Use of Doubled Haploids in the Context of Genetic Resource Exploitation in Maize

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http://www.plantbreeding.iastate.edu/DHF/DHF.htm

Haploids are an effective tool to eliminate recessive genes leading to lethality or sub-vitality. The main application of (doubled) haploids (DHs) is production of completely homozygous and homogeneous lines in short time. Potentially masking genetic variation within lines or families is completely eliminated within DH lines. The usefulness of DH lines is significantly increased over F_{2:3} or F_{3:4} families, due to a significantly enhanced genetic variation among DH lines. Finally, substantial progress has been made in development in novel inducers and for understanding the genetics of haploid induction and response of donor plants to the induction process. The objective of this presentation is to study and discuss possibilities for using DH technology to support the germplasm enhancement in maize (GEM) allelic diversity project (http://www.public.iastate.edu/~usda-gem/). The GEM diversity project using DH lines to evaluate exotic introgressions in elite background taps into a wide diversity of maize races including Highland germplasm. No selection is made for agronomic performance in the ongoing GEM diversity project which may be an advantage to capture novel allelic diversity. Currently, 54 races (including 12 Highland races above 2,500 meters elevation) are represented in the DH materials. As a case study, the level of diversity present at the brown-midrib 3 locus, known to affect cell wall lignification, and its implications will be reported in detail.
Use of Doubled Haploids in the Context of Genetic Resource Exploitation in Maize

Thomas Lübberstedt
- Status Quo Doubled Haploid (DH) Technology

- Challenges Genetic Resources
  - Adaptation
  - Significant Sequence Variation?: Bm3 as case study
  - Linkage Drag

- Efficient Procedures / Outlook
In vivo Haploid Induction

Maternal Inducer

Donor

Paternal Inducer (ig)

F1

F2
<table>
<thead>
<tr>
<th>Step</th>
<th>Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Induction cross</td>
<td>- Inducer availability</td>
</tr>
<tr>
<td></td>
<td>- Induction rate</td>
</tr>
<tr>
<td>Haploid (embryo) selection</td>
<td>- Visual scoring</td>
</tr>
<tr>
<td></td>
<td>- Background effects</td>
</tr>
<tr>
<td></td>
<td>- Inducer DNA introgression ?</td>
</tr>
<tr>
<td>Genome doubling</td>
<td>- Colchicine: Toxic</td>
</tr>
<tr>
<td></td>
<td>- Alternative procedures</td>
</tr>
<tr>
<td></td>
<td>- Background effects</td>
</tr>
<tr>
<td>Selfing of DHs</td>
<td>- Chimera -&gt; partial fertility</td>
</tr>
</tbody>
</table>
Need for Developing Adapted Inducers

Donor (F1 plants)

Inducer

Ames: July 18, 2010
<table>
<thead>
<tr>
<th>Name</th>
<th>Origin</th>
<th>% Haploids</th>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spontaneous ISU (Chase 1952)</td>
<td>0,1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stock 6 USDA (Coe 1959)</td>
<td>2</td>
<td>R-nj</td>
<td></td>
</tr>
<tr>
<td>KEMS, WS14 Russia, France (1980-1990ies)</td>
<td>7</td>
<td>R-nj</td>
<td></td>
</tr>
<tr>
<td>RWS, RWK-76, UH400 U. Hohenheim (2005)</td>
<td>8-10</td>
<td>R-nj</td>
<td></td>
</tr>
<tr>
<td>CAUHOI, CAU5, CAU019 CAU Beijing (2009)</td>
<td>2-10</td>
<td>R-nj, High Oil (CAUHOI)</td>
<td></td>
</tr>
<tr>
<td>PHI1-4 Procera, Romania (2010)</td>
<td>12-14</td>
<td>R-nj, Pl1</td>
<td></td>
</tr>
<tr>
<td>BHI series ISU (2014)</td>
<td>&gt;8</td>
<td>R-nj, Pl1, other Midwest adapted, Popcorn</td>
<td></td>
</tr>
</tbody>
</table>
Haploid Selection Method Overview

