P079

Role of selection and gene conversion in polyploid wheat evolution

Akhunov ED[1,5], Akhunova AR[1,5], Anderson OD[2], Anderson JA[3], Blake N[6], Clegg MT[4], Coleman-Derr D[1], Conley EJ[3], Crossman CC[1], Deal KR[1], Dubcovsky J[1], Gill BS[5], Gu YQ[2], Hadam J[5], Heo HY[6], Huo N[2], Lazo GR[2], Luo MC[1], Ma YQ[1], Matthews DE[7], McGuire PE[8], Morrell P[4], Qualset CO[8], Renfro J[1], Reynolds S[3], Tabanao D[3], Talbert LE[6], Tian C[1], Toleno D[4], Warburton M[9], You FM[1], Zhang W[1], Dvorak J[1]

[1]Plant Sciences, UC Davis, Davis, CA; [2]GGD, USDA/ARS WRRC, Albany, CA;[3]Agronomy and Plant Genetics, University of Minnesota, St. Paul, MN;[4]Ecology and Evolutionary Biology, UC Irvine, Irvine, CA;[5]Plant Pathology, KSU, Manhattan, KS; [6]Plant Sciences and Plant Pathology, Montana State University, Bozeman, MT;[7]USDA-ARS, Cornell University, Ithaca, NY;[8]GRCP, UC Davis, Davis, CA;[9]CIMMYT, Mexico City, Mexico

Functional redundancy in polyploid organisms creates possibilities for the evolution of new gene functions by reducing the selective pressure. To assess the consequences of polyploidization on the evolutionary dynamic of wheat genomes more than 2,000 gene fragments have been sequenced in the populations of polyploid wheat and its diploid ancestors. The divergence between orthologous sequences in polyploid wheat was found to be higher than that in the diploid ancestors. This observation could be explained either by the acceleration of mutation rate after polyploidization or by inter-genomic gene conversion. The latter process would generate shared polymorphism between the wheat genomes. The number of shared mutations between orthologues in the wheat genomes was negligible suggesting that inter-genomic gene conversions are rare. The ratio of non-synonymous (dN) to synonymous (dS) mutation rates in coding sequences was used as a measure of relaxation of purifying selection in polyploid wheat. It was shown that the dN/dS ratio was significantly higher in polyploid wheat compared to that in the diploid ancestors. These results support the hypothesis that the increased divergence between orthologous sequences in polyploid wheat could be attributed to relaxation of purifying selection resulting in acceleration of non-synonymous mutation rate. This process could have contributed to the functional diversity and increased adaptability of polyploid wheat.

P080

Possible horizontal transfer of two subclasses of *Mutator*-like elements within Poaceae

Asakura N[1], Mori N[2], Yoshida S[3], Ohtsuka I[1], Nakamura C[2]

[1]Laboratory of Biology, Faculty of Engineering, Kanagawa University, Yokohama, Japan, [2] Laboratory of Plant Genetics, Graduate School of Agricultural Science, Kobe University, Kobe, Japan, [3]Hyogo Prefectural Research Center for Agriculture, Forestry & Fisheries, Kasai, Japan

Mutator transposons have been studied extensively at molecular level since Mutator trait exhibiting high frequency mutations was first identified in maize. Molecular features and transposition mechanisms of *Mutator* have essentially been_elucidated. Furthermore, these studies have revealed that *Mutator*-like elements (MULEs) are widely distributed in plants and compose a superfamily. MULEs can be divided into several classes based on the coding sequences for transposases. In *Triticum urartu*, a wild einkorn wheat, we identified two distinct MULE subclasses belonging to *Mutator* Class. Similarity of nucleotide sequences between two subclasses was less than 60 %, showing their clear differentiation. We performed a series of study to understand MULE dynamics in Poaceae using these two MULE subclasses as clues. Distribution of two MULE subclasses was investigated among 114 accessions from four subfamilies, i.e., Ehrhartoideae, Pooideae, Panicoideae, Chloridoideae, by PCR using primer sets corresponding to transposases of the two subclasses. Both MULE subclasses showed so-called 'patchy' distribution. Phylogenetic analysis in each MULE subclass was performed by comparing sequences of the PCR-amplicons described above. In both

subclasses, MULEs that were detected from distantly related species exhibited a remarkably high similarity. Phylogenetic relationship of MULEs in both subclasses was partially inconsistent with the presumed phylogeny of the grass species. These results suggested that both MULE subclasses were horizontally transmitted within grass species. Although horizontal transfer of MULEs between rice and *Setaria* has recently become evident, little is known about horizontal transfer of nuclear-encoded genes between higher plants. We report here a case of possible horizontal transfer of MULEs between other grass species.

P081

QTLs for grain color and spike traits in bread wheat and their correspondence in rice genome

Balyan HS, Gupta PK, Kumar A, Kumar J and Singh R

Molecular Biology Laboratory, Department of Genetics and Plant Breeding
Ch. Charan Singh University, Meerut-250004, India

In bread wheat, QTL analyses was conducted (i) for grain colour using an intervarietal RIL population, namely PW-population, derived from the cross PH123 (red grain colour) × WL711 (white grain colour) and (ii) for spike traits (spike length and spikelets per spike) and plot yield using International Triticeae Mapping Initiative population (ITMI*pop*). Besides main-effect QTLs, epistasis was also involved in controlling the genetic variation for the above traits. A major QTL for grain colour explaining up to 36.18% PV was mapped on 3BL in PW-population, and a major QTL was found on 2DS to control three traits including spike length, spikelets per spike and plot yield (explaining from 29.73% to 37.85% PV for individual trait). The QTL for grain color was physically mapped to 37% distal region (3BL1-0.63-1.00) of 3BL, while the QTL for the spike and plot yield was physically mapped to distal bin (2DS5-0.47-1.00) covering 53% region of 2DS. Comparative mapping revealed that the genomic region harbouring QTL for grain colour is orthologous to genomic region proximal to the red pericarp (*Rd*) gene on rice chromosome 1 and the genomic region hrabouring QTL for spike and plot yield could be an orthologue of a major QTL for spikelets per panicle (*qSSP7*) located in a 912.4 kb region of rice chromosome 7. This information may prove useful for high resolution mapping leading to map-based isolation of the above two major QTLs.

