Use of Doubled Haploids in the Context of Genetic Resource Exploitation in Maize

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http://www.plantbreeding.iastate.edu/DHF/DHF.htm

Haploids are an effective tool to eliminate recessive genes leading to lethality or sub-vitality. The main application of (doubled) haploids (DHs) is production of completely homozygous and homogeneous lines in short time. Potentially masking genetic variation within lines or families is completely eliminated within DH lines. The usefulness of DH lines is significantly increased over $F_{2:3}$ or $F_{3:4}$ families, due to a significantly enhanced genetic variation among DH lines. Finally, substantial progress has been made in development in novel inducers and for understanding the genetics of haploid induction and response of donor plants to the induction process. The objective of this presentation is to study and discuss possibilities for using DH technology to support the germplasm enhancement in maize (GEM) allelic diversity project (http://www.public.iastate.edu/~usda-gem/). The GEM diversity project using DH lines to evaluate exotic introgressions in elite background taps into a wide diversity of maize races including Highland germplasm. No selection is made for agronomic performance in the ongoing GEM diversity project which may be an advantage to capture novel allelic diversity. Currently, 54 races (including 12 Highland races above 2,500 meters elevation) are represented in the DH materials. As a case study, the level of diversity present at the brown-midrib 3 locus, known to affect cell wall lignification, and its implications will be reported in detail.

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- **Status Quo Doubled Haploid (DH) Technology**

- **Challenges Genetic Resources**

  - **Adaptation**
  - **Significant Sequence Variation ?: *Bm3* as case study**
  - **Linkage Drag**

- **Efficient Procedures / Outlook**
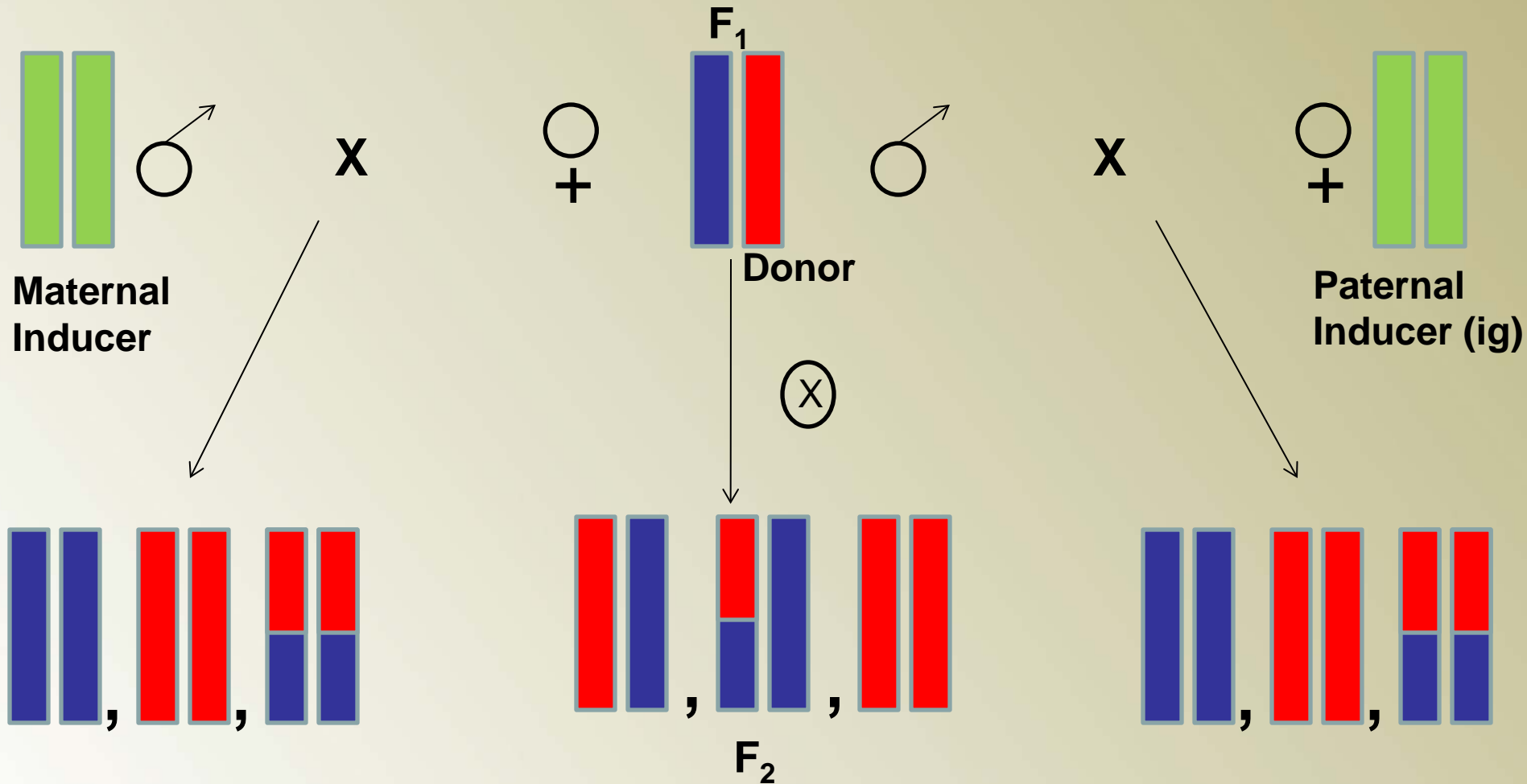
Step	Challenge
Induction cross	**- Inducer availability** **- Induction rate**
Haploid (embryo) selection	**- Visual scoring** **- Background effects** **- Inducer DNA introgression ?**
Genome doubling	**- Colchicine:Toxic** **- Alternative procedures** **- Background effects**
Selfing of DHs	**- Chimera -> partial fertility**

← **Donor (F1 plants)**

← **Inducer**

Ames: July 18, 2010

Maternal Inducer Overview (Incomplete)

| Name	Origin	% Haploids	Features
Spontaneous	ISU (Chase 1952)	0,1	
Stock 6	USDA (Coe 1959)	2	R-nj
KEMS, WS14	Russia, France (1980-1990ies)	7	R-nj
RWS, RWK-76, UH400	U. Hohenheim (2005)	8-10	R-nj
CAUHOI, CAU5, CAU019	CAU Beijing (2009)	2-10	R-nj, High Oil (CAUHOI)
PHI1-4	Procera, Romania (2010)	12-14	R-nj, Pl1
BHI series	ISU (2014)	>8	R-nj, Pl1, other Midwest adapted, Popcorn

