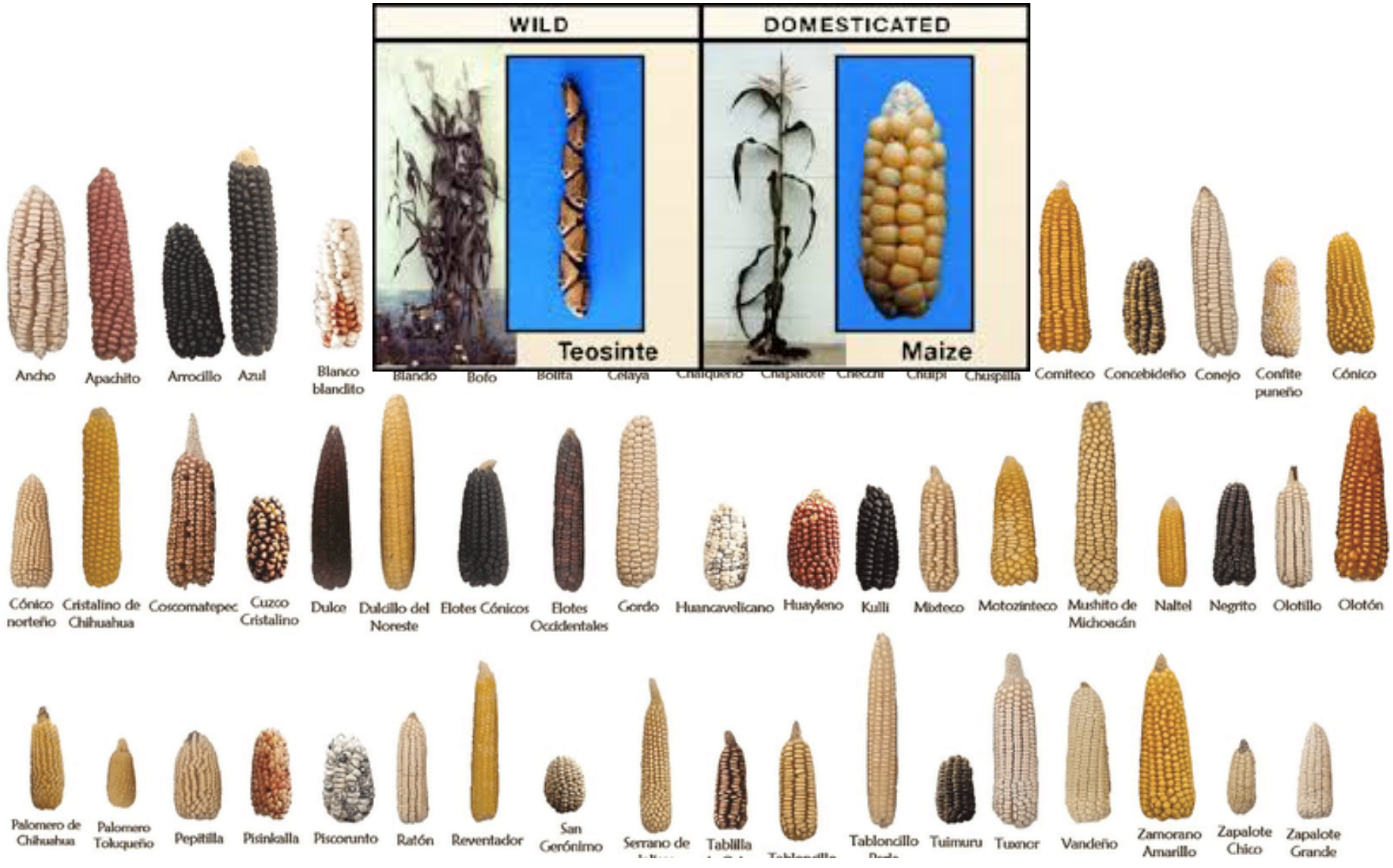
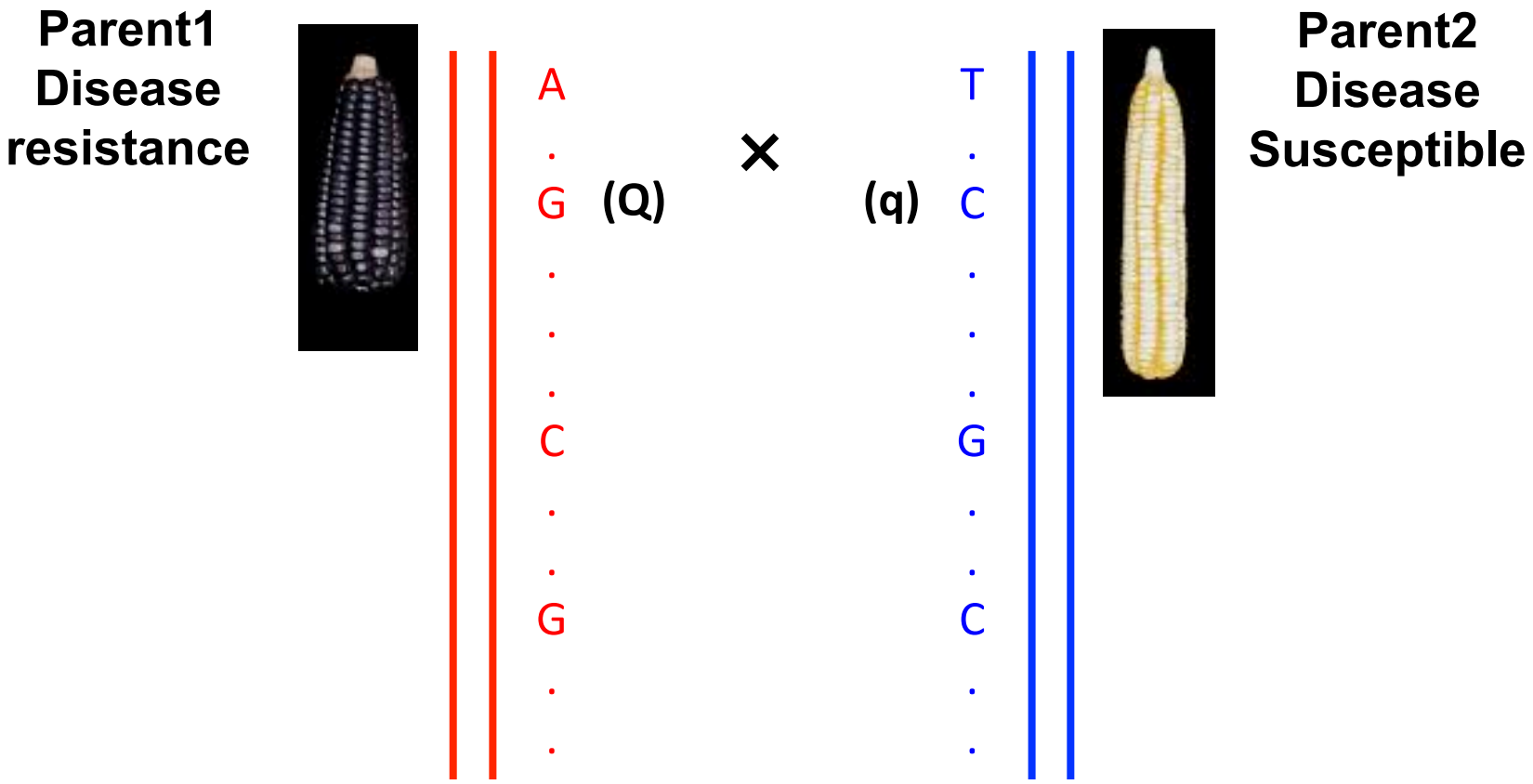
Mutation is a main cause of diversity



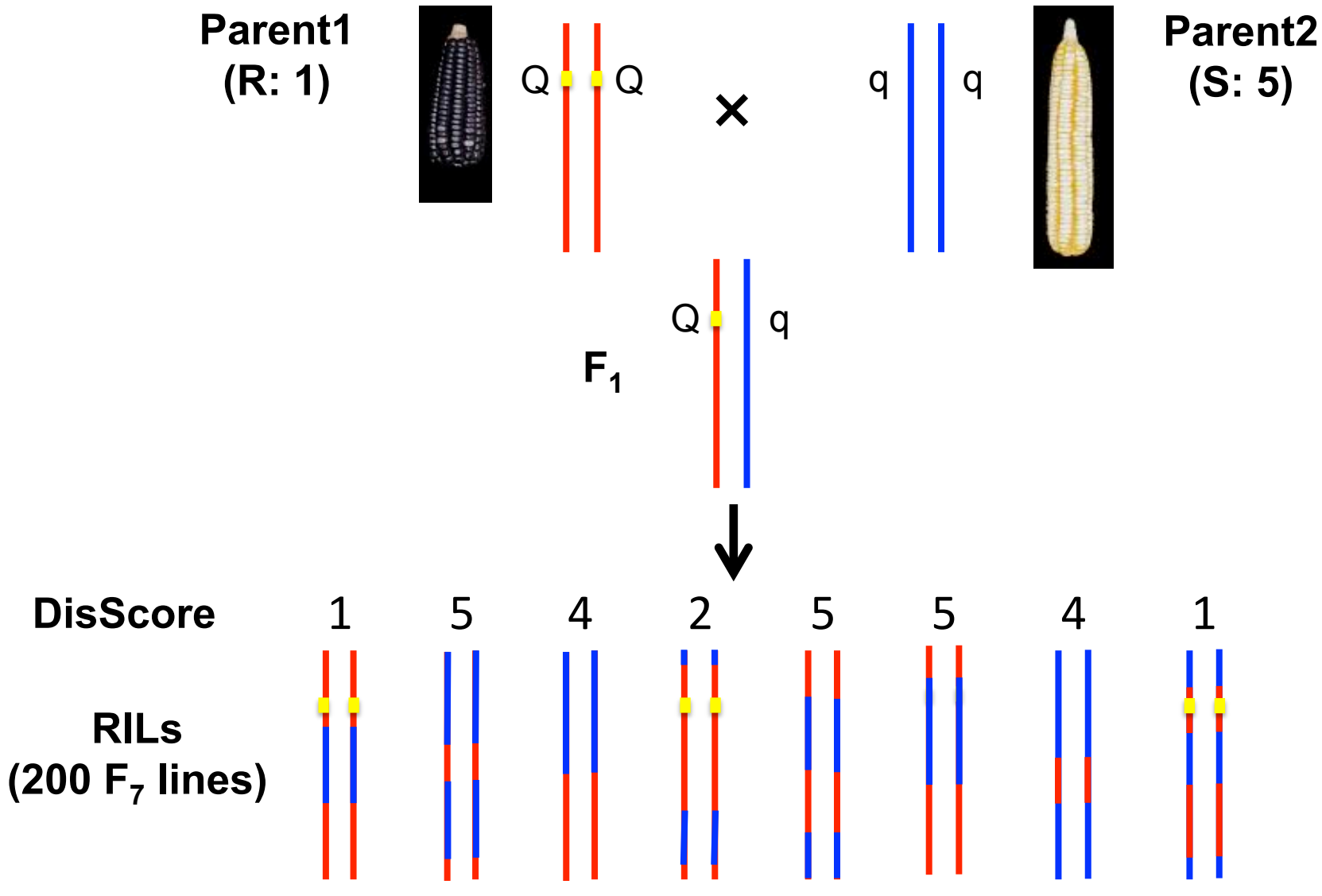
Millions of DNA sequence polymorphisms between two individuals