P082

Towards a Wheat Phenome Atlas and a Phenome Atlas Toolbox: What are they? What progress?

DeLacy IH[1,2], Dieters MJ[1], Crossa J[3,4], Godwin ID[1], Arief V[1], Batley J[1] Davenport G[3,4], Dreisigacker S[3], Edwards D[2], Huttner E[6,7], Lambrides CJ[1], Manes Y[3], Payne T[3], Singh RP[3], Duveiller E[3], Warburton M[3], Wenzl P[6,7], Kilian A[6,7], McLaren G[4,5], Braun H-J[3], Crouch J[3], Ortiz R[3], Basford KE[1,2]

[1]School of Land Crop and Food Sciences, The University of Queensland, Brisbane, [2]Australian Centre for Plant Functional Genomics (ACPFG), [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.

A Phenome Map is a representation of all the regions of a genome that influence heritable phenotypic variation for a trait, and a Phenome Atlas consists of the integration of all available phenome maps with a description of the methodologies that were used to produce the maps. A Phenome Atlas Toolbox is a set of tools and methodologies for producing the Phenome Atlas. The Wheat Phenome Atlas (WPA) will be an integration of phenotypic data (17 million data points for 80 traits from 10,000 international field trials collected over 40 plus years) generated by CIMMYT and partners on approximately 13,000 wheat lines (for which pedigrees are known) with >26 million DArT® marker

data points obtained by genotyping these lines. To generate this amount of phenotypic data would cost over $500 million today. The lines tested in the CIMMYT international nurseries form a plant breeding population and are the result of intense selection among and within a large number of genealogically connected families each with a few (sometimes more) highly related individuals. Plant breeding programs are highly structured populations and this must be catered for in developing the Phenome Atlas. Progress toward the WPA will be reported.

P083

Patterns of linkage disequilibrium in multiple wheat populations

Dreisigacker S[1], Arief V[2], DeLacy IH[2], Davenport G[1], Manes Y[1], Reynolds M[1], Singh R[1], Dieters M[2], Crossa J[1]

[1]*International Maize and Wheat Improvement Center (CIMMYT),*

[2]*School of Land, Crop and Food Sciences, University of Queensland, Brisbane*

Prospects for utilizing whole-genome association analysis in autogamous plant populations appear promising due to the reported high levels of linkage disequilibrium (LD). To determine the optimal strategies for implementing association analysis in wheat (*Triticum aestivum* L. subsp. *aestivum*) we analyzed the structure of LD across the genome in distinct groups of CIMMYT wheat breeding germplasm. LD within a total of 1500 advanced lines bred during the last 2 decades for three different CIMMYT international nurseries (e.g. Elite Spring Wheat Yield Trials, Semi-Arid Wheat Yield Trials, Heat Tolerance Wheat Yield Trials) and pre-breeding materials of 180 synthetic hexaploid wheat lines have been examined. More than 1000 DArT markers were utilized plus additional SSR markers on a smaller set of lines to compare LD measures among marker technologies. We have found that LD is highly variable not only among populations but also between different regions of the genome. LD was distributed in blocks across chromosomes. Long range LD levels were measured. The variable pattern of LD among chromosomes and populations provides a range of estimates of the number of markers that will be needed to capture most haplotype variation for a whole-genome association analysis in wheat.

P084

Physical mapping of chromosomes 3HS and 3DS

Fleury D[1], Shoaei Z[1], Luo M[2], Dvorak J[2], Langridge P[1]

[1] *Australian Center for Plant Functional Genomics, The University of Adelaide, PMB1 Glen Osmond, SA 5064, Australia*

[2] *Department of Plant Sciences, University of California, Davis Davis, CA 95616, USA*

The aim of this Australian International Science Linkages 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. We made a first physical map of chromosome 3S by using the BAC library of *Aegilops tauschii,* the ancestor of D genome. Since the low level of polymorphism limit the anchoring of BAC contigs on wheat genetic maps, we have linked the *Ae. tauschii* physical map on barley genetic maps. Based on BAC-end and EST sequences, new molecular markers have been identified and will be used to tie the physical map to a high resolution genetic map of wheat. We will use two large populations developed in ACPFG, consisting of 300 doubled haploid lines plus 3,000 single seed decent lines (F5) which will give a resolution of less than 0.01cM . The comparison of our data with those from the FP7 project will allow definition of the genetic/physical relationship across the target region in the A, B, D and H genomes.

P085

Analysis of the functional relationships of gametocidal genes

Friebe B, Gill BS
Wheat Genetic and Genomic Resources Center, Department of Plant Pathology, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS 66506-5502, USA

We have recently produced EMS-induced knock-out mutations at the *Gc2* locus that were derived from *Ae. sharonensis*. Our data suggest that the *Gc2* mutant is a knock-out of the gene encoding for the breaking agent. For analyzing the interactions of the *Gc2* mutants with other Gc genes from different *Aegilops* species, we have crossed stocks with functional and mutant *Gc2* genes with stocks having Gc genes from *Ae. speltoides* (T2B-2S, *Gc1*), *Ae. cylindrica* (DA2C^{c}, *GcAe.*$^{cyl.}$), *Ae. triuncialis* (DtA3C^{t}L, *Gc3*), and *Ae. geniculata* (DA4M^{g}, *GcAe.*$^{genic.}$). If the mode of action of *Gc1*, *Gc3*, *GcAe.*$^{cyl.}$, and *GcAe.*$^{genic.}$ is similar to that of *Gc2,* the introduction of a mutant *Gc2* allele that lacks the factor for the breaking agent and only encodes for the protecting agent might restore the fertility in these plants. The presented data show that in none of the cross combinations analyzed does the introduction of a *Gc2* mutant allele rescue plant fertility suggesting that their modes-of-action are different.