Color: R-nj; Red roots

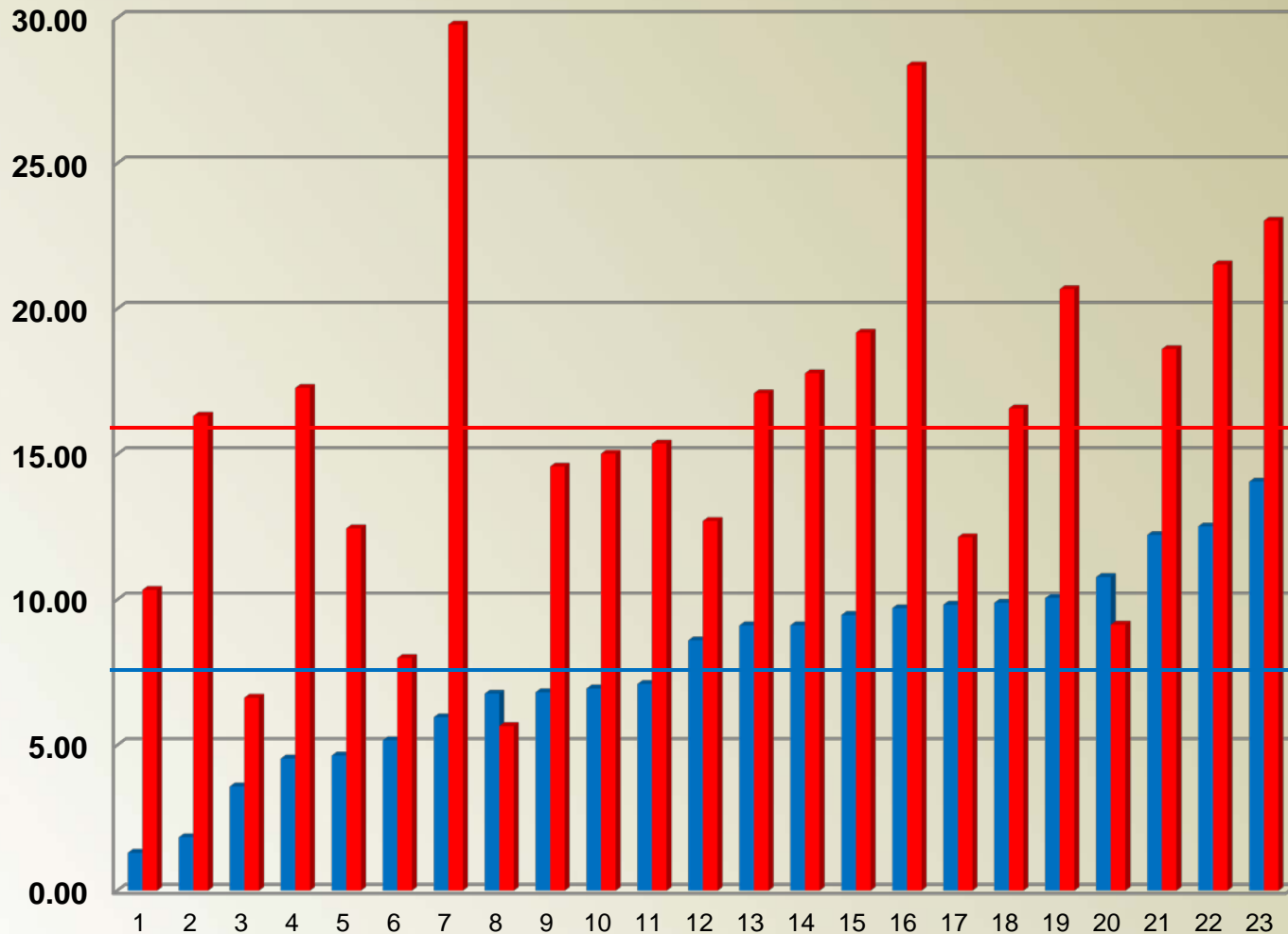**Machine Sorting: NIRS, NMR (High Oil)**
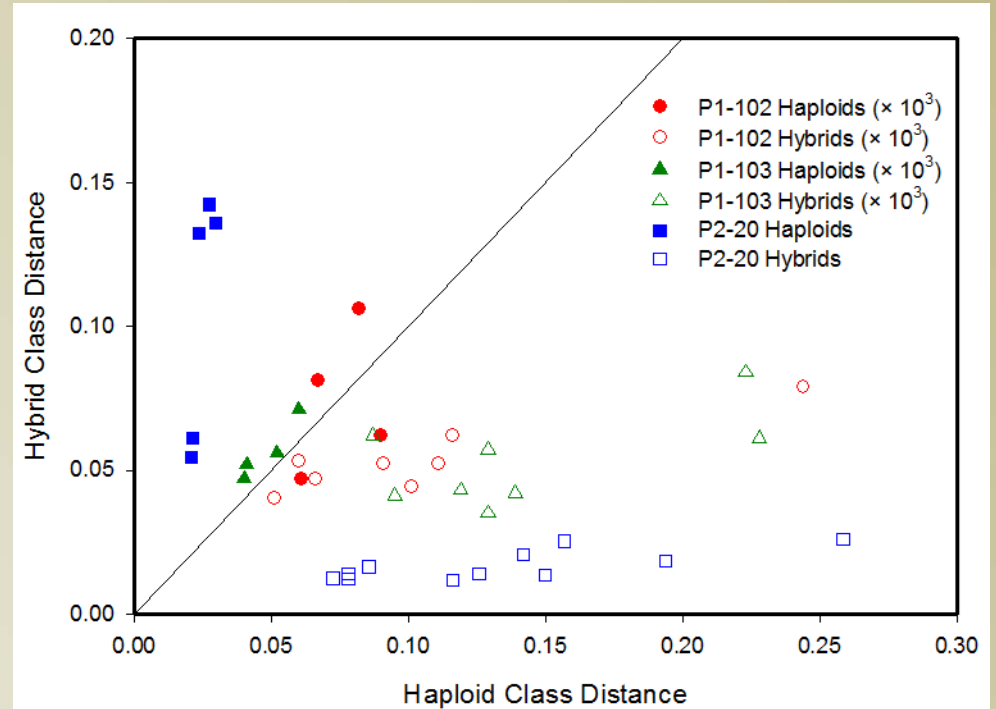
"Phenomic": Weight, MRI (Volume), …

Maize Doubled Haploids: Colchicine Doubling
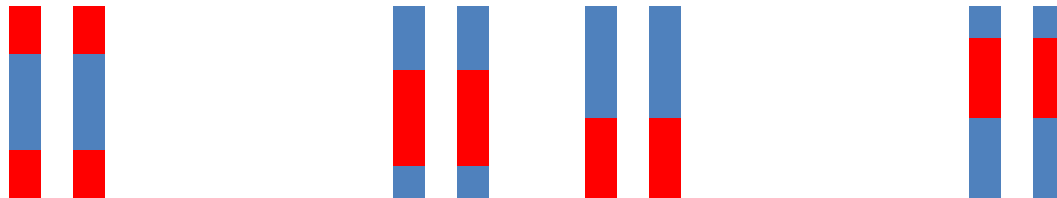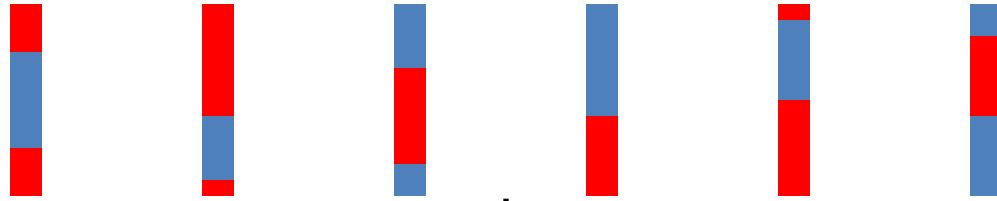
Induction and **"Success Rates"** (Haploid to DH Offspring)