Color: R-nj; Red roots

Machine Sorting: NIRS, NMR (High Oil)

"Phenomic": Weight, MRI (Volume), …

Jones et al., 2012; Smelser et al., unpublished
Maize Doubled Haploids: Colchicine Doubling
Induction and "Success Rates" (Haploid to DH Offspring)

Average Induction (%) 

Average % pollinated

% pollinated is based on no. haploid seed

r = 0.22
Adaptation
Photoperiod Treatment for Exotic x Temperate
Exotic Maize Populations

Recurrent elite parent: B47 or Z51

BC1

DH

Isogenic BC1-DH lines - GEM

http://www.public.iastate.edu/~usda-gem/
Speed in Line Development

Founder line 1 \( \times \) Founder line 2

\[ \text{Selfing} \]

\[ F_1 \]

\[ \text{DH} \Rightarrow \text{Inbred line} \]

\[ \text{Inbred line} \]
Sequence Variation among Exotics:

*Bm3* as Case Study

Are there novel / useful Alleles out there in Exotic Germplasm?
**brown midrib** - mutants

### Morphological:

*brown midrib 3 (bm3-)* mutation

### Agronomical:

<table>
<thead>
<tr>
<th>Trait</th>
<th>Normal</th>
<th>bm3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF lignin</td>
<td>24.5</td>
<td>21.0</td>
</tr>
<tr>
<td>ADL</td>
<td>7.8</td>
<td>5.3</td>
</tr>
<tr>
<td>IVDMD</td>
<td>46.4</td>
<td>54.8</td>
</tr>
</tbody>
</table>

Barriere & Argillier 1993

**But: Pleiotropy!**

### Biochemical:

*Biochemical: Caffeic-O-methyltransferase (Lignin)*
LD between Significantly Associated Polymorphisms in COMT Gene

Brenner et al., 2010, BMC Plant Biol 10:27
Genetic basis of trait correlations

Trait correlation

Linkage
- Linkage disequilibrium
- Genetic Linkage

Pleiotropy
- Intragenic linkage
- True pleiotropy

Chen & Lubberstedt 2010, TIPS 15:454
Molecular Characterization of $Bm3$ locus

• Previous studies on $Bm3$ locus:
  
  – Fontaine and Barriere 2003: 6 elite lines
  – Guillet-Caude et al. 2004: 34 elite lines
  – Zein et al. 2007: 42 elite lines

=> In total 30 distinct full-length $Bm3$ (COMT) allele sequences (NCBI)

• GEM: 55 exotic full-length $Bm3$ alleles (Sanger sequencing)
Brown midrib 3 Full-Length Alleles

55 Exotic Alleles

30 Elite Alleles (70 lines)

All different from each Other & from Elite Alleles

Chen et al., 2014
Higher diversity at AA level of exotic versus elite alleles

NJ tree of exotic AA sequences

NJ tree of elite AA sequences
More polymorphisms in exotic COMT alleles

55 Exotic COMT alleles above: SNP (singleton)

Exon1

10(10)
1(0)
2(0)

77(67)

177(120)

41(2)

138(118)

12(0)

Exon2

70 Elite COMT alleles below: SNP (singleton)

55 Exotic COMT alleles above: Conserved AA / semi-conserved AA / Non-conserved AA

Exon1

2/4/5
0/0/0

3/3/3
0/0/0

Dime

3/4/3
0/1/0

Methyltransferase

30/16/24
3/2/0

1/2/3
0/0/0

70 Elite COMT alleles above: Conserved AA / semi-conserved AA / Non-conserved AA

7 frameshift mutations!
Conclusions

Answer: Yes ! All New !

