Development & Evaluation of FHB Resistant Near-isogenic Lines Using Marker-Assisted Selection

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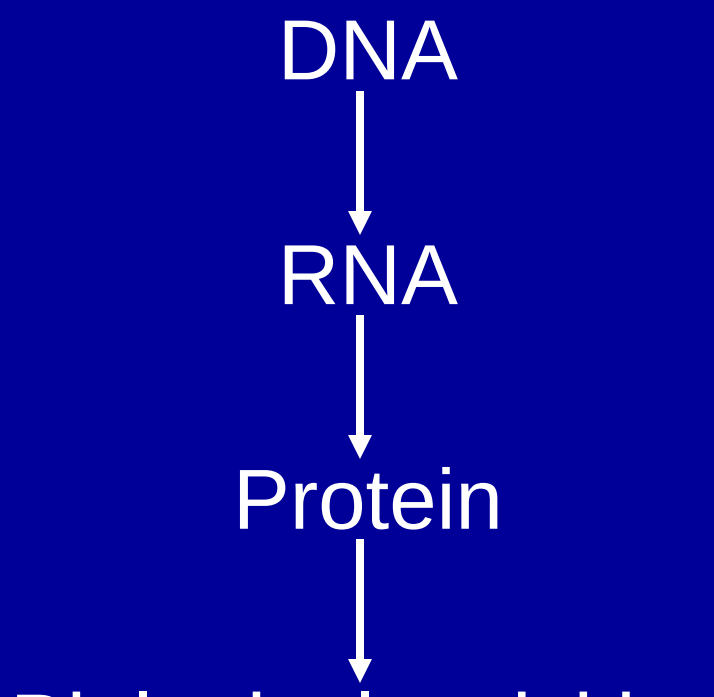
Outlines of This Lecture

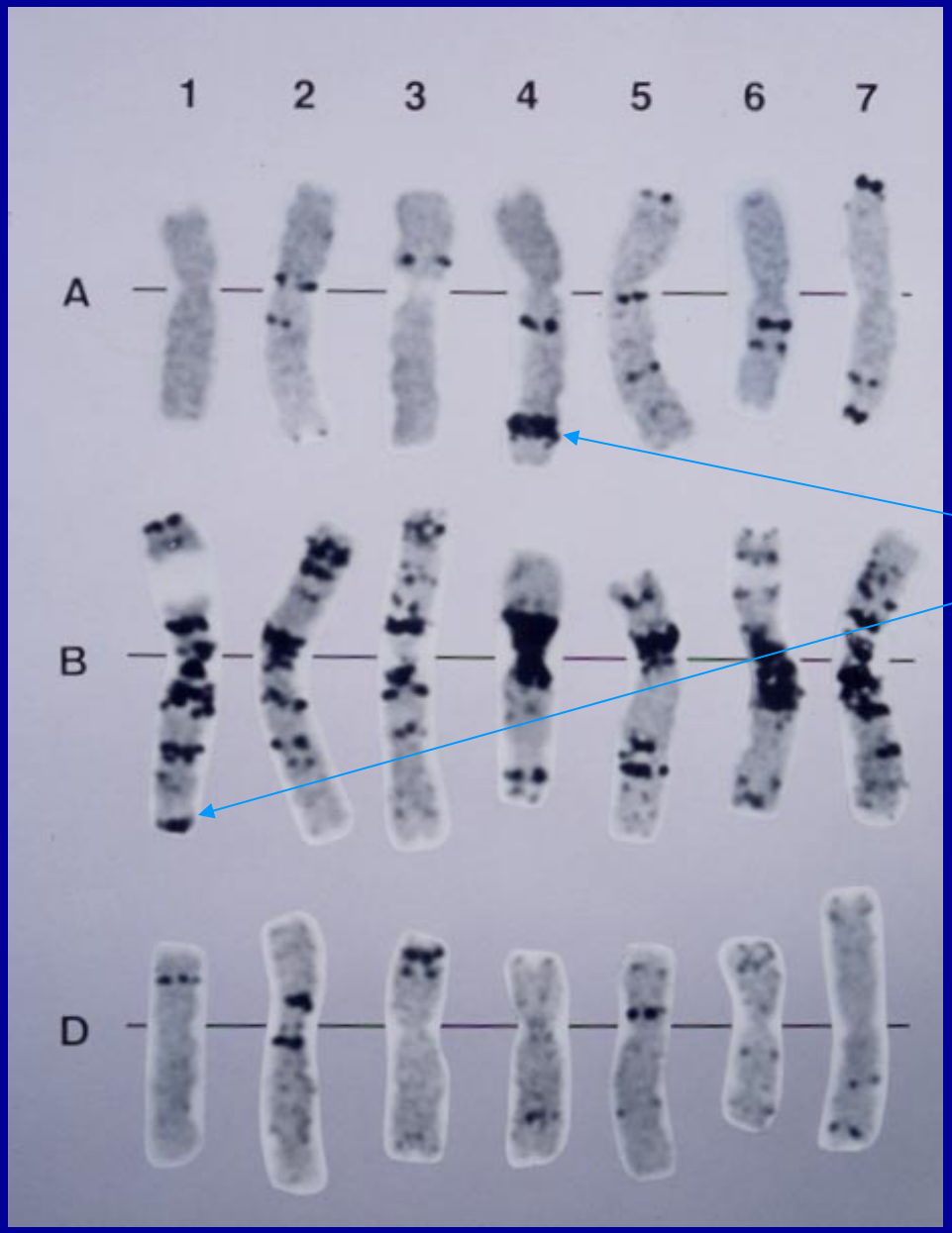
- Markers used and being used in plant breeding
- Rationale for using DNA markers in plant breeding
- Benefits by using DNA markers on selection of Fusarium head blight resistance and development of near-isogenic lines

Information flow



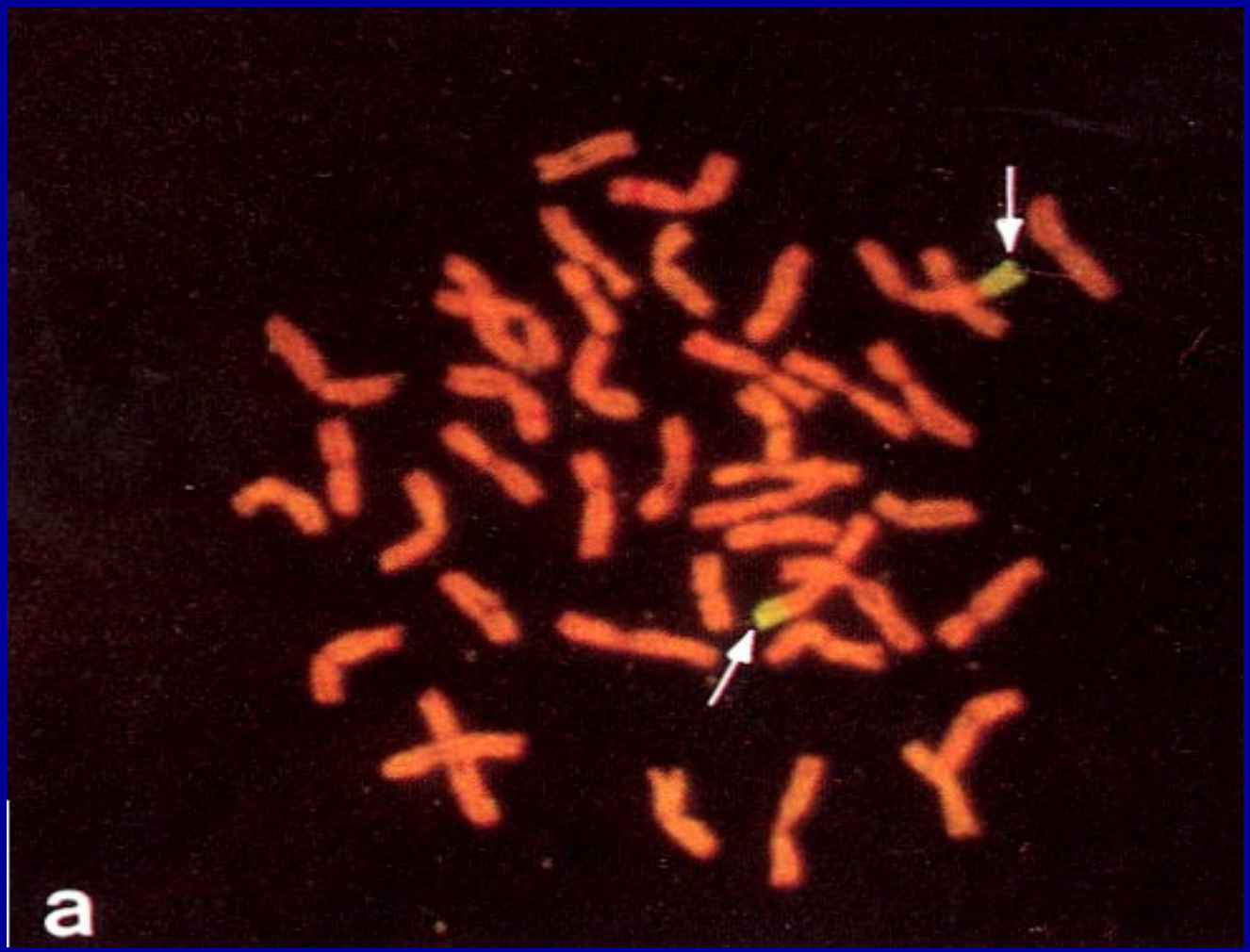
Markers used in selection

- Morphological markers
  - Association between seed size and a seed coat color marker in beans (Sax, 1923)
  - Association between flowering time and flower color in peas (Rasmusson, 1935)
- Chromosome markers
  - C-banding
- Isozyme markers
- DNA markers



