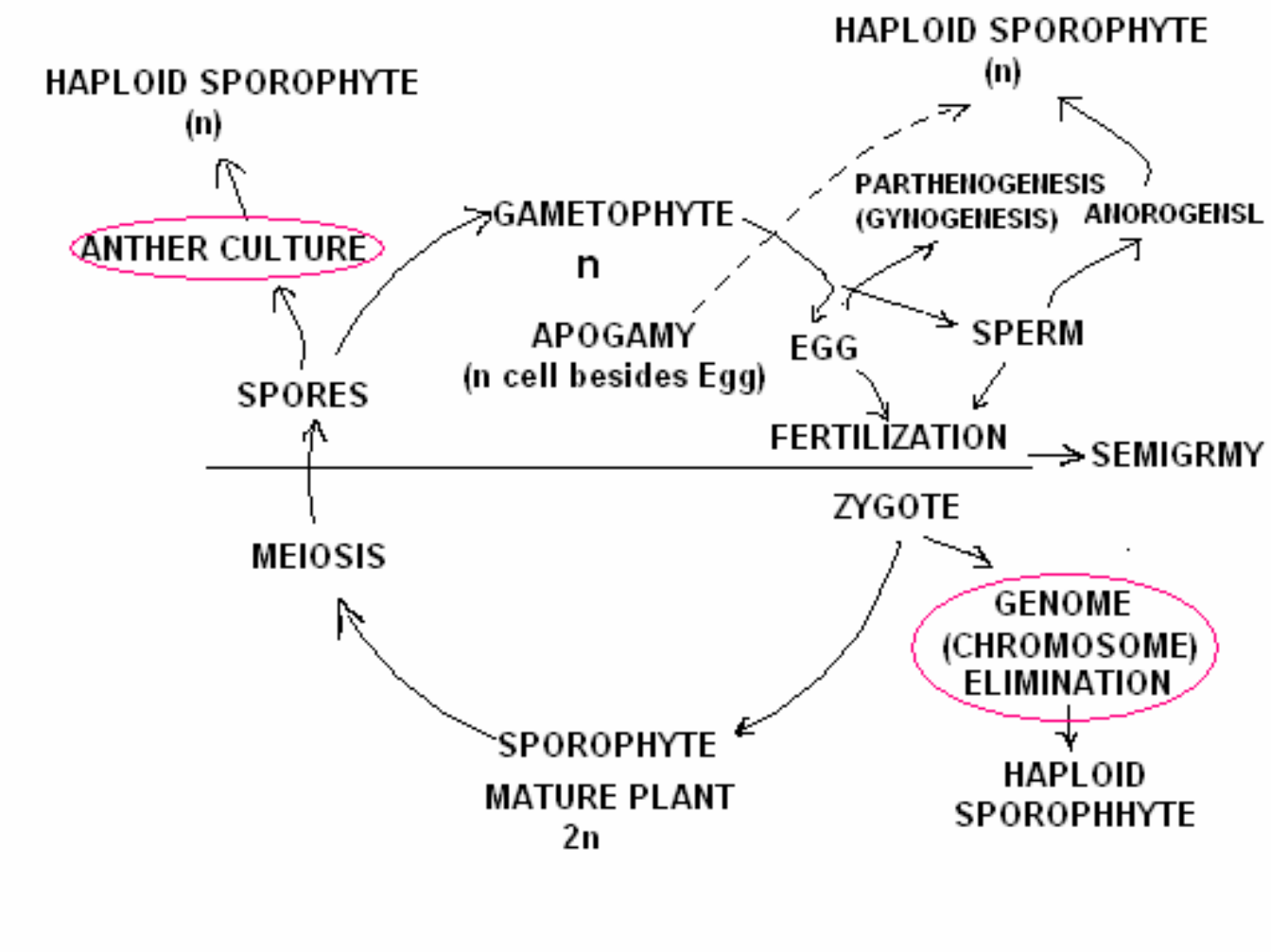
Production of Haploid & Use of Doubled Haploids in Wheat Breeding

