F. M. Bassi, A. Kumar, M. Michalak de J, F. Ghavami, O. Al-Azzam, A. Denton, H. Simkova, J. Dolezel, Y. Gu, J. Dvorak, E. Huttner, A. Kilian, E. Paux, C. Feuillet, O. Riera-Lazarzo, S. S. Maan, S. F. Kianian

Radiation Hybrid: an Alternative Method for QTL Analysis in Wheat

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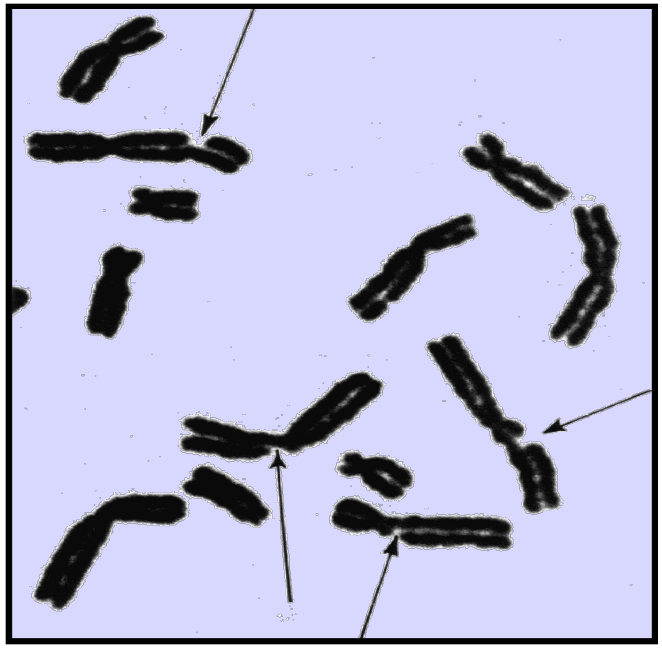
DBI-0822100

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

What is Radiation Hybrid (RH) Mapping?

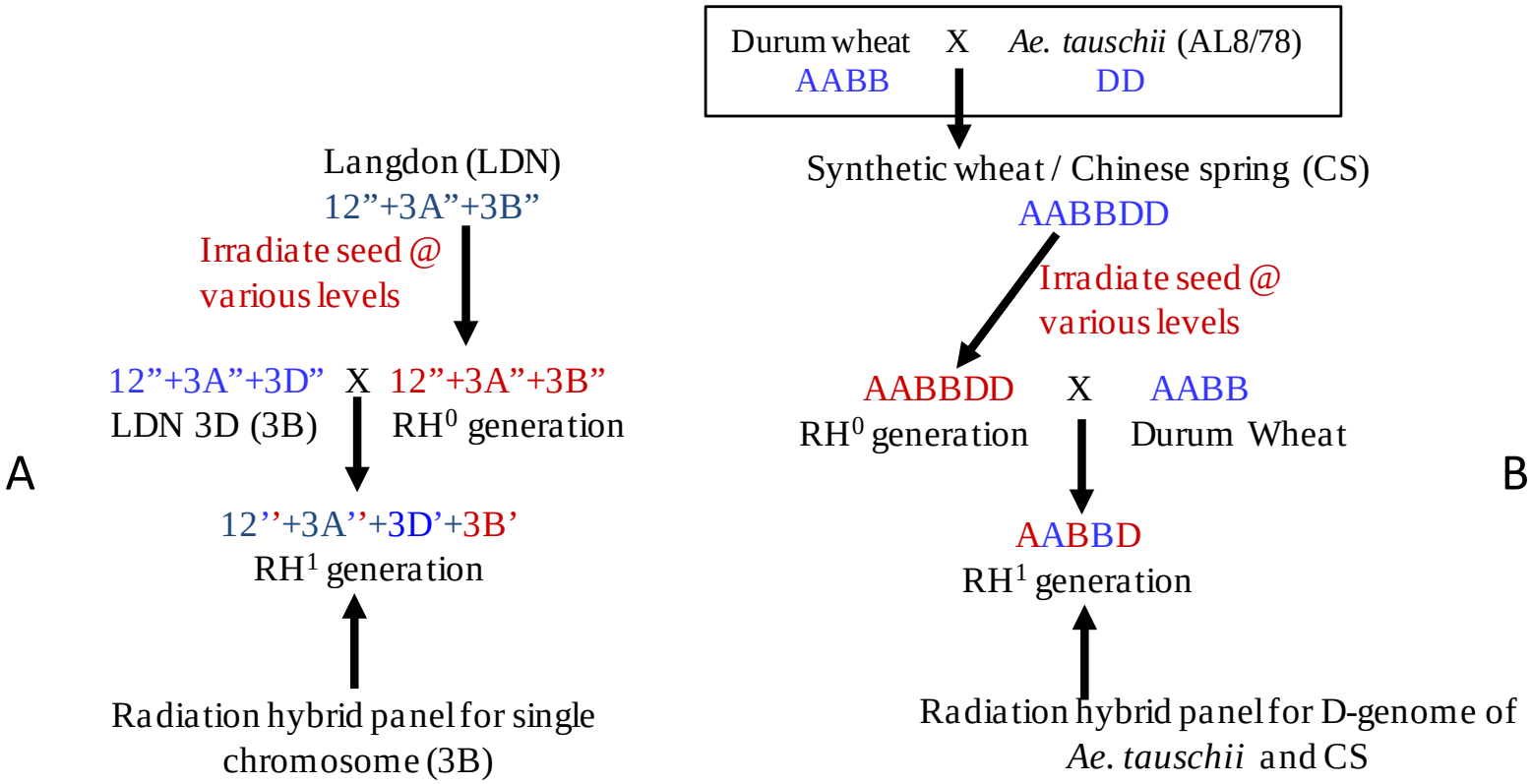
Physical mapping based on radiation induced chromosome breakage and a reconstruction of marker order based on their co-retention pattern

