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ABSTRACT

There is keen interest in constructing a physical map of the 17-Gb bread wheat (*Triticum aestivum* L.) genome as well as its eventual sequencing. Efforts are now underway to develop BAC-based physical maps of chromosomes and genomes of this species. One important component in the construction of physical maps is the alignment and anchoring of contigs to an independently derived marker scaffold (or map). In the case of wheat, mapping methods that are complementary to meiotic recombination will be necessary because of the uneven distribution of meiotic recombination and low correlation between genetic and physical distances. Thus, we have initiated a project to use a method that uses radiation-induced chromosome breakage, rather than meiotic recombination, as a means to induce marker segregation for mapping (known as radiation hybrid (RH) mapping). Here we describe a method to construct D-genome radiation hybrids (DGRHs) as well as a preliminary evaluation of mapping panels with the goal of constructing RH maps of the 4.2-Gb D genome of hexaploid wheat.

OVERALL PROJECT OBJECTIVES

- Develop radiation hybrid mapping populations for D-genome chromosomes
- Develop RH maps of varying resolution and align BAC contigs to the maps using high-throughput protocols
- Develop the database and bio-informatics tools for efficient access to and utilization of the resources generated in this project
- Integrate the knowledge and resources of this project into teaching/training of students at all levels

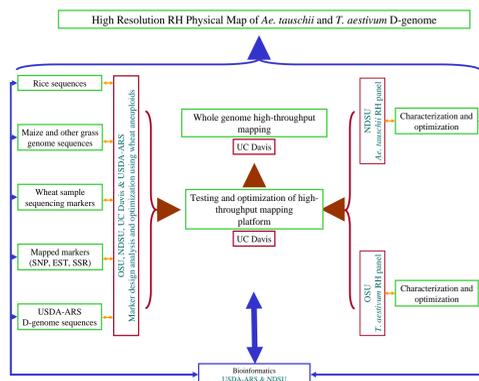


Figure 1: This scheme represents project organization according to the major activities. All encompassing activities relate to education, outreach and bioinformatics. These three components will permeate all work being conducted by this group.

Radiation Hybrid Mapping

This is a proven approach based on radiation-induced chromosome breakage, rather than meiotic recombination, as a means to induce marker segregation for mapping^{1,2}. Using these methods, any given mapping panel member is assayed for the presence or absence of a given marker and markers need not be polymorphic to be mapped.

CURRENT STATUS OF PROJECT ACTIVITIES

Scheme for the Development of D-Genome Radiation Hybrids (DGRHs)

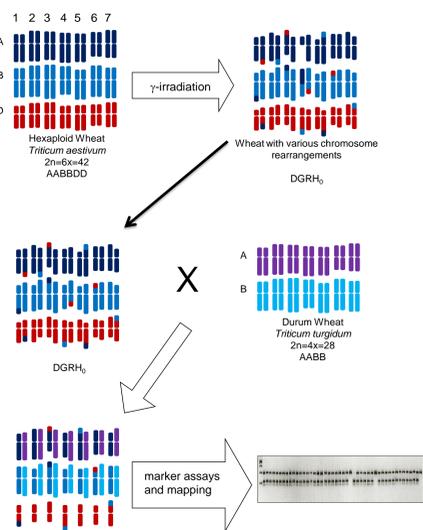


Figure 2: Production of wheat D-genome radiation hybrids (DGRHs). Seeds of the hexaploid wheat (*Triticum aestivum*, 2n=6x=42, AABBDD) 'Chinese Spring' and the synthetic hexaploid wheat LDNxAL8/78 were treated with various doses of gamma-rays, subsequently planted, and surviving plants (DGRH₀) were crossed to the tetraploid wheat (*T. turgidum*, 2n=4x=28, AABB) variety 'Altar 84' or 'Langdon'. Progeny from independent crosses (DGRH₁) are then used to assemble the mapping panel. Important features of this scheme for the production of a mapping panel is that the irradiated D-genome chromosomes will be in the hemizygous condition and lesions on A and B chromosomes from *T. turgidum* will be masked by the presence of normal counterparts from *T. aestivum*. Consequently, this panel will preferentially reveal lesions on D-genome chromosomes. From a mapping perspective, DGRHs permit the simultaneous mapping of all D-genome chromosomes.

