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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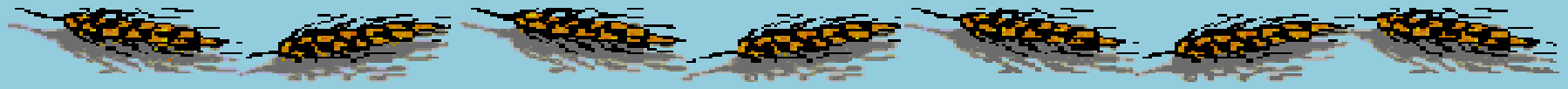
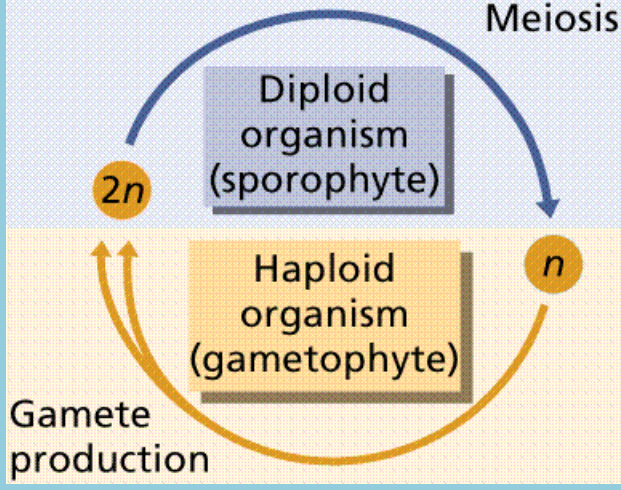
How to Use Doubled-Haploids to Improve Winter Wheat



William Berzonsky & Melanie Caffe-Treml

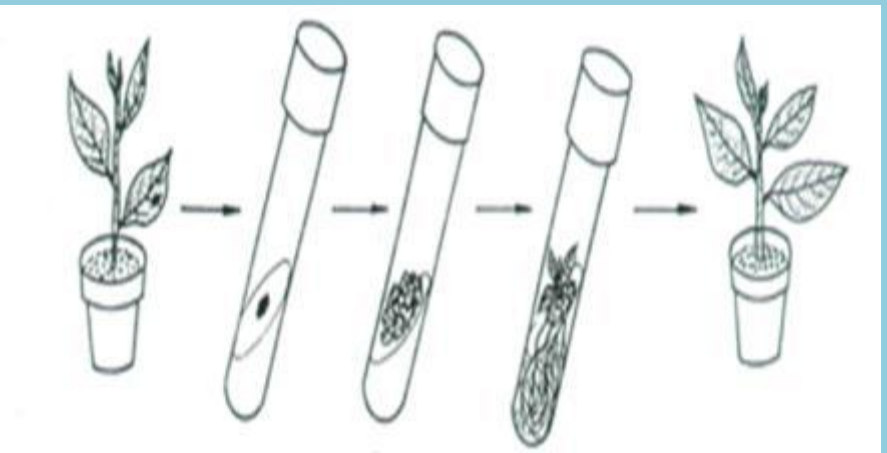
Berzonsky – Strategy in applying DHs to wheat breeding

