Production of Haploid & Use of Doubled Haploids in Wheat Breeding

Jianli Chen
Stages in plant life cycle where haploid can occur or be induced.
What is Haploid and Doubled Haploid?

- **Haploid**: an individuals with the gametic chromosome number in its somatic cells
  - polyhaploid: wheat $n = 3x = 21$; durum $n = 2x = 14$
  - monohaploid: barley $= 1x = 7$
- **Doubled Haploid**: an individual with the doubled chromosome number of the haploid
- **Haploid vs monosomic; doubled haploid vs diploid vs disomic?**
Breeding Using Doubled Haploid System

<table>
<thead>
<tr>
<th>Year</th>
<th>Population</th>
<th>Processes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>Initial crosses made</td>
<td>AABB x aabb</td>
</tr>
<tr>
<td>2nd</td>
<td>F1</td>
<td>AaBb</td>
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<td></td>
<td>Gametes</td>
<td>AB Ab aB ab</td>
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<td></td>
<td>Haploid production</td>
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<td></td>
<td>Cochicine treatment</td>
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<td></td>
<td>Doubled Haploid</td>
<td>(25%) AABB AAbb aaBB aabb</td>
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<tr>
<td>3rd</td>
<td>H1</td>
<td>Head Row Test</td>
</tr>
<tr>
<td>4th-10th</td>
<td>Testing and Cultivar Release</td>
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<tr>
<td>Year</td>
<td>Population</td>
<td>Processes</td>
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<td>1st</td>
<td>Initial crosses made</td>
<td>AABB x aabb</td>
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<tr>
<td>3rd</td>
<td>F2</td>
<td>1AABB 2AABb 1AAbb 2AaBB 4AaBb 2Aabb</td>
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</table>
Backcrossing or Pyramiding

- **Parent**: aabb x AABB
- **F₁**: aabb x AaBb (50%)
- **BC₁F₁**: aabb x AaBb (75%) Aabb aaBb aabb
- **BC₂F₁**: aabb x AaBb (87.5%) Aabb aaBb aabb
- **BC₃F₁**: aabb x AaBb (93.75) Aabb aaBb aabb
- **BC₃F₂**: 1/16 AABB (6.25) DH: AABB (25%)
- **BC₃F₃**: AABB (6.25) AABB (25%)
Breeding Using Wheat x Maize Hybridization

VA01W476

1998  Roane x W14

1999  F1, doubled haploid production

2000  Head row (35 rows)

2001  Observation test (VA01W-476)

2002  Scab regional uniform test

2003  Preliminary test
Advantages of Doubled Haploid Techniques

- Based on gamete selection
- Develop immediate homozygosity, shorten the time to cultivar release
- Provide greater efficiency of selection in plant breeding
- Improve the precision of genetic and mapping studies
- Accelerate gene pyramiding
- Improve efficacy and efficiency in screening for resistance
Application of Doubled Haploid Variety Improvement

- 100% homozygosity of doubled haploid
  - Elimination of dominance variation
  - Much less progeny needed
    - for two desired genes, using DH, you need only grow 4 DH lines instead of growing 16 plants when selfing to obtain desired genotype.
    - for four desired genes, using DH, you need only grow 16 ($2^n$) DH lines instead of growing 256 ($4^n$) plants when selfing.
  - Reduction of 3-5 years for cultivar release
Application of Doubled Haploid Genetic Study

• No risk of heterozygosity
• Quantitative gene inheritance
• Estimation of additive & additive x additive variances
Application of Doubled Haploid Mapping Study

- Permanent population
- no risk of heterozygosity
- can be repeated anytime
- can be used in different Lab
- can be used by different researchers
- data can be accumulated
Methodologies for inducing haploid

- Anther Culture (microspore culture)
- Wide-hybridization mediated chromosome elimination
  - Barley $\times$ H. Bulbosum
  - Wheat $\times$ Maize hybridization
Anther Culture

- Conventional method
- Some success in releasing new cultivars
- Very much genotype dependent
Barley x H. Bulbosum

- Conventional method
- Good haploid production method for barley
- Restricted to Kr$_1$ Kr$_2$ genotypes
Wheat x Maize Hybridization

