Welcome to the How to Use Doubled-Haploids to Improve Winter Wheat Webinar

Today’s Presenters: Drs. Bill Berzonsky & Melanie Caffe-Treml

Presentation
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How to Use Doubled-Haploids to Improve Winter Wheat

William Berzonsky & Melanie Caffe-Treml

Berzonsky – Strategy in applying DHs to wheat breeding

Caffe-Treml – Details of the DH technique
What are Doubled-Haploids (DHs)?
- Plants rapidly moved to a homozygous (2n stage) \textit{without} "traditional" self-pollination
"Traditional" vs. DH Method

Parent 1 x Parent 2

F₁

Selfing

↓

↓

↓

↓

Inbred line

DH

Inbred line
**Self-Pollination Over Generations**

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**“Traditional” Pure-line Breeding Scheme**

<table>
<thead>
<tr>
<th>Year</th>
<th>Procedure</th>
<th>Generation (Homozygosity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cross between parents</td>
<td>F₁ (0%)</td>
</tr>
<tr>
<td>2</td>
<td>Selection between plants</td>
<td>F₂ (50%)</td>
</tr>
<tr>
<td>3</td>
<td>Selection between families</td>
<td>F₃ (75%)</td>
</tr>
<tr>
<td>4</td>
<td>Selection between families</td>
<td>F₄ (87.5%)</td>
</tr>
<tr>
<td>5</td>
<td>Selection between families</td>
<td>F₅ (93.7%)</td>
</tr>
<tr>
<td>6</td>
<td>Selection between pure lines</td>
<td>F₆ (96.8%)</td>
</tr>
<tr>
<td>7</td>
<td>Yield evaluations of lines</td>
<td>F₇ (98.4%)</td>
</tr>
</tbody>
</table>

+ Another 2 years of yield evaluations/seed increases

Total years: 9

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**“Double Haploid” Breeding Scheme**

<table>
<thead>
<tr>
<th>Year</th>
<th>Procedure</th>
<th>Generation (Homozygosity)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cross between parents</td>
<td>F₁ (0%)</td>
</tr>
<tr>
<td>2</td>
<td>Cross F₁ plants with maize*</td>
<td>(100%)</td>
</tr>
<tr>
<td>3</td>
<td>Seed multiplication</td>
<td>(100%)</td>
</tr>
<tr>
<td>4</td>
<td>Selection between pure lines</td>
<td>(100%)</td>
</tr>
<tr>
<td>5</td>
<td>Yield evaluation of lines</td>
<td>(100%)</td>
</tr>
</tbody>
</table>

+ Another 1 or 2 years of yield evaluations/seed increases

Total years: 7

*Added benefit of having homozygous lines for identifying molecular markers
DHs represent an *alternative* to self-pollination and inbreeding for many generations.
Overall Purpose of DH Production is to Sample **Adequate Genetic Variation** from the Initial F$_1$ Cross

*(Initial F$_1$ Cross)*

*Must not* have significant loss of variation during development of DHs

*(DH Lines)*
To realize advantages of DHs in breeding – "Visualize" the breeding process as a factory

Cross Desirable Parents

Evaluations & Selections

Evaluations & Selections

Release Variety ~ 10 to 12 Years
Advantages to Using DHs

- Homozygosity is achieved very rapidly – essentially one step
- “Safe selection" can be practiced on resulting homozygous, true-breeding plants
- Selection is on the basis of additive gene action
- A homozygous source of seed is available for pure-line variety release
- A rapid way to produce inbred lines for more efficient testing of combining ability for hybrids
Improves "Factory"

- **Quality control and efficiency** of factory
- **Time** to product output
- **Sustainability** of factory production
Improving "Parent Building" – Probably Most Valuable Long-term advantage of DH Technique

- Cross Desirable Parents
- Intermate Highest Performers
- Select Highest Performers
- Evaluate Offspring

Advanced Lines, Cycle Again
Disadvantages of Using DHs

- Rapid homozygosity achieved in a single step is often “offset” by the inability to make selections during the traditional inbreeding process.

- Less recombination can occur compared with inbreeding.

- Success is sometimes unpredictable and can consume valuable resources.
Example of Using DHs in Breeding a Self-Pollinator*

Parent 1 \( \times \) Parent 2

\[ F_1 \]

DH Step

Backcross to Parent 1

Now "true-breeding", so select e.g. in GH for disease resistance

DH Project at South Dakota State University

• Initiated to address needs of WCSIA – Bayer/DU Program

• Not producing DHs for service or on a commercial basis (recent estimate for commercial production = $30 per haploid line)

• Focuses on developing winter hardy and disease resistant winter wheat varieties
# Implementation of DH Technique to Wheat Variety Development in Canada*

<table>
<thead>
<tr>
<th>Wheat Class(^+)</th>
<th>Years</th>
<th>No. of Breeding Institutions(^++)</th>
<th>Varieties Released</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWRS</td>
<td>1997 to 2009</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>CWSWS</td>
<td>2001 to 2002</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>CWHWS</td>
<td>2000 to 2006</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>CWES</td>
<td>2004 to 2008</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>CWAD</td>
<td>2010</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CWRW</td>
<td>2010</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CWGP</td>
<td>2008 to 2010</td>
<td>3</td>
<td>6</td>
</tr>
</tbody>
</table>

