Towards high-resolution radiation hybrid-based physical maps of wheat genomes and chromosomes

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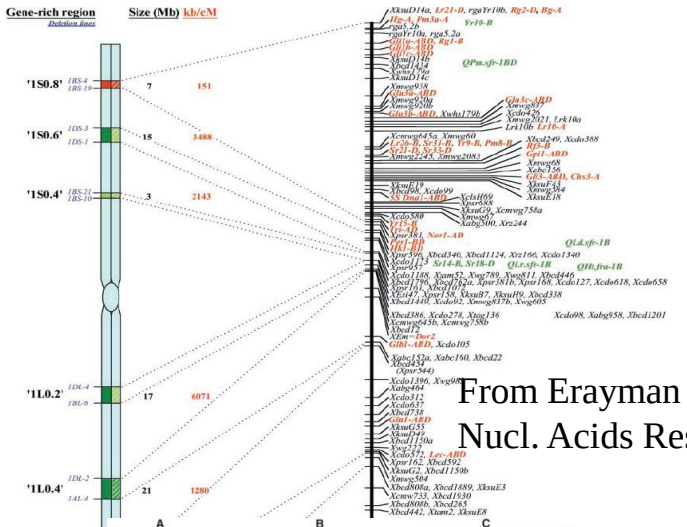
What is Radiation Hybrid (RH) mapping?

Physical mapping based on radiation induced chromosome breakage and a reconstruction of marker order based on their co-retention pattern

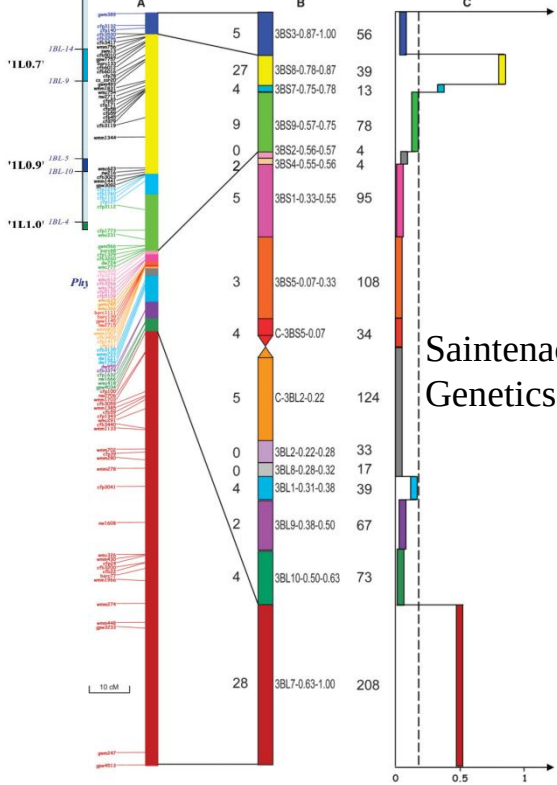
- Radiation breaks chromosomes at random resulting in fragments that carry physically linked loci
- Marker co-retention frequencies (probability of two markers retained or lost with each other) can be used to calculate physical distances

Why RH mapping?

- Non-Polymorphic markers
- Independent of recombinant event
- Higher resolution with small mapping populations
- Resolution can be controlled through radiation doze



From Erayman et al. 2004
Nucl. Acids Res. 32(12)



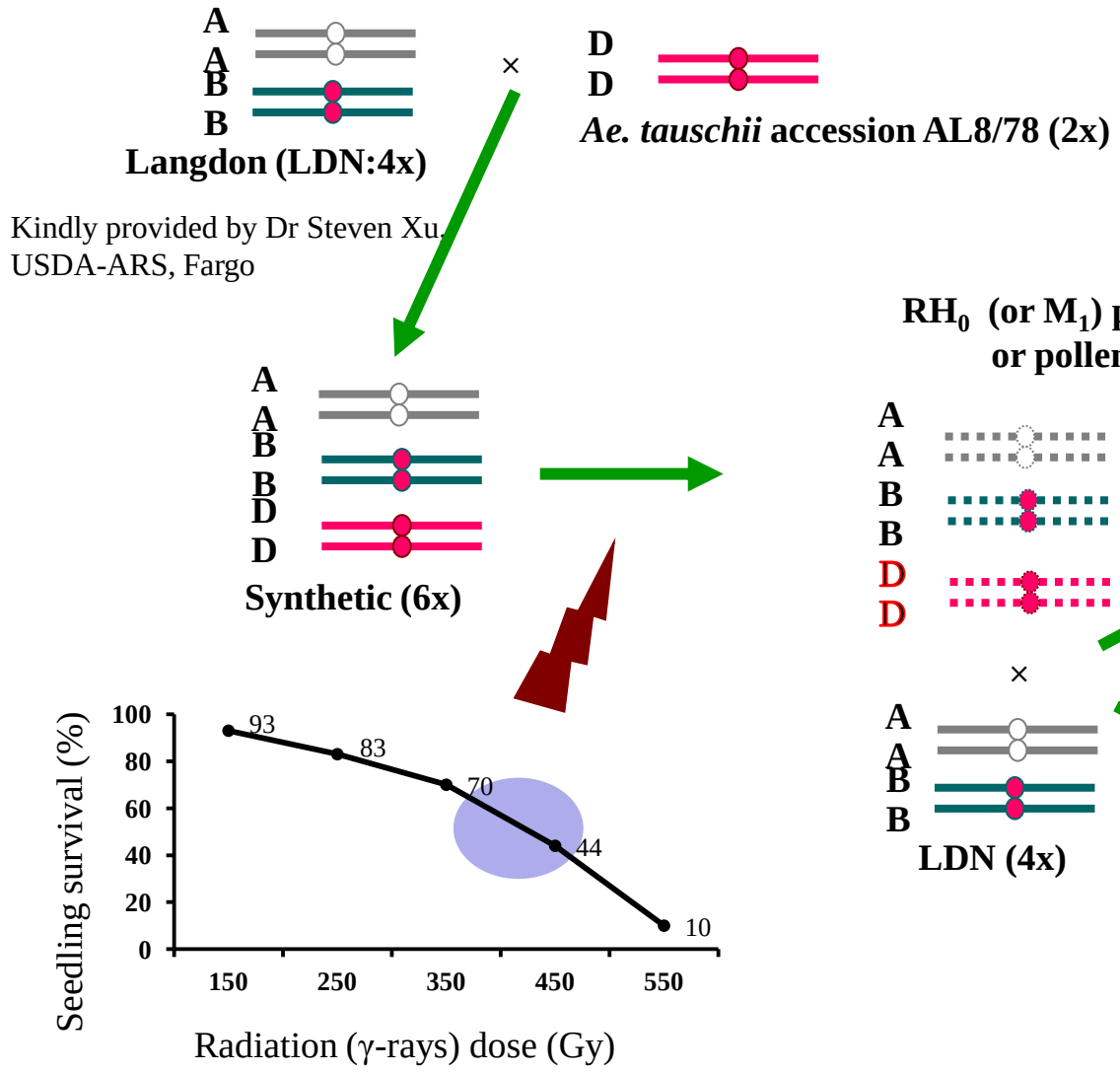
Saintenac et al. 2009
Genetics 181

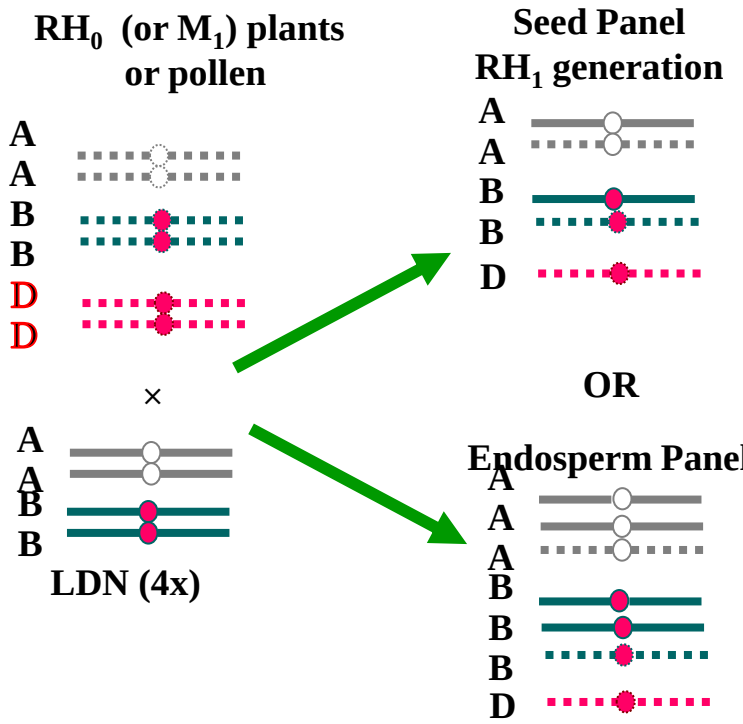
- ~1/4 to 1/3 of the genome around the centromere represents about 1% of recombination on the genetic maps