- Radiation breaks chromosomes at random resulting in loss of 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



http://www.ratical.org/radiation/CNR/PP/chp3.html

Scheme for Development of RH Panel in Wheat



Schematic presentation of the RH population development:

RH Population Development

- **Gamma Ray causes deletions of random size in the genome**

LDN (aneuploid)	LDN (euploid)
1A1A	1A1A
2A2A	2A2A
3A3A	3A3A
4A4A	4A4A
5A5A	5A5A
6A6A	6A6A
7A7A	7A7A
1B1B	1B1B
2B2B	2B2B
--	3B3B
4B4B	4B4B
5B5B	5B5B
6B6B	6B6B
7B7B	7B7B
3D3D	

RH Population Development

- **Gamma Ray causes deletions of random size in the genome**

LDN (aneuploid)

1A1A
2A2A
3A3A
4A4A
5A5A
6A6A
7A7A
1B1B
2B2B
--
4B4B
5B5B
6B6B
7B7B
3D3D

35 Krad Irradiation

LDN (euploid)

1A1A
2A
3A
A
A
A
1
2B
3D
4B4
5B5B
6B6B
7B7B

RH Population Development

- **Gamma Ray causes deletions of random size in the genome**

LDN (aneuploid)	LDN (euploid)
1A1A	1A1A
2A2A	2A2A
3A3A	3A3A
4A4A	4A4A
5A5A	5A5A
6A6A	6A6A
7A7A	7A7A
1B1B	1B1B
2B2B	2B2B
--	3B3B
4B4B	4B4B
5B5B	5B5B
6B6B	6B6B
7B7B	7B7B
3D3D	

RH Population Development

- **Gamma Ray causes deletions of random size in the genome**

RH[1]

1A	1A
2A	2A
3A	3A
4A	4A
5A	5A
6A	6A
7A	7A
1B	1B
2B	2B
	3B
4B	4B
5B	5B
6B	6B
7B	7B
3D	

3B Population Development

- **Population1:**

  35 Krad
  184 RH^1 generated
  92 RH^1 selected (92 markers selection)

- **Population2:**

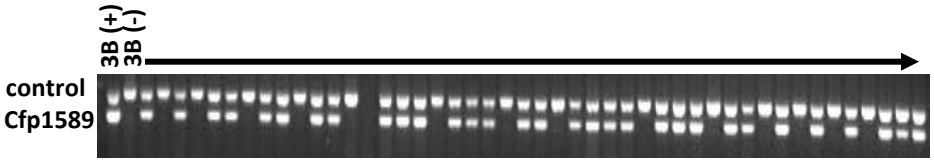
  35 Krad
  698 RH^1 generated

Two Levels Maps

- **Pop1 - 92 RH[1] <u>deep</u> genotyping**

19 SSR
96 ISBP (Cfp)
426 3B DArT

541 markers



Wenzl et al., 2010 TAG 121:465-474

Two Levels Maps

- **Pop1 - 92 RH[1] <u>deep</u> genotyping**

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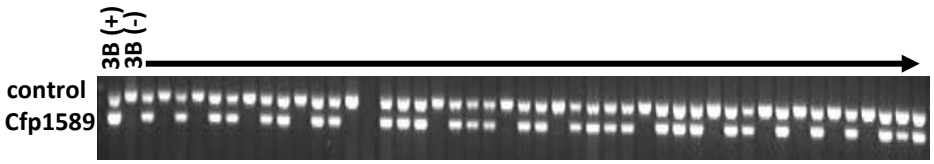


Custom DArT Array with 220 markers/clones

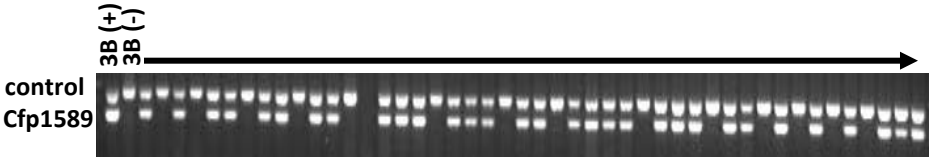
- **Pop2 - 698 RH[1] + 8 Deletion Bins <u>medium</u> genotyping (220 DArTs)**

