

O22

The Big B of Bread wheat – 3B – exploring the structure, function, and evolution of the hexaploid wheat genome

Paux E¹, Sourdille P¹, Salse J¹, Saintenac C¹, Choulet F¹, Leroy P¹, Rudi Appels R², Korol A³, Spielmeier W⁴, Lagudah E⁴, Dolezel J⁵, Bernard M¹, Feuillet C¹

¹INRA-UBP GDEC, Clermont-Ferrand, France, ²State Agricultural and Biotechnology Centre, Murdoch University, Australia, ³Institute of Evolution, University of Haifa, Israel, ⁴CSIRO Plant Industry, Canberra, Australia, ⁵Laboratory of Molecular Cytogenetics and Cytometry, IEB, Olomouc, Czech Republic.

Wheat genomics offers powerful tools to understand the molecular basis of phenotypic variation, accelerate gene cloning and MAS, as well as improve the exploitation of genetic diversity for efficient crop improvement. While rice and maize improvement is profiting already from information derived from genome sequences, wheat is lagging behind without a genome sequence project underway. Physical maps anchored to genetic maps are the substrate for genome sequencing and they provide efficient tools for marker development, map based cloning, QTL mapping, as well as for structural, functional, and comparative genomics studies. In the framework of the IWGSC, we have developed a physical map of chromosome 3B and established the proof of concept for physical mapping of the 21 bread wheat chromosome through a chromosome based approach. The 3B physical map consists of 1,036 contigs with an average size of 783 kb that cover 811 Mb *i.e.* 82% of the chromosome. To date, the physical map is anchored to cytogenetic and genetic maps with 1,397 markers thereby providing a framework for efficient map based cloning and marker development through BAC end and contig sequencing. Application of the 3B physical map for studies of recombination, LD, genome composition, organisation, function, and evolution will be presented.

O23

Genomic shock induced genetic and epigenetic changes in homoeologs of class ABCDE MADS-box genes in hexaploid wheat

Murai K, Shitsukawa N

Fukui Prefectural University, 4-1-1 Matsuoka-kenjojima, Eihei-cho, Fukui 910-1195 Japan

Bread wheat (*Triticum aestivum* L.) is a hexaploid species with A, B and D ancestral genomes. Most bread wheat genes are present in the genome as triplicated homoeologous genes (homoeologs) derived from the ancestral species. There are three possible evolutionary fates for homoeologs as a response of genomic shock through polyploidization: functional diversification, gene silencing, and retention of original or similar function. To understand the effects of genomic shock on genes for floral development, we are investigating genomic structures and expression patterns of class ABCDE MADS-box genes. We identified two class E MADS-box genes in wheat, *WSEP* (*wheat SEPALLATA*) and *WLHS1* (*wheat Leafy Hull Sterile 1*), which are orthologs of *OsMADS45* and *OsMADS1* in rice, respectively (Shitsukawa et al. 2007, *The Plant Cell* 19: 1723-1737). The three homoeologs of *WSEP* showed similar genomic structures and expression profiles. In contrast, the three homoeologs of *WLHS1* showed genetic and epigenetic alterations. The A genome *WLHS1* homoeolog (*WLHS1-A*) had a structural alteration that contained a large novel sequence in place of the K domain sequence. Phylogenetic analysis indicated that the variant *WLHS1-A* appeared in domesticated tetraploid species. A yeast two-hybrid analysis and a transgenic study demonstrated that the *WLHS1-A* protein had no apparent function. The B and D genome homoeologs, *WLHS1-B* and *WLHS1-D* respectively, had an intact MADS-box gene structure, but *WLHS1-B* was predominantly silenced by cytosine methylation. Of the three *WLHS1* homoeologs, only *WLHS1-D* functions in hexaploid wheat. Furthermore we report the genomic structures and expression patterns in homoeologs of other MADS-box genes such as class B MADS-box genes, *WAP3* (*wheat APETALA3*), *WPI-1* (*wheat PISTILLATA-1*) and *WPI-2* in

wheat. Based on the results, we discuss the significance of genomic shock on genes for floral development.

O24

High-resolution radiation hybrid mapping in wheat: an essential tool for the construction of the wheat physical maps

Kianian SF¹, Riera-Lizarazu O², Gu Y³ and Feuillet C⁴

¹ *Department of Plant Sciences, North Dakota State University, Fargo, ND 58105,* ² *Department of Crop and Soil Science, Oregon State University, Corvallis, OR 97331,* ³ *USDA-ARS, Western Regional Research Center, Albany, CA 94710, and* ⁴ *UMR INRA-UBP 1095, 63100 Clermont-Ferrand, France*

Methods for physical mapping of chromosomes, which do not rely on meiotic recombination, are necessary for large genomes like wheat where uneven distribution of recombination and significant variation in genetic to physical distance ratios dramatically affect the capacity to order physical contigs in large portions of the chromosomes. In this context, physical mapping using a radiation hybrid (RH) mapping approach has proved valuable in a number of non-plant and plant systems. RH maps of chromosome 1D, utilizing 87 lines (irradiated with 35 Krad), and chromosome 3B, utilizing 187 lines (99 from 25 Krad and 88 lines from 35 Krad), were generated by mapping different marker classes without the need for polymorphism. Analysis of 1D RH panel with 378 marker loci identified a total of 2,312 obligate breaks for an average resolution of ~199 kb (size of chromosome/total breaks = 464 Mb/2,312 breaks). Remarkably, analysis of several large sequenced segments (3 Mb average size) of chromosome 3B with the 3B RH panels also indicated an average map resolution of ~200 Kb/break. Since this mapping resolution is within the range of BAC contig alignment, these panels have been used to align BAC contigs to regions of chromosomes 1D and 3B and to further refine the location of *species cytoplasm specific* (*scs^{ae}*) locus on chromosome 1D. The *scs^{ae}* locus could not be conventionally mapped in the durum alloplasmic background indicating an added benefit of RH panels.