Average % pollinated

Average Induction (%)

r = 0.22

% pollinated is based on no. haploid seed

Adaptation

Gardner, Blanco et al. 2007

Exotic Maize Populations

BC1

DH

Recurrent elite parent: B47 or Z51
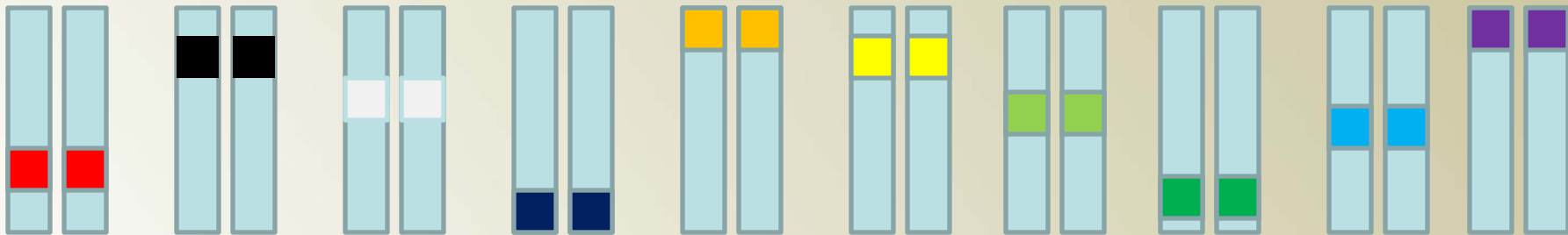
Isogenic BC1-DH lines - GEM

Founder line 1   **x**   **Founder line 2**

F_1

Selfing   **DH**

Inbred line

Inbred line
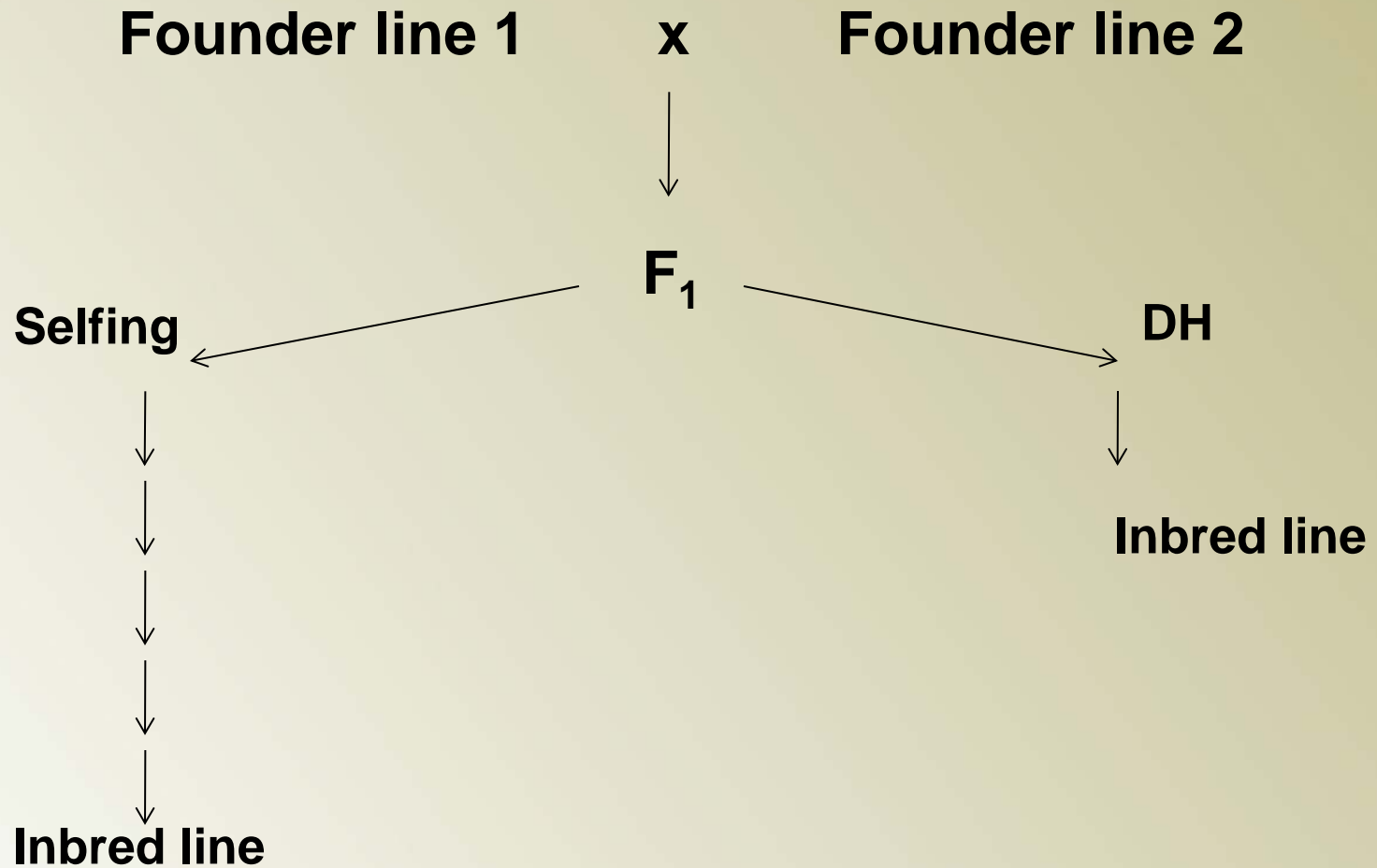
Sequence Variation among Exotics:

Bm3 as Case Study

Are there novel / useful Alleles out there in Exotic Germplasm ?

Morphological:

brown midrib 3 (*bm3-*) **mutation** →

Agronomical:

Trait	Normal	*bm*3
DMF lignin	24.5	21.0
ADL	7.8	5.3
IVDMD	46.4	54.8

Barriere & Argillier 1993

But: Pleiotropy !