Two Levels Maps

- **Pop1 - 92 RH[1] <u>deep</u> genotyping**

  - **19 SSR**
    96 ISBP (Cfp)
    426 3B DArT

    541 markers



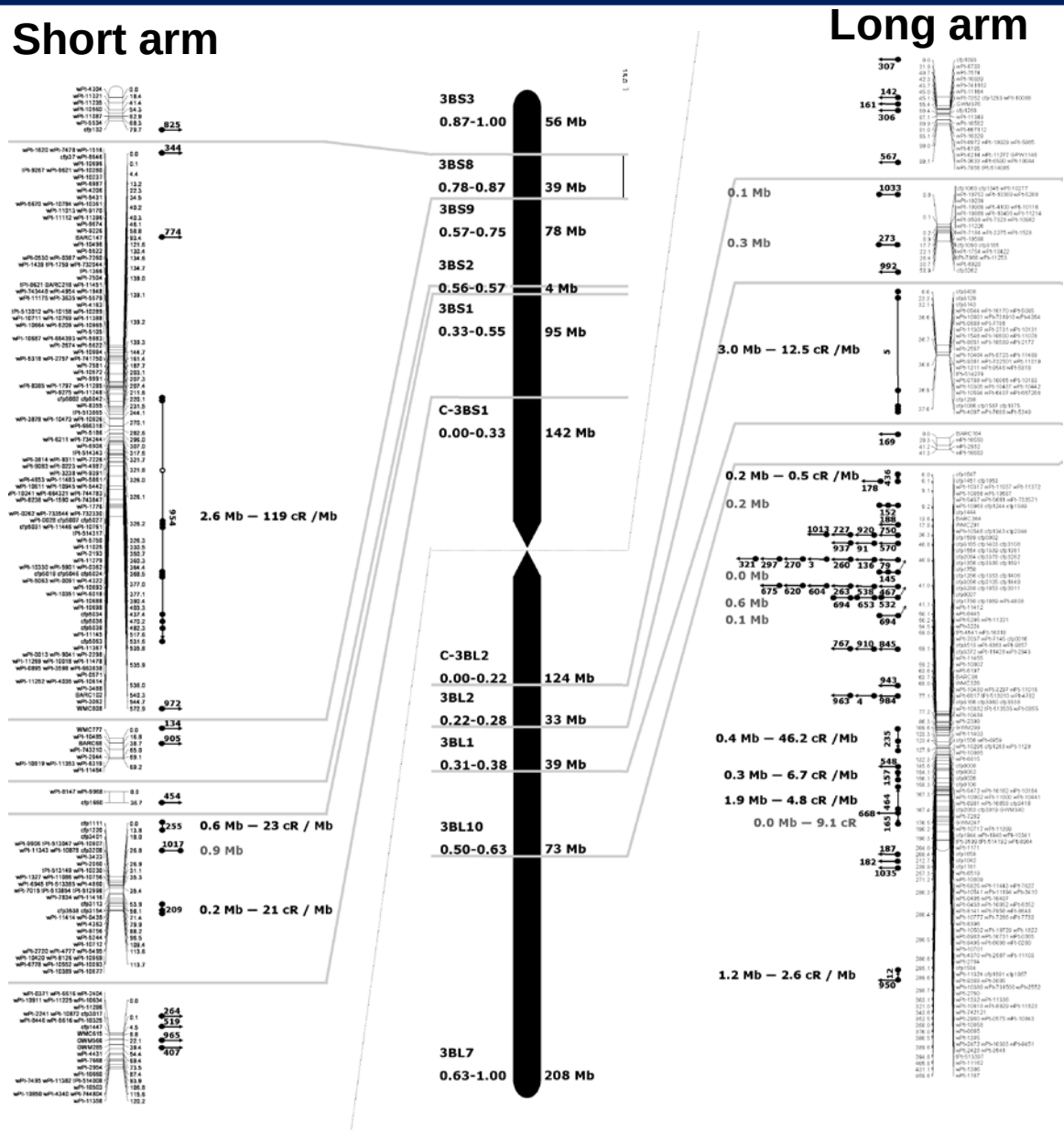
Custom DArT Array with 220 clones

- **Pop2 - 698 RH[1] + 8 Deletion Bins <u>medium</u> genotyping (220 DArTs)**

Selected RH[1] lines will be markers <u>saturated</u> (3B NimbleGen)