Chromosome Markers

Chromosome Markers



Limitations of previous marker system

- Limited number of markers available
- Undesirable effects on phenotype of many of morphological markers

Advantages for using DNA markers

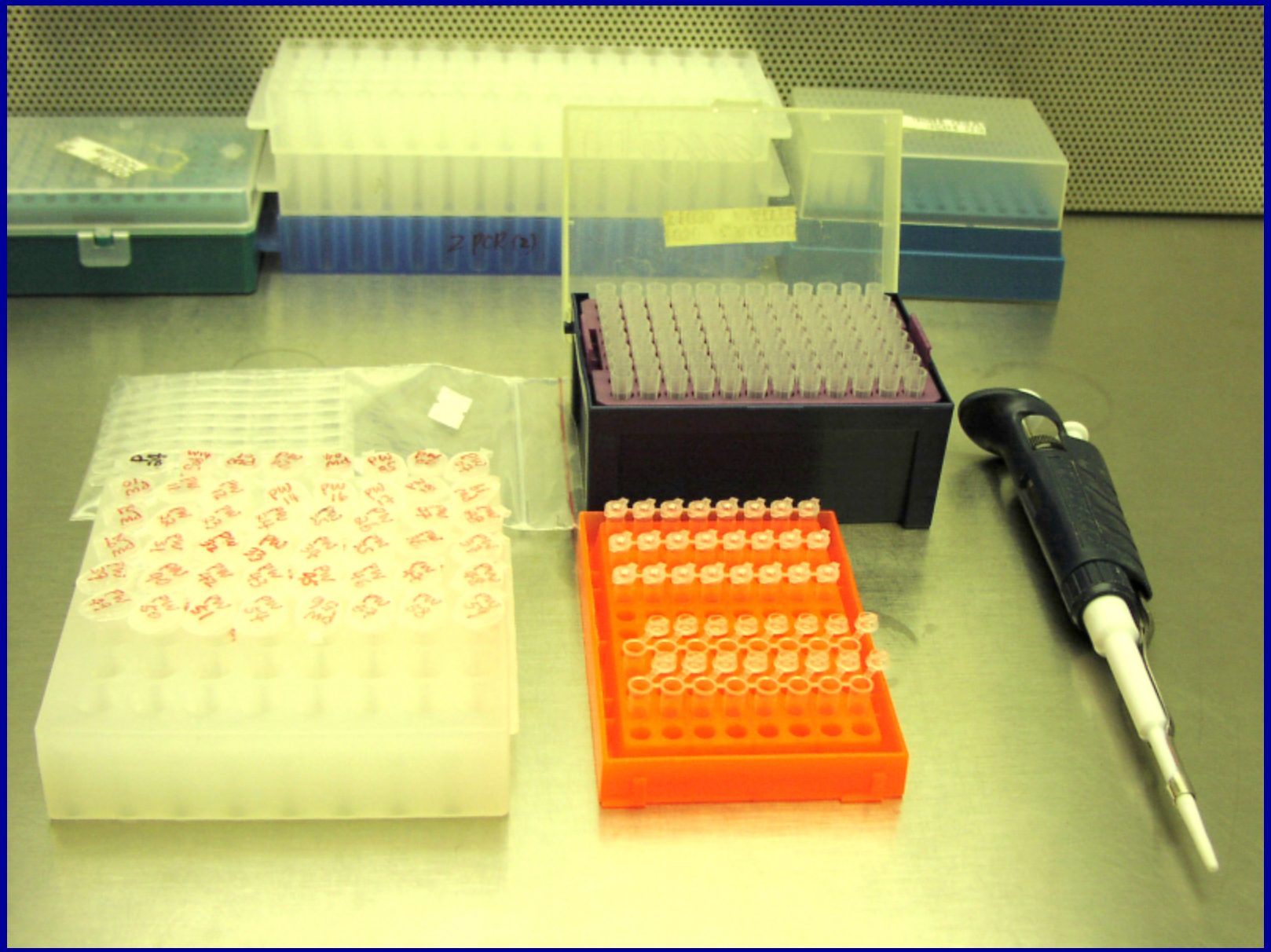
- No phenotype effect
- Well developed markers systems, such as RFLP, RAPD, SSR, AFLP, SNP, etc
- PCR-based marker systems have potential to automation and applied in conventional breeding

What does PCR-based Marker

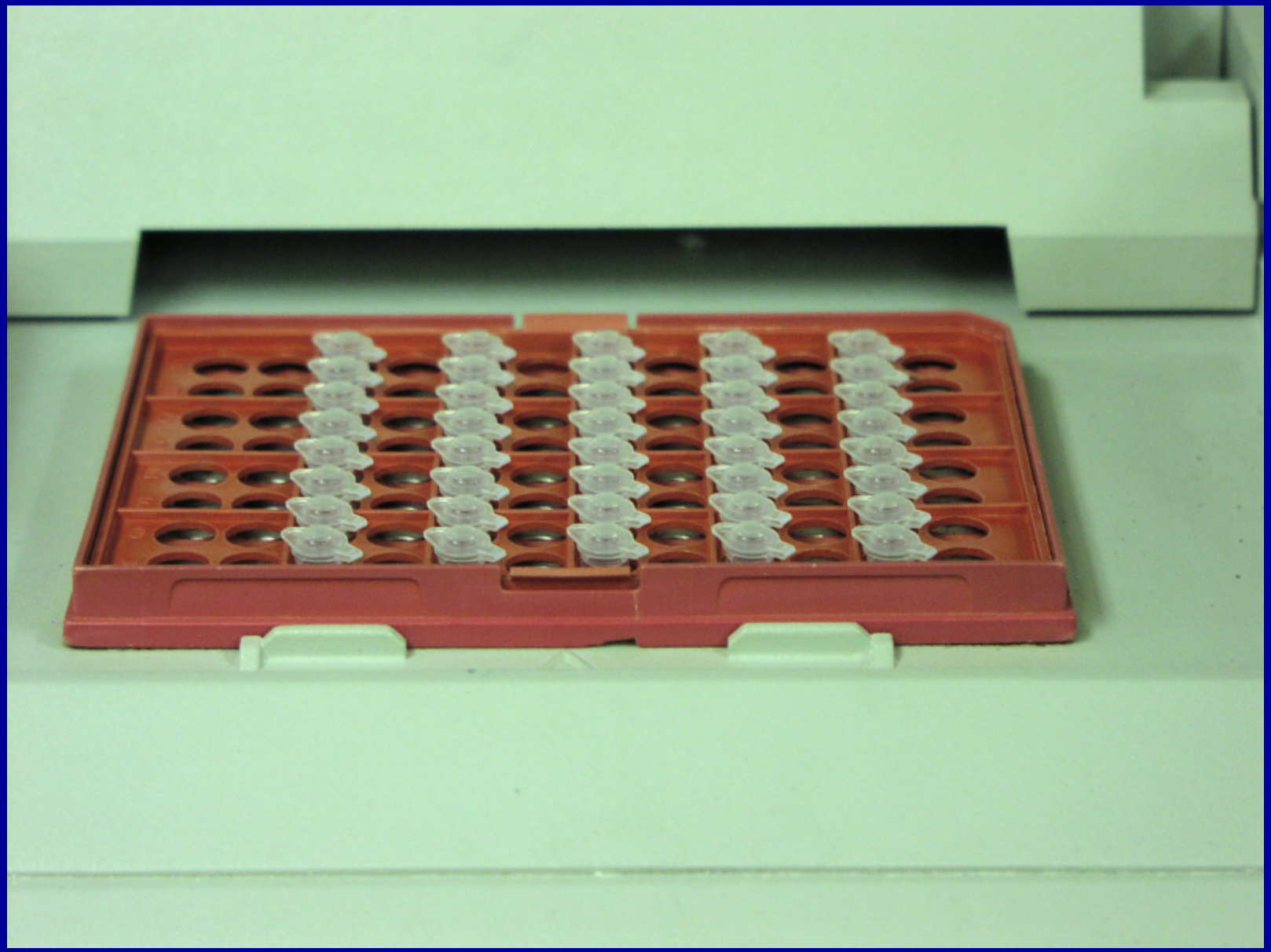
- Less DNA needed
- Easy to assess and automation