Biochemical: Caffeic-O-methyltransferase (Lignin)
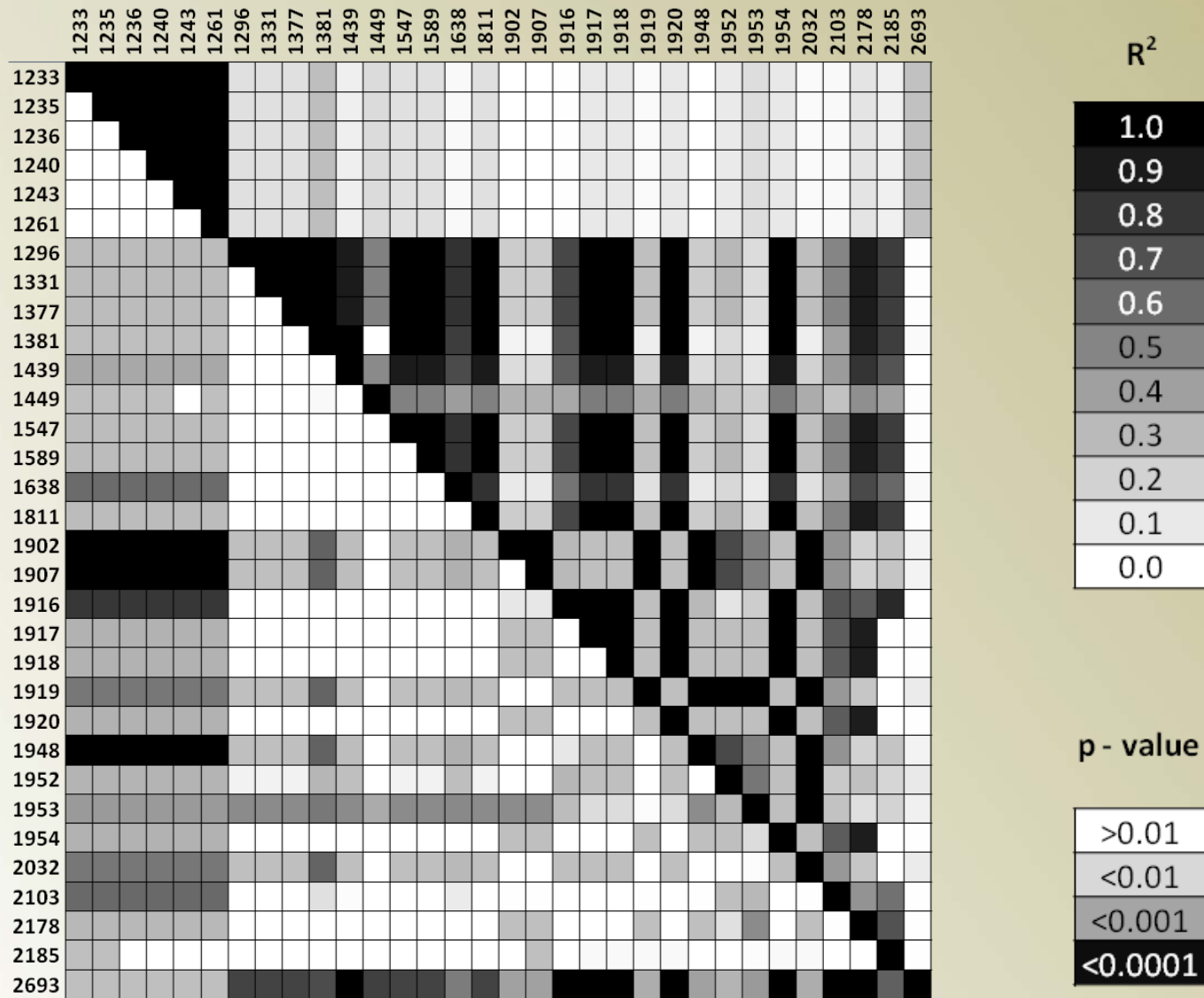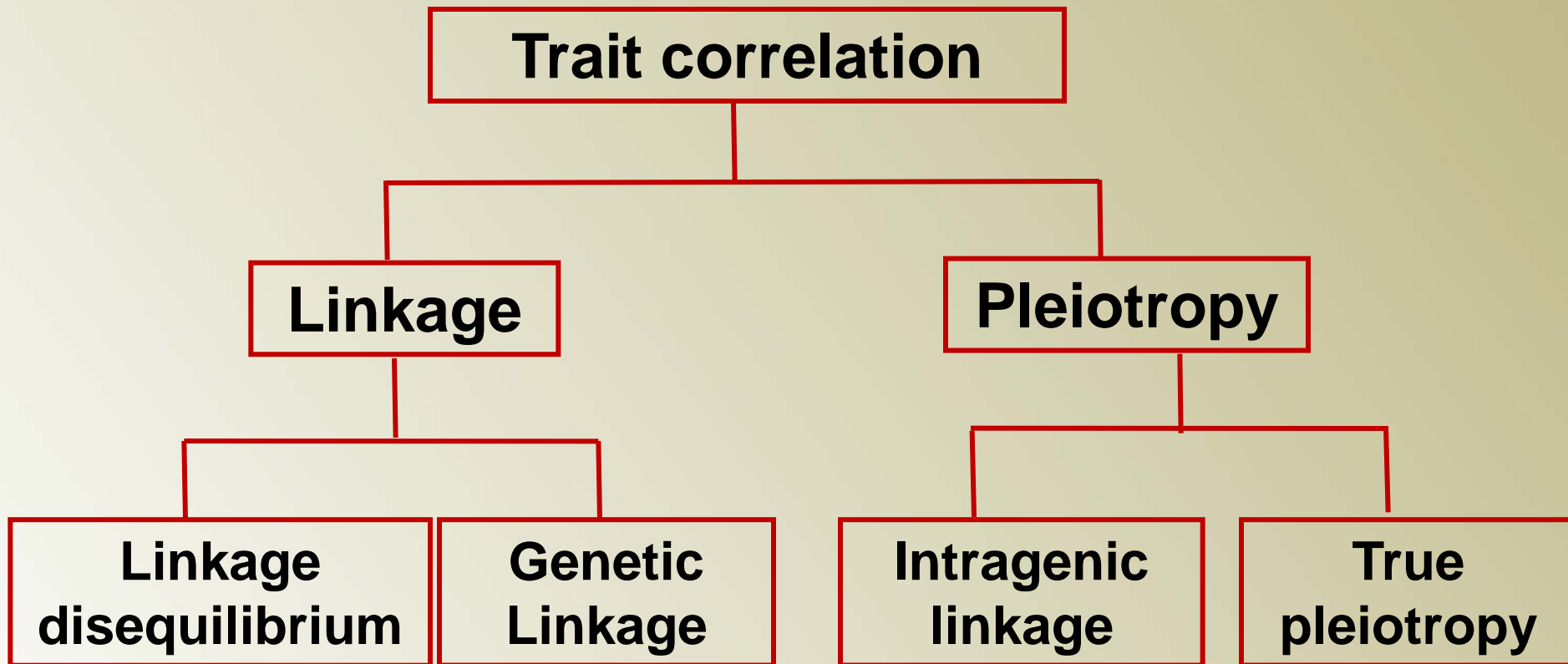
- **Previous studies on *Bm3* locus:**

  - **Fontaine and Barriere 2003: 6 elite lines**
  - **Guillet-Caude et al. 2004: 34 elite lines**
  - **Zein et al. 2007: 42 elite lines**

  => In total 30 distinct full-length *Bm3* (COMT) allele sequences (NCBI)