- High Resolution for Gene-Based Association Analyses
- Discrimination Pleiotropy – Genic Linkage
- Chance for finding “Optimal Haplotypes”
- Deep Genetic Resource for Breeders
Variation for Agronomic Traits

Plant Materials:
- 50 GEM-BC1-DH Lines
- Checks: W604-9S, PHB41, PHZ51, DE811, B73, Mo17

Traits:
- Stover DNDF, Plant Height, Ear Height, Flowering Time, Lodging

Experiments:
- Forage trials: Davenport (Dow), Ames 2010

Genotyping:
- 199 SNP markers
<table>
<thead>
<tr>
<th>Line</th>
<th>DNDF (average)</th>
</tr>
</thead>
<tbody>
<tr>
<td>W604S</td>
<td>53.6</td>
</tr>
<tr>
<td>W605S</td>
<td>46.9</td>
</tr>
<tr>
<td>W606S</td>
<td>43.5</td>
</tr>
<tr>
<td>W607S</td>
<td>44.2</td>
</tr>
<tr>
<td>W609S</td>
<td>46.3</td>
</tr>
<tr>
<td>GEM-Gordo 1</td>
<td>48.8</td>
</tr>
<tr>
<td>GEM-Gordo 2</td>
<td>47.1</td>
</tr>
<tr>
<td>GEM-Gordo 3</td>
<td>47.3</td>
</tr>
<tr>
<td>GEM-Gordo 4</td>
<td>48.8</td>
</tr>
</tbody>
</table>
Mapping in GEM-DH Panel
GEM-DH Overview

- > 600 GEM-DH Lines developed
- Genotyping-by-Sequencing: done for 360 DHLs
- 8000 SNPs: done for 360 DHLs
- Phenotyping: per se 2013, 2014; TC 2015
- Significance of linkage drag> GEM-BC1:3-sublines: 2015
Multiple donors, unbalanced data set

At one locus, different donors, different effects

Genetic Architecture GEM-DH Panel

Exotic mais population constituted of several donors

Cross with elite line

F1

Backcross with elite line

BC1

DH

Elite line
<table>
<thead>
<tr>
<th></th>
<th>PHB47</th>
<th>PHZ51</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of DH lines</td>
<td>203</td>
<td>137</td>
</tr>
<tr>
<td>Excluded Lines (&gt;1% Het)</td>
<td>31</td>
<td>20</td>
</tr>
<tr>
<td>% Donor Expected</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>% Donor (199 SNPs)</td>
<td>88</td>
<td>89</td>
</tr>
<tr>
<td>% Donor (&gt;8000 SNPs)</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>% Donor GBS unimputed</td>
<td>93</td>
<td>92</td>
</tr>
<tr>
<td>% Donor GBS imputed</td>
<td>93</td>
<td>92</td>
</tr>
</tbody>
</table>
## Observed / Expected Crossovers, Recurrent Parent Genome %

<table>
<thead>
<tr>
<th>Crossovers</th>
<th>Obs. (avg)</th>
<th>Exp.</th>
<th>% Rec. Parent</th>
<th>Obs.</th>
<th>Exp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ch1</td>
<td>6.54</td>
<td>2.77</td>
<td>*</td>
<td>0.87</td>
<td>0.75</td>
</tr>
<tr>
<td>Ch2</td>
<td>3.25</td>
<td>2.07</td>
<td>*</td>
<td>0.88</td>
<td>0.75</td>
</tr>
<tr>
<td>Ch3</td>
<td>3.97</td>
<td>2.29</td>
<td>*</td>
<td>0.89</td>
<td>0.75</td>
</tr>
<tr>
<td>Ch4</td>
<td>4.55</td>
<td>1.36</td>
<td>*</td>
<td>0.88</td>
<td>0.75</td>
</tr>
<tr>
<td>Ch5</td>
<td>3.46</td>
<td>1.34</td>
<td>*</td>
<td>0.91</td>
<td>0.75</td>
</tr>
<tr>
<td>Ch6</td>
<td>3.17</td>
<td>0.86</td>
<td>*</td>
<td>0.85</td>
<td>0.75</td>
</tr>
<tr>
<td>Ch7</td>
<td>4.84</td>
<td>1.39</td>
<td>*</td>
<td>0.89</td>
<td>0.75</td>
</tr>
<tr>
<td>Ch8</td>
<td>4.31</td>
<td>1.51</td>
<td>*</td>
<td>0.86</td>
<td>0.75</td>
</tr>
<tr>
<td>Ch9</td>
<td>3.74</td>
<td>1.28</td>
<td>*</td>
<td>0.89</td>
<td>0.75</td>
</tr>
<tr>
<td>Ch10</td>
<td>3.82</td>
<td>1.46</td>
<td>*</td>
<td>0.83</td>
<td>0.75</td>
</tr>
</tbody>
</table>