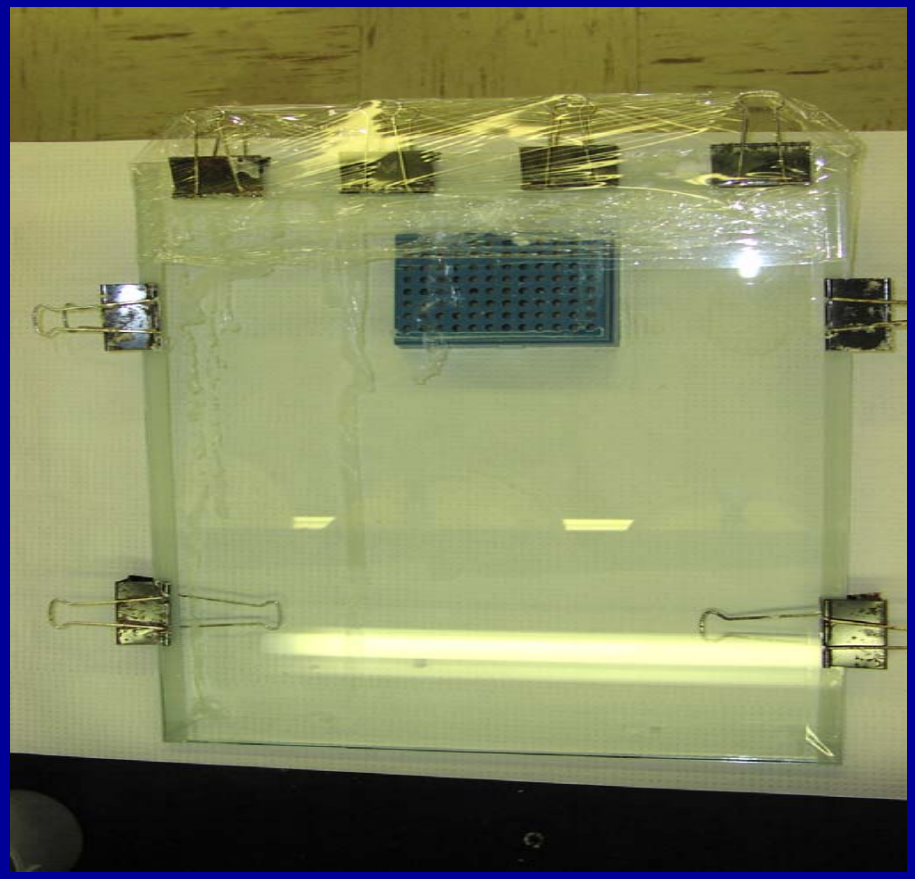

FOR RESEARCH USE ONLY
STOP
GeneAmp®
PCR System 9700
ENTER
CE
VPI & SU INVENTORY
PE Applied Biosystems

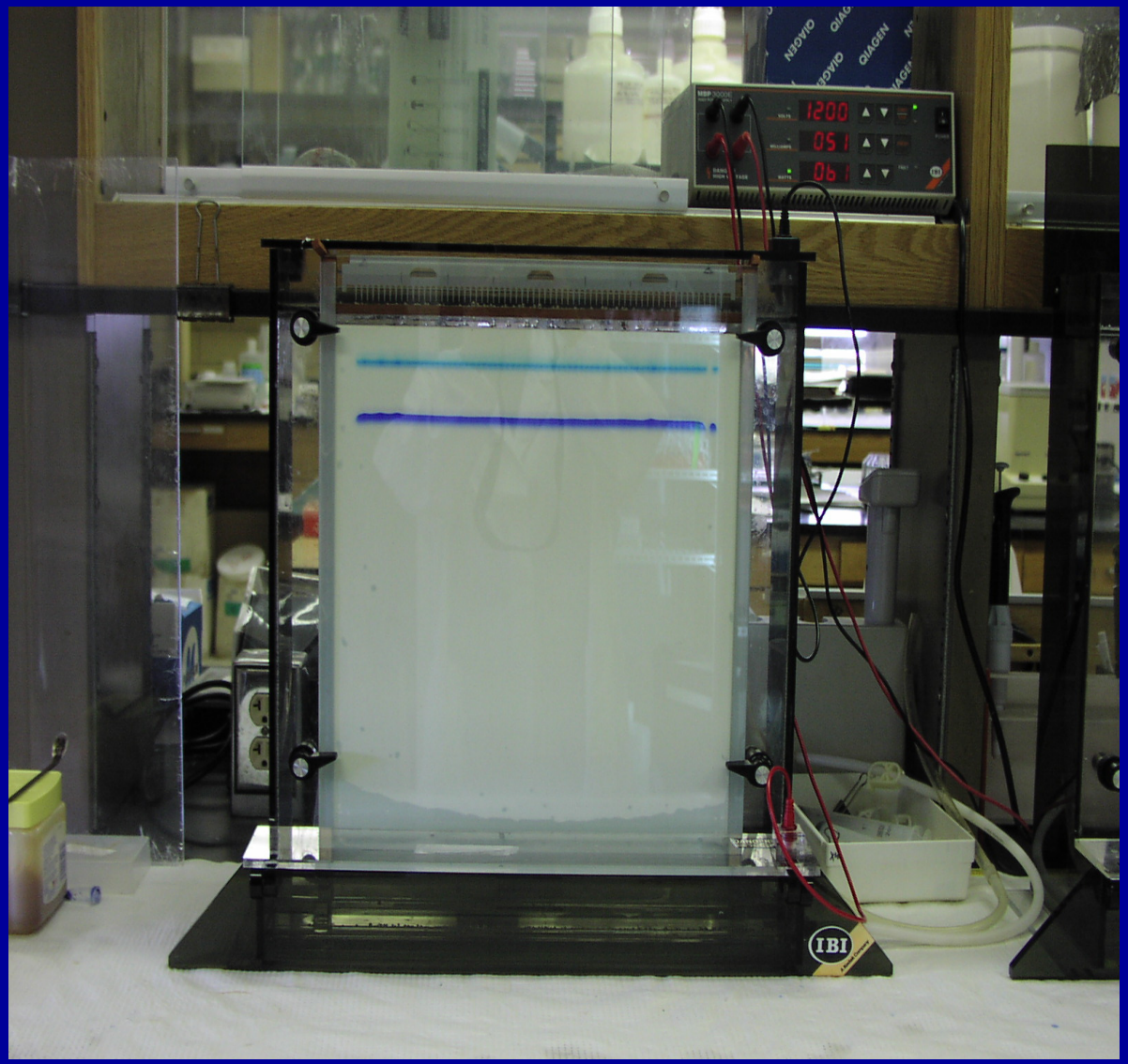
PCR Automation




Property of Virginia Tech
Sample 94.0
94.0 94.0
2:13 0:30 47.0 68.0 68.0
0:30 1:00 7:00 4.0
∞
Pause
Info
F1
F2
F3
F4
F5
POWER