Important tool for BAC contig alignment prior to 'complete' genome sequencing

Development of high density RH maps for D-genomes of *Aegilops tauschii* accession AL8/78 and Chinese Spring

RH_1 panel development for the D-genome of *Ae. tauschii* accession AL8/78 and Chinese Spring wheat





AL8/78-DGRH$_1$ panel

D-genome RH Project

- Two D-genome Radiation hybrid (DGRH) seed panel:
  - ✓ AL8/78 1600 RH_1 lines generated
  - ✓ CS 2565 RH_1 lines generated

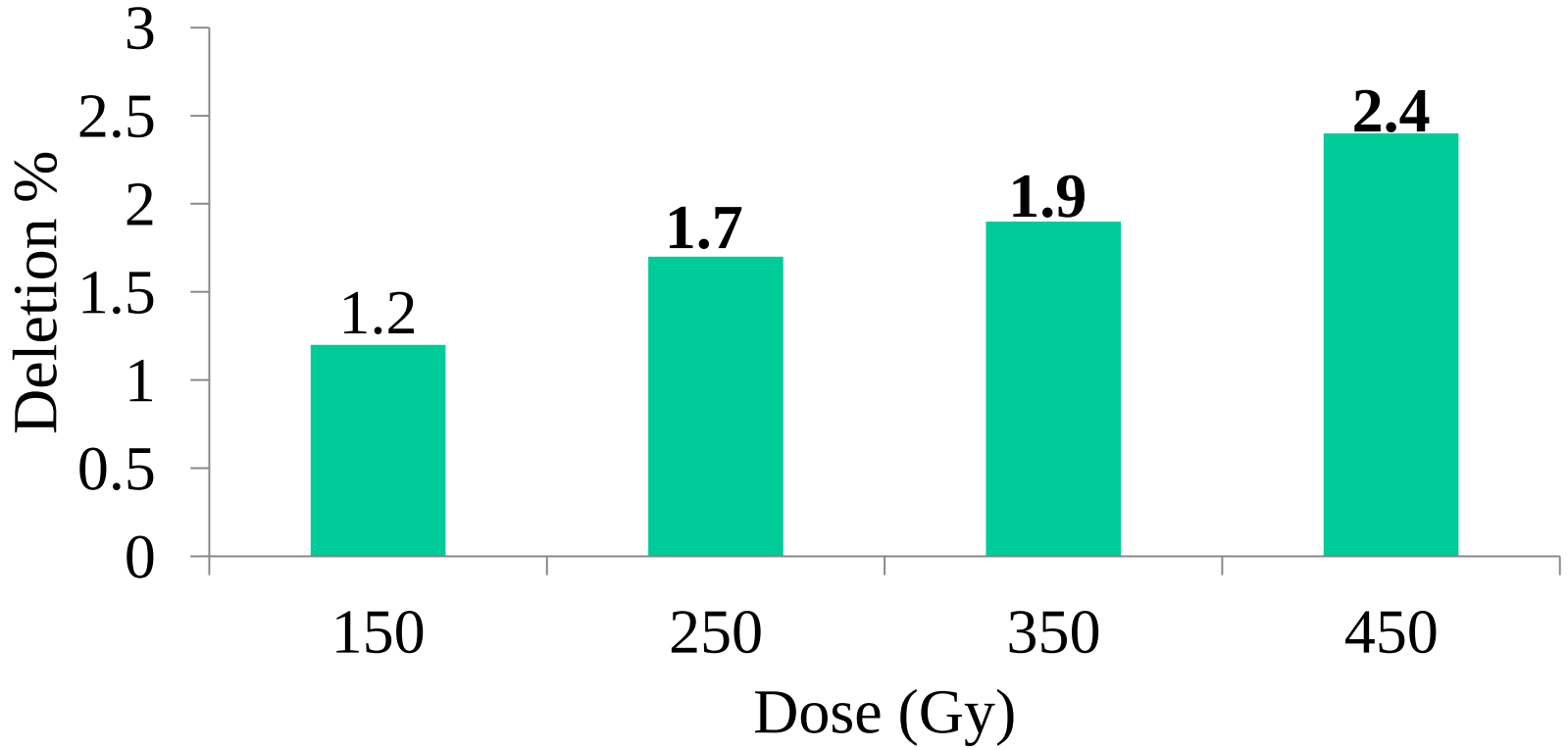
- Several DGRH endosperm & pollen plant panels have been generated

- High-throughput genotyping platforms have been tested (original target 8,000 loci, current target $\geq$ 40,000)

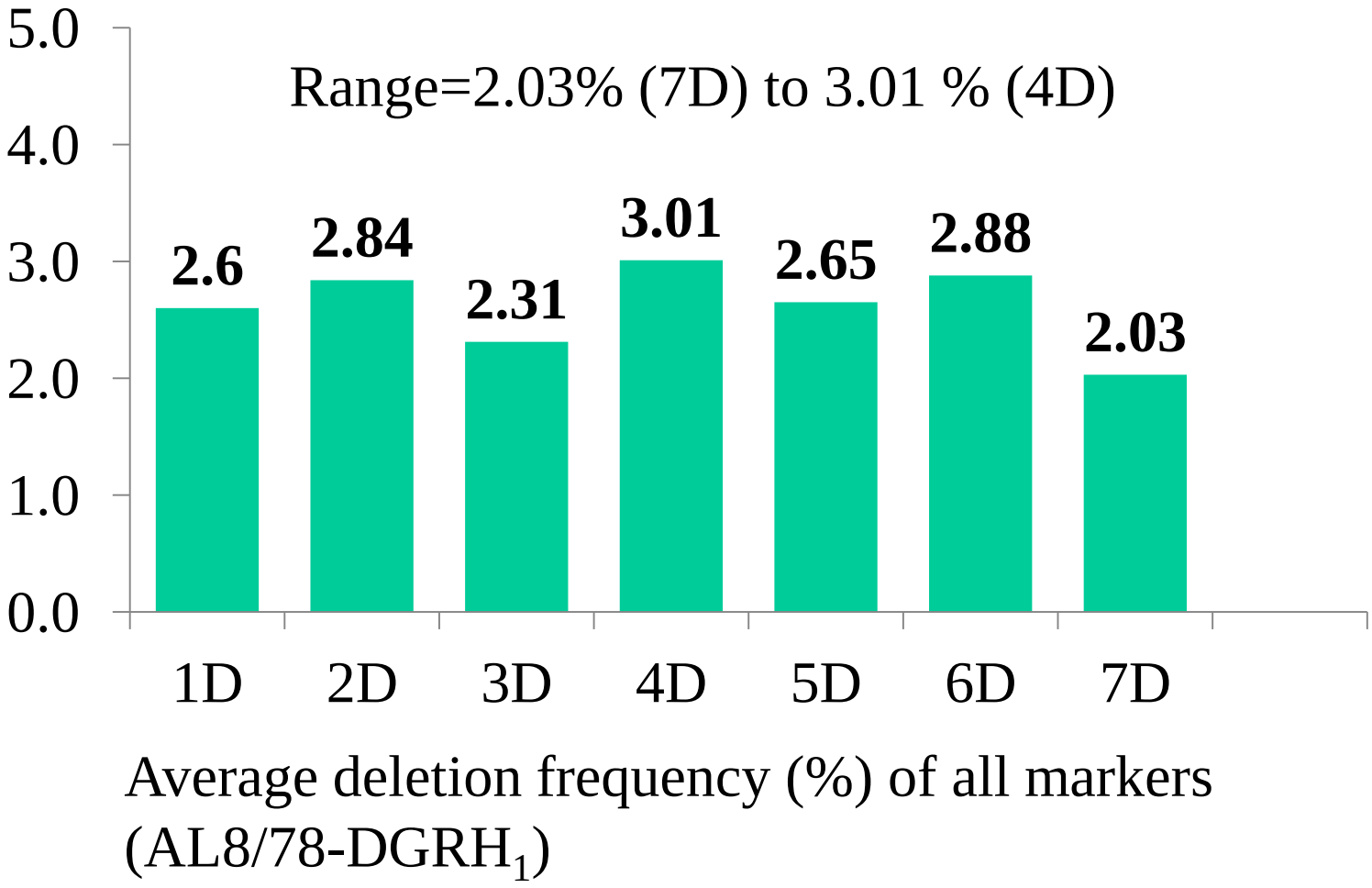
- Bioinformatics tools have been developed to produce RH maps