Pop1 - High Saturation

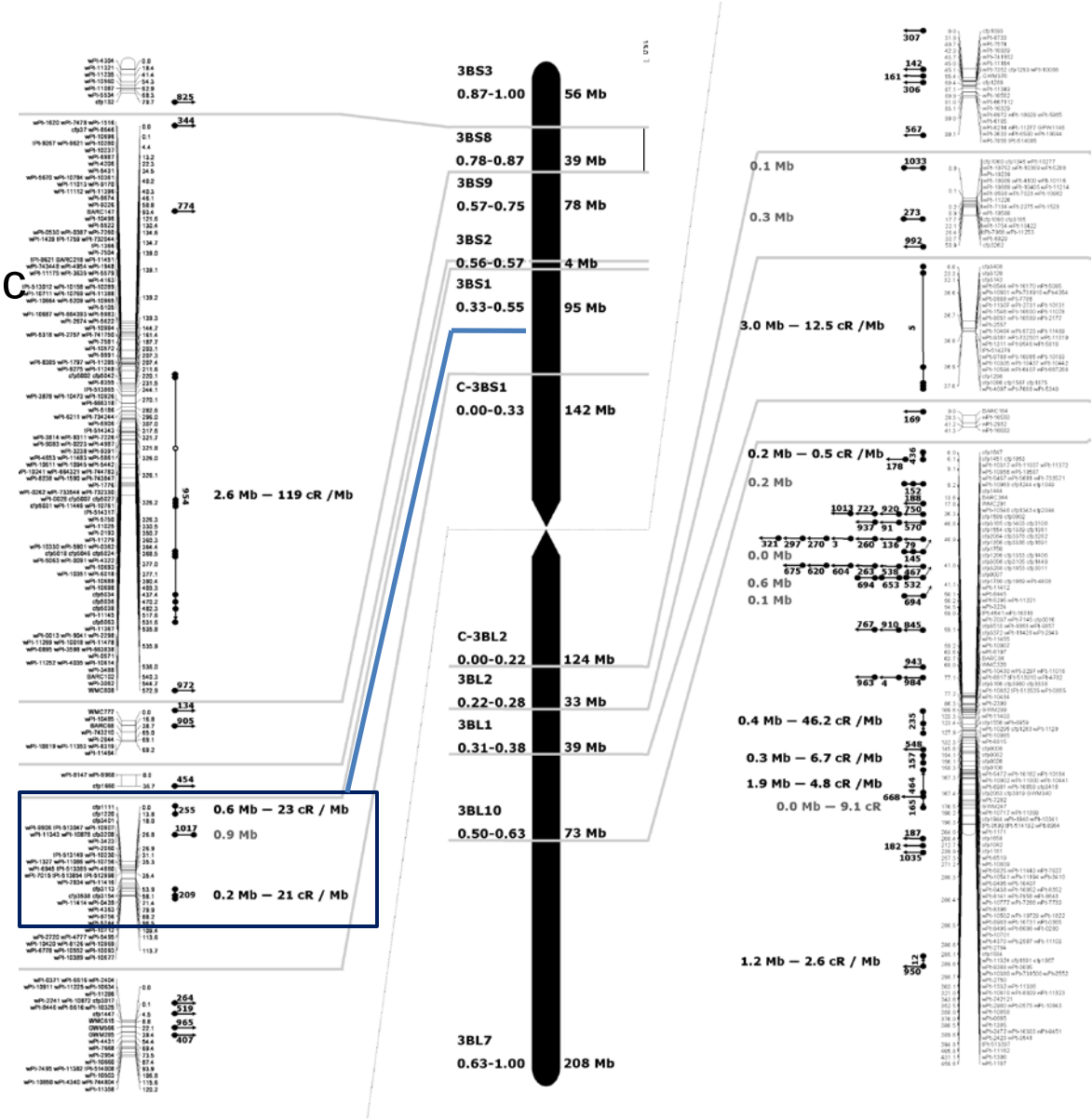
1871.9 cR (92 RH[1])
vs
179.1 cM (376 F_2)



Diversity Arrays Technology Pty Ltd

High Saturation

- ~1/4 to 1/3 of the genome around the centromere represents about 1% of recombination on the genetic maps
- ~30% of the genes are in recombination poor regions