1200
051
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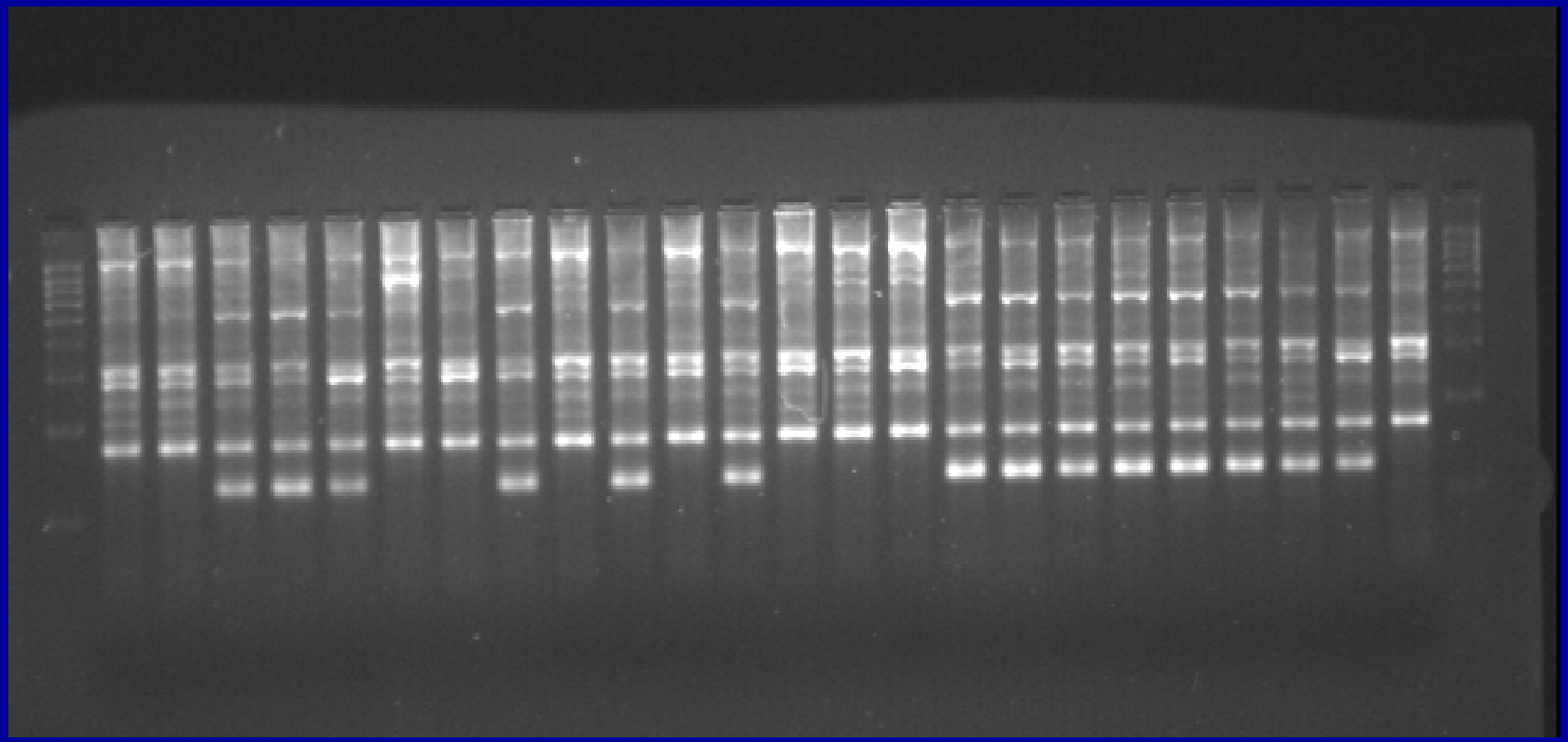
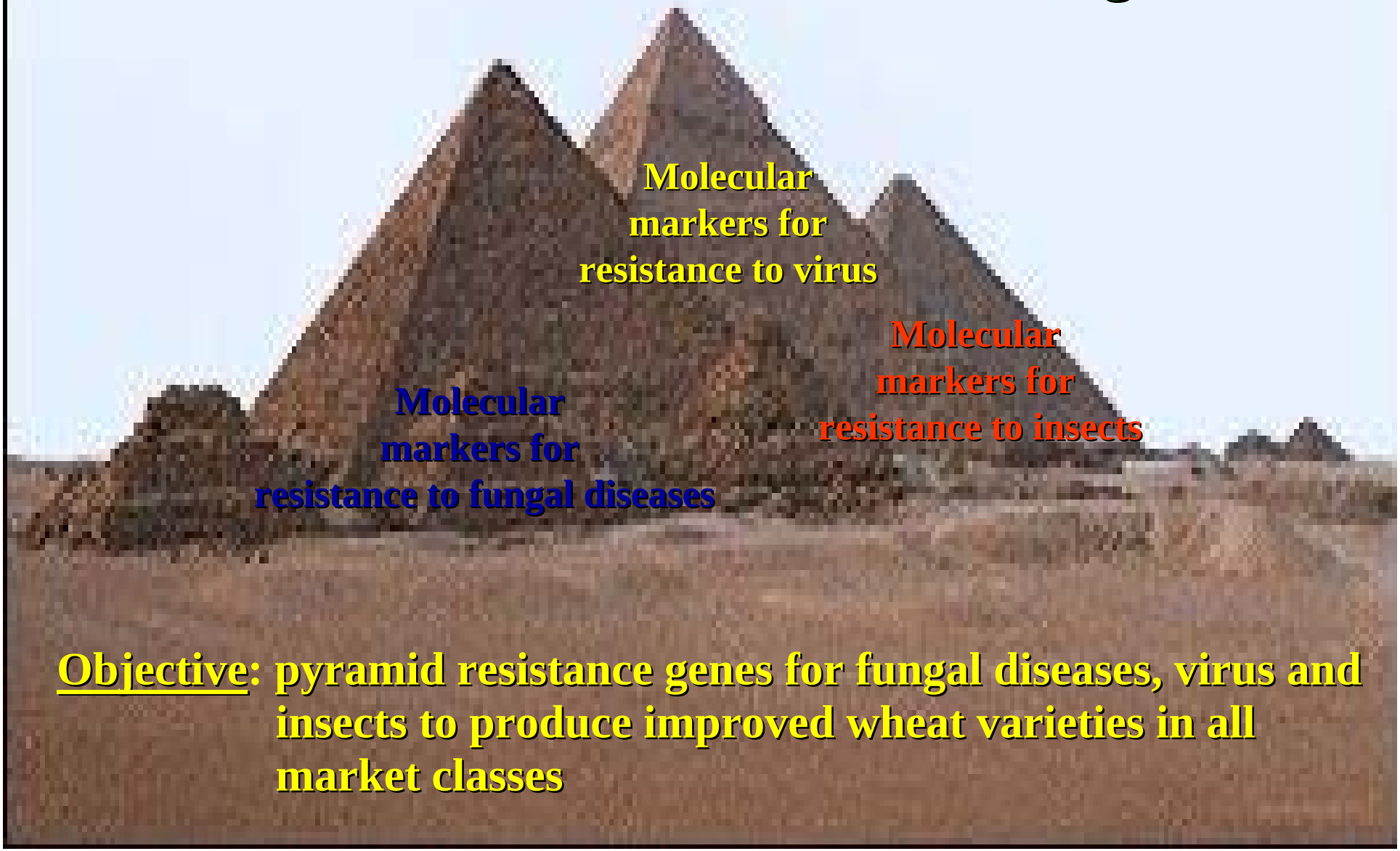
SSR Markers: BARC 55



Applications of DNA Markers in Plant Breeding

- Measure genetic diversity among lines
- Fingerprint lines for legal purposes
- Control genetic purity in the seed production process
- Marker-assisted selection

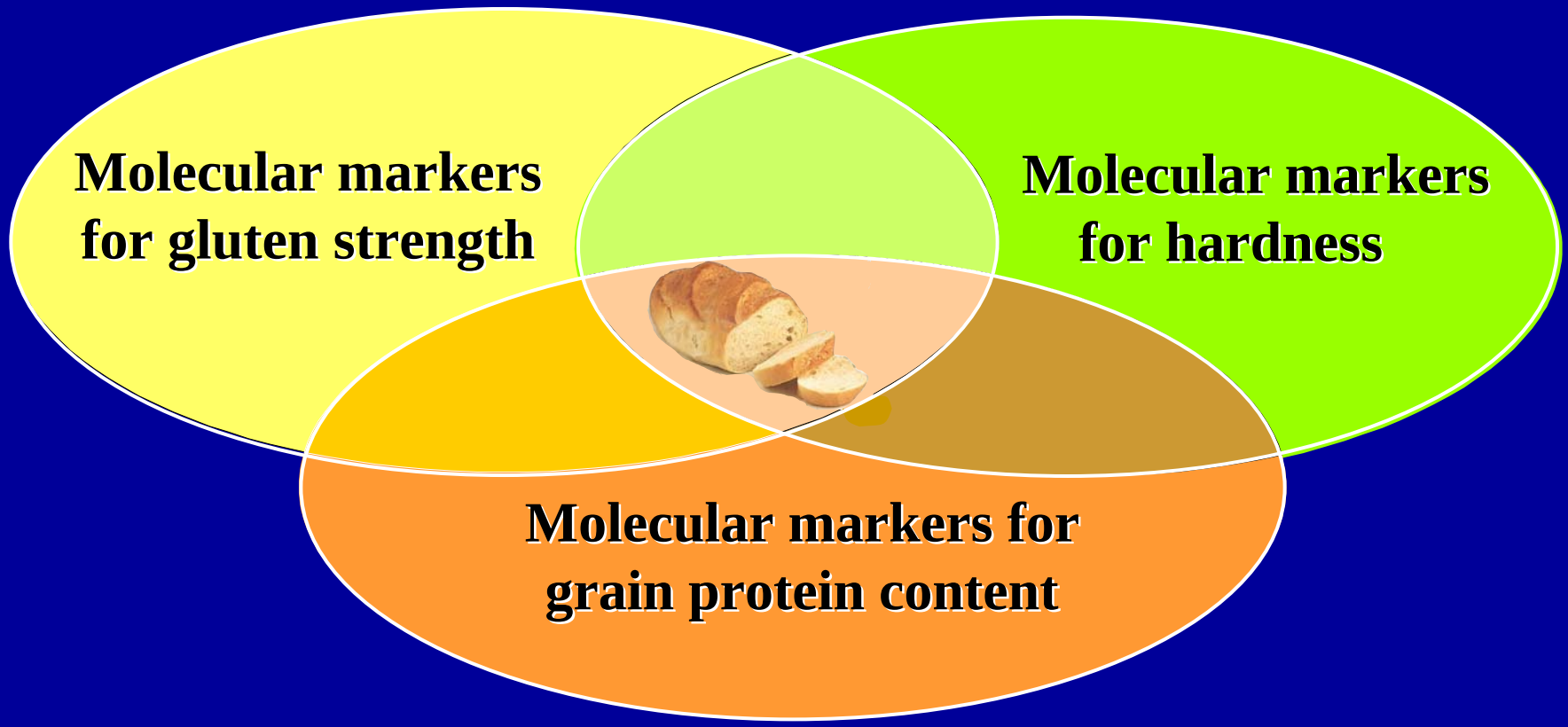
Molecular markers for resistance genes



Objective: pyramid resistance genes for fungal diseases, virus and insects to produce improved wheat varieties in all market classes

Molecular markers for breadmaking quality

Objective: provide an integrated manipulation of breadmaking characteristics using molecular markers



Selected genes

Quality

- **Grain protein content** *QTL.ndsu.6BS*
- **Starch quality** *Waxy mutants*
- **Gluten strength** *Glu-B3, GluA1,B1, D1*
- **Pentosan content**
- **Hardness** *Pinb-D1b, Pinb-A^m1*
- **Pre-harvest sprouting** *QTL.1, 2, 3, 4*
- **Semolina color** *Yp*

Resistance

- **Leaf rust** *Lr37, Lr47, Lr21, Lr39*
- **Stripe Rust** *Yr15, Yr17, Yr5,YrHTAP*
- **Fusarium head blight** *QTLfhs.ndsu.3B, QTL3A*
- **Eyespot** *Pch1, Pch2*
- **BYD Virus** *Bdv2*
- **WSM Virus** *Wsm1*
- **WSSM Virus** *Wss1*
- **Hessian Fly** *H9, H13*
- **Russian Wheat Aphid** *Dn2, Dn4*

Backcrossing Marker Assisted Selection

% of adapted genotype	Procedure
0% +	Adapted Parent X Gene Donor Gene — No selection — Gene
50% +	Adapted Parent X F1 hybrid Gene — No selection — Gene
75% +	Adapted Parent X Back Cross 1 Gene — Select
87% +	Adapted Parent X Back Cross 2 Gene — Select
94% +	Adapted Parent X Back Cross 3 Gene — Select
97% +	Adapted Parent X Back Cross 4 Gene — Select
98%+	Adapted Parent X Back Cross 5 Gene — Select
99%+	Back Cross 6 Self pollination Gene — Select
99%+	

