Chk2. B} + \text{Chk2. n}}{\text{# of secondary checks (n)}}
  \]
  to generate \( \text{Chk2.R}_{i,C_j} \)

- Weight block effects (\( \text{Blk.Eff}_{R_1,C_1} \)) using the slope of the regression between primary check values and secondary check means (\( \text{Chk2.R}_{i,C_j} \))
- Example to follow
Method 3 (M3) Correction, Example

<table>
<thead>
<tr>
<th>Row</th>
<th>Column</th>
<th>Chk1</th>
<th>Chk2.A</th>
<th>Chk2.B</th>
<th>Chk2.R&lt;sub&gt;i&lt;/sub&gt;C&lt;sub&gt;j&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>6.7</td>
<td>4.3</td>
<td>7.1</td>
<td>5.7</td>
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<tr>
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<td>4</td>
<td>9.1</td>
<td>5.1</td>
<td>7.7</td>
<td>6.4</td>
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<tr>
<td>2</td>
<td>2</td>
<td>8.3</td>
<td>3.9</td>
<td>8</td>
<td>5.95</td>
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<tr>
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<td>5</td>
<td>5.9</td>
<td>4.5</td>
<td>7.2</td>
<td>5.85</td>
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<tr>
<td>3</td>
<td>4</td>
<td>7.1</td>
<td>5.3</td>
<td>6.8</td>
<td>6.05</td>
</tr>
</tbody>
</table>

- Plot each block’s **primary check** value against the block’s **secondary check mean** (Chk2.R<sub>i</sub>C<sub>j</sub>)
- Obtain the slope (m) of the regression line of that plot

- Adjust individual plot values by subtracting m*(Blk.Eff<sub>Ri,Cj</sub>)

   Example:
   Assume (Plot<sub>R2,C5</sub>) = 12 and Chk1.GM = 8

   \[
   \text{Adj.M3(Plot}_{R2,C5} = \text{Raw(Plot}_{R2,C5} - m*(Chk1}_{R2,C5} - Chk1.GM)
   \]

   \[
   \rightarrow \text{Adj.M3(Plot}_{R2,C5} = 12 - 0.1613*(5.9 - 8) \rightarrow = 12.34
   \]
Moving Average (Mv.Avg) Correction

- Calculates a covariate for a plot based on the average of surrounding plots
- Covariate used in a statistical model to adjust the plot’s raw value
  - Currently use the R package mvngGrAd()

A covariate for the yellow plot is calculated by averaging the surrounding red plots.

- Location of and the number of plots averaged is adjustable upon consideration of plot dimensions
Relative Efficiency (RE)

• RE is a statistic calculated for each method and is used to compare the effectiveness of methods.

• Variation observed between isogenic lines (inbred lines, F\textsubscript{1} hybrids, clones, etc…) is theoretically due entirely to the environment.

• RE is based upon the variation observed among check lines before and after each adjustment method.

\[
    \text{RE} = \left( \frac{\text{Average variation among secondary checks BEFORE adjustment}}{\text{Average variation among secondary checks AFTER adjustment}} \right) \times 100
\]

• A higher RE is indicative of a better correction procedure for the given trait.
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Screening Germplasm

Huang et al. 2012
Screening Germplasm

Huang et al. 2012
Screening Germplasm

Wild Barley

Resistant
Susceptible

Huang et al. 2012
Screening Germplasm

Huang et al. 2012
### Closed Subpopulation

- Kindred 1944
- Vantage 1947
- Fox, **Parkland, Traill** 1956
- Swan, UM 570 1957
- **Larker, Trophy** 1961
- **NDB112** 1962
- **Dickson** 1964
- Paragon 1969
- Bonanza 1970
- Beacon, Steptoe 1973

### Reopened Subpopulation

- Femina 1984
- Cheri 1987
- 1992 1992
- Chevron
- 1995 1995
- Zhedar 1
- 1996 1996
- Atahualpa, Frederickson
- 1997 1997
- Ac Oxbow
- 1998 1998
- HOR211, PFC88209
- 2000 2000
- BT463, Clho6613
- 2003 2003
- ND20407, ND20493
Breeding for FHB Resistance