P086

Analysis of promoters in transgenic wheat

Furtado A[1], Henry R[1] and Pellegrineschi A[2]
[1]*Molecular Plant Breeding CRC, Centre for Plant Conservation Genetics, Southern Cross University, Lismore,*
[2]*Pioneer Hi-Bred International, Inc, USA.*

The cereal grain is comprised of several tissue types and expressing transgenes in one or more tissue types will be crucial to use the cereal grain as a production platform to express transgenes for various uses. Use of biotechnology to improve the cereal grain for end-use quality or using it as a bioreactor has been amply demonstrated at least for the rice grain. Wheat is an important world food crop and improving it using a transgenic approach would require the use of specific gene/s but also the use of specific promoter elements to control the spatial and temporal expression of the gene/s. Use of one promoter to control expression of several transgenes in a specific plant tissue-type can lead to unpredictable transgene expression due to homology-based transgene silencing. We have studied the rice *Glutelin-B1* promoter, and the *barley alpha-amylase/subtilisin inhibitors* (*isa*) and the *B-hordein* (*B-hor*) promoters in transgenic wheat to investigate if specificity is maintained in a heterologous system. We also report the strength of these promoters to drive *gfp* expression in the wheat grain. Wheat promoters corresponding to the High Molecular Weight glutenin (*HMW-glu*) and the *Early-maturing* (*Em*) genes were also included in this study. Our results indicate that promoters work best in their homologous species and may or may not work in a heterologous species. This data demonstrates that for directing transgene expression in plants it is preferable to use promoters from the same species unless they have been adequately tested in the desired species.

P087

Construction of Euchromatin Enriched Genomic DNA library in wheat and development of STS marker sets

Heo HW[1], Lee SK[2], Oh PR[1], Kim HS[1], Kang MS[1], Park CS[3], Woo SH[4], Hwang JJ[1] and Kim SJ[1]

[1]*National Institute of Crop Science, RDA, Suwon 441-857, Korea*
[2]*Dongguk University, Seoul 100-715, Korea*
[3]*Honam Agricultural Research Institute, NICS, RDA, Iksan 570-080, Korea*
[4]*Chungbuk National University, Cheongju 361-763, Korea*

To develop the EEG(Euchromatin Enriched Genomic DNA) library of wheat, we used Mcr A and Mcr BC system in DH5 alpha bacteria cell line. About three thousands of EEG colonies have been constructed by using junk DNA exclusion. Among the constructed colonies, we analyzed the genetic information of five hundred of colonies using blast search of NCBI and GRAMENE web site. More than two hundred of STS primer sets have been developed using sequencing data of selected colonies. Twenty five percent of designed primer sets have shown polymorphism in wheat germplasm using six endonucleases. These primers could be useful for specific allele tagging in mapping population and germplasm and for the study of functional genomics of wheat.

P088

Quantitative trait loci association with thousand grain weight and test weight in durum wheat (*Triticum turgidum* L. var. durum)

Houshmand S[1], Knox RE[2], Clarke FR[2], Clarke JM[2]

[1]*College of Agriculture, Shahrekord University, Shahrekord, Iran*
[2]*Agriculture and Agri-Food Canada, Semiarid Prairie Agricultural Research Centre, Swift Current, Saskatchewan, Canada*

Thousand grain weight and test weight are complex characters and associate with grain yield and end use quality of durum wheat (*Triticum turgidum* L. var. durum). A doubled haploid population segregating for 1000-grain weight and test weight was developed using the maize pollen method from the cross Kofa/(Kyle*2/ Biodur), wherein the Kofa parent has higher 1000-grain weight and lower test weight compare to another parent, Kyle*2/ Biodur (an F9 line). Thousand grain weight and test weight were determined in replicated field trials grown at three locations, typical of the durum production area of western Canada, in 2000 and 2001(6 environments). Data were analyzed using mixed-model ANOVA to assess differences among genotypes. The parents were screened for polymorphism with 517 wheat microsatellite markers with known chromosomal locations, of which 54 polymorphic markers were employed to genotype the population. The genetic linkage map of the 53 polymorphic loci, that followed a 1:1 ratio of parental bands, covered about 970 cM of the population genome. The QTL analysis was performed by composite interval mapping using the MQTL program. Four QTL affected 1000-grain weight were localized on chromosomes 2A, 2B, 4B and 6B. *QTgw.spa-6B* and *QTgw.spa-2B* were associated with 1000-grain weight at one and two environments, explained 8% and 9% of the phenotypic variation in this trait respectively. QTLs which localized on chromosomes 2A and 4B significantly associated with 1000-grain weight at 3 environments each of them and explained 8-12% and 10-16% of variation of this trait respectively. These two QTLs, located on 2A and 4B, also significantly related to test weight at 2 and 3 environments and explained 10-14% and 8-16% of variation of this trait respectively respectively. Another QTL that was associated with test weight at 2 environments was localized on chromosomes 2A and explained 8-11% of phenotypic variation of test weight.

P089

PCR-based Landmark Unique Gene (PLUG) marker is a useful tool for comparative genomic analysis and BAC clone screening in wheat

Ishikawa G[1], Ashida T[2], Saito M[1], Nasuda S[2], Endo TR[2], Nakamura T[1]
[1] Tohoku National Agriculture Research Center, Morioka, Iwate 020-0198, Japan, [2] Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

PCR-based Landmark Unique Gene (PLUG) markers are EST-PCR markers developed by taking advantage of the orthologous gene conservation between rice and wheat, and of intron polymorphisms among the three orthologous genes of wheat. We have shown the potential of PLUG markers for distinguishing the three wheat orthologs of a template rice gene (Ishikawa et al. 2007). In this study, we designed a total of 960 primer sets from wheat ESTs which showed high similarity with 951 single-copy rice genes. When genomic DNA of Chinese Spring wheat was used as a template, 872 primer sets amplified one to five distinct products. To determine the locations of the products on wheat chromosomes, nullisomic-tetrasomic analyses were conducted. In total, 1,016 products from 533 primer sets were assigned to chromosomes. The number of markers developed for each of the chromosomes ranged from 32 for chromosome 6A to 74 for chromosome 7D, with an average of 48 markers per chromosome. Out of the 533 primer sets, 154 that were assigned to A, B and D genomes were used for deletion-bin mapping. The mapping data from these markers clearly supported previously reported synteny data between wheat and rice gene locations. In addition, using these markers as anchors, we found some large differences in sizes among the corresponding parts of homoeologous chromosomes and identified several novel synteny perturbations. Furthermore, using a PLUG marker as a probe, BAC clones that contain homoeologous regions from A, B and D genomes can be simultaneously picked up from a library.