Characterization of AL8/78-DGRH panel

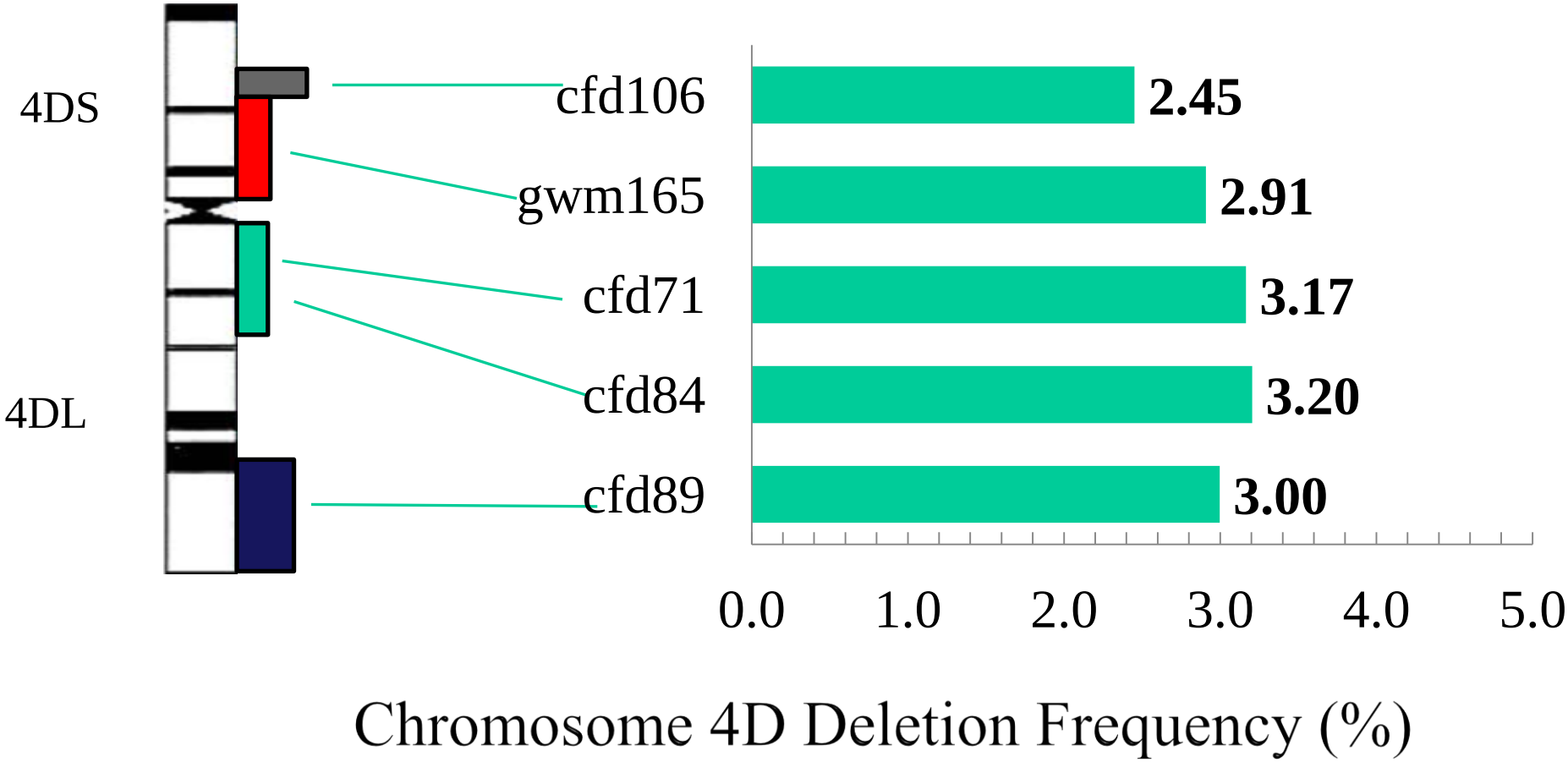
- 35 SSR markers, 5 markers from each of the seven D-genome chromosomes
- Average marker loss= 2.1%



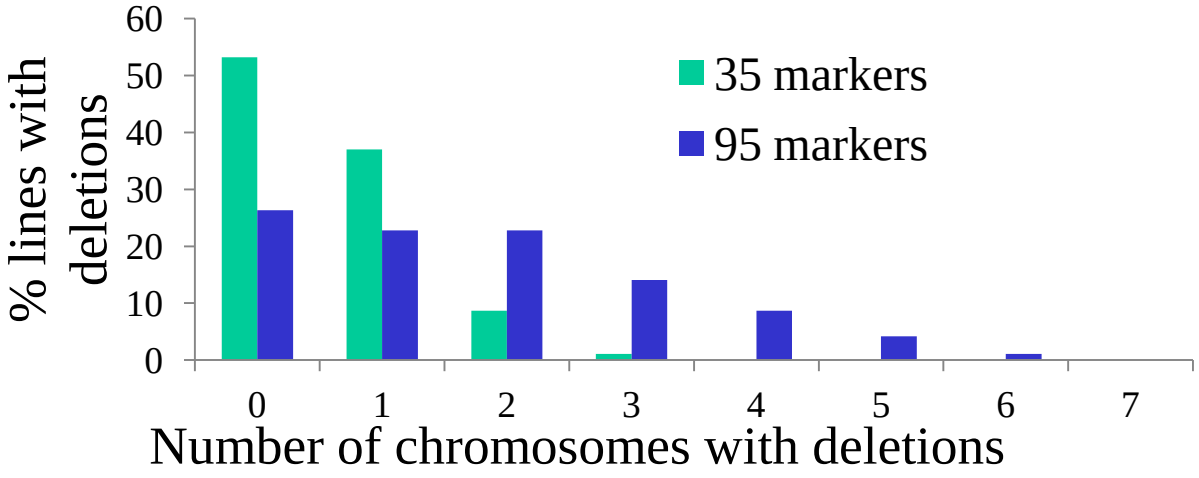
Homogenous marker loss across whole D-genome

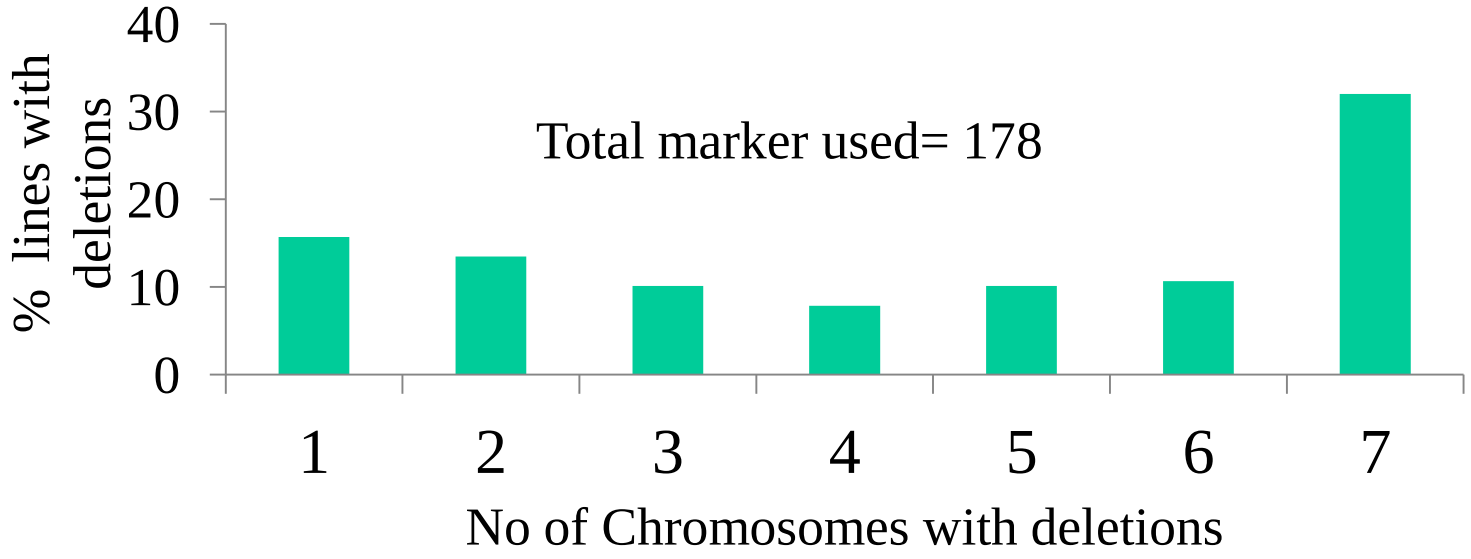


Homogeneous marker loss across the chromosome



Majority of the lines showed deletions for multiple chromosomes





Current status of D-genome RH Project

- Two D-genome Radiation hybrid (DGRH) seed panel:
  - ✓ AL8/78 1600 RH_1 lines generated 368 critical lines selected
  - ✓ CS 2565 RH_1 lines generated 282 critical lines selected

- Several DGRH endosperm & pollen plant panels have been generated

- High-throughput genotyping platforms have been tested (original target 8,000 loci, current target $\geq$ 45,000)