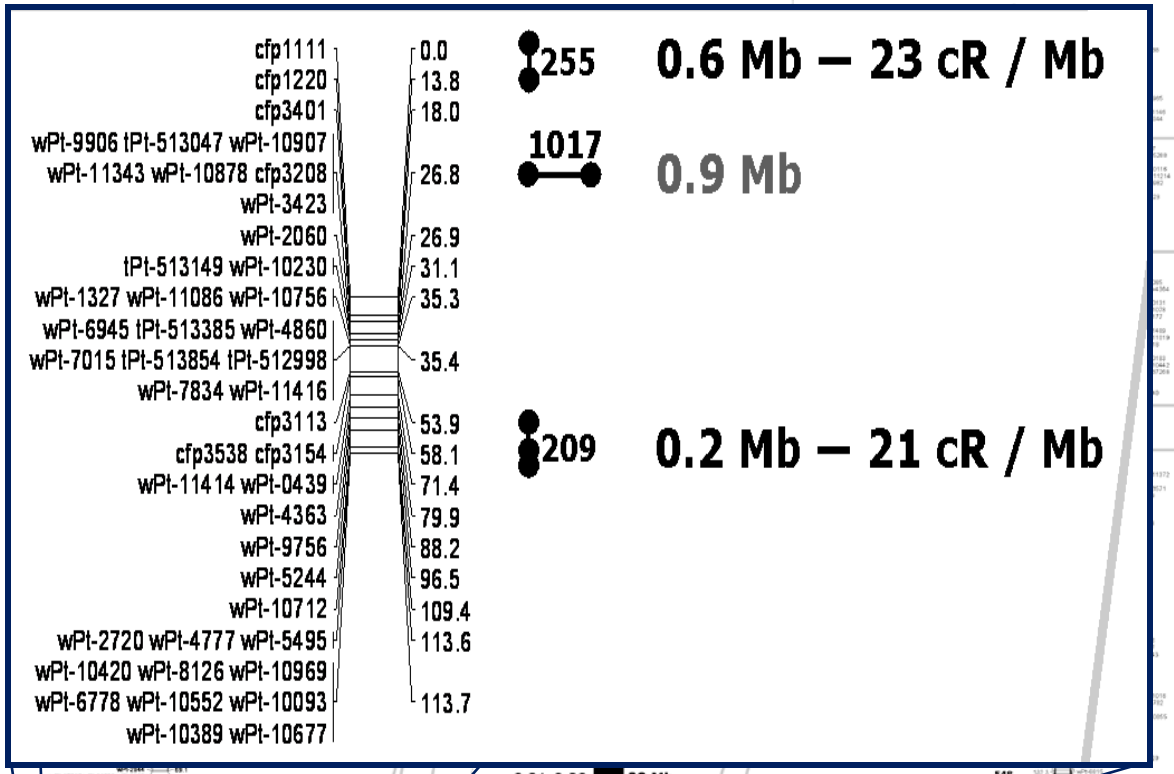


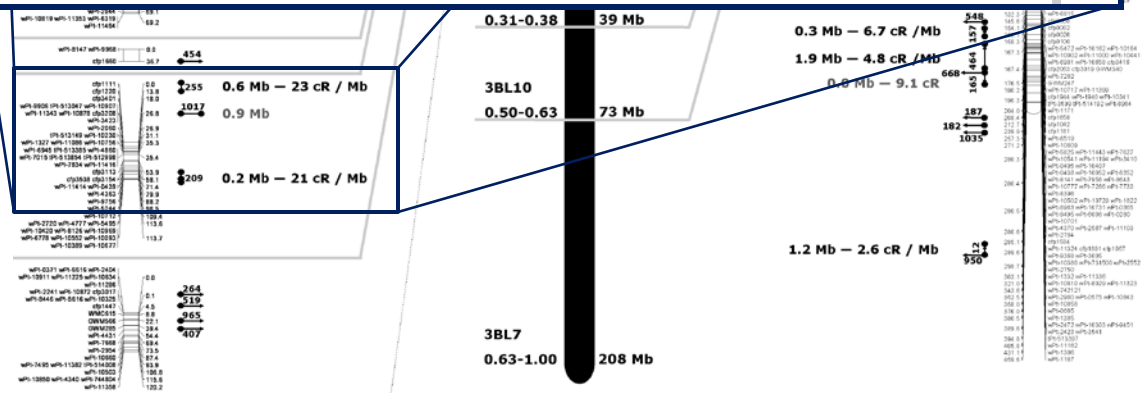
Diversity Arrays Technology Pty Ltd

High Saturation

- 1871.9 cR (92 RH[1])
  vs
- 179.1 cM (376 F_2)

- Contig Orienting:> 1 Mb





Institut National de la Recherche Agronomique

Diversity Arrays Technology Pty Ltd

cM vs cR

- **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

Deletion Bins	3BS3-0.87-1.00	3BS8-0.78-0.87	3BS9-0.57-0.75	3BS2-0.56-0.57	3BS1-0.33-0.55	C-3BS1-0.33	C-3BL2-0.22	3BL2-0.22-0.28	3BL1-0.31-0.50	3BL10-0.50-0.63	3BL7-0.63-1.00	Chr
Bin Size (Mb)	56	39	78	4	95	142	124	33	39	73	208	992
Map Size (cR)	79.7	572.9	69.2	36.7	113.7	115.6	99.1	58.9	37	41.3	459.6	1872
Resolution (Ml	**0.7**	**0.1**	**1.1**	**0.1**	**0.8**	**1.2**	**1.3**	**0.6**	**1.1**	**1.8**	**0.5**	**0.5**
Map Size (cM)	5.2	33.1	13.4	--	1.6	0.9	1.6	1.6	6.2	3.5	104.2	179.1
Resolution (Ml	**10.8**	**1.2**	**5.8**	**--**	**61.3**	**167.1**	**80**	**21.3**	**6.3**	**21.2**	**2**	**5.5**

Saintenac et al., 2009 *Genetics 181:393-403*

Medium Saturation

- **87 bin mapped DArT markers**
- **3008.2 cR**
- **0.33 Mb resolution**
- **326 informative lines (ret. freq. 0.98 – 0.46)**

Population segregating: 31% Sterility

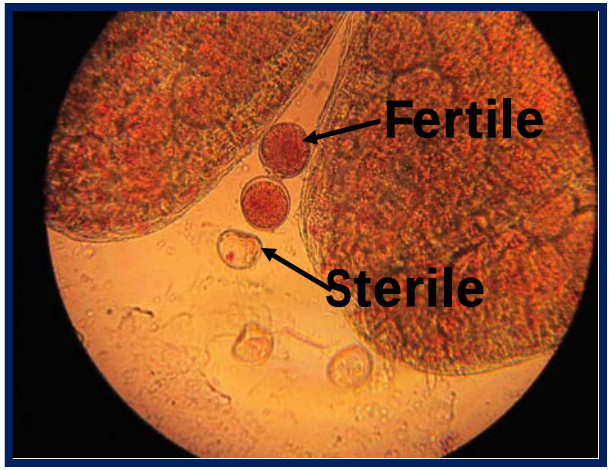
The 3B *fertility* Phenotype

- **Absence of chr. 3B causes sterility**

Genotype	Phenotype	Average seeds/spike	% sterile pollen
Disomic for 3B	Fertile	41 (min 31)	< 10%
Monosomic	Fertile	12 (min 8)	< 30%
Nullisomic	Sterile	2 (max 11)	> 60%

