

# Physical Mapping Resources for Large Plant Genomes: Radiation Hybrids for D-genome of Chinese Spring and *Aegilops tauschii* accession AL8/78

**Ajay Kumar**<sup>1</sup>, Vijay K. Tiwari<sup>2</sup>, Thomas Drader<sup>3</sup>, Omar Al-Azzam<sup>4</sup>, Kristin Simons<sup>1</sup>, Muhammad J. Iqbal<sup>1</sup>, Monika Michalak de Jimenez<sup>1</sup>, Filippo M. Bassi<sup>1</sup>, Farhad Ghavam<sup>1</sup>, Lingli Dong<sup>3</sup>, Yi Wang<sup>3</sup>, Ming-Cheng Luo<sup>5</sup>, Yong Q. Gu<sup>3</sup>, Anne Denton<sup>4</sup>, Gerard Lazo<sup>3</sup>, Jeffrey M. Leonard<sup>2</sup>, Oscar Riera-Lizarazu<sup>2</sup>, and Shahryar F. Kianian<sup>1</sup>

<sup>1</sup> Department of Plant Sciences, North Dakota State University, Fargo, ND 58105, USA; <sup>2</sup> Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97331, USA; <sup>3</sup> USDA-ARS, Western Regional Research Center, Albany, CA 94710, USA; <sup>4</sup> Department of Computer Sciences, North Dakota State University, Fargo, ND 58105, USA; <sup>5</sup> Department of Plant Sciences, University of California, Davis, CA 95616, USA

**NDSU** NORTH DAKOTA STATE UNIVERSITY

[ajayguptajammu@gmail.com](mailto:ajayguptajammu@gmail.com); [s.kianian@ndsu.edu](mailto:s.kianian@ndsu.edu)



## Abstract

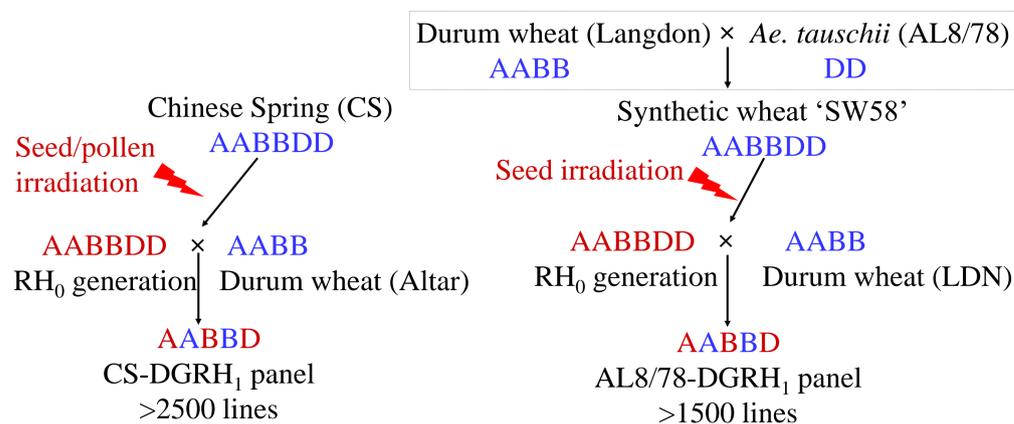
To aid in the development of a complete physical map of wheat, radiation hybrid panels for the D-genome of Chinese Spring (CS) and *Aegilops tauschii* accession AL8/78 were generated and characterized for the presence of deletions. Markers with known physical distances estimated a resolution of < 100 kb for the D-genome panels. Two sets of most informative lines selected from *Ae. tauschii* DGRH<sub>1s</sub> (399 lines) and Chinese Spring DGRH<sub>1s</sub> (300 lines) are being genotyped using NimbleGen array containing ~45,000 RJM and gene based markers to develop high density RH maps which will serve as scaffold for wheat D-genome assembly. A project database (<http://avena.pw.usda.gov/RHmapping/>) provides up-to-date information on tools, data and resources generated from our work.