\(^+\) CWRS = Canadian Western Red Spring, CWSWS = Canadian Western Soft White Spring, CWHWS = Canadian Western Hard White Spring, CWES = Canadian Western Extra Strong, CWAD = Canadian Western Amber Durum, CWRW = Canadian Western Red Winter, Canadian Western General Purpose

\(^++\) AAFC Lethbridge, U. of Manitoba, AAFC U. of Saskatchewan, AAFC Swift Current, Viterra, AAFC Winnipeg

Summary Points – Applying DHs to Breeding

- Must be implemented to achieve a single, focused objective

- Key is to get DH production efficiency to a high level to adequately sample initial $F_1$ genetic variation

- Must have dedicated, full-time personnel and adequate facilities for DH production

- As a breeding "tool", its biggest benefits are long-term, e.g. parent building, improving effectiveness of selection, hastening variety release over years
"Take Home" Message on Strategy of Applying DH Technique

The benefits to applying the technique in wheat are primarily in the short-term improving the power of selection (quality control and efficiency), and in the long-term "building" improved parents (time and sustainability).
Details of the DH Technique

1. Wide Cross
2. Hormone Tmt.
3. Embryo Rescue
4. Colchicine Tmt.
Producing DHs - Maize Pollination Technique

Maize chromosomes are eliminated after fertilization.
Producing DHs - Maize Pollination Technique

Colchicine treat to double the chromosome number of plants

= Doubled-haploids
Step 1: $F_1$ or $F_2$ plants to pollinate

- $F_1$ or $F_2$ plants
- Stagger planting
- Growing environment can impact efficiency
Step 1: Pollen donor plants

- Need to synchronize corn pollen production with wheat flowering time.
- Effect of corn genotype?
Step 1: Emasculation

- Performed on the day before anthesis
- Minimal cutting of the glume
Step 1: Pollination

- Feathery stigma
- Fresh pollen (light yellow)
- Pollen is sprinkled with a brush over the stigma
Step 1: Environmental condition

➤ **Temperature**
Campbell et al (1998) evaluated 3 day/night temperature regimen:
- 17/22°C
- **22/17°C** ➞ Highest embryo formation
- 27/22°C

➤ **Light intensity**
Campbell et al (1998) evaluated 3 light intensity:
- 300µmol m\(^{-2}\) s\(^{-1}\)
- 500µmol m\(^{-2}\) s\(^{-1}\)
- **1000µmol m\(^{-2}\) s\(^{-1}\)** ➞ Highest embryo formation

➤ **Relative humidity**
60-65% (Khan and Ahmad, 2011)
Step 2: Hormone treatments

Hormone treatment required for seed and embryo development.

→ 2,4D

Several methods of applications:
- Spraying
- Dipping of the spike in solution
- Tiller injection
- Detached tillers cultured in a 2,4-D solution
Step 3: Embryo rescue

14 - 15 days after pollination

Self seed

Haploid seed
Step 3: Embryo rescue
Step 3: Embryo rescue

Haploid embryos
Step 3: Haploid plant regeneration

- Commonly used media:
  - Gamborg’s B5 medium
  - Murashige and Skoog basal medium

- Dark/4°C
  → break dormancy

- Dark/room temperature
  → germination

- 16L:8D/room temperature
  → plantlet regeneration
Step 3: Haploid plant regeneration

Haploid plantlets
Step 3: Vernalization

- In soil
  - No risk of contamination
  - Better plant development

- In tube
  - Less space required
  - Number of tubes

7 to 9 weeks at 4°C
Step 4: Colchicine treatment

- Colchicine is an alkaloid obtained from *Colchicum* species. It is very toxic and must be handled with care and appropriate protection.

- Colchicine interferes with microtubule organization and inhibits normal chromosome separation during mitosis, resulting in a cell with double the chromosome number.

- Treatment at the 4-5 tillers stage
Step 4: Colchicine treatment
Step 4: Colchicine treatment
Step 4: Doubled-haploid plants

- Only some tillers will be successfully doubled following colchicine treatment.
- Successfully doubled tillers will set a few seeds.
Efficiency of wheat DH production

- Nb of embryo/100 florets pollinated: 20-30
- Nb of haploid plantlets/100 embryos: 50-70
- Nb of DH/100 haploid plants: 75-85
# Timing of winter wheat DH production

- Vernalization of wheat donor plants: 7 to 9 weeks
- Transfer of wheat to greenhouse: 5 to 6 weeks
- Emasculation: + 1 day
- Pollination: + 1 day
- Hormone treatment: + 14 days
- Embryo rescue: + 14 days
- Embryo germination: + 14 days
- Haploid plantlet regeneration: + 14 days
- Haploid plantlet vernalization: 7 to 9 weeks
- Colchicine treatment: + 1 to 2 months
- DH seed harvest: + 7 to 8 months

**Total:** 14 to 16 months
Summary – Details of the DH Technique

- Technique with proven potential

- Needs optimization:
  
  • Efficiency of production
  • Time-saving

  → Optimizations have to be done for each program based on resources available.
Presentation Available at:

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