Linkage mapping to identify genes controlling a trait

1. Create a segregating population for the target trait **using two parents**
2. **Phenotype** the population for the interested trait
3. Genotype **DNA markers** and **construct genetic linkage map**
4. Perform **marker-trait statistical analysis** to find markers linked to the causal genes

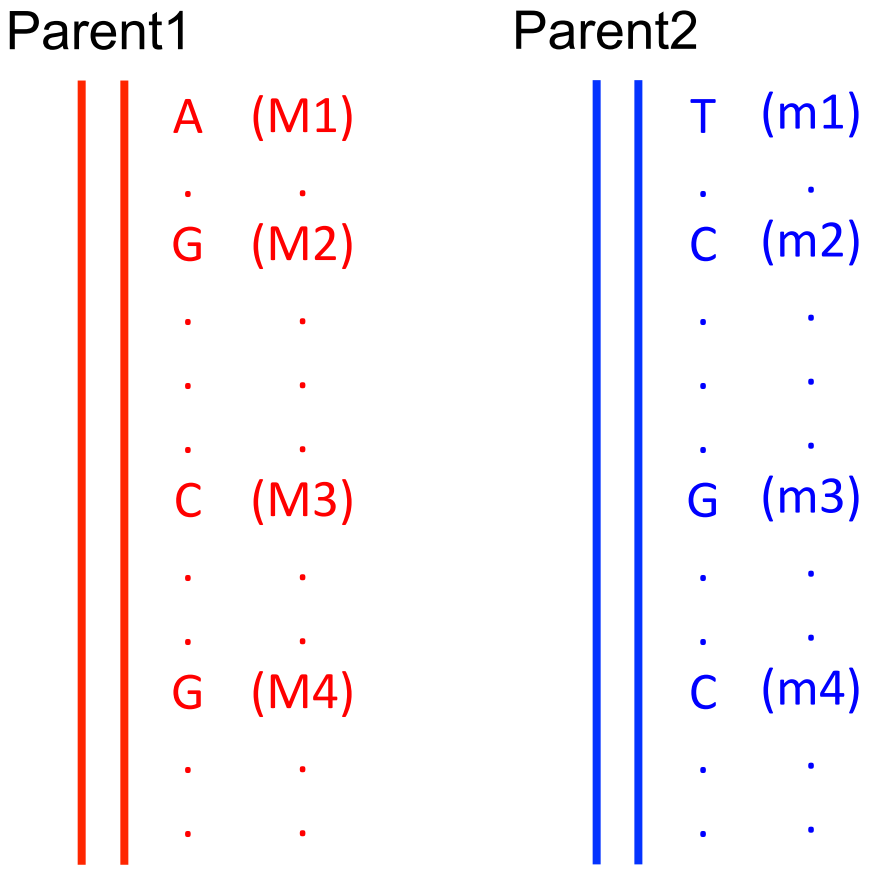
Linkage mapping using bi-parental mapping population



DNA marker: allele and haplotype

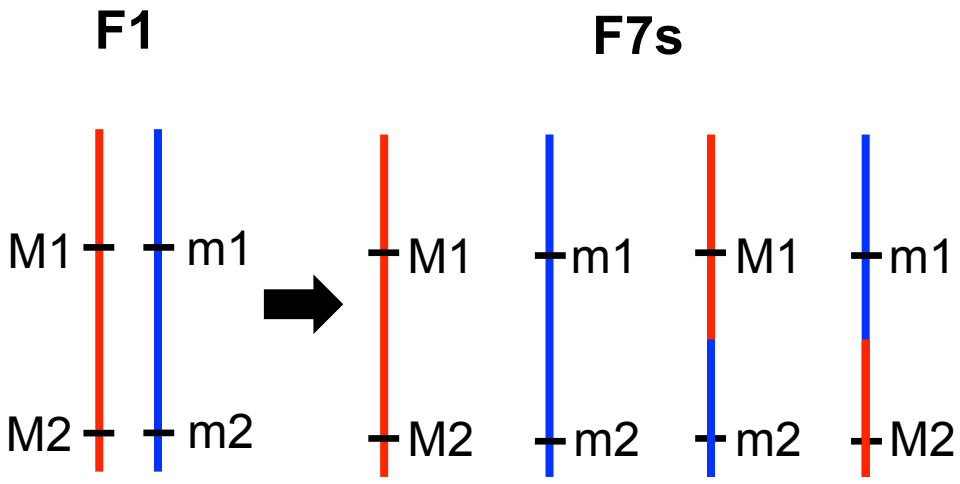
- Allele: a variant form of a given marker (M1 vs m1)
- Haplotype: specific combination of alleles occurring on the same chromosomal segment (M1M2 vs m1m2)

Single Nucleotide Polymorphism (SNP)



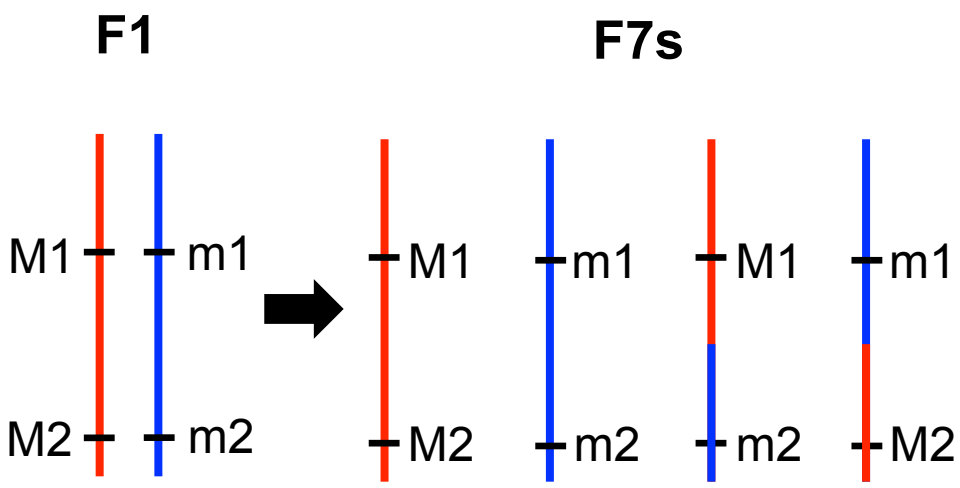
Linkage map construction
--Linkage and homologous recombination

- Two copies of the same chromosome break and rejoin at the same point, which can generate a new haplotype of two or more markers



Genetic distance between two markers on one chromosome

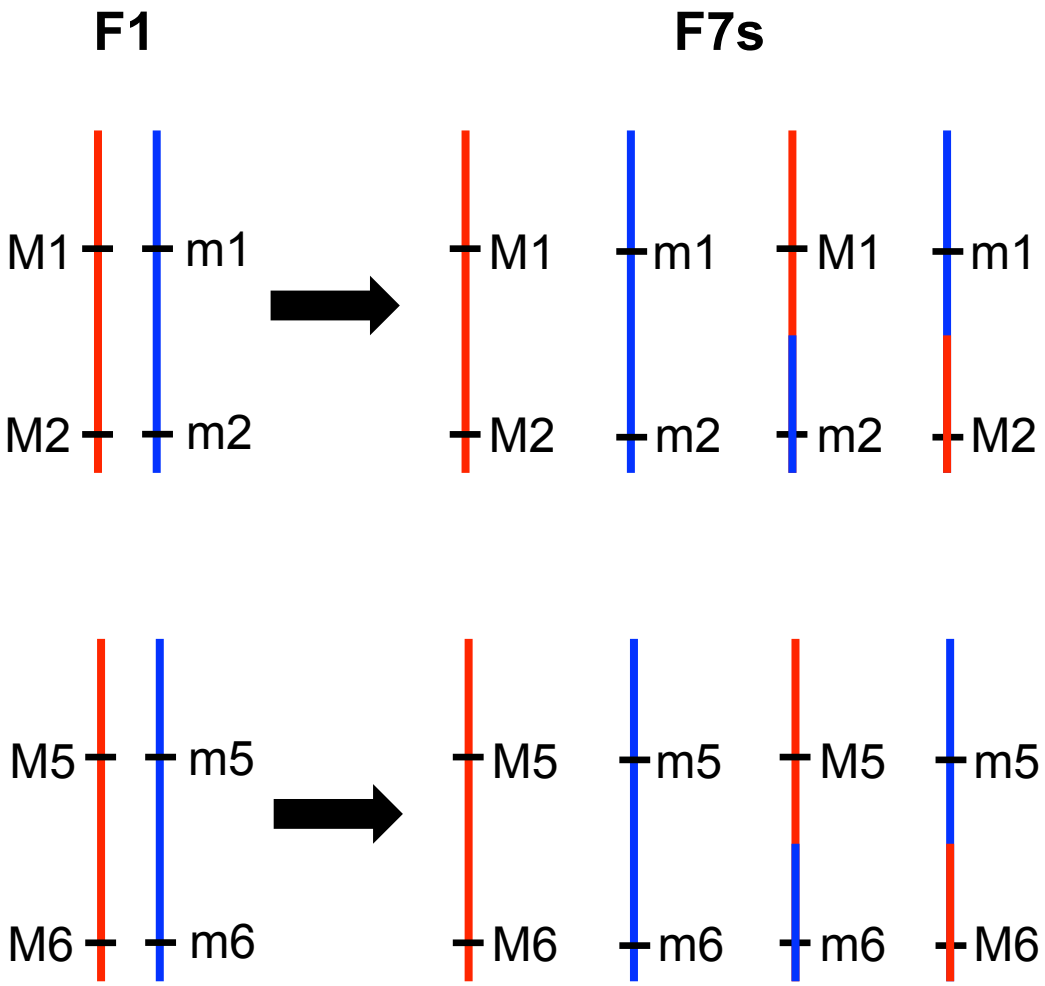
- Recombination rate (r) is percentage of the recombinant haplotypes: r = (M1m2 + m1M2)%; and can be used to estimate genetic distance between markers