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

Year	Population	Processes
1st	Initial crosses made	AABB x aabb
		↓
2nd	F1	AaBb
	Gametes	AB Ab aB ab
		↓ **Haploid production** **Cochicine treatment**
	Doubled Haploid	**(25%)** AABB AAbb aaBB aabb
		↓
3rd	H1	Head Row Test
		↓
4th-10th		Testing and Cultivar Release

Conventional Breeding System

Year	Population	Processes
1st	Initial crosses made	AABB x aabb
2nd	F1	AaBb
	Gametes	AB Ab aB ab
3rd	F2	1AABB 2AABb 1AAbb 2AaBB 4AaBb 2Aabb
		6.25% 1aaBB 2aaBb 1aabb
4th-5th	F3-F4	Advance Progeny: Bulk head selections
6th-7th	F5-F6	Head Row Test
8th-14th		Testing and Cultivar Release

Backcrossing or Pyramiding

- **Parent** aabb x AABB
- F_1 aabb x AaBb (50%)
- BC_1F_1 aabb x AaBb (75%) Aabb aaBb aabb
- BC_2F_1 aabb x AaBb (87.5%) Aabb aaBb aabb
- BC_3F_1 aabb x AaBb (93.75) Aabb aaBb aabb
- BC_3F_2 1/16 AABB (6.25) DH: AABB (25%)
- BC_3F_3 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 herterozygosity
- Quantitative gene inheritance
- Estimation of additive & additive x additive variances

Application of Doubled Haploid Mapping Study

- Permanent population
- no risk of herterozygosity
- 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 x H.Bulbosum
  - Wheat x 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 emasculation