P090

The genomics of stem rust resistance in wheat

Knott DR
Department of Plant Sciences, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, S7N 5A8, Canada

The interaction between wheat (*Triticum aestivum* L.) and wheat stem rust (*Puccinia graminis* Pers. f. sp. *tritici* Eriks. & Henn.) can take many forms. A major division can be made between race-specific and non-race-specific or general resistance. Race-specific resistance involves a gene-for-gene relationship in which a gene product from a gene for resistance in the host interacts with a gene product from a gene for avirulence in the pathogen. The degree of resistance can vary greatly depending on how quickly the gene-for-gene interaction stops rust development. Genes for specific resistance act largely independently of each other. A genotype with two genes for resistance is resistant to all races to which at least one gene conditions resistance. Combinations of two genes that give resistance to the same race may give a somewhat higher degree of resistance than either alone, particularly in field tests.. In recent years, there has been increasing evidence of the importance of suppressors that act against genes for specific resistance. Resistance is often discovered that is not controlled by specific major genes whose individual effects are readily measured but by several genes having relatively small effects. The effects are often additive or even multiplicative. Various names have been applied to this type of resistance – polygenic, partial, slow rusting, adult plant resistance, etc. The resistance is thought to be non-race-specific and, therefore, durable. Although the effect of individual genes is relatively small, because their effects are additive they can result in a high degree of resistance. Since the individual genes have small effects, it is difficult to study them individually to determine whether a gene-foe-gene relationship with the pathogen's genes for avirulence occurs. However, the involvement of several genes in resistance makes the evolution of a gene-for-gene system less likely. The rust resistance phenotype of a wheat plant will depend on the combined effect

of all the genes it carries that have some effect on rust development. On top of this is the effect of the environment, particularly temperature.

P091

The sequence polymorphism of SBEIIa gene in wheat (Triticum sp.)

Konovalov FA, Shaturova AS, Kudryavtsev AM
N.I.Vavilov Institute of General Genetics, 119991 Moscow, Russia

The *SBEIIa* gene in common wheat encodes a starch branching enzyme that converts growing amylose chains to amylopectin tree-like structures and maintains normal amylose/amylopectin ratio in endosperm starch. The mutant plants lacking the activity of all three *SBEIIa* homeologs are expected to have increased amylose content and may be used as a new industrial source of resistant starch. Sequencing of *SBEIIa* copies is an essential step in performing mutation search at DNA level.

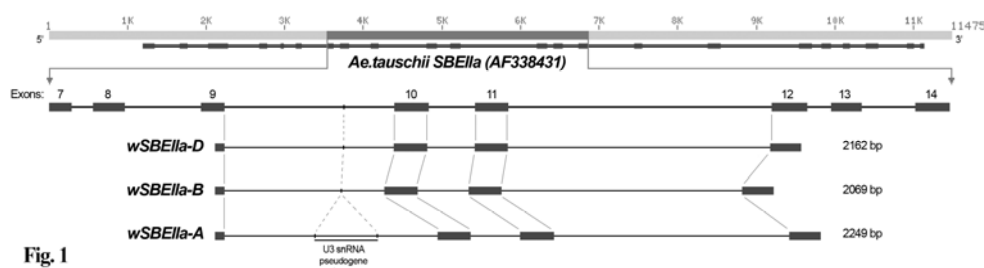

Fig. 1

We have isolated and sequenced three distinct copies of *SBEIIa* fragment (exons 9-12) using PCR with degenerate primers from wheat cv. Chinese Spring DNA and also from the DNA of diploid wheat *T.monococcum* (Genbank entries EU024966–EU024969; Fig.1). One of the inserts in A-subgenome copy intron 9 is similar to wheat U3 snRNA gene (Genbank X63065) and by some features is likely to have arisen as L1-processed pseudogene. In *T.monococcum*, the insert 3'-end also contains a 50-bp deletion that extends to flanking intron sequence. PCR screening of 31 *Triticum* accessions demonstrated that U3-like insert is always present in A-genome, and that the 50-bp deletion is a feature of A^m genome wheats, including *T.boeoticum*, *T.monococcum* and *T.sinskajae*. In A^u genome lineage, from *T.urartu* to various tetra- and hexaploid wheat species, the deletion is absent, confirming that *T.urartu* is an A-genome donor of major cultivated wheat polyploids. It can be speculated that A-genome wheats originated from a single plant in which the integration of snRNA pseudogene by L1 retrotransposon had taken place.