Haplotype	Gamete	Counts
Parental	M1M2	92
Parental	m1m2	88
Recombinant	M1m2	10
Recombinant	m1M2	10

Rec. rate (r)	0.10 (10 cM)

Genetic distance between two markers from different chromosomes



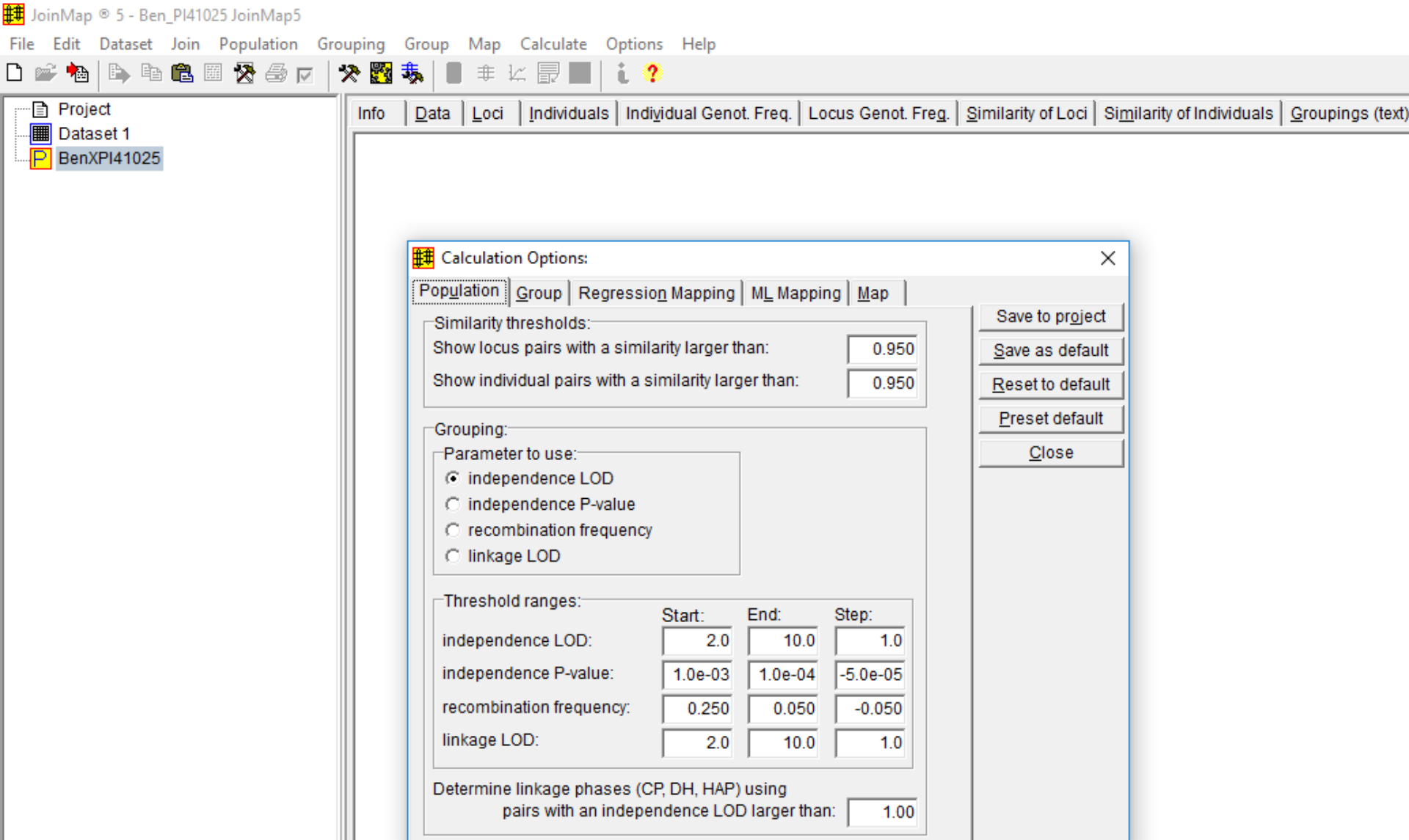
Gamete	Counts
M1M5	50
m1m5	50
M1m5	50
m1M5	50

Rec. rate (r) 0.50 (50 cM)

Grouping of markers based on the pairwise recombination rates

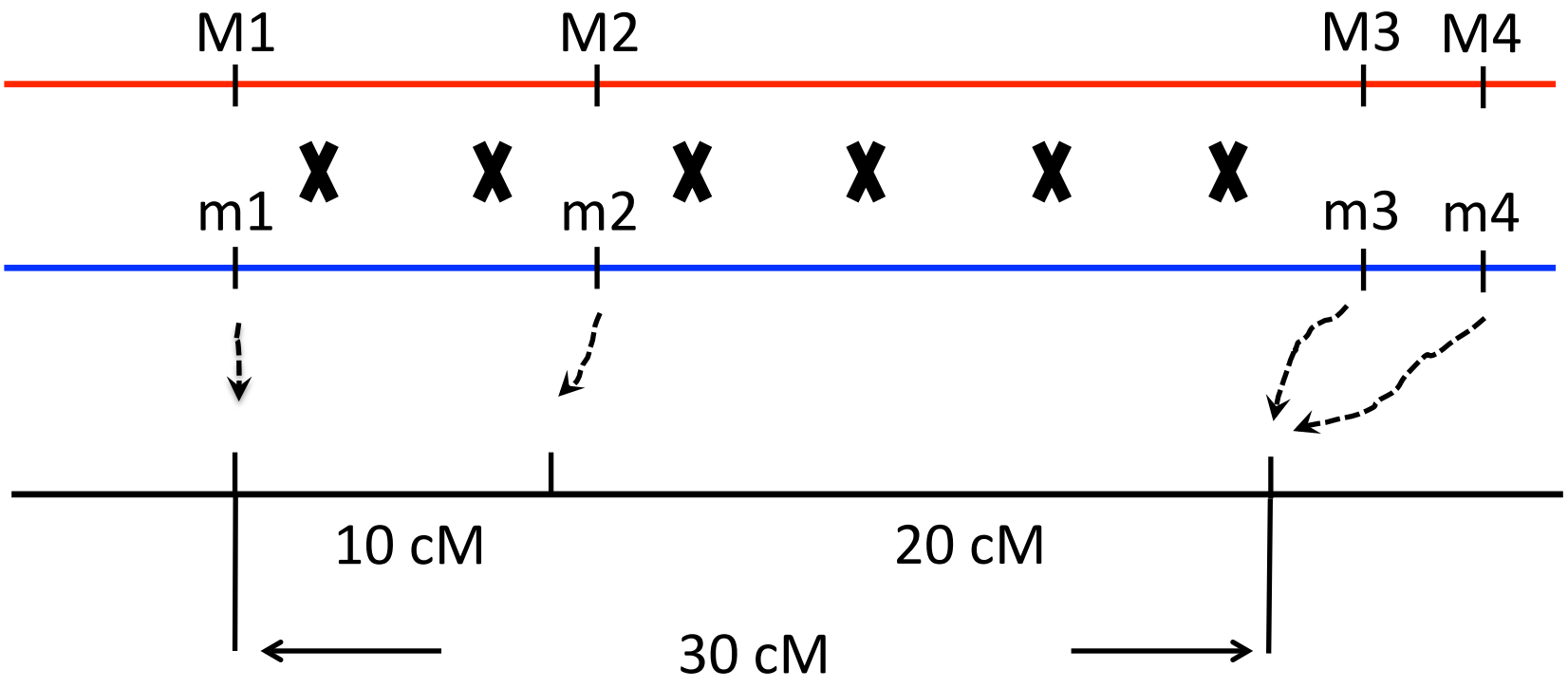
Marker1	Marker2	r	cM	Group
M1	M2	0.1	10	G1
M1	M3	0.3	30	G1
M1	M4	0.3	30	G1
M1	M5	0.5	50	**M5 not in G1**
…	…			
M2	M3	0.2	20	G1
M2	M4	0.2	20	G1
M2	M5	0.5	50	**M5 not in G1**
…	…	…	…	

Calculation Options for grouping



Ordering markers within a linkage group

- Two DNA markers that are physically near to each other are unlikely to be separated during chromosomal crossover; the closer, the more likely to be inherited together



Marker-trait association

	Phenotype	SNP1	SNP2	SNP3	SNP4
Sample 1	**1**	……ACGGT...CGGCA……..TGAT.....AAGGG……			
Sample 2	**1**	……ACGGT…CGGCA……..TGAT.....AAGGG……			
Sample 3	**1**	……ACGGT…CGGCA……..TGAA.....AAGGC……			
Sample 4	**1**	……ACCGT…CGGCA……TGAA.....AAGGC……			
Sample 5	**5**	……ACCGT…CGGCT……..TGAA.....AAGGG……			
Sample 6	**5**	……ACCGT…CGGCT……..TGAA.....AAGGC……			
Sample 7	**5**	……ACCGT…CGGCT……..TGAT.....AAGGC……			
Sample 8	**5**	……ACCGT…CGGCT……..TGAT.....AAGGG……			
……		……			

