Physical mapping of chromosomes 3HS and 3DS

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INTRODUCTION

Physical mapping is the evaluation of the physical distance between markers along a chromosome. It generates large amounts of data useful for studying the structure of the genome, comparison with other genomes and map-based cloning of particular genes. It is also the preliminary step to the sequencing of large and repetitive genomes such as wheat and barley.

The 17 Gb of wheat are organized in 3 homeologous genomes, A, B and D. Due to the high level of similarity between the three genomes, the strategy that has been adopted makes use of a physical map of a specific region based on Bacterial Artificial Chromosome (BAC) libraries specific for a chromosome [1] or for a diploid ancestral parent [2]. A parallel strategy is to link the barley map to wheat. The barley diploid genome is about the same size as the three separate wheat genomes but it has a higher density genetic map, making it a good model.

The aim of our project is to generate fine maps of wheat and barley chromosomes 3 and 7. In association with the European project FP7 TriticeaeGenome, the ultimate goal is to generate physical maps and full genome sequence.

The first step of the strategy is to identify ESTs that map to the target region using deletion lines, the physical map on homeologous chromosome when available, and the barley genetic map (Figure 1). The ESTs are anchored on the BAC contigs of *Aegilops tauschii*, the ancestor of the wheat D genome, and of barley. The selected BACs are end-sequenced to identify more markers and fill the gaps between the contigs. Based on BAC-end and EST sequences, new molecular markers are identified: simple sequence repeat (SSR), single nucleotide polymorphism (SNP) and insertion site-based polymorphism (ISBP). These are used to tie the physical map to a high resolution genetic map of wheat by screening two large populations developed in the ACPFG. These populations consist of 300 doubled haploid lines plus 3,000 single seed decent lines (F5) which will give an estimated resolution of less than 0.01cM. The comparison of our data with those from the

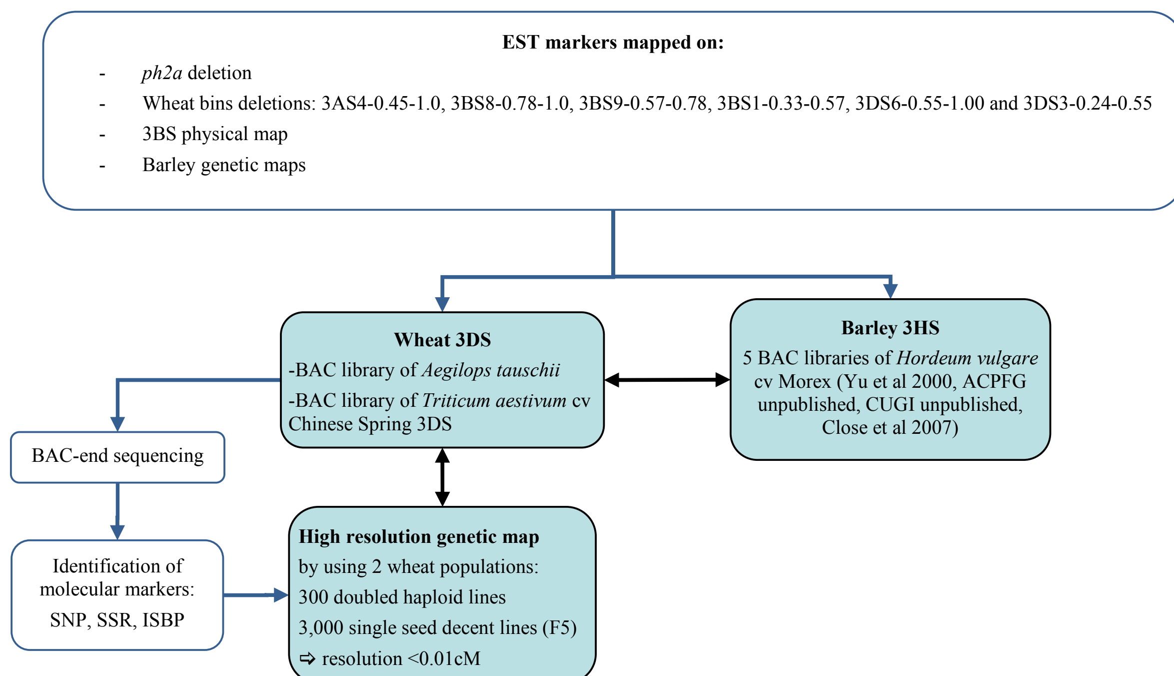

Figure 1. Strategy used for physical mapping of wheat chromosome 3DS.

FP7 project will allow definition of the genetic/physical relationship across the target region in the A, B, D and H genomes.

Our first target region is the telomeric 20 cM of the short arm of Group 3 chromosomes, delineated by an X-ray induced deletion mutation called *ph2a* and estimated to be around 80 Mb in size [3]. We describe here the first assembly of *Ae. tauschii* BACs covering the *Ph2* locus.

MATERIAL AND METHOD

Plant material (*Triticum aestivum* cv Chinese Spring, nullisomic-tetrasomic derivatives) were described in [3].

The wheat EST markers, covering the *Ph2* locus and the syntenous rice region [3], were used to identify the wheat deletion bins of the GrainGenes database (http://rye.pw.usda.gov/cgi-bin/gbrowse/WheatPhysicalESTMaps/?name=Chinese_Spring_Deletion_3D), the homeologous region on chromosome 3BS (Feuillet and Paux, personal communication) and the corresponding single-feature polymorphism (SFP) markers of barley (Waugh and Marshal, personal communication).