## Radiation hybrid (RH) mapping

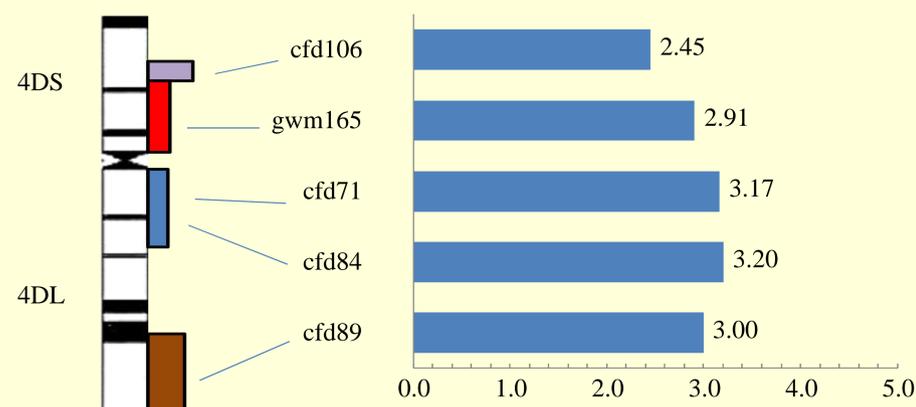
- RH mapping uses radiation to induce chromosomal breaks
- Loci order and physical distances can be determined by co-retention of markers
- Much higher physical resolution than genetic maps can be achieved with fewer lines
- No need for polymorphic markers

## Development and characterization of D-genome RH mapping panel

- RH panels were generated for D-genomes of Chinese Spring (CS) and *Ae. tauschii* accession AL8/78 (Fig. 1)
- A set of 35 SSR markers from across the entire D-genome (5 markers /D-genome chromosome) were used to characterize these RH panels
- Marker loss along a chromosome was homogenous (Fig. 2)
- Characterization with larger set of markers showed that most of the RH lines have deletions
- A major portion of the lines also showed multiple chromosome breaks when characterized with larger marker set
- 399 RH<sub>1</sub> lines with retention frequency of 20-80% for individual D-genome chromosome were selected (critical lines)



**Figure 1:** Scheme for Development of RH Panel for D-genome of Chinese Spring (left) and *Aegilops tauschii* accession AL8/78 (right)



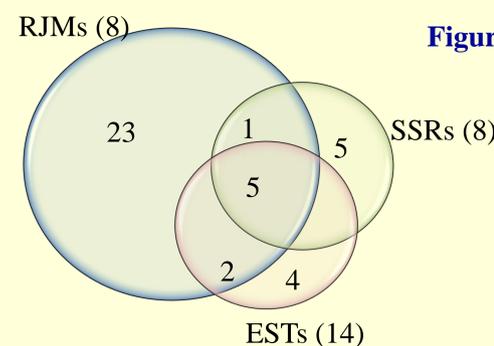
**Figure 2:** Chromosome 4D Marker Loss Frequency

## Mapping resolution of AL8/78-DGRH<sub>1</sub> panel

- Two RJM markers (designed from BAC end sequences) located 400kb apart in centromeric bin of 5DL suggested a resolution of ~100 kb for AL8/78 panel
- This resolution was further confirmed by 3 SSR markers physically mapped to ~3.2Mbp distal bin of 6DS (6DS6-0.99-1.00)

## Different marker systems are required for developing a complete RH map

- More than 80% of the critical lines were detected by only one of the three marker systems (Fig. 3), suggesting the need to use different marker systems for developing a contiguous RH map



**Figure 3:** Comparison of RJM, EST and SSR markers in detecting lines with deletions. Venn diagram shows the number of lines with deletions detected with each marker type in a set of 92 lines of the AL8/78-DGRH<sub>1</sub> panel. Number of markers used from each type are in parenthesis.

## DArT based RH maps of D-genome

- A total of 641 and 764 DArT markers showing were mapped to the seven D-genome chromosomes of AL8/78 and CS respectively
- Using RH mapping almost 10 times more markers were mapped to D-genome compared to genetic maps
- An average of 17:1 map ratio cR/cM was observed for whole D-genome (Table 1)

**Table 1** DArT RH maps of D-genome 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

## Next.....

- A set of ~500 selected most informative lines, each from CS-DGRH<sub>1</sub> and AL8/78-DGRH<sub>1</sub> panel are being genotyped with >30,000 RJM and >15,000 gene based markers using NimbleGen array to develop high-density RH maps
- High-density RH maps will be used to anchor BAC contigs of D-genome to develop a marker scaffold for whole genome assembly

## Available wheat RH resources

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

\* In collaboration with Drs. M. Valarik & J. Dolezel Institute of Experimental Botany, Czech Republic  
\*\* In collaboration with Dr. Odd-Arne Olsen, Norwegian University of Life Sciences, Norway

**The resources of D-genome RH project are available for use by the community under MTA. Please feel free to contact us.**

## Acknowledgements

Thanks are due to Justin Hegstad and Allen Peckrul for extensive help with greenhouse and laboratory work. Funding from the National Science Foundation, Plant Genome Research Program grant No. DBI-082210 to SFK is gratefully acknowledged.