Caffe-Treml – Details of the DH technique

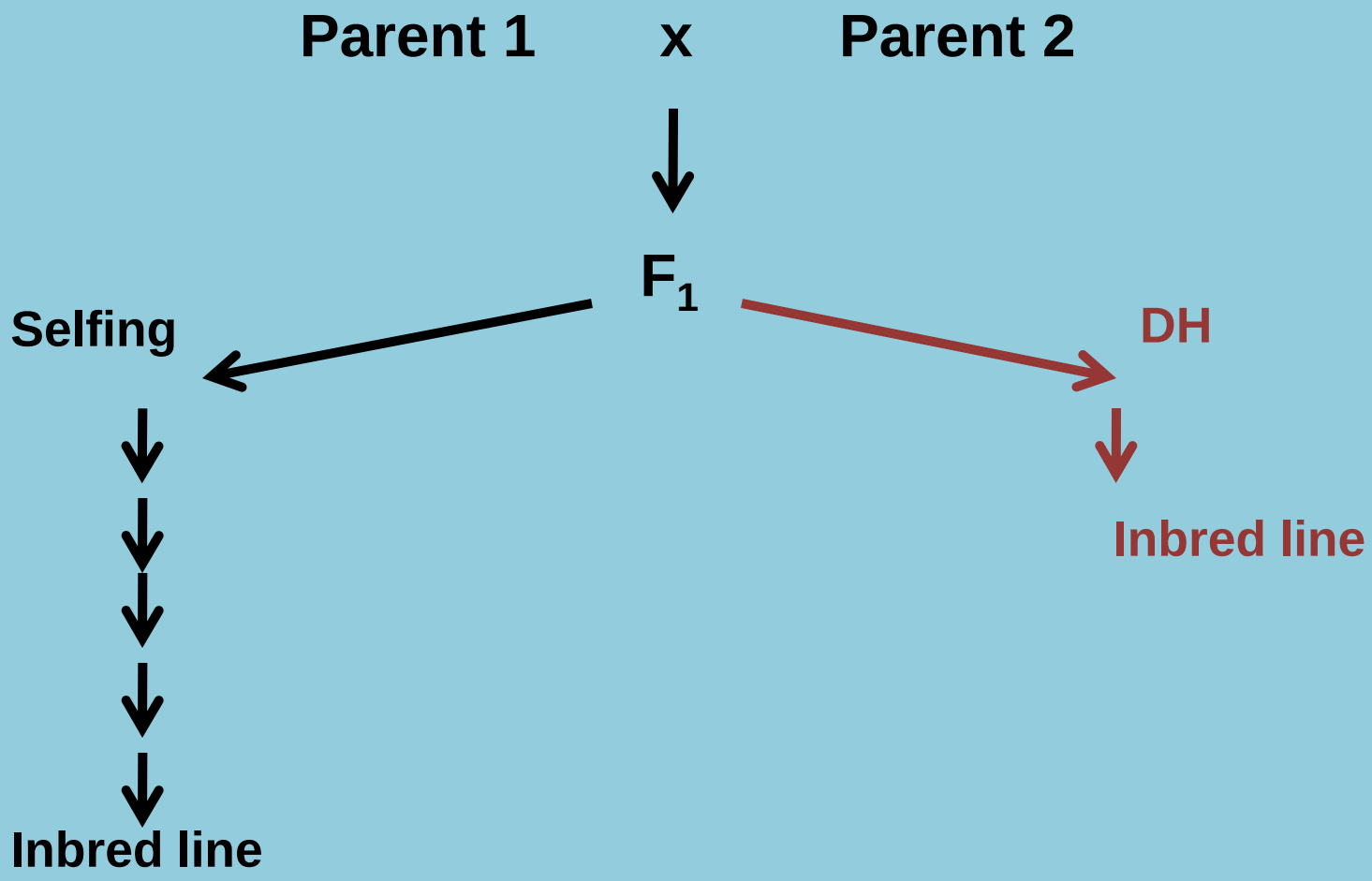


What are Doubled-Haploids (DHs)?

- Plants rapidly moved to a homozygous (2n stage) ***without*** "traditional" self-pollination



"Traditional" vs. DH Method



Self-Pollination Over Generations

DH Method

"Traditional" Pure-line Breeding Scheme

Year	Procedure	Generation (Homozygosity)
1	Cross between parents	F_1 (0%)
2	Selection between plants	F_2 (50%)
3	Selection between families	F_3 (75%)
4	Selection between families	F_4 (87.5%)
5	Selection between families	F_5 (93.7%)
6	Selection between pure lines	F_6 (96.8%)
7	Yield evaluations of lines	F_7 (98.4%)

+ Another 2 years of yield evaluations/seed increases

Total years = 9

"Double Haploid" Breeding Scheme

Year	Procedure	Generation (Homozygosity)
1	Cross between parents	F_1 (0%)
2	**Cross F_1 plants with maize***	(100%)
3	Seed multiplication	(100%)
4	Selection between pure lines	(100%)
5	Yield evaluation of lines	(100%)

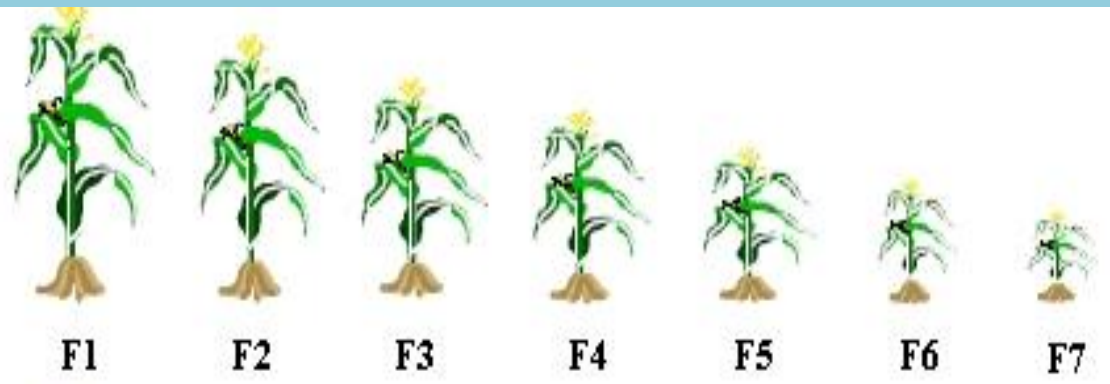
+Another 1 or 2 years of yield evaluations/seed increases