P092

SSR and ISSR markers for assessing DNA arieties of India

Malik R, Kundu S, Sareen S, Kumar R, Shoran J and Mishra B
Directorate of Wheat Research, Post Box No. 158, Karnal-132001, India

SSR and ISSR marker systems were used to study the genetic diversity in bread wheat (Triticum aestivum L.) varieties released for high yield, quality and abiotic stress in India. A set of 27 varieties were screened with 20 wheat microsatellite markers (WMS), covering 15 chromosomes of wheat genome, and 20 UBC series ISSR markers at molecular level. Total 146 allelic variants were detected at 20 loci, ranging from 86 to 210 bp with an average of 7.3 alleles per marker. The occurrence of rare alleles (frequency 33.56 %) was observed for 17 markers. The polymorphism information content (PIC) values of the markers ranged from 0.382 (WMS 186) to 0.826 (WMS 389) with an average of 0.628. During ISSR analysis, total 176 bands were amplified revealing 68.42 % polymorphisms. The DNA fingerprint databases were used for UPGMA based cluster analysis. The genetic relationships estimated by SSR and ISSR markers revealed substantial genetic variability reflecting wide adaptability and applicability of Indian bread wheat cultivars. Both marker types efficiently discriminated all 27 selected varieties at DNA level. However, SSR based

cluster tree grouping of these varieties was found more in agreement with their known origin and pedigree as compare to ISSR cluster analysis. Easy handling, reliability and high information level are the features that justify the utility of SSR and ISSR markers in DNA fingerprinting of wheat for genetic variability analysis.

P093

Gene expression balance among homoeologues and its interdependence on gene dosage in polyploid wheat

Mutti JS, Gill KS

Department of Crop and Soil Sciences, Washington State University, Pullman, WA 99164

Gene duplication by polyploidy (homoeologues) or other means (paralogues) is a prominent feature of angiosperm evolution. We studied gene expression among three homoeologues of hexaploid wheat that evolved from a common progenitor about 3 million years ago (MYA) and came into a common nucleus at different times: ~0.5 and 0.01 MYA. Gene expression corresponding to each homoeologue was identified by sequence comparison of cultivar 'Chinese spring' (CS) ESTs and the results were confirmed by SSCP analysis of RNA using nulli-tetra lines. Of the 632 genes analyzed, 58% were expressed from all three homoeologues, 33% from two, and only 9% were expressed from one of the three homoeologues. The largest percentage of genes (14%) were expressed in anthers and the least (7%) were expressed in pistils. Whereas, the highest number of homoeologues/gene were expressed in roots (1.72 out of 3 homoeologues) and the lowest number were expressed from anthers (1.03 out of 3 homoeologues). In general, the proportion of expressed copies decreased with the increase in homoeologue copy number. The most significant observation was that homoeologues for 87% of the genes showed different expression patterns in different tissues and thus have likely evolved different gene expression controls. About 30% of the genes showed dosage dependence as the expression of homoeologues changed in response to changes in structural copy number

P094

Large scale analysis of expressed genes in common wheat

Ogihara Y[1], Kawaura K[1], Mochida K[2]

[1] *Kihara Institute for Biological Research, Yokohama City University, Japan,* [2] *Plant Science Research Center, RIKEN, Japan*

Because of wheat's huge genome size and polyploidy, we concentrate to conduct large-scale analysis of expressed sequence tags (ESTs) in common wheat. Up to now, 49 cDNA libraries derived from tissues during the wheat life cycle as well as stress-treated tissues were constructed. Several thousands colonies were randomly selected from each of these 49 cDNA libraries and sequenced from both of 5'- and 3' ends. Sequence data of 630,336 ESTs are now available. These ESTs were grouped into about 90 thousands homoeologs and 36 thousands gene clusters with CAP3 and BLAST methods. These contigs were estimated to cover more than 90 % of expressed wheat genes. By comparison of these wheat genes to pseudomolecules of rice, it is shown that about 30 % of genes were plant specific with each other. By computing abundantly expressed ESTs, correlated expression patterns of genes across the tissues (Virtual Display: VD) were monitored. Furthermore, the relationships between gene expression profiles among the stress-induce tissues were inferred using VD, and genes specifically induced and/or suppressed by stresses were able to be selected from the VD. These genes were annotated with the BLAST search. In silico selection of screened genes from VD should provide a powerful tool for functional genomics of cereals. In addition to EST data, we are also accumulating full length cDNA data of Chinese Spring wheat. We extracted total RNAs from 17 tissues during the life cycle and/or stress-treatment, and constructed a full-length cDNA library from these pooled RNAs with the CAP-trapper method. We randomly selected about 20,000 clones from the library, and sequenced from both ends. These sequences were classified into the 7149 gene clusters. Finally,

6,162 clones were selected from the gene clusters to complete the insert sequences. Additionally, further 6,590 clones were selected to carry out entire sequencing after another cycle of end-sequencing of 20,000 clones. These sequence data of full-length cDNAs cover one-third of total expressed genes.

P095

Custom wheat microarray development for analysis of grain quality

Pacey-Miller T, Bundock P, Henry R
Centre for Plant Conservation Genetics, Southern Cross University, Lismore, Australia

Many genes influencing wheat grain quality are expressed during seed development. Custom microarrays have been developed using data produced from previous SAGE (Serial Analysis of Gene Expression) analysis of the wheat genome. A 12K array was designed for each of two time points of the developing wheat grain (14dpa and 30dpa). The arrays contained genes that had shown statistical differences in expression between wheats of varying quality. In addition other genes of specific interest to the authors were included on the slide as were controls. An electrochemical detection system was used for recognition of hybridisation. This process of including only variable genes narrowed the number of data points to be analysed to a more manageable number. This system can therefore be used to analyse a larger number of varieties for genes of interest at a lower cost. This microarray tool should have wide application in wheat quality analysis.