O25

Structure and organization of the wheat genome

Devos, KM

Dept. of Crop and Soil Science, and Dept. of Plant Biology, University of Georgia, Athens, GA 30602, USA

Our knowledge on the structure and the organization of the wheat genome has greatly increased over the past five years, mainly because of large-scale physical mapping of ESTs and sequencing of BAC clones. Nevertheless, because the sequence information obtained during map-based cloning experiments is generally biased towards gene-rich regions, the gene distribution patterns observed in these regions are not representative for the entire wheat genome. We are addressing this gap in our knowledge in two studies. The first study, which has just been completed, involved the sequencing, annotation for gene content and mapping to chromosome bins of 220 randomly selected hexaploid wheat (Chinese Spring) BAC clones. This analysis provides information on how the density and distribution of genes varies along the telomere-centromere axis. To evaluate local organizational patterns within gene-rich and gene-poor regions, megabase regions were selected in *Ae. tauschii* based on the finger print contigs and sequenced. This data also allowed us to assess the extent of colinearity that exists at the DNA sequence level between wheat and *Brachypodium distachyon*. *B. distachyon*, which has a genome size of 320 Mb, is the closest relative of wheat for which whole-genome sequence information is available. With the development of the new generation sequencing technologies such as GS FLX, Illumina and SOLiD, sequencing of the wheat genome is no longer cost-prohibitive, but sequence assembly will be a huge challenge. If colinearity is highly conserved between *B. distachyon*

and wheat, it might be possible to use the *Brachypodium* genome as template to assist in the assembly of the wheat genome. The wheat – *Brachypodium* comparative results spanning several megabases of DNA will be discussed.

O26

Single feature polymorphism discovery using the wheat Affymetrix Gene Chip

Somers DJ, Jordan MC, Banks T

Agriculture and Agri-Food Canada, Cereal Research Centre, 195 Dafoe Rd. Winnipeg, MB, Canada

The Affymetrix GeneChip® Wheat Genome Array holds 605,000 individual 25 bp oligonucleotide probes, or features, which represent >60,000 transcripts. Differences in hybridization affinity to these 25-mers between individuals in a segregating population can be exploited for the development of genetic markers called Single Feature Polymorphisms (SFPs). We are engaged in a project to merge microarray expression profiling and marker development with seed quality and agronomic performance data. Using 80 individuals and parents from a segregating doubled haploid population ('AC Domain' x RL4452) we generated replicated microarray hybridization data and mined it for SFPs having a robust Mendelian segregation characteristic. To date we have identified 100's of SFP markers and have integrated them into an existing SSR map of the population. Further, SFPs are derived from expressed sequences, thus knowledge on the physical location of genes was generated. The usefulness of SFPs in predicting physical location was evaluated using synteny with rice. In addition to SFP genotyping the same data set can be used to identify Expression Level Polymorphisms (ELPs). ELP analysis revealed 2349 probe sets differing in intensity between the genotypes in the population and from these 926 major expression QTL (eQTL) were identified. Integration of the eQTL with the combined SSR/SFP map assists in the discovery of major regulatory regions and in determining whether the eQTL are cis or trans regulated. This information will be useful in exploring the genomic evolution of cereals, in identifying genome regions controlling economically important traits and for devising strategies to sequence complex genomes such as wheat.

O27

Marker/trait associations identified in spring wheat using 25 years of CIMMYT international trials

Arief V¹, DeLacy IH^{1,2}, Dieters MJ¹, Crossa J⁴, Godwin I¹, Batley J¹, Davenport G⁴, Dreisigacker S³, Edwards D², Huttner E^{6,7}, Lambrides C¹, Manes Y³, Payne T³, Singh RP³, Duveiller E³, Warburton M³, Wenzl P^{6,7}, Kilian A^{6,7}, McLaren G^{4,5}, Braun H-J³, Crouch J³, Ortiz R³, Basford KE^{1,2}

¹*School of Land Crop and Food Sciences, The University of Queensland,* ²*Australian Centre for Plant Functional Genomics (ACPGF),* ³*International Maize and Wheat Improvement Center (CIMMYT),* ⁴*Crop Research Informatics Laboratory (CRIL),* ⁵*International Rice Research Institute (IRRI),* ⁶*Diversity Arrays Technology P/L Canberra,* ⁷*Triticarte P/L Canberra.*

This study identified marker/trait associations (MTA) by jointly analysing comprehensive field trials and dense DArT® genome scans (1,447 polymorphic markers). MTA were identified for 21 traits (3 rusts, grain yield, 6 agronomic characters, grain protein, and 10 foliar diseases) using data collected from the first 25 years of CIMMYT's Elite Spring Wheat Yield Trials (ESWYTs). Genotypic data were generated for a set of 645 partially duplicated lines. Population structure was obtained using pedigree information and environment structure was obtained using CIMMYT mega-environments classification. LOD scores were calculated for each marker-trait combination using a t-test for each population and environment structure. All markers with a LOD score greater than three were considered to be potentially associated with traits and displayed using heatmaps. This approach

Tuesday 26 August
Sessions 5 and 6: Genome dynamics

identified numerous associations for each trait studied. DArT[®] genome scans were consistent across duplicated lines and enabled the identification of introgressed segments based on haplotypes. The results of this study improve our understanding of the germplasm used in plant breeding programs, including a better characterization of parents. The results are also useful for selecting new crosses and they provide a path towards 'haplotype' breeding.