Total years: 7

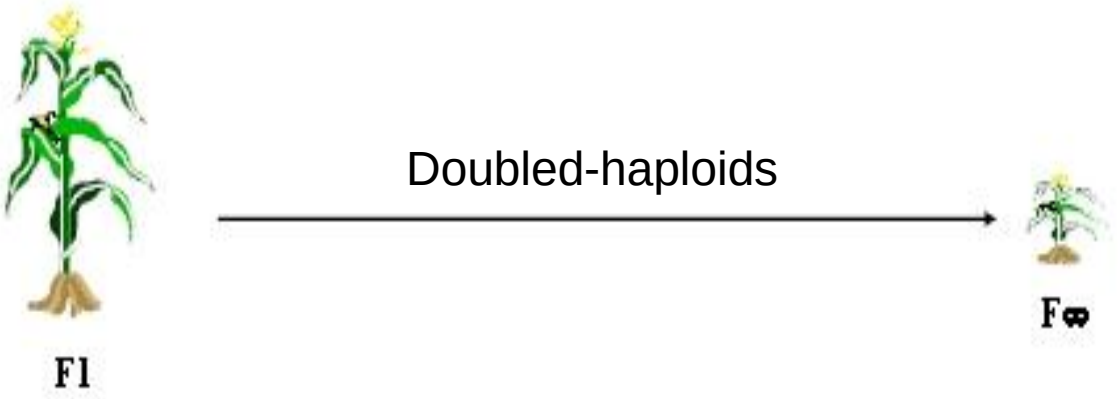
*Added benefit of having homozygous lines for identifying molecular markers

DHs represent an ***alternative*** to self-pollination and inbreeding for many generations

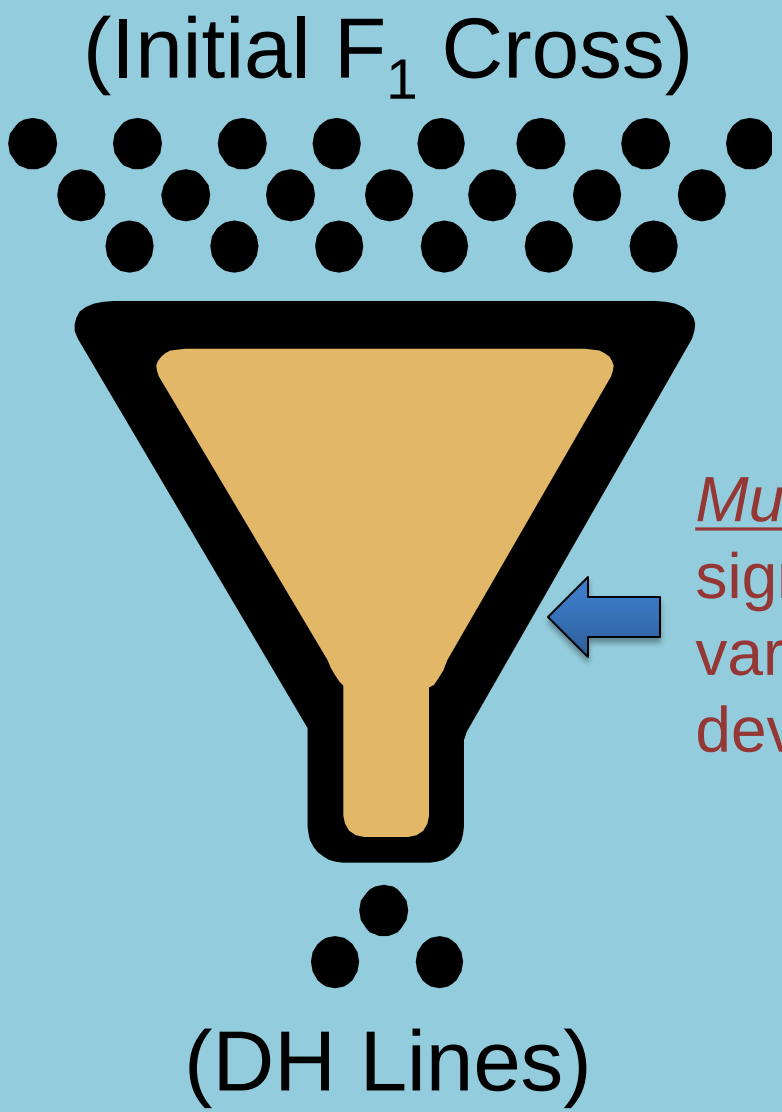
Traditional Inbreeding
7 Years



DH Breeding
2 Years



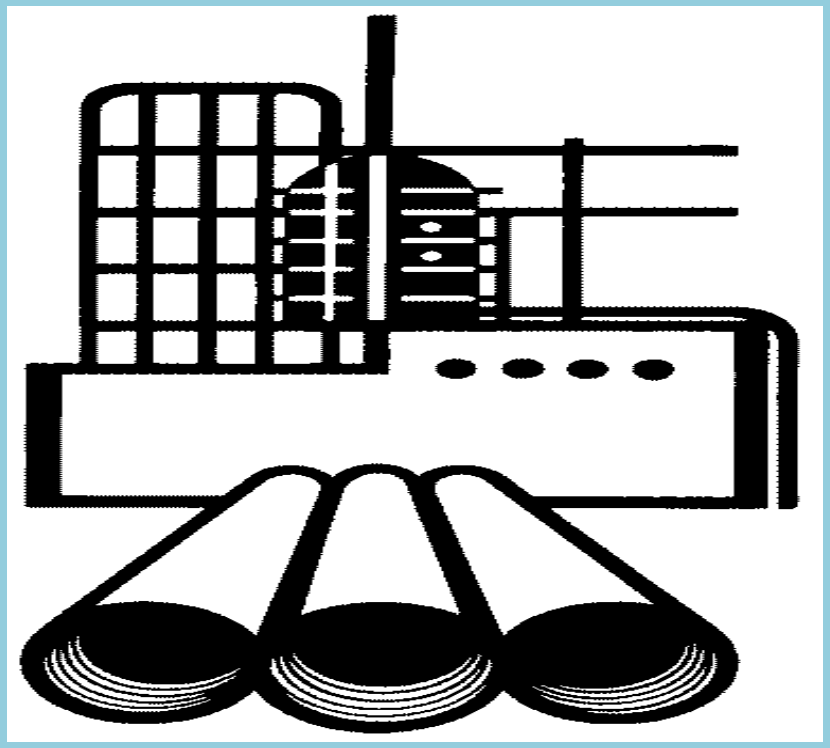
Overall Purpose of DH Production is to Sample Adequate Genetic Variation from the Initial F_1 Cross