Marker-trait analysis of linkage mapping

$$y_{ij} = bx_j + e_{ij}$$

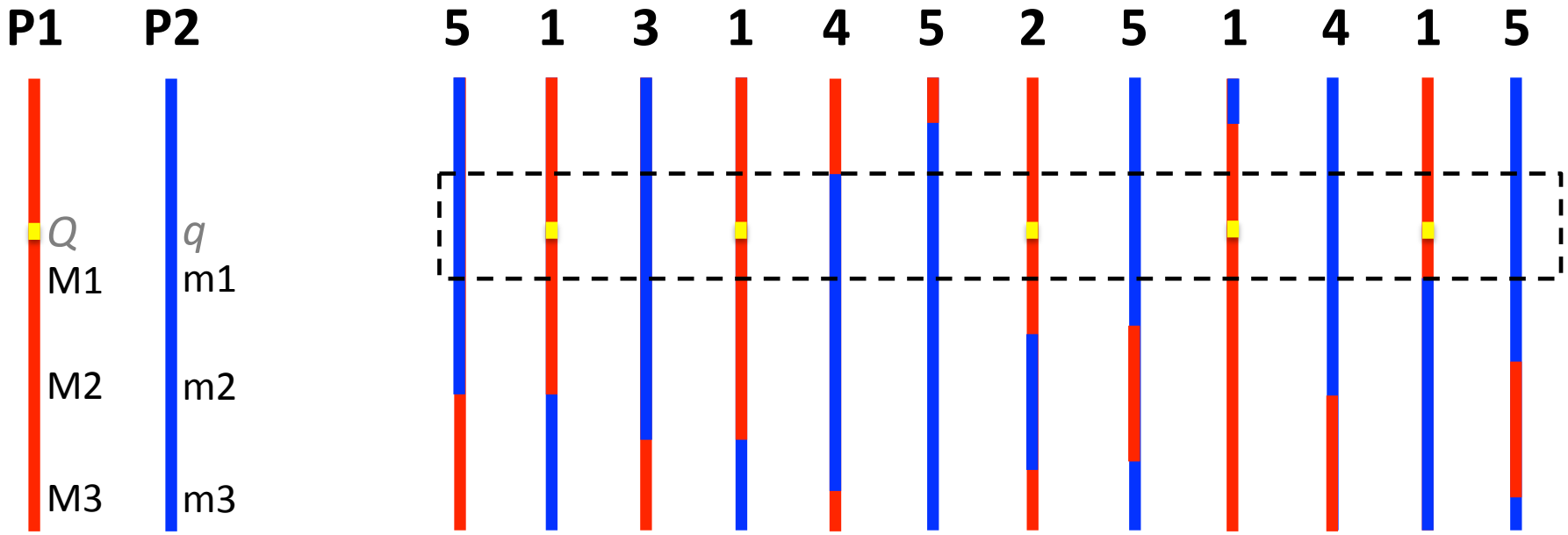
- *Ho*: μ(MM) = μ(mm) vs *H1*: μ(MM) ≠ μ(mm)
- μ(MM) is the mean disease resistance score of the individuals with genotype of MM for a marker
- μ(mm) is the mean disease resistance score of the individuals with genotype of mm for a marker
- When a marker and the gene (Q) are not linked, μ(MM) = μ(mm) and then b=0

(Zeng, 1994)

Single marker association analysis

	y	X
Entry	**Disease Score**	**MM (1) vs mm (0)**
1	2	1
2	5	0
3	1	1
4	4	0
5	3	0
6	2	1
…	…	….

Marker-trait analysis of linkage mapping



SNPs	Marker 1		Marker 3	
Marker Allele (Gene allele)	M1 (100) (95Q and 5q)	m1 (100) (95q and 5Q)	M3 (100) (50 Q and 50 q)	m3 (100) (50 Q and 50 q)
Mean resistance	1.2	4.5	2.45	2.48
p-value	0.0005		0.2	

Fusarium Head Blight (FHB) in wheat

- FHB, caused by *Fusarium graminearum*, is a devastating disease of wheat worldwide
- FHB causes both severe loss of grain yield and quality
- Epidemics of FHB from 1993 to 1997 in Northern Great Plains; over one billion dollars loss to wheat industry in 1993 (McMullen et al., 1997); all commercial cultivars were susceptible
- Developing and growing resistant cultivars is the most efficient mean to minimize the negative effects of the disease

Waldron et al., 1999

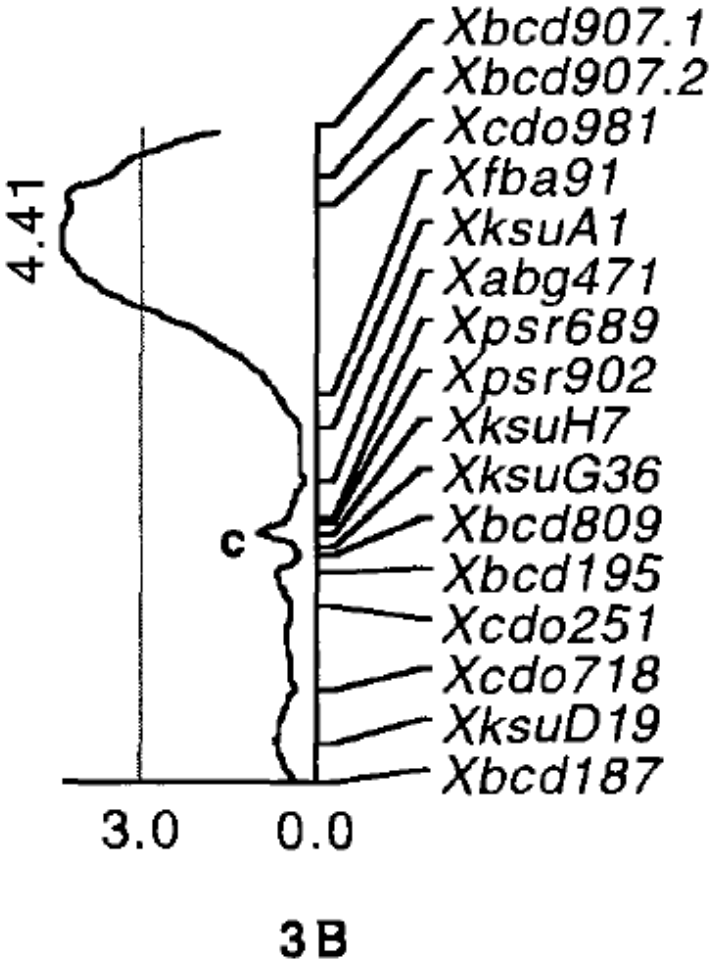
Linkage mapping of FHB resistance in Wheat

- Create a population segregating for FHB resistance
  - A population of 112 F_5-derived recombinant inbred lines (RILs) developed by single seed descent from the spring wheat cross 'Sumai3'/'Stoa'
  - Sumai3 is a Chinese cultivar with high resistance to FHB
  - Stoa (susceptible to FHB) is a hard red spring cultivar released by North Dakota State University in 1984
- Phenotyping of FHB resistance
  - Evaluated in two experiments, each with three replications, 1994 and 1995

Waldron et al., 1999

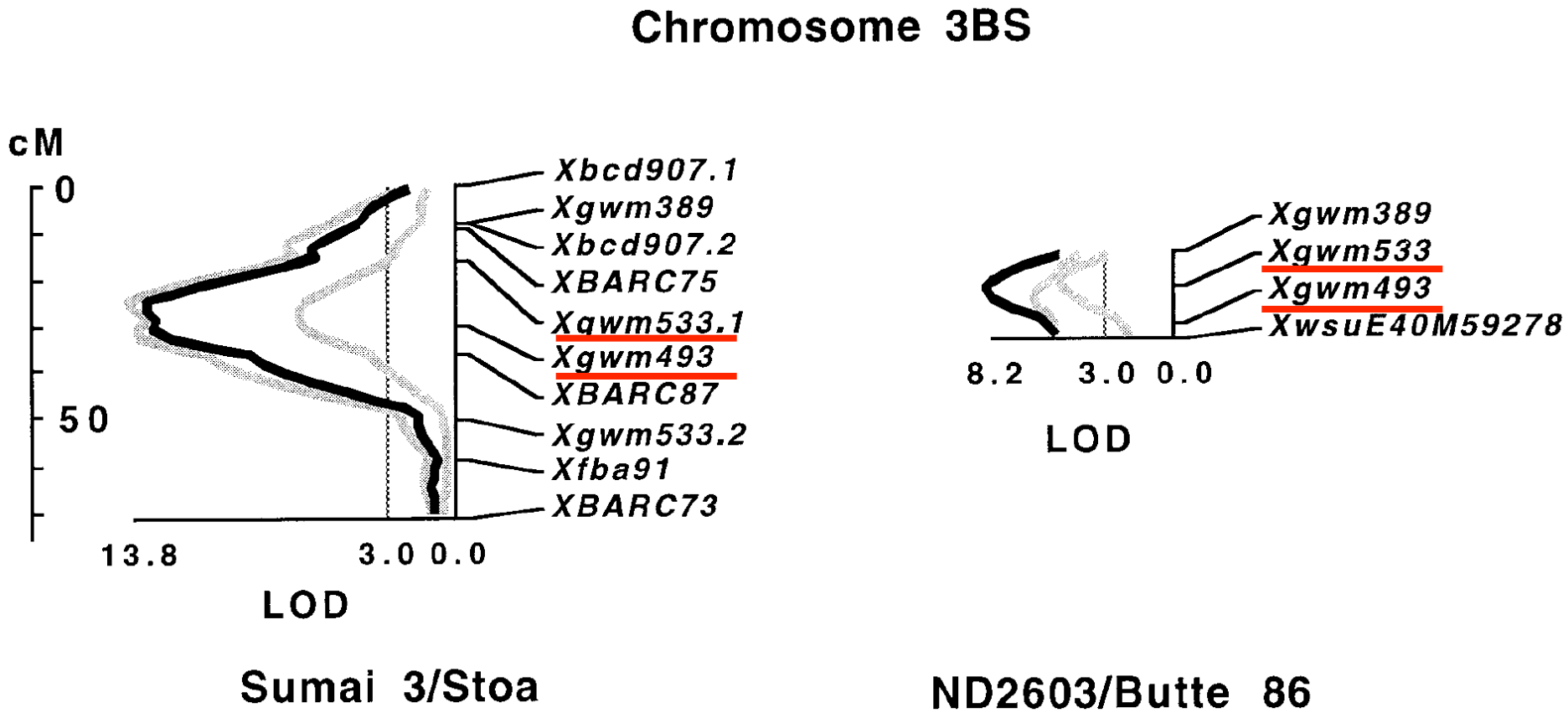
Linkage mapping located a major gene in a large interval on Chr3B