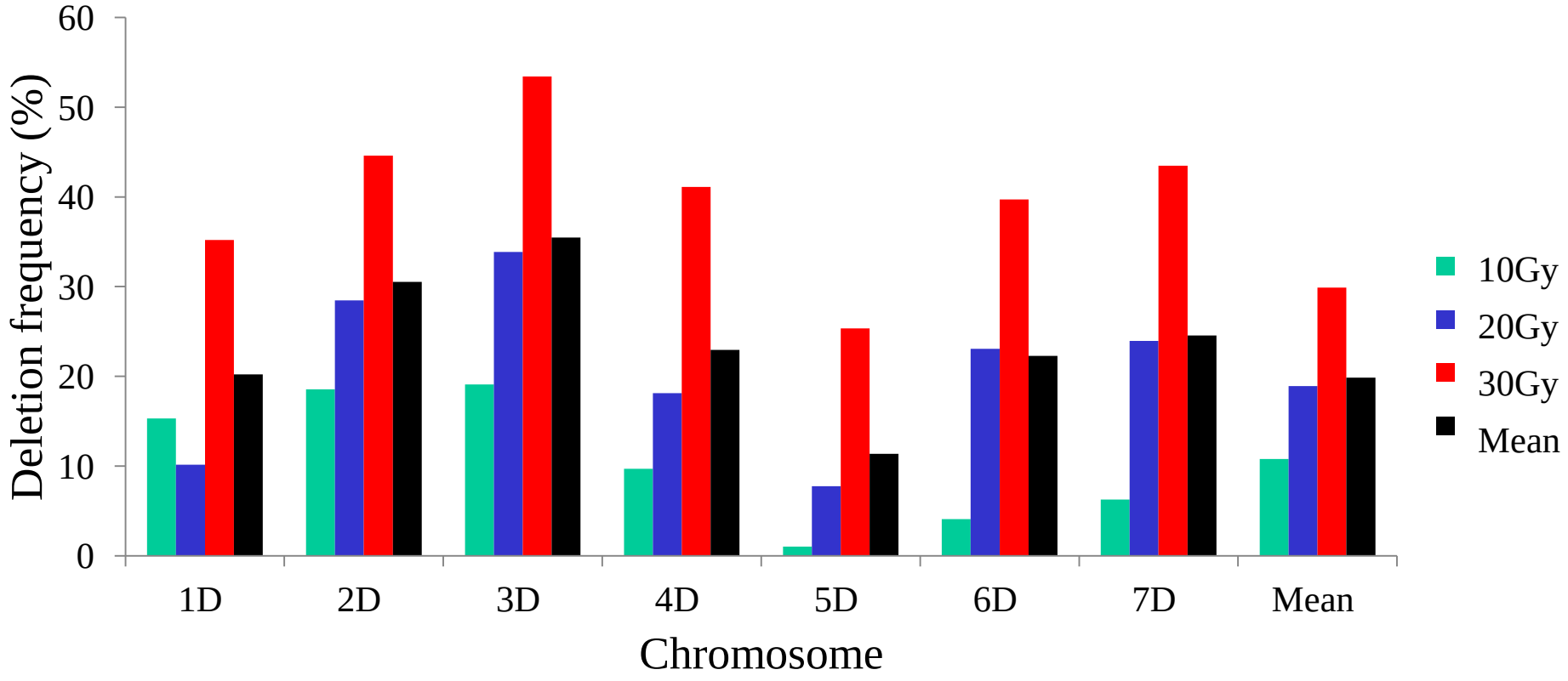
- Bioinformatics tools have been developed to produce RH maps

Additional D-genome RH panels (DGRH)

- DGRH endosperm panel
  - ✓ CS 1,000 samples
  - ✓ AL8/78 640 samples
- DGRH pollen panel
  - ✓ Cs 500

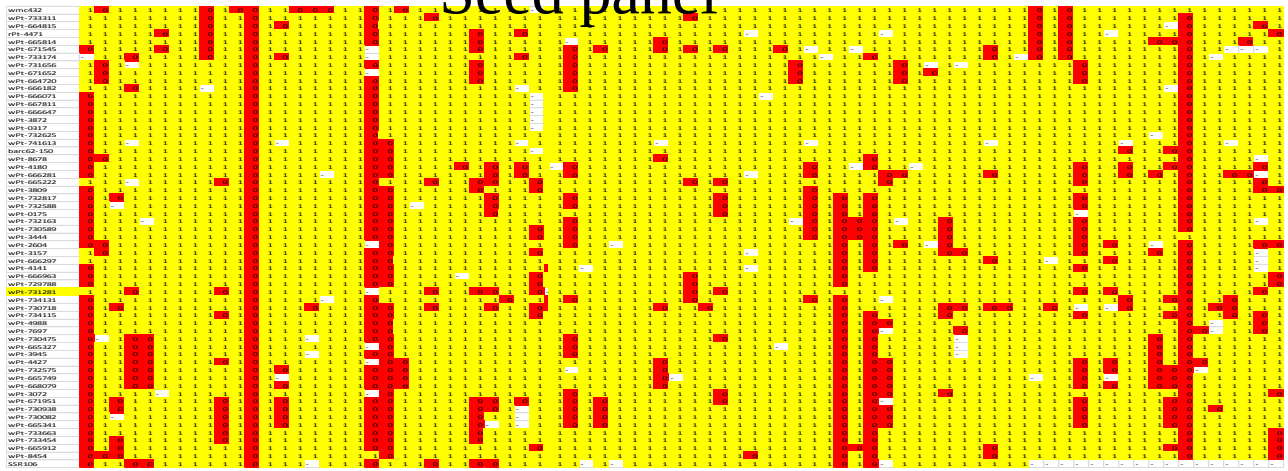
Characterization of endosperm panel

Deletion frequency = 10.8% (10 Gy), 18.9% (20Gy), 29.9% (30GY)