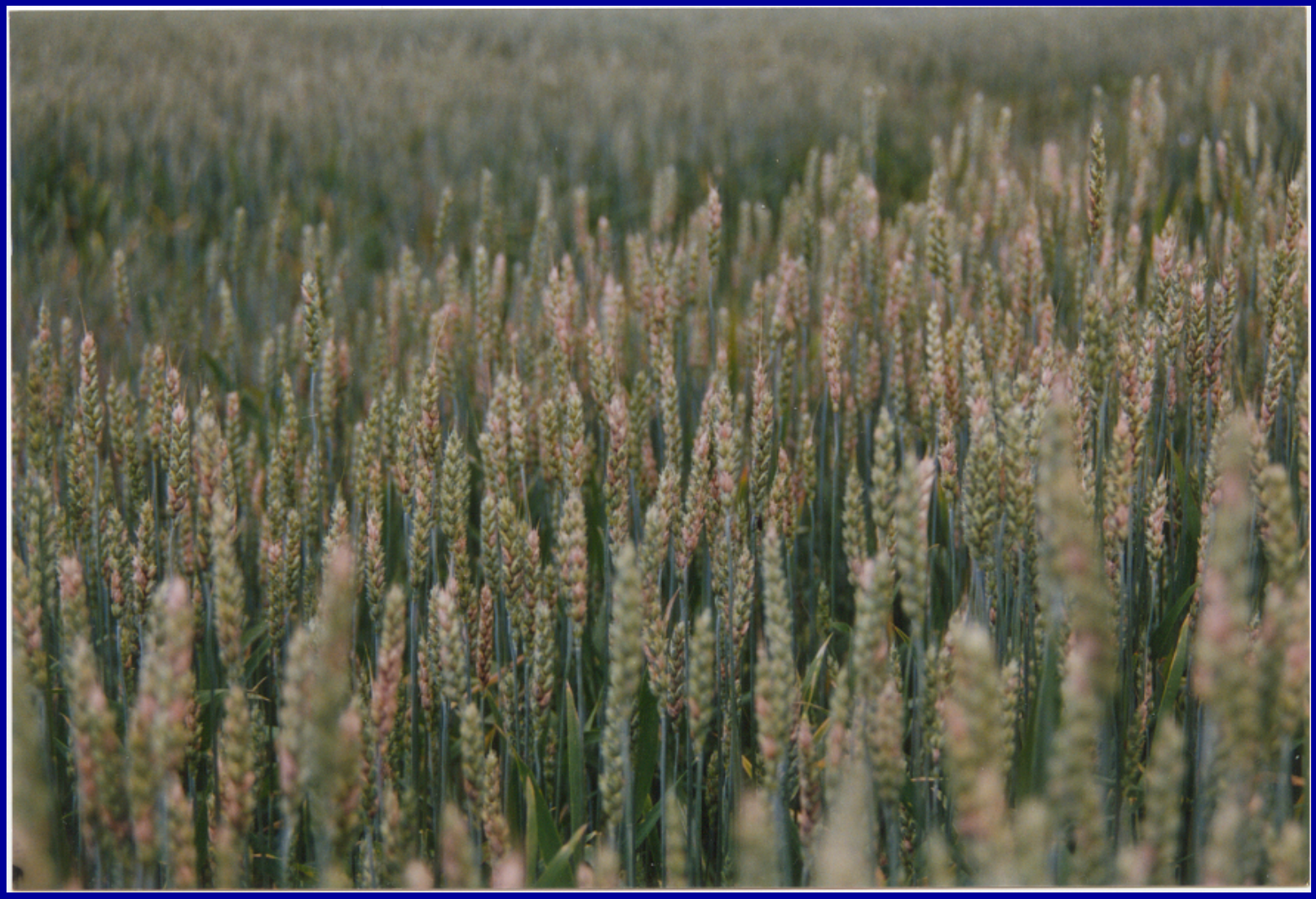
Practical Considerations in MAS

- Choice of molecular markers
  - DNA quality and quantity required
  - Degree of polymorphism
  - Reliability
  - Difficulty of assay
- The value, ease and the cost of measurement, and nature of genetic control of your gene determine the way in which molecular markers may be the most effectively used in a breeding program
- Availability of genes fine-mapped (minimized C.O. BTW marker alleles and YFG)

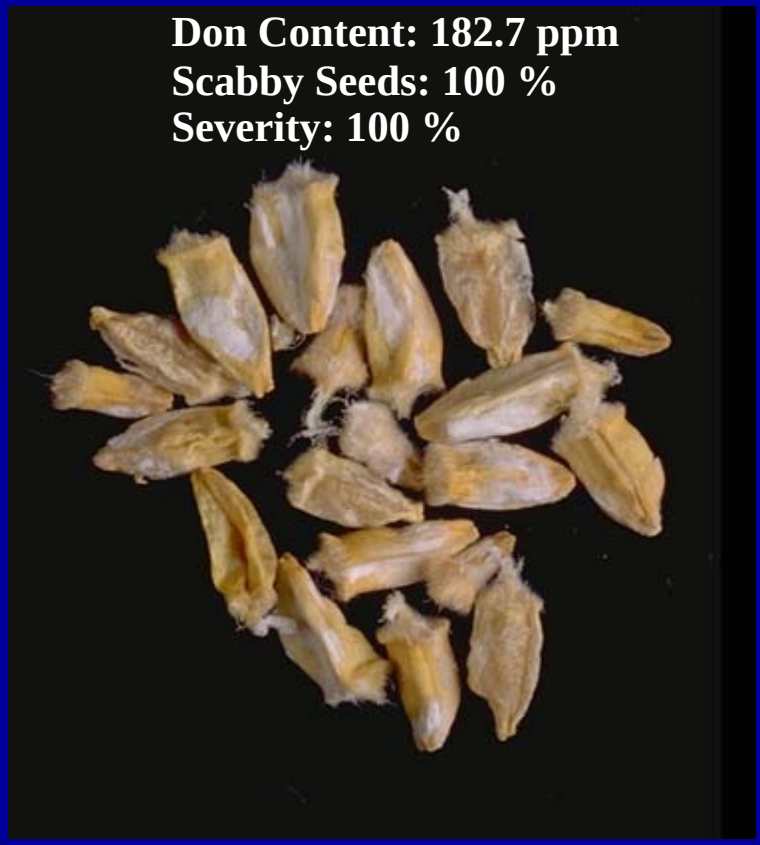
QTL Mapping of FHB Resistance

Backcrossing Marker Assisted Selection

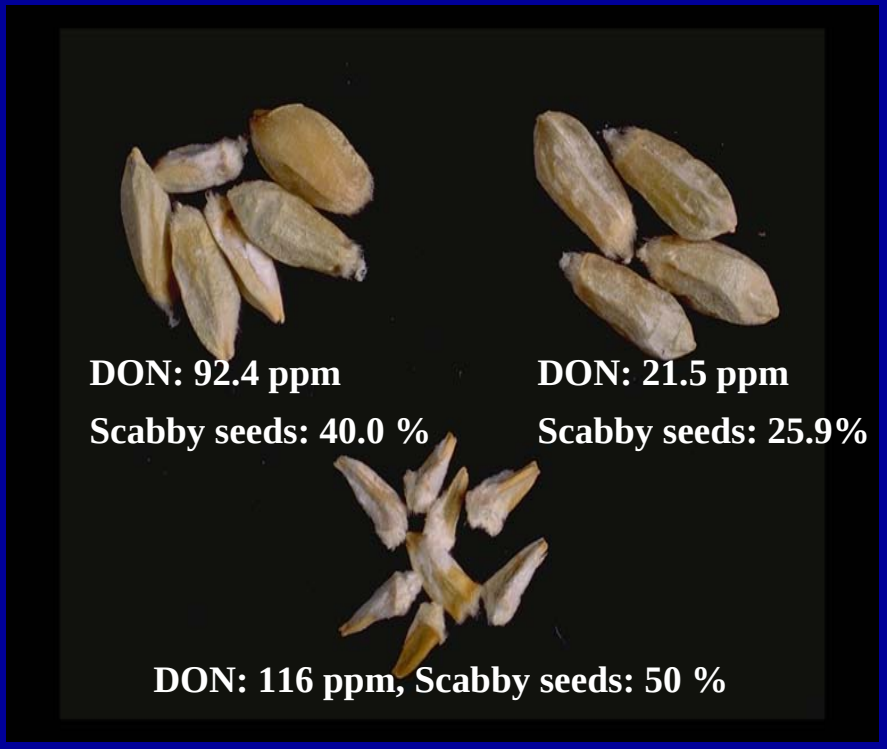
Infection Occurred in Field Nursery



Individuals Susceptible to Type II, III and IV Resistance



Moderately Resistant to Susceptible Individuals with Varied Scabby Seeds and Varied DON Content