- **GEM: 55 exotic full-length *Bm3* alleles (Sanger sequencing)**

Chen et al., 2014

55 Exotic Alleles

30 Elite Alleles (70 lines)
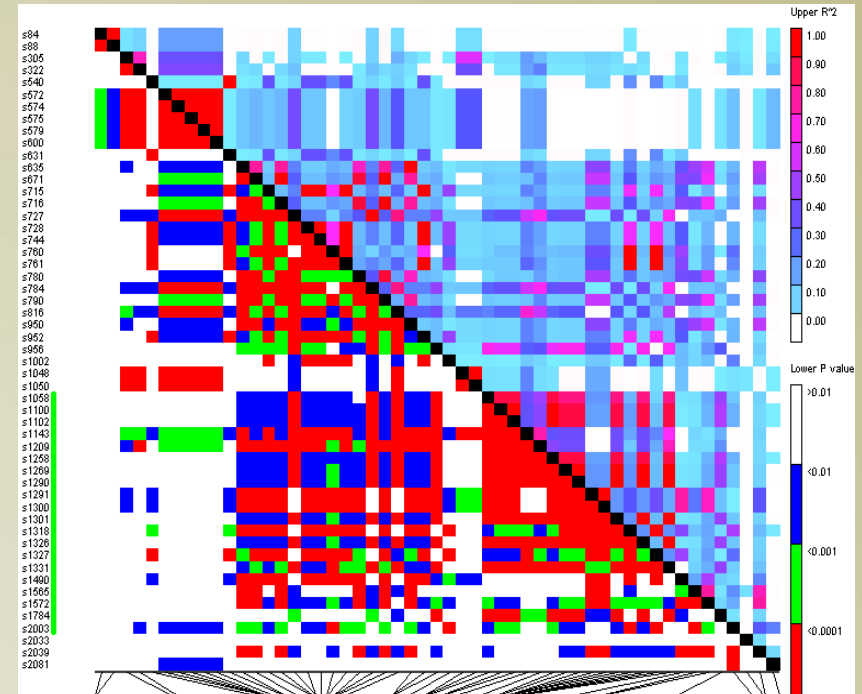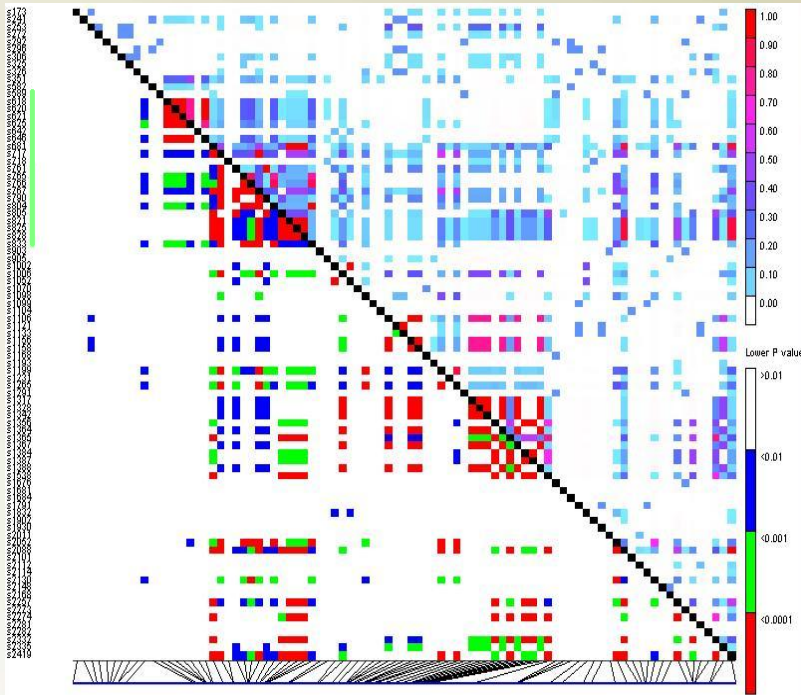
All different from each
Other & from Elite Alleles

Chen et al., 2014
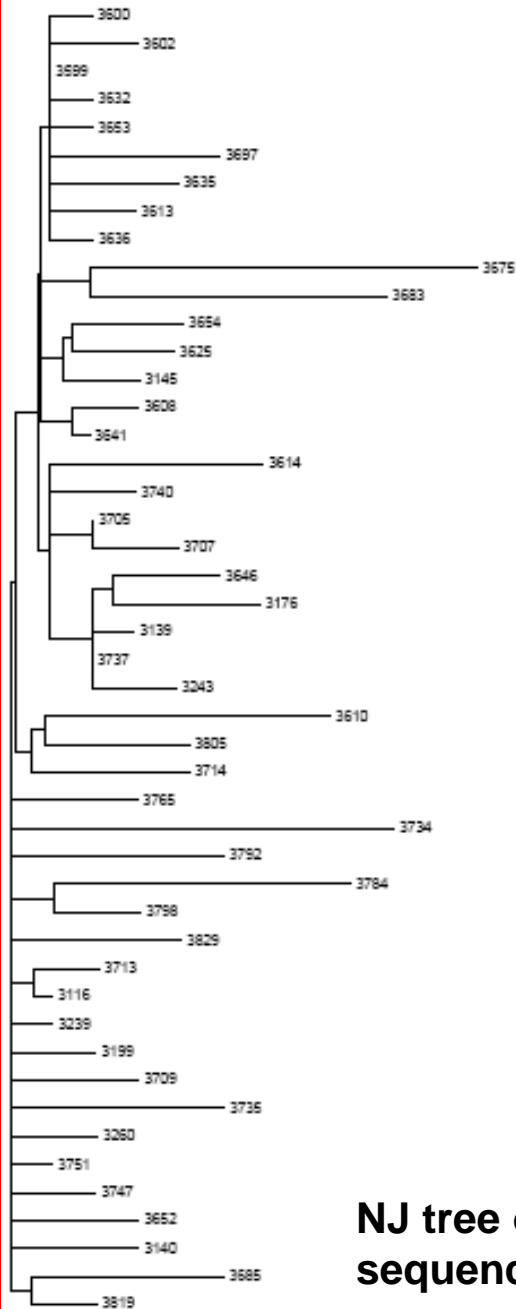
NJ tree of exotic AA sequences
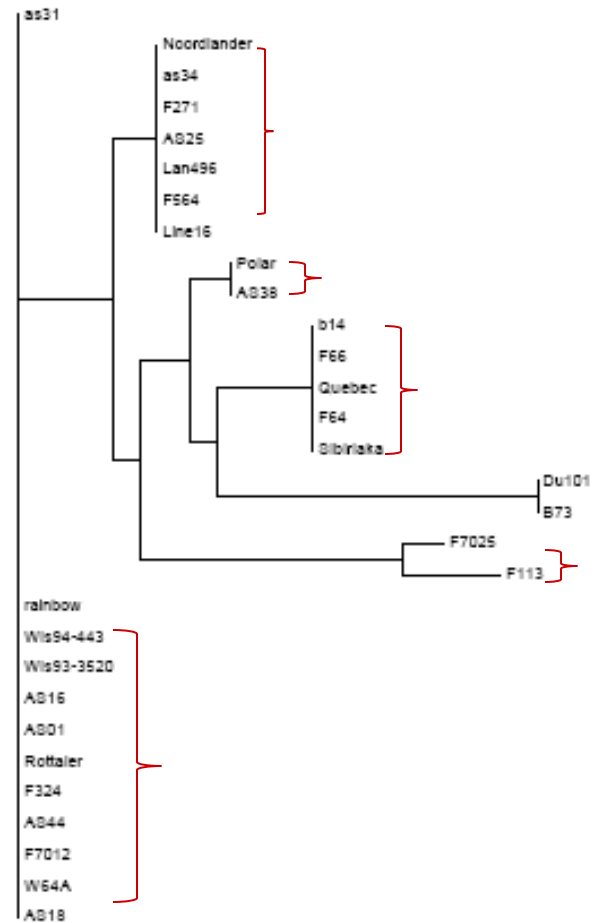
NJ tree of elite AA sequences

More polymorphisms in exotic COMT alleles

55 Exotic COMT alleles above: SNP (singleton)

77(67)