Must not have significant loss of variation during development of DHs

To realize advantages of DHs in breeding – "Visualize" the breeding process as a factory

Cross Desirable Parents

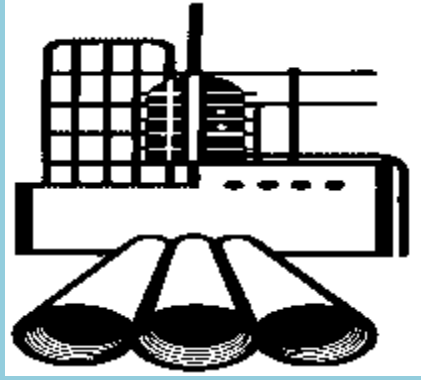


Evaluations & Selections

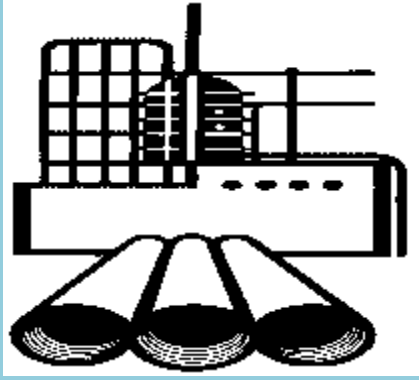
Evaluations & Selections

Release Variety ~ *10 to 12 Years*

Advantages to Using DHs



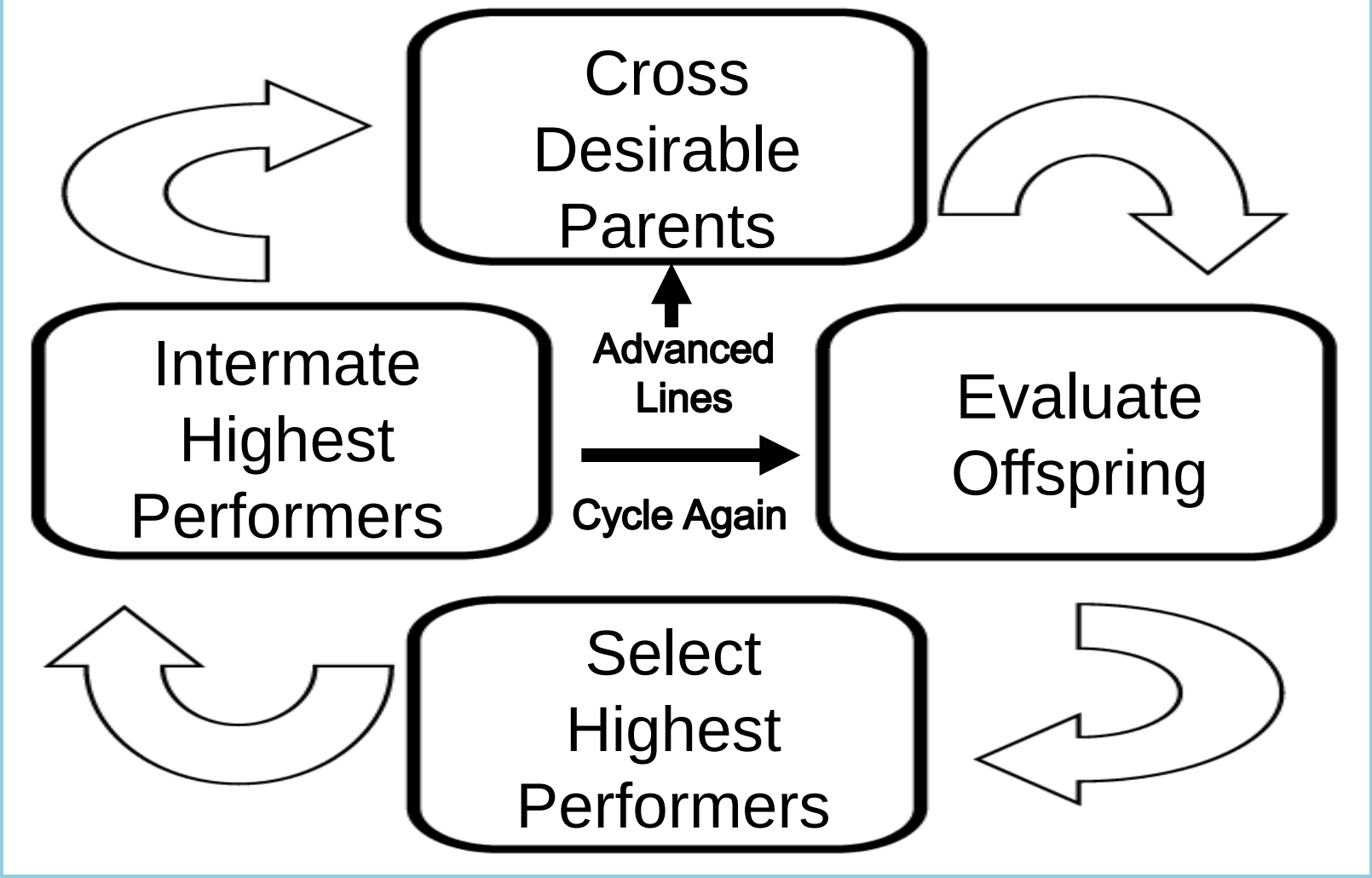
- Homozygosity is achieved very rapidly – essentially one step
- “Safe selection" can be practiced on resulting homozygous, true-breeding plants
- Selection is on the basis of additive gene action
- A homozygous source of seed is available for pure-line variety release
- A rapid way to produce inbred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