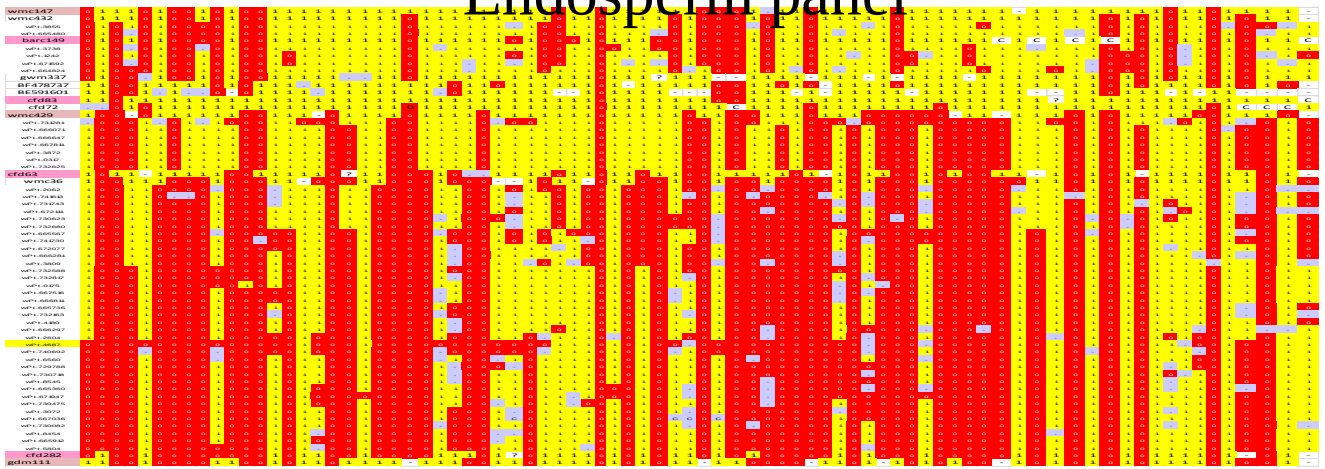


Combination of seed-endosperm panel for complete map

Seed panel



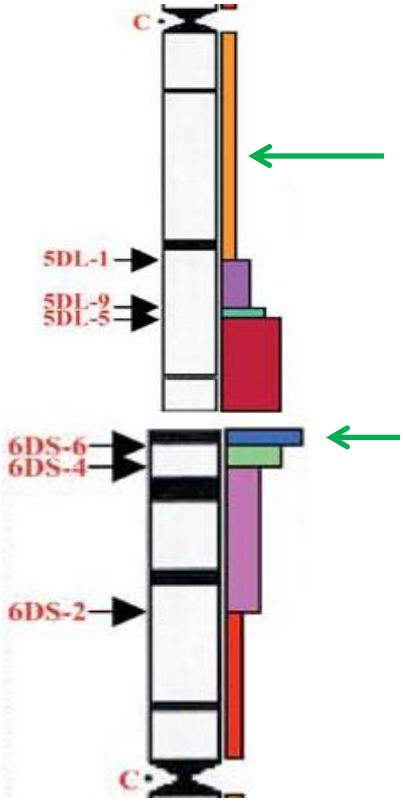
Endosperm panel



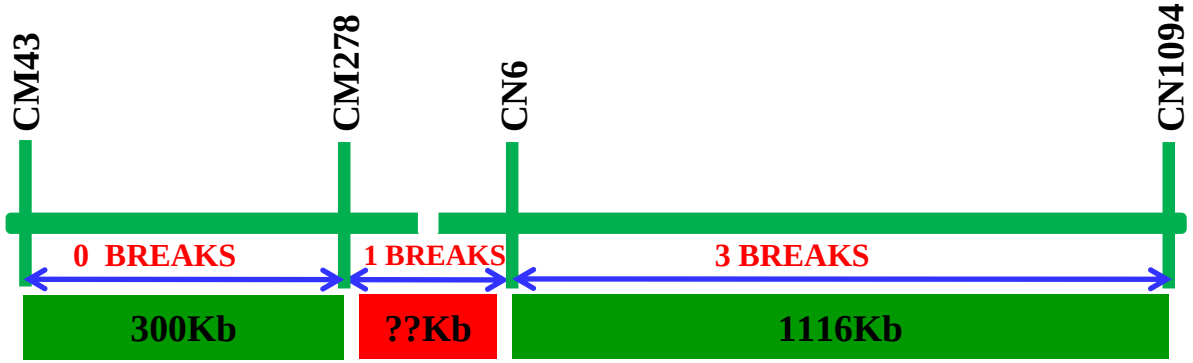
Red= marker absent

Yellow= marker present

Resolution of $DGRH_1$ panels



- Four RH lines with break between these two markers (400kb apart), suggest a resolution of ~100 kb for this panel
- 3 SSRs located in 3.2 Mb region detected 23 obligate breaks
- Mapping resolution of <140 kb.



3DS contig (CS panel)

➢**Average resolution using 282 lines: 372Kb**

DArT based RH maps of D-genome

- A total of 641 and 764 were mapped to the seven D-genome chromosomes of AL8/78 and CS respectively
- Using RH mapping almost 7 times more markers were mapped to D-genome compared to genetic maps

Higher resolution than genetic maps (cR:cM= 17:1)

Table First generation RH maps of all 7 D-genome chromosomes of AL8/78

Chromosome	Markers Mapped	Unique Loci	Total Length (cR)	Marker Density (cR/marker)	cR:cM
1D	59	52	1278	24.6	11:1
2D	51	46	1543	33.5	14:1
3D	108	78	2634	33.8	33:1
4D	49	42	1116	26.6	12:1
5D	58	57	1786	31.3	15:1
6D	72	55	1771	32.2	16:1
7D	218	152	3095	20.4	20:1
Average					**17:1**

DArT based RH maps of D-genome

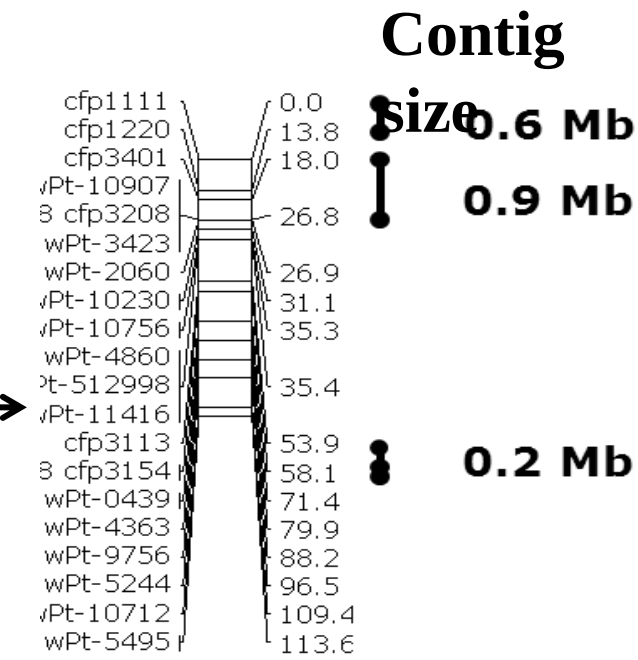
Resolution in centromeric regions = 84 times higher than genetic maps

Chr.	bin	Size (Mb)	length (cR_{2000})	Resolution Mb/cR_{2000}	Resolution (Mb)	Resolution Mb/cM	cR_{2000}/cM ratio
3DS	3DS3-0.24-0.55 to 3DS6-0.55-1.00	243.96	591.5	0.41	0.94	8.1	19.7
3DL	3DL3-0.81-1.00	85.31	303.8	0.28	0.68	2.1	7.5
7DS	7DS4-0.61-1.0	224.25	815.4	0.27	0.75	2.9	10.7
C-7D	C-7DS4-0.36 to C-7DL5-0.30	240.96	813.6	0.29	0.45	11.1	84.2
7DL	7DL3-0.82-1.00	68.6	186.2	0.36	0.74	1.75	4.8

3B-Radaition hybrid map

- 92 3B-RH lines characterized with 540 markers
- **Resolution in centromeric regions = 85.6 times higher than genetic maps**

Chr.	RH map size (cR)	Genetic map size (cM)	Map size ratio (cR cM^{-1})
3BS telom.	79.7	5.2	15.3
3BS	755.7	48.1	15.7
Centrom.	214.7	2.5	85.9
3BL	137.2	11.3	12.1
3BL telom.	459.6	104.2	4.4
3B chrom.	1871.9	179.1	10.5



Kumar et al., accepted BMC Genomics

Current status of D-genome RH panels (DGRH)

Selection of critical lines

Total= 1,231

- DGRH seed panel:
  - ✓ AL8/78 399 critical lines selected
  - ✓ CS 282 critical lines selected

- DGRH endosperm panel:
  - ✓ AL8/78 200 critical lines selected
  - ✓ CS 200 critical lines selected

- DGRH pollen panel:
  - ✓ CS 150 critical lines selected

Current status of D-genome RH Project

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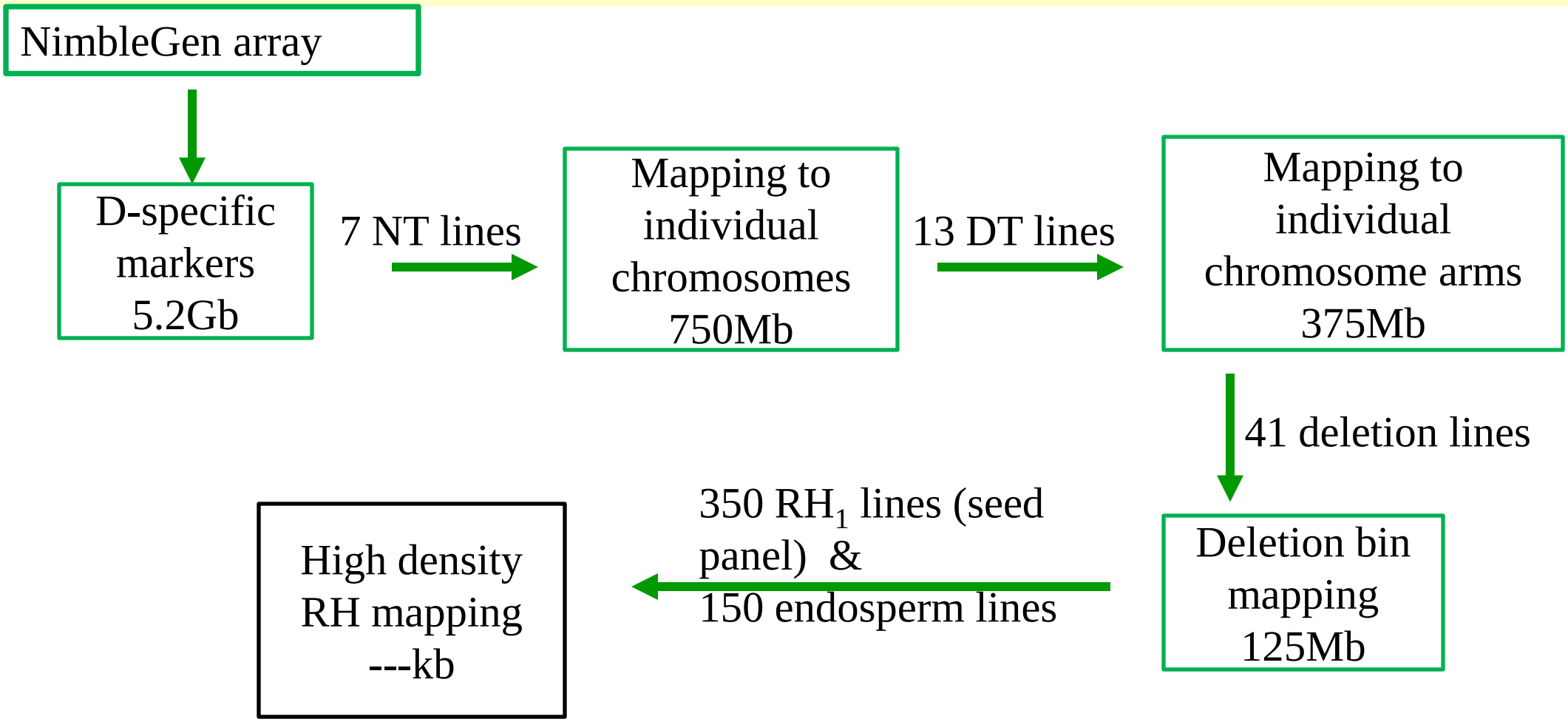
- Several DGRH endosperm & pollen plant panels have been generated