10(10) / 1(0)   **Exon1**   177(120) / 41(2)   138(118)   **Exon2**

2(0)   12(0)
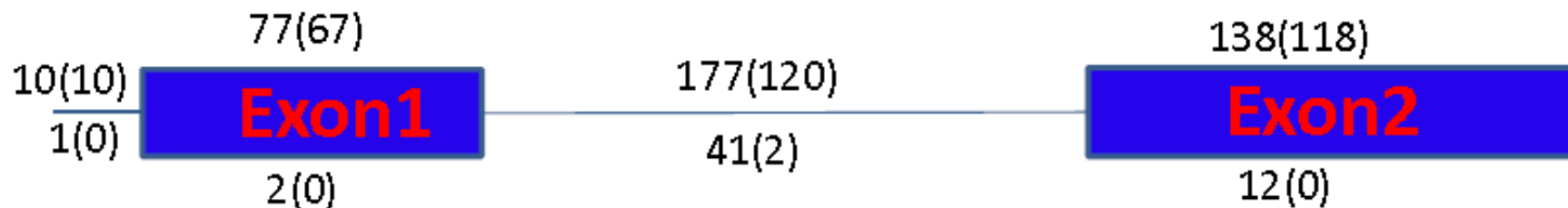
70 Elite COMT alleles below: SNP (singleton)

---

55 Exotic COMT alleles above: Conserved AA / semi-conserved AA / Non-conserved AA

3/3/3

2/4/5   **Dime**   3/4/3   30/16/24   **Methyltransferase**   1/2/3

0/0/0   0/1/0   3/2/0   0/0/0

0/0/0
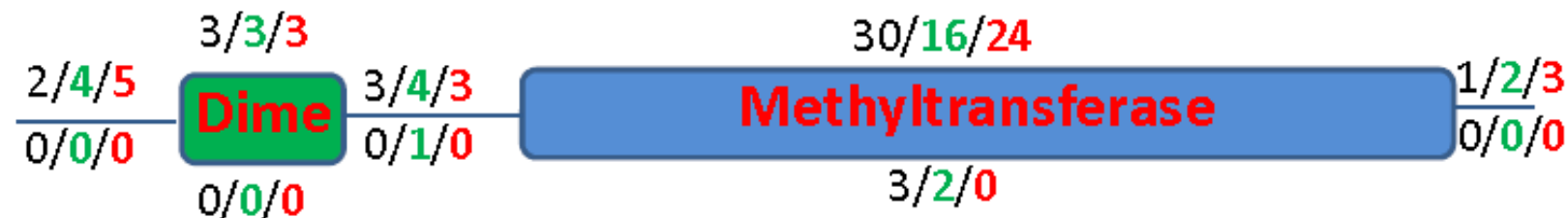
70 Elite COMT alleles above: Conserved AA / semi-conserved AA / Non-conserved AA

Conclusions

Answer: Yes ! All New !

- **High Resolution for Gene-Based Association Analyses**

- **Discrimination Pleiotropy – Genic Linkage**

- **Chance for finding "Optimal Haplotypes"**

- **Deep Genetic Resource for Breeders**

Variation for Agronomic Traits

Plant Materials:

- **50 GEM-BC1-DH Lines**
- **Checks: W604-9S, PHB41, PHZ51, DE811, B73, Mo17**

Traits:

- **Stover DNDF, Plant Height, Ear Height, Flowering Time, Lodging**

Experiments:

- **Forage trials: Davenport (Dow), Ames 2010**

Genotyping:

- **199 SNP markers**

Stover Cell-Wall Digestibility of Checks vs. GEM-DHs

Line	DNDF (average)
W604S	53.6
W605S	46.9
W606S	43.5
W607S	44.2
W609S	46.3
GEM-Gordo 1	48.8
GEM-Gordo 2	47.1
GEM-Gordo 3	47.3
GEM-Gordo 4	48.8

Mapping in GEM-DH Panel

- > 600 GEM-DH Lines developed

- Genotyping-by-Sequencing: done for 360 DHLs

- 8000 SNPs: done for 360 DHLs

- Phenotyping: per se 2013, 2014; TC 2015

- Significance of linkage drag> GEM-BC1:3-sublines: 2015

Multiple donors, unbalanced data set
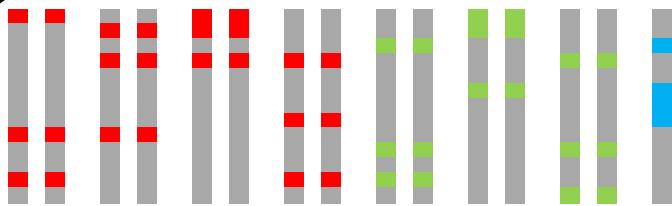
GEM

At one locus, different different effect

Exotic mais population constituted of several donors

X

Elite line

Cross with elite line
F1
Backcross with elite line
BC1
DH

GEM-BC1-DH Genotyping

	PHB47	PHZ51
No. of DH lines	203	137
Excluded Lines (>1% Het)	31	20
% Donor Expected	75	75
% Donor (199 SNPs)	88	89
% Donor (>8000 SNPs)	85	85
% Donor GBS unimputed	93	92
% Donor GBS imputed	93	92

Observed / Expected Crossovers, Recurrent Parent Genome %

	Crossovers			% Rec. Parent		
	Obs. (avg)	Exp.		Obs.	Exp.	
Ch1	6.54	2.77	*	0.87	0.75	*
Ch2	3.25	2.07	*	0.88	0.75	*
Ch3	3.97	2.29	*	0.89	0.75	*
Ch4	4.55	1.36	*	0.88	0.75	*
Ch5	3.46	1.34	*	0.91	0.75	*
Ch6	3.17	0.86	*	0.85	0.75	*
Ch7	4.84	1.39	*	0.89	0.75	*
Ch8	4.31	1.51	*	0.86	0.75	*
Ch9	3.74	1.28	*	0.89	0.75	*
Ch10	3.82	1.46	*	0.83	0.75	ns