Radiation Hybrid Panel Development and Evaluation

- Two DGRH panels:
 - LDNxAL8/78 165 DGRH₁ lines analyzed
 - Chinese Spring 184 DGRH₁ lines analyzed
 - LDNxAL8/78 2,000 DGRH₀ being crossed
 - Chinese Spring 995 DGRH₀ being crossed
- Final target is 1,000 RH1 lines per panel
- Markers that have been used include SSR, gene-based, and DARTs
- Target 10,000 loci mapped on critical lines using high-throughput protocols

Characterization of Some D-Genome Radiation Hybrids (DGRHs)

Table 1: DNA marker retention in DGRHs from CS and LDNxAL8/78

Panel	Radiation dose (krad)	Marker retention % per plant (range)	% of individuals containing all of the tested markers
CS DGRH	15	99.6 (90-100)	95.7
	35	96.0 (65-100)	53.9
AL8/78 DGRH	15	94.7 (83.4-100)	27.9
	35	94.9 (64.3-100)	31.1

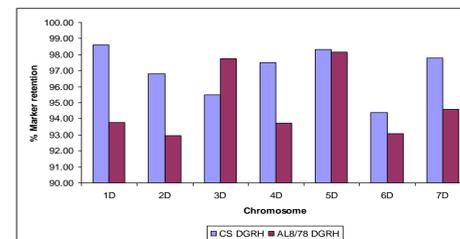


Figure 3: DNA marker retention frequencies of D-genome chromosomes in two DGRH Panels

Table 2: Distribution of critical lines for DGRH panels from LDNxAL8/78 and Chinese Spring

Number Of Chromosomes	LDNxAL8/78	Chinese Spring
Critical lines for 1 chromosome	21.8%	28%
Critical lines for 2 chromosomes	13.9%	12%
Critical lines for 3 chromosomes	12.9%	4%
Critical lines for 4 chromosomes	6.9%	1%
Critical lines for 5 chromosomes	4%	0%
Critical lines for 6 chromosomes	1%	0%
Critical lines for 7 chromosomes	0%	0%

Regardless of radiation dose, the DGRH panels from LDNxAL8/78 showed greater indices of chromosome breakage (lower marker retention rates and greater proportions of critical lines) compared to the panels from Chinese Spring (Tables 1 and 2, Figure 3). Additional materials are being evaluated to see if this discrepancy is due to sampling error or genotypic effects. The pattern of radiation-induced breakage of D-genome chromosomes was assessed by comparing marker retention rates in various D-genome chromosomes. In the panel from Chinese Spring, marker retention rates among D-genome chromosomes were homogeneous except for chromosome 6D (lower retention rates than expected). In the LDNx8/78 marker retention rates were also homogeneous except for chromosomes 3D and 5D (higher retention rates than expected). Overall, marker retention for a given D-genome chromosome in these panels was homogeneous (Figure 3). This suggests that radiation-induced breakage appears to be even along D-genome chromosomes.

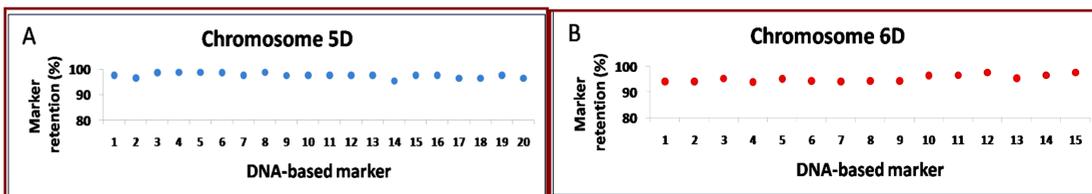


Figure 4: Average retention frequencies (%) for DNA markers for wheat chromosomes 5D (A) and 6D (B). Marker loci for chromosome 5D from 1 to 20 correspond in order to those shown in figure 5A, number 1 being *Xbarc130* and number 20 corresponding to *Xgwm565*. Markers for chromosome 6D from 1 to 15 correspond in order to those shown in figure 5B, number 1 being *Xcfd49* and number 15 corresponding to *Xwmc773*.

