SESSION VI: PHYSICAL MAPPING AND MAP-BASED CLONING — YOUNG RESEARCHERS

Physical mapping of the wheat and Triticeae genomes using single gene FISH.

Tatiana V. Danilova, Bernd Friebe, and Bikram S. Gill. Wheat Genetic and Genomic Resources Center, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506, USA.

The cytogenetic structure of wheat, *Triticum aestivum* (2n=6x=42, AABBDD), was analyzed intensively during the last century. Chromosomes were identified based on meiotic pairing affinities during the 1920s, by aneuploidy during the 1950s, banding techniques in the 1970s, and *in situ* hybridization with repeated DNA probes during the 1980s, which also allowed the mapping of genes to chromosomes and chromosomal arms. The chromosome bin physical maps of expressed sequence tags (ESTs) were developed in the 2000s using deletion stocks. Because of the large sizes of deletion bins, loci within the bins cannot be ordered. Moreover, most of these resources do not exist in wild species, which hinders their exploitation in crop improvement. Fluorescent *in situ* hybridization (FISH) allows mapping of particular sequences to specific chromosomal regions including those with low recombination rates as well as studying chromosomal rearrangements. FISH with wheat tandem repeats can be used for chromosome identification, but the distribution of repetitive elements varies among homoeologous chromosomes within a species or between species. Single-gene FISH can be a useful and tool for genome physical mapping and studying chromosome rearrangements in wheat and its relatives. The genic regions are highly conserved and homoeologous genomes of Triticeae are largely collinear. In our study, to develop a single gene FISH probe, the cytosolic acetyl-CoA carboxylase gene (*Acc-2*) was selected, and the probe was hybridized to chromosomes of bread wheat, *T. urartu*, *T. monoccocum*, *Aegilops speltoides*, *Ae. tauschii*, *T. turgidum*, and *T. timopheevii*. Additional cDNA FISH probes were developed and used for chromosome identification. The *Acc-2* probe was detected on the long arms of each of the group-3 homoeologous chromosomes, on 5DL and 4AL of bread wheat, and on homoeologous and nonhomoeologous chromosomes of the diploid and tetraploid species. In all the species tested, FISH detected more *Acc-2* gene sites or pseudogenes than those detected by PCR or Southern analysis. The *Acc-2* FISH mapping detected chromosome translocations in some of the wild species. The present study demonstrates the usefulness of the FISH technique for physical mapping of genic sequences in wheat and will have broad applications in genome analysis of the Triticeae.

Assembly of chromosome 1BS physical map and its utilization for positional cloning of disease resistance genes in wheat.

Dina Raats [1], Zeev Frenke [1], Tamar Krugman [1], Itay Dodek [1], Jaroslav Dolezel [2], Hana Simkova [2], Helene Berges [3], Federica Cattonaro [4], Thomas Wicker [5], Etienne Paux [6], Catherine Feuillet [6], Abraham Korol [1], and Tzion Fahima [1].

[1] Institute of Evolution and Department of Evolutionary and Environmental Biology, University of Haifa, Haifa, Israel; [2] Institute of Experimental Botany, Olomouc, Czech Republic; [3] INRA–CNRGV, Castanet Tolosan, France; [4] Institutodi Genomica Applicata, Italy; [5] Institute of Plant Biology, University of Zurich, Switzerland; and [6] INRA, Genetics, Diversity and Ecophysiology of Cereals, Clermont-Ferrand, France.

A physical map of chromosome 1BS was constructed within the framework of the European consortium Triticeae Genome designed to develop physical maps of wheat and barley group 1 and 3 chromosomes. Fingerprinted BAC clones from a 1BS-specific library were assembled by LTC software into 385 contigs covering 274 Mb (87%) of 1BS (Sulston 10-25). These contigs were then re-organized (Sulston 10-15) into 52 long supercontigs (1–22 Mb, each), covering ~259 Mb (~82%) of 1BS. Verification steps were conducted using BAC-end sequences. Hybridization of a Nimblegen 40K wheat expression array with 57 MTP pools resulted in the assignment of wheat unigenes to 1BS physical supercontigs. The orientation and order of the supercontigs was determined based on parallel synteny between *Brachypodium* Bd2 and wheat 1BS unigenes. About 600 markers representing 400 different genes were assigned to individual BACs composing the 1BS physical map. Around 300 of these genes were *in silico* anchored to Group 1S Genome Zipper and their deletion bin position was estimated. The assembled 1BS physical map is now being utilized for positional cloning of disease resistance genes derived from wild emmer wheat, *Triticum turgidum* subsp. *dicoccoides*. *YrH52* is conferring broad spectrum resistance to stripe rust, one of the most destructive diseases of wheat. A large mapping population segregating for *YrH52* was developed. Comparative genomic analysis was used to anchor the *YrH52* interval to the colinear region on the *Brachypodium* Bd2, rice Os5, and sorghum Sb9 chromosomes. Screening of the 1BS MTP pools with *YrH52*