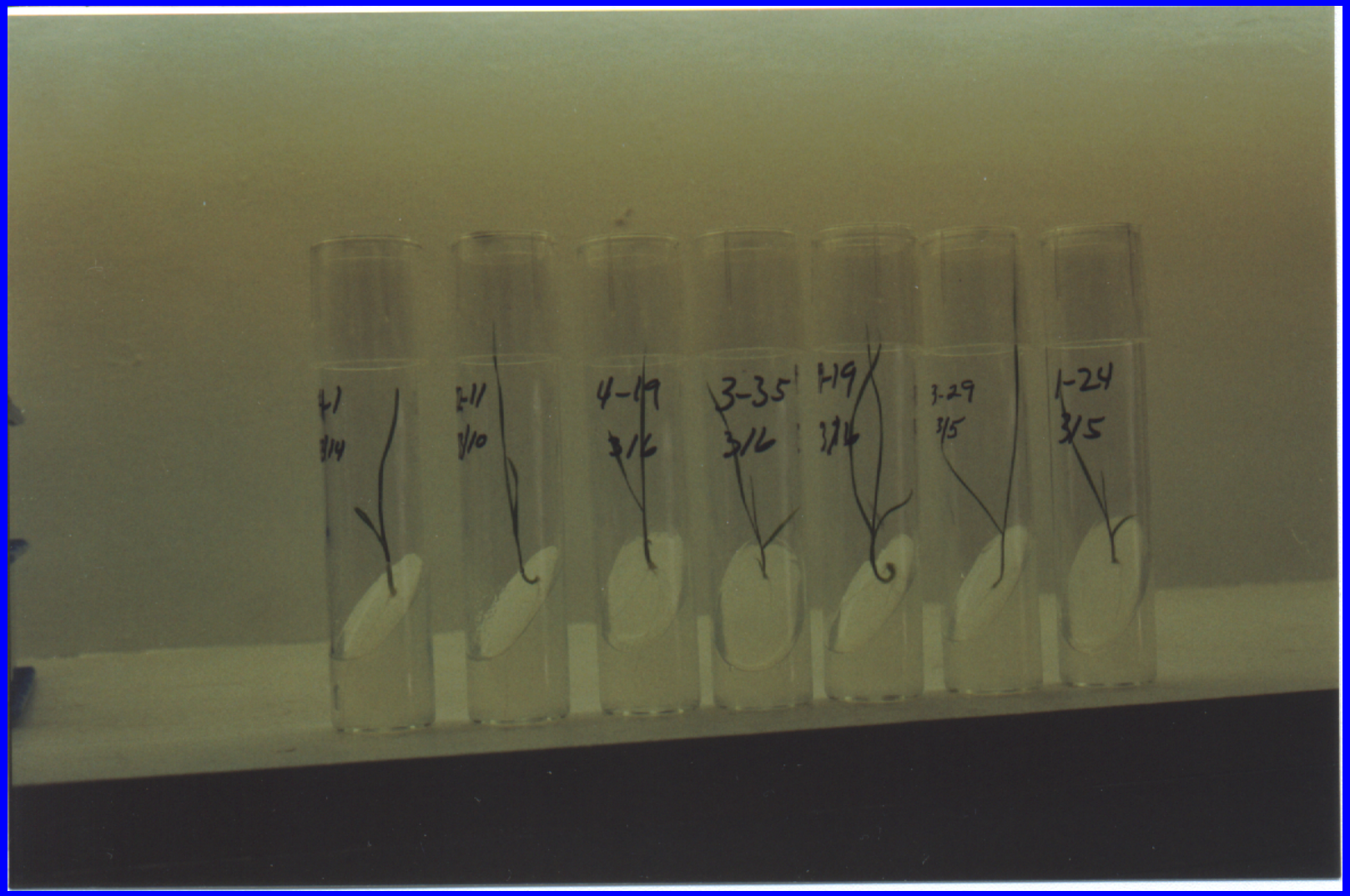
2,4 - D Treatment

- 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^{o}C in dark
- Grow above embryos at 20 - 25^{o}C, 12 hrs of fluorescent light (1-2 weeks)