Distribution of Introgressions

Brenner et al. 2012

Applying science to fuel and feed our global society

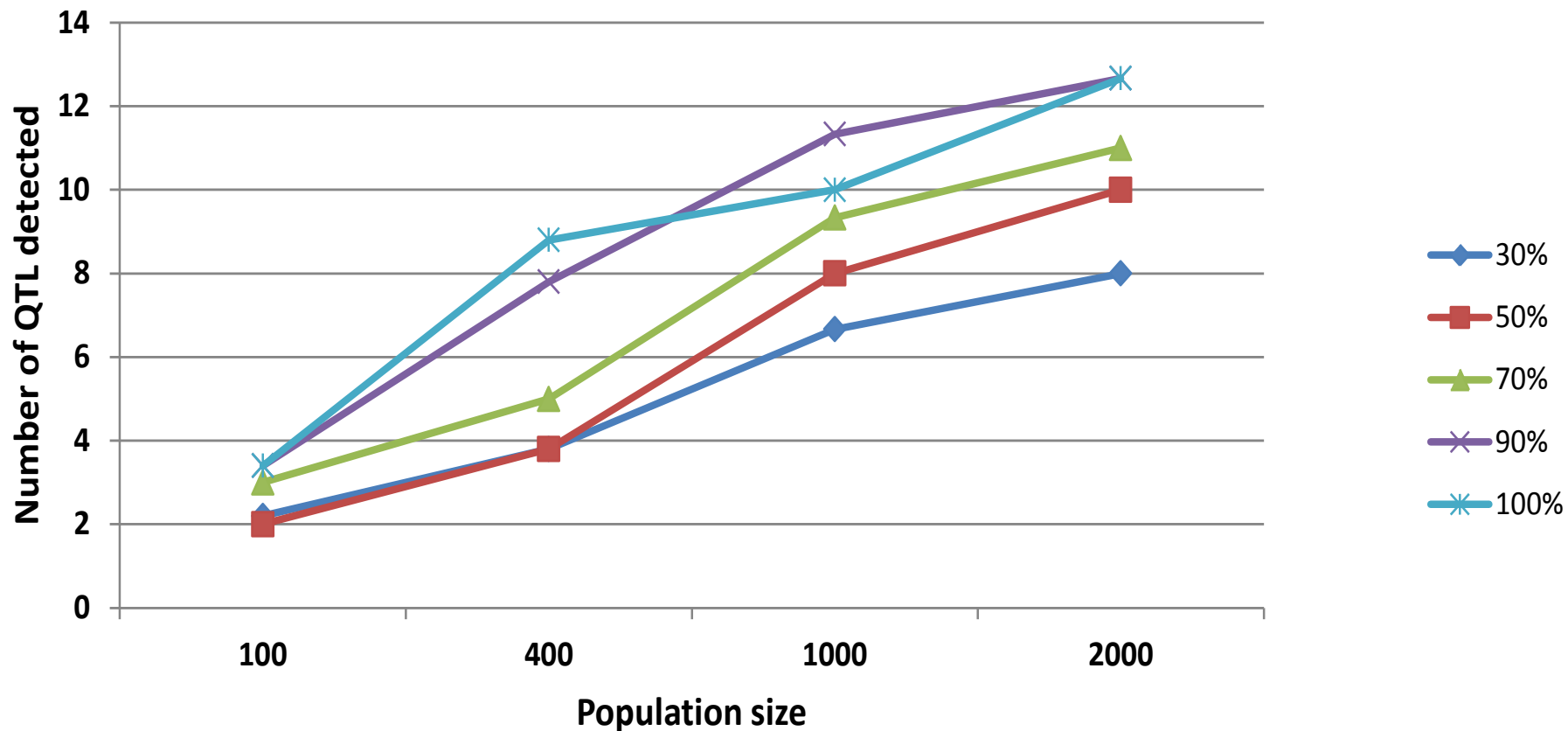▸ **Plant height as « model » trait**

Number of QTL	15
Genetic variance explained	100%, each QTL explaining between 2% and 30% of the genetic variance
Mean	200 cm
h²	90% or 50%

▸ **Simulation of different scenarios regarding QTL alleles:**

◦ Simulation of different proportions ψ of donor lines carrying an allele whose effect is different from the elite line.

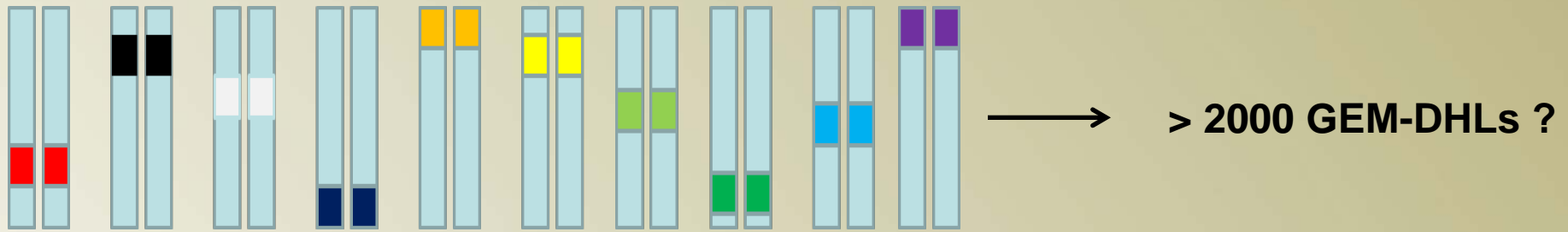◦ Simulation of multiple alleles whose effects are comprised between –a and +a for 3 QTL (= « multiple allele » QTL)

Number of QTL detected per proportion of donor lines carrying QTL allele and population size (Method BIM, h² = 90%; marker coverage = 10 cM))
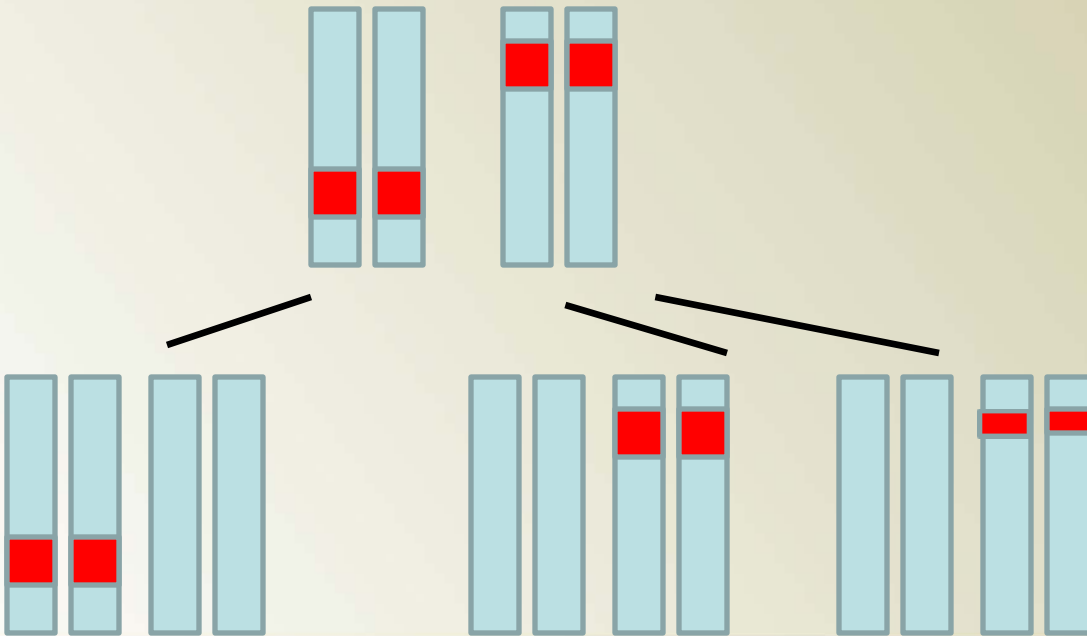
Impact of Linkage Drag

% Exotic

Exp (Obs.)

0

100

25 (17)

6

6

```
Recurrent parent
RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR   Chrom 1

RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR         Chrom 2


Exotic inbred
00000----000000----0-0-00---00------0----0----0---0-  Chrom 1

--00--00--00---000-0-0-0-00---00----000----0+++      Chrom 2


GEM-BC1-DH
RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0---0-  Chrom 1

RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR-0+++        Chrom 2


GEM-BC1:3-DHa
RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR0----0---0-  Chrom 1

RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR        Chrom 2


GEM-BC1:3-DHb
RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR   Chrom 1

RRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRRR-0+++        Chrom 2
```

> 2000 GEM-DHLs ?

GEM-DH-BC1:3 Sublines ?