- The best single marker, Xcdo981, was mapped on chromosome 3BS



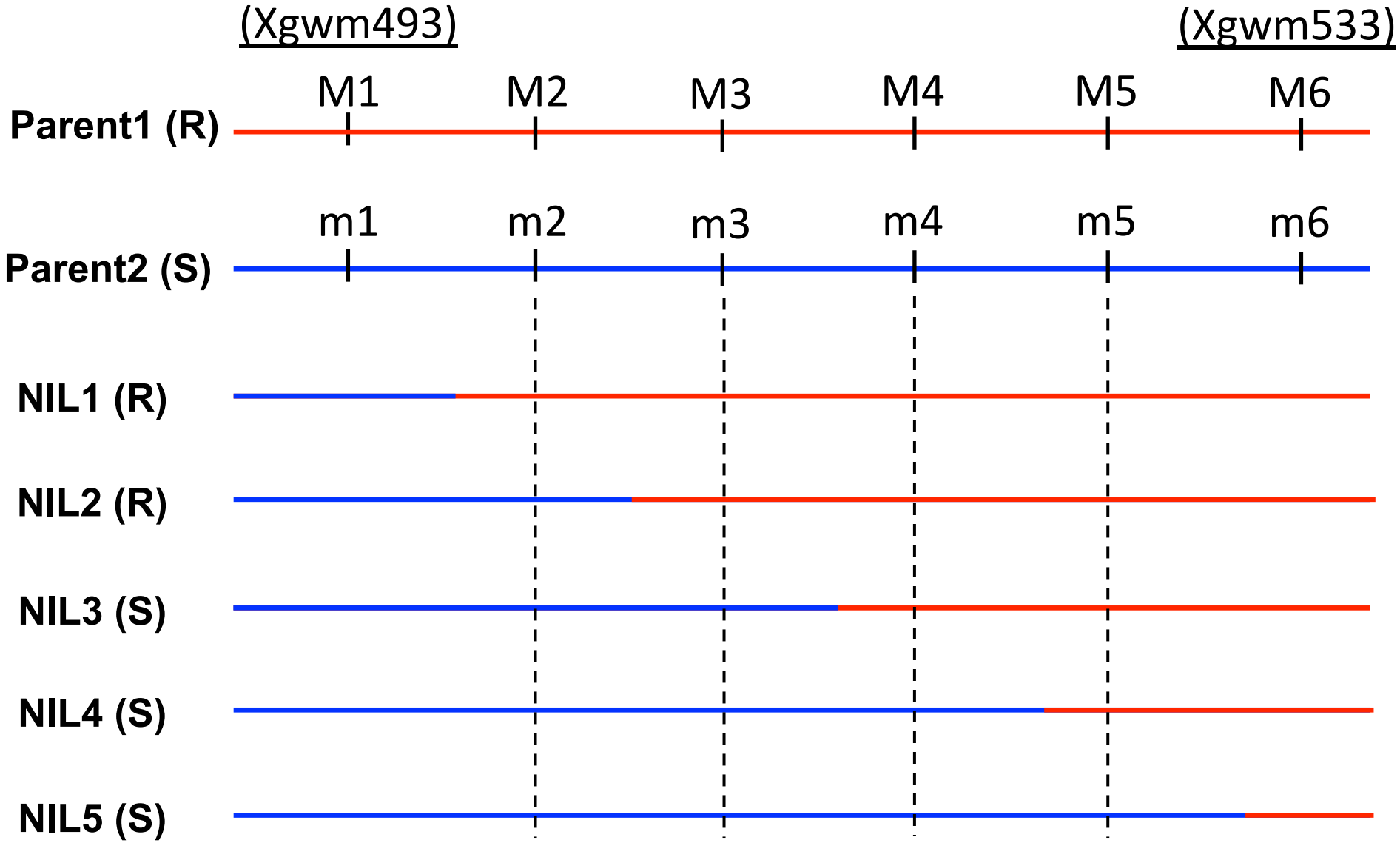
Waldron et al., 1999

Linkage mapping located a major gene in a large interval on Chr3B (many genes are located in the region)

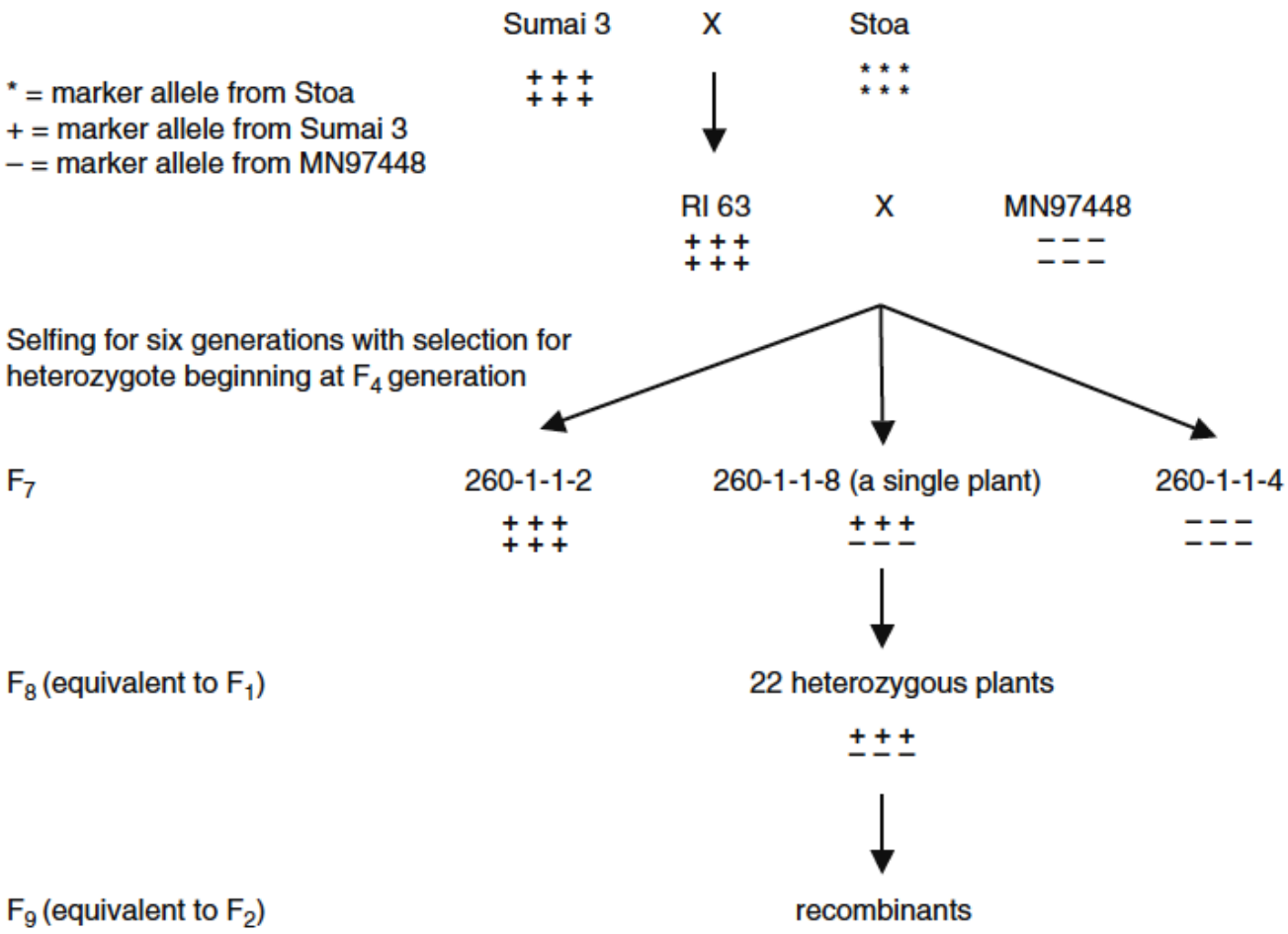


Anderson et al., 2001

Fine mapping of the Chr3B candidate region



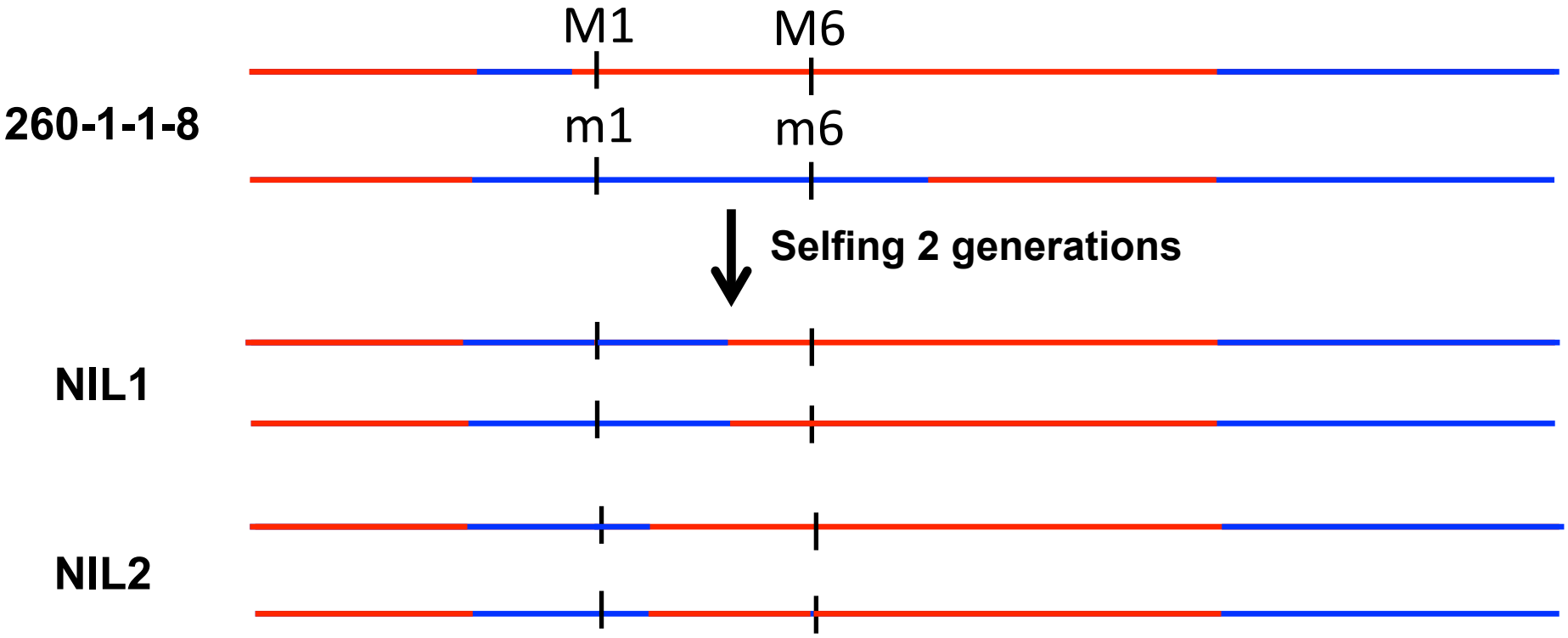
Develop near isogenic lines (NILs) for fine mapping of the Chr3B genomic region