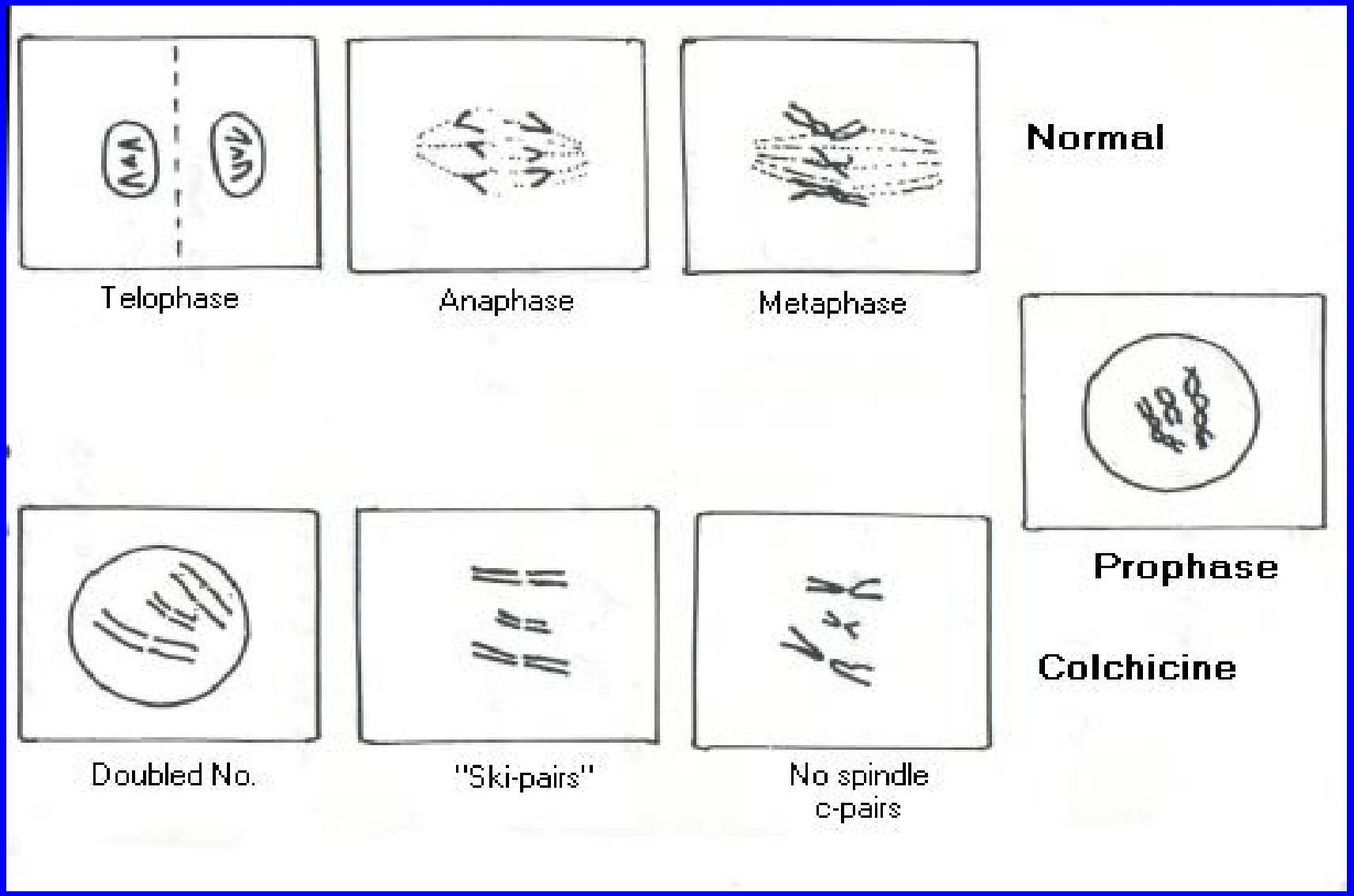
Embryo Germination



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.