- High-throughput genotyping platforms have been tested (original target 8,000 loci, current target $\geq$ 45,000)

- Bioinformatics tools have been developed to produce RH maps

Mapping approach



NimbleGen array for D-genome
~45,000 markers

- 30,900 wheat repeat junction markers specific to D-genome

- 15,324 gene markers representing 6,330 genes specific to D-genome

- 23,891 markers mapped to individual chromosomes, chromosome arms and deletions bins using NT, DT and deletion lines

- ~1,200 selected RH lines are being genotyped

Drader et al. IWGSC presentation

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Bioinformatics Tool Development

The resources of D-genome RH project are available for use by the community under MTA

Please feel free to contact us

Radiation hybrids for physical mapping of other chromosomes

Chromosome 3B RH Panel

- 1871.9 cR (92 RH[1])

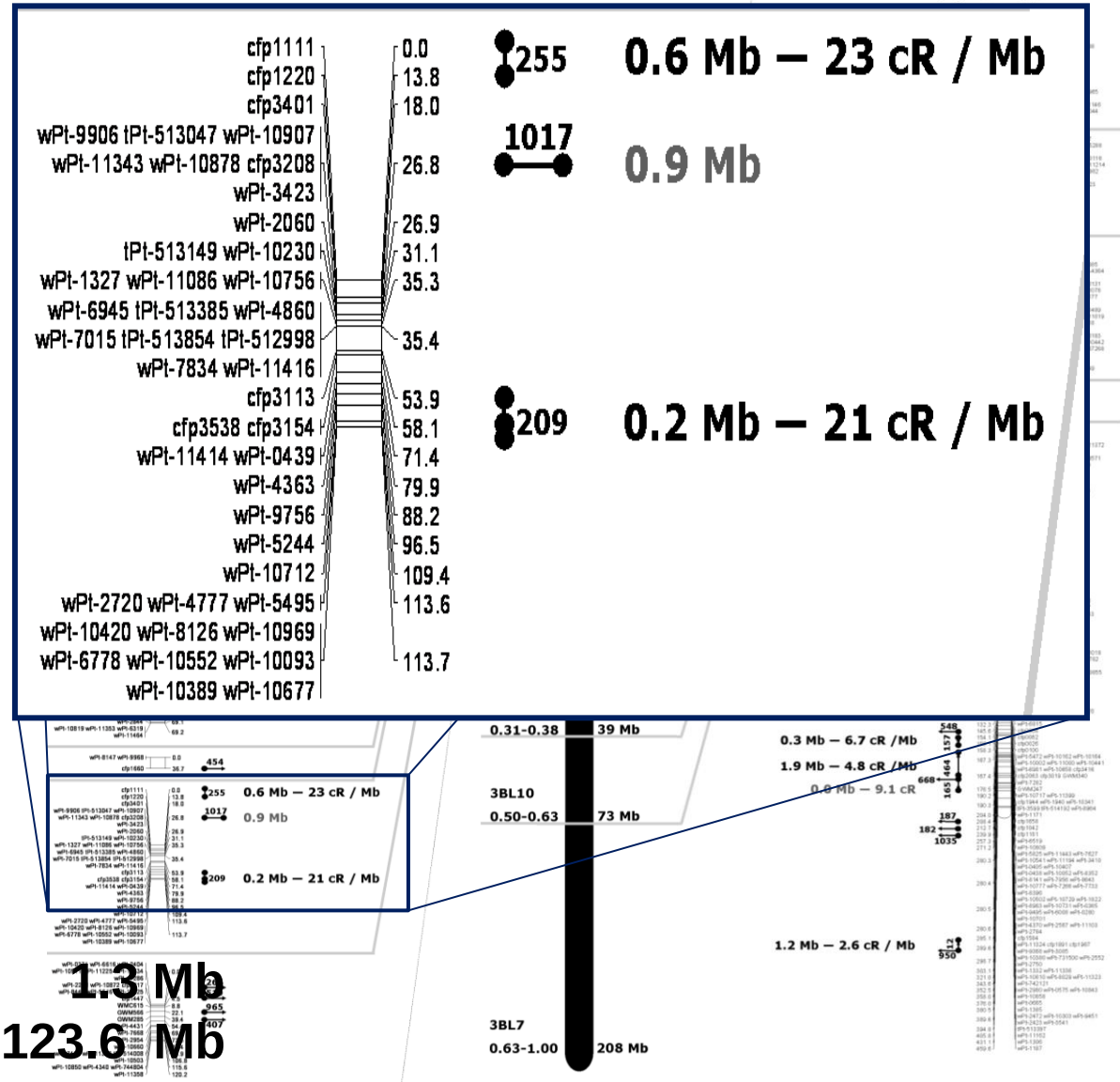
vs

- 179.1 cM (376 F_2)