P096

Real-Time PCR, a tool for the analysis and quantitation of WIS2-1A retrotransposon in hulled wheat

Mondini L, Porceddu E, Pagnotta MA
Department of Agrobiology and Agrochemistry. University of Tuscia,
Via S.DeLellis, 01100 Viterbo (Italy) pagnotta@unitus.it

The Real-time PCR technique was adopted to assess genetic variability present in five accessions of einkorn (*T. monococcum*, 2n = 2x = 14), emmer (*T. turgidum*, 2n = 4x = 28) and spelt (*T. spelta*, 2n = 6x = 42). A simple Real-time PCR assay, based on SYBRGreen I dye, was employed to detected the copies number of one of the most important retrotransposon present in *Triticum* genomes, WIS2-1A. It is the first retrotransposon found in wheat and was primarily observed as an insertion into a High-Molecular-Weight (HMW) storage protein gene in *T. aestivum*. It represents an ancient DNA element that probably was already present in the common diploid ancestor of the *Triticae* tribe. In the present work, it has been developed and optimized a Real-time PCR assay which has permitted to detect the presence of retrotransposon; moreover, it has provided an accurate quantitative analysis. Significant differences were observed in the WIS2-1A copies number both among species and among accessions within species. Furthermore, as expected, the lowest copy number was observed for *T. monococcum* which represent the diploid level present among hulled wheats. On the other hand, a similar number of copies it has been observed in *T. dicoccum* (tetraploid) and in *T. spelta* (exaploid). Even if in previous studies in barley was observed, a strong correlation between the retrotransposons copy number and genome size, recently in wheat it has been demonstrated that the wheat genome A have the higher transposable elements content than those genomes B and D. Therefore, this work confirms previous results where it has been observed that the A ancestral genome may have under-gone differential genome expansion caused by Class I elements prior to speciation of the tetraploid wheat ancestor; hence that the amount of retrotransposon is not linearly linked to the ploidy level of the wheat species.

P097

Insertion site-based polymorphism: A Swiss army knife for wheat genomics

Paux E[1], Saintenac C[1], Choulet F[1], Faure S[1], Roger D[2], Chevalier K[1], Paux K[1], Cakir M[3], Gandon B[2], David J[4], Nevo E[5], Bernard M[1], Sourdille P[1], Feuillet C[1]

[1] UMR GDEC 1095, INRA, 63100 Clermont-Ferrand, France, [2] Limagrain Verneuil Holding, 63204 Riom, France, [3] State Agricultural Biotechnology Center, Murdoch University, Murdoch WA 6150, Australia, [4] UMR DGPC, INRA, 34130 Mauguio, France, [5] Institute of Evolution, University of Haifa, Israel

Transposable, elements (TEs) are prevalent in most plant genomes. They are ubiquitous, in high copy numbers, evenly distributed in the genome, in both hetero- and euchromatin, and show insertional polymorphism both within and between species. In wheat, TEs account for more than 80% of the genome. From a quantitative perspective, it is easy to see that they are the most significant factors in determining the genome structure. It is also likely that TEs have driven wheat genome evolution in diverse ways, including genome expansion and contraction, segmental duplication, and exon shuffling. Therefore, TE-based molecular markers represent ideal tools to study the structure and evolution of the hexaploid wheat genome. We have recently demonstrated the potential of using BAC-end sequences from specific wheat chromosomes for developing TE-based markers. The Insertion Site-Based Polymorphism (ISBP) markers are based on the PCR amplification of a sequence spanning a junction between a TE and a flanking sequence using specific primers designed in both sequences. Since TEs are evenly distributed, ISBP markers have no distribution bias along wheat chromosomes. Moreover, they are adapted to a wide range of detection techniques and show a high level of inter-and intraspecific polymorphism even in the elite wheat pool. These features make ISBPs very powerful tools for genomics analyses in wheat. Examples of their applications for cytogenetic, genetic and physical mapping, evolution and phylogenetic studies, recombination and linkage disequilibrium analyses as well as marker-assisted selection will be presented.

P098

A rice centromeric sequence is conserved between wheat and rice, as well as between monocots and dicots

Qi LL, Friebe B, Gill BS

Wheat Genetic and Genomic Resources Center, Department of Plant Pathology, Kansas State University, Manhattan, KS 66506-5502, USA

The rice centromeric clone 6730.t11, located in the kinetochore region of rice chromosome 8 centromere, was mapped to the centromeric regions of wheat group-7 chromosomes by Southern hybridization. PCR amplification with RT-PCR (Reverse Transcription-PCR) primer of 6730.t11 was conducted in genomic DNA isolated from Triticeae species, including *T. uratu*, *T. monococcum subsp. monococcum and subsp. aegilopoides*, *Ae. speitoides*, *Ae. tauschii*, barley, rye, and *H. villosa*; the rice cultivars (*O. sativa* subsp. *Japonica*) 'Nipponbare' and (*O. sativa* subsp. *Indica*) 'IRRB7'; maize; soybean; tomato; and *Arabidopsis*. A 211-bp sequence was amplified from Nipponbare, which showed 100% identity to the sequenced rice genomic DNA covered by the 6730.t11 RT-PCR primer. Of eight plasmid clones of PCR products sequenced from IRRB7, six had the same 211-bp sequence as found in Nipponbare. Two clones had a 202-bp sequence that shared 100 percent and 87 percent similarity in the first 38 and the last 72 nucleotides, respectively, with the 211-bp sequence amplified from Nipponbare. Surprisingly, the 202-bp sequence amplified from IRRB7 was found in all monocot and dicot species used in this study except Nipponbare. Sequence similarity ranged from 99% to 100% when compared to the 202-bp sequence in IRRB7. A PCR-amplified fragment from genomic DNA of Chinese Spring (CS) wheat using RT-PCR primer of the clone 6730.t11 was mapped to the same region as the clone 6730.t11 in wheat. The RT-PCR results from CS cDNA with primers of 6730.t11 indicated that the rice centromeric gene was expressed in wheat leaf tissue. Our data demonstrate

strong selection pressure for the conservation of genes in the kinetochore region, although their functional role remains to be established.