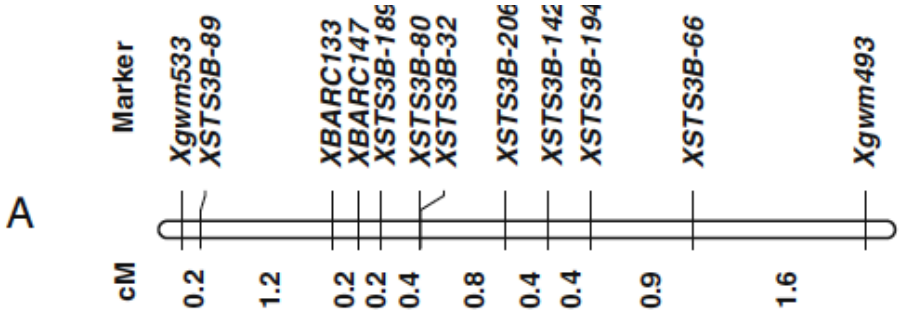
Liu et al., 2006

Develop near isogenic lines (NILs) for fine mapping

- Among the 3,156 plants screened for recombinants with the two SSR marker loci, Xgwm533 and Xgwm493
- Total of 382 recombinants were genotyped with two more SSR markers, BARC133 and BARC147, and eight STS markers



Fine mapping of the Chr3B candidate region



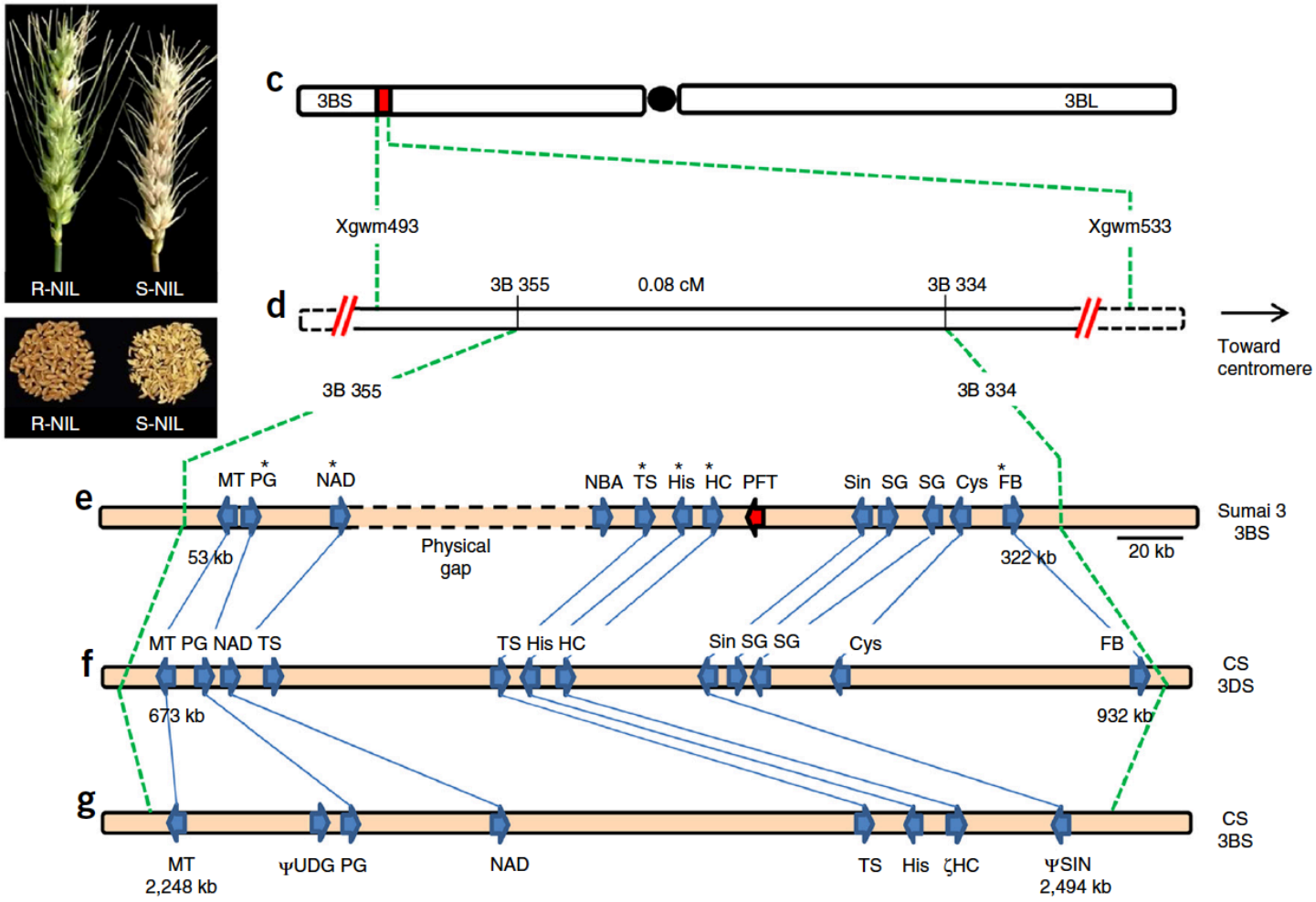
B

Line	%FHB UMN	%FHB KSU
HR4	55 (S)	73 (S)
HR41	40 (S)	
HR46	57 (S)	
HR45	41 (S)	66 (S)
HR56	3 (R)	9 (R)
HR68	6 (R)	
HR63	4 (R)	
HR74	7 (R)	
HR11	4 (R)	
HR20	5 (R)	
HR26	7 (R)	
HR37	37 (S)	70 (S)
HR77	49 (S)	
HR78	53 (S)	
HR85	61 (S)	76 (S)
260-1-1-2	5 (R)	10 (R)
260-1-1-4	47 (S)	64 (S)
$LSD_{0.05}$	13	9

Location of *Fhb1*

Liu et al., 2006

Linkage mapping and map-based clone of *Fhb1* in wheat



Rawat et al., 2016

Selection of *Fhb1* candidate gene

- Six genes (*) of the thirteen had been ruled out previously by gene complementation
- Expression analyses were performed in spikes, inoculated with *Fusarium* macroconidia, of resistant near-isogenic line (R-NIL) and susceptible NIL (S-NIL). The genes *PFT* and *NBA* were expressed only in R-NIL and not in S-NIL, whereas the other genes had similar expression patterns in both the NILs
- *NBA* was present in a susceptible haplotype containing cultivars Nanda 2419, Jingzhou 1 and Emai 6
- Therefore, excluded *NBA* and considered *PFT* as the putative candidate for *Fhb1*

Rawat et al., 2016

Validation of the *Fhb1* candidate gene, *PFT*

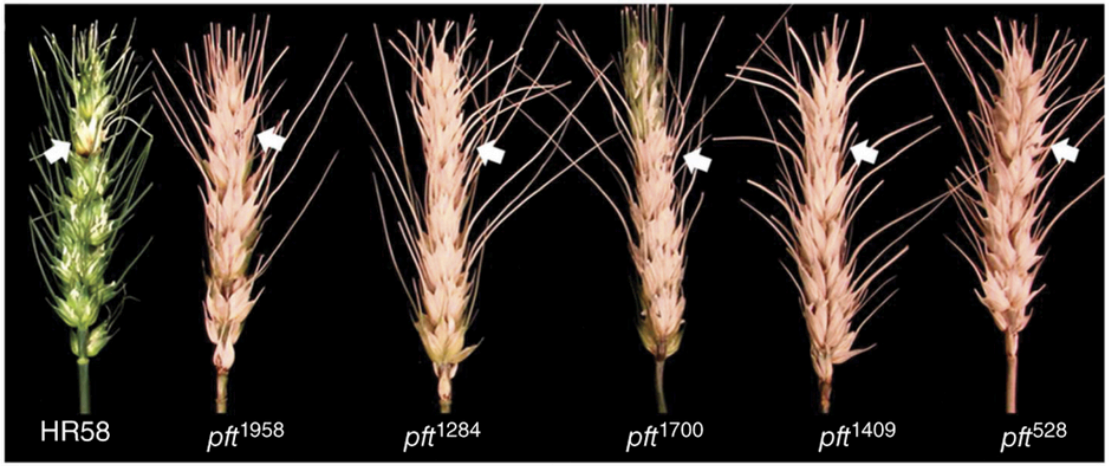
- *PFT* is a 3,472-bp gene with two exons generating a 1,437-bp mRNA

1. Assessed the candidacy of *PFT* for *Fhb1* using induced mutants
2. Gene silencing by using RNA interference (RNAi)
3. Gene complementation by transformation