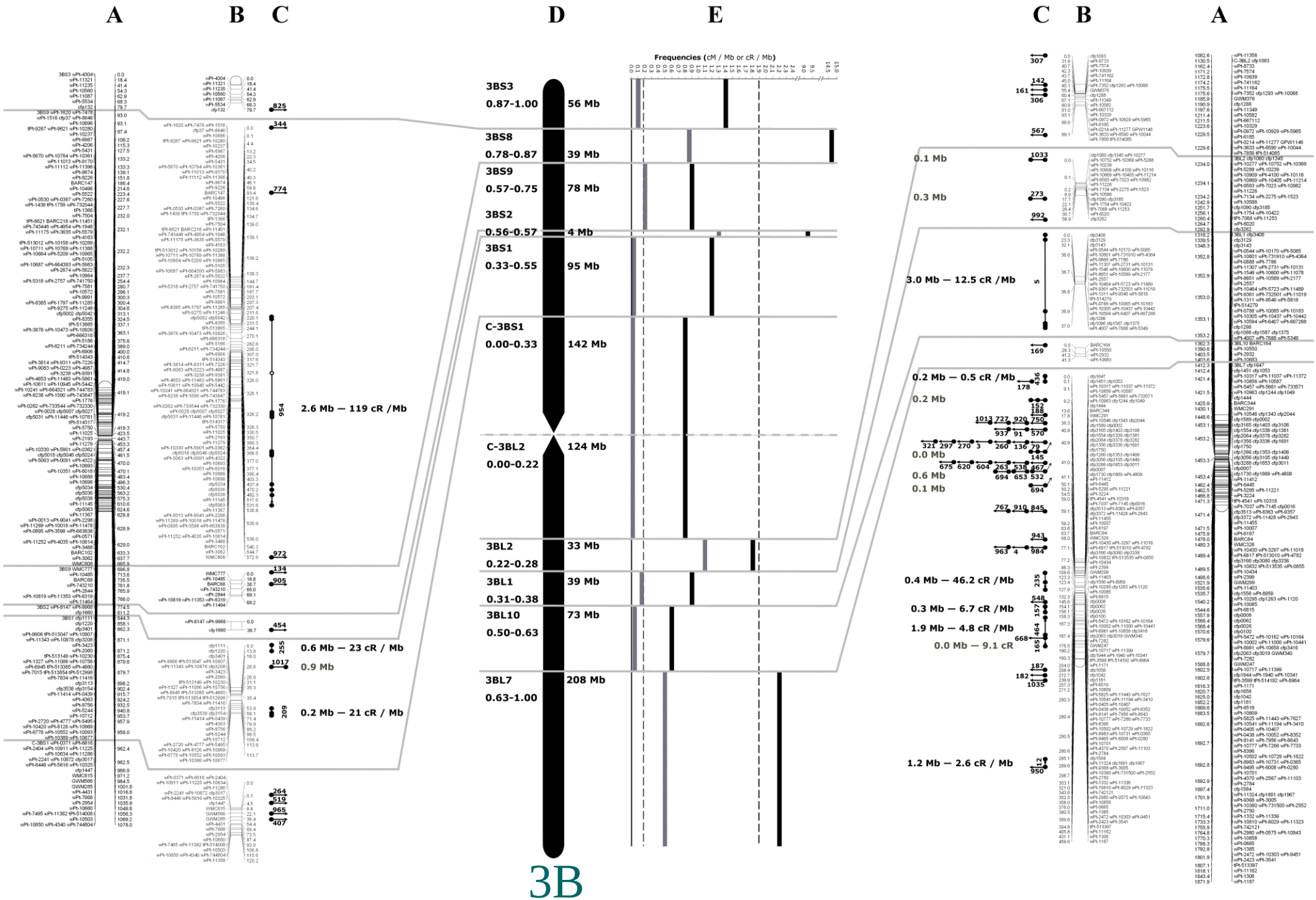
- Contig Orienting > 1 Mb

- 92 RH[1] resolution: 0.5 Mb
- 376 F_2 resolution: 5.5 Mb

Centromere RH[1]: 1.3 Mb
F_2: 123.6 Mb



Institut National de la Recherche Agronomique


A
B
C
D
E
C
B
A
Frequencies (cM / Mb or cR / Mb)
3BS3
0.87-1.00
56 Mb
3BS8
0.78-0.87
39 Mb
3BS9
0.57-0.75
78 Mb
3BS2
0.56-0.57
4 Mb
3BS1
0.33-0.55
95 Mb
C-3BS1
0.00-0.33
142 Mb
C-3BL2
0.00-0.22
124 Mb
3BL2
0.22-0.28
33 Mb
3BL1
0.31-0.38
39 Mb
3BL10
0.50-0.63
73 Mb
3BL7
0.63-1.00
208 Mb
2.6 Mb — 119 cR /Mb
0.6 Mb — 23 cR / Mb
0.9 Mb
0.2 Mb — 21 cR / Mb
0.1 Mb
0.3 Mb
3.0 Mb — 12.5 cR /Mb
0.2 Mb — 0.5 cR /Mb
0.2 Mb
0.0 Mb
0.6 Mb
0.1 Mb
0.4 Mb — 46.2 cR /Mb
0.3 Mb — 6.7 cR /Mb
1.9 Mb — 4.8 cR /Mb
0.0 Mb — 9.1 cR
1.2 Mb — 2.6 cR /Mb
3B

Radiation hybrid panel for 7B physical mapping

Irradiate seeds at 25,35,45 krad

Langdon 7D(7B) × Langdon

Chr 1-6: AABB Chr 1-7: AABB

Chr 7: AADD

1200 RH_1 panel for chromosome 7B

Chr 1-6: AABB

Chr 7 : AABD

300RH_1 lines with retention frequency 10-90% were seelected

RH panel for A and B-genome of wheat

Seed panel (Total) = 1373 RH_1 lines

-1136 (350Gy)

- 101 (450 Gy)

Deletion frequency = 1.7%

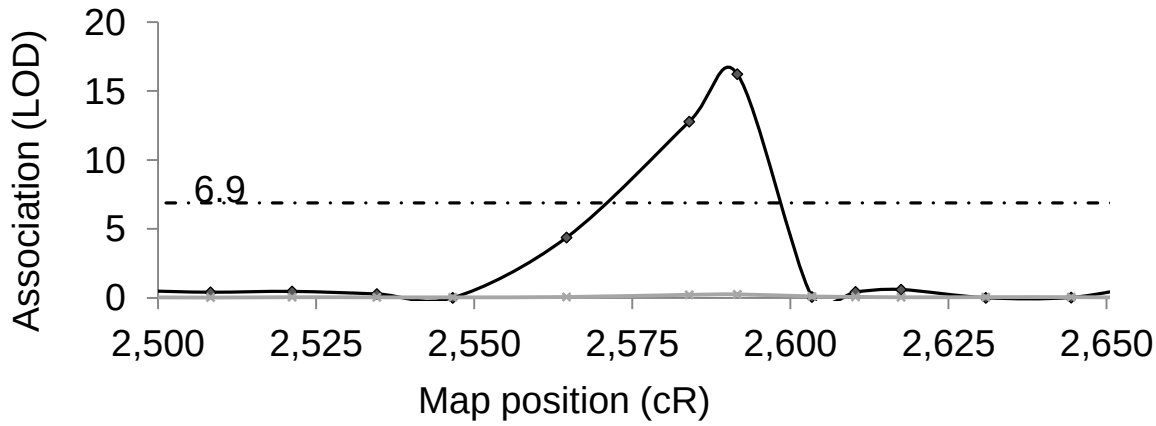
Pollen panel (Total) = 180 RH_1 lines

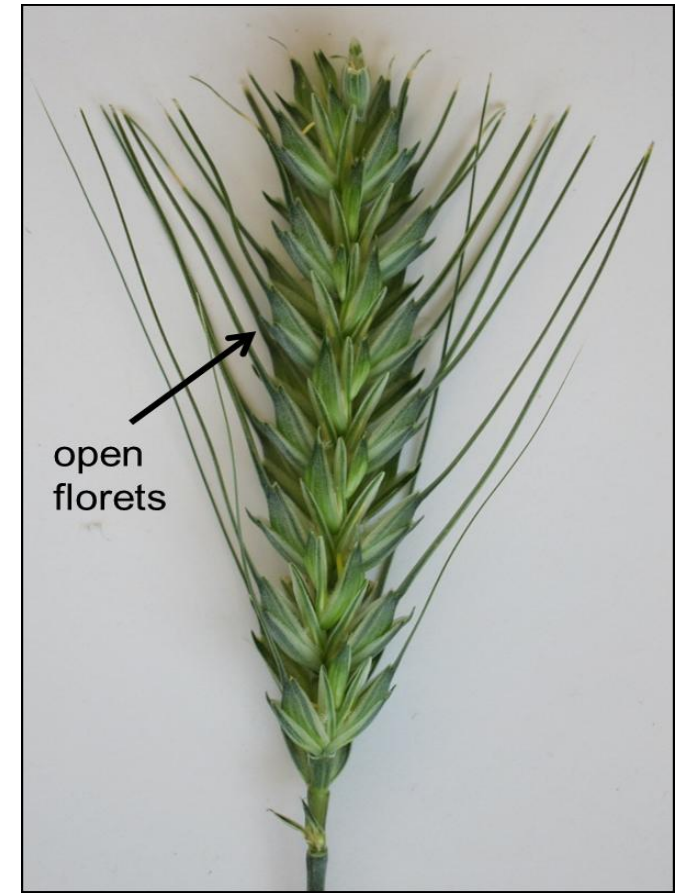
Deletion frequency = 9.6 % (10Gy) and 14.5 (15 Gy)

Radiation hybrids: application to gene cloning

3B-radiation hybrids for mapping/cloning gene for sterility

- 696 3B-RH lines characterized with 140 DArT markers
- 336 selected lines provide a calc. 50 kb resolution