P099

Molecular phylogeny of a LTR copia retrotransposon family in *Triticum aestivum* reveals recent transposition activity

Ragupathy R [1,2], **Cloutier S** [2]
[1] *Dept.of Plant Science, FGS, University of Manitoba, Winnipeg, Canada R3T 2N2*
[2] *Agriculture and Agri-Food Canada, Cereal Research Centre, 195 Dafoe Road, Winnipeg, Canada R3T 2M9*

Retrotransposons play a significant role in evolution affecting both genome structure and function. In this study, we characterized a LTR copia retroelement family that was responsible for the duplication of the HMW-GS Bx7 gene. According to the 80/80/80 rule of transposable element naming convention, this element was novel and we named it Sasanda_EU157184-1. Screening of the high density filters of a hexaploid wheat BAC library with a LTR-specific probe identified ~1075 positive clones representing an estimated copy number of 334 elements per haploid genome. Among them, 242 clones showing strong hybridization signals were isolated. To ensure isolation of complete elements, screening with a RT domain probe identified 133 clones. Sequencing of the left and right LTR as well as the RT domain was carried out on this subset. Phylogenetic inference was obtained from a data set consisting of 101 RT, 100 left LTR and 104 right LTR sequences representing 233, 501 and 460 active sites, respectively. Neighbour Joining tree constructed using the Kimura-2 parameter method with a substitution rate of $2x10^{-8}$ /synonymous site /year indicated that the element is at least 1.2 to 1.8 million years old with at least seven sub-families. The insertion times of 89 complete elements estimated based on the divergence between their two LTRs also indicated bursts of amplification from 1.7 million years ago (MYA) to now. One member approximately dated 2.9 ± 0.4 MYA coinciding with the divergence of *Triticum* and *Aegilops* 3 MYA. Interestingly, in 49 elements, the left and right LTRs were identical indicating recent transposition activity. To our knowledge, this is the first report of a single element family in wheat genome with such a high number of complete elements that have not accumulated mutations in the LTRs. The retroelement can be used to develop markers like SSAP, REMAP and IRAP which are ideal for genotyping studies.

P100

Flavanone 3-hydroxylase genes in *Triticum aestivum* L.

Khlestkina EK [1], **Röder MS** [2], **Salina EA** [1]
[1] *Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, 630090, Russia, e-mail: khlest@bionet.nsc.ru,*
[2] *Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Corrensstr. 3, D-06466 Gatersleben, Germany*

PCR-based cloning of *F3H* (flavanone 3-hydroxylase) genomic sequences from hexaploid wheat (*Triticum aestivum* L.) and its putative diploid progenitors was used as a platform for further localization, genetic mapping and expression analysis of four *F3H* gene copies in *T. aestivum*, based on development and exploitation of gene copy-specific PCR primers. Three homoeologous *F3H* gene loci were mapped on the long arms of homoeologous group 2 chromosomes closely linked to microsatellite loci *Xgwm0877* or *Xgwm1067*, whereas one non-homoeologous locus (*F3H-2B2*) was mapped about 40 centimorgans distal, on chromosome 2BL. DNA sequence of *F3H-2B2* differs significantly from those of the other three genes, which is accompanied by the silencing of this copy in wheat anthocyanin-colored coleoptiles in contrast to active expression of the three homoeologs. Co-location of wheat *Rc-1* (red coleoptiles) genes and loci regulating expression of the three *F3H* homoeologs on chromosomes 7B and 7D was shown. Quantitative examination of temporal

expression of the three homoeologous *F3H* genes in genotypes carrying different homoeologous dominant *Rc-1* alleles was performed. No significant functional difference between homoeologous *F3H* genes was found whereas significant distinction of the total *F3H* expression levels in genotypes carrying different homoeologous dominant *Rc-1* alleles was observed. Overall, no genome-specific relationships between wheat regulatory and structural homoeologous genes were found in the present study.

P101

Composition and location of wheat BAC sequences marked by *Aegilops Speltoides* subtelomeric repeats

Salina EA[1], Sergeeva EM[1], Adonina IG[1], Shcherban AB[1], Belcram H[2], Huneau C[2], Chalhoub B[2]

[1]*Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia,* [2]*Organization and evolution of plant genomes, Unité de Recherche en Génomique végétale (URGV), Evry, France.*

Telomeric and subtelomeric regions are essential for genome stability and faithful chromosome replication. We characterized wheat BAC clones containing Spelt1 and Spelt52 sequences that are the members of subtelomeric tandemly repetitive families of wheat B/G genomes and *Aegilops* section *Sitopsis*. Screening of the *Triticum aestivum* BAC library with Spelt1 and Spelt52 probes was performed. Totally 9 clones were isolated; one of them, clone 2050O8 was localized mainly on the distal parts of wheat and *Aegilops* chromosomes by *in situ* hybridization. The distribution of other clones pointed to the presence of different types of repetitive sequences in BACs. The clone 2050O8 was sequenced and the obtained sequence 119,737 bp was annotated. The sequence consist of about 32.1% of transposable elements (TE), 11.6% Spelt52 and other non-TE-repeats and 6.9% non-TE-related genes. DNA transposons are predominant and make up 24.3% of entire BAC-clone, whereas retroelements account for only 7.8% clone length. Full-length CACTA transposon Caspar covers 11,667 bp, encoding a transposase and CTG-2 proteins, alone accounts for 40 % of the DNA transposons. Comparing the data of *in situ* hybridization of clone 2050O8 and its fragment with BLAST search against wheat mapped EST contigs, we established the location of 2050O8 on the end of 4BL. Based on the degree of sequence conservation and results of *in situ* hybridization with transposase and CTG-2 sequences as probes, the CACTA transposon Caspar is shown to tend to accumulate in distal regions of *Triticum* and *Aegilops* and its divergence correlates with the cereal genomes evolution.

P102

Cloning and characterization of small RNAs from wheat (*Triticum aestivum* L.)