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Type I  resistance to initial infection
Type II  resistance to spread “in the head”

Wheat

Barley

APSNET.ORG
Parents with contrasting phenotypes crossed

Populations are selfed and advanced for multiple generations

Mapping population

Bi-parental mapping

Assessment data collected in breeding trials

Assess more than two alleles per locus

Multiple generations of recombination

Evaluate allelic effects in relevant breeding background

More rapid translation to marker-based breeding strategies

\[ Y = XB + Sa + Qv + Zu + e \]
FHB QTL - Bi-Parental

- Heading Date
- Vrs1 2-row / 6-row spike
- Cleistogamy
- Grain Protein Senescence

Chevron
- de la Pena et al., 1999
- Ma et al., 2000
- Canci et al., 2004

Frederickson
- Mesfin et al., 2003

Cl4196
- Horsley et al., 2006

Zhedar 2
- Dahleen et al., 2003

Gobernadora
- Zhu et al., 1999

Russian 6
- Hori et al., 2005

Harbin 2row
- Hori et al. 2006, Sato et al. 2008
## FHB QTL - Association Mapping

<table>
<thead>
<tr>
<th>Trait</th>
<th>Chrom</th>
<th>cM</th>
<th>MN</th>
<th>BA</th>
<th>N2</th>
<th>N6</th>
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<tbody>
<tr>
<td>DON</td>
<td>1H</td>
<td>88</td>
<td>1.0</td>
<td>0.90</td>
<td>0.74</td>
<td>0.99</td>
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<tr>
<td>DON</td>
<td>2H</td>
<td>125-132</td>
<td>0.54</td>
<td>0.55</td>
<td>0.64</td>
<td>0.12</td>
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<tr>
<td>DON</td>
<td>3H</td>
<td>52-65</td>
<td>0.19</td>
<td>0.57</td>
<td>0.95</td>
<td>0.00</td>
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<tr>
<td>DON</td>
<td>4H</td>
<td>3</td>
<td>0.17</td>
<td>0.49</td>
<td>0.98</td>
<td>0.02</td>
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<tr>
<td>DON</td>
<td>4H</td>
<td>21-36</td>
<td>0.33</td>
<td>0.58</td>
<td>0.99</td>
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<td>4H</td>
<td>40-61</td>
<td>0.29</td>
<td>0.32</td>
<td>0.47</td>
<td>0.51</td>
</tr>
<tr>
<td>DON</td>
<td>5H</td>
<td>190-192</td>
<td>0.85</td>
<td>0.81</td>
<td>0.95</td>
<td>0.54</td>
</tr>
<tr>
<td>DON</td>
<td>6H</td>
<td>42-67</td>
<td>0.02</td>
<td>0.36</td>
<td>0.73</td>
<td>0.10</td>
</tr>
</tbody>
</table>

Identified in previous mapping studies

Massman et al., 2011
Validation of Association Mapping QTL using Near-Isogenic Lines (NIL)

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>NIL Study</th>
<th>AM Study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NIL family</td>
<td>DON</td>
</tr>
<tr>
<td>FEG149-18</td>
<td>4 3</td>
<td>-10%*</td>
</tr>
<tr>
<td>ND25697</td>
<td>1 1</td>
<td>-15%**</td>
</tr>
</tbody>
</table>

Complicated – multiple alleles or haplotypes at a QTL
Most QTL identified in AM study were not validated with NILs
The size of the effect in NILs was often less than predicted in the AM study

Navarра and Smith, 2013
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UM Barley Breeding Program

Breeding Scheme

1. Crossing:
   - A x B
   - F1

2. Inbreeding:
   - F2
   - F3
   - F4 Winter Nursery
   - F4:5 Plant Rows

3. Evaluation:
   - Winter Nursery Increase
     - 1st year trial
     - 2nd year trial
   - AMBA
   - Pilot Malt
   - Plant Brew

