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

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

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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.

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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*

flanking markers resulted in the identification of BAC supercontigs that cover 19.2 Mb of the *YrH52* gene region. Further work is underway to refine the physical map and identify candidate gene(s) for *YrH52*. A similar strategy is employed for positional cloning of *Yr15* and *YrG303*, also derived from *T. turgidum* subsp. *dicoccoides*. Collaboration with other groups was established to promote the positional cloning of other wheat genes that reside on 1BS. These results demonstrate the importance of the genomic resources developed by TriticeaeGenome consortium for accelerating positional cloning of target genes in the complex genome of wheat.

High-resolution mapping of areas of low recombination and polymorphism containing the hexaploid wheat loci Pis1 and C.

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Gene mapping and map-based cloning studies in wheat are often complicated by lack of marker polymorphism and/or recombination. Radiation hybrid mapping can address both problems as it relies on radiation-induced breaks to order markers. Due to the nature of RH panels markers are scored as presence/absence, eliminating the need for polymorphism. We utilized this approach to physically map genes based on multiple overlapping deletions induced by γ irradiation. *Pis1* is a wheat floral mutant producing three fully functional pistils per floret instead of the usual single pistil. Compactum (*C*) is responsible for club head phenotype and maps to the low-recombination, pericentromeric region of 2D. The location of *C* with respect to the centromere is not well established. *Pis1* has proven difficult to map in an F_2 population due to lack of marker polymorphism. In this study, independent RH mapping panels were assembled for both genes (282 RH lines for each of *Pist1* and *Compactum*). For each gene, a set of 94 lines were selected and characterized with ESTs and SSRs, specific to the targeted regions. We mapped *Pist1* gene on chromosome 2D (deletion bin 2DL-9) using 14 SSRs and 27 ESTs. Total map distance was 145.0 cR1500 covering ~98 Mb. Even in this region of a recombination hot spot, a cM/cR1500 ratio was found as 1:8; with a mapping resolution of ~750 kb. The closest ESTs flanking *Pist1* are co-segregating and spanned only six rice genes. *Pis1*-linked ESTs were then mapped on a genetic mapping F_2 (Multiovary/Winsome) population. For the *Compactum* locus, 25 ESTs and 16 SSRs were used in mapping. A total map length created was 158.8 cR1500 with average marker retention of 80% and map resolution of ~710 kb. The cM/cR1500 ratio in this region of chromosome was found to be 1:60. Two ESTs flanking *C* locus on this RH map span 15 rice genes. ESTs flanking the *C* locus were mapped on two different bi-parental genetic populations to confirm the outcomes of RH mapping. Putative candidates are being used for developing gene specific markers and work on fine mapping and their eventual cloning is underway.

The rye gene Pm8 conferring resistance to wheat powdery mildew is a homologue of the wheat powdery mildew resistance gene Pm3.

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During wheat breeding, chromosomes of the rye (*Secale cereale* L.) genome have been introgressed into the wheat genome to enhance tolerance to biotic and abiotic stresses. The most widely used wheat–rye translocation nowadays is the 1RS chromosome arm derived from the rye cultivar Petkus carrying three rust resistance genes and the powdery mildew resistance gene *Pm8*. In wheat, *Pm* genes mediate resistance against powdery mildew, a major fungal pathogen. *Pm8* was mapped at the same gene-rich region on the short arm of wheat homoeologous group-1 chromosome as the powdery mildew resistance gene *Pm3* of hexaploid wheat and was, therefore, proposed to be an ortholog of *Pm3*. Southern hybridization analysis showed that there is a sequence in rye and in *Pm8* wheat lines with homology to the *Pm3* promoter region. In a homology-based cloning approach based on primers derived from the cloned wheat powdery mildew resist-