All lines are from Durum wheat Langdon

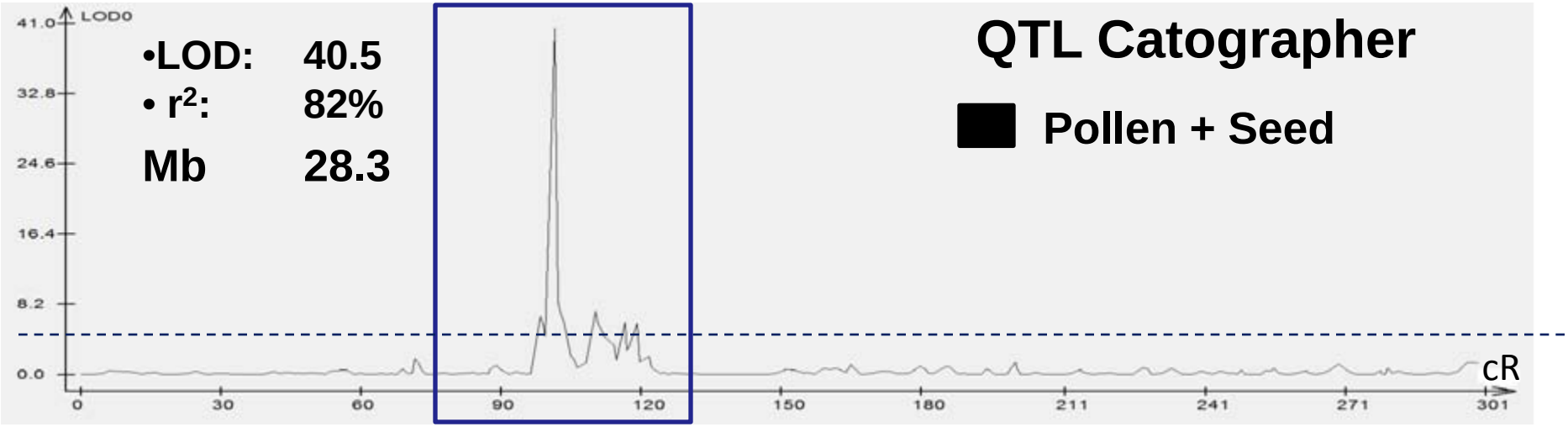
- **Gamma deletion of *fertility* causes sterility**



Sears ER, 1954 Res. Bull. Missouri Agric. Exp. Stn. 572

QTL analysis for *fertility* in RH

- No allelic variation required (Wt vs K.O.)
- Composite Interval Mapping – 1,000 Permutations



Limitations:

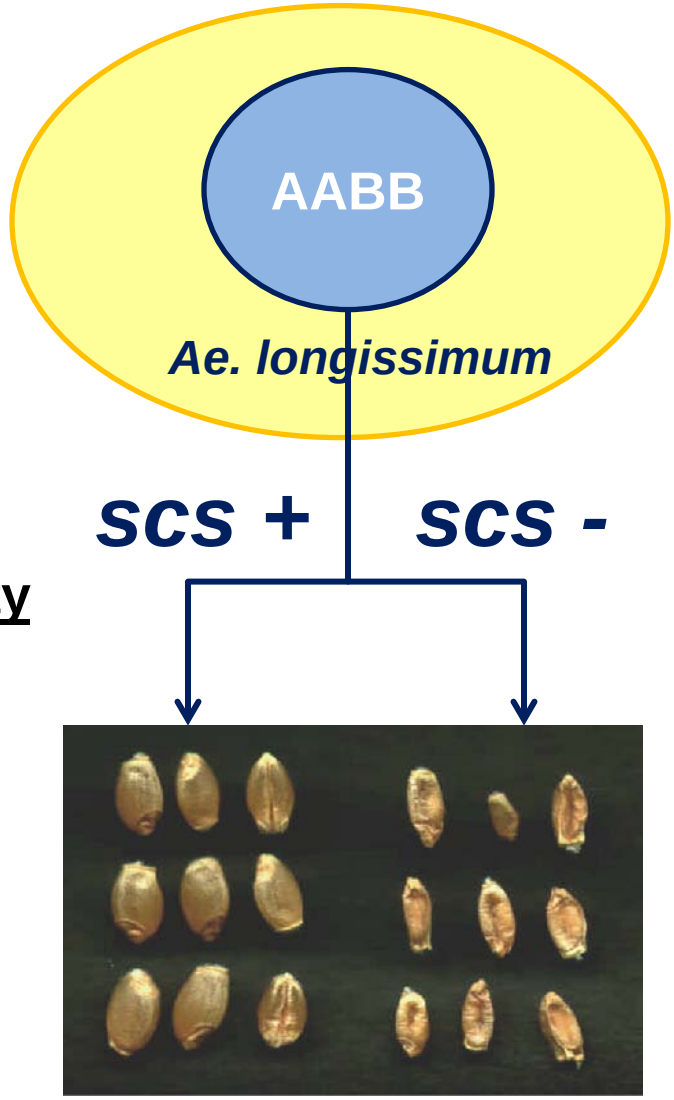
> Single plant phenotyping (No Rep, High E. effect,..)