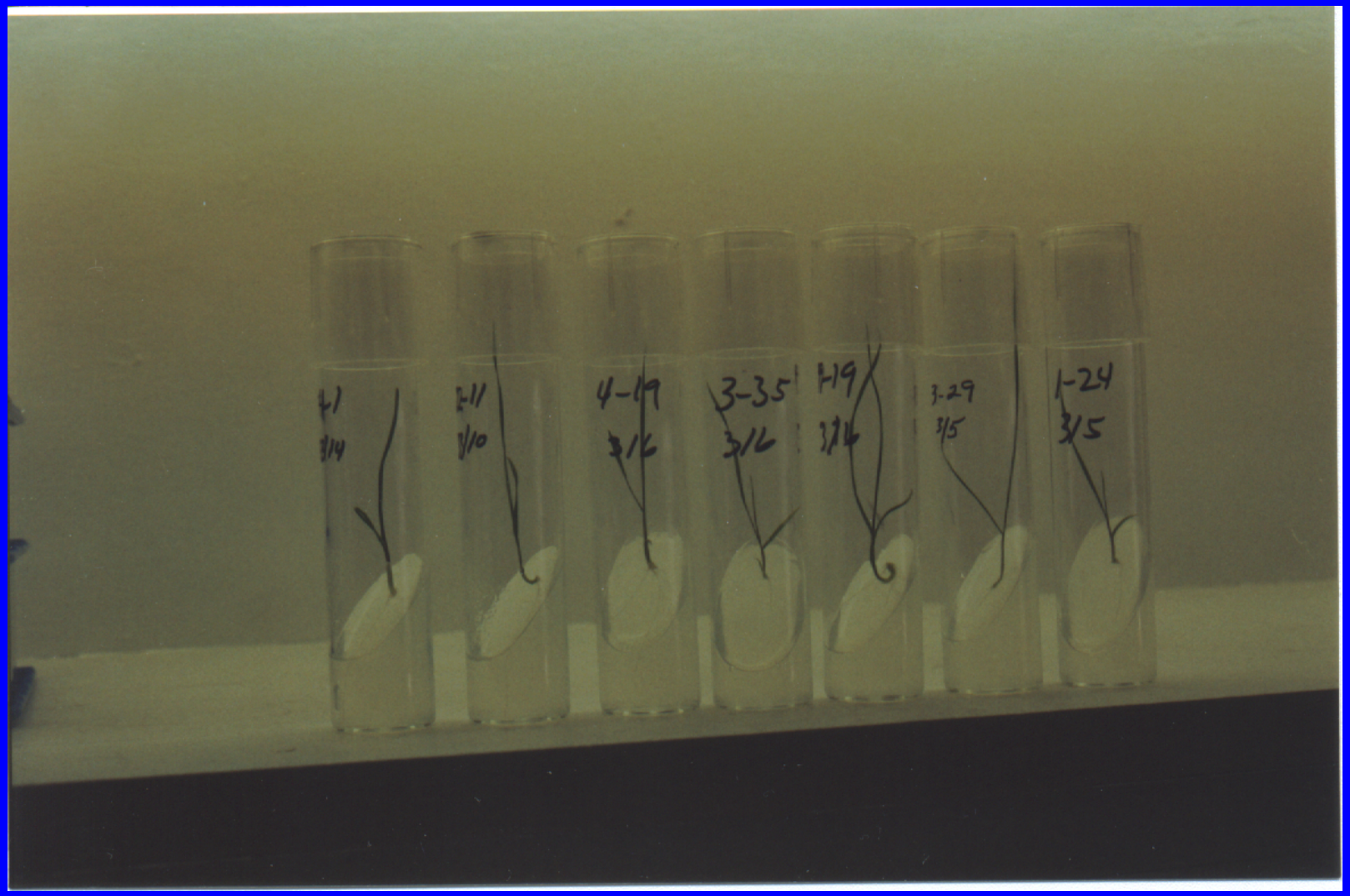
Normal versus Cochicine Mitosis



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

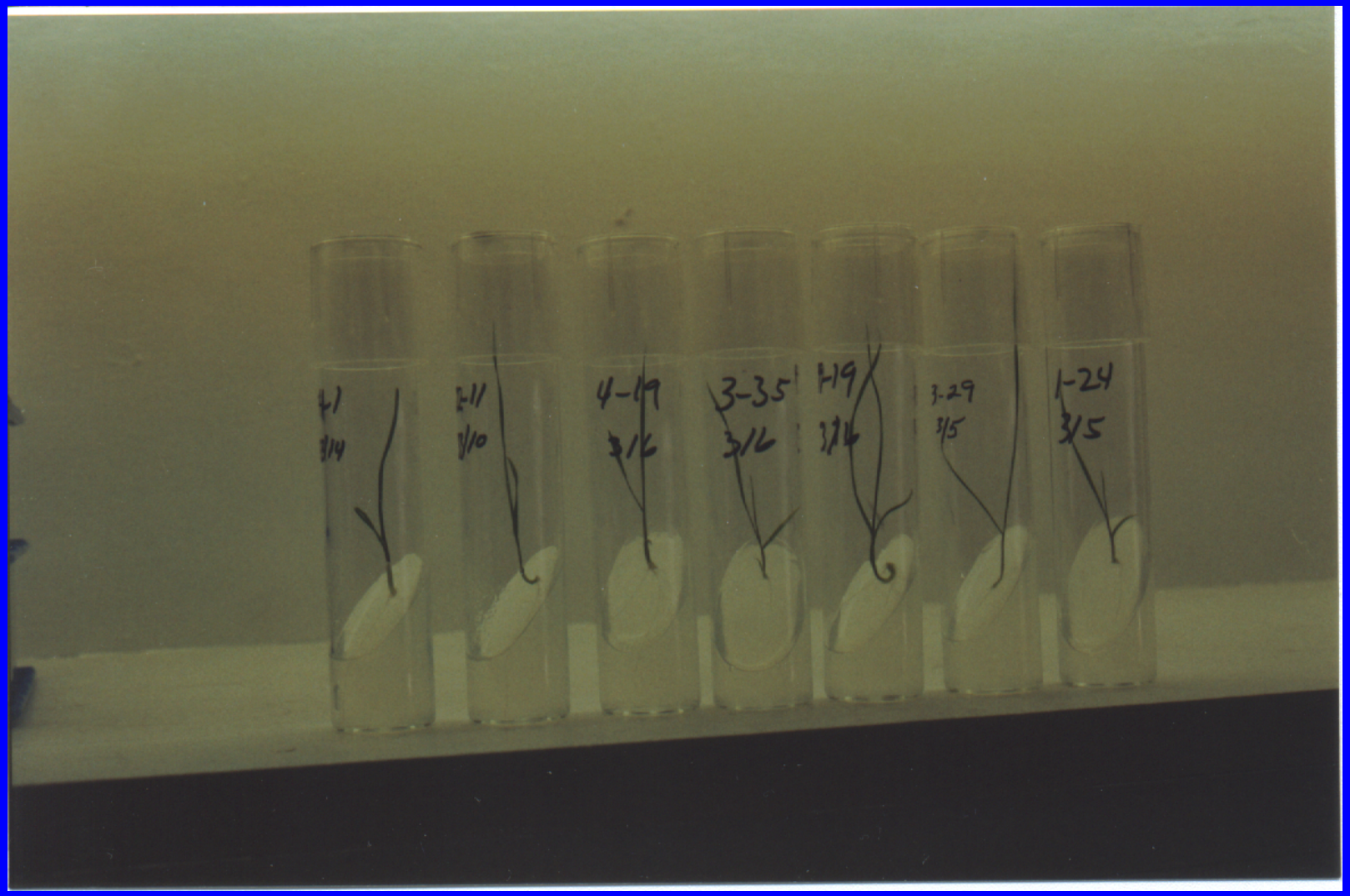
Vernalization of Haploid



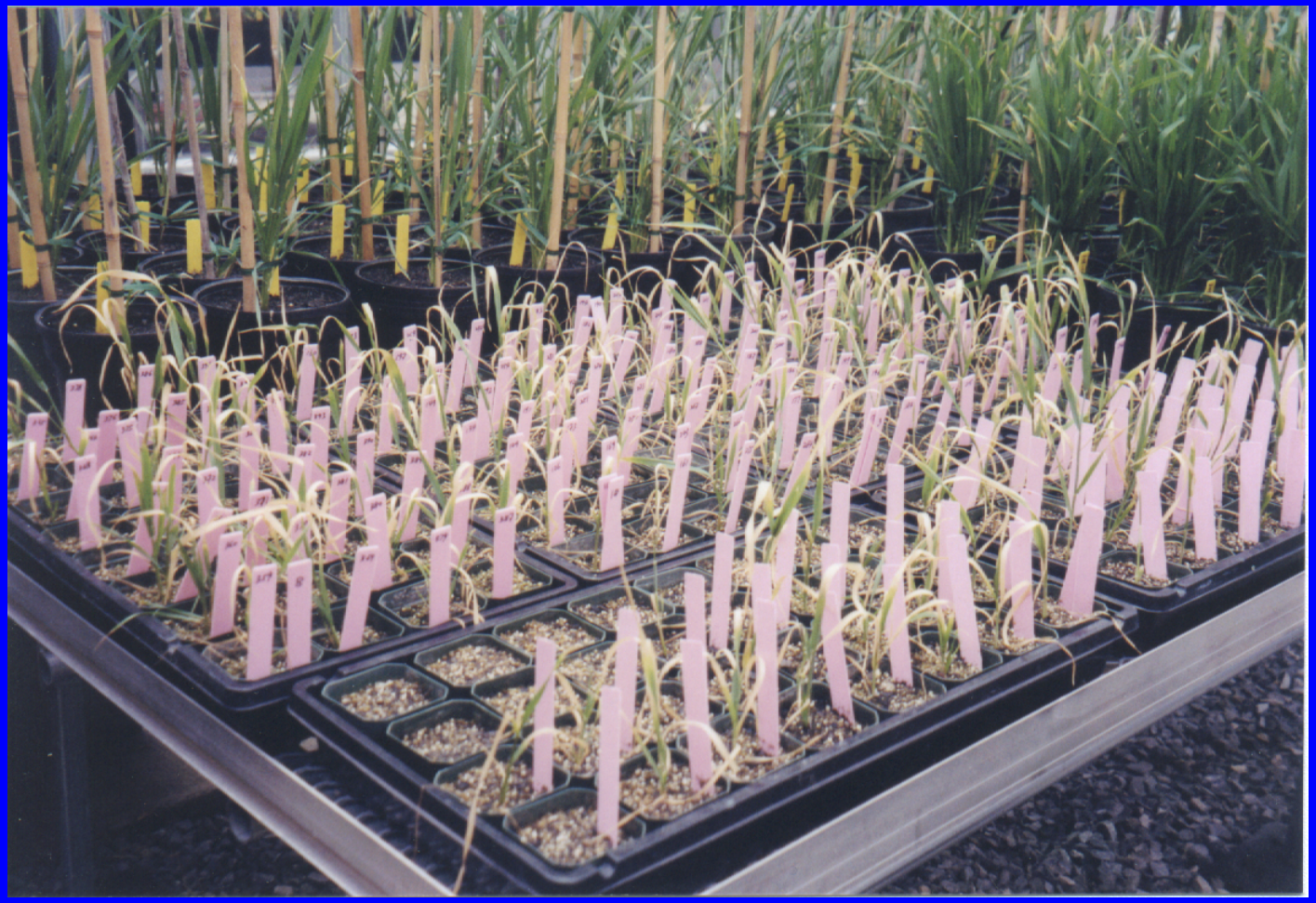
Colchicine Treatment

- 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 Colchcine 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

- F_1 from winter x winter crosses
  - Grow the first set of corn four weeks after winter wheat is put into vernalization chamber
- F_1 from spring x spring or winter x spring crosses
  - Grow the first set of corn two weeks after planting wheat
- Growing conditions: 80-87^{o}F with 16 hrs photo period provided by high intensity lights in greenhouse
- Grow 40 to 60 F_1 seeds to get 200 to 300 doubled fertile haploid (1-2 fertile green plants per emasculated head)

Table 1. Haploid production by wheat x maize hybridization in 12 wheat F1 crosses.