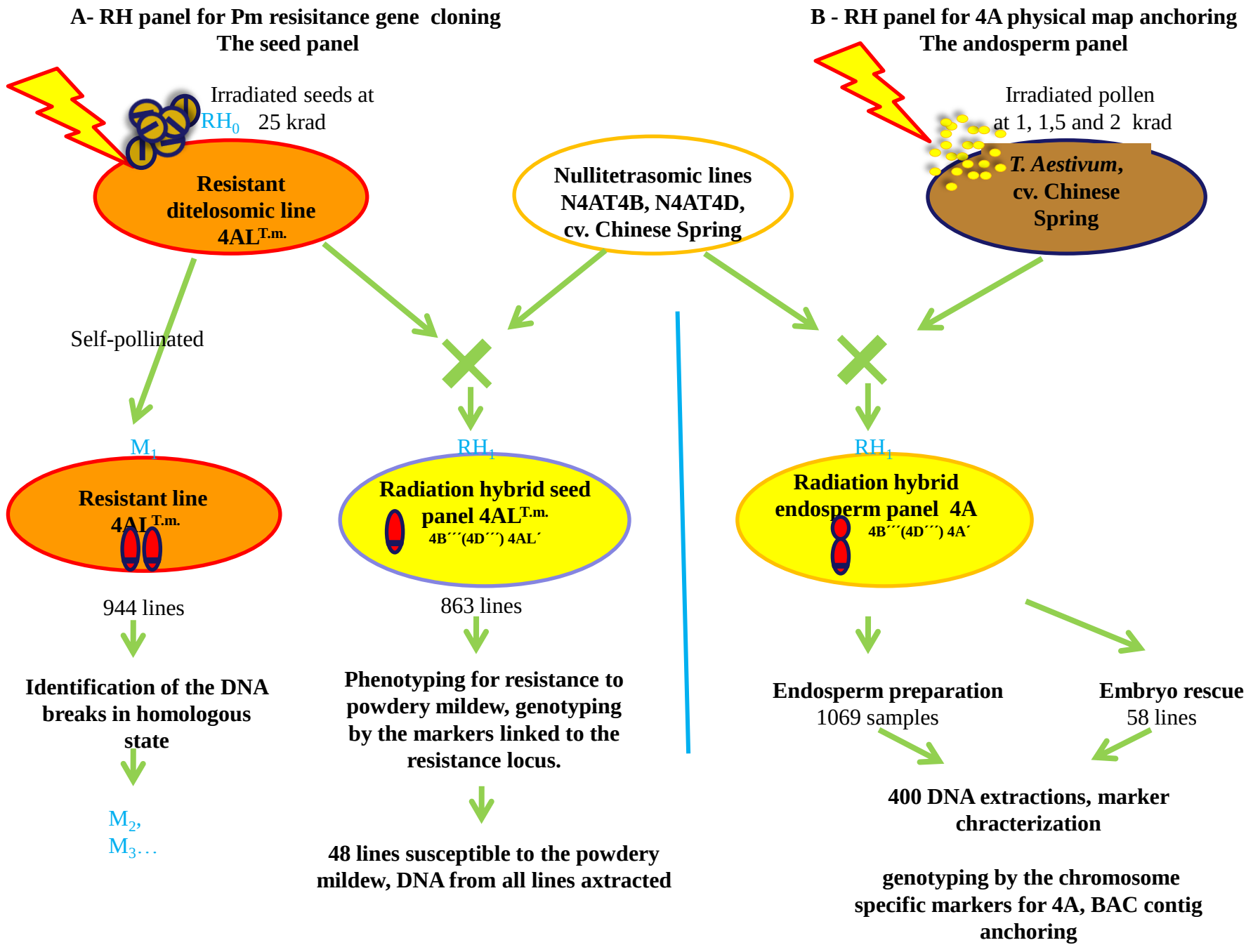




4A specific radiation hybrid panels for cloning of powdery mildew resistance gene *QPm-tut-4A* and 4A specific physical maps anchoring

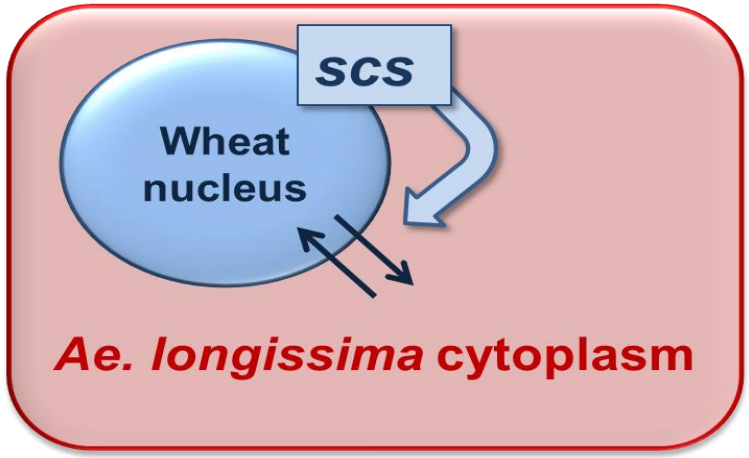
Monika Kladivová[1], Ajay Kumar[2], Shahryar F. Kianian[2], Diana Posti[3], Irena Jakobson[3], Hilma Peusha[3], Kadri Järve[3], Ljudmilla Timofejeva[3], Barbora Klocová[1], Jaroslav Doležel[1], Miroslav Valárik[1]

[1] Centre of the Region Haná, Institute of Experimental Botany, Sokolovská 6, Olomouc 77200, Czech Republic
[2] Department of Plant Sciences, North Dakota State University, Fargo, Loftsgard Hall 470G,ND 58108, USA
[3] Department of Gene Technology, Tallinn University of Technology, Akadeemia tee 15, Tallinn 19086, Estonia


A- RH panel for Pm resisitance gene cloning
The seed panel
Irradiated seeds at
RH0 25 krad
Resistant ditelosomic line 4AL T.m.
Nullitetrasomic lines N4AT4B, N4AT4D, cv. Chinese Spring
B - RH panel for 4A physical map anchoring
The andosperm panel
Irradiated pollen at 1, 1,5 and 2 krad
T. Aestivum, cv. Chinese Spring
Self-pollinated
M1
RH1
RH1
Resistant line 4AL T.m.
Radiation hybrid seed panel 4AL T.m.
4B´´´(4D´´´) 4AL´
Radiation hybrid endosperm panel 4A
4B´´´(4D´´´) 4A´
944 lines
863 lines
Identification of the DNA breaks in homologous state
M2,
M3…
Phenotyping for resistance to powdery mildew, genotyping by the markers linked to the resistance locus.
48 lines susceptible to the powdery mildew, DNA from all lines axtracted
Endosperm preparation
1069 samples
Embryo rescue
58 lines
400 DNA extractions, marker chracterization
genotyping by the chromosome specific markers for 4A, BAC contig anchoring

1D-RH segregates for the *scs* gene

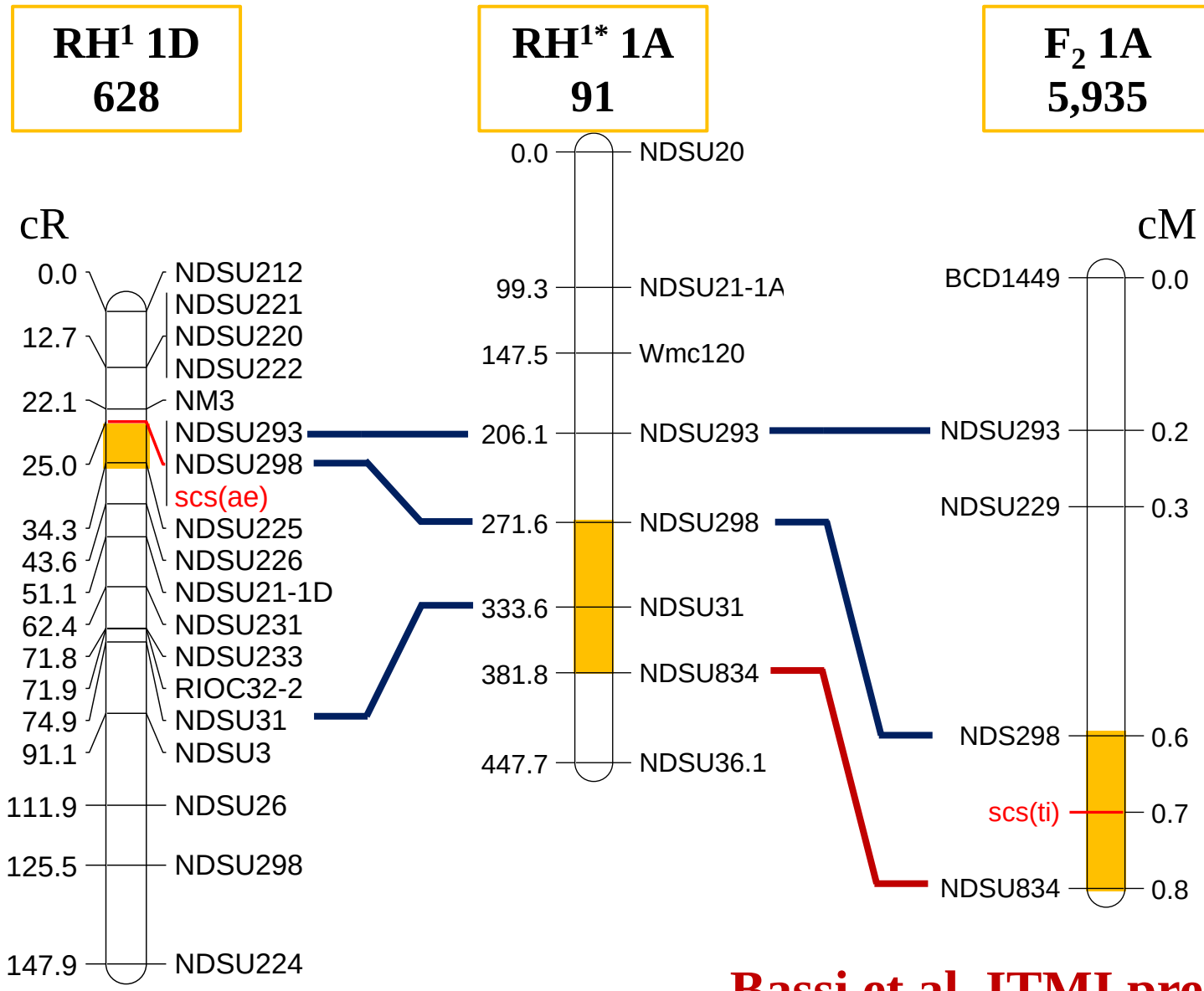
- *Species cytoplasm specific* controls nuclear-cytoplasm interactions
- 1,300 1D-RH lines segregating for shriveled and plump seeds



scs ***Δscs***

Hossain et al., 2004. Genetics

1A to 1D Conservation



Bassi et al. ITMI presentation

Summary

Radiation hybrids are efficient tool for :

Physical mapping of chromosomes/chromosomal regions

Cloning genes (particularly from low recombination regions)

http://avena.pw.usda.gov/RHmapping/










Thanks!