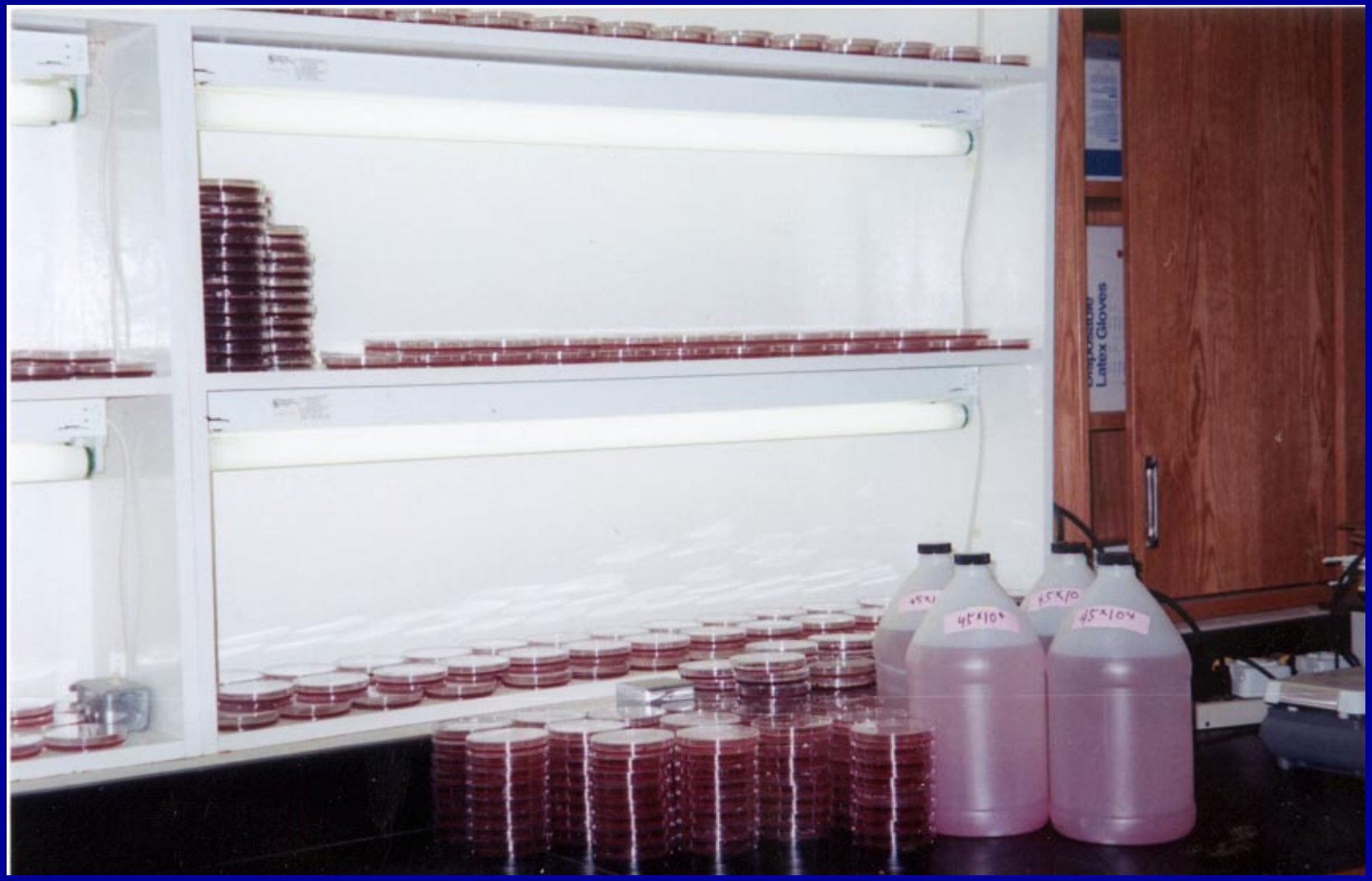


Field Inoculums Production: Mason Jar Method

Scab Research in Field Nurseries

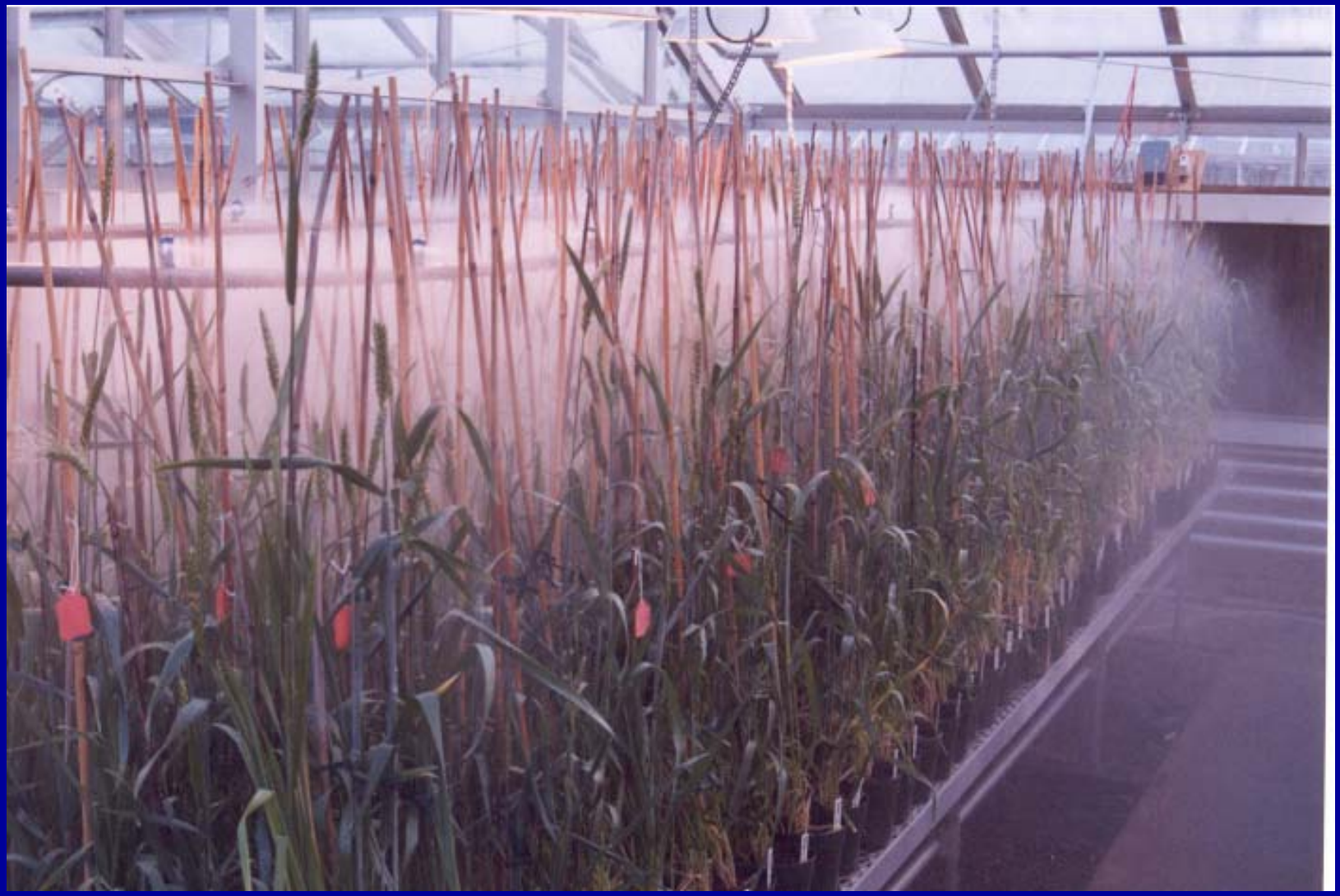
Early Generation Selection for Scab Resistance

Coniadial Production: Petridish Method



Tools for Greenhouse Inoculation

Screening for Type II Scab Resistance



Chromosome Locations

Aneuploid Analysis		Marker Analysis	
Sumai 3	Other	Sumai 3	Other
1B(2),	1B(3), 1D(2)		
2A(2), 2B(1)	2A(1), 2B(3)	2AL	2AS
3B(1), 3D(1)	3A(2), 3B(2), 3D(3)	3AL, 3BS	3AL, 3BS
	4A(1), 4B(2), 4D(2)	4BS	
5A(2)	5A(4), 5B(3), 5D(3)		5AL
6B(1), 6D(2)	6A(1), 6B(5), 6D(1)	6AS	6BS
7A(1), 7D(2)	7A(5), 7B(2), 7D(1)		7BS

Update on Scab QTL mapping

- QTL on 3BS is available.
- QTL on 5AL is being saturated.
- Other minor QTLs are being identified.
- Does interactions exist among these QTLs???

Steps in MAS

- Identify associations between marker alleles and your favor gene
- Use these associations to develop improved lines or populations

Mapping Populations

- Segregation populations, such as F2 & F2:3
- Recombination inbred lines
- Doubled haploid lines
- Near-isogenic Lines