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Brenner et al. 2012
Applying science to fuel and feed our global society

Distribution of Introgressions

Ch 3

Ch 4

IOWA STATE UNIVERSITY
Department of Agronomy
Crop, Soil, and Environmental Sciences

Brenner et al. 2012

Applying science to fuel and feed our global society
Plant height as « model » trait

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of QTL</td>
<td>15</td>
</tr>
<tr>
<td>Genetic variance explained</td>
<td>100%, each QTL explaining between 2% and 30% of the genetic variance</td>
</tr>
<tr>
<td>Mean</td>
<td>200 cm</td>
</tr>
<tr>
<td>$h^2$</td>
<td>90% or 50%</td>
</tr>
</tbody>
</table>

Simulation of different scenarios regarding QTL alleles:

- Simulation of different proportions $\psi$ of donor lines carrying an allele whose effect is different from the elite line.

- Simulation of multiple alleles whose effects are comprised between $-a$ and $+a$ for 3 QTL (= « multiple allele » QTL)
Number of QTL detected per proportion of donor lines carrying QTL allele and population size (Method BIM, $h^2 = 90\%$; marker coverage = 10 cM))
Impact of Linkage Drag
### How & When to Evaluate Genetic Resources: BC1 vs BC3

<table>
<thead>
<tr>
<th>% Exotic Exp (Obs.)</th>
<th>Recurrent parent</th>
<th>Exotic inbred</th>
<th>GEM–BC1–DH</th>
<th>GEM–BC1: 3–DHa</th>
<th>GEM–BC1: 3–DHb</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR</td>
<td>00000----000000----0-0-00----00-------0-----0-----0----0-</td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0----0----0-</td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0----0----0-</td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0----0----0-</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td>--00----0--00-----00-----00-----00-----00-----00-----00-----000-----0+++</td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0----0----0-</td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0----0----0-</td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0----0----0-</td>
</tr>
<tr>
<td>25 (17)</td>
<td></td>
<td></td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0----0----0-</td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0----0----0-</td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0----0----0-</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0----0----0-</td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0----0----0-</td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0----0----0-</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0----0----0-</td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0----0----0-</td>
<td>RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0----0----0-</td>
</tr>
</tbody>
</table>

Chrom 1

Chrom 2
How to Find Needle in GEM Stack

> 2000 GEM-DHLs?

GEM-DH-BC1:3 Sublines?
Efficient Procedures & Outlook
The DH Process: Inducibility

81 entries scored

De La Fuente, unpublished
The DH Process: Spontaneous Doubling

- Observed >50% spontaneous doubling on 2 lines
- Attempted self pollinations were successful as were cross pollinations
- Observed fertility of F₁ in greenhouse
- Female fertility is not an issue

De La Fuente, unpublished
GEM with Improved Inducers and Recurrent Parents

**Improved Inducers:**

- Higher Induction Rates
- Automated Sorting

**Improved Recurrent Parents:**

- Substantially increased Inducibility
- No Need for Colchicine Treatment
What Are The Questions?

- Is there useful genetic variation?
- What is impact of linkage drag?
- What is impact selective sweeps?
- Impact of background / recurrent parent (epistasis)?
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DH Facility
http://www.plantbreeding.iastate.edu/DHF/DHF.htm

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ThomasL@iastate.edu

Chase 1947, 2011