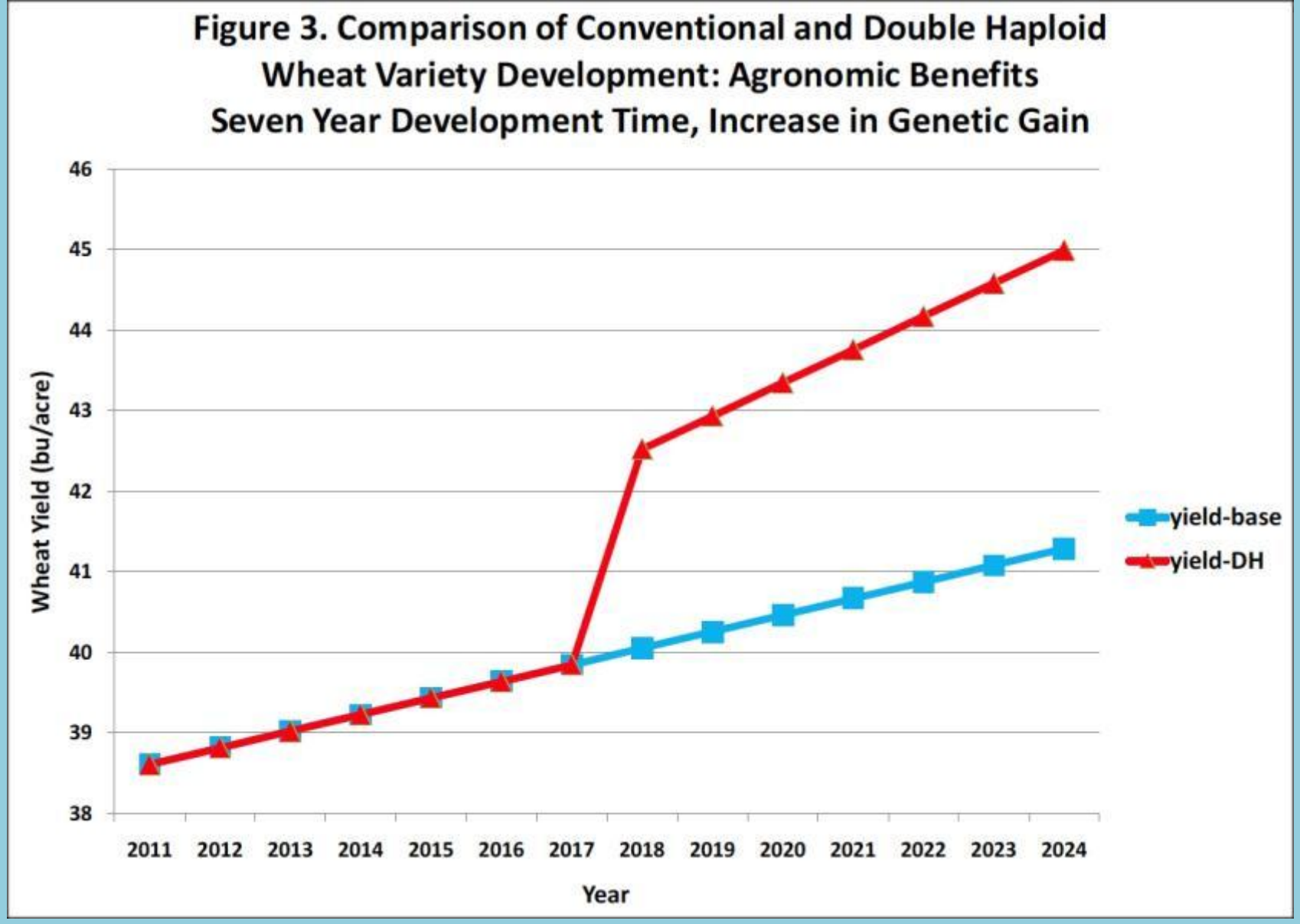


Improves "Factory"