4. Release

Six-row FHB

- 100 crosses
- 16,000 F2’s
- 1,600 F5’s
- 240 PYT’s
- 40 2nd Year
- 15 3rd Year
- 4 Industry Testing

FHB field screening
10,000 plots per year
Variety Release Quest (2010)

UM Breeding Scheme
Marker-based breeding

UM Breeding Scheme

Crossing
A x B
↓
F1

Inbreeding
F2
↓
F3
↓
F4 Winter Nursery
↓
F4:5 Plant Rows

Evaluation
Winter Nursery Increase
1st year trial
2nd year trial

Yr 1

Yr 2

Yr 3-4

Yr 5-9

AMBA
Pilot Malt
Plant Brew

3rd year trial
4th year trial
5th year trial
6th year trial

RELEASE
Number of lines with the favorable allele selected from an initial population of 1,000

<table>
<thead>
<tr>
<th>Number of QTL</th>
<th>Allele Fixation</th>
<th>Allele Enrichment</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>F2</td>
<td>F3</td>
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<tr>
<td>1</td>
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<td>375</td>
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<td>4</td>
<td>4</td>
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<td>5</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>3</td>
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Breeding for FHB Resistance

I  Measuring FHB and DON
II  Screening Germplasm
III  Genetics of Disease Resistance
IV  UM Breeding Scheme
V  Genomics Resources for Breeding
Marker Assisted Selection
Genomic (Genome-Wide) Selection
UM Breeding Program

Current Scheme

- Crossing: A x B → F1
- Inbreeding: F1 → F2 → F3 → F4 Winter Nursery → F4:5 Plant Rows
- Evaluation: Winter Nursery Increase
- Yr 1: 1st year trial
- Yr 2: 2nd year trial
- Yr 3-4: 3rd year trial, 4th year trial
- Yr 5-9: 5th year trial, 6th year trial
- AMBA, Pilot Malt, Plant Brew
- RELEASE

Numbers of Breeding Lines

- 10,000s
- 1,000s
- 100's
- 10's
- 1

Breeding Cycle

Genomics Resources

- High Throughput: HD, Ht
- High Heritability: Disease
- Simple Inheritance: Yield, Quality
- Inexpensive: Low Throughput
- Low Heritability: Complex Inheritance
- Expensive
UM Breeding Program

Current Scheme

- Crossing: A × B → F1
- Inbreeding: F2 → F3 → F4 Winter Nursery → F4:5 Plant Rows
- Evaluation: Winter Nursery Increase 1st year trial
- 2nd year trial
- AMBA
- Pilot Malt
- Plant Brew
- RELEASE

Genomic Selection

- Crossing: A × B → F1
- Inbreeding: F2 → F3
- Evaluation: F4 Winter Nursery → 1st year trial
- 2nd year trial: AMBA, Pilot Malt, Plant Brew
- 3rd year trial
- 4th year trial
- 5th year trial
- 6th year trial
- RELEASE

Genomics Resources
Breeding gains for PYT breeding lines

Phenotypic Selection
Genomic Selection

Genomics Resources
Genomic Resources

9,000 SNP Marker Map

Centralized Public Database
Triticeae Tollbox
http://triticeaetoolbox.org/

USDA Genotyping Centers

Genomics Resources
Some Take Home Messages

Robust field screening is absolutely necessary

Evaluate current varieties and advanced breeding lines and identify most susceptible

Selection for FHB resistance needs to be done in concert with selection for agronomic and quality traits

Marker assisted selection for several modest effect QTL

Genomic selection for disease resistance AND agronomic and malting quality traits. Take advantage of “CAP” resources and infrastructure.