Rawat et al., 2016

EMS induced mutants of the gene *PFT*

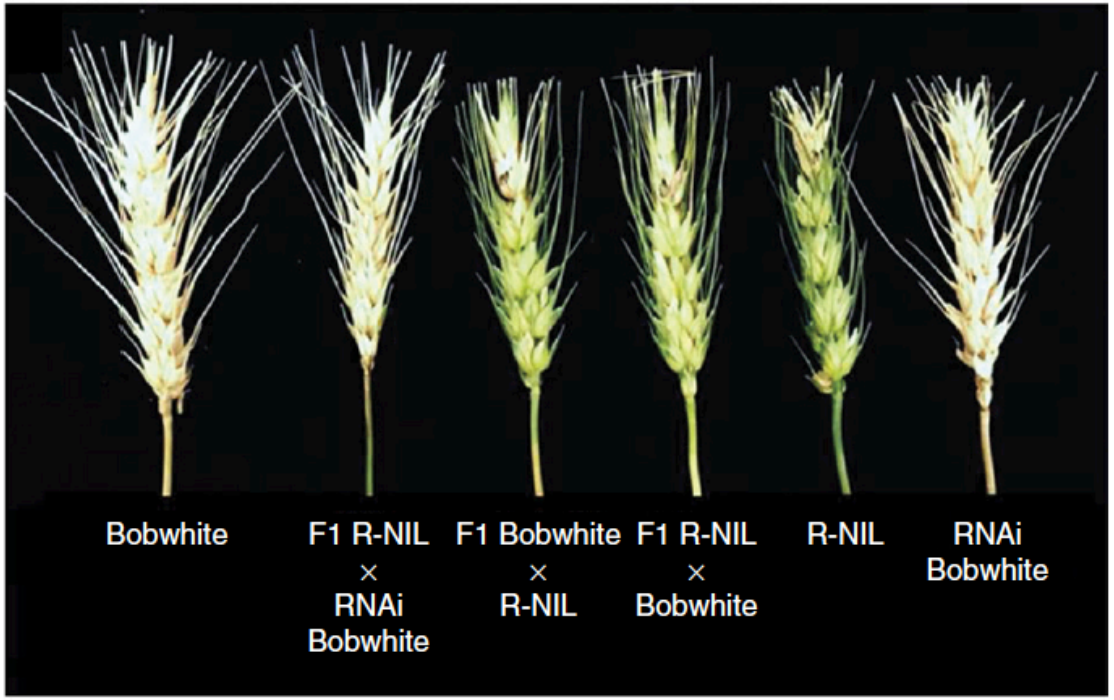
- Five mutations of *PFT* caused the plants to be susceptible to FHB
- Sequencing of exons of all the other genes in the *Fhb1* region in the susceptible mutants revealed no mutations



Rawat et al., 2016

Gene silencing with small RNA

- Sumai3 and the R-NIL with *Fhb1* were not amenable to tissue culture and thus, were not responsive to transformation
- F1 plants from reciprocal crosses of the R-NIL and Bobwhite



Rawat et al., 2016

Validation of *PFT* using transgenic lines

- Generated transgenic plants expressing the *PFT* gene in wheat cultivar Fielder

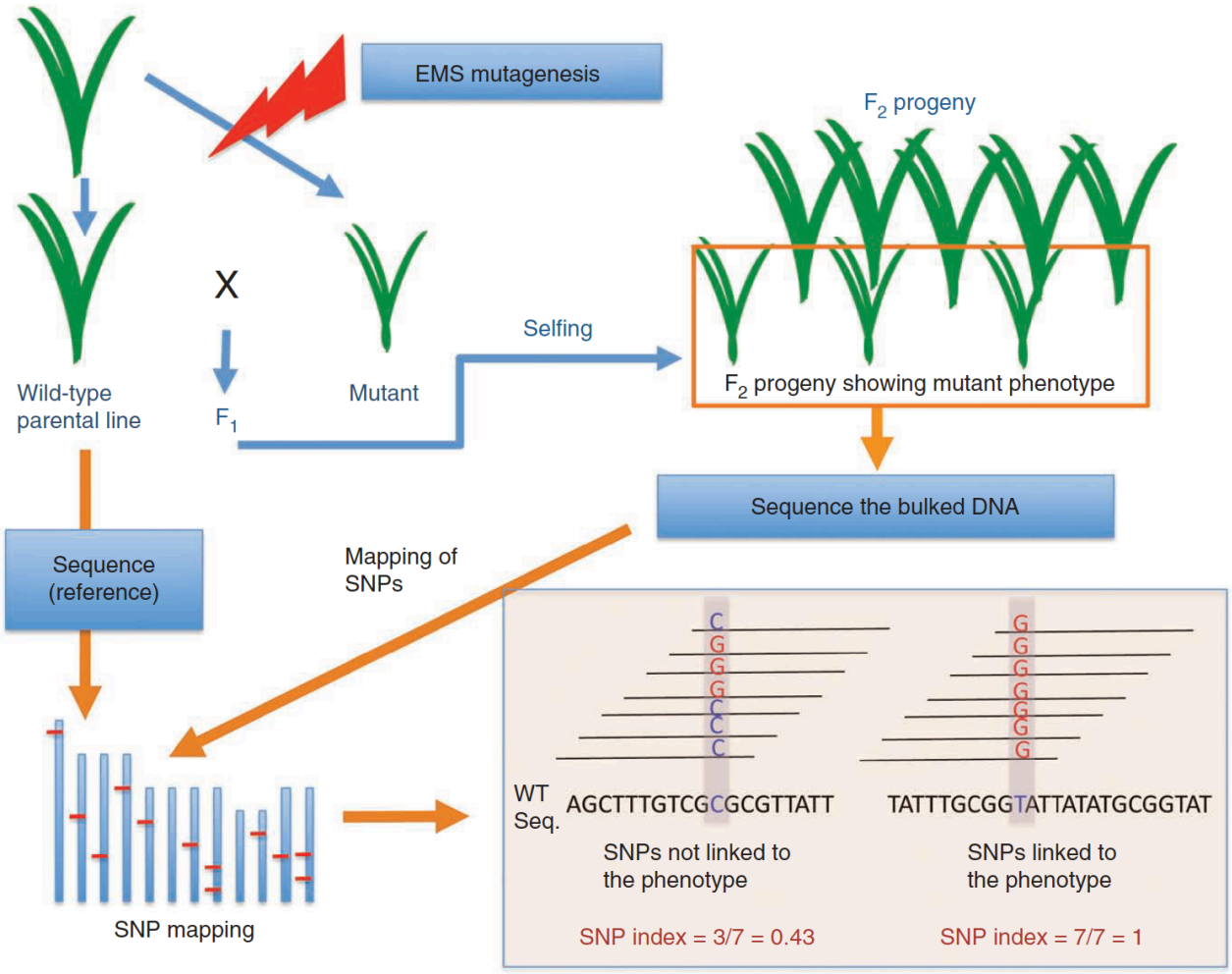
Rawat et al., 2016

Linkage mapping using a bi-parental mapping population to clone gene

- Initial linkage mapping
  1) Create a segregating population for a target trait using two parents
  2) Phenotype the population for the target trait
  3) Genotype the population for molecular markers and construct linkage map
  4) Perform marker-trait statistical analysis to find markers linked to the causal gene
- Fine mapping
- Candidate gene validation

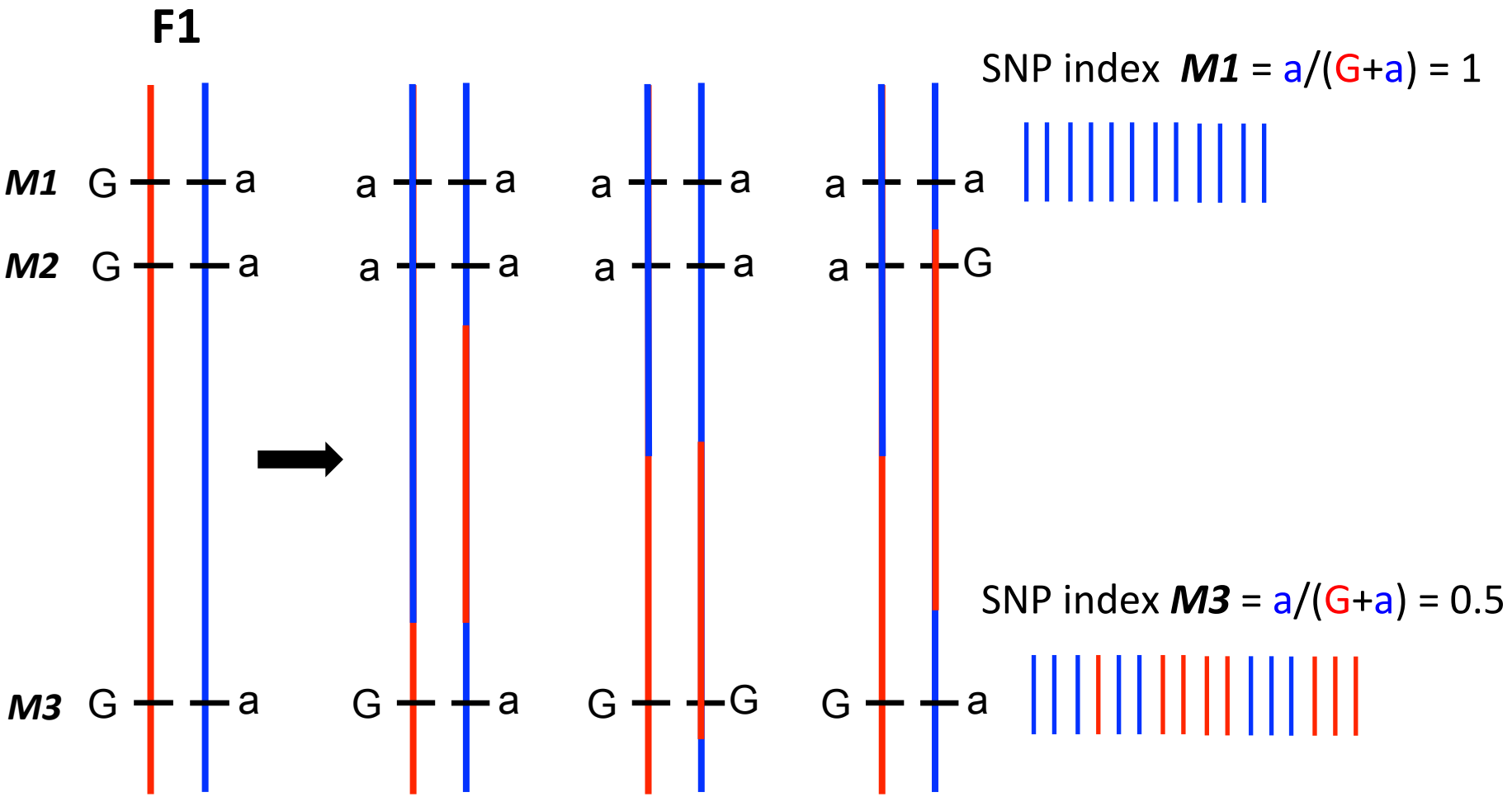
MutMap: rapid gene isolation using a cross between a mutant and wild type