- *Quality control and efficiency* of factory
- *Time* to product output
- *Sustainability* of factory production

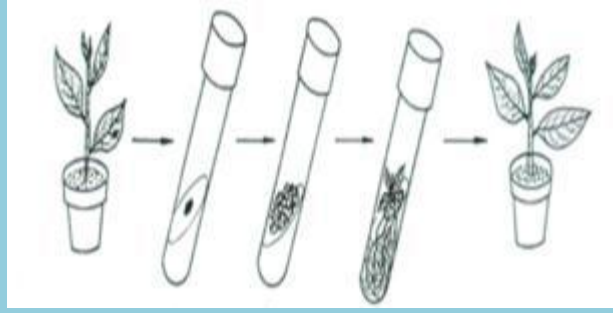
Improving "Parent Building" – Probably Most Valuable Long-term advantage of DH Technique





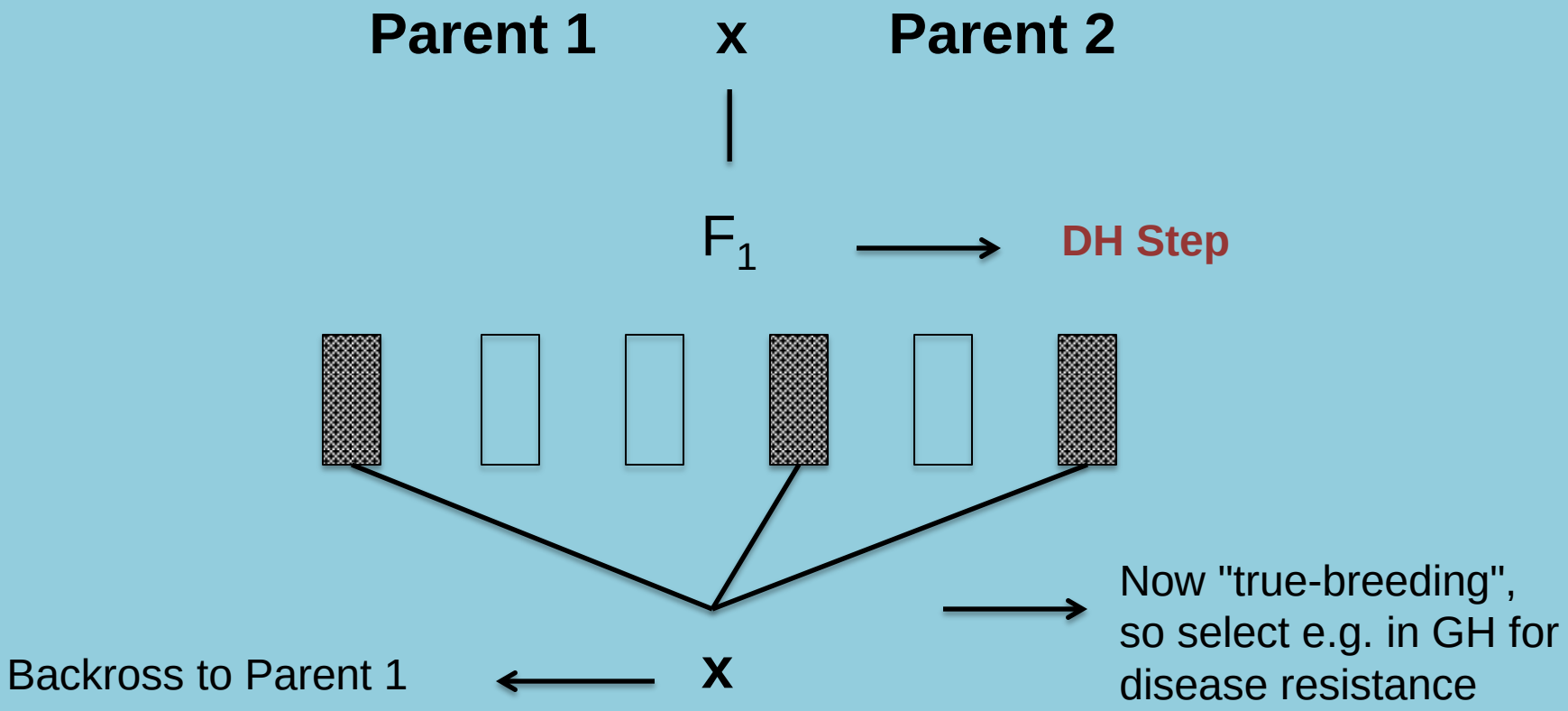
From: Barkley, A. and F.G. Chumley. A doubled haploid laboratory for Kansas wheat breeding: An economic analysis of biotechnology option, March 15, 2011.