Sun Qixin, Yao Yingyin, Ni Zhongfu, Guo Ganggang Du Jinkun

Department of Plant Genetics and Breeding, China Agricultural University, Beijing, 100094, China

Endogenous microRNAs (miRNA) and small interfering RNAs (siRNAs) are emerging as important regulators of eukaryotic gene expression by guiding mRNA cleavage, translational inhibition, or chromatin modification. Up to date, identification of small RNAs has been limited to a few model plant species. Here, to identify miRNAs and siRNAs in wheat (*Triticum aestivum* L.), which is one of the most important cereal crops worldwide, a small RNA library was constructed. By 454 high throughput sequencing, we identified 58 miRNAs comprising 43 miRNA families. Thirty five miRNAs belong to 20 conserved miRNA families. Remaining 23 miRNAs are novel in wheat and more importantly, 4 of these new miRNAs appear to be monocot-specific. Northern blot analysis indicated that some of the new wheat miRNAs are preferentially expressed in certain tissues. Based on sequence homology, we predicted 46 potential targets. Thus we have identified a large number of

monocot-specific and wheat-specific miRNAs. These results indicated that both conserved and wheat-specific miRNAs play important roles in wheat growth and development, stress responses and other physiological processes.In addition to miRNA, a total of 2076 siRNAs were also identified. Most of them can be mapped to non-coding genes, whereas, a substantial part originated from the CDS region of protein-coding genes. This study also led to the discovery of 5 nat-siRNAs, which were experimentally confirmed. We further showed that 4 wheat siRNAs were strongly responsive to various stress treatments, such as cold, heat and salinity. In summary, this study provides a first large scale cloning and characterization of wheat miRNAs, siRNAs and their predicted targets, which can serve as a foundation for future functional studies.

P103

Variation of repetitive elements in sibling wheat cultivars containing 1BL.1RS translocation

Tang ZX[1], Fu SL[1], Ren ZL[1,2], Zhang HQ[1]

[1]State Key Laboratory of Plant Breeding and Genetics, Sichuan Agriculture University, Ya'an, Sichuan 625014, China; [2]School of Life Science and Technology, University of Electronic Science and Technology of China, Chengdu 610054, China.

Three kinds of rye-specific repetitive DNA sequence pSc119.1, pSc20H and pAW161, from three sibling wheat cultivars containing 1BL.1RS translocation, were investigated by sequencing and alignment analysis. pSc20H and pAW161 are more conservative than pSc119.1. Nonrandom nucleotide substitution throughout the sequence pSc119.1 was found. Furthermore, the three cultivars were derived from a single F_4 wheat plant. PCR analysis using 210 wheat SSR markers indicates that the wheat A, B and D genomes have highly genetic identity among the three cultivars. We presume that the variation of repetitive DNA may be related to the variability for some agricultural traits among the three cultivars. Different repetitive elements may play different roles in genomic system. The results in this study have given better understanding of genetic variation of 1BL.1RS translocation and imply that the variation of different repetitive DNA sequences may be used as a new kind of molecular marker to select elite genotype in wheat breeding program.

P104

The relation of grain yield and quantitative traits in *Tritipyrum*, a new salt tolerant amphiploid, with *Triticale* and Iranian wheat

Tohidi-nejad E[1], Shahsavand Hassani H[1], Madani H[2] , Kamyab M[1]

[1]Bahonar university Kerman, Iran, [2] Islamic Azad University, Arak, Iran

Many different characteristic in breeding and selection programs, have considered by breeders to determine the potential of genetically different lines and cultivars. Tritipyrum, a new salt tolerant amphyploide, which is the third handmade cereal after triticale and triturdeum considered as cultivating in saline soils. To study the superior characters of this crop in comparison with triticale and wheat for cultivating in saline soil and to determine the relation ship between grain yield, its components and morphological characters, three statistical analysis including, XYZ, factor analysis and coefficient correlation were used. Cluster analyses also were used to classify cultivars and lines (13 Tritipyrum lines, 14 Iranian wheat cultivars, and 5 Triticale lines). Result revealed that the grain yield of all these three amphiploids correlated with chaff weight, biological yield, number of grain per spike, plant high and number of spike let per spike, also in tritipyrum it was positively correlated with flag leaf length, number of spikes and tillers. 6 factors were determined by using factor analysis of wheat and tritipyrum and 4 factors for triticale. These factors were corresponded to source and sink potential and the relation between them and plant height. Genotypes were classified by cluster analysis in 4 groups. A large amount of grain yield was for 2nd group which involves tritipyrum lines.

P105

Genomic and RNA divergences of *Revolver* transposon-like gene offer chromosome tags in Triticeae

Tomita M, Tanaka E
Molecular Genetics Laboratory, Faculty of Agriculture, Tottori University, Tottori 680-8553, Japan

Revolver is a new class of transposon-like gene composing the Triticeae genome[1,2]. By RT-PCR and TA cloning, *Revolver* cDNAs were obtained from self-fertile rye and several Triticeae species. The total lengths of the *Revolver* cDNAs are 665 to 723 bp, and they are classified into three sub-families wherein the regions of the second and third exons are almost identical, while the region of the first exon exhibits the low homology of 60% among the families due to duplication or deletion. Such a length divergence of *Revolver* is effective to develop rye chromosome markers. PCR was performed using the 3'-flanking region of *Revolver*-2 as a single primer, and 4 types DNA (2.3 kb, 2.8 kb, 3.3 kb and 4.3 kb) were amplified from the rye genome, but nothing was amplified from the wheat genome. These variants assumed to be non-autonomous elements of *Revolver* have the downstream region of the second intron, but they have structural modifications at the 5' side and first exon region as in the cDNAs. Moreover, when PCR was performed with the same primer using rye chromosome addition wheat lines, 4 types DNA were recovered from 1R, 5R, 6R and 7R addition lines, respectively. By the PCR primers comprising the sequences flanking to each element of *Revolver* scattering in the genome, the chromosome on which each *Revolver* is located can be determined or tagged. Furthermore, an anchored AFLP approach, called as Sequence-Specific Amplification Polymorphism (SSAP) or transposon display, was applied to exploit the variation in the sequences flanking the insertion site of *Revolver* and to establish *Revolver*-based rye DNA markers in a wheat background. The regions between *Revolver* elements and adjacent *Eco*RI-cleaved host sites were amplified by a *Revolver* primer labeled with fluorescent dye and an *Eco*RI adaptor primer. The SSAP autoradiograph from an inbred rye and callus derived from it showed numerous DNA fragments including a few callus-specific SSAP, which means mobilization of *Revolver*.

1. Tomita, M. et al. (2008) DNA Research 15:49-62, 2. Tomita, M. (2008) US patent 20050091710