> 3B is monosomic

The *scs* Phenotype

- ***Species Cytoplasm Specific***

from *T. aestivum*	***1D***
from *T. timophevii*	***1A***



<u>Restores Nuclear-Cytoplasm compatability</u>

Hossain et al., 2004 *Genome 47:206-214*

Phenotyping by BackCrossing

- **RH^1 1D developed before phenotyping**

RH^1 1D

scs

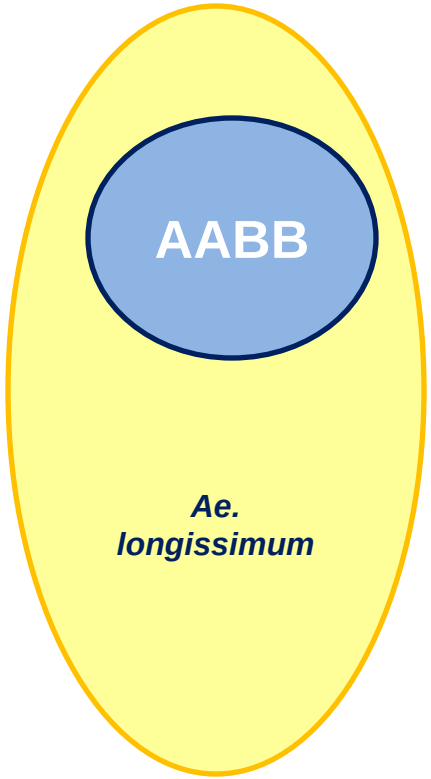
or

scs

Phenotyping by BackCrossing

- **RH^1 1D developed before phenotyping**

RH^1 1D	**(lo)*scs*$^{ti-}$**
scs	***scs***
or	**or**
scs	**-**



Phenotyping by BackCrossing

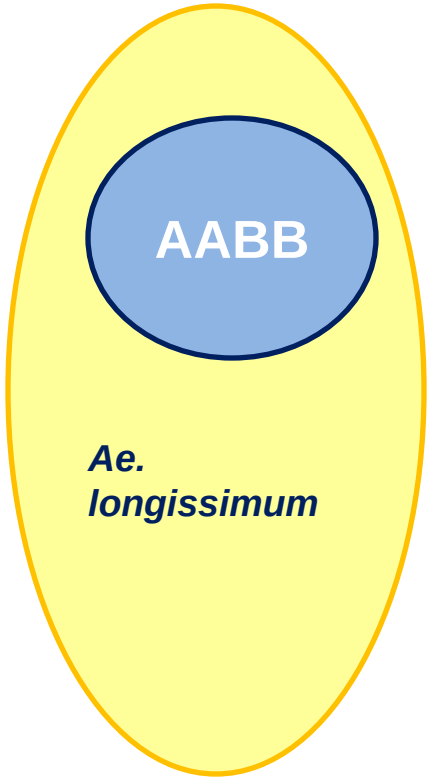
- **RH[1] 1D developed before phenotyping**