• An alternative to anther culture and the *H. bulbosum* system in wheat
• First reported by Laurie and Bennett (1986)
• Less genotype-dependent response
• Higher efficacy (Kisana et al., 1993)
  – 2-3 times greater than anther culture
  – Save 4-6 weeks in obtaining the same age haploid green plants
• Less Variation (Kisana et al., 1993)
  – Fewer aneuploids or chromosomal abnormalities
  – Fewer spontaneous regeneration
Procedures of Wheat x Maize Hybridization

- Emasculation
- Pollination
- 2,4-D treatment
- Embryo culture
- Colchicine treatment
- Doubled haploid production
Emasculation

- Cut the glume tips a little
- Reduce damage to glume and stigmas as much as possible.
Pollination

- Fresh pollen
- Pollinate 1-2 days after emasculcation
2,4 - D Treatment

- Concentration: 100 mM
- Timing: 24-36 hrs after pollination
- Method: merge entire head into 2,4 – D solution
Embryo Culture

- Excise embryos from developing seeds 12-16 days after pollination and place them on Gamborg’s B5 or MS basal medium
- Treat rescued embryos for 5 days at 4-8°C in dark
- Grow above embryos at 20-25°C, 12 hrs of fluorescent light (1-2 weeks)
Embryo Germination
Doubled Haploid Production

- Colchicine treatment: Colchicine prevents cell division by inactivating the spindle mechanism. It doesn’t affect chromosome replication, but does prolong the time for mitosis.

- Cells at meristematic stage are sensitive to the treatment; therefore, a mixture of haploid and doubled haploid will be obtained.
Normal versus Cothicine Mitosis

Telophase  |  Anaphase  |  Metaphase
---|---|---
Doubled No. | "Ski-pairs" | No spindle c-pairs

Normal
Prophases
Colchicine
Colchicine Treatment

- Colchicine solution: 1g colchicine powder (1%), 20 ml dimethylsulphoxide (DMSO), and 10 drop of Tween 20 per liter
- **Post - treatment after vernalization**
  - take more time but may save more haploid plants
  - vernalize green plants right after regeneration
  - transfer haploid plants to vermiculite after 6-8 weeks
  - treat them with colchicine solution when plants become healthy
Vernalization of Haploid
Colchicine Treatment

- Colchicine solution: 1g colchicine powder (1%), 20 ml dimethylsulphoxide (DMSO), and 10 drop of Tween 20 per liter
- **Direct – treatment**
  - save time but may lose some haploid plants
- **Post - treatment after vernalization**
  - take more time but may save more haploid plants
  - vernalize green plants right after regeneration
  - transfer haploid plants to vermiculite after 6-8 weeks
  - treat them with colchicine solution when plants become healthy
Before Colchicine Treatment
After Colchicine Treatment
Doubled Haploid Production

- Temperature
- Moisture
- Light
- Nutrition
- Seeds production
Factors?

• Pre-hybridization
• Genotype difference
• Pre-cold treatment
• Apply favorable condition
Pre-hybridization

- **F1 from winter x winter crosses**
  - Grow the first set of corn four weeks after winter wheat is put into vernalization chamber

- **F1 from spring x spring or winter x spring crosses**
  - Grow the first set of corn two weeks after planting wheat

- **Growing conditions:** 80-87°F with 16 hrs photo period provided by high intensity lights in greenhouse

- Grow 40 to 60 F1 seeds to get 200 to 300 doubled fertile haploid (1-2 fertile green plants per emasculated head)
<table>
<thead>
<tr>
<th>Pedigree</th>
<th>Florets Pollinated</th>
<th>Seeds Developed</th>
<th>Embryos Rescued</th>
<th>Green Plants</th>
<th>% B/A</th>
<th>% C/B</th>
<th>% D/C</th>
<th>% D/A</th>
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<tr>
<td>MADISON/ERNIE</td>
<td>814</td>
<td>704</td>
<td>174</td>
<td>60</td>
<td>86.49</td>
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<td>958</td>
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<td>24.84</td>
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<td>ROANE/MADISON</td>
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<td>291</td>
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<td>C.V. (%)</td>
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<tr>
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<td>D/C</td>
<td>D/A</td>
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<td>Between crosses with PION 2684 and Madison</td>
<td>1</td>
<td>490.980***</td>
<td>19.404*</td>
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<tr>
<td>Within crosses with either PION 2684 or Madison</td>
<td>4</td>
<td>106.088**</td>
<td>8.352</td>
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<td>Error</td>
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</table>