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

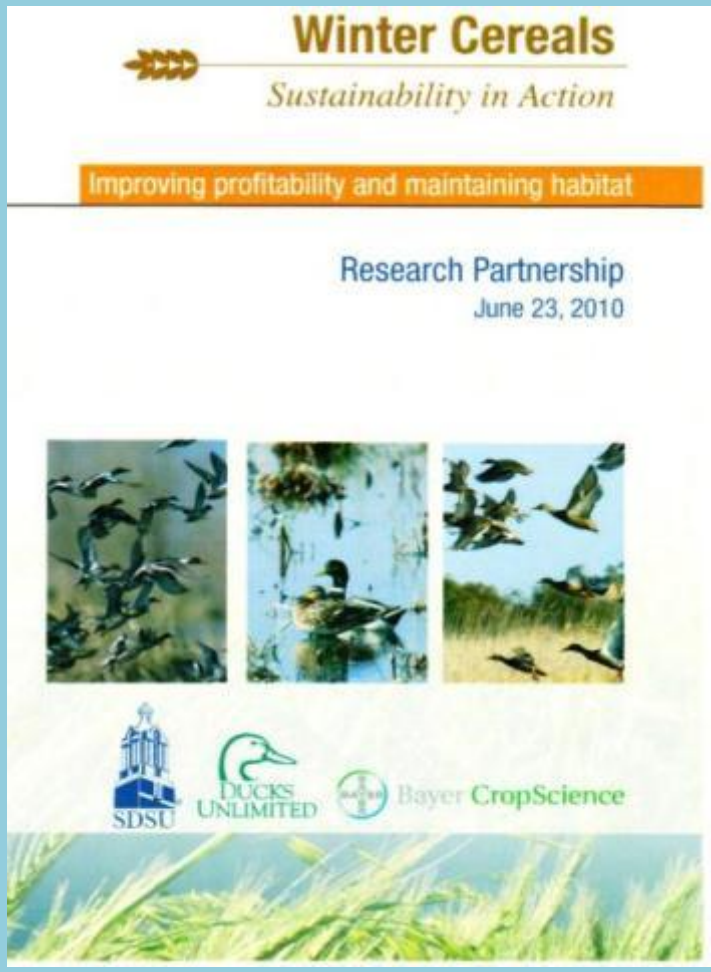
Example of Using DHs in Breeding a Self-Pollinator*



*From: **Fig. 2.** B. Fouroughi Wehr and G. Wenzel. 1990. Recurrent selection alternating with haploid steps — a rapid breeding procedure for combining agronomic traits in inbreeders. Theor. Appl. Gen. 80:564-568.

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*

Wheat Class[+]	Years	No. of Breeding Institutions[++]	Varieties Released
CWRS	1997 to 2009	4	10
CWSWS	2001 to 2002	1	2
CWHWS	2000 to 2006	1	3
CWES	2004 to 2008	1	2
CWAD	2010	1	1
CWRW	2010	1	1
CWGP	2008 to 2010	3	6

[+] 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

* Derived from: Table 2. in DePauw, R.M. et al. 2011. New breeding tools impact Canadian commercial farmer fields. Czech. J. Genet. Plant Breed. 47:S28-S34.

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

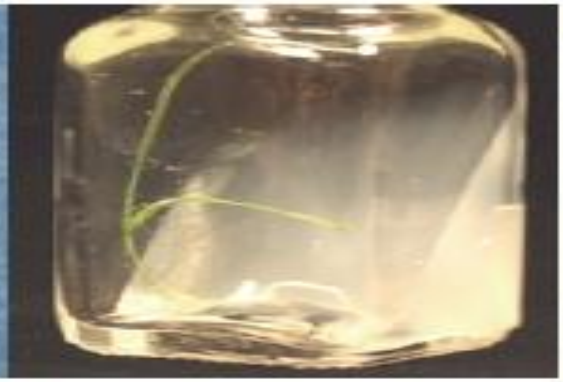
"Take Home" Message
on Strategy of Applying DH Technique