RH[1] 1D × **(lo)*scs*[ti-]**

scs **3:1** ***scs***

or **or**

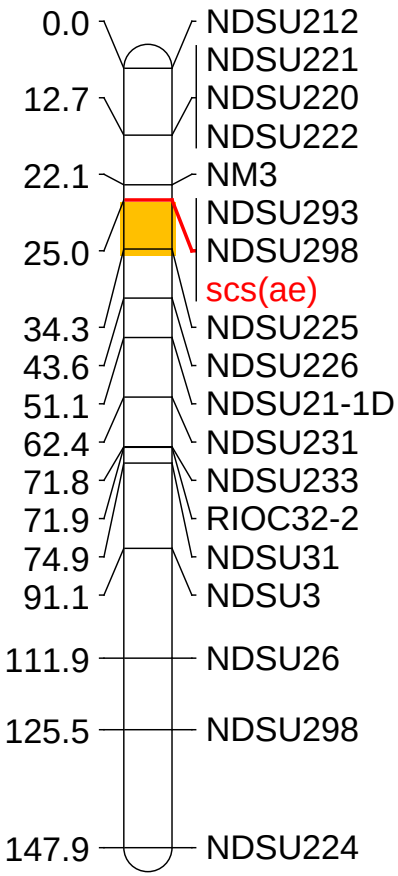
scs **1:1** **-**



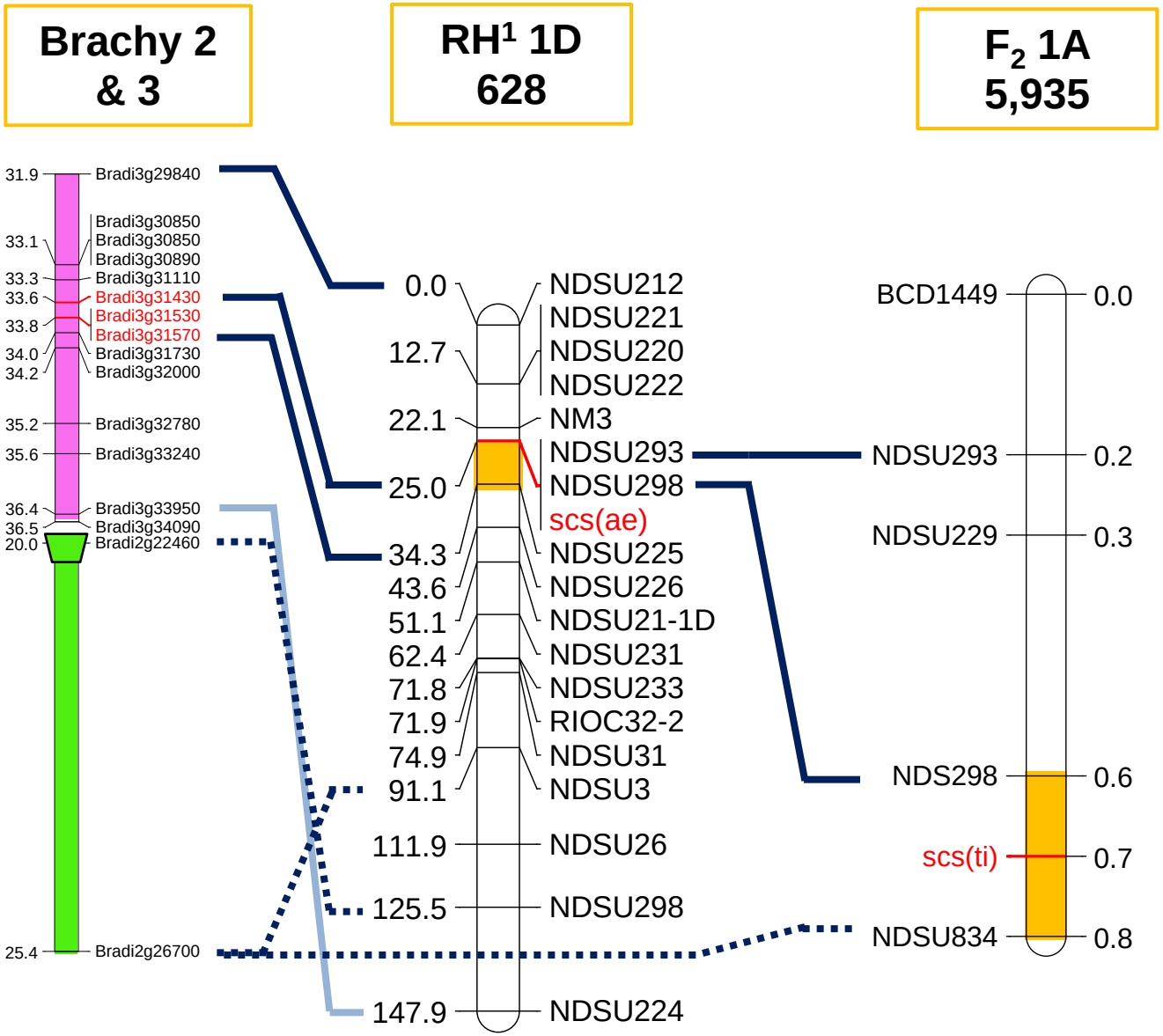
F_2 1A vs RH^1 1D *scs* Conservation

RH^1 1D
628

- **0.3 Mb resolution**



F_2 1A vs RH^1 1D *scs* Conservation



Conclusions

- RH Mapping population development is rapid (~6 months)
- RH mapping is independent of recombination
- Marker system is not constrained by polymorphism
- The limitations of phenotyping can be overcome through selfing or back-crossing
- Very high map resolution (0.3 - 0.5 Mb) can be achieved with small populations
- QTL analysis is feasible as we demonstrated for fertility gene on 3B
- Positional cloning is feasible with limited resources

Available RH Resources

Chromosomes	Species	RH lines available	Purpose
Single chromosome panel			
1A, 1B, 1D, 3B, 4A*, 7B**	Durum , Hexaploid	1,000-3,500	Physical mapping/ Cloning gene (s)
Whole genome panel			
A and B genome	Durum, Hexaploid	3,000	Physical mapping/Field phenotyping
D-genome	*Ae. tauschii* (Synthetic), Chinese spring	>4,000	Physical mapping

* In collaboration with Drs. M. Valisek and J. Dolzel at Institute of Experimental Botany, Czech Republic

**In collaboration with Dr. Odd-Arne Olsen at The Norwegian University of Life Sciences

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- **A. Denton**
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- **S. F. Kianian**

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Wheat Germplasm Enhancement NDSU

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

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