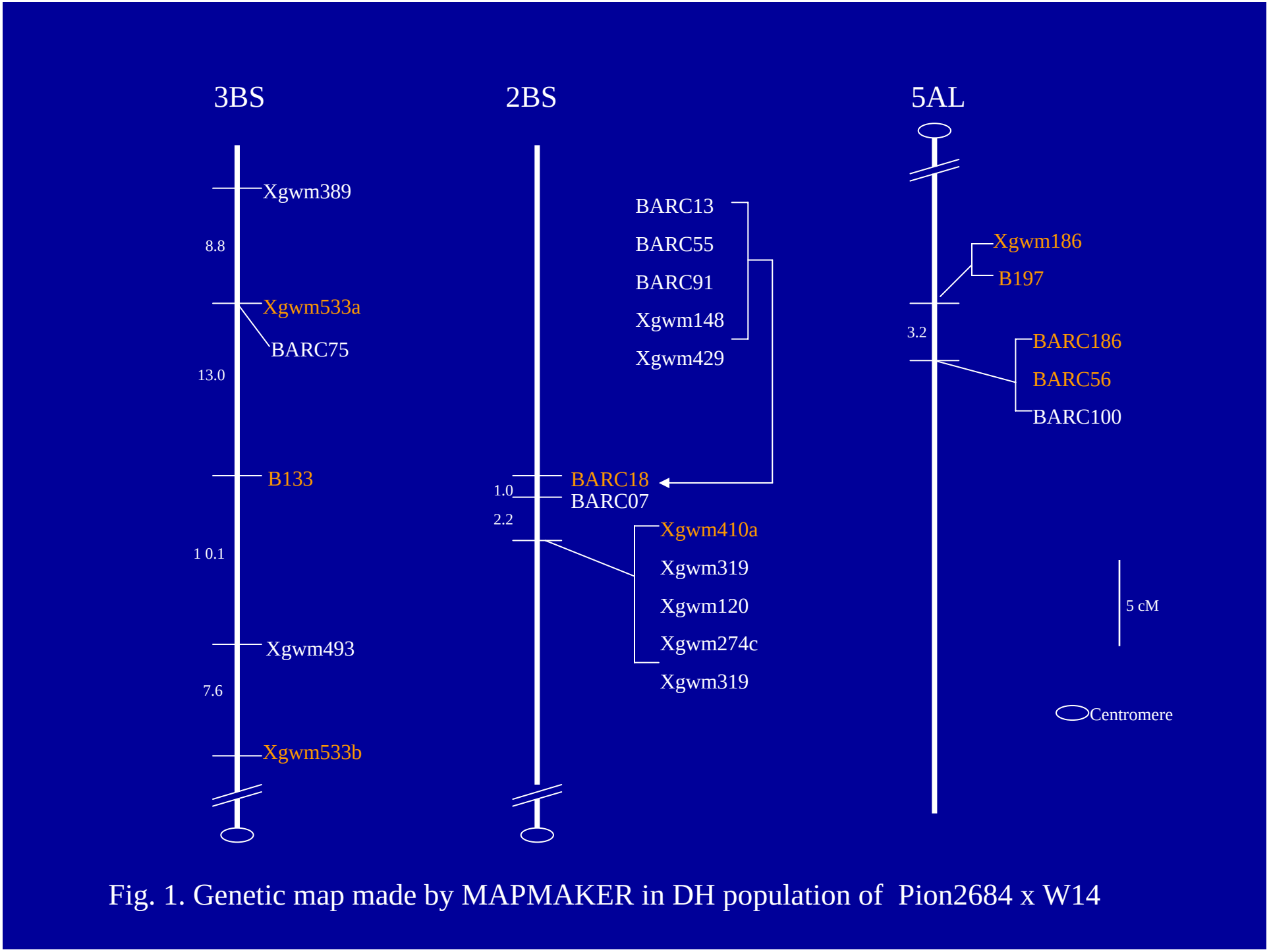
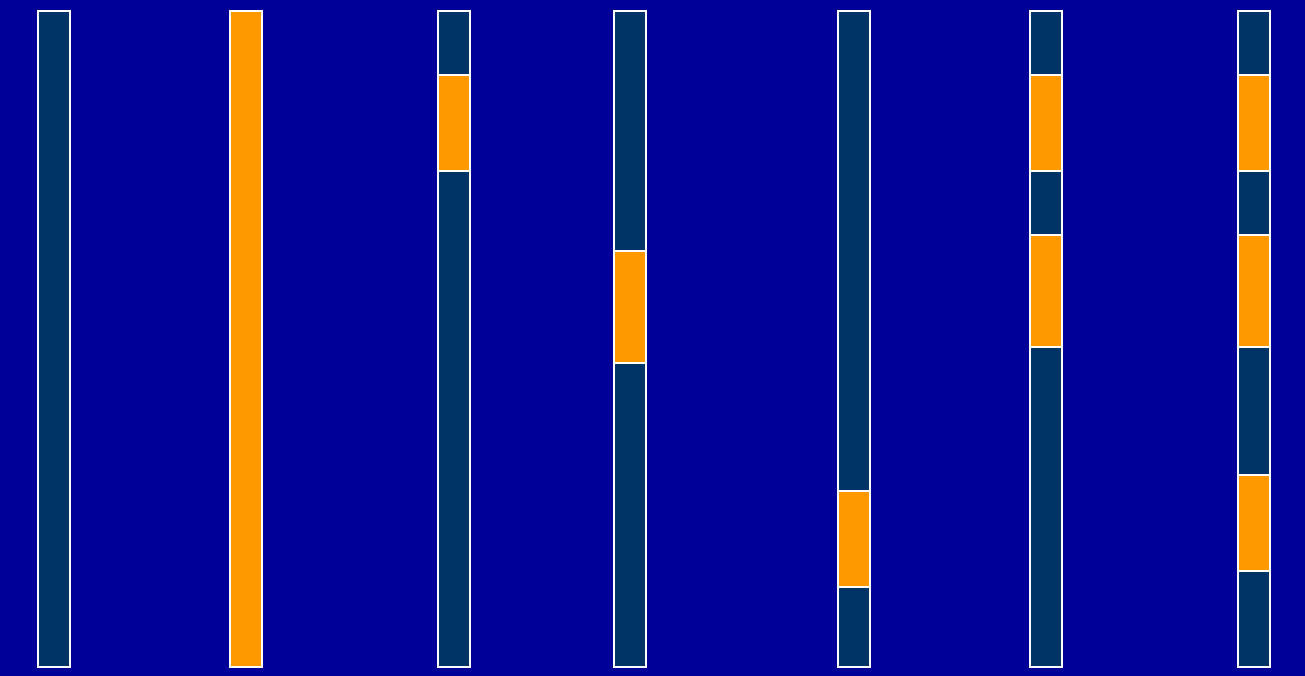


Fig. 1. Genetic map made by MAPMAKER in DH population of Pion2684 x W14

Development of Near Isogenic Lines for 3BS QTL in W14



Backcrossing marker assisted selection

Traits controlled by small numbers of QTL

- MAS can be used to monitor the incorporation of the desirable alleles from the donor source,
- MAS of the recurrent parent can speed the recovery of the recurrent parent genotype

Traits controlled by large numbers of QTL, some with small effect, the probability of identifying marker loci linked to all the QTL is low. When selection with marker loci will be effective? Is selection index helpful?