* p< 0.05, ** p< 0.01, *** p< 0.001

D/C: The percentage of embryo germination; D/A: The percentage of haploid regeneration based on the number of florets emasculated.
Haploid Production in Twelve Wheat F$_1$ Populations

- Significant genotype differences were found in F$_1$ crosses for the efficiency of haploid production, based on the percentage of embryo germination and the percentage of haploid green plants regenerated.
- Roane, a scab-tolerant variety, and Pioneer 2684 were the best parents for doubled haploid production.
Table 3. Effect of pre-cold treatment for haploid regeneration with wheat x maize hybridization in two wheat F1 crosses conducted in 1999 and 2000*, Blacksburg, Virginia.

<table>
<thead>
<tr>
<th>Pedigree</th>
<th>Seeds Excised (No.)</th>
<th>Embryos Rescued (No.)</th>
<th>Haploid Obtained (No.)</th>
<th>Embryo Formation (%)</th>
<th>Haploid Regeneration (%)</th>
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</thead>
<tbody>
<tr>
<td>Madison x W14</td>
<td>452</td>
<td>2202</td>
<td>48</td>
<td>320</td>
<td>12</td>
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<tr>
<td>Pioneer 2684 x W14</td>
<td>587</td>
<td>1473</td>
<td>140</td>
<td>258</td>
<td>61</td>
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<tr>
<td>Total</td>
<td>1049</td>
<td>3675</td>
<td>188</td>
<td>578</td>
<td>73</td>
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<tr>
<td>Average</td>
<td></td>
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</tbody>
</table>

* All embryos were given pre-cold treatment in 2000 greenhouse test.
Efficiency of Haploid Production

- Fertilization: 80 - 85%
- **Embryo formation**: 20 - 30%
- **Embryo germination**: 45 - 60%
- **Haploid green plants**: 8 - 15%
- Doubling efficiency: 80 - 85%
- **Doubled haploid plants**: 6 - 10%
Technical Difficulties

- Unusual regeneration (13%)
  - Regeneration without shoot
  - Regeneration with glass or white shoot
  - Regeneration without roots

- Various Loss during colchicine treatment (30%)

- Five fertile plants were obtained per 100 florets pollinated (average 2 doubled haploid plants per pollinated head)
Increase the Efficacy of Wheat x Maize Method

• To increase embryo formation:
  – Select genotype for both wheat $F_1$ and corn
  – Apply optimal temperature and light regimes for plant growth and reproduction in both wheat and corn
  – Handle spikes carefully during emasculation
  – Optimal timing of 2,4-D post-treatment

• To increase embryo regeneration:
  – Pre-cold treatment of embryos

• To increase the efficacy of colchicine treatment
  – Appropriate tiller stage
Increase the Efficiency of Wheat x Maize Method

• Reduce unusual regeneration (13%)
  – Pre-cold treatment for embryos

• Reduce loss during colchicine treatment and transfer shock (30%)
  – Healthy haploid plants (Colchicine agar medium or liquid medium)
  – Prevent contamination during growth (Vermiculite)

• Increase seed set
Summary of Wheat x Maize System

• Superior to other systems in wheat
• Potential to improve this technique for practical use in breeding programs
• Desirable method to generate genetic and mapping populations
• Economical application for special targeted traits
• Some drawbacks need to be improved
Some Drawbacks with Doubled Haploid Production

• GENERAL:
  – more expensive: expertise, facilities
  – restriction on number of crosses

• SPECIFIC:
  – mutagenic treatment
  – genotype dependent in anther culture or microspore culture and *bulbosum* method
  – need improvement of haploid regeneration frequency