The DNA extraction and southern blot analyses were performed according to [4]. Primers were designed by using Primer 3 program to obtain one-fragment PCR product of 200-400 bp length and exon-specific when possible. The fragments were purified and used as probes on DNA from nullisomic-tetrasomic lines using Southern blotting. The low-copy probes were then hybridized on filters to screen the BAC library. The BAC filters of *Ae. tauschii* were obtained from Jan Dvorak (Department of Agronomy and Range Science University of California, Davis, USA).

The anchoring of the markers on the positive BAC clones was validated by PCR on the overlapped clones according to the assembly on http://wheatdb.ucdavis.edu:8080/wheatdb/. Then the clones were reassembled and end-merged when single copy markers were located at the end of the contigs. The BAC were assembled in contigs by using the FPC program [5]. One CB unit corresponds to 1.9 kb (Ming-Cheng Luo, personal communication).

RESULTS AND DISCUSSION

A first physical map of the *Ph2* locus was built with 123 wheat ESTs. These included 70 fragments that detected low copy sequences on nullisomic-tetrasomic lines of wheat and were used to probe the *Ae. tauschii* BAC filters. The positive clones and the clones assembled on the same contigs were then screened by PCR with the 53 remaining markers. A total of 89 markers were anchored onto 24 contigs (Table 1). The average size of the contig was 709 kb. The physical map of 996 clones covered about 17.7 Mb of the region. Considering that the *Ph2* region is about 80 Mb, the map covered about one third of the target region.

Table 1. Description of the *Ae. tauchii* physical map of the *Ph2* region. CB refers to Consensus Band of the fingerprint. The BACs were assembled with the FPC program.

	total	Average per contig	min	max
contigs	24			
clones	996	39.8	8	94
marker	89	3.6	1	10
Size (CB units)	9,334	373.4	99	836
Size (kb)	17,735	709	188	1,588

Because the genome organization is likely to be highly similar between 3DS and 3BS, we ordered the *Ae. tauschii* contigs according to the physical map of the 3BS chromosome (Figure 2). Where corresponding information was not available for 3BS, the contigs were ordered to minimize the differences with the rice genomic sequence.

In order to tie the *Ae. tauschii* physical map to the barley genetic map, we compared the wheat EST sequences to the SFP markers of the barley genetic map. A Blastn of the 123 wheat ESTs identified 30 barley ESTs. Among these, 17 ESTs allowed us to link 14 *Ae. tauschii* physical contigs to 7 bins of the barley genetic map (Figure 1). This preliminary result showed a similar order of the markers with a possible translocation or duplication of the segment 3H_04 (or a part of it) in *Ae. tauschii* 3DS.

Additional data will be necessary for a syntenic study of the 3HS and 3DS chromosomes. The *Ae. tauschii* contigs will be extended using markers identified on the BAC-end sequences and new markers from the *Brachypodium* genomic sequences (http://www.brachypodium.org/). The comparison with the barley genome structure will be extended by integrating the physical map to the 1,000-loci transcript map of barley [6].

ACKNOWLEDGEMENTS

This work is supported by the University of Adelaide, the Australian Research Council, the Grains Research and Development Corporation, the Government of South Australia and the International Science Linkages CG120174. We thank Margaret Pallotta for supplying wheat nullisomic-tetrasomic DNA and Southern membranes, Etienne Paux and Catherine Feuillet for the markers information of 3BS physical map, Alina Akhunova and Karin Deal for providing the *Ae. tauschii* BAC clones.

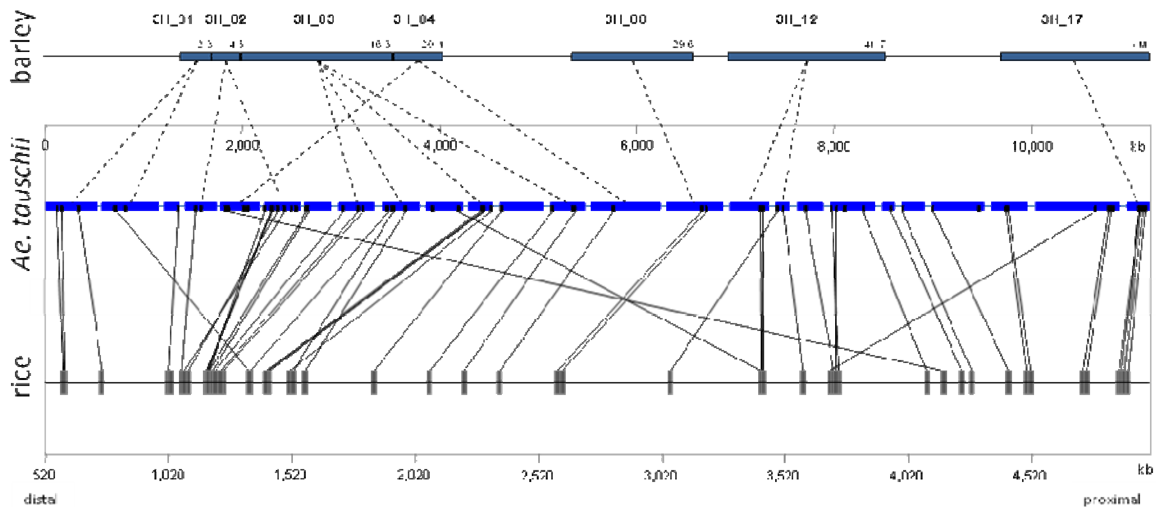

Figure 2. Physical map of the *Ph2* region in *Ae. tauschii* and the syntenous region in the rice genome and barley genetic map.

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