Resistant varieties will only be effective if they are grown using best management practices.
USWBSI Barley Coordinated Project

**B CAP RESEARCH ACTIVITIES**

**MANAGEMENT TOOLS**

Obj 6 c
Best MGMT Practices

Outreach

**IMPACT**
Adoption of BMP
Lower DON in barley
Stable barley supply

Obj 5
Pathogen Colonization DON

Obj 4 a
Host Gene Discovery

Obj 1
Pre-breeding & mapping

Obj 3
Breeding

Obj 2
QTL Validation & Fine Mapping

Obj 4 b
Transformation

Obj 6 b
Uniform Fungicide Trials

Obj 6 a
Validate/Refine DON Model

Disease Forecasting Models

SCAB SMART Field Days Bulletins

Residue Mgmt

Varieties

Fungicides

Pre-breeding & mapping

SCAB SMART Field Days
Bulletins

Host Gene Discovery

QTL Validation & Fine Mapping

Valid/Refine DON Model

Validate/Refine DON Model
Project Members / Collaborators / Support

**Barley Project**
- Ed Schiefelbein
- Guillermo Velasquez
- Karen Beaubien
- Ahmad Sallam
- Stephanie Navarra
- Vikas Vikram
- Leticia Kumar
- Danelle Dykema
- Jon Massman
- Lex Nduulu

**University of Minnesota**
- Gary Muehlbauer
- Jochum Wiersma
- Ruth Dill-Macky
- Brian Steffenson
- Yanhong Dong
- Galen Thompson
- George Nelson

**Other Institutions**
- Richard Horsley, NDSU;
- Paul Schwarz, NDSU
- Shiaoman Chao, USDA;
- Blake Cooper, Busch Ag. Res., Inc
- Flavio Capettini, ICARDA
- Jean-Luc Jannink, USDA
- Aaron Lorenz, Nebraska University

---

USDA-AFRI
NIFA

U.S. Wheat & Barley Scab Initiative

SMALL GRAINS INITIATIVE

American Malting Barley Association
Questions
Genomic Selection

Training population

<table>
<thead>
<tr>
<th>Line</th>
<th>Yield</th>
<th>Mrk 1</th>
<th>Mrk 2</th>
<th>...</th>
<th>Mrk p</th>
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Selection candidates

<table>
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<tr>
<th>Line</th>
<th>Yield</th>
<th>Mrk 1</th>
<th>Mrk 2</th>
<th>...</th>
<th>Mrk p</th>
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<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
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<td>Line B</td>
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<td>1</td>
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<tr>
<td>Line C</td>
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<td>1</td>
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<td>Line D</td>
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<td>...</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

Model training

\[ y_i = \sum_{j=1}^{p} b_j x_{i,j} \]

Prediction

\[ \text{GEBV}_i = \sum_{j=1}^{p} \hat{b}_j x_{i,j} \]

Parent selection

<table>
<thead>
<tr>
<th>Line</th>
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<th>Mrk 2</th>
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<td>1</td>
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<td>Line D</td>
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<td>...</td>
<td>23</td>
<td>1</td>
<td>1</td>
<td></td>
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</tbody>
</table>

Basic framework

GEBV = genomic estimated breeding value
GPC1 associated with remobilization of grain protein (GP) and senescence

Karl x Lewis
Lacey x Chevron

GP
Low
High

Maturity
Late
Int.
Early

Distelfeld et al., 2008
Genomic selection

- Make crosses and advance generations
- Genotype selection candidates
- Test varieties and release
- Advance lines with highest GEBV
- New Germplasm
- Updated Model
- Updated Model
- Advance lines informative for model improvement
- (Training population)
- Model Training Cycle
- Train prediction model
- Phenotype (lines have already been genotyped)

Heffner, Sorrells, Jannink, Crop Science 2009
Traits Correlated with FHB

- 2(2H)
  - Heading Date
  - Vrs1
  - FHB
  - Russian 6
  - Chevron
  - Frederickson
  - Zhedar 2
  - CI 4780

- 6(6H)
  - FHB
  - Grain Protein
  - Kernel Discoloration
  - Frederickson
  - Zhedar 2
  - CI 4780
  - Chevron
  - Frederickson
  - Hor211