Additional Characterization of Chromosomes 5D and 6D in DGRHs from Chinese Spring

The integrity of two D-genome chromosomes (5D and 6D) in CS DGRHs from the 35-krad treatment was assessed in greater detail by assaying these materials with 15 to 20 markers (Fig. 4 and 5).

- Chromosome breakage along each chromosome was homogeneous (Figure 4).
- A subset of 13 DGRHs for chromosome 5D (Fig. 5A) was used to dissect chromosome 5D into 16 distinct bins.
- Marker retention study in this subpanel revealed DGRHs with 1 to 8 obligate breaks per chromosome
- A subset of 15 DGRHs were found for chromosome 6D (Fig. 5B). This subpanel allowed the dissection of chromosome 6D into 13 distinct bins. Analysis showed DGRHs with 1 to 5 obligate breaks per chromosome
- A comparison of radiation hybrid data with genetic and deletion-based maps of chromosome 5D and 6D, showed that radiation hybrids provide useful and complementary information. Namely, markers in areas of low recombination or in deletion-based bins can be ordered with radiation hybrid data.

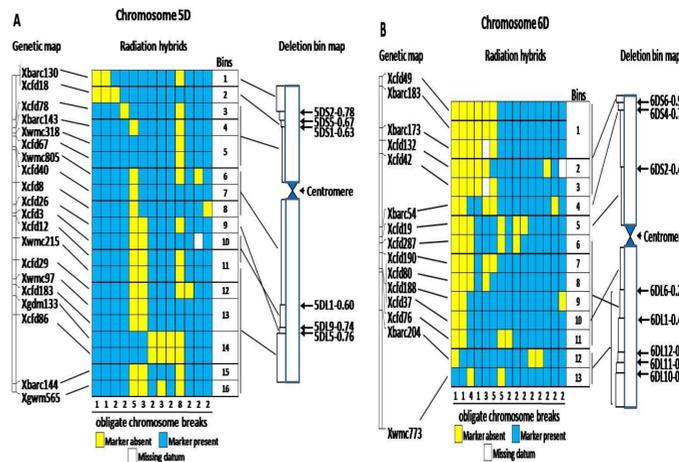


Figure 5: DNA-marker-based dissection of wheat chromosomes 5D (A) and 6D (B) using D-genome radiation hybrids (DGRHs). The dissection of each chromosome is based on the pattern of marker retention or loss in a panel. Solid blue squares indicate that a marker is present in a given DGRH. Solid yellow squares indicate that a marker was absent in a given DGRH. Empty squares indicate missing data. The order of the DNA markers is based on linkage maps (left of each figure) reported by Somers et al.³. The correspondence between linkage maps and DGRH data is shown by connecting lines and DGRH data is shown by connecting lines. The deletion bin maps (right of each figure) are based on Sourdille et al.⁴. The correspondence between DGRH data and the deletion bin maps is shown by connecting lines.

Current Activities

- We are in the process of constructing additional mapping panels, evaluating their quality, and assembling panels of varying levels of resolution.
- Retrotransposon element junctions (REJ) are under evaluation as a new single-copy and locus-specific marker system for hexaploid wheat.
- Various marker platforms are being explored for high throughput genotyping.
- Various bioinformatics tools to store, mine, and analyze data are also under development

References

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- Kalavacharla et al. Genetics: 173: 1089–1099 (2006).
- Somers DJ, Isaac P, Edwards K: Theor Appl Genet 109:1105-1114 (2004).
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