- Rice: about 2,000 point mutations per mutant induced by EMS



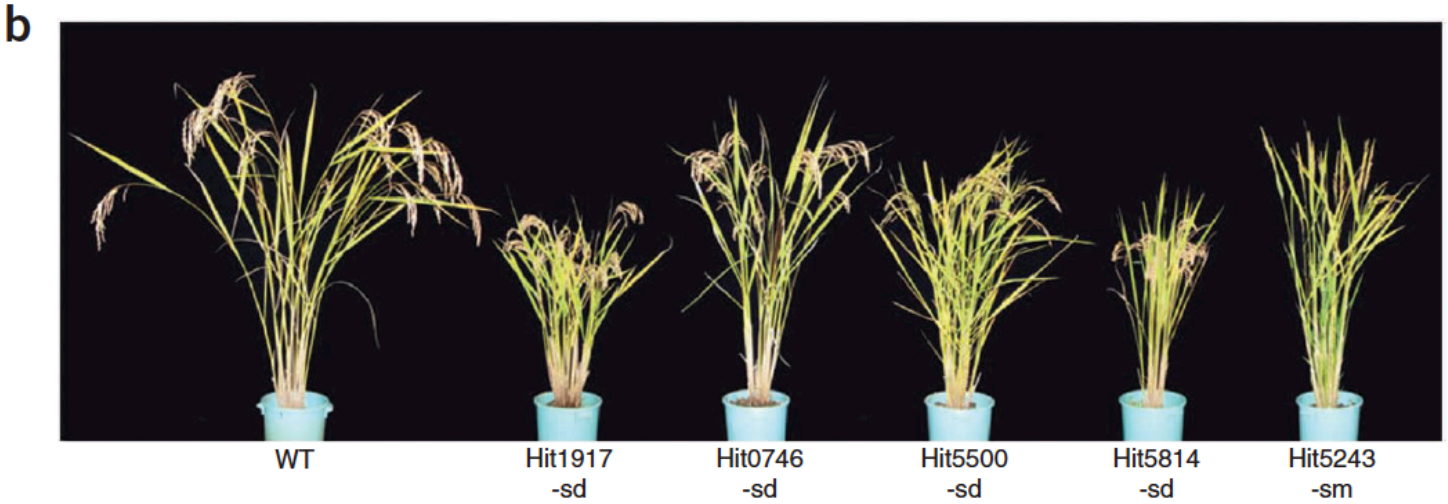
Abe et al., 2012

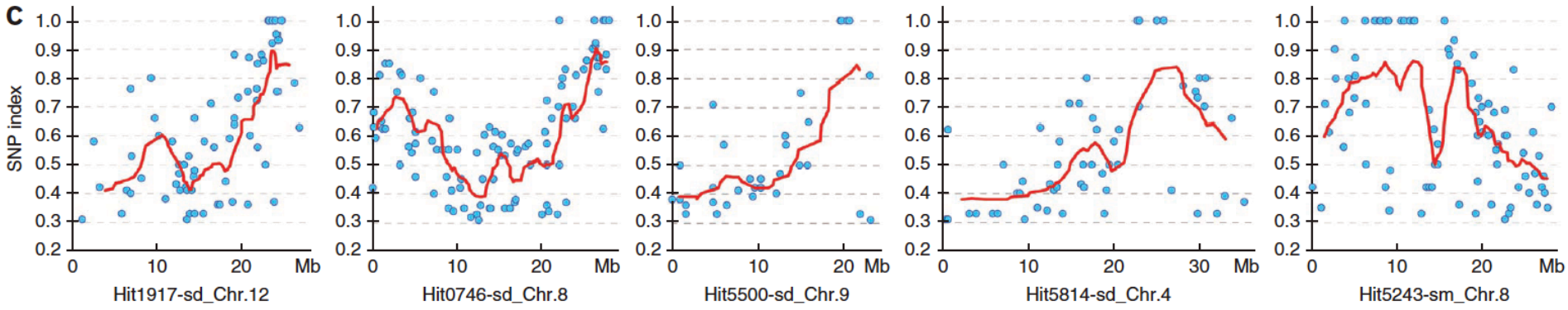
SNP index is frequency of mutant allele in a group of individuals with mutant phenotype a/(G+a) If the causal mutation is ***M1***



Abe et al., 2012

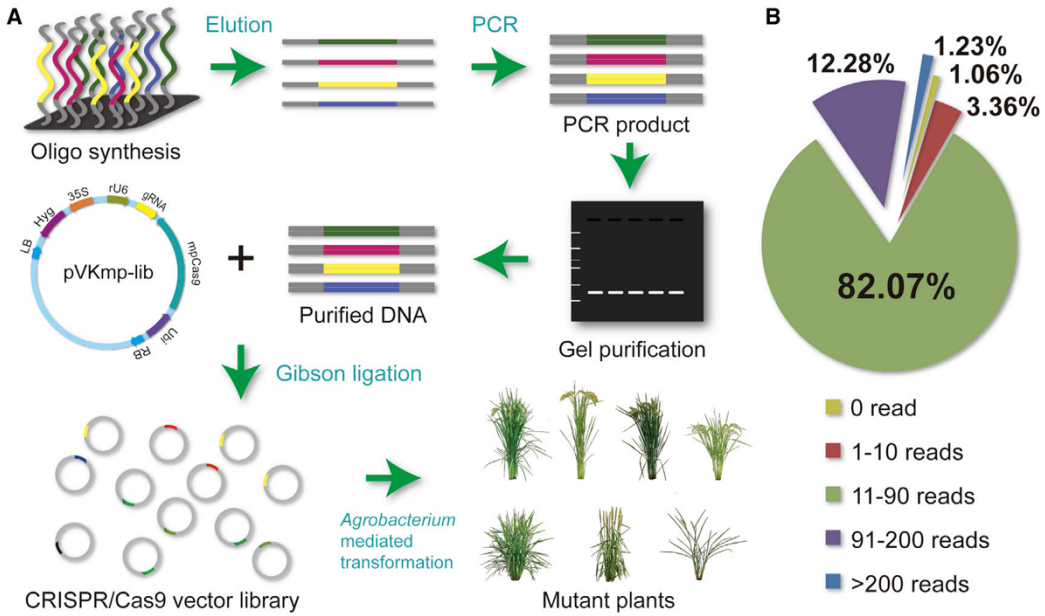
Identification of genomic regions harboring causal mutations for five rice mutants using MutMap

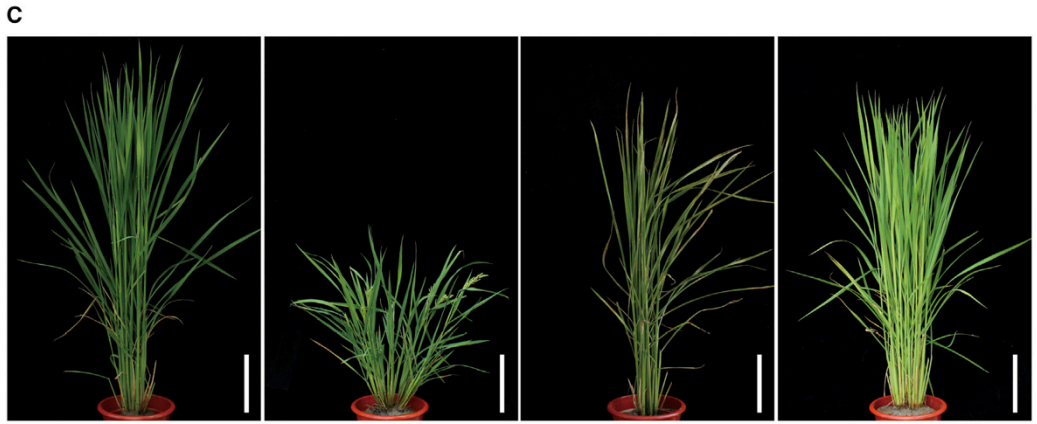




Abe et al., 2012

Construction of a genome-wide mutant library in Rice using CRISPR/Cas9





Meng et al., 2017

What need to know for final exam

- What is linkage mapping?
- What is fine mapping?
- Basic procedure of linkage mapping and fine mapping
- Methods used in validation of a candidate gene
- Basic procedure of MutMap

References

- Abe, A., Kosugi, S., Yoshida, K., Natsume, S., Takagi, H., Kanzaki, H., Matsumura, H., Yoshida, K., Mitsuoka, C., Tamiru, M. and Innan, H., 2012. Genome sequencing reveals agronomically important loci in rice using MutMap. *Nature biotechnology*, *30*(2), pp.174-178.
- Anderson, J. A., Stack, R. W., Liu, S., Waldron, B. L., Fjeld, A. D., Coyne, C., ... & Frohberg, R. C. (2001). DNA markers for Fusarium head blight resistance QTLs in two wheat populations. *Theoretical and Applied Genetics*, *102*(8), 1164-1168.
- Galagedara, N., Liu, Y., Fiedler, J., Shi, G., Chiao, S., Xu, S.S., Faris, J.D., Li, X. and Liu, Z., 2020. Genome-wide association mapping of tan spot resistance in a worldwide collection of durum wheat. *Theoretical and Applied Genetics*, pp.1-11.
- Meng, X., Yu, H., Zhang, Y., Zhuang, F., Song, X., Gao, S., ... & Li, J. (2017). Construction of a genome-wide mutant library in rice using CRISPR/Cas9. *Mol. Plant*, *10*(9), 1238-1241.
- Rawat, N., Pumphrey, M.O., Liu, S., Zhang, X., Tiwari, V.K., Ando, K., Trick, H.N., Bockus, W.W., Akhunov, E., Anderson, J.A. and Gill, B.S., 2016. Wheat Fhb1 encodes a chimeric lectin with agglutinin domains and a pore-forming toxin-like domain conferring resistance to Fusarium head blight. *Nature genetics*, *48*(12), pp.1576-1580.
- Waldron, B.L., Moreno-Sevilla, B., Anderson, J.A., Stack, R.W. and Frohberg, R.C., 1999. RFLP mapping of QTL for Fusarium head blight resistance in wheat. *Crop Science*, *39*(3), pp. 805-811.