Pedigree	Florets Pollinated A	Seeds Developed B	Embryos Rescued C	Green Plants D	% B/A	% C/B	% D/C	% D/A
MADISON/ERNIE	814	704	174	60	86.49	24.72	34.48	7.37
PION2684/ERNIE	1151	958	238	121	83.23	24.84	50.84	10.51
ROANE/MADISON	1569	1286	291	135	81.96	22.63	46.39	8.6
ROANE/PION2684	1730	1518	304	176	87.75	20.03	57.89	10.17
SHAAN85-2/MADISON	957	788	136	60	82.34	17.26	44.12	6.27
PION2684/SHAAN85-2	1040	717	130	76	68.94	18.13	58.46	7.31
VR95B717/MADISON	1300	1146	138	54	88.15	12.04	39.13	4.15
VR95B717/PION2684	1300	1015	159	77	78.08	15.67	48.43	5.92
MADISON/W14	612	452	48	12	73.86	10.62	25.00	1.96
PION2684/W14	729	587	140	61	80.52	23.85	43.57	8.37
ROANE /W14	616	502	109	63	81.49	21.71	57.80	10.23
FREEDOM / PION 2684	1709	1534	387	126	89.76	25.23	32.56	7.37
Total	13527	11207	2254	1021				
Mean					82.87	20.13	45.21	7.54
Crosses with PION2684	5950	4795	971	511	**79.50**	**20.50**	**51.84**	**8.46**
Crosses with MADISON	5252	4376	787	321	**82.56**	**17.45**	**37.82**	**5.67**
DIFFERENCES					**-3.06**	**3.05**	**14.02**	**2.79**
C.V. (%)					7.93	22.76	5.84	21.71
HERITABILITY					0	0.05	0.93	0.59
LSD (0.05)					8.67	5.82	3.53	2.07

Table 2. Analysis of variance for frequency of embryo germination and green plants regeneration.

Comparison	df	Mean Square	
		D/C	D/A
Between crosses with PION 2684 and Madison	1	490.980***	19.404*
Within crosses with either PION 2684 or Madison	4	106.088**	8.352
Error	4	6.864	2.352
Total	9		

* 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 F_1 crosses conducted in 1999 and 2000*, Blacksburg, Virginia.

Pedigree	Seeds Excised (No.)		Embryos Rescued (No.)		Haploid Obtained (No.)		Embryo Formation (%)		Haploid Regeneration (%)	
	1999	2000	1999	2000	1999	2000	1999	2000	1999	2000
Madison x W14	452	2202	48	320	12	144	11	15	25	45
Pioneer 2684 x W14	587	1473	140	258	61	144	24	18	44	56
Total	1049	3675	188	578	73	288				
Average							17.5	16.5	34.5	50.5

* 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