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 ----------



---------- Embryo Rescue ----------

3

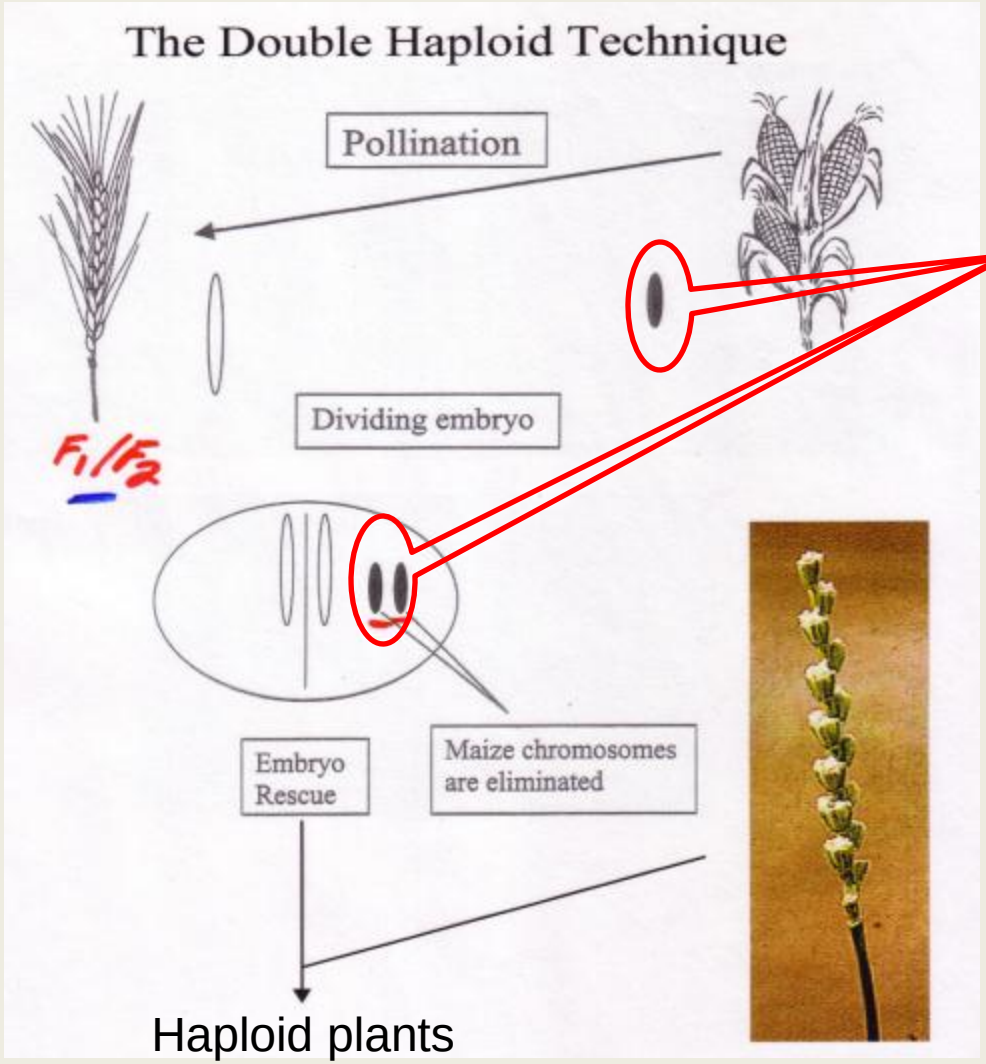
2

--------- Hormone Tmt.----------

---- Colchicine Tmt.-----

4

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
- 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

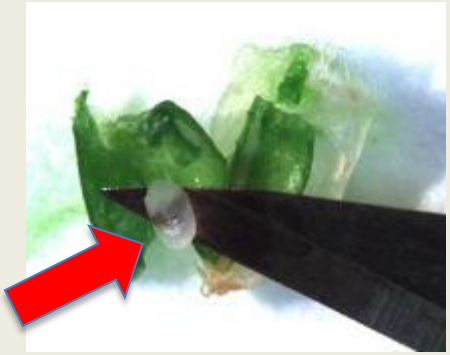
Self seed

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

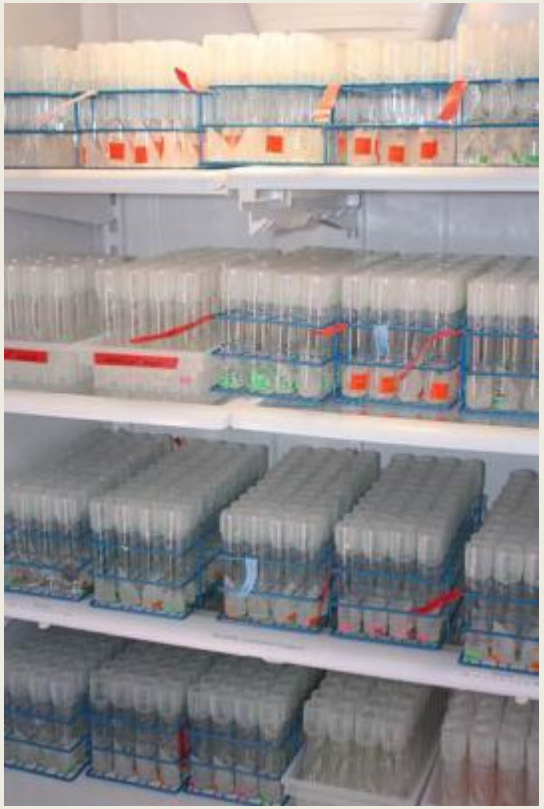
Step 3: Vernalization

- 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

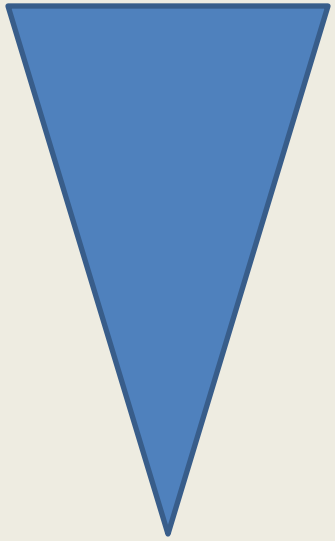
Step 4: Colchicine treatment

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	
• Pollination	+ 1 day
• Hormone treatment	+ 1 day
• 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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