Efficient Procedures & Outlook

The DH Process: Inducibility
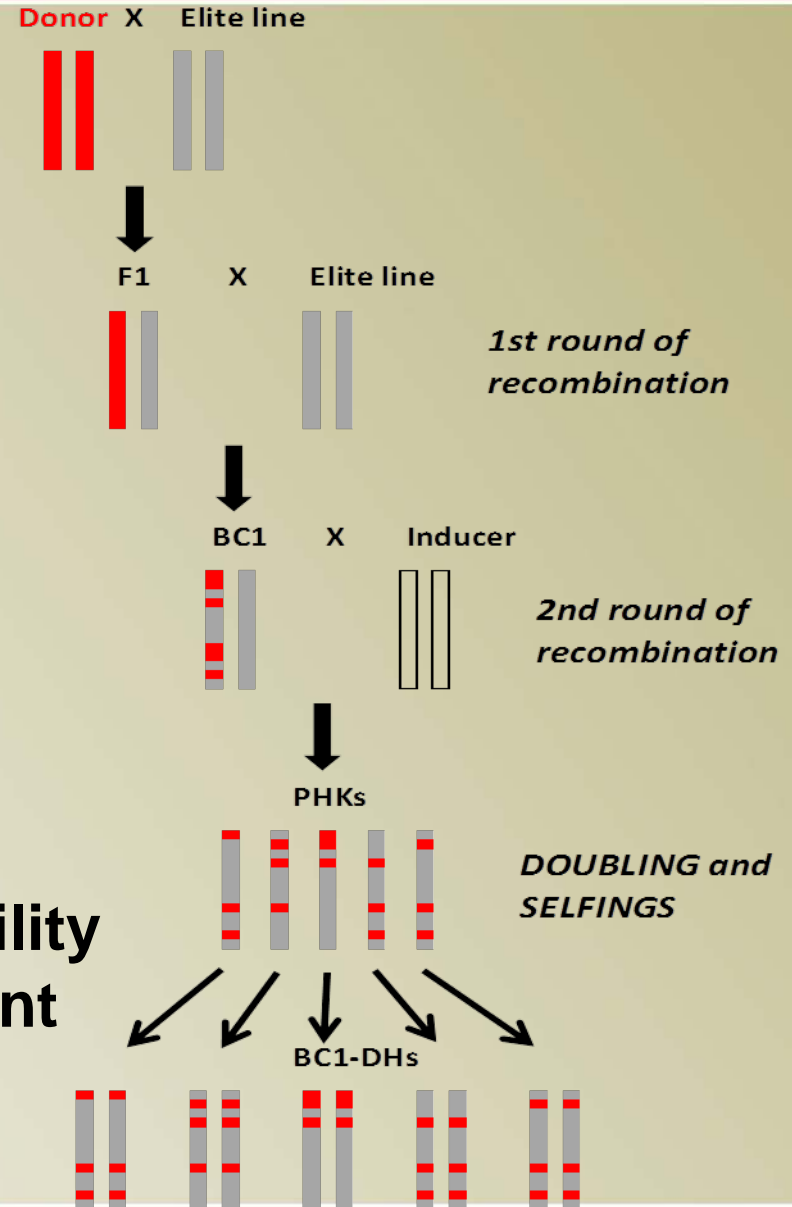

De La Fuente, unpublished
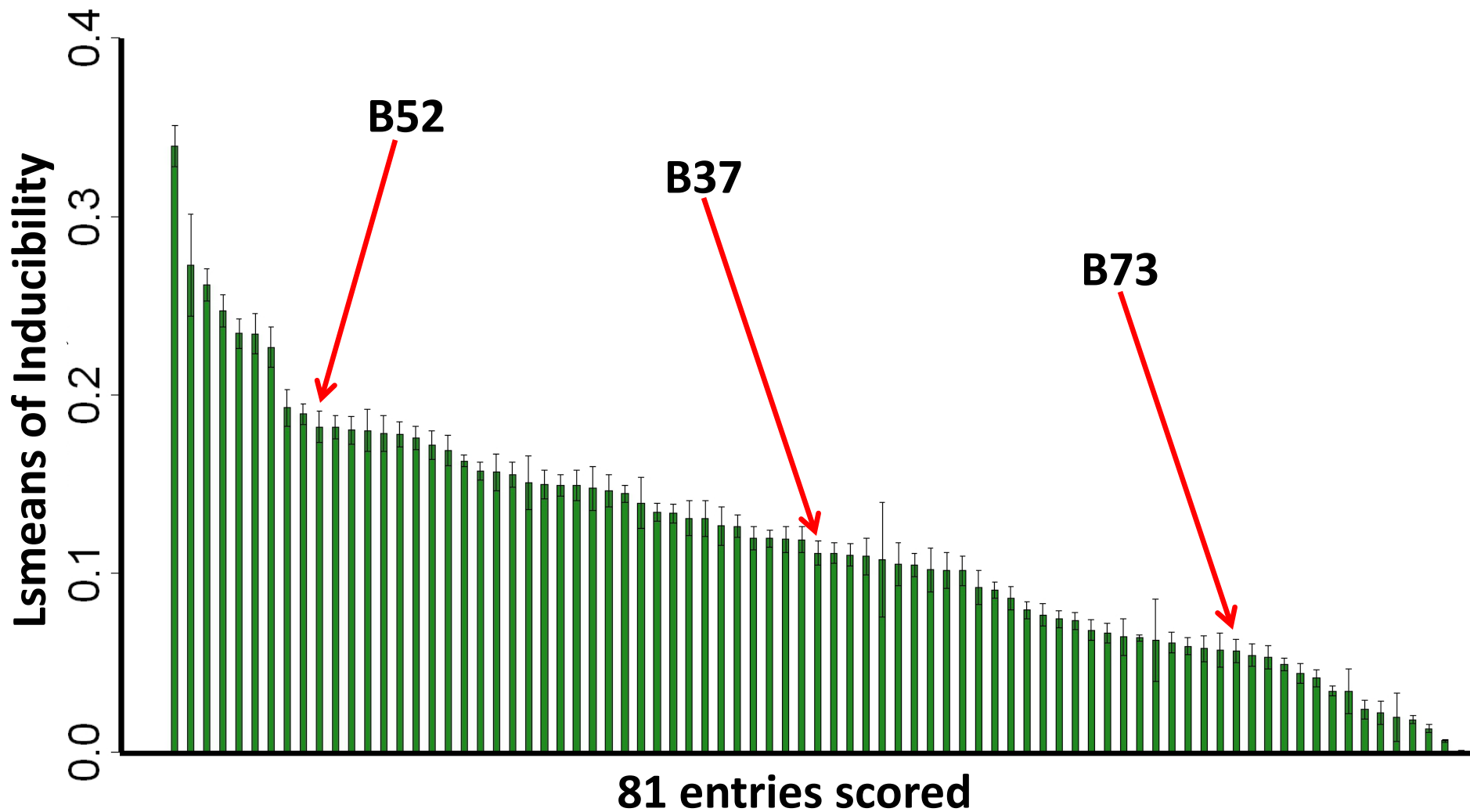
The DH Process: Spontaneous Doubling

- Observed >50% spontaneous doubling on 2 lines

- Attempted self pollinations were successful as were cross pollinations

- Observed fertility of F_1 in greenhouse

- Female fertility is not an issue

De La Fuente, unpublished

Improved Inducers:

- **Higher Induction Rates**
- **Automated Sorting**

Improved Recurrent Parents:

- **Substantially increased Inducibility**
- **No Need for Colchicine Treatment**

IOWA STATE UNIVERSITY
Department of Agronomy
Crop, Soil, and Environmental Sciences

- Is there useful genetic variation ?

- What is impact of linkage drag ?

- What is impact selective sweeps ?

- Impact of background / recurrent parent (epistasis) ?

Acknowledgments

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DH Facility

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Chase 1947, 2011