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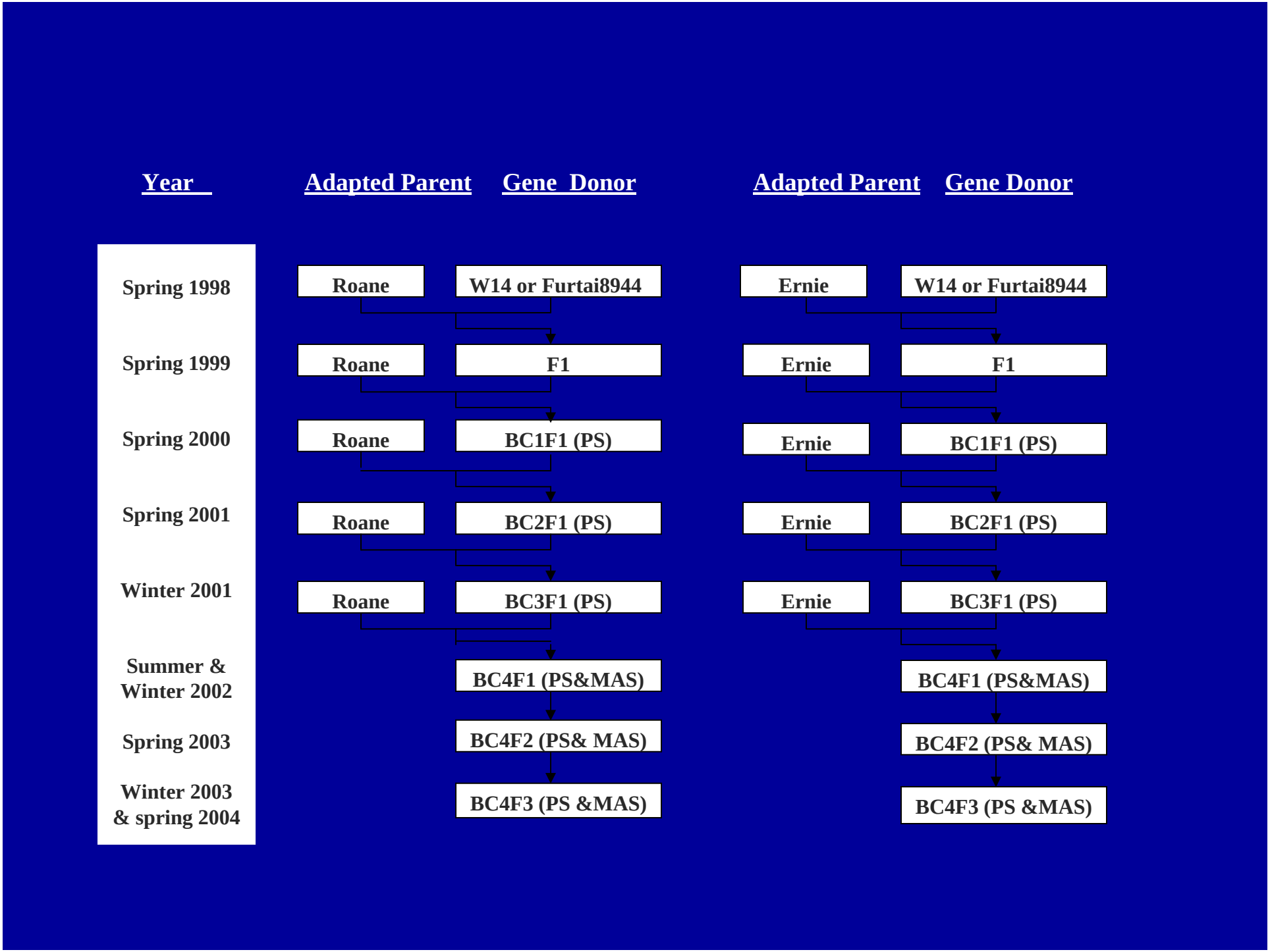
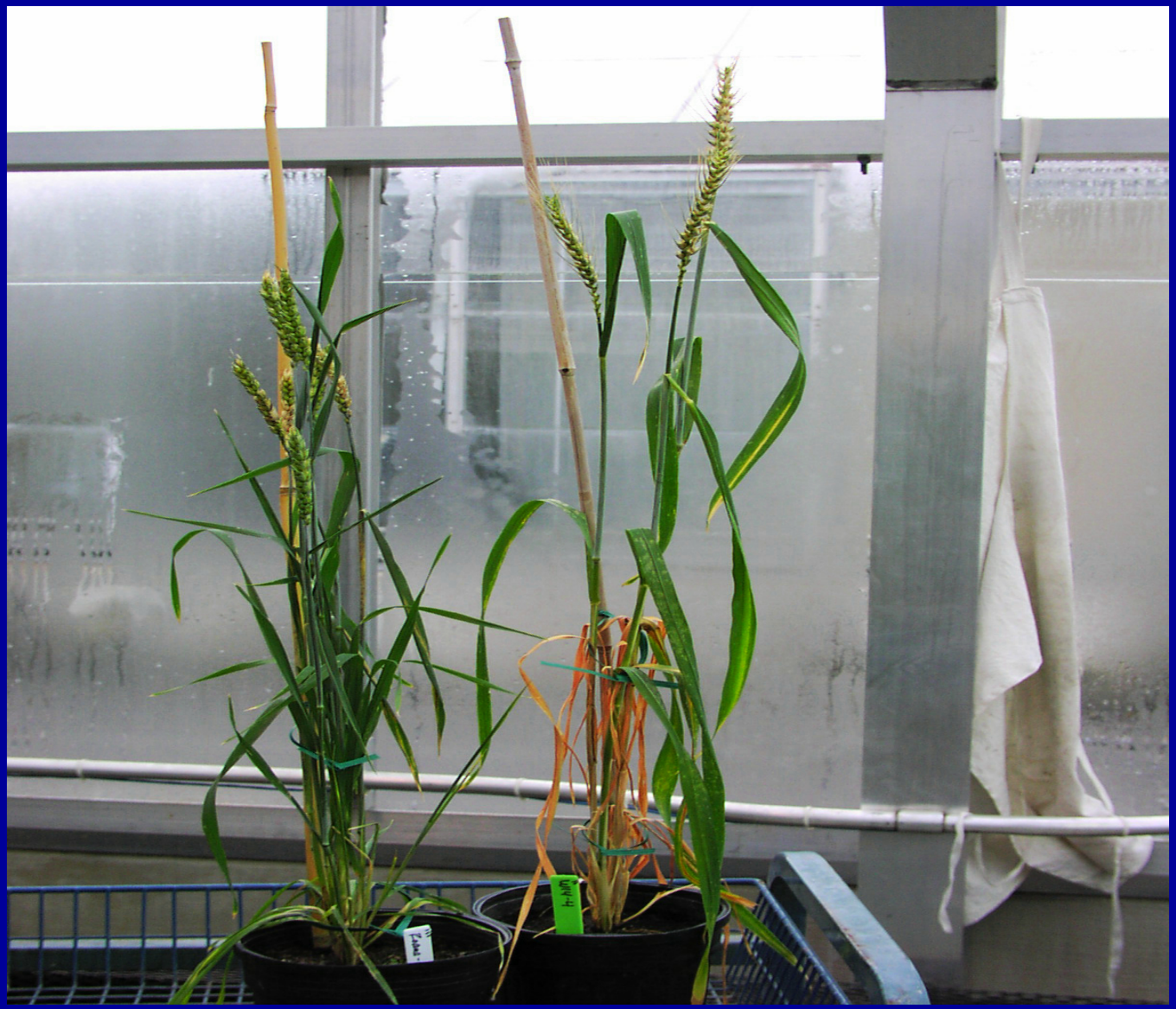

Year
Adapted Parent
Gene Donor
Adapted Parent
Gene Donor
Spring 1998
Roane
W14 or Furtai8944
Ernie
W14 or Furtai8944
Spring 1999
Roane
F1
Ernie
F1
Spring 2000
Roane
BC1F1 (PS)
Ernie
BC1F1 (PS)
Spring 2001
Roane
BC2F1 (PS)
Ernie
BC2F1 (PS)
Winter 2001
Roane
BC3F1 (PS)
Ernie
BC3F1 (PS)
Summer & Winter 2002
BC4F1 (PS&MAS)
BC4F1 (PS&MAS)
Spring 2003
BC4F2 (PS& MAS)
BC4F2 (PS& MAS)
Winter 2003 & spring 2004
BC4F3 (PS &MAS)
BC4F3 (PS &MAS)



Roane

3007-8-5-4

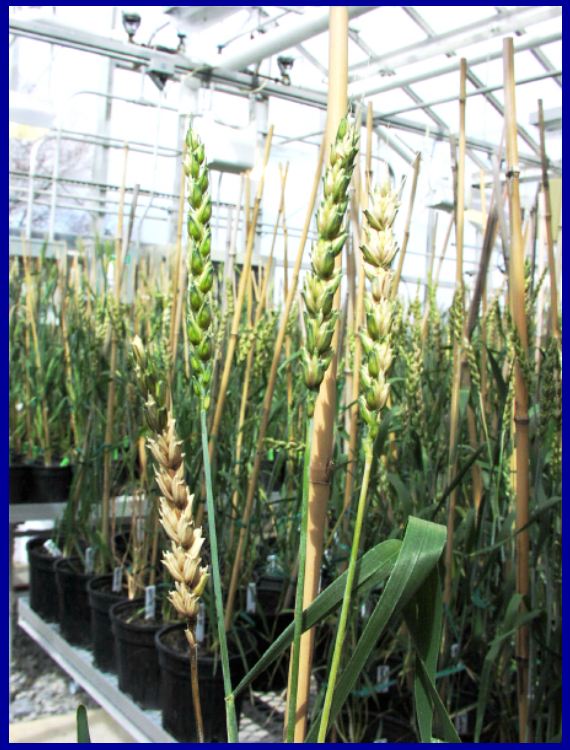
W14

Roane

3008-8-5-5

W14

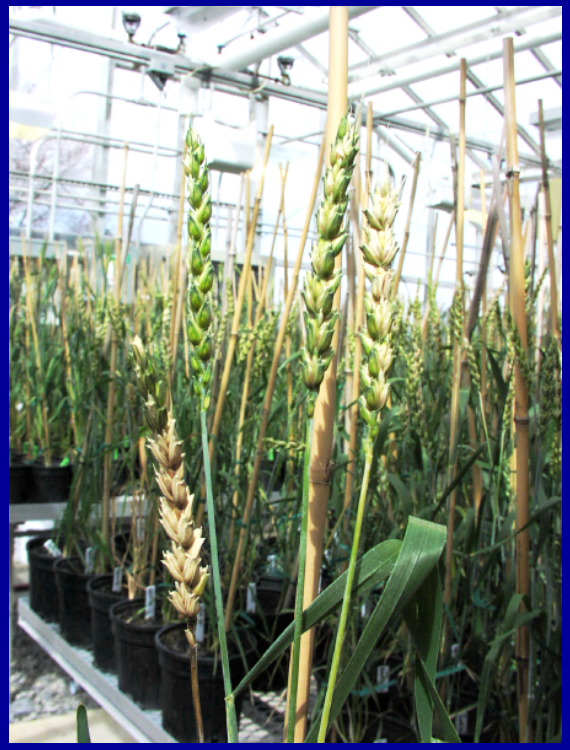
Ernie



5012-10-2-3

W14

Ernie



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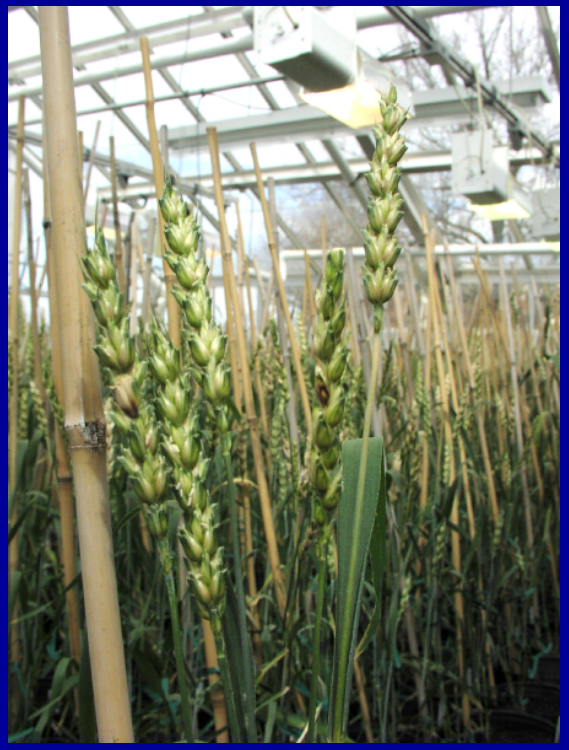

W14

Thank You !!!